Independent Expert Scientific Committee
on Coal Seam Gas and Large Coal Mining Development

Australian Government
Department of the Environment and Energy

Research to inform the assessment of ecohydrological responses to coal seam gas extraction and coal mining

This report was commissioned by the Department of the Environment and Energy on the advice of the Independent Expert Scientific Committee on Coal Seam Gas and Large Coal Mining Development (IESC). It was prepared by researchers from the Australian Rivers Institute, Griffith University, the Connected Waters Initiative Research Centre, University of New South Wales, the Murray-Darling Freshwater Research Centre, La Trobe University, Subterranean Ecology Pty Ltd, The University of Queensland, the Centre for Ecosystem Management, Edith Cowan University and Yorb Pty Ltd.

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Contents

Abbreviations

General abbreviations	Description
CDP	Cellulose decomposition potential
CSG	Coal seam gas
CSGCM	Coal seam gas and coal mining
CTFR	Cotton tensile force ratio
Dbh	Diameter at breast height
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EIS	Environmental Impact Statement
EPBC Act	Environment Protection and Biodiversity Conservation Act 1999
FDOM	Fluorescent dissolved organic matter
FPOM	Fine particulate organic matter
GDE	Groundwater dependent ecosystem
GDV	Groundwater dependent vegetation
HEF	Hydrologic exchange flows
IESC	Independent Expert Scientific Committee on Coal Seam Gas and Large Coal Mining Development
K	Decay rate
LMM	Linear Mixed-effects Model
NDVI	Normalised Difference Vegetation Index
NDVW	Normalised Difference Wetness Index
NSW	New South Wales
MLR	Multiple Linear Regression
OM	Organic matter
OWS	Office of Water Science
PARAFAC	Parallel Factor Analysis (here applied to fluorescence data)
Qld	Queensland
RS	Remote sensing
RV	Remnant vegetation
TDS	Total dissolved solids
VHH	Vertical hydraulic head difference
y	Year

Glossary

Term	Description
Adventitious Roots	Roots that arise from an organ other than the root, usually a stem and sometimes a leaf. Common in plants that are subject to flooding, to improve oxygen exchange.
Aerenchyma	Spongy tissue with large air spaces found between the cells of the stems and leaves of aquatic plants to allow circulation of gases.
Allochthonous	Having an external or terrestrial (for freshwater ecosystems) origin.
Anthropogenic	Relating to, or resulting from, the influence of humans on nature.
Aquatic autotrophs	Organisms that generate organic compounds from inorganic material [generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis)] in streams, rivers, lakes, and wetlands.
Aquatic ecosystems	Are those that depend on flows, or periodic or sustained inundation/waterlogging for their ecological integrity (e.g. lakes, wetlands, rivers, aquifers, saltmarshes and estuaries) but do not generally include marine waters.
Aquatic macroinvertebrate	Organisms without backbones, which are visible to the eye without the aid of a microscope, and that live on, under, and around rocks and sediment on the bottoms of lakes, rivers, and streams.
Aquifer	Rock or sediment in formation, group of formations or part of a formation, that is saturated and sufficiently permeable to transmit quantities of water to wells and springs.
Assemblage Beta Diversity (BD)	Variability in assemblage composition among sampling units for a given area.
Autochthonous	Having an internal origin (autotrophic production in freshwater ecosystems).
Autotroph	Organism that produces complex organic compounds from simple substances present in its surroundings, generally using energy from light or inorganic chemical reactions.
Baseflow	The portion of streamflow not influenced by precipitation or overland flow but from deep subsurface flow paths and delayed shallow subsurface flow.
Biodiversity	Variation of life at all levels of biological organisation (molecular, genetic, species, and ecosystems) within a given area.
Biofilm	A diverse assemblage of bacteria, fungi, and algae, which exists in complex, three-dimensional matrices attached to benthic and other substrates.
Biogeochemistry	The chemical, physical, geological, and biological processes and reactions that shape the composition of the natural environment (in this report mainly referring to the hydrosphere).
Canopy cover	The proportion of the forest floor covered by the vertical projection of the tree crowns from the trees in upper vegetation layer within a forest or woodland.
Capillary fringe	Part of the unsaturated zone where groundwater is rising up from the water table by capillary forces.
Chlorophyll a	A green pigment, present in all green plants and in cyanobacteria, which is responsible for the absorption of light to provide energy for photosynthesis.
Consumer	An organism that has obtained resources (i.e. energy) from other organisms.
Crenophile	An organism preferring spring environments and unlikely to persist elsewhere.
Cumulative impacts	Combined effects of multiple, potentially interacting threats.
Cumulative risk assessment	Analysis, characterization, and quantification of the combined risks to the environment from multiple stressors.
Dendrometer	A device for monitoring variations in the girth of a tree trunk, usually a steel band that fits around the trunk and measures changes in stem diameter, either manually or automatically.
Denitrification	The microbial transformation of nitrate (NO_3^-) and nitrite (NO_2^-) by reduction to gaseous molecular nitrogen ($N_2(g)$).
Diatoms	Algae with distinctive, transparent cell walls made of silica.
Diel	Of or relating to a 24-hour period, especially a regular daily cycle, as of the physiology or behaviour of an organism.
Discharge spring (also referred to as 'artesian' spring)	Groundwater outflow from confined underground aquifers in which the water has had an extremely long residence time and where the water reaches the ground surface via faults in the strata overlying the aquifer or from thin or exposed parts of the aquifer.
Dissolved organic matter	A broad classification for organic molecules of carbon, hydrogen, nitrogen, and phosphorus with varied origin and composition within aquatic systems. A practical definition of dissolved is all substances that pass through filter pores below 0.45 micrometers diameter.
Diversity	See 'Taxonomic diversity'
Drought avoiders	Plants that use adaptive strategies to avoid drought.
Drought tolerators	Plants that have developed mechanisms that allow them to tolerate dry conditions.
Dry gravel bed	An area of coarse sediment and gravel, usually within the parafluvial zone.

Term	Description
Ecohydrology	Study of the availability and movement of water through the environment and ecosystems. See also hydroecology.
Ecology	The branch of biology that deals with the relations of organisms to one another and to their physical surroundings.
Ecosystem functions	The biological, geochemical and physical processes that take place within an ecosystem.
Ecosystem services	Are the 'services' provided by natural (and semi-natural) ecosystems - 'ecosystem service providers' - that benefit, sustain and support the well-being of people.
Endemism	See 'Taxonomic endemism'
Ephemeral stream	A category of temporary stream in which water is present only after unpredictable rainfall and runoff. Surface water dries within days to weeks of filling and can support only short-lived aquatic life (adapted from Boulton et al. 2014).
Epigean	Meaning 'of the surface' (terrestrial organisms or habitats)
Epilithon	Freshwater organisms attached or clinging to stones and rocks.
Floodplain	The area of flat land adjacent to a stream or river that floods during periods of high river overbank flows.
Floristics	The branch of phytogeography concerned with the study of plant species present in an area.
Flow path	The route that water passes through in surface (including organic soil horizons) and subsurface environments.
Flow regime	The flow regime of flowing water systems has five components: magnitude, frequency, duration, timing and rate of change. Modification of flow has cascading effects on the ecological integrity of streams (Poff et al. 1997).
Food web	A diagram of the trophic links among species in an ecosystem – essentially who eats what.
Geomorphology	The study of the physical features of the surface of the earth and their relation to its geological structures.
Graminoid	Herbaceous plant with a grass-like morphology usually from the plant families Poaceae, Cyperaceae and Restionaceae.
Groundwater	Water occurring naturally below ground level (whether in an aquifer or other low-permeability material), or water occurring at a place below ground that has been pumped, diverted or released to that place for storage. It does not include water held in underground tanks, pipes or other works.
Habitat	An ecological or environmental area that is inhabited by a particular species. It is the natural environment in which an organism lives, or the physical environment that surrounds (influences and is utilized by) a species population.
Humic	Produced by biodegradation of dead organic matter, humic substances are the major organic constituents of soil (humus), peat and coal. humic substances are also a major organic constituent of many upland streams, dystrophic lakes, and ocean water.
Hydrochemistry	Refers to the resulting chemical composition of water due to chemical, physical, and biological processes (often used interchangeably with 'water quality').
Hydroecology (ecohydrology)	The simplest definitions of ecohydrology and hydroecology consider either: (1) the scientific overlap between the fields of hydrology and ecology or (2) the impact of hydrology on ecosystems or *vice versa*. See also ecohydrology.
Hydrogeology	The branch of geology dealing with the waters below the earth's surface and with the geological aspects of surface waters
Hydrology	The study of the movement, distribution and quality of water on Earth and other planets, including the hydrologic cycle, water resources and environmental water allocations.
Hydrotype	Functionally similar plant species with shared requirements for and adaptions to specific hydrological conditions.
Hyporheic zone	The portion of the groundwater interface in streams where a mixture of surface water and groundwater can be found.
Hyporheos	The assemblage of organisms (including micro-organisms) which inhabits the hyporheic zone
Immobilisation	The incorporation of inorganic and organic matter (carbon and nutrients) into living tissue.
Intermittent stream	A category of temporary stream with alternate flowing and non-flowing periods that may not be seasonal.
Invertebrates	Multicellular animals without backbones (vertebral column). Includes terrestrial and aquatic animals such as insects, crustaceans and arachnids (all arthropods), as well as molluscs, annelid worms, nematodes, jellyfish, sponges and corals. Does not include unicellular microorganisms such as protozoans and bacteria.
Isotope	A chemical element having the same atomic number as another (i.e. the same number of nuclear protons), but having a different atomic mass (i.e. a different number of nuclear neutrons).
Lacustrine	Relating to a lake.
Leaf stomata	Pores found in the epidermis of plant leaves that allow the exchange of water and gases.
Littoral zone	The part of a sea, lake or river that is close to the shore.
Lotic	Relating to running or flowing waters, as compared with standing (lentic) waters.

Term	Description
Mineralisation	The conversion of organic matter to inorganic matter by abiotic and biotic processes.
Mixing model	Mathematical models with fixed and random effects to determine the proportions of various sources in a mixture. Commonly used in stable isotope analysis.
Morphology	The physical form and structure of an organism or one of its parts.
Morpho-species, morpho-taxon	A species (or taxon) distinguished from others only by its morphology. Typically applied during para-taxonomic sorting and identification, for specimens in unsuitable condition for the observation of species level characters, or for organisms where the current state of taxonomic knowledge is insufficient or unknown.
Multi-dimensional scaling	Mathematical multivariate (ordination) technique for visualizing the level of similarity of individual cases or objects of a dataset using a number of variables.
NOx	The sum of nitrate (NO_3^-) and nitrite (NO_2^-) concentrations.
Organic matter processing	The biological (i.e. consumption) and physical (i.e. fragmentation due to flowing water) degradaton of organic substances.
Palustrine	Relating to a small wetland that is non-riverine or non-channel and can be vegetated throughout. They include billabongs, swamps, bogs, springs, soaks.
Parafluvial zone	The area of the bankfull channel that is usually scoured annually by flooding.
Parataxonomy	Sorting of specimens into recognizable taxonomic units (e.g. higher taxonomic levels such as family or order, or morpho-species) based upon morphology, without the use of taxonomic keys or expert knowledge.
Particulate organic matter (POM)	Material of plant or animal origin that is suspended in water (e.g. leaves, sticks, bark). May be further defined as coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM).
Perennial stream	A stream with continuous flowing water for the majority (e.g. 90%) of the time.
Periphyton	Freshwater organisms (generally confined to small autotrophs) attached or clinging to submerged substrata in an aquatic ecosystem.
Phreatophyte	Plants that habitually obtain water from groundwater, via the zone of saturation, either directly or through the capillary fringe above the water table.
Phylogenetic diversity (PD)	A measure of diversity based on units of phylogenetic variation (instead of taxa). Areas with high PD may represent centres of current speciation and may be important areas to protect for maintenance of evolutionary processes. High PD could arise by having a high number of closely related taxa or by having few taxa that are phylogenetically divergent from one another.
Phylogenetic endemism (PE)	A measure of the degree to which elements of evolutionary history are spatially restricted in space. PE combines the phylogenetic diversity (PD) and taxonomic endemism (TE) measures to identify areas to which substantial components of phylogenetic diversity are restricted.
Phylogeny	Branching diagram or "tree" showing the inferred evolutionary relationships among various taxa based upon similarities and differences in their physical or genetic characteristics
Plant life history	The longevity and reproductive traits of plants (e.g. annuals, perennials, ephemerals)
Pool	A slow moving, often stagnant section of a stream or river, with deeper water than adjacent habitats.
Primary producers	Organisms in an ecosystem that produce biomass from inorganic compounds. In almost all cases these are photosynthetically active organisms.
Recharge spring (also referred to as 'non-artesian' spring)	Groundwater outflow from overflow from unconfined aquifers (generally associated with outcropping sandstones). The water generally has a relatively short residence time compared with confined underground aquifers (except in western GAB) and water flow rates are affected by recent rainfall events.
Resilience	Capacity of a species (or other entity) to recover from disturbance.
Resistance	Capacity of a species (or other entity) to withstand a disturbance.
Rheophilous	Having an affinity for, or thriving in, flowing water.
Riffle	A shallow section of a stream or river with rapid current and a surface broken by gravel, rubble or boulders.
Riparian	From the Latin word meaning of or belonging to the bank of the river, and refers to the biotic communities living along streams. The riparian zone can be thought of as the interface or ecotone between terrestrial and aquatic systems.
Sclerophyll	Plants with hard, leathery, evergreen foliage that is specially adapted to prevent moisture loss and cope with low soil fertility (e.g. Eucalyptus).
Seston	Microorganisms and non-living matter swimming or floating in a water body.
Soil structure	The arrangement of the solid parts of the soil and of the pore space located between them. It is determined by how individual soil granules clump, bind together and aggregate.
Soil texture	The relative proportion by weight of the mineral soil (% sand, silt, and clay) for soil particles <2mm.
Species	A taxonomic rank that is the basic rank of biological classification. Commonly, a group of organisms capable of interbreeding and producing fertile offspring.

Term	Description
Species richness	The total number of species in a given area or sample, the most commonly used measure of biodiversity.
Spring	A vent or vents where the natural groundwater outflow forms a single spring wetland. See also 'watercourse spring'.
Spring complex	Collection of springs all related to a particular location and hydrogeological feature(s).
Spring group	Multiple springs all related to the same hydrogeological structure and probably the same aquifer (or groundwater source) and hydrochemistry (at the present time).
Stable isotope	An isotope that does not decay into other elements. An isotope of an element has the same number of protons as another isotope of that element, but a differing number of neutrons. Stable isotope analysis can be used in ecological studies to trace chemical movement through the environment.
Streambed	The channel bottom of a stream, river or creek; the physical confine of the normal water flow.
Stygo/ Stygal	Prefix referring to groundwater habitats or organisms.
Stygobite	Stygobites are specialised obligate subterranean aquatic species (or taxa) that occur in all kinds of groundwater habitats, and sometimes may be found very close to the surface including the hyporheic zone.
Stygofauna	Aquatic fauna inhabiting the various types of groundwater. The term 'stygofauna' embraces, implicitly, three categories (stygobites, stygophiles, stygoxenes) which can be used to further define aquatic subterranean fauna.
Stygomorphy	Any morphological, physiological, or behavioural feature that characterizes aquatic subterranean taxa. Common (stygo-)morphologic traits include: reduction of eyes, pigment, elongation of appendages, enhancement of non-optic sensory structures.
Stygophile	Collectively, facultative subterranean aquatic species (or taxa) which can complete their life-cycles either underground or in epigean habitats. Hypogean and epigean populations may simultaneously exist, and individuals may be able to move between them.
Stygoxene	Collectively, aquatic species (or taxa) that occur incidentally or 'accidentally' in subterranean habitats. They generally cannot complete their entire life-cycles underground, and individuals may frequently move between the surface and underground habitats for foraging and sheltering, or to complete part of their reproductive cycles.
Supergroup	Major regional clusters of spring complexes with some consistent hydrogeological characteristics.
Supra littoral	The landward zone adjacent to the littoral zone that only very occasionally is flooded.
Taxa	(Singular taxon) is any taxonomic group or rank, generally used as a grouping of organisms that belong to different species but the same family or genus.
Taxonomic diversity (H')	An index that incorporates the number of taxa and the evenness of the distribution of individuals across species.
Taxonomic endemism (TE)	An index of endemism identifying areas where taxa with restricted ranges are concentrated. This index ranges from one, where all taxa in a given area have broad geographical ranges, to infinity, with large values indicating the presence of taxa with range-size rarity (i.e. areas with high endemism).
Taxonomic richness (S)	Number of taxa in a given area (also referred to as alpha diversity).
Taxonomy	The scientific classification of organisms into named groups based either on shared characteristics or on evolutionary relationships.
Temporary stream	A stream that flows less than 90% of the time.
Therophytes	Plants that complete their life cycle in a short period when conditions are favourable and survive harsh conditions as seeds.
Tree basal area	The area of a given section of land that is occupied by the cross-section of tree trunks and stems at the base.
Typology	A scheme for categorising entities into distinct groups such that those entities belonging to a type share common attributes, but in combinations that differ from other types.
Vent	Point of natural groundwater discharge at the ground surface.
Vertisols	Clay-rich soils that shrink and swell with changes in moisture content. During dry periods, the soil volume shrinks, and deep wide cracks form. The soil volume then expands as it wets.
Water quality	The physical, chemical and biological attributes of water that affect its ability to sustain environmental values.
Water use efficiency	A measure of a plant's capacity to convert water into biomass that includes the use of water stored in the soil, groundwater and rainfall during the growing season.
Watercourse spring	A section of a watercourse where groundwater from an aquifer enters the stream through the streambed. This includes waterholes and flowing sections of streams dependent on groundwater. Sometimes also referred to as a baseflow-fed section of a watercourse.
Watertable	The upper surface of a body of groundwater occurring in an unconfined aquifer. At the watertable, pore water pressure equals atmospheric pressure.

Term	Description
Xylem	Specialized plants cells for the transport of water and dissolved minerals from the roots to the leaves.
δ^{13}C	A measure of the ratio of stable isotopes ^{13}C: ^{12}C, reported in parts per thousand (per mil, ‰).
δ^{15}N	A measure of the ratio of stable isotopes ^{15}N: ^{14}N, reported in parts per thousand (per mil, ‰).

Executive summary

Background

In 2012, the Australian Government established an Independent Expert Scientific Committee on Coal Seam Gas and Large Coal Mining Development (IESC) to provide scientific advice to government regulators on the impacts that coal seam gas (CSG) extraction and large coal mining development may have on Australia's water resources. The IESC is supported by the Office of Water Science (OWS) within the Australian Government Department of the Environment and Energy. The IESC's Information Guidelines (IESC 2015) outline the information requirements considered necessary by the IESC to enable delivery of robust scientific advice to government regulators on the water-related impacts of CSG and large coal mining developments.

This project was commissioned by the OWS to undertake research to address critical ecology research priorities concerning ecohydrological responses to CSG extraction and coal mining. The ecology research priorities were developed by the OWS (under their Ecosystems and Water Research theme) in consultation with the IESC and informed by a workshop "Modelling water-related ecological responses to coal seam gas extraction and coal mining" (Commonwealth of Australia 2015[1]). This project delivers an integrated programme of research that addresses key elements within each of the four major components identified in the OWS ecology research priorities. The project was undertaken by a collaborative, interdisciplinary team of Australia's leading freshwater researchers. The project was hosted by Griffith University's Australian Rivers Institute, in partnership with the University of New South Wales, Edith Cowan University, Subterranean Ecology Pty Ltd and Yorb Pty Ltd.

Research aims and project structure

The aim of this project, *Research to inform the assessment of ecohydrological responses to coal seam gas extraction and coal mining*, was to provide information to support assessments of the water-related impacts of coal seam gas (CSG) extraction and coal mining. The project design focussed on developing a quantitative understanding of ecological responses across gradients of surface-groundwater interaction as a basis to understand and predict potential consequence of groundwater drawdown associated with CSG extraction and coal mining.

The program is constructed around four major research Components:

Component 1 – *Ecohydrology of groundwater dependent terrestrial vegetation. (Short title: Groundwater dependent vegetation).*

> Objectives - to understand the impacts of altered groundwater regimes on groundwater-dependent vegetation in different biophysical settings.

Component 2 – *Ecological values of baseflow and surface water-groundwater connectivity regimes in non-perennial streams. (Short title: Baseflows).*

> Objectives - to quantify and describe the ecological values of non-perennial waterways, the contributions from groundwater in maintaining those values, and the potential threats to those values resulting from altered surface-groundwater connectivity, including the ability for systems to recover from periods of altered groundwater-surface water connectivity.

Component 3 – *Hydrogeochemistry, biogeochemical processes and the hyporheos / stygofauna (Short title: Subsurface ecology).*

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[1] Commonwealth of Australia (2015). *Modelling water-related ecological responses to coal seam gas extraction and coal mining.* Report prepared by Auricht Projects and CSIRO for the Department of the Environment, Commonwealth of Australia, Canberra.

Objectives - to understand how changes in groundwater-surface water connectivity, hyporheic exchange and biogeochemical conditions affect the ecological conditions for hyporheos and stygofauna.

Component 4 – *Biodiversity and risk assessment of Great Artesian Basin spring wetlands (Short title: GAB springs).*

Objectives - to conceptualise and quantify the environmental determinants of GAB spring wetland species composition and biodiversity patterns and how resident species respond to cumulative impacts of groundwater drawdown and other threats.

Report Structure

This report begins with a brief context-setting description of the range of hydrological conditions within streams that are likely to be impacted by CSG extraction and coal mining activities. We also present a basic conceptual synthesis of the role that water plays in different stream ecosystems at different temporal and spatial scales. The subsequent sections of the report are focused on the four major research components. A summary of the rationale, approaches used, and key findings for each research component is presented below.

Component 1 – Groundwater dependent vegetation

The role of groundwater in supporting vegetation is poorly understood and therefore so are the potential impacts of a reduction in groundwater availability. We aimed to understand the characteristics of plants that utilise groundwater so we can predict plant responses to groundwater availability. We used a combination of on-ground measurements of plant communities and remote sensing of vegetation patterns. We characterised vegetation communities as a mix of plant functional types (hydrotypes) that emphasises the plant-hydrology linkages relevant in recognising the likelihood of groundwater use. In most landscapes as depth to groundwater increases and therefore accessibility decreases, the proportion of hydrotypes in the plant community adapted to wet conditions (and the number of phreatophytes) will decline. The typology provides us with a convenient first step in identifying the water sources important to maintaining vegetation.

We provide a decision tree for broadly categorising plants within a community into hydrotype functional groups, and identifying potential phreatophytes. The decision tree uses life history traits (annual or perennials) and life form (woody or herbaceous) as well as habitat, depth to groundwater, duration of inundation and drought adaptation to identify the different hydrotypes. Consequently, this type of approach can be used to assess potential ecological impacts of groundwater drawdown as a result of coal seam gas (CSG) and coal mining developments in a variety of biophysical settings.

Our results suggest that it is possible to predict, for management purposes, the importance of groundwater to a given plant community. This prediction can be based on a number of easily observable factors including landscape position, climate, soil, geology, groundwater depth and the proportions of plant hydrotypes within a plant community. The development of this typology will enable measurement of the proportions of different hydrotypes within a habitat, which will provide an indication of the degree of reliance on groundwater. Measurements of plant hydrotypes, stem diameter changes and water isotopes ($\delta^{18}O$ and $\delta^{2}H$) of river red gum trees, in riparian areas and on the floodplain, provides multiple lines of evidence that are strongly suggestive that where groundwater is close to the surface, these trees are utilising groundwater for at least some of their water requirements.

We also developed and applied a web-based toolset to support the analyses of remote sensing imagery to model relationships between vegetation greenness, climate data and groundwater level dynamics. This toolset adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. It was implemented in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications. The analysis showed that in study areas the NDWI plant wetness index is more sensitive to the variability in depth to groundwater than the NDVI plant greenness index. However, both remote sensing indices are substantially lower where groundwater is deep. This remote sensing analysis can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation. Small-scale on-ground data can also be used to ‘ground-truth’ these remotely sensed data. The combination of on-ground studies and remote sensing will provide a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales.

Component 2 – Baseflows

Reduced baseflow permanence can have a destabilising effect on stream ecosystems by reducing algal biomass, by altering the breakdown rates of organic matter, and through the elimination of top predators. We examined the effects of reduced baseflow permanence on a range of ecological indicators by sampling in wetter and drier periods and among streams with varying degrees of intermittency. We quantified organic matter processing (leaf litter and cellulose breakdown rates), the cycling of carbon and nitrogen through food-webs (using stable isotopes of nitrogen and carbon), and the composition of benthic macroinvertebrate and fish assemblages. Processing of organic matter was consistently greater in hyporheic than surface habitats, and both leaf and cotton processing was greater in surface habitats subject to persistent saturated conditions. Algae were an important energy source for aquatic consumers in wetter periods, with no evidence of reliance on sampled food sources in drier periods. The intermittent streams studied contained a diverse biotic assemblage of fish and macroinvertebrates, and continual subsurface baseflow appeared to be instrumental in sustaining these assemblages. Our findings support existing research challenging the commonly held assumption that intermittent streams do not contribute to local and regional aquatic biodiversity. Longer-term patterns in flow variability are an important predictor of fish assemblage structure. Streams with a lower discharge will likely be more intermittent and have low wetted habitat persistence and longitudinal connectivity, which may act as a broad-scale filter in determining fish species composition and assemblage structure. However, groundwater contributions to baseflow may play an important role in sustaining fish populations by buffering against periods of flow cessation by maintaining residual wetted habitats. Furthermore, our results highlight that intermittent river systems are important locations for detrital processes, and that the hyporheic zone sustains critical ecosystem processes even when surface flow-paths cease. Given the susceptibility of algae to drying, and the likely importance of continual subsurface baseflow for maintaining fish and macroinvertebrate communities, groundwater contributions to baseflow may be vital for sustaining stream ecosystem functioning and structure.

Component 3 – Subsurface ecology

The hyporheic zone (the shallow zone of saturated sediments beneath the beds of streams and rivers, where stream and groundwater interact) is a focal point of stream and groundwater hydroecological processes including the degradation of organic matter, biogeochemical cycling of organic carbon and nutrients, and a rich diversity of aquatic invertebrates (hyporheos). The taxonomic richness and functional diversity of groundwater ecosystems (including aquifers and hyporheic zone) are globally well known, and Australia is a global hotspot of groundwater diversity. However, the functional aspects and ecosystem services contributed by this remarkable hidden biodiversity are poorly understood. While stygofauna in aquifers is considered in current EIS policy and regulations in some Australian states, hyporheic ecosystems have to date been ignored. We sought to characterise hydrological, biogeochemical and detrital processes in the hyporheic zone, and to understand how variations in groundwater-surface water connectivity, hyporheic exchange and biogeochemical conditions influence the ecological conditions for hyporheos and stygofauna. We examined the effects of reduced surface and baseflow permanence by sampling in wet and dry periods and among streams of varying degrees of intermittency and perenniality. Multidisciplinary suites of methods were employed spanning hydrology, hydrochemistry, stream and groundwater ecology. A main objective was to integrate and synthesize the multidisciplinary findings into conceptual models to aid understanding of ecological responses to groundwater drawdown in streams due to environmental changes or human activities.

Our study confirmed that groundwater is essential for sustaining baseflow, hydroecological processes and ecosystem structure and function in surface and subsurface (hyporheic) habitats in both intermittent and perennial streams. We also confirmed that the hyporheic zone plays an important role in stream biogeochemical and detrital processes, including in intermittent streams when surface flows have ceased.

Biogeochemical processing of nutrients and organic carbon (OC), and redox hydrochemistry, were principally related to the interplay between groundwater discharge and hyporheic exchange gradients. Hyporheic downwelling and stream losing conditions were characterised by chemically reducing conditions. Hyporheic upwelling and stream gaining hydrochemical conditions were dependent on the length and/or residence time along hyporheic flow paths, as well as the influence of regional groundwater discharge mixing with hyporheic exchange flows. In the Maules Catchment, localised discharge of oxic regional groundwater exerted a strong influence on hyporheic biogeochemical processes by sustaining aerobic biogeochemical processes to a greater degree than would be expected if groundwater discharge was anoxic. Generally, we found that nitrate/ammonium ratios initially increased along hyporheic flow paths, presumably due to nitrification coupled to organic matter mineralisation, and then decreased as denitrification set in. As the length and/or residence time along hyporheic flow paths increased, hydrochemical conditions became more reduced and progressed from oxic to suboxic (DO < 0.5 mg/l and NO_3 < 0.5 mg/l) and then anoxic (DO < 0.5 mg/l) leading to reduced ionic species including ferrous iron and manganese. We found that the onset of iron-oxide reduction coincided with release of phosphorus and a reduction in nitrogen/ phosphorus ratios.

Our results strongly indicate that intermittent streams with groundwater baseflow component are ecologically important as they harbor a rich biodiversity in both surface and subsurface (hyporheic) habitats. Obligate groundwater invertebrates (stygobites) are an ecological group that are ‘signature’ indicators of baseflow connectivity between streams and permanent groundwater (aquifers), and they respond to changes in groundwater condition and quality (DO and redox state), hyporheic exchange and stream flow regime. In both the Bremer and Maules catchments we found a rich diversity of invertebrates in the hyporheic zone, including streams with only a thin alluvial cover (sediment depth < 1.0m) overlying relatively impermeable strata. Hyporheos assemblage structure and abundance were highly variable over small spatial scales, and at different time periods of sampling. In our study sites, oxic conditions, irrespective of flow regime class and hyporheic exchange direction, supported the existence of hyporheos. In both Bremer and Maules Catchments, suboxic conditions (DO < 0.5 mg/L) were associated with depletion of invertebrate richness and abundance, and invertebrate absence was correlated with severely hypoxic / anoxic conditions and reduced ionic species (especially iron). We found limited evidence of associations between hyporheic diversity and measures of organic carbon, namely negative correlations of richness and abundance with processed and unprocessed humics in the Maules catchment only.

Our study confirmed the fundamental relevance of the hydrological interconnectivity between streams and groundwater, and groundwater connectivity may confer resistance and resilience in stream ecosystems, the narrow vertical range of the hyporheic zone means that relatively small groundwater drawdown may have a locally significant impact on stream ecosystem function. Groundwater recession/drawdown reduces baseflow and changes hyporheic exchange flows, which may deplete ecological values and the hydroecological functioning of streams and associated groundwater-dependent ecosystems (GDEs). The very narrow vertical range (generally < 1 to 2 m depth) of the hyporheic zone means that only small amounts of groundwater drawdown may have a significant impact on the ecological functioning and baseflow connectivity of streams. Ecosystem assets and services that are potentially depleted by groundwater drawdown include biodiversity, detrital processes, and important biogeochemical processes mediated by microbes such as carbon and nutrient transformations. Conceptual models were useful for integrating and synthesizing the multidisciplinary findings to aid understanding of hydroecological responses in streams due to changes in groundwater regime.

Component 4 – GAB springs

Spring wetlands are a characteristic groundwater-dependent feature of the Great Artesian Basin and have great ecological, scientific and socio-economic significance. Due to prolonged isolation, many GAB springs contain rare and endemic species of plants and animals that have undergone significant local genetic differentiation and speciation. The springs are also important as drought refuge areas for much wildlife and as wetlands for migratory birds. However, development of the GAB over the past century by human activity has seen an overall decline in the flows from springs in many areas. The ecological consequences of groundwater decline for GAB springs and the resident biota may be substantial but difficult to detect and potentially difficult to reverse. Furthermore, impacts of groundwater drawdown could greatly increase vulnerability to other existing and future threatening processes such as land use, direct human modifications, livestock damage, introduced species and climate change.

Using available literature, ecological theory, existing databases, unpublished data and expert knowledge, we conceptualised and quantified the environmental determinants of GAB spring endemic species composition and biodiversity patterns (within the fish, molluscs, crustaceans and plants) and assessed how they respond to cumulative impacts of human activities and other stressors. We found that many species endemic to GAB springs have extremely restricted distributions, have very few populations within conservation reserves, and have high exposure to a range of threats. Many of these taxa are assessed as being highly vulnerable (i.e. low resistance and/or resilience) and therefore may be at substantial risk from threats associated with groundwater drawdown, introduced plants and animals and range of other stressors. We report which spring complexes are exposed to the greatest number and intensity of threats, how vulnerability to threats varies among taxa and which complexes are at the highest risk (i.e. contain a high number of vulnerable species and are exposed to the highest threat intensities). Our cumulative risk assessment for the subset of 76 spring complexes containing one or more endemic taxa revealed that the Pelican Creek complex (containing Edgbaston and Myross Springs) in the Barcaldine supergroup in Queensland was at substantially higher risk of threats than all others. This complex is exposed to all five threats with the highest cumulative intensity and contains 26 species with representatives from all four taxon groups that were vulnerable to all five threats. Thus, the Pelican Creek complex can be considered to be of extremely high conservation concern. Other complexes at relatively high risk included Moses complex (Barcaldine supergroup), Yowah Mud complex (Eulo supergroup), Dalhousie springs complex (Dalhousie supergroup) and 15 complexes from the Lake Eyre supergroup. We synthesise on-ground management and monitoring for GAB springs identified as being at highest risk. We also provide some views, informed by our assessment, on where GAB springs research and conservation management might focus.

These included:

- A need to improve our taxonomic understanding of GAB spring flora and fauna to help to preserve its evolutionary potential
- An opportunity to update conservation listings of GAB-dependent species based on our assessment which highlights the scientific basis for a re-evaluation of taxa belonging to the ‘community of native species dependent on natural discharge of groundwater from the GAB’ listed as ‘Endangered’ under the EPBC Act
- A need to broaden the focus of the biodiversity assessment to other organisms and increase the spatial resolution of the assessments to enable more effective conservation management of springs and their resident biodiversity
- A requirement to improve collaboration across research effort to facilitate identification of knowledge gaps and research priorities
- A focus on the ecology of species and their responses to threats to improve our ability to prioritise species and locations for conservation management
- The importance of strategic, coordinated, inclusive and committed management to address current and future threats to some GAB springs and their endemic species which are clearly at high risk
- Improved science to establish ecological responses to threats and benefits of actions
- A call to action as many on-ground management actions to mitigate threats can be implemented now – the high concentration of endemic species in relatively small areas means that it should be feasible to identify appropriate threat management strategies and successfully implement them.

Application of the research

The report includes a synthesis of potential applications of the research and a collated list of key findings for each research component. To facilitate uptake of the research, we evaluate the inferential strength and potential broader application of the research by outlining key considerations when transferring our findings to other situations and scales. We detail specific issues associated with transferability and scaling for each of the key ecological response themes examined in the project (relating to biogeochemistry, ecosystem processes, groundwater dependent vegetation, hyporheic fauna, macroinvertebrates and fish). We argue that our research findings, if appropriately applied, can strengthen the scientific basis for environmental assessments of new CSG or large coal mining development proposals (either greenfield or brownfield). We also outline how our methods and research findings can inform the development and implementation of monitoring programs aimed at assessing performance of proposed measures to mitigate potential impacts of CSG or large coal mining development. In particular, we highlight ways in which some of our findings and research methods offer potential for monitoring impacts of water-related threats associated with CSG extraction and coal mining.

Project outcomes

We expect that the methods and key findings from our research will be valuable for OWS, IESC, proponents of coal seam gas (CSG) or large coal mining development projects, the broader scientific community and other stakeholders. The expected outcomes from the project are:

- improved identification and understanding of the potential water-related ecological responses to coal seam gas extraction and coal mining in Australia
- information that will strengthen IESC advice on project proposals concerning the adequacy of methods of prediction, assessment and mitigation of impacts on:
  - subsurface biogeochemistry and water quality,
  - groundwater dependent vegetation,
  - ecological values of non-perennial streams (including ecosystem processes, and hyporehic and surface fauna)
  - GAB springs and the biodiversity they sustain
- strengthened regulator and industry understanding of:
  - the water-related requirements of, and impacts on, groundwater dependent vegetation
  - the ecological importance of groundwater discharge and surface water-groundwater connectivity regimes in non-perennial streams
  - the ecological relevance of subsurface fauna and biogeochemistry
  - the biodiversity and conservation values of GAB spring wetlands and the vulnerability of resident taxa to hydrological changes and other threats
- research that complements the bioregional assessments and other research activities coordinated by OWS that will collectively improve the knowledge base regarding the potential water-related impacts of CSG extraction and coal mining.
- a rigorous foundation for future research to fill critical research gaps.
- improved capacity to evaluate ecological and biodiversity values of an area subject to proposed mining development relative to elsewhere
- information for future updates to conservation listing of GAB discharge spring ecological communities (and individual species) by DoE Threatened Species Scientific Committee

1 Introduction

In 2012, the Australian Government established an Independent Expert Scientific Committee on Coal Seam Gas and Large Coal Mining Development (IESC) to provide scientific advice to government regulators on the impacts that coal seam gas (CSG) extraction and large coal mining development may have on Australia's water resources. The IESC is supported by the Office of Water Science (OWS) within the Australian Government Department of the Environment and Energy. The IESC's Information Guidelines (IESC 2015) outline the information requirements considered necessary by the IESC to enable delivery of robust scientific advice to government regulators on the water-related impacts of CSG and large coal mining developments.

The aim of this project, *Ecohydrological responses to coal seam gas extraction and coal mining*, was not only to provide information to support assessments of the water-related impacts of CSG extraction and coal mining but also to provide a case study of interdisciplinary working, discuss suitable field methods and analyses and provide guidance on integrated assessment of ecosystem responses and on application of methods and analyses in other catchments and regions.

The project builds on the outcomes of an expert workshop held in 2014 which considered approaches to ecological conceptual modelling that may assist assessment of the water-related ecological impacts of CSG extraction and coal mining development (Commonwealth of Australia 2015). Knowledge gaps and research questions identified by workshop participants informed the scope of the present study.

The research program has been designed to support assessments of CSG extraction and coal mining development proposals and does not directly examine the ecological impacts of particular existing or proposed developments. Instead, the experimental design focusses on developing a quantitative understanding of ecological responses across gradients of surface-groundwater interaction as a basis to understand and predict potential consequence of groundwater drawdown associated with CSG extraction and coal mining.

The program is constructed around four major research components focused in three study areas (Figure 1). Research components 1 – 3 are conducted in the Bremer River (south-east Queensland) and Maules Creek (upper Namoi River basin, central New South Wales, research component 4 is focused on the Great Artesian Basin (inland Australia). Each research component is introduced below.

Component 1 – Ecohydrology of groundwater-dependent terrestrial vegetation. (Short title: Groundwater dependent vegetation).

> Objectives - to understand the impacts of altered groundwater regimes as a result of CSG extraction and coal mining developments on groundwater-dependent vegetation in different biophysical settings.

Component 2 – Ecological values of baseflow and surface water-groundwater connectivity regimes in non-perennial streams. (Short title: Baseflows).

> Objectives - to quantify and describe the ecological values of non-perennial waterways, the contributions from groundwater in maintaining those values, and the potential threats to those values resulting from altered surface-groundwater connectivity, including the ability for systems to recover from periods of altered groundwater-surface water connectivity.

Component 3 – Hydrogeochemistry, biogeochemical processes and the hyporheos / stygofauna (Short title: Subsurface ecology).

> Objectives - to understand how changes in groundwater-surface water connectivity, hyporheic exchange and biogeochemical conditions affect the ecological conditions for hyporheos and stygofauna.

Component 4 – Biodiversity and risk assessment of Great Artesian Basin spring wetlands (Short title: GAB springs).

> Objectives - to conceptualise and quantify the environmental determinants of GAB spring wetland species composition and biodiversity patterns and how resident species respond to cumulative impacts of groundwater drawdown and other threats.

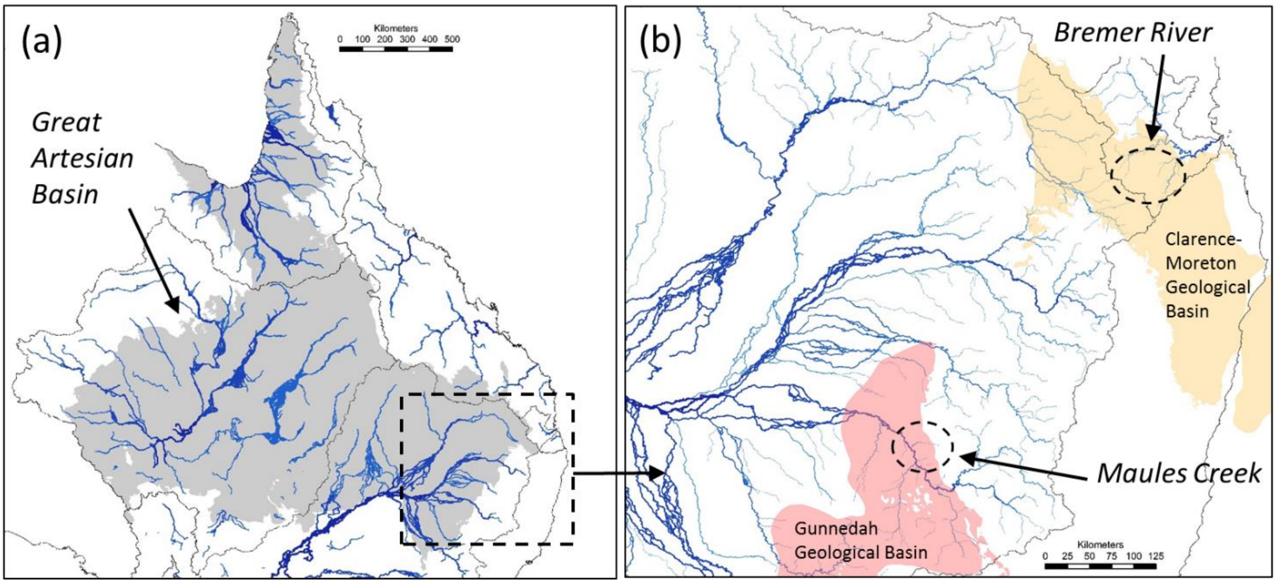


Figure 1: Main study areas including (a) the Great Artesian Basin and (b), the Bremer River (south-east Queensland) and Maules Creek (upper Namoi River basin, central New South Wales). Major geological basins in the vicinity of the Bremer River and Maules Creek are also shown.

This report begins (following section) with setting the hydrological context for the range of conditions within streams that are likely to be impacted on by CSG extraction and coal mining activities. We present a basic conceptual synthesis of the role that water plays in different stream ecosystems at different temporal and spatial scales. The subsequent sections of this report are focused on the key research components and their results. The report concludes with a summary of potential applications of the research and implications for assessment and monitoring of water-related ecological responses to CSG extraction and coal mining development.

1.1 Conceptual framework for understanding potential water-related impacts of CSG extraction and coal mining

Coal seam gas extraction and coal mining can cause a complex range of hydrogeological and hydrochemical impacts and elicit a variety of ecohydrological responses in groundwater-influenced ecosystems. In this project, we focus on the ecohydrological responses in surface and subsurface ecosystems to groundwater drawdown, a pervasive impact of coal seam gas extraction and coal mining. A series of conceptual representations of potential physical, hydrochemical and ecological responses to groundwater lowering is considered. This conceptual framework informed the development of hypotheses and the overall research plan.

Coal seam gas extraction and coal mining activities are widespread and occur in a range of landscape settings, but low-relief, sedimentary landscapes dominate the major coal-bearing regions. These landscapes can have complex geology with local and regional aquifers that are highly variable in their hydrogeological parameters (e.g. grain size distribution, permeability and porosity) across broad spatial extents and with depth. Our research focuses on systems where surface water and groundwater are hydraulically connected, whereby abstraction from the groundwater system can affect the quantity (and quality) of the surface water system (SKM 2011).

Within this broad definition of connected water systems, our research program is primarily focused on examining ecological responses to the various stages of transition from a connected surface-groundwater water system to a disconnected surface-groundwater system, as this transition reflects increasing levels of impact of groundwater drawdown associated with CSG extraction and coal mining. Brunner et al. (2009) illustrate five stages of connectivity based on the hydraulic connection of the stream with the underlying aquifer. Our research primarily focused on the surface and near-surface expression of groundwater and the longitudinal extent or continuity of that expression, as well as hyporheic exchange flows and the connectivity between groundwater and stream flow. We have adopted a terminology for surface-groundwater interaction regimes that combines the cross-section descriptions of Brunner et al. (2009) with a typology described by SKM (2011), which includes the notion of longitudinal connection. In this way, we can apply the four-dimensional representation (longitudinal, vertical, lateral and temporal) of lotic systems. We conceptualise five basic states of the surface-groundwater system (Figure 2):

a) Gaining with perennial surface flow: A gaining stream (the groundwater hydraulic gradient is toward the stream), with continuous or near continuous surface flow (Figure 2a). In a gaining stream with perennial surface flow, water exchange through the streambed is dominated by regional groundwater discharge, but variations in the streambed geometry and pressure distribution will support local hyporheic exchange (local down- and upwelling over relative short time and length scales). The surface water quality is dominated by aerobic processes associated with turbulent aeration due to free flow over riffles.

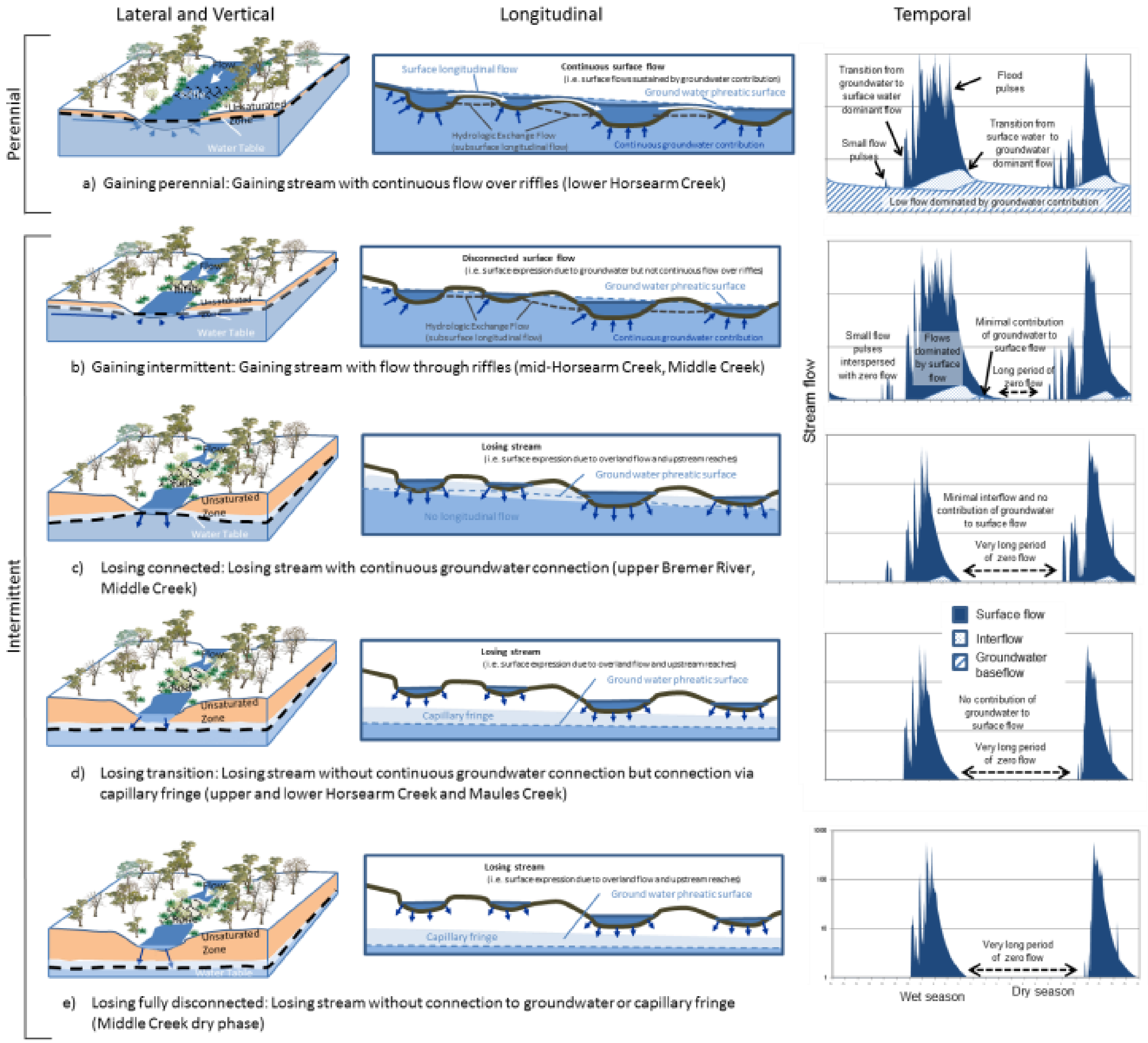
b) Gaining with intermittent surface flow: A gaining stream, where stream pools are supplied by the groundwater (upwelling) and are permanent or near permanent, but where surface flow over riffles is due only to surface inflow related to storms. At other times flow between pools is via the subsurface (Figure 2b).

c) Losing-connected with intermittent surface flow: A losing stream (hydraulic gradient is from the stream to the groundwater) where intermittent surface flow results from overland flow and from upstream reaches (Figure 2c). Compared to a gaining stream with intermittent surface flow (Figure 2b), a losing stream with intermittent surface flow has a net loss of water through the hyporheic zone (downwelling). Where the region between the streambed and the groundwater is fully saturated, the surface-groundwater system is hydraulically connected.

d) Losing-transition (Brunner et al. 2009) with intermittent surface flow: A losing stream where there is an unsaturated zone between the streambed and the water table (partially disconnected) but the capillary fringe extends from the water table to the streambed. Surface flow is intermittent and is from periodic overland flow and from upstream reaches (Figure 2d). Riparian and floodplain vegetation may be able to access groundwater even though it is disconnected from the stream. However, further lowering of the water table may render this water source unavailable for vegetation.

e) Losing fully disconnected: A losing stream where there is an unsaturated zone between the streambed and groundwater (disconnected) as in Figure 2d but where the capillary fringe extending up from the water table does not intersect the streambed (Figure 2e). Further lowering of the water table in a losing-disconnected stream (Figure 2c) has little effect on the rate of water loss from the surface water system.

All the types of surface-groundwater connections represented in Figure 2 naturally occur within stream networks. The focus of this research program is to consider the ecological implications of the transition from one surface-groundwater configuration to the next (summarised in Table 1) as an analogue for progressive groundwater drawdown due to CSG extraction and coal mining activities.



a) Gaining perennial: Gaining stream with continuous flow over riffles (lower Horsearm Creek)

b) Gaining intermittent: Gaining stream with flow through riffles (mid-Horsearm Creek, Middle Creek)

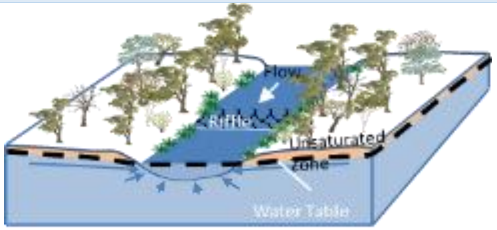
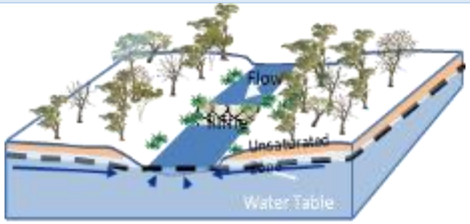
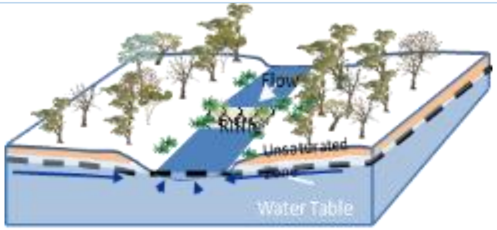
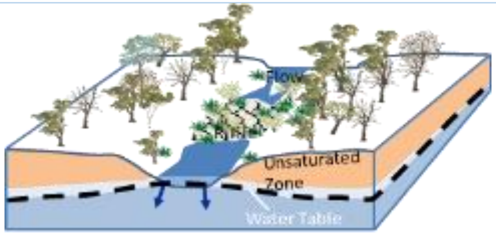
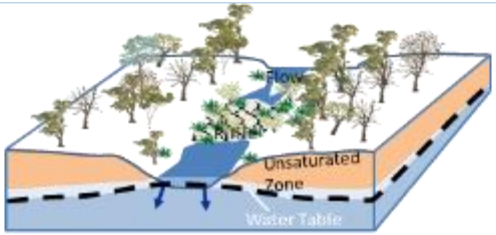
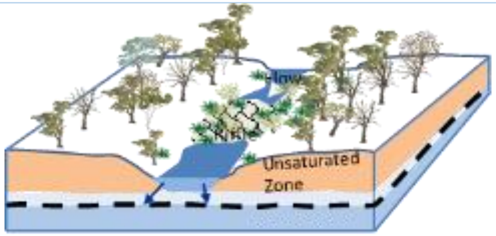
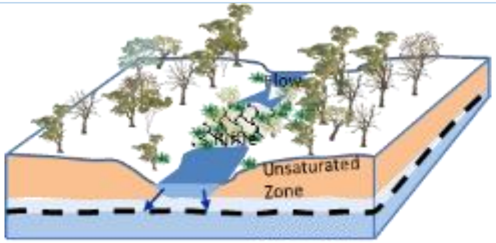
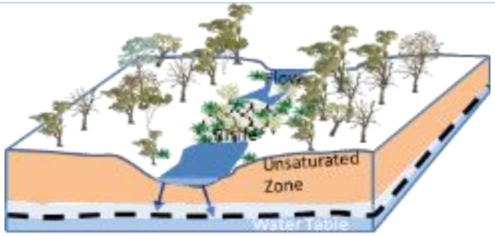
c) Losing connected: Losing stream with continuous groundwater connection (upper Bremer River, Middle Creek)

d) Losing transition: Losing stream without continuous groundwater connection but connection via capillary fringe (upper and lower Horsearm Creek and Maules Creek)

e) Losing fully disconnected: Losing stream without connection to groundwater or capillary fringe (Middle Creek dry phase)

Figure 2: Conceptualisation of streams with different surface-groundwater configurations represented in four dimensions (vertical, lateral, longitudinal and temporal, respectively). a) Gaining with perennial surface flow, b) Gaining with intermittent surface flow, c) Losing-connected with intermittent surface flow, d) Losing-transition with intermittent surface flow, and e) Losing fully disconnected.

Table 1: Key physical changes that result from transition from one surface-groundwater configuration to the next as may be expected to occur in response to groundwater drawdown. Study sites characterising each transition state is also listed for each case.

Case	Initial State	End State	Description of key physical changes expected
1	 Sites: Upper Bremer River Perennial section of Horsearm Creek	 Sites: Upper Bremer River Upper section of Horsearm Creek	Transition from gaining perennial stream to a gaining stream with intermittent surface flow. Contribution from groundwater remains but surface flow over riffles ceases periodically.
2	 Sites: Upper Bremer River Upper section of Horsearm Creek	 Sites: Middle Creek (following flow event) Lower Maules Creek (not examined)	Transition from gaining stream with intermittent surface flow to a losing stream with intermittent surface flow. Flow reversal in hyporheic zone from upwelling to downwelling. Increasing duration of no-surface-flow periods.
3	 Sites: Upper Bremer River Middle Creek (following flow event)	 Sites: Maules and Middle creeks (following dry period) Lower Maules Creek (not examined)	Transition from losing stream with intermittent surface flow to losing-transition stream. This case is intended to represent the effect of large scale groundwater drawdown and its effects on near stream and floodplain vegetation. Increasing intermittency. Reduced accessibility of vegetation to groundwater.
4	 Sites: Maules Creek and Middle creeks (following dry period)	 Sites: Lower Maules Creek (not examined)Middle Creek (after prolonged dry period)	Transition from losing-transition stream to losing-disconnected stream. This case is to consider the effect of large scale groundwater drawdown and its effects on near stream and floodplain vegetation. Increasing intermittency. Reduced accessibility of vegetation to groundwater.

1.2 Conceptualisation and framing of research questions

Building on the conceptual models presented in Figure 2 and Table 1, we here conceptualise expected ecological responses to changes in groundwater regimes such as those that may result from groundwater drawdown. This conceptualisation forms the basis for development of the research questions tested in this project and is intended to facilitate synthesis, integration and future application of research findings.

Consider a gaining perennial stream (Figure 2a). The temporal flow pattern (right of Figure 2(a)) shows continuous flow with a significant contribution from groundwater. The water chemistry is dominated by the combination of continuous groundwater inflow and the highly aerobic conditions created by flow over riffles (Figure 3a). For gaining perennial streams the hyporheic exchange will be strong in localised zones as driven by velocity and pressure effects, but relative to the overall flow down the creek the hyporheic contribution as well as regional groundwater discharge will be small (compared to a gaining stream with intermittent surface flow Figure 3b, c). The hyporheic flow paths will be shorter/faster with limited time for streambed nitrification which means that the amount of oxidised N (NO_x) contribution from upwelling will be smaller than for streams without continuous surface flow (Figure 3b, c). Due to the surface water flow, the nutrient distribution will be well mixed in the stream. Consequently, algae, which are an important energy source in many aquatic food webs, would grow more uniformly along the stream as opposed to their growth being restricted to near the upwelling zones in a gaining stream with intermittent surface flow.

Lowering of groundwater levels and a transition from gaining (a) to losing (c) in Figure 3 would lead to an overall reduction in the amount of NO_x inasmuch as longer flow paths allow for N removal by denitrification. This may cause N-limitation and limit algal production. However, longer flow paths and hence longer residence times would lead to increased time for organic matter mineralisation which for redox-reactions past denitrification would lead to a release of ammonium (NH_4^+) from dissolved organic carbon (DOC) mineralisation.

If the groundwater table below a stream is sufficiently deep, changes in the groundwater table position effectively do not alter the infiltration rate through the streambed; this is referred to as a disconnected system (Brunner et al. 2009). For a shallower water table a saturated groundwater mound with a capillary fringe may maintain a zone of saturated connectivity between the groundwater table and the streambed. A lowering of the groundwater table from this level may cause a transition state prior to full disconnection when the groundwater table and capillary fringe completely disconnect from the streambed. Brunner et al. (2009) found that the the critical groundwater level at which full disconnection occurs is related to aquifer thickness and conductivity, stream width, depth and the hydraulic conductivity of the streambed sediments. While the vertical extent of the capillary fringe may be quite narrow in permeable sediments such as sands and gravels (in the order of a few to tens of centimetres, in fine grained silty/clayey sediments the fringe may be several meters thick (Liu et al. 2014). We have included the transition state (Figure 1d) in our conceptual models because it is considered important in the hydrogeological literature and because the saturation and moisture status of streambed sediments are critical to ecosystem processes in the hyporheic zone. Even if the capillary fringe is narrow, as in coarse sediments, we consider that the moisture status of streambed sediments, and their capacity to retain that moisture (for weeks, months, years) are important factors to consider for hyporheic ecosystem functioning and its resistance and resilience to groundwater recession.

In the case of groundwater drawdown leading to a net losing stream (Figure 2c, d, e) the surface hydrology is dominated by surface inflows, with a short period of baseflow generated by storm events (i.e. as bank storage). In the case of pools no longer being supported by horizontal subsurface flows the stream becomes ephemeral with occasional remnant pools following surface-water flows. The aquatic ecological processes are limited for this stream condition (Figure 3d).

To consider riparian and floodplain vegetation, we need to take a broader lateral view (Figure 4). In the case of lateral flow in a gaining stream, groundwater discharge into the channel is localised or continuous along the reach and occurs throughout periods of low runoff and channel flow. High flows in the channel lead to localised recharge and bank storage with increasing groundwater levels near the channel. Larger flow events leading to over-bank flow may result in groundwater recharge and rising groundwater levels over a larger part of the floodplain. With increasing distance away from the bank and floodplain, water tables are deeper and recharge from streams does not occur, leaving localised rainfall and runoff as the major sources of recharge. Soil water contribution to losing streams is limited to short-lived return flow derived from local bank storage created during periods of high flow and high rainfall.

These spatial and temporal patterns in water distribution and recharge processes across the riparian and upland landscape determine the availability of water to vegetation and consequently are reflected in plant distribution, composition, productivity and relative dependence on groundwater (Figure 4). Hydrophytes (plants preferring wet areas with comparatively long periods of flood inundation) dominate the low-lying riparian and floodplain areas where flow is either permanent or over most of the year, soil moisture is regularly recharged and water tables are shallow. Groundwater use (inferred dependence) and plant productivity are high as a consequence of perennial discharge/accessibility. In upland areas hydrophyte habitat does not occur and xerophytes dominate. Groundwater dependence is reduced but still present in lower slope habitats. Greater reliance on localised rainfall recharge/runoff results in lower productivity as the growth period is shortened due to increased seasonality of available water.

Drawdown of groundwater due to CSG extraction and coal mining may result in reduced/lost groundwater discharge to the channel (shift to an intermittent flow model) and lower accessibility to water tables across all habitats (Figure 5). This fundamental change in the hydrological habitats for vegetation will have the greatest impact on vegetation dependent on groundwater and will result in reduced abundance/loss of hydrophytes and greater importance of xerophytes. Increased seasonality of available water will restrict periods of maximum vegetative growth and result in reduced riparian and floodplain net primary production. The change in upland vegetation (less dependent on groundwater) will be less with productivity expected to remain the same unless reduction in rainfall recharge also occurs.

In Component 4, we present a conceptualisation of seasonal variation in natural functioning discharge springs and the cosmopolitan, aquatic, and semi-aquatic species they sustain. We also conceptualise potential responses of discharge springs and their resident species to the cumulative impacts of groundwater drawdown and interactions with other threats.

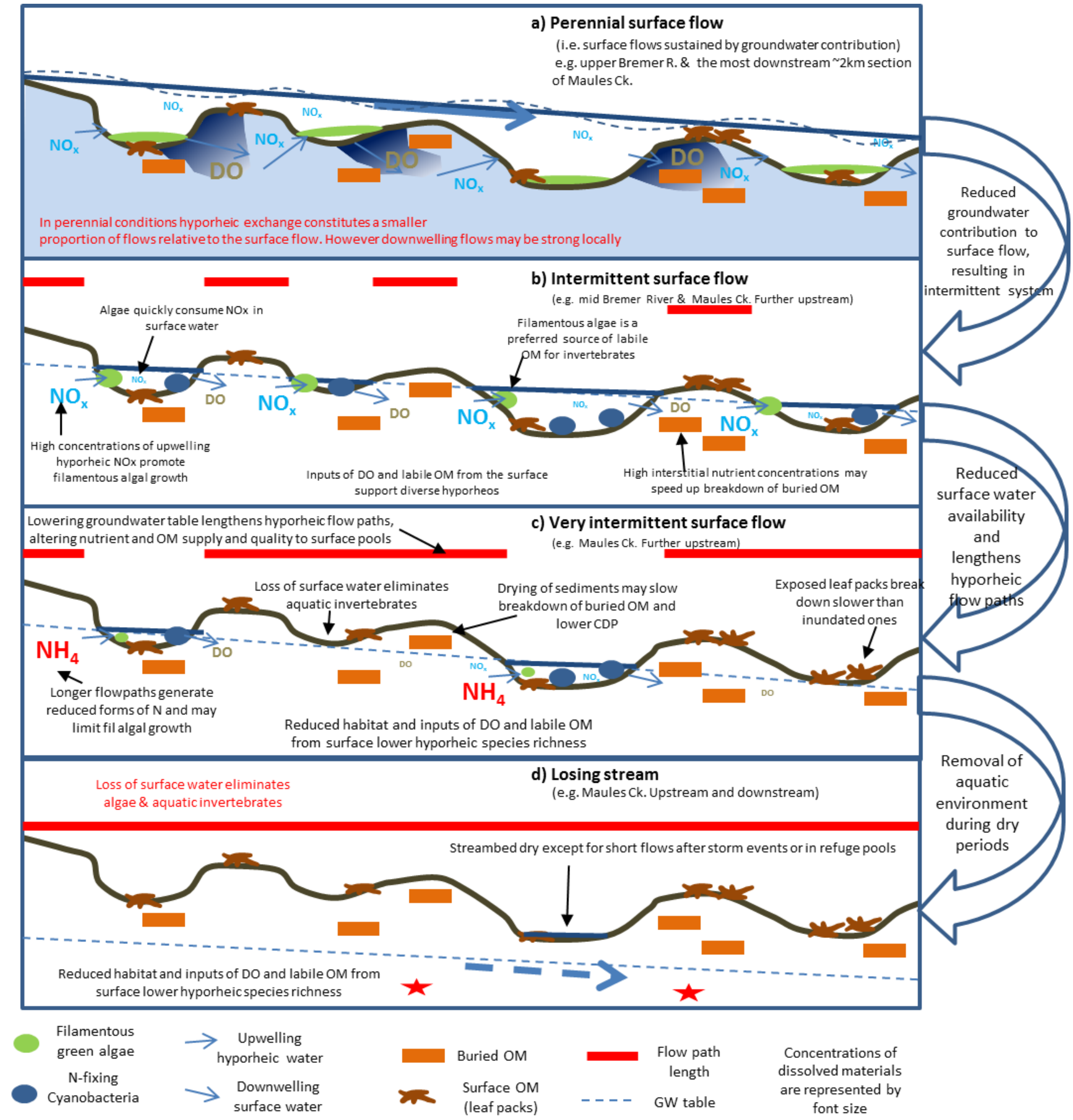


Figure 3: Conceptualised longitudinal in-stream and hyporheic-zone hydrochemical conditions for four stages of progressive groundwater drawdown. This scenario is one where the groundwater is predominantly oxidised (as observed in the Maules Creek catchment). a) Perennial surface flow: Subsurface flow is a combination of regional groundwater discharge (e.g. baseflow) and hyporheic exchange (local upwelling and downwelling). Short hyporheic flow paths and oxic conditions favour rich and abundant hyporheos; b) Intermittent surface flow (when not in flood), and some regional groundwater discharge maintain pools. Flow between pools is via subsurface hyporheic flow paths that are now longer than in scenario a). The longer subsurface flow paths (and residence times) allow redox reactions to progress towards more reduced conditions with nitrate reduction and some manganese-oxide and iron-oxide reduction. c) Further reduction in the groundwater level limits surface water expression and flow is now predominantly along subsurface hyporheic flow paths. The redox conditions are now further reduced with iron-oxide reduction, sulfate reduction and methanogenesis. The iron-oxide reduction leads to release of phosphate from the dissolving iron-oxide surfaces; d) For further lowering of the water table surface expressions of groundwater is lost and subsurface flows are dominated by regional groundwater flows and after a transition period the subsurface hydrochemistry may resemble the regional conditions in the aquifer.

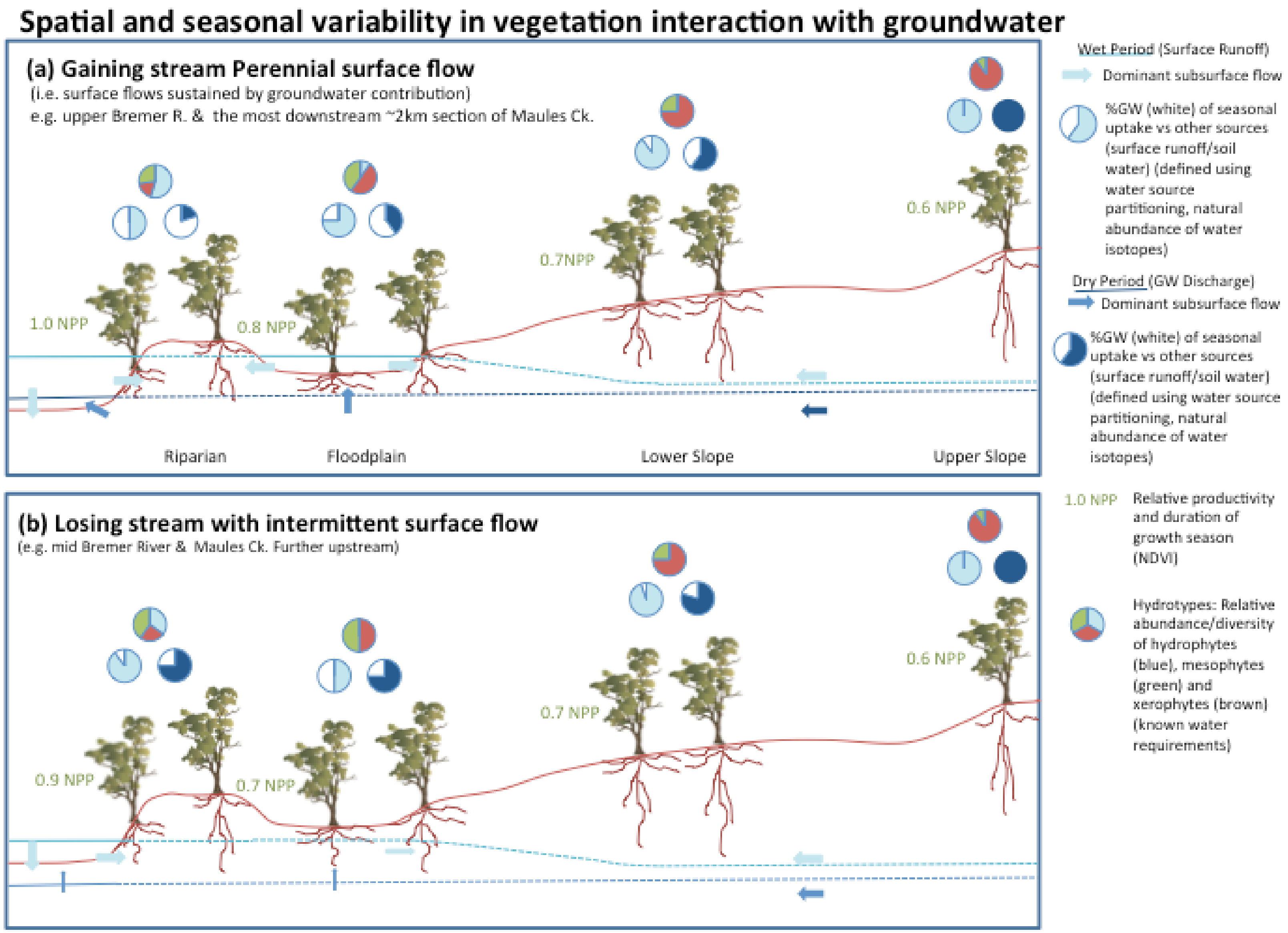
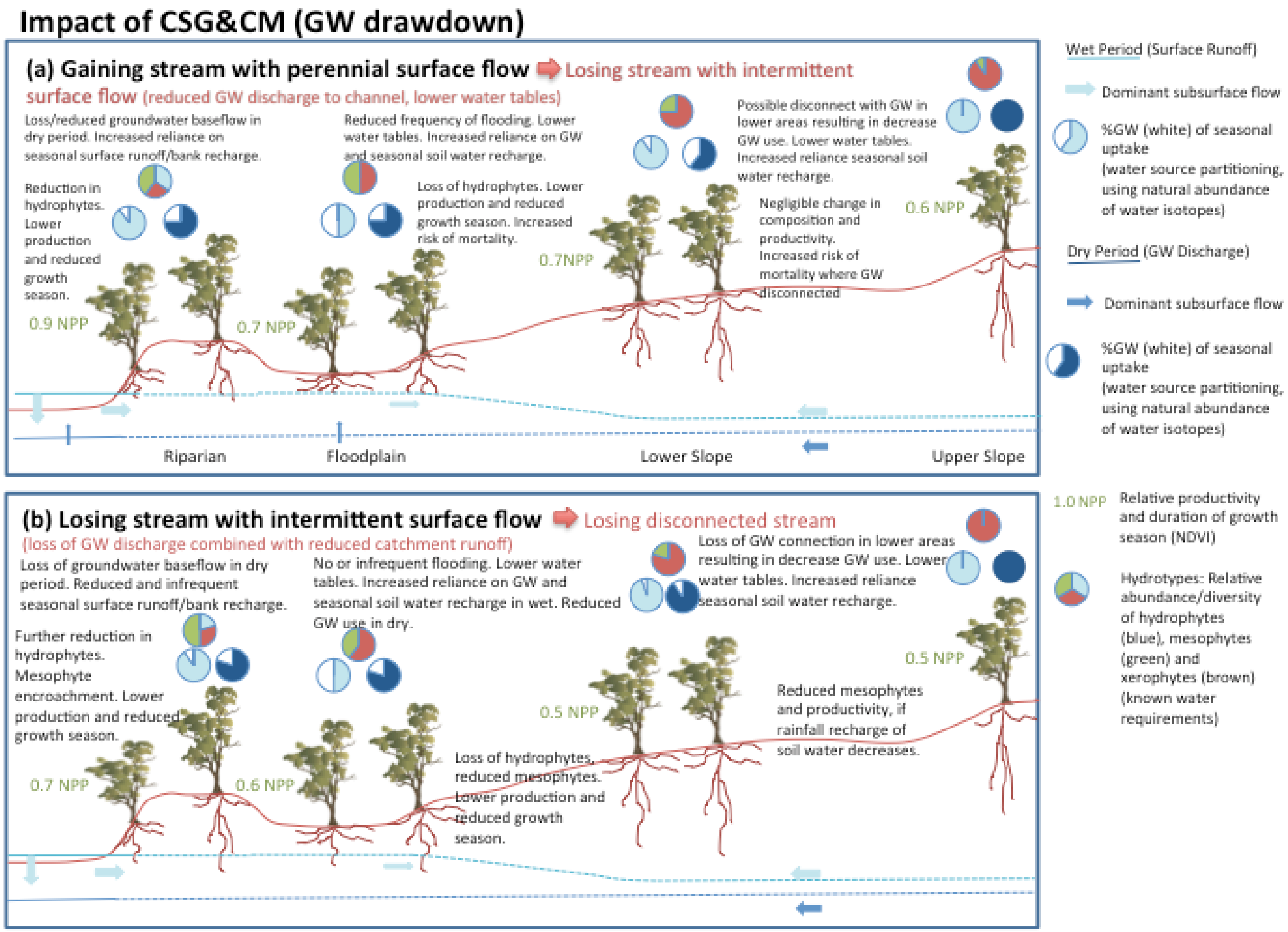


Figure 4: Natural spatial and seasonal variation in groundwater dependent vegetation for a gaining perennial stream (a) and for a losing disconnected stream (b).



Impact of CSG&CM (GW drawdown)

(a) Gaining stream with perennial surface flow ➡ Losing stream with intermittent surface flow (reduced GW discharge to channel, lower water tables)

Loss/reduced groundwater baseflow in dry period. Increased reliance on seasonal surface runoff/bank recharge.

Reduction in hydrophytes. Lower production and reduced growth season.

Reduced frequency of flooding. Lower water tables. Increased reliance on GW and seasonal soil water recharge.

Loss of hydrophytes. Lower production and reduced growth season. Increased risk of mortality.

Possible disconnect with GW in lower areas resulting in decrease GW use. Lower water tables. Increased reliance seasonal soil water recharge.

Negligible change in composition and productivity. Increased risk of mortality where GW disconnected

(b) Losing stream with intermittent surface flow ➡ Losing disconnected stream (loss of GW discharge combined with reduced catchment runoff)

Loss of groundwater baseflow in dry period. Reduced and infrequent seasonal surface runoff/bank recharge.

Further reduction in hydrophytes. Mesophyte encroachment. Lower production and reduced growth season.

No or infrequent flooding. Lower water tables. Increased reliance on GW and seasonal soil water recharge in wet. Reduced GW use in dry.

Loss of hydrophytes, reduced mesophytes. Lower production and reduced growth season.

Loss of GW connection in lower areas resulting in decrease GW use. Lower water tables. Increased reliance seasonal soil water recharge.

Reduced mesophytes and productivity, if rainfall recharge of soil water decreases.

Wet Period (Surface Runoff)

Dominant subsurface flow

%GW (white) of seasonal uptake (water source partitioning, using natural abundance of water isotopes)

Dry Period (GW Discharge)

Dominant subsurface flow

%GW (white) of seasonal uptake (water source partitioning, using natural abundance of water isotopes)

1.0 NPP Relative productivity and duration of growth season (NDVI)

Hydrotypes: Relative abundance/diversity of hydrophytes (blue), mesophytes (green) and xerophytes (brown) (known water requirements)

Figure 5: Consequences of groundwater drawdown on vegetation. (a) shows the end state of transition from a gaining stream (see Figure 4a) to a losing stream (see Figure 4b). (b) describes transitioning from a stream with connected surface-groundwater to a disconnected stream.

1.3 Research questions to test

This study is structured around a series of research questions listed in Table 2. Subsequent sections of this report refer back to these research questions.

Table 2: Key Research Questions and approaches to answering them with reference to the relevant research Component.

Research Question	Research Component	Summary of approach for addressing Research Question
1a). What are the characteristics of plants that utilise groundwater and under what environmental conditions do they occur (typology of phreatophytic vegetation)?	1	Recent research on long-term change in phreatophytic vegetation composition revealed the dynamics between functional classes of plant species and hydrological gradients defined by accessibility to groundwater. The characteristics of vegetation responses are defined by the hydrological state of the environment and whether it is altered by artificial drawdown and reduction in recharge. However, a general typology of vegetation and groundwater interactions and responses to groundwater drawdown does not exist. Previous studies of terrestrial groundwater-dependent vegetation have found a dynamic interaction between deep roots and groundwater, suggesting mature trees have the capacity to adapt to declining water tables. Whether this interaction is common to all phreatophytes in different biophysical settings is not known. Root redistribution may afford tolerance to short-term drawdown in water tables but protracted and rapid groundwater declines are known to result in phreatophyte water deficit stress and mortality and prevent vegetation resilience. The information outlined in the previous sentences formed the basis for our development of a typology.
1b). Are there functionally similar plants that share groundwater requirements (hydrotypes) and what is their distribution in relation to groundwater availability?	1	The landscape gradient from stream to upland formed the basis of the sampling design, and responses of different functional plant types to potential groundwater drawdown are inferred from this space-for-time analysis. The change in the hydrological environments for vegetation will have the greatest impact on vegetation dependent on groundwater and will result in reduced abundance/loss of hydrophytes and greater importance of xerophytes. Initial vegetation surveys are conducted at each site to determine composition, population structure of potentially phreatophytic species and biomass indices/productivity. Species are classified into hydrotypes according to the preliminary typology. These field surveys of hydrotypes and plant productivity in different landscape positions also provide the ground-truthing for the remote sensing aspect of this project. On-ground surveys are complemented by a search of the national and international literature to develop a typology of hydrological environments and associated vegetation. This review asks fundamental questions including how to define phreatophytic vegetation, what are the types of phreatophytes, and what types of landscapes they occur in.
2). Is plant distribution and response (groundwater use and productivity) to groundwater availability predictable?	1	Previous studies of trees growing above shallow groundwater have revealed a dynamic interaction between deep roots and groundwater, suggesting mature trees have the capacity to adapt to declining water tables. Whether this interaction is common to all plants that access groundwater in different biophysical settings is not known. We used dendrometer bands with attached data loggers, placed on the trunk of overstorey trees (*Eucalyptus camaldulensis*) to measure their diel and seasonal hydrological state and growth in the different landscape positions (riparian and floodplain) over the study period. To determine where these trees are sourcing their water, we measured stable isotopes of oxygen (δ^{18}O) and hydrogen (δ^{2}H) extracted from xylem water from tree branches and from soil samples from the unsaturated soil layers, as well as groundwater.
3). How do vegetation and groundwater interactions vary spatially and temporally	1	Remote sensing is used to quantify the spatiotemporal variation in vegetation type and growth dynamics relative to water availability from

Research Question	Research Component	Summary of approach for addressing Research Question
across the landscape? This includes identification of the determinants of vegetation recovery following altered groundwater regimes in different biophysical settings and at different scales, from remote sensing (RS) data.		local (e.g. stream reach) to landscape (e.g. catchment) scales. Plant productivity and groundwater relationships are drawn from existing knowledge and by applying new remote sensing methods to determine ecohydrological patterns. Archival data sets in the form of remotely sensed data are used to reveal longer-term temporal patterns and establish consistency of interactions with groundwater
4) How does stream flow regime and degree of substrate saturation affect organic matter processing in surface habitats (riffle, pool, and dry gravel bar) and the hyporheic zone?	2,3	Organic matter processing was assessed by two complementary methods: leaf litter incubations and cotton strip assays. Leaf litter packs were deployed in coarse-mesh and fine-mesh bags to distinguish the effect of macroinvertebrate shredders. The design involved replicates deployed in riffle, pool, and dry gravel surface habitats, as well as the hyporheic zone, and under a range of flow regime and substrate saturation conditions (saturated, moist, dry). In the Maules Creek catchment, two field sampling campaigns were undertaken for leaf litter incubations and cotton strip assays. In the upper Bremer River Catchment, two leaf litter incubations and four cotton-strip assays were undertaken.
5) How does stream flow regime affect invertebrate diversity and abundance in surface habitats (riffle and pool) and the hyporheic zone?	2,3	Invertebrate community structure and composition in pool and riffle habitats was assessed by taking replicate kick net samples from each of these two invertebrate habitat types. Hyporheic samples were collected from shallow and deep piezometers (see below).
6) How does stream flow regime affect basal resource use and food web structure in surface and hyporheic environments?	2,3	Stable isotope analysis of C and N was used to quantify the basal resource use and trophic position of surface and subsurface consumers.
7) How does stream flow regime affect fish diversity and abundance?	2	Fish in riffles and pools were sampled in dry and wet phase in the upper Bremer River catchment sites.
8) How does stream flow regime and degree of substrate saturation affect nutrient (N) biogeochemical processes and organic matter Carbon (C) degradation rates in the hyporheic zone?	3	Nutrient (N) biogeochemical processes and organic matter carbon (C) degradation rates in the hyporheic zone were characterised by surface water and hyporheic pore water sampling. At Maules Creek this was done from the range of longitudinal surface water-groundwater hydrological regimes (from intermittent losing over perennial gaining to perennial losing). For the Bremer River this was done at five individual streams. The biogeochemical processes were characterised by measuring nutrients, redox, and organic and inorganic carbon in the context of local hyporheic exchange flow paths (downwelling-upwelling) and regional groundwater discharge.
9). How does stream flow regime, hyporheic exchange and degree of substrate saturation affect invertebrate assemblages, and their associations with dissolved oxygen (DO) and hydrochemical processes in the hyporheic zone?	3	Invertebrate faunal communities in the hyporheic zone were sampled with temporary piezometers installed in paired shallow (40 cm) and deep (approx. 80 cm) sub-sites (where possible), and in a range of flow-regime and hyporheic-exchange conditions. At Maules Creek three surface flow regimes were sampled - intermittent losing, perennial gaining and perennial losing. At Bremer River five streams with a range of flow regime expressions (characterised during study) were sampled. Invertebrate communities were identified and characterised, initially on higher level (family) taxonomy and degree of groundwater dependency (stygobite, stygophile, stygoxene), to identify assemblage patterns related to stream flow regime and hyporheic exchange. Abiotic variables were measured in each piezometer from which invertebrates were sampled. Abiotic variables measured included flow regime, hyporheic exchange (via vertical hydraulic gradient), field physico-chemistry, major ions, nutrients, DO and redox sensitive species, DOC and FDOM (dissolved organic matter fluorescence). Associations between biotic and abiotic variables were explored and characterized initially with multivariate pattern analytical methods. Potential biotic and abiotic indicators of hyporheic ecosystem functioning were identified.

Research Question	Research Component	Summary of approach for addressing Research Question
10). What is the degree of hydrologic connectivity, hydro chemical and invertebrate assemblage similarities, between the hyporheic zone and regional aquifer at Maules Creek, and what are the implications of this with respect to understanding and managing groundwater drawdown impacts on hyporheic ecosystems?	3	Fifteen groundwater bores located in the regional aquifers alongside Middle Creekand Maules Creek were sampled for stygofauna and hydrochemistry. The bores were equipped with water level loggers to record groundwater level fluctuations that were related to adjacent stream flow conditions. The hydrograph, hydrochemistry and invertebrate sampling results were analysed to evaluate the degree of hydroecological connectivity between the streams, hyporheic zone and the regional aquifer. Two field sampling campaigns were conducted: Sept-Oct 2015 and Feb-Mar 2016.
11). What is the current state of knowledge available concerning freshwater biodiversity endemic to GAB springs, with respect to taxonomy, distribution, population status, population connectivity, ecology and threats and how does this knowledge vary among taxa?	4	We undertook a comprehensive and systematic literature review, supplemented with assessments and review by relevant experts and on-the-ground managers of spring wetlands and spring-dependent species as well as a semi-quantitative assessment of information availability for each taxa.
12). What are the key environmental determinants of the distribution and biodiversity patterns (e.g. taxonomic and phylogenetic richness, diversity and endemism) of taxa endemic to GAB springs?	4	We used species distribution data to quantify spatial variation in biodiversity values of spring complexes. We used a range of explanatory statistical modelling methods to quantify relationships of spatial variation in biodiversity and species composition to a set of ecologically-relevant environmental attributes describing spring complexes.
13). How well do existing conservation measures (i.e. representation within protected areas, inclusion in species' conservation status listings) align with our new assessment of the taxonomy and present-day distribution of GAB endemic taxa?	4	We evaluated current conservation status listing of GAB endemics (using various state, federal and international listings) with respect to our new data on species distributions to identify those species worthy of consideration for elevated conservation status. We also quantified representation of spring complexes and endemic spring taxa within existing protected areas to evaluate current levels of protection and identify gaps.
14). How does relative exposure to the different threats posed by groundwater drawdown and other stressors vary among GAB spring complexes?	4	We quantified the relative exposure of each GAB spring complex to five major current threats including aquifer (groundwater) drawdown, human modification of springs, disturbance from livestock and feral animals, introduced flora and introduced aquatic fauna. This analysis was undertaken with existing readily available data layers and Arc GIS. From this analysis we identified spring complexes that are exposed to the greatest number and intensity of threats.
15). How does vulnerability to threats posed by groundwater drawdown and other stressors vary among endemic flora and fauna of GAB spring wetlands?	4	We used expert elicitation to assess taxa vulnerability to each threat. Our approach incorporated measures of resistance, resilience and certainty and was informed by literature, ecological theory, unpublished data and expert knowledge.
16). Which GAB spring complexes are at the highest cumulative risk (i.e. contain high number of vulnerable species and are exposed to the highest threat intensities)?	4	We used a well-established and robust cumulative risk assessment method to estimate cumulative risks to spring complexes from multiple threats. The approach combined threat exposure data with taxon occurrences and certainty-weighted taxon vulnerability to threats to assess the overall risk for each complex. From these data, we evaluated: 1) which complexes are exposed to the greatest number and intensity of threats; 2) how vulnerability to threats varies among species; and, 3) which complexes are at the highest risk (i.e. contain high number of vulnerable species and are exposed to the highest threat intensities).
17). What on-ground management and monitoring activities are appropriate for managing threats in GAB spring complexes identified as being at highest risk?	4	We reviewed the literature to synthesise on-ground management and monitoring to ameliorate or mitigate threats to GAB springs.

1.4 References

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2 Component 1 – Groundwater dependent vegetation

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2.1 Introduction

Plants maximise their water-use efficiency through the use of the most readily available water and so plant communities are likely to have a highly complex dependence on water from different sources. These can include soil-stored water in most instances, and deeper, less accessible groundwater sources under drying conditions. Groundwater sources are particularly important for plants in areas where rainfall is low, either annually or seasonally, and when drought conditions prevail for all or some part of the year. Therefore, plants may access shallow groundwater on a permanent, seasonal or episodic basis and different species assemblages may develop that are characteristic of the predominant groundwater / surface water hydrological regime (Cassanova 2010). Phreatophytes are plants that habitually obtain water from groundwater, viz. the zone of saturation, either directly or indirectly through the capillary fringe above the water table (Meinzer 1927). The distribution of phreatophytic plants generally reflects an adaptation to the dry conditions of arid or semi-arid regions in which they occur (Thomas 2014), but phreatophytes can also occur in other temperate or tropical regions where there are periods of seasonal drought.

The role of groundwater in supporting vegetation across hydrological gradients and in different biophysical settings is poorly understood and knowledge is usually site specific and based on the detailed study of only a few species. Recent research on long-term changes in phreatophytic vegetation composition (Sommer & Froend 2014) revealed the dynamics between functional classes of plant species and hydrological gradients, defined by accessibility to groundwater. However, a general typology of vegetation and groundwater interactions does not exist. Consequently, attempts to assess potential ecological impacts of groundwater drawdown as a result of coal seam gas (CSG) and coal mining developments in a variety of biophysical settings have been hampered by a lack of accepted conceptual models of groundwater-dependent vegetation ecology.

This project has developed a typology of plant functional types in relation to groundwater and how this and other hydrological mechanisms structure plant communities. In developing a typology, the project has identified functionally similar plant species or communities with shared groundwater requirements (hydrotypes) and investigated whether hydrotypes in different biophysical settings share responses to changes in hydrological regime (Sommer & Froend 2014). Critical to the development of the typology and prediction of the response of plant communities to groundwater drawdown is a more detailed understanding of the relationship of plants and the hydrological environment. Plant functional types combine attributes of plant physiology, plant communities and ecosystem processes so that they provide an integration of species and environmental interactions (Diaz et al. 1997). Growth forms such as annual and perennial, herbs and woody plants, are indicators of responses to the environment such as capture of light, nutrients and water and of the links between key plant traits and plant response and function

(Chapin 1993). Ecologically, wetland plants have morphological and physiological responses to water availability and can be described according to where their photosynthetic parts are in relation to the water: submerged, floating, emergent, semi-terrestrial and terrestrial (Brock & Casanova 1997). It is expected that the natural spatial and temporal patterns in water distribution and recharge across the lowland and upland landscape determine the availability of water to vegetation and consequently are reflected in plant distribution, composition, productivity and relative dependence on groundwater. In this project, we used a combination of field studies and desktop modeling to develop a classification of hydrotypes within plant communities according to their groundwater dependencies. This typology will assist in assessing potential ecological impacts of changes to groundwater availability, as the proportion of different hydrotypes within a habitat will provide an indication of the degree of reliance on groundwater. Furthermore, detected changes over time in the proportion of different hydrotypes in a community will indicate possible changes in the hydrological regime (e.g. groundwater drawdown) operating within that area.

In general, there are uncertainties regarding the potential for recovery of groundwater-dependent vegetation exposed to a reduction in groundwater availability. Previous studies of trees growing above shallow groundwater have identified a dynamic interaction between deep roots and groundwater (Guevara et al. 2010, Vonlanthen et al. 2011, Canham et al. 2012), suggesting mature trees have the capacity to adapt to declining water tables (Richardson et al. 2011). Whether this interaction is common to all plants that access groundwater in different biophysical settings is not known. The water source partitioning of key plant hydrotypes in these different biophysical settings can be determined through analysis of the natural abundance of stable isotopes of water (δ^{18}O and δ^{2}H), in an effort to represent the relative importance of groundwater as a plant water source in different hydrological conditions. The abundance of the naturally occurring stable isotopes of water is a standard tool to determine the source of water used by plants (Dawson and Ehleringer, 1991, Adams and Grierson, 2001). For this project, we examined the spatial variability of the isotopes δ^{18}O and δ^{2}H in xylem water, soil water and groundwater along transects spanning the elevation gradient of the riparian zone and the floodplain, in perennial and intermittent sections of the stream. Groundwater use can be determined by comparing xylem δ^{18}O and δ^{2}H to soil water and groundwater isotope ratios for the different landscape positions. Research on riparian and floodplain trees using this stable isotope methodology in different environments in Australia has provided evidence for trees using various combinations of groundwater, rainfall-derived shallow soil water, and stream water (Thorburn and Walker, 1994, Lamontagne et al. 2005, O'Grady et al. 2006, Pfautsch et al. 2015). This project component explored the use of groundwater by vegetation across an ecohydrological gradient extending from terrestrial to freshwater ecosystems.

The use of archival data sets in the form of remotely sensed data can reveal longer-term temporal patterns and consistency of interactions with groundwater in different reaches and catchments. Remote sensing can be used to quantify the spatiotemporal variation in vegetation type and growth dynamics relative to water availability from local (e.g. stream reach) to landscape (e.g. catchment) scales. Plant productivity and groundwater relationships can be drawn from existing knowledge (e.g. Zolfaghar et al. 2014) and the application of new remote-sensing methods (Barron et al. 2014) to determine ecohydrological patterns over time and between different habitat types (riparian, floodplain, lower and upper slope). Remote sensing data can help delineate groundwater-dependent ecosystems and the analysis of their dependency on various elements of the water regime (groundwater, surface water, soil water or purely rainfall). We use a combination of remotely sensed data and data describing water regime, allowing assessment of the extent of groundwater dependence of remanent vegetation at a catchment scale. Remote sensing (RS) techniques can also help in the characterisation of the groundwater environmental functions and the effect of changes in groundwater regime on groundwater-dependent environmental assets. Particular advantages of RS techniques include the availability of long-term historical satellite datasets and the ability to monitor large areas at low cost. The combination of on-ground studies and remote sensing provides a critical linkage in understanding how plant communities may respond to groundwater drawdown at the local and catchment scales.

The key research questions to address in developing a typology of interactions are:

Research Question 1a: What are the characteristics of plants that utilise groundwater and under what environmental conditions do they occur (typology of phreatophytic vegetation)?

Research Question 1b: Are there functionally similar plants that share groundwater requirements (hydrotypes) and what is their distribution in relation to groundwater availability?

Research Question 2: Is plant distribution and response (groundwater use and productivity) to groundwater availability predictable?

Research Question 3: How do vegetation and groundwater interactions vary spatially and temporally across the landscape? This includes identification of the determinants of vegetation recovery following altered groundwater regimes in different biophysical settings and at different scales, from remote sensing (RS) data.

2.2 Methods

Research Question 1a - Typology of phreatophytic vegetation –A review of the published scientific literature was used to provide an understanding of how key environmental factors such as climate, geology, hydrology and soils shape the development of particular plant functional types and communities. The first step was to identify functionally similar plant species or communities with shared groundwater requirements (hydrotypes). We developed a typology of hydrotypes based on their reported distribution in terms of climate, geology, landscape position, soil characteristics and plant traits. Plant ecological, physiological and morphological traits that are reported in the literature as characteristic of phreatophytes were used to identify situations where there is likely to be dependency on groundwater. Using the developed typology we estimated the proportion of different hydrotypes (including phreatophytes) that occur within a given plant community and made inferences concerning how groundwater use by plants varies spatially and temporally across the landscape. From this review, we develop a decision tree for categorising plants into particular hydrotype functional groups.

Research Question 1b – Landscape distribution of plant hydrotypes and productivity - Field surveys for this study were undertaken at riparian, floodplain and terrestrial sites within the Maules Creek catchment in northern NSW and the Bremer River catchment in south-east Queensland (see supplementary Table 45 for a listing of the locations of all sites). Plant communities in the Maules Ck and Bremer River catchments were surveyed in October 2015 and March 2016. On each occasion, in each catchment, we sampled in five different landscape positions including riparian and floodplain sites on perennial river sections and a riparian and floodplain sites on intermittent river sections. The fifth landscape position included sites in upslope terrestrial areas. The rationale for choosing this sampling strategy was to have a gradient of groundwater depth across sites at both river systems. Riparian perennial sites had the shallowest depth to groundwater, indicated by the water level in the permanent stream pools at these sites (confirmed by water isotope signatures of the pools being similar to groundwater signatures, see section 2a). Floodplain sites close to the perennial pools (100 – 200m distant) were considered to have the next shallowest groundwater followed by sites further up the catchment at the intermittent riparian sites and then the nearby floodplain sites (so called, ‘intermittent floodplain’), where groundwater is deeper or shallower for only short periods (Figure 6). Terrestrial sites were upslope from the riparian and floodplain sites and considered to be sites where the vegetation is disconnected from the groundwater. Depth-to-groundwater data are limited at the study sites (particularly for the Bremer River catchment) but the data available do support our assumptions about differences between landscape positions (Maules Creek sites in particular). This gradient, as a representation of expected interactions and responses to water availability, across the landscape and inferred over time, forms the basis of the sampling design and vegetation responses to potential groundwater drawdown (Figure 6).

A total of 25 sites were surveyed at Maules Creek and 27 sites at the Bremer River, representing the five landscape positions within each catchment. At each site a 20m x 20m overstorey plot were arbitrarily placed to be representative of local riparian conditions, making sure to include the lowest point on the riparian bank at the 5m mark of the plot. Within each 20m x 20m plot we recorded the stem diameter at breast height (dbh) of all woody species with a dbh >2cm. Nested within each overstorey plot, a 10m x 10m sub-plot was established where all plants were identified and given a percentage foliage projected cover score for the plot. Nine canopy photos were taken in a grid pattern across the 10 x 10m sub-plot and converted to canopy and foliage cover with MatLab Image Processing Toolbox (MacFarlane & Ogden 2012). Plot overstorey basal area for each site was calculated from dbh measurements. As a measure of tree productivity, three leaf litter traps were installed at one site for each of the landscape positions. Litter was collected from each trap during sampling trips and the contents of each trap was dried (80° C for 24 hrs) and weighed. Litter traps were set at the Maules Creek sites only as this was the site of more intensive sampling and hence more frequent field trips for the collection of traps.

All species recorded in each sub-plot were assigned to a hydrotype based on attributes from a comprehensive review of the literature on plant functional characteristics relative to hydrological habitat, and the proportion of each hydrotype within each landscape position was calculated for both catchments. Literature was sourced from a systematic search of relevant online databases (i.e. Web of Science, Scopus and Google Scholar). Patterns in species and hydrotype community composition across landscape positions and catchments were assessed with multivariate ordination using distance-based redundancy analysis (dbRDA). The SIMPER routine was used to identify the species primarily providing the discrimination between and within sample groups (in this case, landscape position). All % foliage cover data for plant species within plots was transformed (log (x+1)) and all analyses were conducted with the Primer multivariate statistics package (Clarke & Gorley 2006). Differences in tree canopy, basal area and litter fall between landscape positions and, for litter fall, seasons, were analysed with analysis of variance (ANOVA) followed by Fisher's PLSD tests to discern differences between factors.

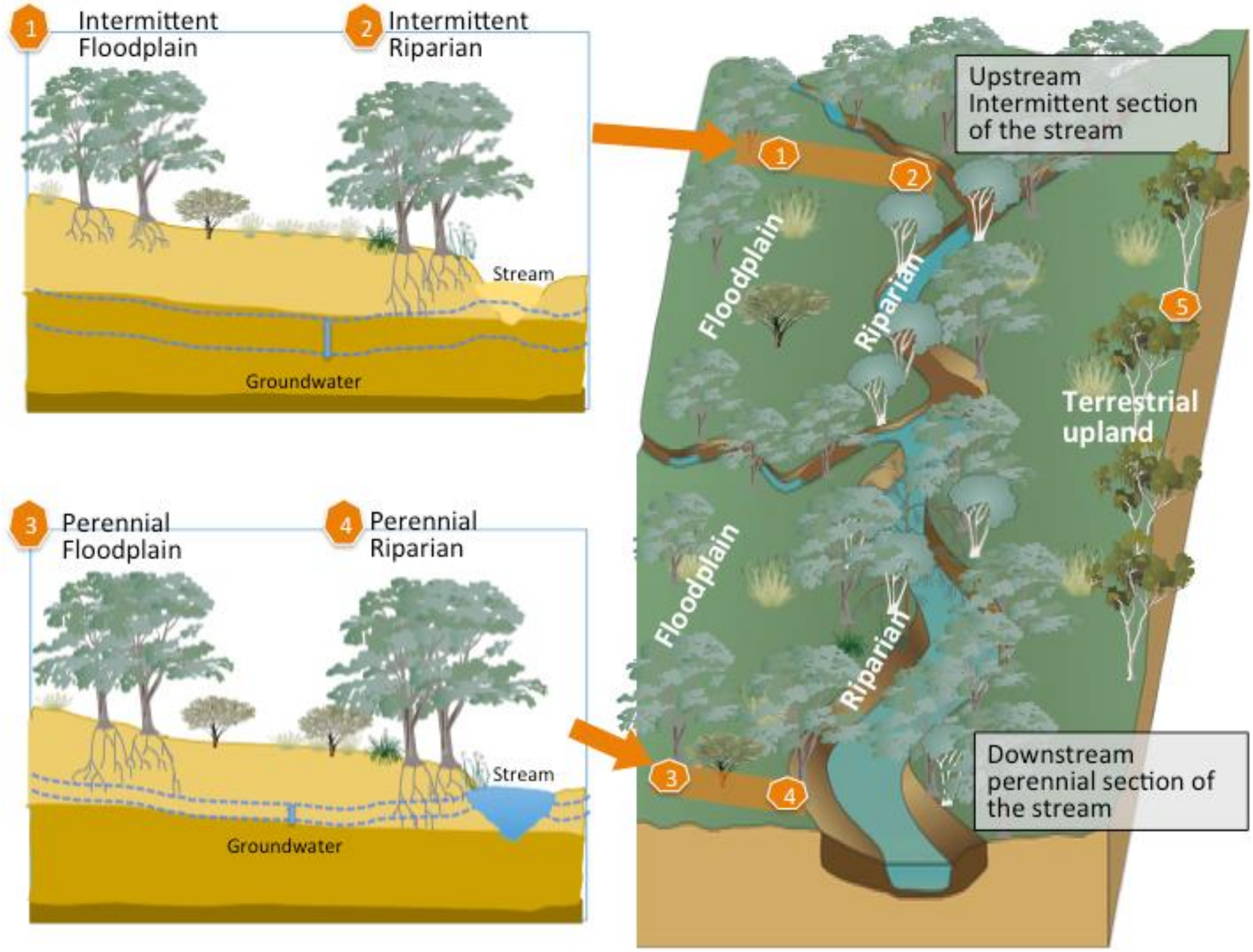

Figure 6: Schematic of field survey layout for vegetation surveys and tree measurements at the Maules Creek and Bremer River sites. Vegetation plots (and at Maules Creek, dendrometer and water isotope data) were established at riparian and adjacent floodplain sites in perennial and intermittent river sections. These plots generally corresponded with depth to groundwater at the sites with intermittent floodplain (1) having greatest depth to groundwater and intermittent riparian (2) a large seasonal range in groundwater depth and perennial sites (3 & 4) with shallower more consistent depth to groundwater. Vegetation plots were also established at terrestrial (upland) sites (5) where groundwater is generally too deep for interaction with plants.

Research Question 2 – Predicting groundwater use of plants across landscapes - At Maules Creek we fitted dendrometer bands with an attached data logger to the trunk of three trees at each of a perennial riparian, perennial floodplain, intermittent riparian and intermittent floodplain site (Figure 7). The dendrometers measured and logged stem diameter changes (mm) continuously, at one hourly intervals. The stem diameter dataset was used to assess the diel and seasonal hydrological state and growth of overstorey trees in the different landscape positions over the study period (350 days between October 2015 and September 2016). Stem increment data were compared visually with meterological and stream flow conditions over the study period, to assess the extent and timing of tree responses to rainfall or groundwater.

To determine from where trees (*Eucalyptus camaldulensis*) on riparian and floodplain sites at Maules Creek are sourcing their water, we measured stable isotopes of oxygen (δ^{18}O) and hydrogen (δ^{2}H) extracted from xylem water from tree branches and from soil samples from the unsaturated soil layers, as well as groundwater and stream pool water. At each tree where a dendrometer band was installed, we also collected samples of small tree branches (5mm dia. x 10 mm length) from the northern sun-lit mid-canopy on each of three trees at each landscape position. Soil samples were taken near each sampled tree at 0.25m intervals down to 1.5m depth where possible with a hand auger. Plant and soil samples were put on ice and taken back to the laboratory where water was extracted via cryogenic distillation (Turner et al. 2001). Collections were made in October 2015, March 2016 and June 2016. Extracted water was analysed for δ^{18}O and δ^{2}H isotopes to compare isotope ratios of plant and soil water with values for rainwater, stream water and groundwater (data collected as part of research in Component 3 and supplied by M. Andersen & H. Rutlidge). Water samples with similar isotope ratios are likely to come from the same source and therefore provide insight to whether tree water comes from soil water (rainfall) vs groundwater. By using an isotopic mixing model (IsoSource) (Phillips & Gregg 2003), we quantified the proportion of water in tree xylem that is contributed by each sampled environmental source. The range in % contributions (1st - 99th percentiles) and mean value of each water source (groundwater, deep and shallow soil water) at each site and sampling time was determined. The mixing model results were based on isotopic proportional increments of 2% and a tolerance of 2% (Phillips & Gregg 2003). Sources with a low 99th percentile value are not likely to be important for leaf water at that site, whereas sources with high 1st and 99th percentile values show strong constraint and high probability of being an important water source for trees (Benstead et al. 2006).

Figure 7: Setting up a dendrometer band and logger for measuring changes in tree stem diameter over diel and seasonal time periods. Inset shows detail of dendrometer and logger. The species is Eucalyptus camaldulensis situated at the perennial riparian site at Maules Creek.

Research Question 3 - Using remote sensing to assess the spatial and temporal dependency of plant communities on groundwater

The groundwater-dependent vegetation (GDV) relationship with water regime (and particularly with groundwater) results in distinct signatures in remotely sensed surface reflectance images, which differs from surrounding areas and varies through time. Remotely sensed reflectance data allow analysis of 'active greenness' provided by the multispectral NDVI (Normalised Difference Vegetation Index) time series. Time series of RS data are well suited for exploration of differences in the vegetation 'phenology', or cyclical growth rate, of terrestrial vegetation with and without access to groundwater. This exploration of differences makes use of time series of NDVI and NDWI (Normalised Difference Wetness Index) as indicators of vegetation greenness and wetness patterns, respectively. In this study, we mainly use Landsat data, which has the longest record of data acquisition. RS provides rapid and spatially extensive techniques for the assessment of vegetation structure and function, and can also provide insights into relationships between vegetation structure and function and climate variables (Eamus et al. 2015). The distinct GDV signatures are manifested in plant density, greenness or specific spatial arrangement, given that plant density associated with leaf area index is positively correlated with water availability (Glenn et al. 2008). This report presents the outcomes of the analysis undertaken within the Maules Creek and Bremer River catchments. As more on-ground information was available for the Maules Creek study area, a wider range of analyses was carried out for this region. Some interpretations presented in this report were based on general knowledge of the eco-hydrological conditions in the region. We provide here a brief outline of the methods used for development of a remote sensing technique to assess groundwater-dependent vegetation.

A full description of the image capture methods and analysis, along with CSIRO-GDV methodology and procedures are provided in the full Remote Sensing report (see Section 7.1, Appendix 1 for executive summary and bibliographic details.). This includes the devlopment and application of a web-based toolset which adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. The toolset can be implemented using the supplied javascript code (see Appendix B "GEE-CSIRO-GDV scripts" of Section 7.1, Appendix 1) in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications. The developed GEE-CSIRO-GDV tools include:

Tool 1 - creating image thumbnails for image quality control;
Tool 2 - identification of dates of cloud-free Landsat imagery from the GEE remote sensing database and generation of cloud free NDVI and NDWI data cubes covering the selected time periods;
Tool 3 - NDVI and NDWI time series extraction at selected point locations;
Tool 4 - Principal Component Analysis of multi-temporal NDVI and NDWI datasets;
Tool 5 - Multiple Linear Regression Analysis to model relationships between NDVI temporal profiles at each NDVI grid pixel location and local climate parameters (e.g. temperature, rainfall).

All available cloud-free images were geometrically rectified, terrain-corrected, radiometrically calibrated and converted to surface reflectance (Masek et al. 2006). Landsat 5 Thematic Mapper (TM) and Landsat 8 Operational Land Imager (OLI) data covering the study sites were downloaded from the Google Earth Engine (GEE) RS database (https://earthengine.google.com) for the period between 1986 and 2015 (in total, 169 and 121 datasets for Maules Creek and Bremer River catchments, respectively). For further processing and analysis, the imagery was converted into two sets of multi-date stacks of band reflectance grids. According to the number of the optical Landsat bands, six data cubes (one for each band) were created for each of the study sites. The CSIRO-GDV methodology is a suite of procedures developed under the Pilbara Water Resources Assessment project (McFarlane 2015) to delineate GDVs, which assesses their sensitivity to climate variability and explores relationships between vegetation greenness, groundwater levels and streamflow (where data were available) (Barron and Emelyanova 2015). The methodology is based on a combined analysis of available information on geological and hydrogeological data, climate variability, water regime and groundwater dependent ecosystem (GDE) ecohydrological characteristics, and the spatiotemporal analysis of Landsat data. This methodology allows the capture of specifics of the temporal and spatial variability of various land cover types and extrapolating local knowledge to the larger scales available for the historical period. Due to the spatial resolution of available RS products (e.g., Landsat imagery with 30m by 30m spatial resolution) with longer records, the developed methodology is more suitable for delineation and characterisation of vegetation communities or habitats rather than for individual plants.

For quantitative assessment of greenness and wetness we used NDVI and NDWI grids derived from the Landsat data cube. NDVI is used for indirect evaluation of vegetation greenness (Townsend and Justice 1986, Tucker 1986). The NDVI allows assessment of density and vigour of green areas on the surface. The NDWI was proposed by Gao (1996) to evaluate vegetation liquid water within the leaves. NDWI is less sensitive to atmospheric scattering effects than NDVI and provides additional and independent information about vegetation water content (Gao 1996).

Riparian vegetation is characterised by a consistently higher level of greenness across long-term (multi-year) periods in comparison with other types of vegetation with seasonal access to water (e.g. annual pasture, etc.). Therefore, a multi-date NDVI dataset (NDVI data cube) contains information about greenness dynamics over the considered period. Extraction of such information is achieved by the application of principal components analysis (PCA) (Jolliffe 2002) to the NDVI data cube. PCA allows transformation of the original NDVI data cube into a new dataset of principal component (PC) grids. The lower order PCs represent most correlated information between image dates (Richards 1993, Lunetta and Elvidge 1998, Canty 2010), in other words, temporally invariant regions of the landscape. The higher order PCs indicate land cover changes. It was expected that PC1 and PC2 grids produced from the multi-temporal NDVI dataset (NDVI PC1 and NDVI PC2, hereafter) would provide information for mapping temporally invariant vegetation areas characterized by high NDVI values, such as groundwater dependent vegetation (GDV) (see Section 7.1, Appendix 1: Table 43).

Dependence of NDVI (or vegetation greenness) on climate conditions was investigated by modelling relationships between NDVI and climate variables with the multiple linear regression (MLR) analysis method:

$$NDVI = a_0 + \sum_{i=1}^{N} a_i CP_i \qquad (4)$$

Where $N = 14$ is the number of climate variables (CP_i , $i = 1,2, \dots, N$), and a_0 and a_i are the Intercept and MLR coefficients, respectively. Climate variables used for modelling in this study included mean values of daily maximum temperature ($CP_1 = Tmax_m$; $CP_8 = Tmax_l$); pan evaporation ($CP_2 = Evap_m$; $CP_9 = Evap_l$); vapour pressure ($CP_3 = Vp_m$; $CP_{10} = Vp_l$); vapour pressure deficit ($CP_4 = VpD_m$; $CP_{11} = VpD_l$); relative humidity at maximum temperature ($CP_5 = RHmaxT_m$; $CP_{12} = RHmaxT_l$); solar radiation ($CP_6 = Rad_m$; $CP_{13} = Rad_l$) and rainfall ($CP_7 = RF_m$; $CP_{14} = RF_l$). These were all estimated over two selected periods of time to model immediate and longer-term effects. The immediate and long-term periods covered 15 days and a year, respectively. Labels '*m*' and '*l*' in climate variables names stand for immediate and annual time periods, respectively.

Temporal profiles of NDVI were extracted from the multi-temporal NDVI dataset created for applying PCA at each 30 m by 30 m pixel of the image covering the analysis area. Equation (4) was then applied for each of these NDVI profiles and normalised values of the climate variables. Normalisation of the climate variables allowed quantitative assessment of their contribution into the MLR model (4) by comparison of the MLR coefficients' magnitudes: it was assumed that the larger the magnitude the more influential the climate variable was. In addition, the sign of a coefficient's value allowed us to conclude whether a particular climate variable contribution was negative or positive. The MLR coefficients were assessed over the entire study area within the Maules or Bremer catchments, within the remnant vegetation mask and at the vegetation observation points. At the entire area scale, the ISODATA clustering technique (Ball and Hall 1967) was applied to the set of 15 grids containing the intercept a_0 and MLR coefficients a_i to identify six clusters of various land cover types with similar dependence on climate. It was assumed that remnant vegetation patches will be grouped into one or at least two clusters with distinctive statistical characteristics compared to the other clusters regardless of their total number, i.e., six MLR clusters, as in this study, or more. The mean coefficients values of the six MLR clusters were then estimated and their magnitudes and signs compared as described above. To investigate ability of the MLR coefficients to distinguish various remnant vegetation communities, the ISODATA method was applied only at the image pixels located within the remnant vegetation mask. According to the remnant vegetation conceptual model (see Section 7.1, Appendix 1), it was expected that the clustering procedure will allow us to distinguish at least six MLR remnant vegetation (MLR RV) clusters.

The combination of on-ground studies and remote sensing used in this study provides a critical link in understanding how plant communities respond to groundwater drawdown at the local and catchment scales. The analysis of Landsat data allows the capture of specifics of the temporal and spatial variability of broad land cover types and extrapolating local knowledge to the larger scales available for the historical period, when satellite images are available. For this study the remnant vegetation classes (riparian, floodplain, terrestrial) distinguished from the remote sensing analysis were found to be in good agreement with on-ground observations. Based on the fieldwork the remnant vegetation was observed either in the immediate vicinity of the creek channels (riparian vegetation) or within the floodplains. Such broad vegetation classes were also associated with locations, where the stream flow was either intermittent or permanent. The remaining remnant vegetation was classified as terrestrial, also in agreement with field observations.

2.3 Results & Discussion

2.3.1 Typology of phreatophytic vegetation

The interrelationships among key environmental elements such as climate, geology, landscape and soils has a strong influence on the plant attributes that will determine the distribution and abundance of groundwater-dependent plants and phreatophytes (*Figure 8*). We distinguish here between groundwater dependence and phreatophytes because relative groundwater dependence of different plant species is related to habitat use and plant functional characteristics (defining hydrotypes), maintained by significant groundwater discharge. Phreatophytes are a sub-group of these hydrotypes that maintain a subsurface root interaction with the capillary fringe and saturated zone of the groundwater. For example, riparian ecosystems can be groundwater dependent and contain both hydrophytes and phreatophytes. Influences on the development of phreatophytes within plant communities occur at multiple scales. Regional-scale variation in climate and geology, landscape-scale variation in landform and groundwater systems, and local-scale variation in soil and micro-climate interact with the intrinsic traits of plants to influence the distribution and production of phreatophytic species. The climatic environments where phreatophytes have been recorded are diverse and include most regions of the world (Table 3). However, the majority of reported studies on phreatophytes are from arid and semi-arid and Mediterranean-type climates where a combination of annual and/or seasonal drought and accessible, good quality groundwater is available. Underlying geology generally provides the conditions for the development of shallow aquifers accessible to the roots of phreatophytic vegetation and includes alluvial and aeolian sediments, sedimentary rocks, particularly karst, and fractured volcanics such as basalt. These conditions can create different groundwater systems from shallow alluvial to deep regional aquifers (Figure 8). The common requirement for phreatophytes is groundwater shallow enough for roots to access and a soil profile or rock fissures that allows penetration by plant roots. Soil depth and texture are likely to be particularly important in the development of root-groundwater interactions. A deep soil profile with soil texture and structure that readily retains moisture is likely to preclude the necessity for extensive development of deeper roots to meet water requirements, except in extremely dry habitats such as in deserts where rainfall is insufficient to replenish soil water supplies. Phreatophytes with a high dependency on groundwater are most common on soil types that do not readily retain water, such as deep sands. True phreatophytes are commonly perennial trees and shrubs (Table 3) with particular ecological, physiological, reproductive and morphological attributes (Figure 8) that enhance the ability of the plants to access groundwater (Eamus et al. 2015), for example, perennial woody plants with secondary growth allowing the development of sinker roots that can penetrate deep into the soil to access groundwater.

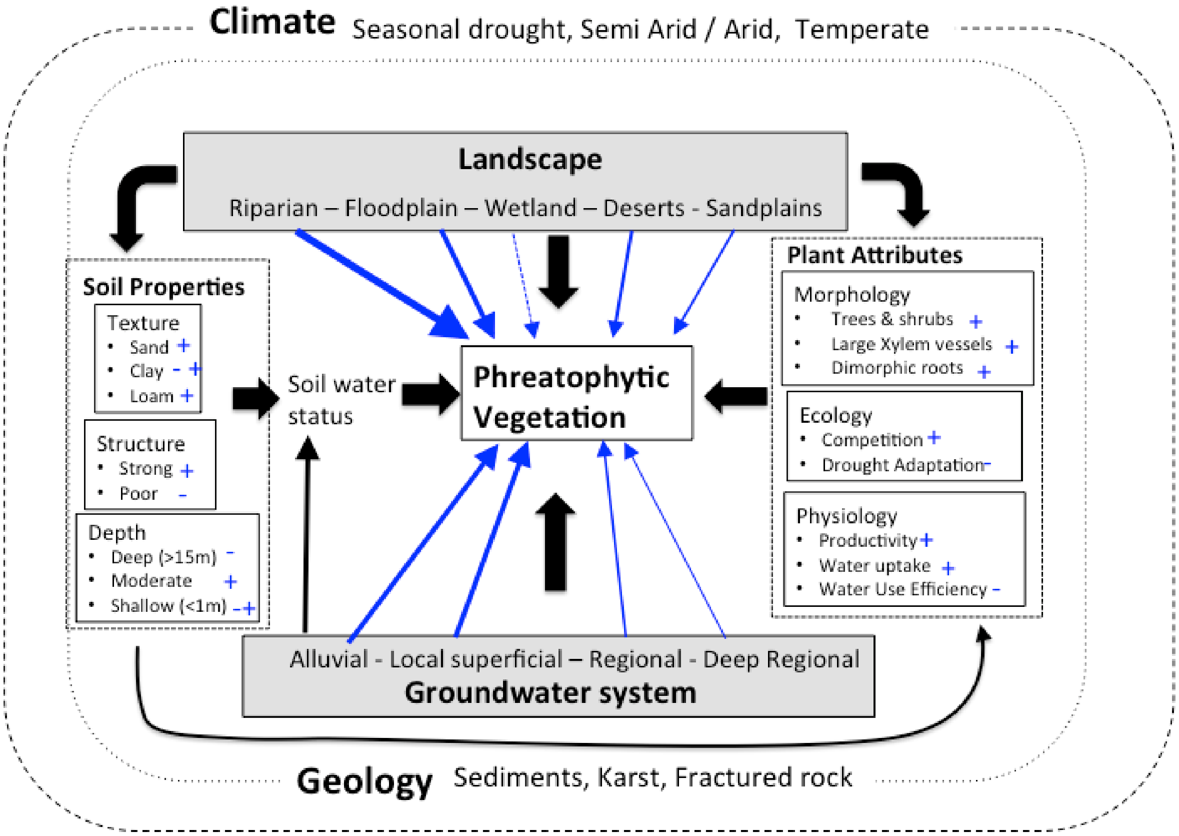

Figure 8: Conceptual model of the environmental drivers and landscape attributes and their relationships to phreatophyte vegetation that influence the development of conditions under which phreatophytic vegetation types may occur. Influences on the development of phreatophytes within plant communities occur at multiple scales including climate and geology as overriding influences at the regional scale, habitat and groundwater systems at the landscape scale and soil and plant traits at the local scale. Thickness of arrows indicates the relative strength of the influence and plus and minus signs indicate whether influence is mostly positive or negative.

The types of habitats where phreatophytes can occur include a wide variety of landscapes, geology and climates.Some examples are provided in Table 4. Riparian areas are the most readily identifiable phreatophytic landscape type and where most research has been based, particularly in south-western USA (eg. Meinzer 1927, Robinson 1952, Stromberg et al. 1996, Smith et al. 1998, Snyder & Williams 2000). Rivers are in the water gaining parts of the landscape and generally have shallow alluvial soils and sediments with shallow groundwater. Adjacent to the river, floodplains are common habitats with features that allow the development of phreatophytes. Seasonally episodic surface flooding over alluvial sediments recharges soil water and groundwater in the wet season. In desert habitats phreatophytes can make up a large proportion of plant communities as rainfall is usually insufficient to maintain perennial trees and shrubs and additional water sources are needed. Desert phreatophytes often have unique adaptations to the dry environment including extensive deep root systems to source deep water from local and regional aquifers. Examples include plants of the Namib desert of southern Africa with uniquely adapted species such as the shrubs *Welwitschia mirabilis* and *Acanthosioyos horridus* (Canadell et al. 1996, Cowling et al. 1997). Desert oases are generally a surface expression of the groundwater and can contain hydrophytes and obligate phreatophytes such as palm trees and perennial grasses such as *Phragmites australis* that would not normally occur in the surrounding arid environments (Bruelheide et al. 2010).

Ecological adaptations of plants to seasonal or episodic drought periods can be either via those that have developed mechanisms that allow them to tolerate dry conditions (drought tolerators) (plant hydrotypes 1, 2 and 3 in *Table 5*) or those that use adaptive strategies to avoid drought (drought avoiders) (plant hydrotypes 4, 5, and 6 in *Table 5*). Drought tolerators can physiologically shut down to prevent desiccation during drought periods. This shut-down can be through mechanisms such as control of leaf stomata opening, or possessing few and hidden stomata to reduce water loss from leaves, or the capacity to store water within the plant (e.g. cactus). Plants with these characteristics can shut down when conditions don't suit during drought, enabling them to occupy a wide range of climates and topographic positions in the landscape. Drought avoiders are either annual plants or plants that are able to access water at all times. These plants usually have higher summer water potentials and water loss and low stomatal control, and are therefore restricted topographically and climatically to areas where water is available all year round (Groom 2004). Phreatophytes are considered classic drought avoiders with their deep or dimorphic root systems that let them reach water sources deep in soil profiles or tap into the capillary fringe above the water table when soil moisture is depleted or hard to access (Ehleringer et al., 1991; Dawson and Pate, 1996). The ability to access shallow groundwater means these plants are less restricted by low rainwater and soil water availability and are able to photosynthesise and grow for longer periods during periods with no rainfall, provided that groundwater is accessible.

Table 3: Summary of the number of published references (108) by region, climate, habitat and life form of phreatophytes indicating the major regions, climate, and habitat where they have been studied and the major plant life-forms. Data are taken from Table 1 in Thomas (2015) and supplemented with data from additional literature. Numbers are biased towards regions (and their flora) where there has been greater research effort.

Region	No	Climate	No	Habitat	No	Life form	No
S.-W. USA	38	Semi-Arid	45	Riparian	25	Trees	47
Central Asia	13	Arid	34	Desert plain	14	Shrubs	33
S. Australia	11	Mediterranean	19	Floodplain	12	Perennial Grasses	4
S. Africa	10	Tropical	12	Sand plain	12	Perennial Herbs	1
Mediterranean	9	Temperate	9	Woodland	8		
China	5	Sub-alpine	1	Saline areas	3		
N. Australia	4						
N. Africa	4						
S. America	4						
N. USA	3						

Table 4: Typology of hydrogeomorphic environments where phreatophytes may occur.

Habitat	Geology/ Lithology	Climate	Ground-water depth	Existing hydrology	Vegetation-groundwater dependencies	Example	Reference
Riparian	Alluvial Sediments	Wet-dry Tropical, Sub-tropical, Mediterranean semi-arid,	Shallow alluvial	Perennial surface flow. Bank recharge in wet. Shallow WT in dry. Consistent soil recharge.	Hydrophytes dominant. High % groundwater use, High productivity, long growth season.	Riparian forests	Thorburn & Walker 1993, Lamontagne et al 2005, Lite & Stromberg 2005, O'Grady et al 2006, Chen et al. 2015.
Floodplain	Alluvial Sediments	Tropical, Semi-arid, temperate	Shallow alluvial, local regional	Seasonal-episodic surface flooding. Recharge in wet. Shallow WT in dry.	Hydrophytes present. High-moderate %groundwater use, high productivity, long growth season.	Floodplain forests	Thorburn & Walker 1993, O'Grady et al. 2009, Stromberg & Merritt 2016, Pfautsch et al 2015
Coastal sandplain	Sedimentary	Mediterranean	Shallow to moderate	porous sand shallow aquifer	High proportion of phreatophytes, varies along a catena	Swan Coastal Plain, *Banksia* spp. Coastal heath and woodlands	Sommer & Froend 2010, Zencich et al. 2002
Coastal dunes	Sedimentary	Mediterranean, Sub-tropical	Shallow to deep	Poor soil water retention, Perched shallow groundwater.	High proportion of phreatophytes, varies along a catena	Coastal dunes & wetlands	Barrabas et al. 1999, Rutherford et al. 2013
Desert sand dunes,	Sedimentary	Arid	Moderate to deep	Little soil water storage, deep regional aquifer	Highly specialised arid zone plants with deep taproots	Namib desert, South Africa	Cowling et al. 1997, Canadell et al. 1996
Desert oasis	Sedimentary	Arid	shallow	Surface expression of regional groundwater	Hydrophytes and aquatic plants in proxity to open water, reliance on groundwater along catena	Silt loam soil, catena	Vonlanthen et al. 2011, Bruelheide et al. 2010.
Savanna plain	Sedimentary	Mediterranean	Shallow to moderate	Summer or prolonged drought, low soil moisture storage capacity	*Quercus suber* with dimorphic root system	Central Portugal	David et al. 2013, Pinto et al. 2014
Groundwater discharge springs	Sedimentary	Mediterranean, Sub-tropical	shallow	Artesian springs	Hydrophytes and aquatic plants in proximity to springs	Great Artesian Basin, eastern Australia, Fens, glaciated Midwest and Northeast USA	Bedford & Godwin 2003, Fensham et al 2004
High elevation lacustrine plain.	Permeable volcanic rock	Montane Sub-tropical, seasonal rainfall	moderate	deep local aquifer with localized discharge areas	Mixed plant communities around discharge sites	Central mountains Mexico, deep groundwater discharge areas	Duraz & Farvolden 1989

Table 5: Typology of groundwater-dependent vegetation including phreatophytes.

	Plant Hydrotype[1]	Plant Type/Characteristics	Response to drought	Range of habitats	Example taxa	References
1	Hydrophyte[2]	Aquatic submerged/floating, reliant on surface water expression of groundwater. Annual or perennial herbs. Usually ephemeral and exist as dormant seeds, tubers or spores in sediments until wetland is inundated.	Drought avoider, growing only in surface water or surface expression of groundwater. Exist as dormant seeds, tubers. Groundwater range 0-0.25m	Perennial palustrine, lacustrine wetlands, streams	Nymphaea, Hydrilla, Vallisineria spp., Utricularia spp.	Den Hartog & van der Velde 1988; Boutin & Keddy 1993; Brock 1994, Cassanova 2011
2	Helophyte[2, 3]	Aquatic, semi-aquatic emergent plants. Transition between aquatic and terrestrial areas. Graminoid, or perennial herbs spreading roots or rhizomes, aerenchyma cells. Tolerant of seasonal drying of surface water as long the water table is not below the rooting zone for an extended period	Drought avoider or drought tolerator and many die back to rhizomes or dormant seed during dry periods. Groundwater range 0-1m.	Perennial palustrine, lacustrine wetlands, streams, floodplains, desert oasis, mound springs	Phragmites australis, Enchinochloa polystachya, Eleocharis spp., Cyperus spp., Juncus spp., Scirpus spp.	Pieadade et al. 1991, Boutin & Keddy 1993, Brock 1994, Vonlanthen et al. 2011, Bruelheide et al. 2010.
3	Mesophyte[2,3]	Usually trees and shrubs that are flooding tolerant, aerenchyma, adventitious roots and shallow roots. Able to survive on soil moisture only if water table is inaccessible during dry periods but can also tolerate periods of inundation and waterlogged soils. Development of arenchyma cells in roots, large xylem.	Drought avoider via high water use. Groundwater range 0-5m	Riparian, wetlands, floodplains, desert oasis.	Prosopis spp., Tamarix ramossina, Populus euphratica, Melaleuca spp., Eucaluptus camaldulensis	Stromberg et al. 1996, Stromberg & Merritt 2015; Vonlanthen et al. 2011, Bruelheide et al. 2010, Lamontagne et al. 2005
4	Semi-mesophyte[3]	Drought tolerant, some waterlogging tolerance, dimorphic roots, trees and shrubs. Able to access groundwater from the capillary fringe but can access soil water when available and possesses physiological mechanisms to cope with drought. High to moderate productivity.	Drought avoider accessing soil water. Groundwater range 2-10m	Riparian, floodplain, woodlands, savanna, sedimentary plains	Banksia spp., Quercus sp Acer negundo, Eucalyptus largeflorens	Sommer & Froend 2010, Zencich et al 2002; David et al. 2013, Pinto et al. 2014, Lamontagne et al. 2005.
5	Semi-xerophytes[3]	Sclerophyll, drought tolerant dimorphic roots, possesses physiological mechanisms to cope with drought. Can access deeper groundwater via sinker roots. Moderate productivity.	Drought tolerator, physiological shutdown in dry conditions, under certain conditions will access groundwater. Groundwater range 5-30m	Woodlands, floodplain, coastal sand dunes, savanna plains.	Eucalyptus vitrix on semi-arid floodplain, Tamarix aphylla, Banksia attenuata, Prosopis veluntina	Synder & Williams 2000 Lite & Stromberg 2005 Vonlanthen et al. 2011, Pfautsch et al 2015;
6	Xerophytes	Trees and shrubs, spreading root system, sclerophyll leaves and reduced leaves, small diameter xylem, low productivity.	Drought tolerator, physiological shutdown under dry conditions. Soil water dependent. Groundwater range 2- >30m	Upslope, terrestrial environments.	Quercus turbinella, Carnegiea gigantea, Acacia anuera	Neilson & Wullstien 1985, Barcikowski & Nobel 1984, Pressland 1973,

[1] The functional traits that distinguish different plant hydrotypes relating to physiology, morphology and ecology (*Figure 8*, *Table 4*). Traits can include: 1. life cycle, 2. root system, 3. cell development, 4. productivity, 5. leaf morphology, and 6. reproduction. The various responses of hydrotypes to drought (*Table 4*) include: 1. drought avoider (water saving, spending), 2. drought tolerator (avoider by accessing groundwater, or tolerator by shutting down), semi-aquatic emergents includes species described as 'matrix and interstitial' by Boutin & Keddy (1993).

[2] Groundwater dependent plants. [3] potential phreatophytic plants.

We have used the general plant life form and life history functional types (Raunkaier 1934, Dansereau 1959, Hutchinson 1975) as well as wetland plant types (Boutin & Keddy 1993, Brock & Cassanova 1997, Keddy 2000) and dryland riparian plant characteristics to develop a typology of hydrotypes with different functional/physiological traits representing adaptions specific to hydrological conditions, and in our case groundwater use (Figure 9 and Figure 10, Table 5). Our typology comprises six functional types that reflect a gradient of groundwater dependency and includes: aquatic (hydrophytes) and semi-aquatic (helophytes) plants; terrestrial plants that are tolerant of some flooding and waterlogging (mesophytes, semi- mesophytes); dryland plants that are drought avoiders with roots that can access the capillary fringe of the groundwater (semi-xerophytes); and, dryland plants that are drought tolerators that have morphological and physiological features that allow them to survive long periods without water (xerophytes) (Table 5, Figure 9). These hydrotypes can range from fully aquatic plants in permanently flooded wetlands where the groundwater levels are higher than the land surface for much of the year, to plants adapted to habitats that have only seasonally or episodically available soil water (xerophytes). Functional hydrotypes are likely to experience spatial and temporal variability in frequency of contact with, and extent of use of groundwater, and plasticity in groundwater dependence may also occur between and within species.

The criteria used to define the hydrotypes are broad and intended to provide an indication of the potential for the occurrence of phreatophytes within a plant community (Figure 9). Criteria used for identifying hydrotypes included life history (annual or perennial), life form (woody or herbaceous), landscape position (aquatic, littoral, terrestrial), depth to groundwater and drought adaptation (drought avoider or drought tolerator) (see Table 5). Plasticity in ecological adaptations and local conditions (ecotypes) between and within species means that there is likely to be considerable overlap across hydrotypes. This overlap is particularly likely for transition groups such as semi-mesophytes and semi-xerophytes. Therefore, we have distinguished six general hydrotypes that comprise the following:

1. *Hydrophytes* are aquatic plants that are dependent on some semi-permanent surface water in low-lying areas that are commonly are maintained by surface water and groundwater in varying proportions. These plants occur in the sub-littoral zone with seasonal surface water and therefore tend to be annuals which exist as dormant seeds or spores in sediments or perennials with a perennial storage organ, such as tubers, rhizomes, etc. Hydrophytes can be fully aquatic and submerged (e.g. *Myriophyllum* spp.), floating *(Azolla* spp.) or attached with floating leaves (*Nymphaea* spp.).

2. *Helophytes* are plants that dominate the littoral zone boundaries of low-lying wetland areas where surface water is present for some part of the year (den Hartog & van der Velde 1988). These plants are also described as 'matrix' species by Boutin and Keddy (1993), capable of vigorous clonal spread. Helophytic plants tolerate a wide range of water depths and periods of inundation. This group can grow in semi-permanent standing water but also can be relatively tolerant of drying soil conditions, as long the water table is not below the rooting zone for an extended period. Graminoids are typical helophytes from the Poaceae (eg. *Phragmites* spp.) or Cyperaceae (eg. *Cyperus* spp.) families, possessing rhizomes, stolons or other below-ground perenniating organs.

3. *Mesophytes* generally occupy littoral and supra-littoral areas and can be phreatophytic. Seasonal drying of soil water for at least some part of the year is offset by accessing shallow water tables. Mesophytes are tolerant of flooded conditions for extended periods and may also require groundwater to be accessible to allow root development under aerated soil conditions. Mesophytes can be phreatophytic wetland trees and shrubs that tolerate flooding and exploit shallow groundwater in the dry part of the year to maintain long periods of growth

and reproduction. They are typically obligate phreatophytes with shallower maximum rooting depths (Figure 9) and limited physiological capacity to tolerate prolonged drought and disconnection from shallow water tables.

4. *Semi-mesophytes* can be either obligate or facultative phreatophytes that are opportunistic in their use of groundwater. They will develop root systems according to the distribution of water, groundwater depth and soil water stores, and have greater maximum rooting depths than mesophytes. Dimorphic root systems are common in this hydrotype, enabling these plants to access shallow and deep water sources. Some species can also tolerate short periods of inundation and waterlogged soils, and most are more tolerant of drought than mesophytes.

5. *Semi-xerophytes* occur at the transition between flooding-tolerant and drought-tolerant xerophytes (Table 5, Figure 9). Semi-xerophytes can either be obligate (rare) or facultative phreatophytes accessing deep soil. Drier conditions and less soil water storage make accessing groundwater through deep sinker roots important for growth and reproduction during seasonal or episodic drought (Figure 9). These plants are able to access water from the capillary fringe of the water table if it is shallow enough but can survive in areas with no groundwater influence by accessing deep soil water when available and possessing physiological mechanisms to cope with drought.

6. *Xerophytes* are drought-tolerant plants that are unlikely to be dependent on groundwater to maintain growth and reproduction. Drought tolerance is typically through various physiological and morphological traits (Table 5). They can grow in areas with no groundwater influence where plants must obtain their water from the soil profile via direct rainfall infiltration only. Plants usually either require extensive root systems to explore a large volume of soil and/or possess mechanisms to minimize water loss, such as becoming dormant during drought periods (Ehleringer et al. 1991, Smith et al. 1997).

In most landscapes, as depth to groundwater increases and therefore accessibility decreases, the proportion of hydrotypes in the plant community adapted to wet conditions (and the prevalence of phreatophytes) will decline (Figure 10). The proportion of phreatophytic plants is usually highest at the lowest part of the landscape such as the bottom of a slope in a river valley or wetland basin. There is however, a range of groundwater depths at which phreatophytes occur and this range increases across the different hydrotypes (Table 5, Figure 10). In upper-slope terrestrial areas where groundwater is very deep (>15m), almost all plants will be xerophytes or drought tolerators that derive their water predominately or exclusively from the unsaturated soil and will have traits indicative of drought tolerance (Table 5).

Use of hydrotypes to define plant communities in terms of hydrological preferences and (inferred) groundwater dependency, highlights the importance of vegetation-hydrology linkages. We provide a decision tree for categorising plants into hydrotype functional groups (Figure 11). The decision tree starts with life history traits (annual or perennials) and life form (woody or herbaceous) and utilises information on habitat use, depth to groundwater, duration of inundation and drought adaption to identify the hydrotype (such as high water use efficiency or sunken stomata, for drought tolerator xerophytes). Groundwater dependent vegetation is likely to be dominated by hydrophytes, helophytes, mesophytes and semi-mesophytes and phreatophytes are most likely to be either mesophytes, semi-mesophytes or semi-xerophytes, and in some situations helophytes (Figure 11). The diagram also places the hydrotypes according to their relationship with depth to groundwater and duration of surface water inundation. Other plants groups that are not directly influenced by groundwater or surface water hydrology include terrestrial perennial herbs such as geophytes and terrestrial annual herbs including therophytes.

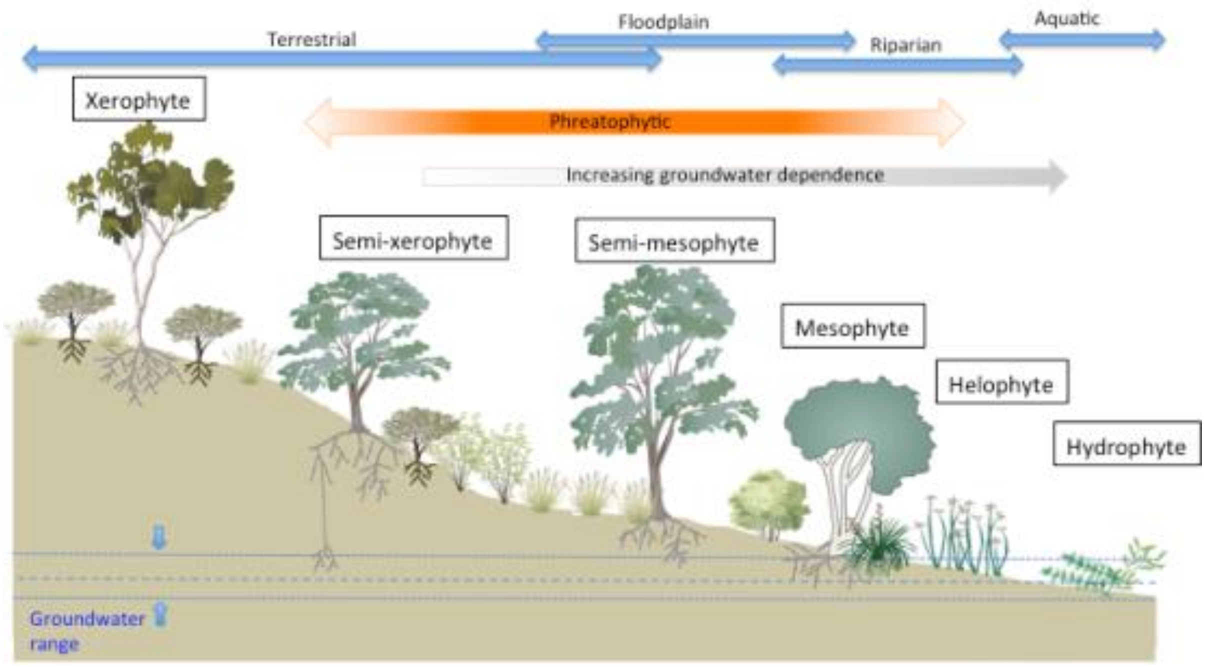

Figure 9: Relationship between plant hydrotypes and depth to groundwater and the range of landscape positions and hydrotypes that include phreatophytes. Hydrophytes are aquatic plants that are dependent on surface water expression of groundwater. Helophytes and mesophytes occur where groundwater is seasonally at or close to the surface. Semi-mesophytes and semi-xerophytes occupy the transitional areas between terrestrial and wetland habitats and can tolerate drought conditions and may access groundwater. Xerophytes have no access to groundwater and thus are dependent on rainwater and able to tolerate drought conditions. Phreatophytes include semi-xerophytes, semi-mesophytes, mesophytes and, in some situations, helophytes.

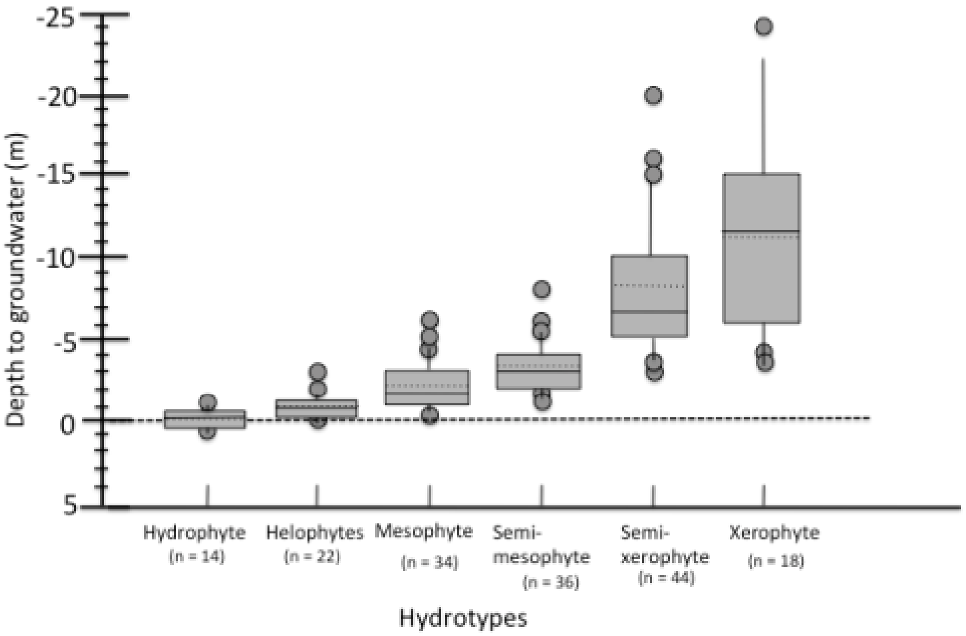

Figure 10: Box plots of data gathered from 168 articles in the international literature on the range in depths to groundwater at which different plant hydrotypes including phreatophytes (mesophytes, facultive mesophytes and semi-xerophytes) are found. Number of studies cited for each hydrotype is given in parentheses. The data highlight both the degree of overlap in depth to groundwater for different hydrotypes and increasing range in depth to groundwater preferences from hydrophytes to xerophytes.

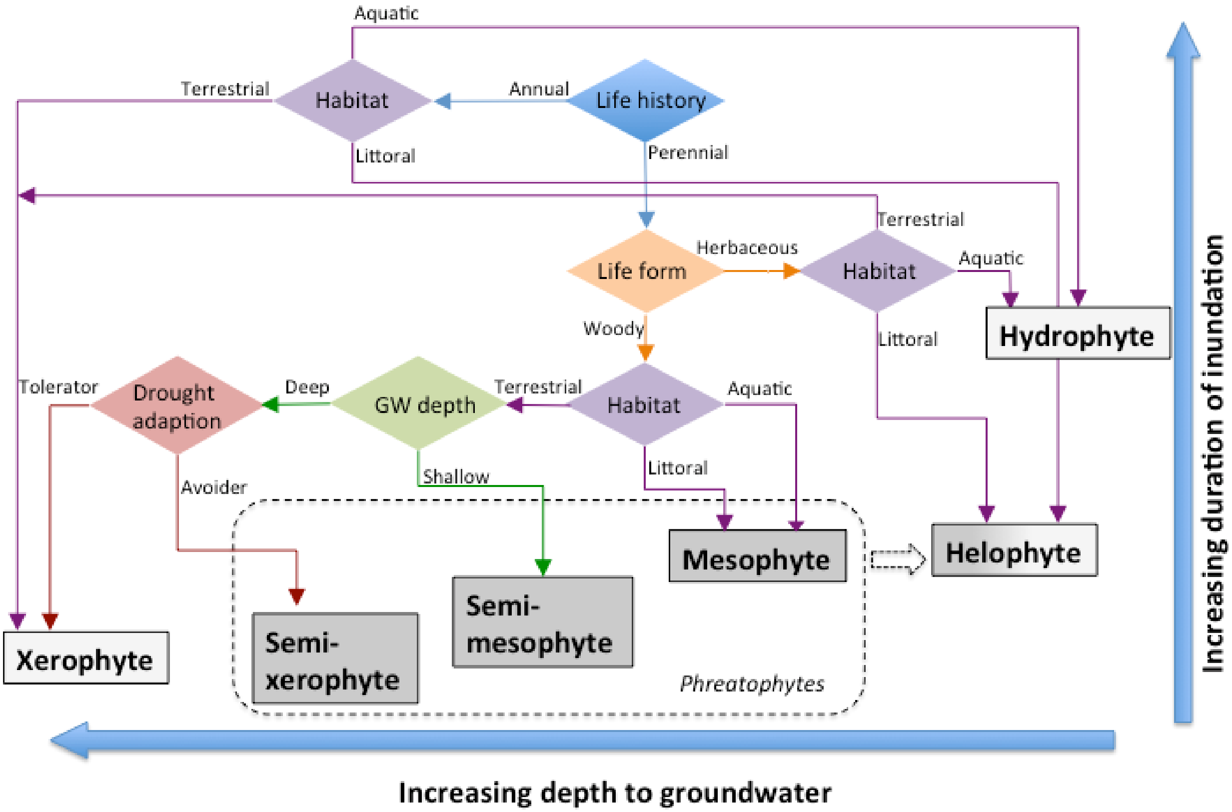

Figure 11: Decision tree for categorising plants into particular hydrotype functional groups. This starts with life history traits (annual or perennials) and life form (woody or herbaceous). Plants can then be categorised according to habitat or position in the landscape and for terrestrial woody plants depth to GW. For terrestrial woody plants where GW is deep hydrotypes can be distinguished according to their drought adaption strategy such as high water use efficiency or morphological features such as sunken stomata, etc, for drought tolerator xerophytes. Drought avoiding plants (semi-xerophytes) require deep sinker roots to access deep GW, have a longer growing period in the dry season and low water use efficiency. Hydrotypes that are most likely to contain some phreatophytes are highlighted (dashed line box, and dashed arrow indicating that in some situations helophytes can also be phreatophytic). The diagram also places the hydrotypes according to their relationship with depth to GW and duration of surface water inundation. Other plants groups that are not related to groundwater and therefore not included here include terrestrial perennial herbs such as geophytes and terrestrial annual herbs including therophytes.

2.3.2 Landscape distribution of plant hydrotypes and productivity

Vegetation plot surveys undertaken between October 2015 and March 2016 produced a total of 112 species at the 27 Maules Creek sites and 118 species at the 25 Bremer River sites, with 32 and 27 exotic species, respectively (supplementary Table 46). Surveys indicated the primary floristic difference between the plant communities for Maules Creek and Bremer River (Figure 13). Ordination of plant species composition data revealed that, for the Maules Creek sites, there was clear separation in plant communities between riparian and floodplain landscape positions and between perennial and intermittent riparian sites. Indicator species for each landscape position for each river are given in Table 6. River red gum (*Eucalyptus camaldulensis*) was a dominant tree for both riparian and floodplain sites at Maules Creek, while River Oak (*Casuarina cunninghamiana*) and Black Tea-tree (*Melaleuca bracteata*) were common riparian trees at both Maules Creek and the Bremer River (Table 6). Distance-based redundancy analysis for each study catchment confirmed the plant community differences for the different landscape positions, particularly for Maules Creek where lower rainfall is likely to create greater distinctions in plant community types, based on water availability (Figure 12). This difference in plant communities is emphasised by the vector plots indicating the influence of hydrotype composition on the separation between sites. Vector plots showed that hydrophytes, helophytes and mesophytes were associated with riparian sites while xerophytes and semi-xerophytes mostly associated with the drier terrestrial and intermittent floodplain sites (Figure 12). In terms of potential groundwater-dependent vegetation, 41% of the plant cover at the riparian perennial sites, and 52% for intermittent riparian sites were for plant hydrotypes that are likely to be groundwater dependent (Table 5, Figure 13). At floodplain sites, groundwater-dependent species made up 19% of foliage cover and no groundwater dependent species were recorded at the terrestrial sites (Figure 13). These figures show that the hydrophytes and helophytes were found only in the wetter landscape positions and mesophytes are also most abundant in the riparian and floodplain areas (Figure 13).

We measured standing biomass (as basal area per ha), canopy cover and litter fall of overstorey species in different landscape positions at the Maules Creek and Bremer River study sites. Data indicate similar tree basal area across landscape positions with only terrestrial sites having significantly lower basal area (F=4.32, p=0.02) *(Figure 14).* Basal area at the perennial riparian sites at Maules Creek was higher than at other sites but because of high variability this was not significant (F= 2.1 p=0.08). Canopy cover was similar across landscape positions but generally higher in the Bremer catchment, likely due to higher rainfall and some rainforest elements in the plant communities in this catchment, particularly in the riparian zone. Litterfall results for Maules Creek were patchy due to the loss of a number of traps attributed to cattle and wind disturbance. Litterfall rates varied across sites from 308 g m^{-2} yr^{-1} at the perennial floodplain site to 644 g m^{-2} yr^{-1} at the intermittent riparian site (Figure 15). There were also higher rates of litterfall over the summer period (October to March) than at other times of the year (F=8.23, p=0.01) (Figure 15).

River red gum *(E. camaldulensis)* was the dominant tree species on perennial riparian and floodplain sites at Maules Ck with by far the highest basal area, dbh and number of trees (Table 7). This species also occurred at the intermittent sites but with lower biomass and canopy cover. River red gums are considered mesophytes and able to tolerate high water tables and flooded conditions and are widespread throughout most of Australia as riparian and floodplain trees. The other prominent overstorey species were the obligate riparian species River Oak (*Casuarina cunninghamiana)* and Black tea-tree (*Melaeuca bracteata)*. On the intermittent floodplain and terrestrial sites, native cypress pine (*Callitris glaucophylla)* was the most abundant overstorey tree, although total tree basal area was much lower than at riparian and floodplain perennial sites (Table 7). This species can be considered a xerophyte with features indicating adaptation to dry conditions. At the Bremer sites the riparian trees with the highest biomass were C. *cunninghamiana* and *M. bracteata* with *M. bracteata* also common on floodplain sites (Table 7). As for Maules Creek sites, the dominant overstorey trees were similar between the terrestrial and floodplain intermittent sites on the Bremer River. These were the forest red gum (*E. tereticornis*) and Carbeen (*Corymbia tessellaris*). These species occur across a wide variety of habitats and can be considered semi-xerophytes. The obligate riparian tree *Melaleuca viminea* was a common riparian species that appears restricted to streams with perennial pools.

Table 6: Top five indicator species (Simper results) for each landscape position at the Maules Creek and Bremer River study sites.

Landscape	Maules Creek			Bremer River		
	Species	Abun.	% Cont.	Species	Abun.	% Cont.
Riparian Perennial	*Eucalyptus camaldulensis*	5.1	28.7	*Casuarina cunninghamiana*	3.6	17.7
	Cyperus sp	2.3	12.3	*Melaleuca viminalis*	3.0	12.2
	Leptochloa digitata	3.1	9.9	*Melaleuca bracteata*	3.4	12.1
	Pericarya sp	1.4	6.4	*Cynodon dactylis*	2.8	10.3
		2.3	5.3	*Aegeratina denophora*	1.9	7.2
Floodplain Perennial	*Eucalyptus camaldulensis*	4.3	20.3	*Lantana camara*	6.3	26.9
	Geijera parviflora	4.4	19.1	*Melaleuca bracteata*	5.4	26.5
	Aristida sp	3.8	15.2	*Macfadyena unguis-cati*	3.9	12.8
	Hyparrhenia hirta	3.7	10.7	*Cynodon dactylis*	4.2	8.4
	Astrostipa sp	2.6	8.2	*Lantana camara*	6.3	26.9
Riparian Intermittent	*Melaleuca bracteata*	3.9	13.9	*Melaleuca bracteata*	4.7	29.5
	Eucalyptus camaldulensis	3.2	13.5	*Aegeratina denophora*	2.4	13.6
	Casuarina cunninghamiana	2.7	10.0	*Cynodon dactylis*	2.4	8.8
	Abutilon oxycarpum	2.0	6.5	*Lantana camara*	2.1	7.4
	Callitris glaucophylla	2.3	5.6	*Lomandra longifolia*	1.9	6.6
Floodplain Intermittent	*Astrostipa* sp	2.9	21.3	*Imperata cylindica*	3.8	19.9
	Callitris glaucophylla	3.9	19.4	*Bothriochloa sp*	2.7	17.6
	Eucalyptus camaldulensis	3.5	18.3	*Themeda australis*	2.3	11.4
	Aristida sp	2.8	11.8	*Corymbia tessellaris*	2.8	9.7
	Alectryon oleifolius	2.0	4.7	*Eucalyptus tereticornis*	1.8	4.4
Terrestrial	*Callitris glaucophylla*	3.5	27.7	*Bothriochloa* sp	6.7	44.8
	Triodia sp	3.2	19.5	*Eucalyptus tereticornis*	4.1	28.6
	Aristida sp	3.1	19.5	*Heteropogon contortus*	3.5	11.4
	Themeda australis	1.5	9.8	*Eucalyptus melanophloia*	2.2	7.4
	Eucalyptus dealbata	2.3	8.6			

Table 7: Average number of trees, diameter at breast height (dbh) and basal area (BA) of the dominant tree species for each landscape position at Maules Creek and the Bremer River sites.

Site	Landscape Position	Stream Hydrology	Main Species	Mean no. trees	Mean dbh (cm)	BA m^2 ha^{-1}
Maules	riparian	perennial	*Eucalyptus camaldulensis*	17	38.67	18.99
			Melaleuca bracteata	4	4.33	0.45
Maules	floodplain	perennial	*Eucalyptus camaldulensis*	9	34.00	7.55
			Geijera parviflora	14	8.03	0.76
Maules	riparian	intermittent	*Eucalyptus camaldulensis*	3	26.50	3.05
			Casuarina cunninghamiana	11	31.55	5.36
			Melaleuca bracteata	15	15.13	3.14
Maules	floodplain	intermittent	*Eucalyptus camaldulensis*	2	25.80	1.30
			Callitris glaucophylla	17	8.68	2.48
Maules	terrestrial	dry	*Callitris glaucophylla*	18	19.20	1.43
			Eucalyptus dealbata	5	18.3	1.61
Bremer	riparian	perennial	*Casuarina cunninghamiana*	9	26.20	4.51
			Melaleuca viminea	11	11.50	0.26
			Melaleuca bracteata	16	22.00	6.23
Bremer	floodplain	perennial	*Melaleuca bracteata*	9	14.50	5.00
			Eucalyptus tereticornis	1.5	44.00	6.00
Bremer	riparian	intermittent	*Melaleuca bracteata*	31	21.30	8.96
			Casuarina cunninghamiana	1.5	16.80	0.26
			Eucalyptus tereticornis	0.5	46.70	3.20
Bremer	floodplain	intermittent	*Corymbia tesselaris*	18	12.10	2.59
			Melaleuca bracteata	9	20.80	10.44
			Eucalyptus tereticornis	4	7.70	0.71
Bremer	terrestrial	Perennial	*Eucalyptus tereticornis*	8	24.90	2.87
			Corymbia tesselaris	0.5	6.90	0.06

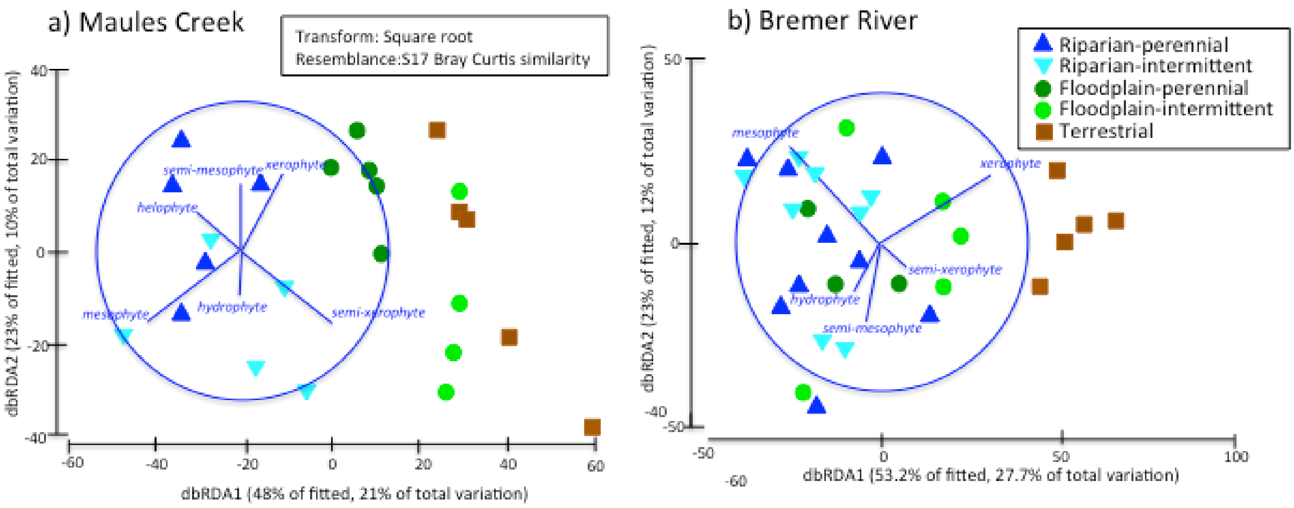

Figure 12: Distance based redundancy analysis of the floristics for different landscape positions (terrestrial, perennial and intermittent riparian and floodplain) for the (a) Maules Creek and (b) Bremer River sites. Each data point represents a site and the proximity of points in ordination space indicates greater similarity in plant communities between sites. Blue vector lines show the relationship of the different hydrotypes to the distribution of sites, with length of the line indicating the strength of the relationship (only vectors with r > 0.3 are shown).

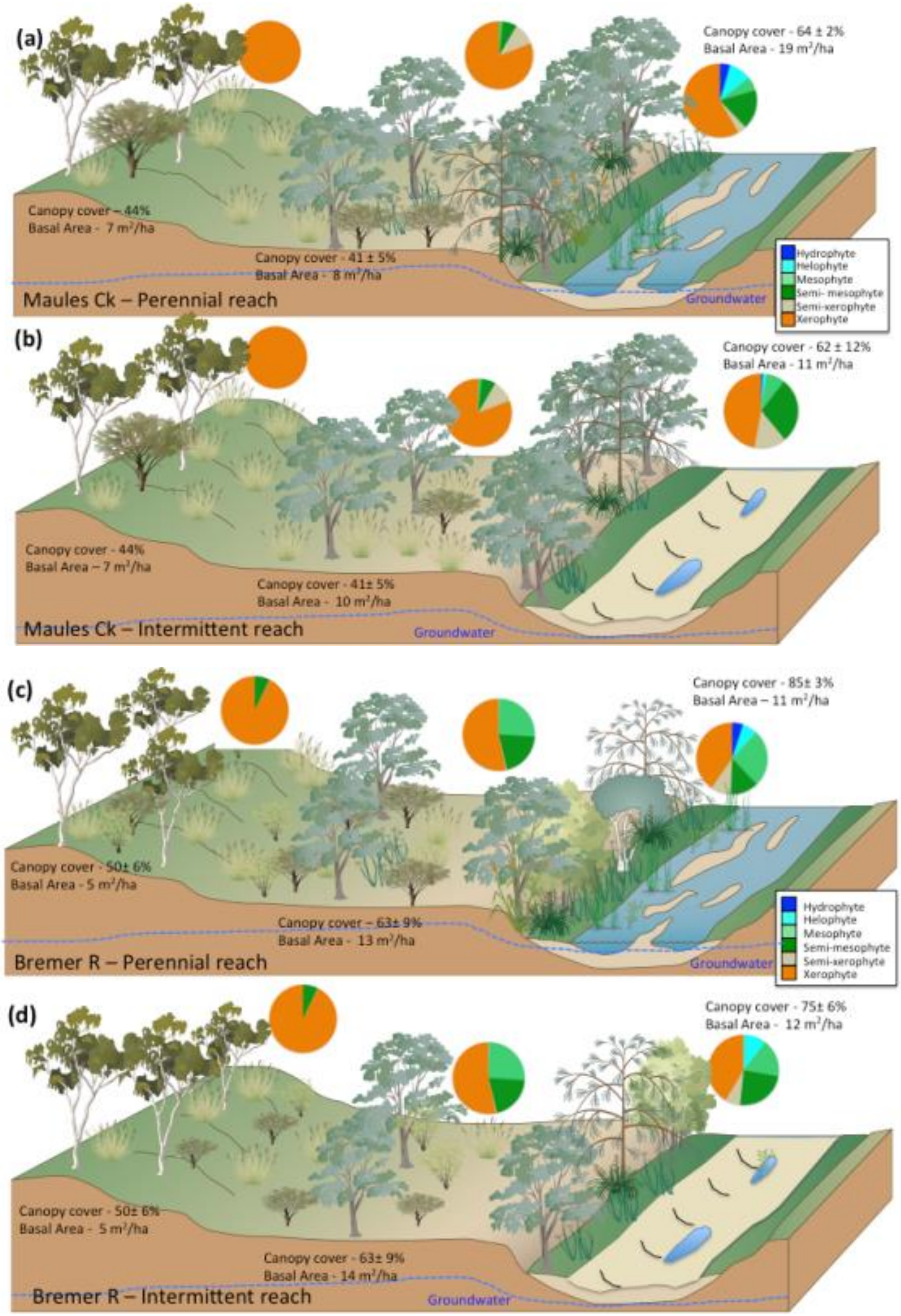

Figure 13: Schematic of an (a) perennial and (b) intermittent reach of the Maules Creek and (c) a perennial and (d) intermittent reach of the Bremer River sites, respectively. The schematics summarise the relationship between landscape position and plant hydrotypes (see Table 3) in each flow regime type in each catchment. Pie graphs indicate the proportion of plant abundance for each hydrotype for each landscape position. Also given are indicators of plant productivity (canopy cover, basal area) for each landscape position.

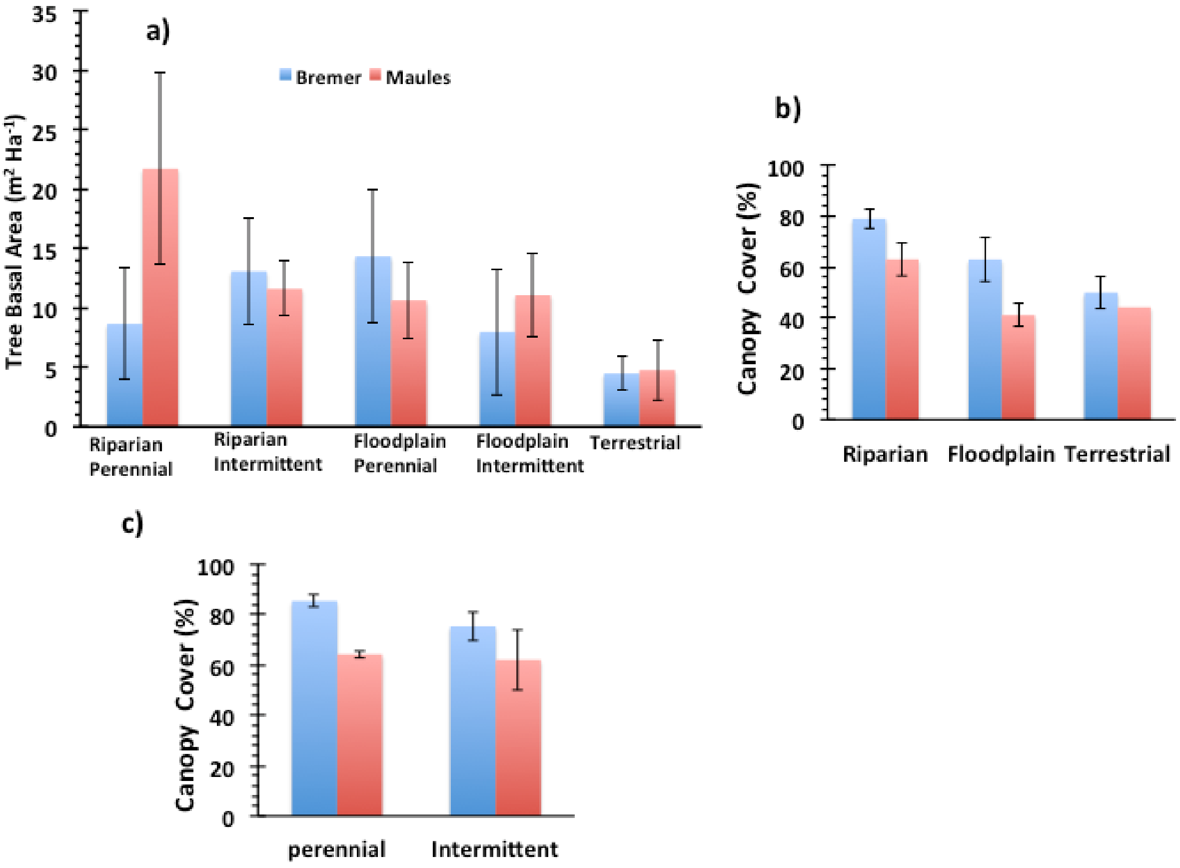

Figure 14: (a) Tree basal area for each landscape position and study catchment, (b) Tree canopy cover for each landscape position, (c) Tree canopy cover for perennial and intermittent landscape positions. Data are means ±SE for five plots in each landscape position at the Maules Creek and Bremer River study sites.

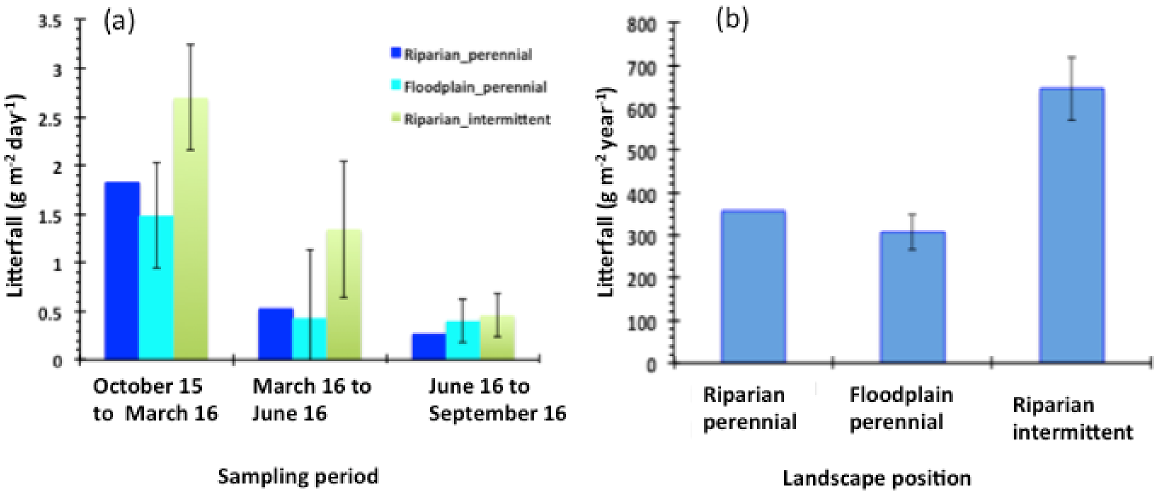

Figure 15: (a) Differences in mean (± SE) daily litter fall for different landscape positions at Maules Creek sites over the three sampling times which represented different seasons including summer (October to March), Autumn (March to June) and Winter (June to September). (b) Mean (± SE) annual litter fall for the different landscape positions.

2.3.3 Predicting groundwater use of plants across landscapes

Annual rainfall at Maules Creek over the study period was 21% above the long-term average (620mm). But rainfall varied across the study period with the late summer, early autumn period (February to April) below average at 56% of long-term average (132 mm) for these months. In contrast, June to September was 214% above the long-term average for these months (124 mm) (Figure 16). The dendrometer traces of changes in stem diameter of red gum (E. camaldulensis) trees over the 12-month study period (October 2015 to September 2016) indicate seasonal variation in hydrological state and growth among landscape positions (Figure 16). The traces indicate the short-term responses (hydration) of trees to rainfall events and longer-term changes in stem diameter (growth) over the study period. Trees at the Maules Creek perennial riparian and floodplain sites showed sustained growth over the study period. Average growth rate was 8.4 mm yr^{-1} for perennial riparian trees and 4.1 mm yr^{-1} for floodplain perennial trees. For the intermittent sites, the riparian tree growth was 1.4 mm yr^{-1} and floodplain trees showed a negative growth over the study period of -0.2 mm yr^{-1}. These growth rates for the different landscape positions accorded with average depth to groundwater at each site, with perennial sites that had shallower depth to groundwater (2 to 7 m), having more rapid growth than trees higher up in the catchment at the intermittent sites (Figure 16). Negative growth of the intermittent floodplain trees related to much greater depth to groundwater (>18 m) and suggests these trees were not phreatophytic. Interestingly, these trees showed some recovery in stem diameter in the latter stages of the study period when there was above-average rainfall in the area of Maules Creek, suggesting dependence on rainfall recharge of soil. Tree response to rainfall events and periods also varied among landscape positions (Figure 17, Table 8).

We analysed the response of all tree diameter traces to a single large rainfall event, a sustained period of rainfall and a prolonged drought period (Figure 17). Responses of trees in the perennial riparian and floodplain positions were much greater than for the intermittent sites in terms of the rate, size and duration of the stem increment (Table 8). During a sustained rainfall period, riparian perennial trees showed the least variation in response to rainfall and, during a sustained drought period, showed a lower rate of decline (drought slope) in stem diameter (Figure 17, Table 8). These differences in stem diameter responses are also evident when considering the diel traces for trees in each landscape position during a period immediately before and after a rainfall event (Figure 18a, b) and during a drought period (Figure 18c, d). These traces clearly show the diel expansion and contraction of the trees stems as they transpire and rehydrate. They also show greater responses of trees in the perennial riparian and floodplain landscapes compared with the intermittent sites. These results suggest that the trees at the riparian perennial site (in particular, but also the floodplain perennial site) had higher growth rates over the year and larger diel responses indicating higher short-term growth rates. Although these trees have responded to rainfall events (and corresponding flow), they show less response to drought periods, suggesting they are less dependent on rainfall and may also be accessing groundwater continuously. To a lesser extent, dendrometer traces also suggest riparian trees at the intermittent site may also be accessing groundwater at some periods during the year.

The δ^2H and δ^{18}O water isotope signature for leaves, soil, groundwater and stream water at the different landscape positions (Figure 19) corroborate the results from the dendrometer data. The isotope study shows in particular the separation of leaf water and soil water from the perennial riparian site. Mixing model results (Table 9) indicate that trees mostly obtain water from deep soil sources and groundwater at perennial sites where groundwater is relatively shallow. For all landscape positions leaf isotope values were most similar to groundwater and deep soil water in October when rainfall for the previous two months had been well below average. Water δ^2H and δ^{18}O values for leaves were very different than groundwater for samples taken in March during a time of extended dry, hot conditions (Figure 20a, b).

Soil water isotope values showed similar trends over the study period as the values for leaves although the seasonal changes were not as great (Figure 20c, d). Soil water δ^{18}O values were more enriched near the soil surface reflecting an increase in fractionation through the effects of evaporation (Figure 21). Isotope values were more positive in upper than in lower soil layers and became more depleted with depth and δ^{18}O and δ^{2}H values at 75-100 cm soil depth were similar to those of groundwater at the perennial sites (Figure 21). Mixing model results show that tree water is likely to come from multiple sources and that this varies with landscape position and time of year (Table 9). Strong groundwater links to plant water are seen for trees in the riparian perennial site in March and June (groundwater depth 2-3m) and for floodplain perennial (groundwater depth 5-7m) and riparian intermittent sites (groundwater depth 6-8m) during the October sampling period. The results also suggest that groundwater is likely to make up some part of the water obtained for most of the year in three of the four landscape positions with relatively shallow groundwater. Although mixing model results suggest that there is some groundwater use by trees in the intermittent floodplain site, this is unlikely given the depth of groundwater (>18 m) and the dendrometer data. Groundwater samples were not taken at precisely the same times as leaf samples, but groundwater isotope signatures did not vary greatly, compared with leaf and soil water values, over the study period at Maules Creek. Groundwater isotope values were also similar to perennial pool water, indicating that evaporation from the pool water is rapidly replaced by groundwater inflow.

We also matched leaf water δ^{18}O values with maximum daily stem expansion from dendrometer readings for the surveyed trees at each sampling time (Figure 22). The measurement of the diel maximum stem expansion gives an indication of the level of hydration of the tree for a given 24-hr period so that we can assess the relationship of tree hydration with tree water isotope values. For all landscape positions, diel maximum stem expansion, as a measure of tree hydration, showed highest values in June (winter) and lowest values in March (late summer). Riparian trees adjacent to the perennial pool showed the highest stem expansion across all dates and expansion was higher in March than June at the other sites (Figure 21). Seasonal changes in water source availability that produced enriched δ^{18}O values of the plant water were generally accompanied by higher maximum stem expansion values for all landscape positions. For all sites, leaf water δ^{18}O values were significantly enriched (higher) in March than at the other sampling times and maximum diel stem expansion was significantly greater in June and for perennial sites compared with intermittent sites.

Table 8: Statistics for the dendrometer traces of trees in each landscape position showing average tree responses to different rainfall patterns including storm event[1], period of frequent rainfall[2], and period of drought[3]. Also shown is the average growth increment[4] for the study period and average daily rate[5] for each landscape position at Maules Creek.

Location	Response slope[1]	Response max.[1] (mm/day)	Period of response[1] (days)	Decline slope[1]	Std Dev. for rain period[2]	Drought slope[3]	Increment (mm/year)[4]	Rate (mm/day)[5]
Riparian Perennial	0.087	0.85	18	-0.027	0.184	0.0003	8.4	0.024
Floodplain Perennial	0.258	1.4	16	-0.118	0.278	-0.017	4.1	0.012
Riparian Intermittent	0.061	0.33	9	-0.051	0.254	-0.021	1.4	0.005
Floodplain Intermittent	0.053	0.39	10	-0.082	0.269	-0.034	-0.2	-0.00016

Table 9: Stable Isotopes (δ^{18}O and δ^{2}H) mixing model results for the potential contribution to plant water of various sources including groundwater, deep soil water (75-100 cm) and shallow soil water (5-10 cm), at each sampling time at Maules Creek. Groundwater values are from monitoring bores closest to the sampled trees. Numbers in bold show strong constraint and high probability of being an important water source for the sampled trees.

	Landscape	Riparian perennial	Floodplain perennial	Riparian intermittent	Floodplain intermittent
Month	Source	Plant water Range, 1- 50 -99 percentile	Plant water Range, 1- 50 -99 percentile	Plant water Range, 1- 50 -99 percentile	Plant water Range, 1- 50 -99 percentile
October 2015	Groundwater	0 - 8 -25	**26 - 44 - 64**	**74 - 86 - 98**	0 - 37 - 86
	Deep soil	**65 - 80 - 98**	0 - 26 - 50	0 - 4 - 16	0 - 26 - 60
	Shallow soil	0 - 10 - 35	0 - 28 - 60	0 - 8 - 24	**12 - 36 - 58**
March 2016	Groundwater	**50 - 60 - 80**	0 - 29 - 75	0 - 9 - 24	0 - 3 - 15
	Deep soil	6 - 24 - 40	0 - 34 - 80	**66 - 79 - 96**	**44 - 87 - 98**
	Shallow soil	0 - 8 - 24	0 - 35 - 90	0 - 11 - 30	0 - 9 - 20
June 2016	Groundwater	**17 - 40 - 81**	0 - 35 - 76	0 - 29 - 72	0 - 31 - 60
	Deep soil	0 - 29 - 31	**22 - 56 - 96**	**27 - 56 - 78**	0 - 16 - 39
	Shallow soil	0 - 31 - 63	0 - 7 - 17	0 - 15 - 36	2 - 51 - 96

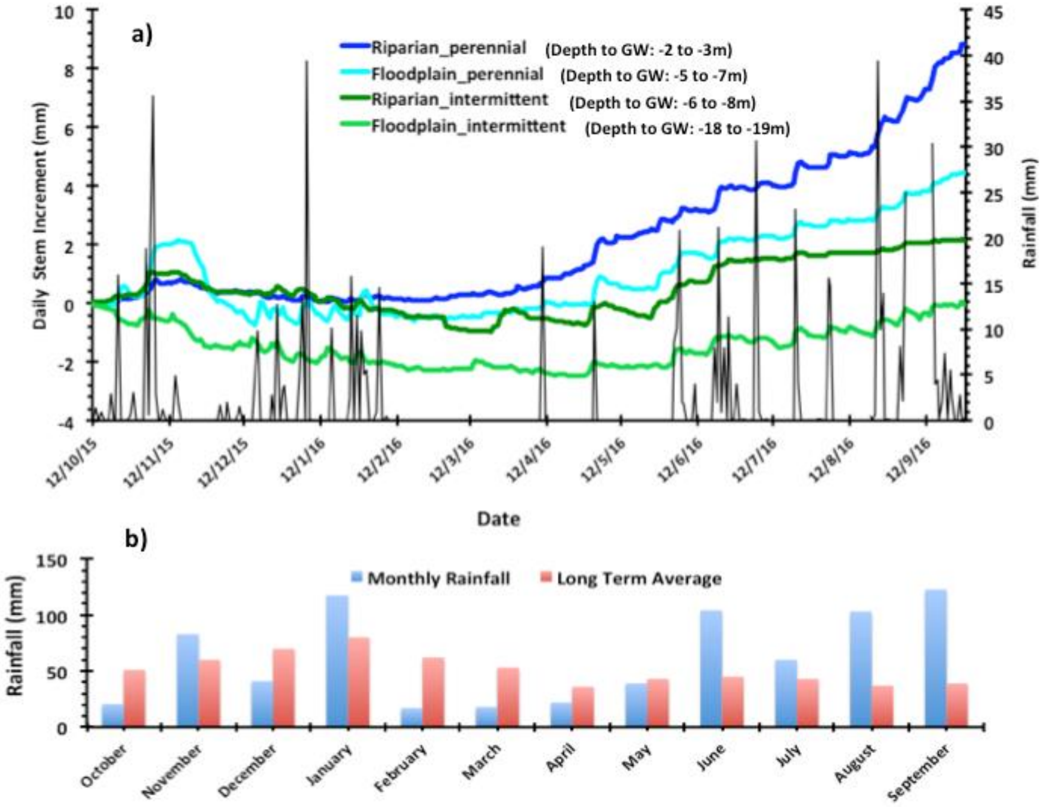

Figure 16: (a) Traces of changes in stem diameter of Eucalyptus camaldulensis trees (averages for 3 trees at each site) and daily rainfall over the study period indicating seasonal hydrological state and growth of red gum trees in different landscape positions with logged dendrometer bands. Average annual growth rate for riparian perennial trees was 8.4 mm yr^{-1}; floodplain perennial was 4.1 mm yr^{-1}; riparian intermittent was 1.4 mm yr^{-1} and floodplain intermittent trees -0.2 mm yr^{-1}. (b) Annual rainfall over the study period (October 2015 to September 2016) was 21% above the long-term average (620mm). But rainfall varied across the study period with the late summer, early autumn period (February to April) below average with 56% of long term average (132 mm) for these months. In contrast, June to September was 214% above the long-term average for these months (124 mm).

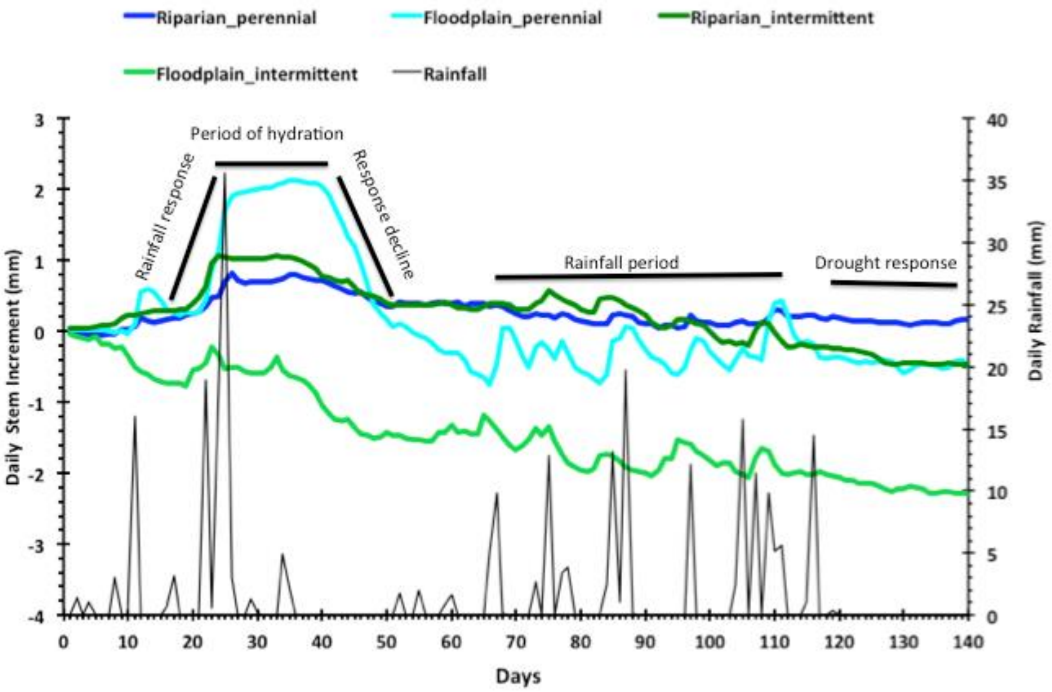

Figure 17: Analysis of the dendrometer growth curves over six months indicating different responses to rainfall events for trees in different landscape positions at Maules Creek. We analysed the response of tree diameter traces to a single large rainfall event, a sustained period of rainfall and a prolonged drought period.

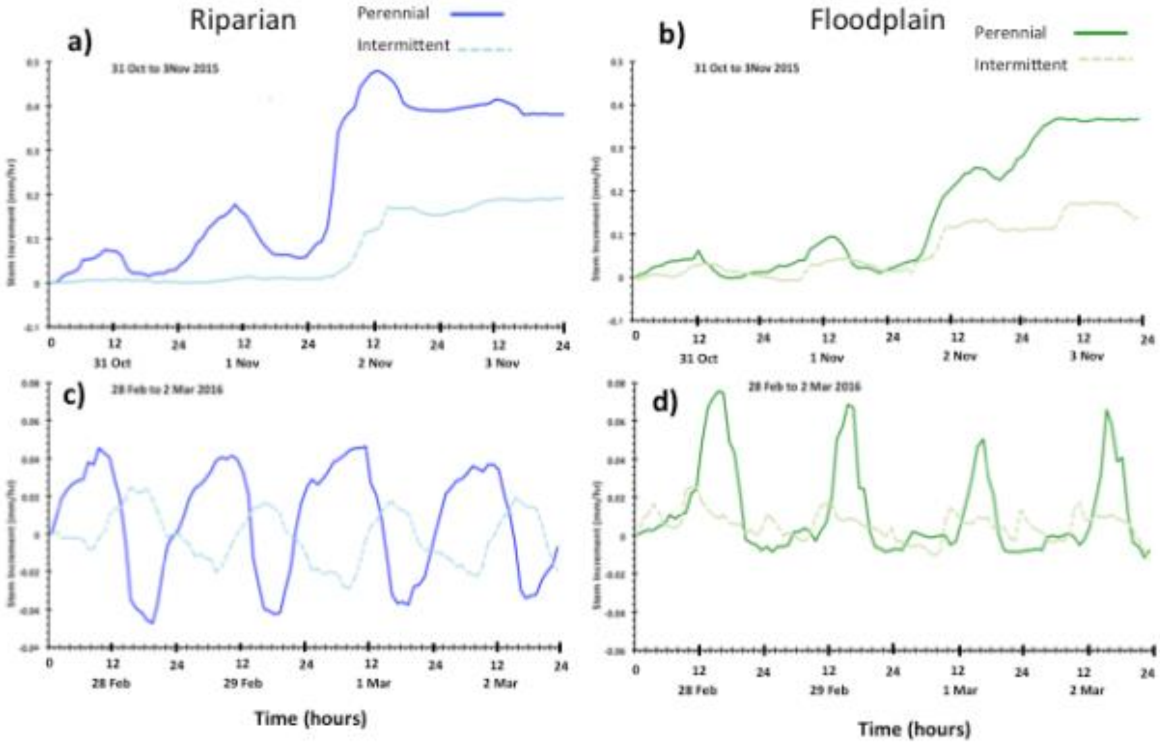

Figure 18: Diel stem diameter of E. camaldulensis trees in the different landscape positions at Maules Creek in response to a rainfall event 2 days prior when 18 mm rain fell over 48 hours (a) riparian trees from perennial and intermittent sites and (b) floodplain trees from perennial and intermittent sites. (c & d) Diel stem diameter response of trees after an extended drought period (38 days of no rain) for (c) riparian perennial and intermittent sites and (d) floodplain perennial and intermittent sites.

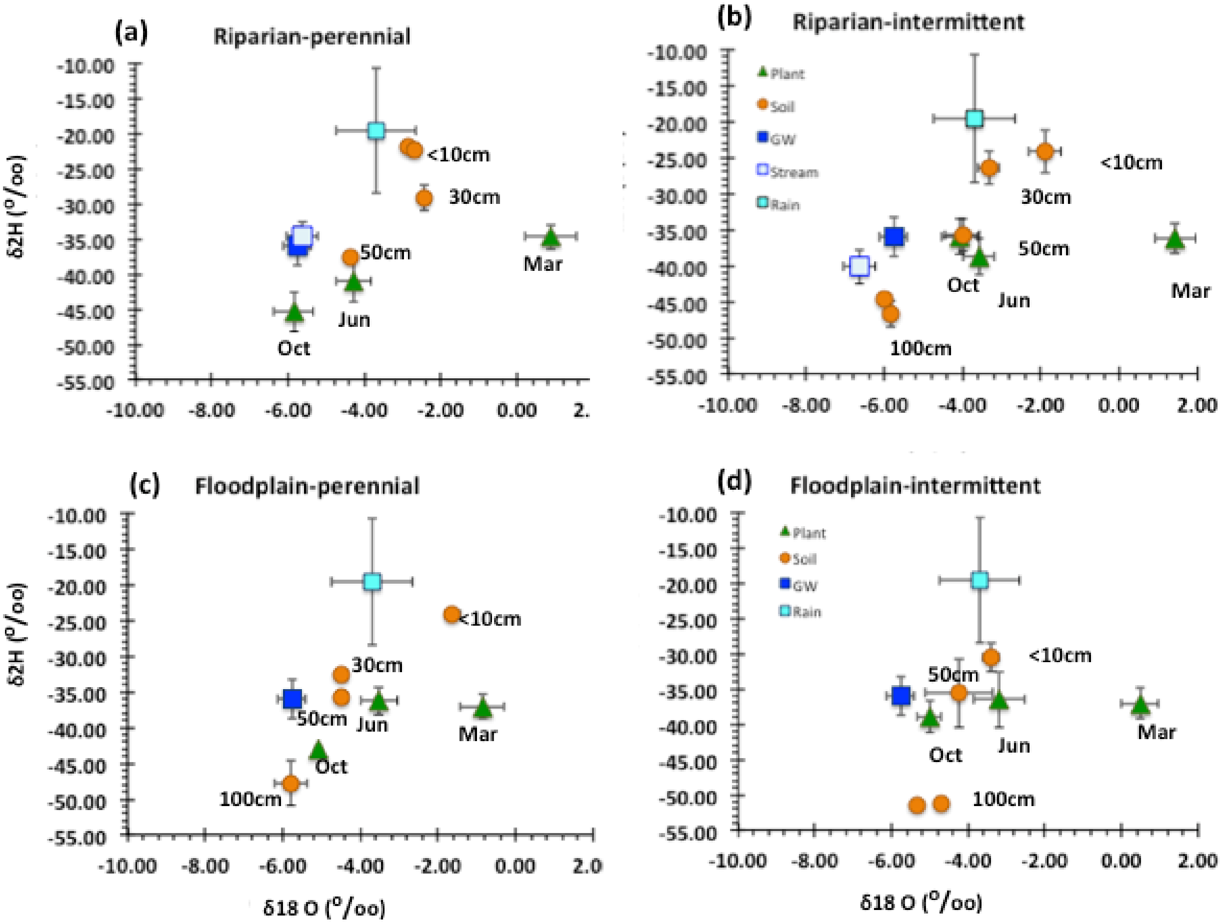

Figure 19: Biplots of water stable isotope composition (δ^{18}O and δ^{2}H) from water in tree leaves (green triangles), soil water (brown circles), groundwater (dark blue squares), pool water (light blue squares) and rainfall from sites at each of the different landscape positions at the Maules Creek study site. (a) Riparian perennial, (b) Riparian intermittent, (c) Floodplain perennial and (d) Floodplain intermittent. SI values for leaves are the mean of 3 samples from 3 trees at each site for each sampling time (October 2015, March 2016 and June 2016) ±SE. SI values for soil samples are means of 3 samples for each soil depth (cm) and each sampling time. Groundwater samples are means of 6-10 monitoring bores at the study sites and rain water and stream water samples were opportunistically collected during field sampling

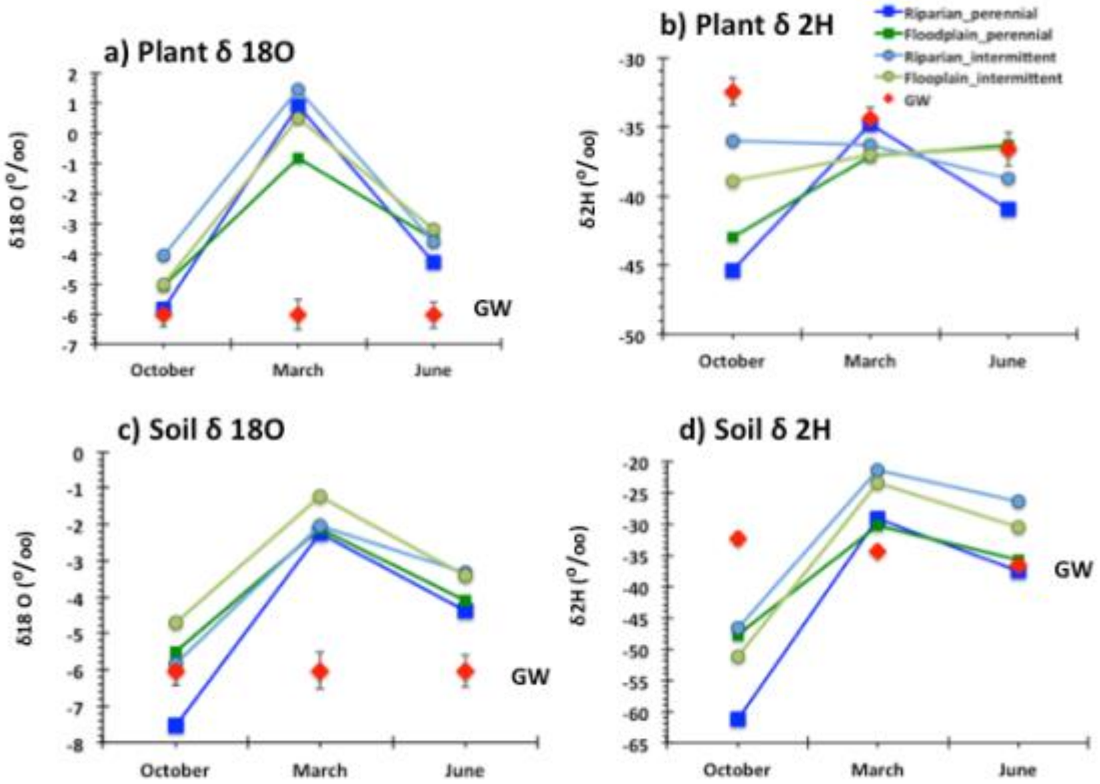

Figure 20: Changes in leaf stem water (a) $\delta^{18}O$ and (b) $\delta^{2}H$ isotope values at each sampling time (October 2015, March 2016 and June 2016), for 3 samples for each of 3 trees in each landscape position at Maules Creek. Changes in soil (c) $\delta^{18}O$ and (d) $\delta^{2}H$ isotope values at each sampling time. For reference, groundwater isotope values are given on each graph during the study period (red diamonds).

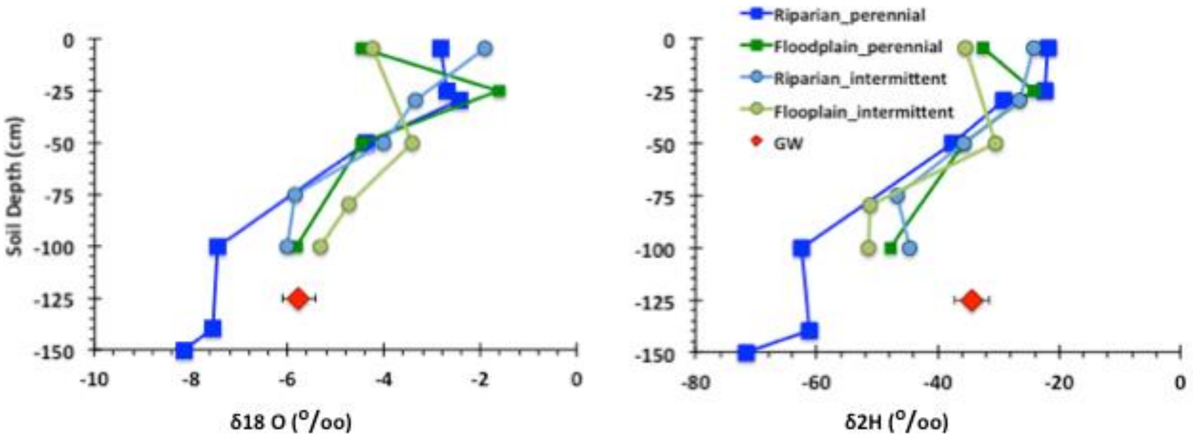

Figure 21: Relationship between soil depth and soil water a) $\delta^{18}O$ and b) $\delta^{2}H$ isotope values from Maules Creek samples. Reference average (±SE) groundwater isotope values are given on each graph (red diamond). Soil water $\delta^{18}O$ and $\delta^{2}H$ isotope values were more positive in upper than in lower soil layers, and became more negative with depth.

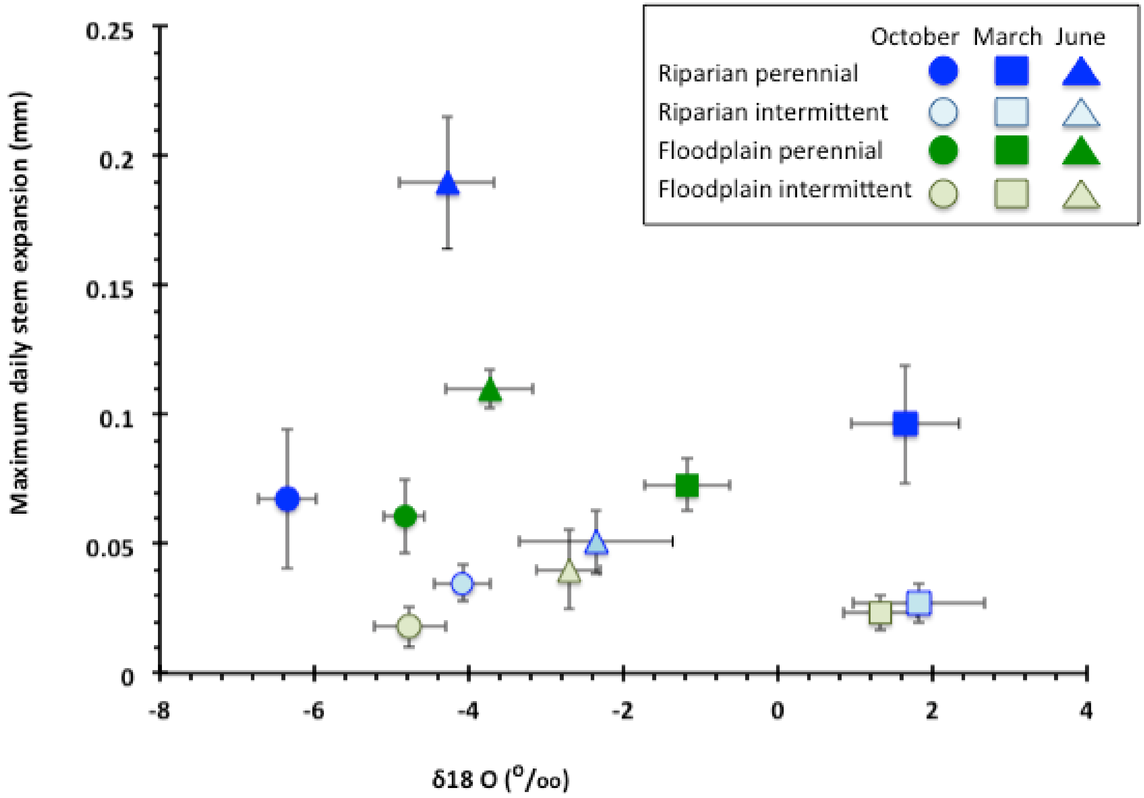

Figure 22: Daily maximum stem diameter expansion and δ^{18}0 values of tree leaf water for each month for E. camaldulesis trees in the different landscape positions at Maules Creek. Dark blue are trees at the riparian perennial site (mean ±SE of 3 trees); dark green are trees at the floodplain perennial site; light blue are trees at the riparian intermittent sites; light green are trees at the floodplain intermittent site. October 2015 sampling (circles), March 2016 sampling (squares), June 2016 sampling (triangles). For all landscape positions, diel maximum stem expansion, as a measure of tree hydration, showed highest values in June (winter) and lowest values in March (late summer). Riparian trees adjacent to the perennial pool showed the highest stem expansion across all dates and increments were higher in March than in winter at the other sites. Seasonal changes in water source availability that produced enriched δ^{18}O values of plants were generally accompanied by higher stem expansion for all landscape positions. For all sites leaf water δ^{18}O values were significantly enriched (higher) in March than at the other sampling times and maximum diel stem expansion was significantly greater in June and for perennial sites compared with intermittent sites.

2.3.4 Using remote sensing to assess the spatial and temporal dependency of plant communities on groundwater

For the Maules Creek study area we present the NDVI PC1 and PC2 grids colour-coded to highlight the areas of the highest magnitude of both positive and negative PC1 and PC2 values by the most intense colour tone (Figure 23). Visual analysis of the spatial distribution of these values on the NDVI PC1 grids allows us to distinguish regions of persistently green (dark green) land cover from the patches of bare soil indicated by red colour (Figure 23). The results of the ISODATA clustering applied to both the NDVI and NDWI PC1 and PC2 grids (Figure 23) within the delineated area delineated the spatial extent of remnant vegetation including riparian, floodplain and terrestrial (Figure 24). Seven types (classes) were determined in terms of their eco-hydrological conditions. These classes included: 1) open water, 2) riparian vegetation along perennial streams; 3) vegetation within floodplains; 4) riparian vegetation along intermittent streams; 5, 6) two vegetation classes outside of the riverine systems in terrestrial areas; and 7) one indistinguished class (Table 10, Figure 23).

The statistical analysis of NDVI and NDWI was undertaken for the identified RV classes. Mean, minimum and maximum NDVI and NDWI values at individual pixels within each class were estimated and their ranges (Figure 25). Within the study area the NDWI range indicated that this index is more sensitive to the variability in depth to groundwater compared to NDVI. However, both RS indices are substantially lower where groundwater is deep. The variability of NDVI and NDWI time series at the vegetation observation points grouped accordingly to their RV classes with the NDVI ranges lower than NDWI ranges (Figure 25). NDVI and NDWI value ranges gradually reduce from class 2 to class 6 but the proportion of the negative NDWI values increased from class 4 to class 6 (Figure 26), which is likely to be indicative of the less persistent access of vegetation to water.

A scatterplot of intercepts and slopes estimated by the linear regressions between NDVI and NDWI (Figure 27) shows that high value intercepts are associated with relatively lower slopes, indicating the high and persistent NDVI (vegetation greenness) with lower dependence on NDWI. On the other hand, the lower values of intercepts and higher slope values allow inferring a greater dependency of NDVI values on NDWI and show lower vegetation greenness under drier conditions (NDWI<0). Hence it appears that vegetation with an access to groundwater or permanent surface water is likely to be characterised by high intercept and low slope values in linear regressions describing relationship between NDVI and NDWI. Seasonal variability of NDVI values for all observation points within each RV class show the mean monthly NDVI are highest in the early winter (June-July) and the lowest in late spring – early summer (November-December) (Figure 28).

NDVI dependence on climatic conditions was assessed for each of the MLR clusters. The remnant vegetation extent is mainly included in cluster 4 and partially in cluster 6 (Figure 29). The red (cluster 4) and green (cluster 6) curves are the least variable and relatively low (close to zero) values of all MLR coefficients (Figure 29). All clusters exhibit similar mean MLR coefficients value distributions for all seven immediate climate variables. Among the other immediate climate parameters, pan evaporation shows the largest negative impact on NDVI. In comparison, the NDVI response to the annual climate parameters is different across various clusters. Clusters 1 and 2, for example, show a relatively large negative dependence on annual maximum temperature, pan evaporation and vapour pressure, while the other four clusters are positively correlated with annual maximum temperature and vapour pressure. Clusters 1, 2 and 6 indicate a relatively high dependence on the annual vapour pressure deficit climate parameter in comparison with the other clusters. It appears that remnant vegetation is independent from both immediate and annual rainfall. Analysis of the MLR RV coefficients at the observation points also demonstrated a greater dependence of NDVI on annual climate parameters rather than on the immediate meteorological conditions (Figure 30). The largest (negative) impact on NDVI among the immediate climate parameters was identified for pan evaporation. A dependence of NDVI on the annual climate parameters such as vapour pressure (positive), vapour pressure deficit (positive), maximum temperature (negative) and pan evaporation (negative) is gradually reduced from cluster 2 to cluster 6 and is minimal for terrestrial vegetation (clusters 5 and 6) (Figure 30).

Available monitoring data clearly indicate the effect of groundwater abstraction on groundwater levels (Figure 31). In addition to the effect of the interannual rainfall variability, this results in an overall greater groundwater downward trends than in the area unaffected by groundwater use for irrigation (Figure 31). These trends were modelled for all groundwater monitoring locations by a linear regression and measured by the regression slope (Figure 32a). The bores affected by groundwater abstraction are characterized by slopes greater than 0.05. In addition, the historical trend of the NDVI temporal profiles modelled by a linear regression was measured by the regression slope (Figure 32b). The comparison of trends in the groundwater levels and NDVI was inconclusive as the slope values for NDVI are insignificant and the trends' direction (positive or negative) inconsistent. The available long-term groundwater monitoring data were not sufficient to draw a statistically justifiable conclusion on the effect of the depth to groundwater on NDVI. However, Figure 33 illustrates that it is likely that the RV greenness is higher where groundwater is shallow. This is consistent with the adapted conceptual model of eco-hydrological conditions of the RV vegetation classes.

Some short-term groundwater monitoring data were available in the proximity of the study site vegetation observation points. The annual average depth to the watertable in the recently installed project monitoring bores was used to infer the relationship between the depth to groundwater, NDVI and NDWI values (Figure 34). The relationship illustrates that NDWI appears to be more sensitive to the depth to groundwater when compared to NDVI. However, both RS indices are substantially lower where groundwater is deep. This is also illustrated in the long-term dynamics of the groundwater depth and NDVI at a selected location, where groundwater is not affected by abstraction (Figure 35a, b). A greater depth to groundwater (green) is indicated for the period of 1992 to 1995 and mostly in the mid 2000s. This greater depth to groundwater corresponds to a relative reduction in NDVI during the same periods. The effect of the groundwater abstraction on remnant vegetation was not detected with the study area, though some changes were observed along the Maules Creek downstream. In contrast, Figure 35c and d illustrate local NDVI changes at a bore where the groundwater level was affected by irrigation. The reduction in both the depth to groundwater and NDVI value are greater during the dry periods compared with the area unaffected by groundwater abstraction. The groundwater levels at this bore vary from 4 mBGL to more than 20 mBGL (Figure 35). A particularly substantial groundwater drawdown (yellow in Figure 35) occurred in mid 2000s drought and particularly during the summer when groundwater use for irrigation is in greater demand. This is in a contrast to the area unaffected by abstraction, where groundwater level drawdown and reduction in NDVI values was not as great (Figure 35a, b).

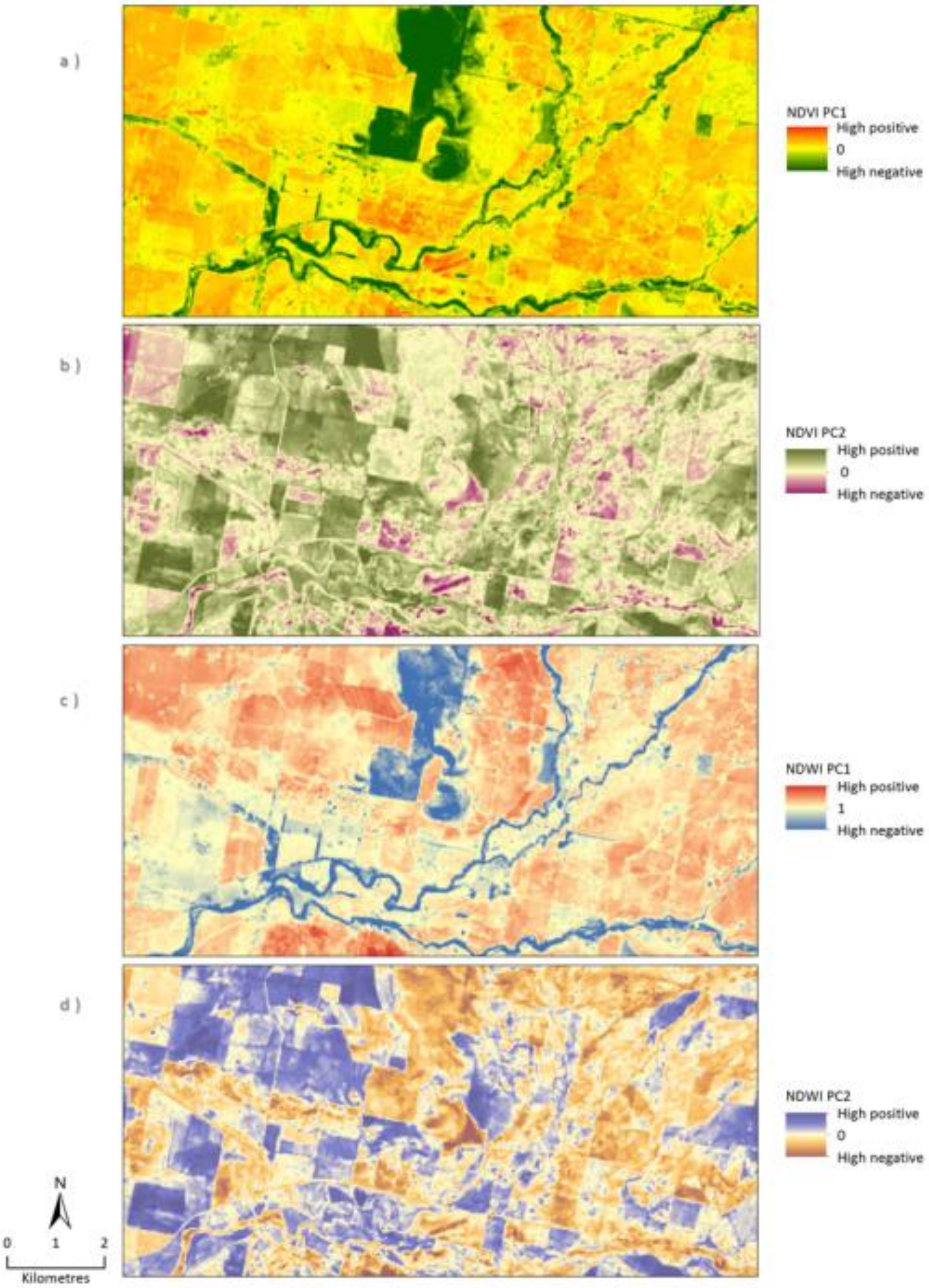

Figure 23: Maules Creek NDVI and NDWI Principal Components: (a) NDVI PC1, (b) NDVI PC2, (c) NDWI PC1, (d) NDWI PC2.

Table 10:Conceptual vegetation classes identified within the Maules study area.

Landscape	**Stream Type**		
	Perennial	Intermittent	not in the proximity of a stream
Water	1	-	-
Riparian	2	3	-
Floodplain	3	4	-
Terrestrial	-	-	5, 6
Other	-	-	7

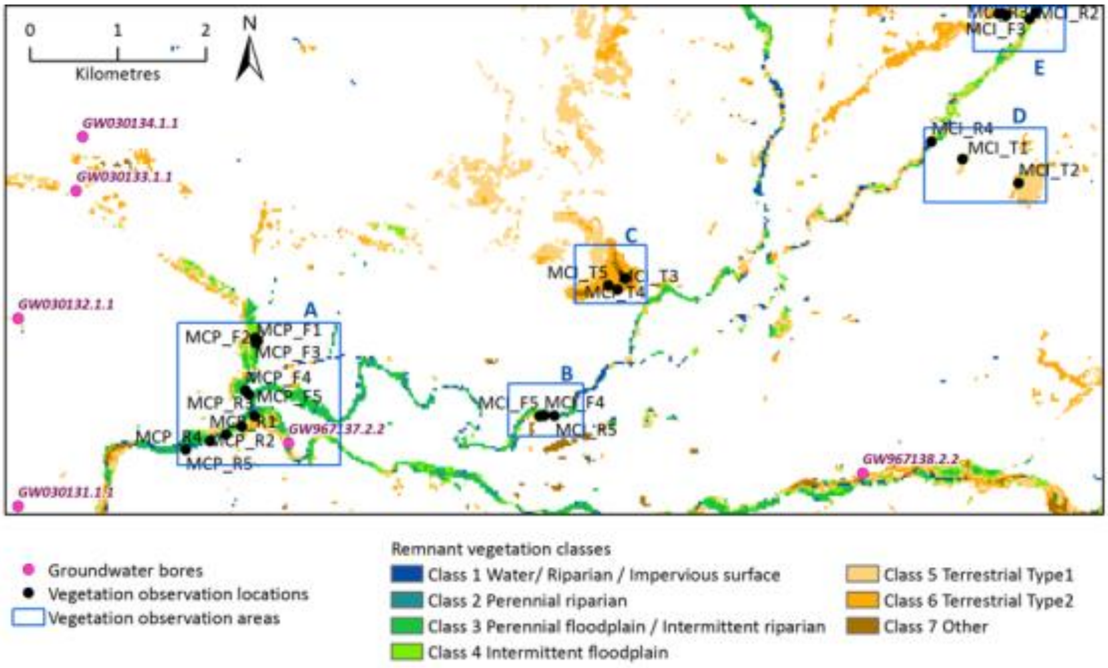

Figure 24: Identified remnant vegetation classes at the Maules Creek study area.

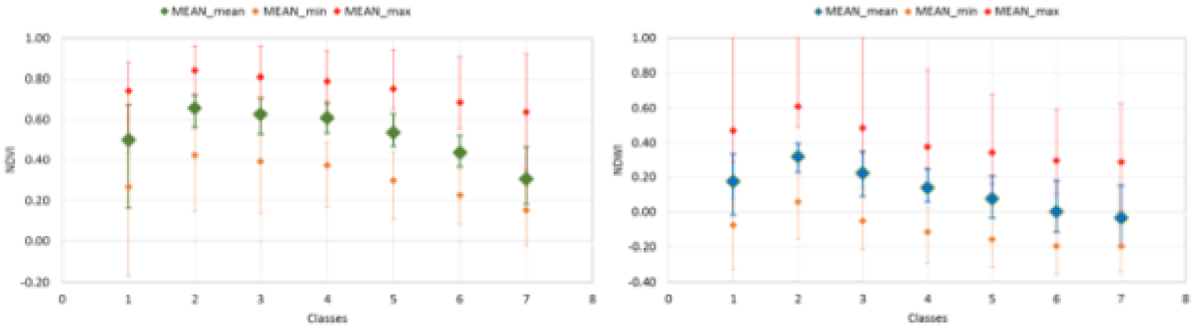

Figure 25: Ranges of NDVI (left panel) and NDWI (right panel) for the identified classes of remnant vegetation. MEAN_mean is the mean pixel values of multi-date NDVIs or NDWIs averaged for each class; MEAN_min is the minimum pixel values of multi-date NDVIs or NDWIs averaged for each class; and MEAN_max is the maximum pixel values of multi-date NDVIs or NDWIs averaged for each class.

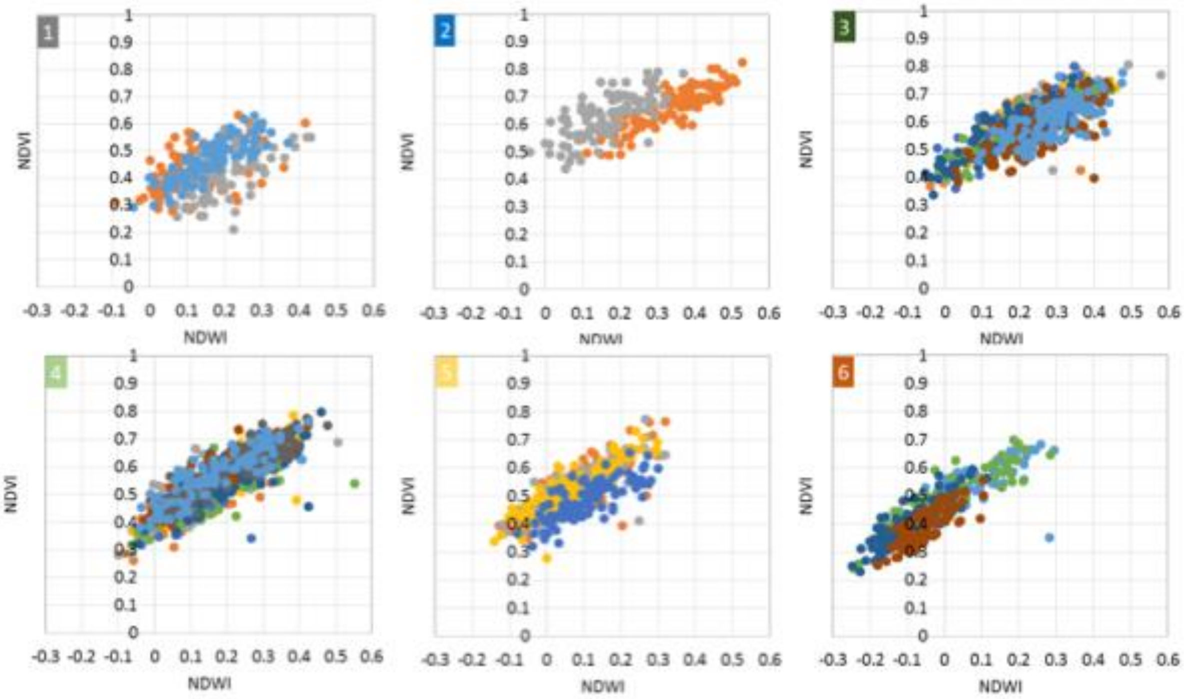

Figure 26: Scatter plots of NDVI and NDWI extracted at the vegetation observation points, grouped for six remnant vegetation classes. Colour coding of points are random, just to illustrate that each plot contains information from more than one point.

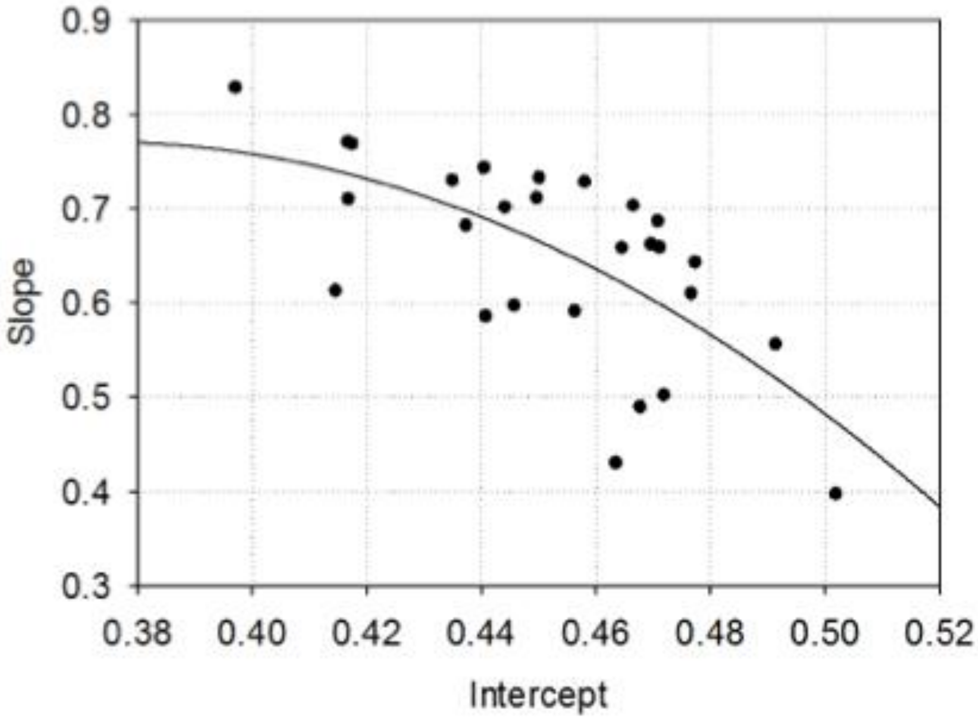

Figure 27: Relationship between the values of the intercepts and slopes estimated from the NDVI and NDWI scatter plots shown in Figure 26. The likelihood of vegetation dependency on groundwater increases with an increase in the intercept values and reduction in the slope values.

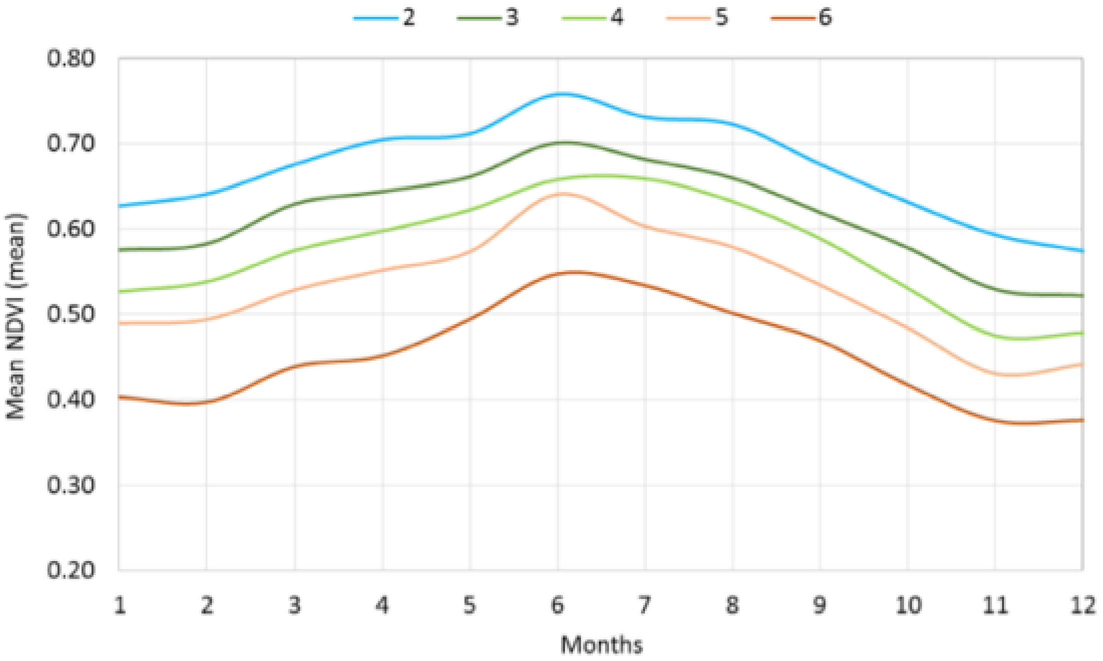

Figure 28: Mean monthly NDVI at the individual vegetation observation points averaged for each remnant vegetation class. Seasonal variability of NDVI values for all observation points within each RV class show the mean monthly NDVI are highest in the early winter (June-July) and the lowest in late spring – early summer (November-December).

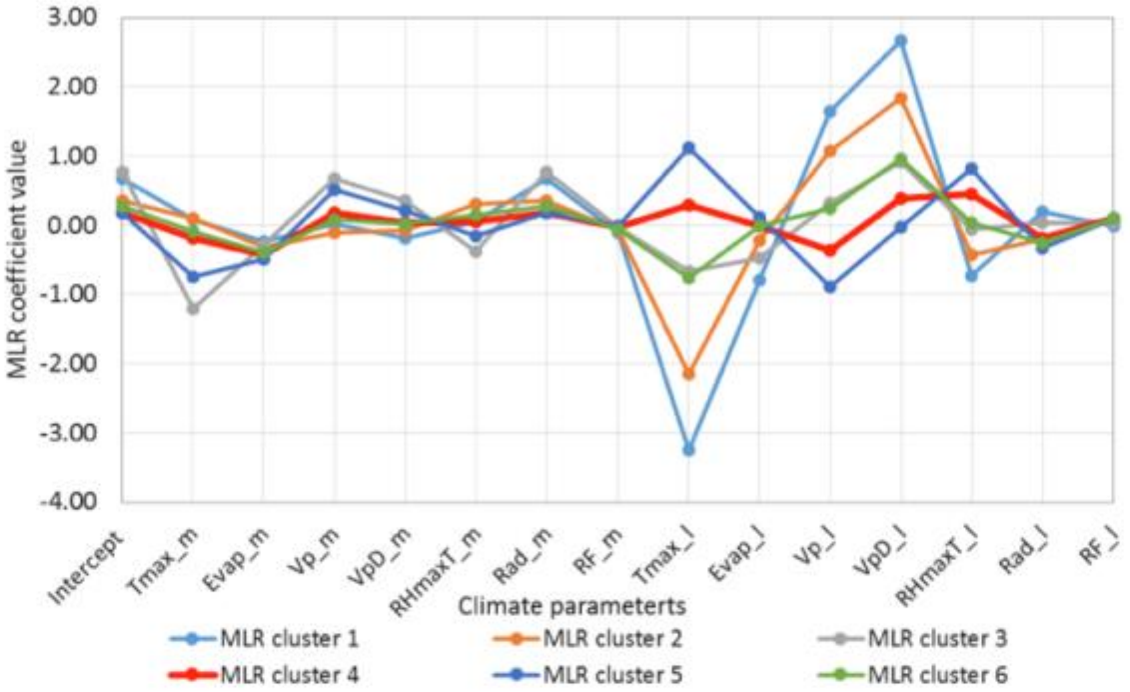

Figure 29: Mean values of MLR cluster's coefficients. Labels 'm' and 'l' indicate immediate and annual climate conditions. Abbreviations include Tmax = daily maximum temperature; Evap = pan evaporation; Vp = vapour pressure; VpD_m = vapour pressure deficit; RHmaxT_m = relative humidity at maximum temperature; Rad = solar radiation; RF = rainfall.

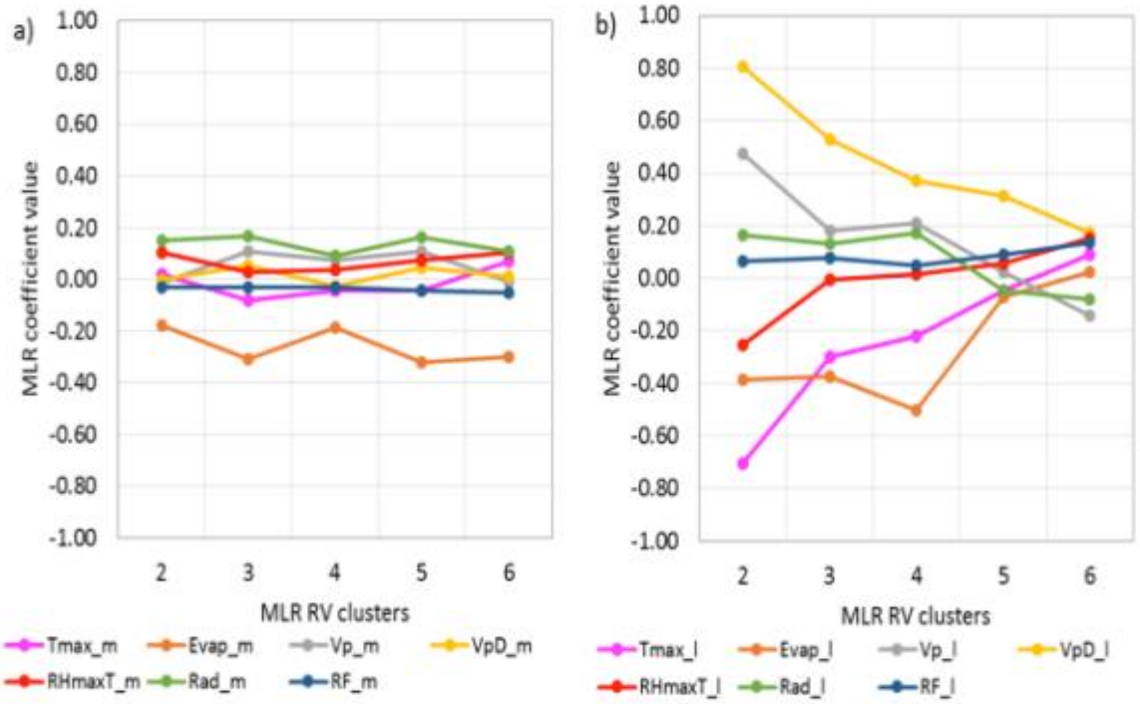

Figure 30: Multiple linear regression (MLR) coefficients for the immediate (a) and annual (b) periods at the observation points averaged within the MLR remnant vegetation (RV) clusters. Abbreviations include Tmax = daily maximum temperature; Evap = pan evaporation; Vp = vapour pressure; VpD_m = vapour pressure deficit; RHmaxT_m = relative humidity at maximum temperature; Rad = solar radiation; RF = rainfall. Labels 'm' and 'l' indicate immediate and annual climate conditions.

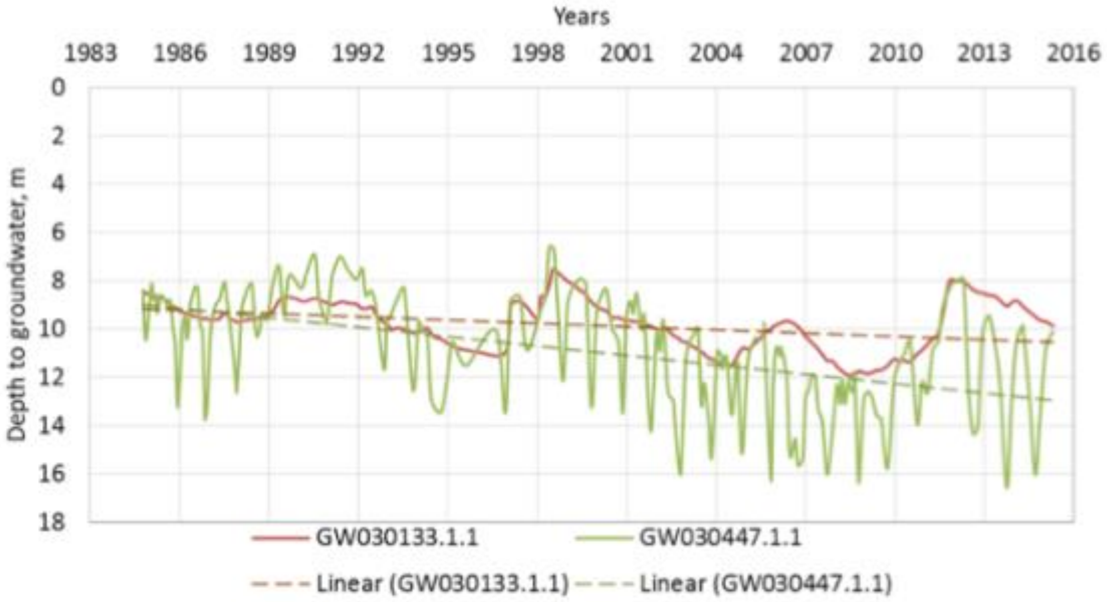

Figure 31: Depth to groundwater at two individual bores and the long-term downward linear trends. GW030133.1.1 is located in an area not affected by groundwater abstraction and GW030447.1.1 in an area of irrigation.

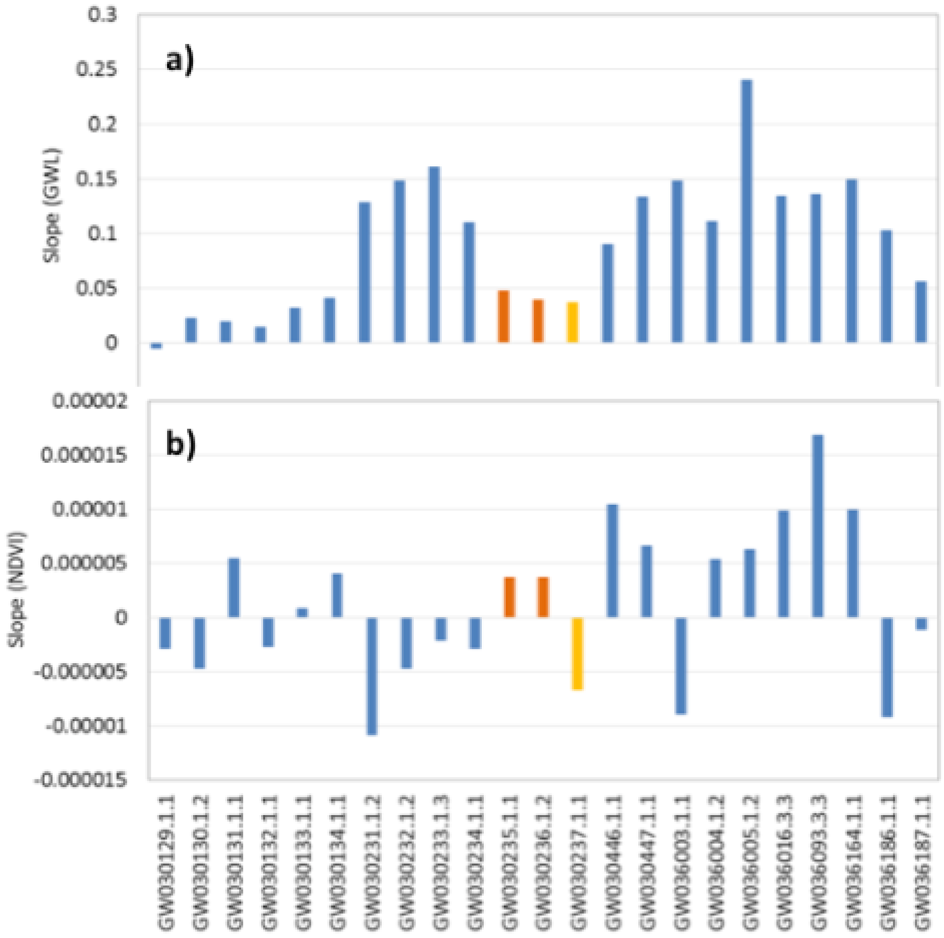

Figure 32: Trends in (a) depth to groundwater and (b) NDVI extracted at the individual bores. Orange and yellow columns are for bores located within the study area remnant vegetation classes 5 (yellow) and 6 (orange). For (a) the bores affected by groundwater abstraction are characterized by slopes greater than 0.05.

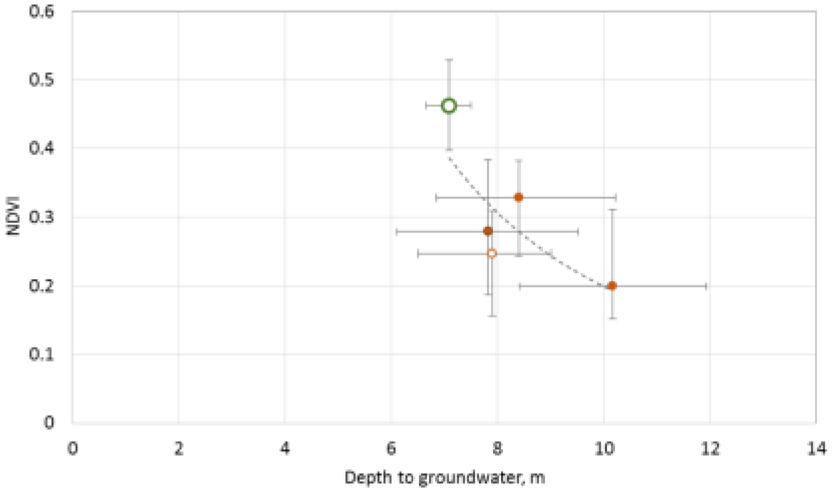

Figure 33: The relationship between NDVI and the depth to groundwater at individual bores within the identified RV classes. NDVI and the depth to groundwater were estimated as means of annual minimum values, and the error bars show their interannual variability (SD). RV classes 3 and 6 are shown in green and brown, respectively. The “empty” symbols are related to the bores with short periods of groundwater level monitoring.

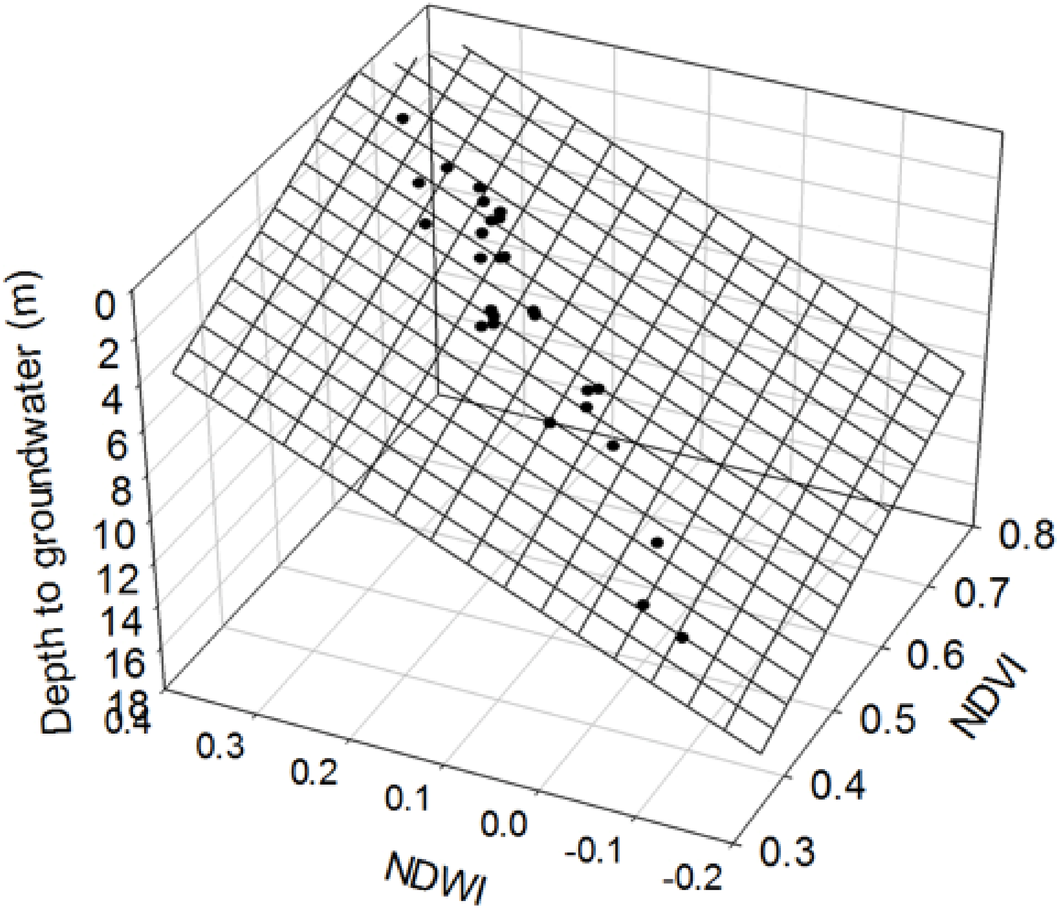

Figure 34: The relationship between the depth to groundwater, NDVI and NDWI of the remnant vegetation, showing lower NDVI and NDWI values where groundwater is deeper. The variability (or range) in NDVI values at locations with different groundwater depths appear to be lower than the variability in NDWI values. This indicates that NDWI is more sensitive to the depth to groundwater.

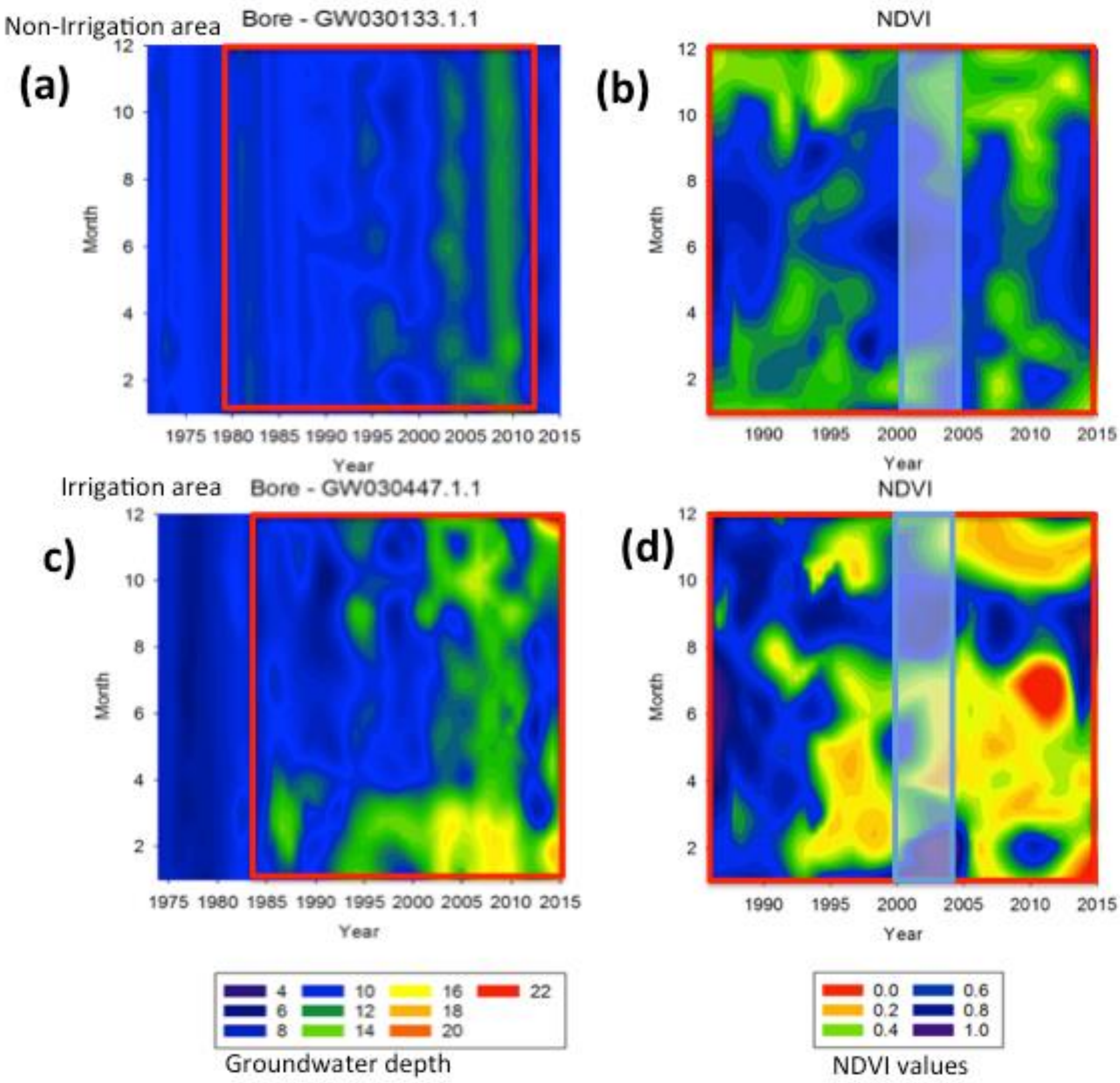

Figure 35: Long term variation in groundwater level (m) (a) at a monitoring bore at a location unaffected by groundwater abstraction for irrigation and (b) NDVI at that location (bore GW0310133.1.1) and (c) long term variation in groundwater level at a monitoring bore at a location affected by groundwater abstraction for irrigation and (d) NDVI at that location (bore GW030447.1.1). For (a) and (c) colours represent depth to groundwater and in (b) and (d) NDVI. Red outline indicates the period when Landsat data were available, blue outline indicates the gap in available Landsat data.

2.4 Major Findings

Research Question 1a, b - What are the characteristics of the environment and the plants that utilise groundwater (typology of hydrotypes and phreatophytic vegetation)?

The climatic environments where phreatophytes have been recorded are diverse with the majority of reported studies on phreatophytes from arid and semi-arid and Mediterranean-type climates where a combination of annual and/or seasonal drought and accessible, good-quality groundwater is available. Underlying geology generally provides the conditions for the development of shallow aquifers accessible to phreatophytic vegetation to grow and includes alluvial and aeolian sediments, sedimentary rocks, particularly karst, and fractured volcanics such as basalt. True phreatophytes are commonly perennial trees and shrubs with particular ecological, physiological, reproductive and morphological attributes (Figure 8) that enhance the ability of the plants to access groundwater.

This project has characterised plant communities as a mix of hydrotypes indicative of hydrological requirements and associated with landscape position and other environmental factors. In particular, this typology emphasizes the plant-hydrology linkages relevant in recognizing the likelihood of groundwater use. These hydrotypes can range from fully aquatic plants (hydrophytes) in permanently flooded wetlands where the groundwater levels are higher than the land surface for much of the year, to plants adapted to habitats that have only seasonally or episodically available soil water (xerophytes). In most landscapes as depth to groundwater increases and therefore accessibility decreases, the proportion of hydrotypes in the plant community adapted to wet conditions (and the number of phreatophytes) will decline. The typology provides us with a convenient first step in identifying the water sources important to maintaining vegetation and the potential for groundwater dependence for EIA. Caveats on the development of a plant hydrotypes typology are the requirement for good taxonomic and ecological knowledge of species, as well as information on ecophysiological traits and responses (Casanova 2011). However, the typology developed here works well as a general guide to categories of hydrological plant types in a continuum of groundwater dependence at the plant community level.

Our results suggest that it is possible to predict for management purposes, the importance of groundwater to a given plant community. This prediction can be based on a number of relatively easily observable factors including landscape position, climate, soil, geology, groundwater depth and proportion of plant hydrotypes within a plant community. However, the degree of certainty will increase with the level of knowledge of plant traits and physiology, such as root morphology, xylem anatomy, water use efficiency and productivity (see Eamus et al. 2006). Use of hydrotypes to define plant communities in terms of groundwater dependency, and the likely presence of phreatophytes, provides a practical way of assessing the importance of these functional types within a landscape. We provide a decision tree for broadly categorising plants within a community into hydrotype functional groups, and identifying potential phreatophytes (see Figure 11). The decision tree uses life history traits (annual or perennials) and life form (woody or herbaceous) as well as habitat, depth to groundwater, duration of inundation and drought adaption to identify the different hydrotypes. Consequently, this type of approach can be used to assess potential ecological impacts of groundwater drawdown as a result of coal seam gas (CSG) and coal mining developments in a variety of biophysical settings. This is important given our limited knowledge of the relationship between hydrology and plant community composition and a lack of accepted conceptual models of groundwater dependent vegetation ecology.

Our field studies confirmed the desktop analysis of hydrotypes and showed that hydrophytes, helophytes and mesophytes were associated with the wetter environment of riparian sites, while xerophytes and semi-xerophytes mostly associated with the drier terrestrial and intermittent floodplain sites where deep groundwater is not readily accessible to plants. Hydrophytes and helophytes were found only in the wetter landscape positions and mesophytes are also most abundant in the riparian and floodplain areas. We suggest that our approach of developing a typology of plant hydrotypes can be used as part of a field assessment of plant community vulnerability to changes in hydrology such as groundwater drawdown. The development of a typology of hydrotypes will assist in assessing potential ecological impacts of changes to groundwater availability, as measuring the proportion of different hydrotypes within a habitat will provide an indication of the degree of reliance on groundwater. Furthermore, detected changes over time in the proportion of different hydrotypes in a community will indicate possible changes in the hydrological regime (e.g. groundwater drawdown) operating within that area.

Research Question 2 - Distribution of plant hydrotypes and productivity: predicting groundwater use of plants across landscapes.

Our results indicate the importance of antecedent climate (particularly rainfall) and hydrological conditions for the ecohydrological state of the trees and from where they are accessing water. When there has been abundant rainfall and stream flow, trees tend to be well hydrated and accessing their water mainly from soil water. Annual rainfall over the study period (October 2015 to September 2016) was 21% above the long-term average (620mm) for the Maules Creek area. However, this rainfall varied across the study period with the late summer-early autumn period (February to April) below average with 56% of the long-term average and June to September was 214% above the long-term average for these months. Therefore, in terms of rainfall and stream flow, the study period can be considered atypical with the rainfall seasons reversed with what is, on average, the driest part of the year being the wettest during our study. Therefore, although this deviation from average rainfall does not reduce the validity of our findings, comparison or transfer of our findings to other years or regions would be most valid for situations with similar rainfall conditions as experienced during our study.

Our dendrometer study of red gum trees in different landscape positions suggests that sites with shallow groundwater for most of the year had greater growth rates over the year and larger daily stem changes indicating greater short-term growth rates. Although these trees have responded to rainfall events (and corresponding flow), they show less response to drought periods, suggesting they are less dependent on rainfall and may also be accessing groundwater continuously. Riparian trees at the intermittent site, where groundwater is seasonally deeper, may also be accessing groundwater at some periods during the year. Stable isotope analysis provided further evidence that there were strong links to groundwater for trees in the riparian area of Maules Creek at the site of a perennial pool as well for trees on the nearby floodplain where groundwater is deeper (5-7m). At the sites further up the catchment where stream flow and shallow groundwater are seasonally intermittent, trees close to the stream (groundwater depth 6-8m) appeared to be accessing groundwater during the October sampling period.

Although the data presented here are not conclusive as to which trees are using groundwater, and where or when, there are multiple lines of evidence that strongly suggest that red gum trees (*E. camaldulensis*) in riparian areas and on the floodplain, where groundwater is relatively close to the surface, are utilizing groundwater for some of their water requirements. This finding confirms results of other studies within Australia where river red gum growing in riparian areas have been shown to be making use of groundwater (Thorburn and Walker 1994, Lamontagne et al. 2005). The degree of dependence on groundwater is likely to vary considerably among locations and times of year and for individual trees. The ability of some plant species to change their physiological and morphological responses to changing hydrologic conditions makes the categorising of phenotypically plastic species as phreatophytic challenging. For example, *Populus* spp. may have denser wood, more cavitation-resistant xylem and slower growth if established in drier environments and may be less vulnerable to abrupt groundwater decline than individuals growing in wetter areas (Stromberg & Merritt 2016).

Plants using groundwater (phreatophytes) are generally restricted to environments with permanent groundwater supplies that are within a depth accessible to plant roots. Therefore, phreatophytes can occur in most habitats where groundwater is shallow enough for roots to reach and plants opportunistically use groundwater to a greater or lesser extent when the energetic balance is favourable to extracting deeper soil water at the capillary fringe of the water table. It is generally assumed therefore that if plants can access groundwater then some plants within the ecosystem will develop some degree of dependence. Therefore, phreatophytic behaviour seems to be more related to the prevailing environmental conditions than to the capabilities of a given plant species or type (Thomas 2012). In addition, trees are likely to maximize the exploitation of the environmental resources by using the topsoil water during most of the year and groundwater in the dry summer (David et al. 2013).

As for the critical question of whether mature trees have the capacity to adapt to declining water tables, this is likely to be most dependent on the rates of groundwater drawdown and recovery. Root redistribution may afford tolerance to short-term drawdown in water tables but protracted and rapid groundwater declines are known to result in phreatophyte water deficit stress and mortality (Shafroth et al. 2002, Froend & Sommer 2010, Barron et.al. 2014a) and reduce vegetation resilience (Sommer & Froend, 2011). The nature of dependence on groundwater relative to other sources of water is important in differentiating these responses to changes in groundwater availability. Therefore, to assess potential groundwater use by plant communities we suggest that ideally sources of tree water use must be assessed over several different seasons and years. Clearly this is rarely practical for environmental impact assessments and a possible compromise is to substitute space for time, so that assessment of groundwater use by trees can be done at different locations with a gradient of groundwater depths. However, if the trajectory of environmental change is faster than vegetation adaptability, plants are likely to have physiological limits to how quickly root extension can keep pace with rapid groundwater drawdown through extraction (Zencich et al., 2002).

Research Question 3 - How do vegetation and groundwater interactions vary spatially and temporally across the landscape within catchments?

Most of the identified remnant vegetation in the Maules Creek study area is associated with the riverine systems, including riparian vegetation and vegetation established on the floodplains. Here, the depth to groundwater reduces from the north-west to the south-east of the study area. Following this pattern, the ephemeral Maules Creek becomes perennial, providing annual recharge to groundwater in the north-west and being a gaining stream in the south-east. As a result, all riparian and floodplain vegetation in the study area is likely to have access to groundwater, though at various depths: more than 8 m below ground level (BGL) in the north-west and within a few metres BGL in the south-east. Decadal variability of rainfall in the study area led to some reduction in vegetation greenness during the particularly dry periods (such as during the Millennium Drought of mid 2000s). During this time the groundwater levels also dropped, in some bores up to 4 m. However, both groundwater levels and vegetation greenness recovered in the wetter periods following the droughts. This recovery is likely to indicate that vegetation is well adapted to the decadal variability of the climatic conditions and variation in water availability associated with it. Groundwater is locally used for irrigation, which has an effect on groundwater levels in the south-west of the study area outside the riverine systems. Seasonal changes in the groundwater level may be more than 7 m, and there is a long-term trend in the groundwater drawdown in this area. However, this trend has not had an impact on vegetation greenness within the study area. All analysis indicated that vegetation wetness (NDWI) is a more sensitive variable than NDVI to spatiotemporal variations in groundwater levels. This great sensitivity of NDWI can potentially be due to a greater effect of the depth to groundwater on evapotranspiration rates by plants rather than on their greenness.

In summary, decadal and seasonal variations in groundwater levels, even when induced by groundwater use for irrigation, do not have detectable impacts on remnant vegetation from the riverine systems. This lack of impact is likely to be due to the position in the landscape of riparian vegetation, within groundwater discharge zone or localised groundwater discharge zone associated with the riverine systems. Unless groundwater abstraction, climate change or their combination led to substantial changes in surface and groundwater interaction processes (e.g. reversing streams from gaining to losing), seasonal river flow and regional groundwater discharge provide a sufficient water source for vegetation within the riverine systems.

The methods developed in this project allowed the use of remote sensing imagery for the delineation of remnant vegetation in the two study areas at Maules Creek in northern NSW and Bremer River in south-east Queensland. This use of RS imagery included the determination and characterization of eco-hydrological classes of the delineated remnant vegetation (including riparian perennial, floodplain and riparian intermittent and terrestrial vegetation classes) and assessment of seasonal and long-term variability of the remnant vegetation greenness at the vegetation observation points. Modelling relationships between the remnant vegetation greenness and climate data and the analysis of the relationship between the remnant vegetation greenness and groundwater level dynamics indicated that vegetation with an access to groundwater or permanent surface water is likely to be characterised by high intercept and low slope values in linear regression models describing the relationship between vegetation greenness (NDVI) and wetness (NDWI).

The mean monthly NDVI estimated over the period of Landsat data availability (1985-2015) is highest in the early winter (June-July) and the lowest in late spring – early summer (November-December) and NDWI is more sensitive to the depth to groundwater compared to NDVI. However, both RS indices are substantially lower where groundwater is deep. Changes in groundwater levels due to groundwater abstraction for irrigation do not affect remnant vegetation within the study area.

This remote sensing analysis can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation at the landscape scale. Small-scale on-ground observations can also be used to ‘ground-truth’ these remotely sensed data. The combination of on-ground studies and remote sensing will provide a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales.

We also developed and applied a web-based toolset to support the analyses (see Section 7.1, Appendix 1 for full description). This toolset adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. It It can be implemented using the supplied javascript code in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications. The developed GEE-CSIRO-GDV tools include:

Tool 1 - creating image thumbnails for image quality control;
Tool 2 - identification of dates of cloud-free Landsat imagery from the GEE remote sensing database and generation of cloud free NDVI and NDWI data cubes covering the selected time periods;
Tool 3 - NDVI and NDWI time series extraction at selected point locations;
Tool 4 - Principal Component Analysis of multi-temporal NDVI and NDWI datasets;
Tool 5 - Multiple Linear Regression Analysis to model relationships between NDVI temporal profiles at each NDVI grid pixel location and local climate parameters (e.g. temperature, rainfall).

Any agency or researcher can use the scripts to analyse the groundwater dependent vegetation in their region of interest. However, the user will require experience using Javascript code in GEE. Contact the authors for details.

2.5 References Component 1

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3 Component 2 – Ecological values of baseflow and surface water-groundwater connectivity regimes in non-perennial streams. (Short title: Baseflows).

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3.2 General introduction

3.2.1 Purpose of study

This research component aims to understand how reductions in baseflow affect the structure and function of surface and hyporheic environments in intermittent and perennial streams. We examine the effects of reduced baseflow permanence on organic carbon cycling (litter and cellulose processing), the composition of benthic invertebrate and fish assemblages, and cycling of carbon and nitrogen through food-webs (using stable isotopes). The effect of reduced baseflow permanence is investigated by sampling in wet and dry periods and among streams of varying degrees of intermittency. The work was conducted in two field locations (upper Bremer River and Maules Creek catchments).

3.2.2 Background

Baseflow is the component of total stream and river flow that is derived from subsurface sources (Boulton and Hancock, 2006), and thus is most apparent during periods of reduced surface runoff. Baseflow sources include groundwater, bank-storage and unsaturated lateral inflows (Hatton and Evans, 1998), but baseflow is generally dominated by groundwater. Baseflow contributions to rivers can strongly influence their hydrologic characteristics, by either helping to maintain permanent flow and/or decreasing the duration of cease-to-flow periods between rainfall events, on a regular basis or during extreme droughts (Hall, 1968; Tallaksen, 1995). Baseflows play a pivotal role in maintaining river ecosystems during dry periods, and while it is not only rivers with a high baseflow contribution that may be considered groundwater dependent (Boulton and Hancock, 2006), the ecological reliance on baseflows may also provide a rough indicator of groundwater dependency (Hatton and Evans, 1998).

Low-flow periods are ecologically important components of the hydrological cycle of all river systems (Poff *et al.*, 1997; Bunn and Arthington, 2002; Rolls *et al.*, 2012; King *et al.*, 2015). Rolls et al. (2012), building on earlier work (e.g., McMahon and Finlayson 2003, Boulton and Hancock 2006), characterised low-flow ecological patterns and processes as including: (i) the physical extent of aquatic habitats; (ii) the physical and chemical conditions within those habitats; (iii) the sources of and processing of materials and energy occurring within different habitats; and (iv) the diversity, and degrees of connectivity among habitat patches. Further, King et al. (2015) conceptualised and reviewed the hydro-ecological consequences of changes to low flow regimes due to surface- and groundwater extraction. They predicted changes to aquatic habitat connectivity and availability, reductions in dry-season persistence of in-channel refugia, reductions in water quality during dry–wet and wet–dry transition periods, decoupling of wet- and dry-season flows, and the cumulative negative effects on groundwater-dependent species and whole ecosystems. Research on the ecological roles of low flows, has tended to focus on perennial streams; however, more recent research is beginning to highlight the ubiquity and ecological importance of intermittent streams and our relatively poor understanding of their low-flow ecology and responses to anthropogenic flow reductions (Datry *et al.*, 2014; Leigh *et al.*, 2016; Leigh and Datry, 2016).

The effects of reductions in surface flow on the availability and quality of aquatic habitats through space and time are relevant in both perennial and intermittent streams. For example, in perennial streams the low-flow magnitude will determine the minimum area of riffle habitats that is maintained during dry spells, and which are prone to drying. These habitats support diverse invertebrate assemblages, with many (rheophilous) taxa dependent on flowing water. Such taxa respond to reductions in riffle area by concentrating their distribution within the remaining flowing areas (Dewson *et al.*, 2007b), although over time diversity may be reduced (McKay and King, 2006). Some riffle fauna may also exploit local hyporheic refuges during drying, or recolonise from such areas if subsurface flows persist in the absence of surface water (Boulton, 2003; James *et al.*, 2008). Continuous surface flow (i.e. baseflow derived from groundwater) can also buffer temperature extremes relative to the surrounding air (Leigh *et al.*, 2015) and help maintain higher dissolved oxygen concentrations and lower salinities, both of which can be critical to the survival of numerous vertebrate and invertebrate taxa in wetted habitats. Areas of groundwater and hyporheic upwelling within pools can also provide critical thermal refuges for fish (Ebersole *et al.*, 2001).

In naturally intermittent streams, baseflows can play an important role in maintaining aquatic habitats and has consequences for biological structure. For example, groundwater contributions can maintain pool habitats long after surface flows have ceased, either through sustained subsurface flows within the stream channel itself, from localised upwelling of groundwater as springs, or via deep pools intercepting the regional water table (Davis *et al.*, 2013a). The relative contribution of these different mechanisms will vary with climate, geology, and channel morphology and substrate type. Even in rivers with no subsurface flow or groundwater connections, variation in the duration of cease-to-flow spells caused by longer-term climate cycles can greatly influence the amount of surface habitat that persists (Perry and Bond, 2009). Numerous studies have also demonstrated the negative effects of decreasing flow permanence and pool persistence on fish diversity and abundance (Labbe and Fausch, 2000; Freeman *et al.*, 2013). Reductions in pool area also increase densities of aquatic biota, leading to crowding and increased likelihood of negative density-dependent effects. For example fish may decline in condition from intra- and inter-specific competition (Magoulick and Kobza, 2003), and be exposed to increased rates of predation and parasitism (Medeiros and Maltchik, 1999; Bond, 2004; Maceda-Veiga *et al.*, 2009).

Variation in baseflow and periods of low-flow also have consequences for stream and sediment biogeochemical characteristics and the rates of important ecosystem processes. For instance, baseflow variability determines the vertical exchange rates and fluxes of water, materials (sediment, inorganic and organic nutrients), dissolved oxygen (DO), and energy (dissolved and particulate organic carbon) along surface and subsurface flow-paths (see Component 3). Where surface flow enters subsurface flow paths via downwelling zones, gradients in DO and dissolved organic carbon (DOC) can influence both habitat suitability for hyporheic fauna, as well as patterns of nutrient transformations, such that subsequent upwelling of zones contribute to altered surface-water chemistry and changes in microbial and algal activity. These characteristics may also have flow on effects for surface-dwelling fauna, especially for species that require flowing, oxygenated water (Boulton and Hancock, 2006). Intermittent flow can also lead to pre-conditioning of allochthonous leaf litter, via irradiation and anoxic conditions, altering subsequent breakdown rates and contributions of carbon to the aquatic food-web (Dieter *et al.*, 2011). While the majority of studies examining the ecological effects of flow permanency on aquatic biota focus on patterns of distribution and abundance, Sabo et al. (2010) found that food chain length increased in streams with greater baseflow and lower flow variability, suggesting that flow intermittency has a destabilising effect on food webs, and reduces food-chain length through the elimination of top predators. In addition, Jardine et al. (2012) found that flow regime intermittency and connectivity were major determinants of consumer-resource coupling in riverine ecosystems. Consumer-resource coupling assesses the energetic contribution of primary producers (autochthonous C source) and/or terrestrial organic matter (allochthonous C source) to organisms in a food web, and understanding how this varies in periods of different flow conditions can help evaluate the potential effects of reduced baseflow for aquatic food webs (Bunn *et al.*, 2003).

While the overall importance of baseflows to surface water ecosystems is well established, much of our current understanding is drawn from work in normally perennial systems, where periods of intermittent flow are uncommon. In fact, some recent reviews, (e.g. Dewson et al. 2007b) intentionally omit naturally intermittent rivers due to the perceived additional complexities in interpreting ecosystem responses to baseflow reductions. Yet, a number of studies have pointed to the sensitivity of intermittent systems to the loss of baseflows (Boulton and Hancock, 2006; King *et al.*, 2015). Given that the majority of Australian rivers are intermittent (Kennard *et al.*, 2010), there is arguably a pressing need to better understand the role of baseflows in supporting ecosystem patterns and processes within intermittent as well as perennial streams, especially within areas subjected to potential groundwater drawdowns. This topic is the focus for the Component 2 work plan. As will become obvious below, we note that many of our hypotheses regarding the impacts of baseflow reductions in intermittent streams parallel those from perennial stream systems.

3.3 Hypothesised responses to reduced baseflow

The expected impacts of groundwater drawdown will vary depending on both the natural hydrologic regime (e.g. perennial versus intermittent), the degree to which baseflow contributions from groundwater contribute to that regime (e.g. low versus high baseflow contribution), and the magnitude of groundwater drawdown occurring (e.g. 0-5 m versus >5 m reduction). For the purposes of this project, we focus on potential effects of relatively low levels of drawdown (<5 m) in small to mid-sized intermittent to perennial streams with varying degrees of baseflow contributions to surface flow. In all cases, the hydrologic impact of baseflow reductions will be an increase in periods without surface flow.

Our hypotheses focus on four ecological responses relating to biotic community structure and ecosystem processes:

1. organic matter decomposition
2. basal resource use and food-web structure (including surface-subsurface fauna)
3. fish assemblage composition and abundance

4. invertebrate assemblage composition and abundance

Based on our earlier review of the literature, we predict the following ecological responses:

- variation in stream flow regime and the degree of substrate saturation will influence organic matter processing in surface and hyporheic environments, with greater decomposition in wetter experimental periods and areas.

- reductions in the availability and quality of autochthonous and allochthonous carbon sources in low-flow periods will alter basal resource use and food-web structure in surface and hyporheic environments.

- variation in surface flow, and changes in longitudinal connectivity and pool persistence over time, will influence fish assemblage composition and abundances.

- variation in surface flow, and associated changes in aquatic habitat persistence over time, will influence macroinvertebrate richness and abundances.

3.4 Study site characteristics in the upper Bremer River catchment

3.4.1 Study region and sites

Five intermittent streams where chosen in south-east Queensland (SEQ) as study sites (Figure 36). All study streams occur within the headwaters of the Bremer River catchment (region referred to as 'upper Bremer River catchment' from this point forward). This region has a sub-tropical climate with annual average rainfall ranging from 944 to 1028 mm in our study area (Table 11). Catchment areas of study streams ranged from 5.97 to 45.3 km^2 and modelled mean annual flow ranged from 504 to 3429 ML (Table 11). Sites were minimally disturbed by human activities (river disturbances scores $\leq$ 0.1), although some catchment clearing has occurred in all five sub-catchments (particularly in Wild Cattle Creek and Reynolds Creek; Table 11) and evidence of cattle disturbance (trampling and cow pats) was apparent at all sites. All sites were well shaded by high riparian vegetation cover. Modelled monthly flow data from January 1900 to September 2016 indicate that our study streams have a flow regime typical of SEQ, with discharge lowest from late winter to early spring (August to November) and peaking in late summer to early autumn (Figure 37). Despite these seasonal patterns, there can be substantial year-to-year variation in the timing of high and low flows due to the varying influence of tropical and temperate weather systems (Kennard *et al.*, 2010; Mackay *et al.*, 2014). The study period (2015-2016) typified this strong inter-annual variation (Figure 37), with major differences in the monthly timing of high and low flows each year, but all within the range of long-term monthly variation.

Three study sites were also chosen in the Maules Creek catchment in northern New South Wales. These sites are the main focus of Component 3; however, chlorophyll *a* and food web characteristics were assessed and included in Component 2. In the Maules Creek catchment, sites were selected to represent a losing intermittent section (Middle Creek), a perennial gaining section (Horsearm Creek), and a perennial losing section (Maules Creek). Details of study sites within the Maules Creek catchment are described in Component 3.

Table 11. Stream reach and catchment information for the five study streams in the upper Bremer River catchment (source: Stein et al. (2014). The River Disturbance Index (RDI – Stein et al. 2002) synthesises the spatial extent and potential magnitude of impact from multiple human disturbances indicators (including land uses and flow alteration) and ranges from 0 to 1 (least to most disturbed, respectively). ^{222}Rn, mean radon-222 gas concentration (n=3; Bq m^{-3}) measured once in July 2015.

Site	Catchment area (Km^2)	Reach Elevation (m asl)	Mean annual temperature (^{o}C)	Annual rainfall (mm)	Mean annual discharge (ML)	RDI	Historical catchment natural forest cover (%)	Existing catchment natural forest cover (%)	Mean ^{222}Rn
Bremer River	5.97	284	17.8	954	504	0.10	6	4.86	2863
Warrill Creek	19.9	202	18.2	944	1406	0.08	52.7	39.5	1980
Coulson Creek	15.6	240	18.0	995	1561	0.05	77.8	57.4	3247
Wild Cattle Creek	45.3	165	18.3	1023	3707	0.07	57.0	28.6	2343
Reynolds Creek	38.2	172	18.3	1028	3429	0.09	73.8	19.1	2963

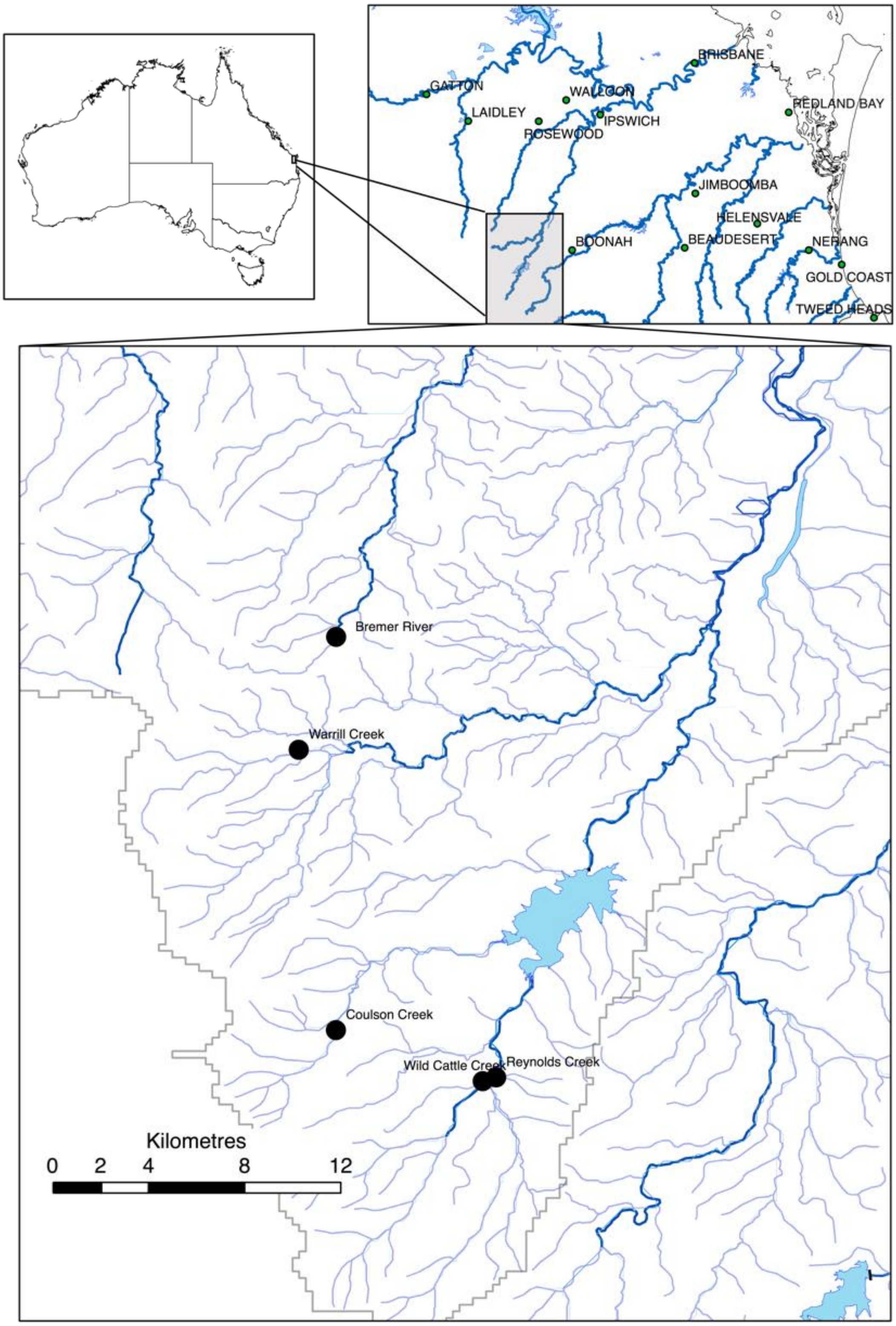

Figure 36. Location of the five upper Bremer River catchment study sites in south-east Queensland.

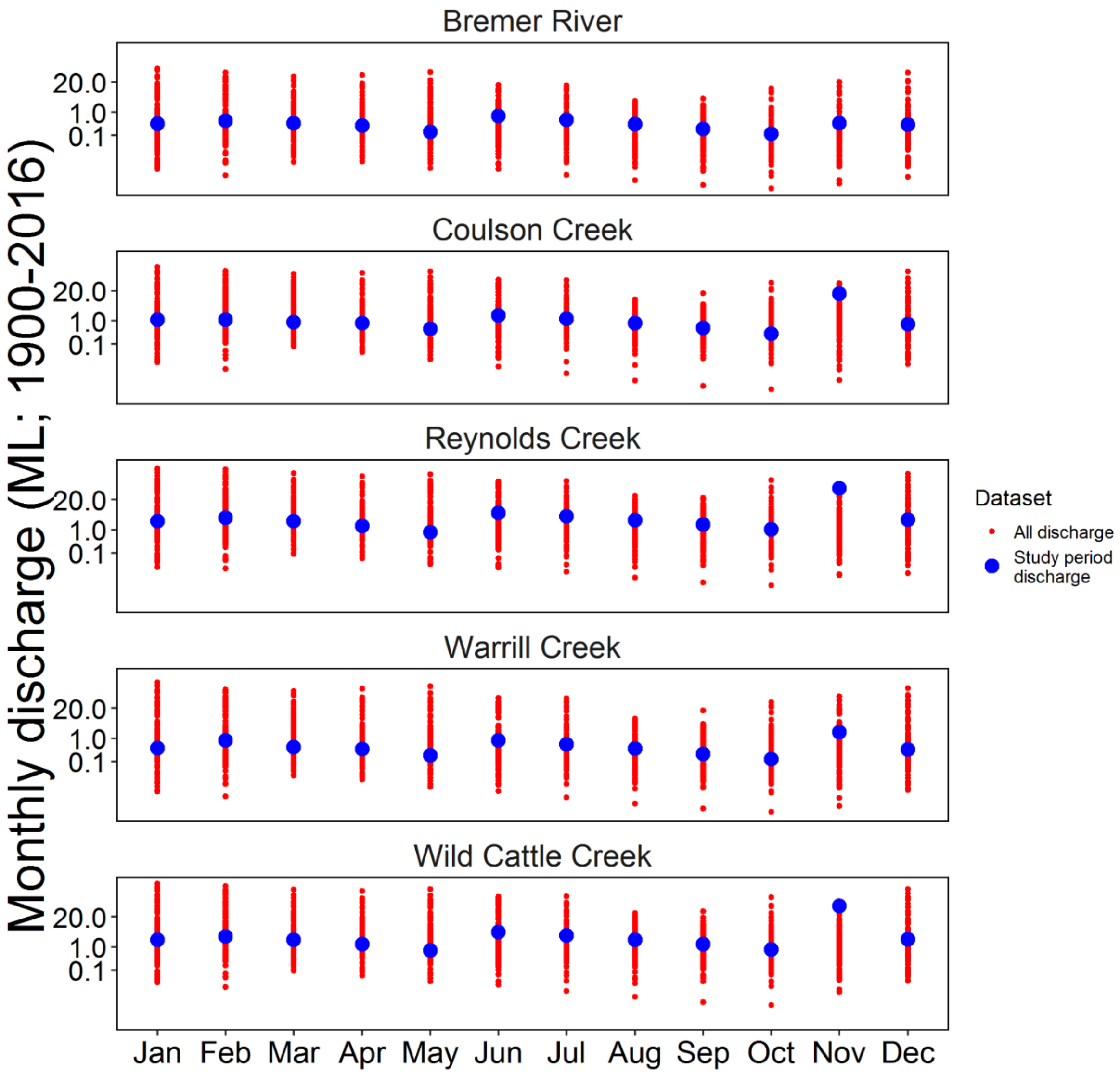

Figure 37. The modelled mean monthly discharge (mega litres; ML) from January 1900 to September 2016 for the five study streams. The monthly discharge data for the study period (June 2015 – May 2016) is overlain in blue. Data sourced from a national monthly water balance model developed through the Australian Water Availability Project (Raupach et al., 2008).

3.4.2 Estimating temporal variation in flow

Because our study sites were ungauged and modelled monthly discharge is insufficient to examine fine-scale and short-term temporal variation in stream discharge, we monitored variation in pool water height at each site using water Level TROLL 400 Data Loggers (In-Situ, Fort Collins, USA), and used this as a proxy for discharge. A higher pool water height was assumed to be positively related with channel inundation. Traditional methods for monitoring flow, such as v-notch weirs, are not suitable for many intermittent streams and rivers due to their braided morphology and often substantial volume of subsurface flow. Pool water height data revealed that temporal patterns in flow varied substantially among sites, although the timing of high-flow events was generally similar (Figure 38). The average pool water height during each experiment or survey period is summarised in Figure 39. Although conducted in September (i.e. traditionally the dry season), the first food-web sampling trip was characterised by a high pool water height, whereas the second sampling trip in March 2016 was characterised by dry conditions (Figure 39). Consequently, we treated the food web sampling trip in early September 2015 as the wetter period and the March 2016 sampling trip as the drier period.

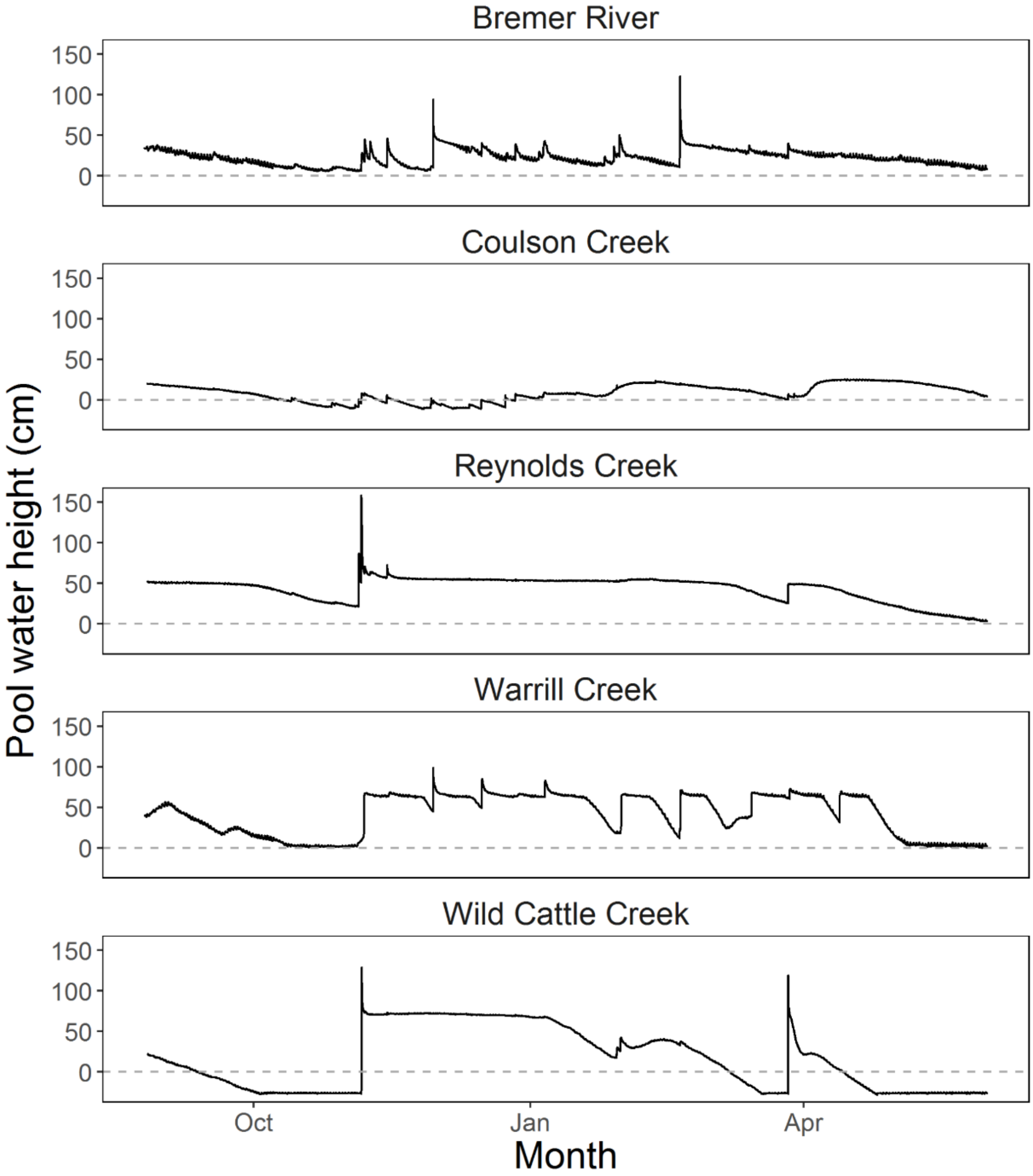

Figure 38. The pool water height, recorded every 15 minutes, at each study stream in the upper Bremer River catchment over the study period. The bottom of each pool is indicated by a dashed line at depth zero. Negative values occur because the base of each water height logger was lower than the pool bottom.

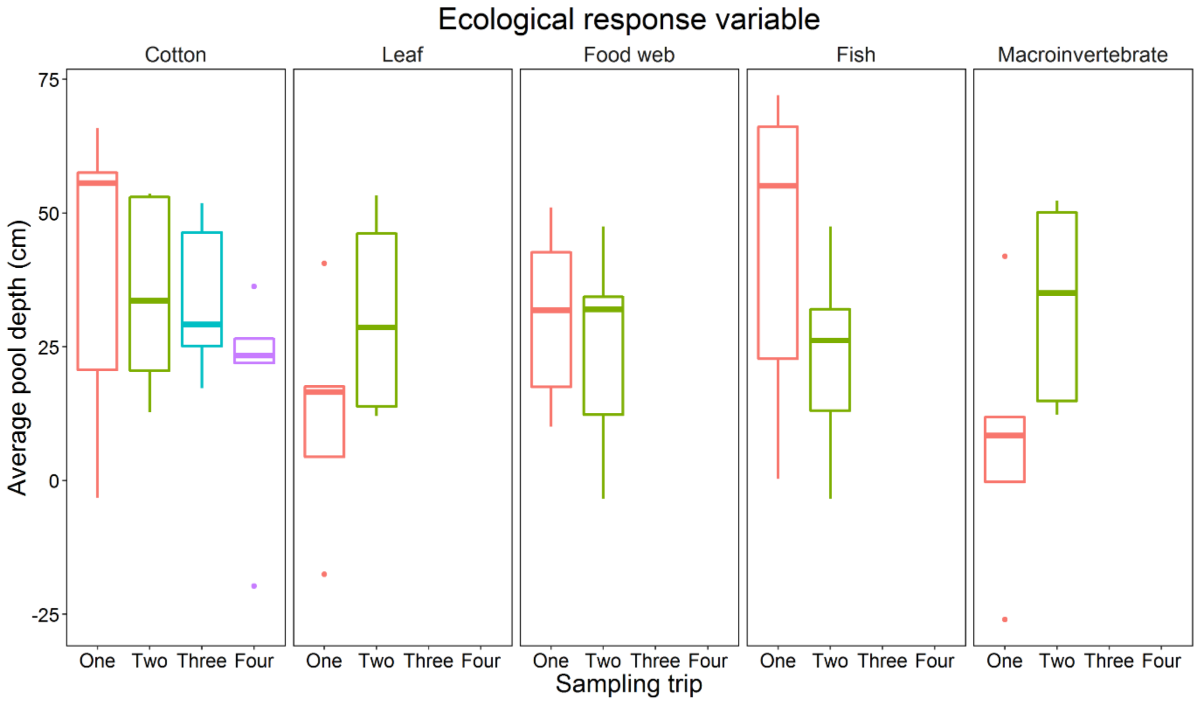

Figure 39. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the average pool depth at the five study streams in the upper Bremer River catchment (n=5 per box-whisker) during the cotton strip incubations (Cotton), leaf litter incubations (Leaf), food web sampling (Food web), fish surveys (Fish), and macroinvertebrate surveys (Macroinvertebrate) at the five study streams in the upper Bremer River catchment.

3.4.3 Groundwater contributions to flow

Interactions between groundwater and surface water play a fundamental role in the functioning of river ecosystems. In particular, groundwater discharge to rivers can make up a major component of baseflow that maintains surface and subsurface aquatic habitats during dry periods. We used concentrations of radon-222 (^{222}Rn) to determine whether groundwater was contributing to stream flow among our study sites. ^{222}Rn is produced in the subsurface by the radioactive decay of uranium-series isotopes, is the most stable isotope of radon gas, and has a short half-life of 3.82 days (period after which the initial radioactivity of radon is halved). After groundwater containing ^{222}Rn discharges to surface waters, ^{222}Rn concentrations decrease due to gas exchange with the atmosphere and its short half-life. High ^{222}Rn concentrations are thus observed only in surface waters characterised by high groundwater inflows, and ^{222}Rn is commonly used to detect groundwater (Cook *et al.*, 2003). High concentrations of ^{222}Rn were observed at all study sites in the Bremer River catchment, indicating that all sites have substantial groundwater contributions to flow (Table 11).

3.4.4 Water quality and chlorophyll

Water samples for physical and chemical analysis were collected during each sampling period at each study stream. A ProDSS multiparameter sampling instrument (YSI Incorporated, Yellow Springs, USA) was used to measure pH, water temperature (°C), dissolved oxygen (mg L^{-1}), turbidity (NTU), and conductivity (µS cm^{-1}). The ProDSS multiparameter was calibrated every 6 months. We collected water samples to measure ambient concentrations of dissolved organic carbon (DOC), dissolved organic N (DON), nitrate (NO_3^-), ammonium (NH_4^+), total dissolved N (TDN), and soluble reactive phosphorus (SRP). Water samples were filtered in the field (0.45 µm nylon membrane filters; Sarstedt) and transferred to a freezer within 6 hours.

For sites in the upper Bremer River catchment and the Maules Creek catchment, we assessed the ambient variation of chlorophyll *a* of seston (organisms and non-living matter swimming or floating in a water body) and periphyton (attached to rocks and woody debris). To measure seston chlorophyll *a*, triplicate samples of known volumes of surface water were filtered on 0.45-mm glass-fibre filters. For the chlorophyll *a* of periphyton, triplicate samples of known areas of submerged rocks and woody debris were scrubbed and the sample rinsed into small plastic zip-lock bags. The samples were then filtered on 0.45-mm glass-fibre filters. Glass-fibre filters were then frozen immediately for analysis for chlorophyll *a* in the laboratory.

Analysis of nutrients and chlorophyll *a* by a NATA-accredited laboratory followed standard analytical procedures. Dissolved organic carbon (DOC) was analysed with the combustion catalytic oxidation method (method APHA 5310D). Total nitrogen (TN) and total phosphorus (TP) were analysed via the persulphate digestion (APHA 4500), and NO_3^-, NH_4^+, and SRP were analysed with a SEAL Analytical AutoAnalyzer 3 (Porvair Sciences, Wrexham, UK). DON was calculated by subtracting DIN (NO_3^- + NH_4^+) from TN. Chlorophyll *a* was extracted in 100% ethanol and measured spectrophotometrically (Steinman *et al.*, 2006).

3.4.4.1 Water physicochemical and nutrient characteristics

Substantial spatial and temporal variation was evident in the physical and chemical variables of the upper Bremer River catchment study sites (Figure 40a, b). In particular, turbidity and pH were high in all sampling sites during July 2015 relative to other sampling times (Figure 40a, b). Concentrations of DOC were generally low throughout the study streams, varying from 0.9 to 3.7 mg L^{-1}. DIN concentrations were also generally low (mean = 19.8 µg L^{-1}), varying from 1.9 to 75 µg L^{-1}. SRP concentrations varied from 9 to 79 µg L^{-1}, with an average value of 28 µg L^{-1} across streams and sampling dates.

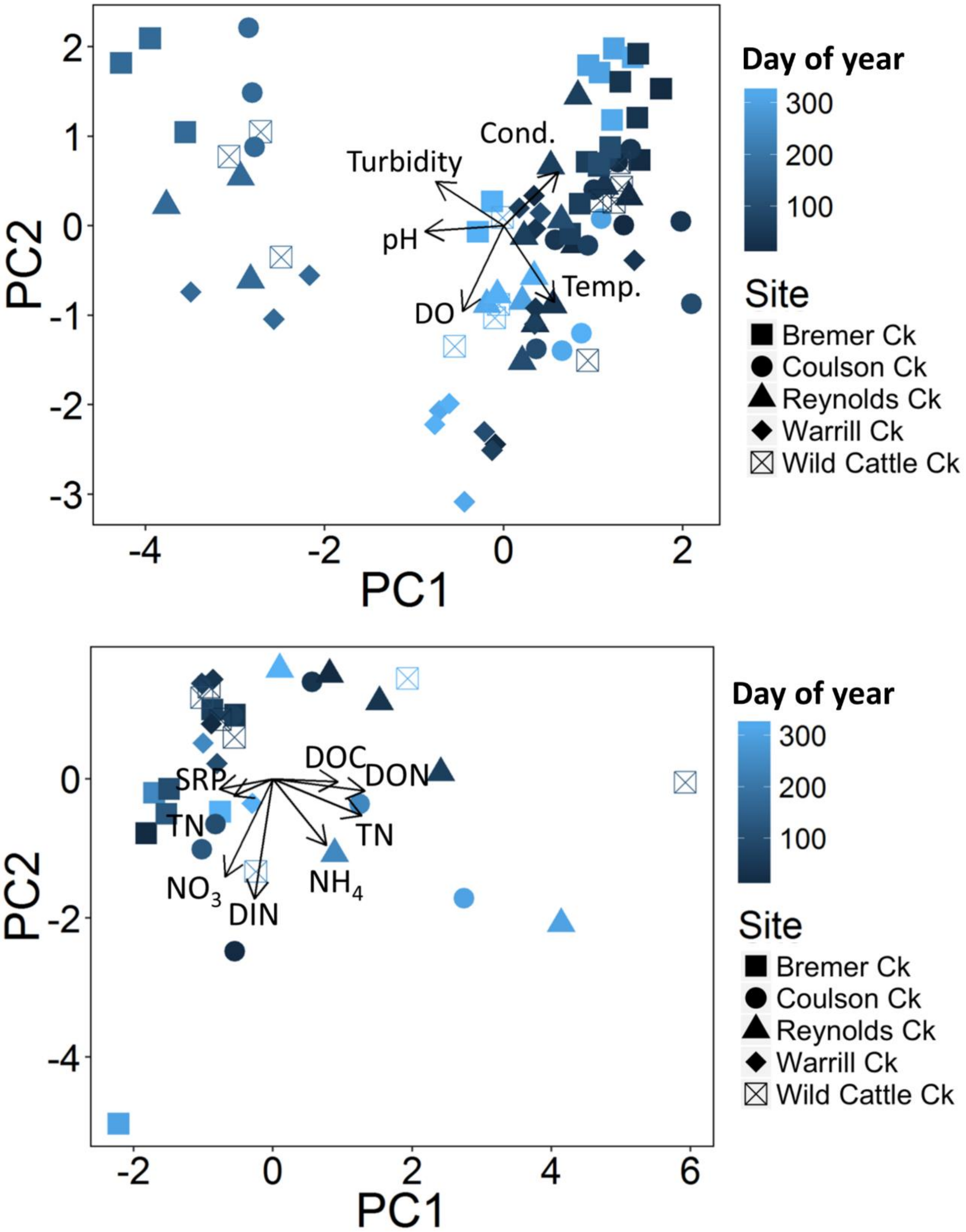

Figure 40. Plots of the first two principal components from two separate principal components analyses (PCA) of the a) physical and chemical, and b) nutrient variables measured at each date (from July/Oct 2015 until April 2016) in study streams in the upper Bremer River catchment. For the physical and chemical PCA, PC1 and PC2 accounted for 51% and 28%, respectively, of the spatio-temporal variation. For the nutrient PCA, PC1 and PC2 accounted for 38% and 25%, respectively, of the spatio-temporal variation. DO, dissolved oxygen; Cond., electrical conductivity; temp., water temperature. DOC, dissolved organic carbon; DIN, dissolved organic nitrogen; SRP, soluble reactive phosphorus; DON, dissolved organic nitrogen; TN, total nitrogen; TP, total phosphorus; NO_3, nitrate; NH_4, ammonium.

3.4.4.2 Chlorophyll *a* characteristics

Overall, there were no consistent patterns in chlorophyll *a* concentrations of seston or periphyton between wet and dry sampling periods. This finding may suggest that algal dynamics are highly site-dependent, with variability related to the patchiness of residual wetted habitats, frequency and duration of drying, and local biogeochemical conditions. It is also unclear the extent to which our limited sampling frequency reflects temporal variation in algal production over shorter and longer time frames. In the upper Bremer River catchment, seasonal differences in the chlorophyll *a* concentrations from periphyton samples were site-dependent (Figure 41a; one-way ANOVA for each site), with periphyton chlorophyll *a* greatest in the wet sampling period for Reynolds Creek ($F = 51.9$, $p = 0.002$) and during the dry sampling period for Warrill Creek ($F = 14.2$, $p = 0.02$). There were no seasonal differences in periphyton chlorophyll *a* for Wild Cattle Creek ($F = 8$, $p = 0.2$), Bremer River ($F = 0.4$, $p = 0.6$), and Coulson Creek ($F = 0.3$, $p = 0.6$) (Figure 42a). At the Maules Creek catchment study sites, chlorophyll *a* concentrations from periphyton samples were similar among wet and dry sampling periods in those sites where water was present (Figure 41b; $p > 0.05$, two-way ANOVA). At Maules Creek, there were no significant differences in average chlorophyll *a* concentrations from periphyton samples among study sites (Figure 41b; $p > 0.05$, two-way ANOVA). Algae were not present in both habitats at Middle Creek, and within the riffle habitat at Maules Creek, during the dry sampling due to cessation of flow. Lower chlorophyll *a* concentrations of periphyton were evident in riffle than in pool habitats at Maules Creek ($p = 0.03$). Seston chlorophyll *a* was highest at Maules Creek (i.e. perennial losing site) and lowest at Warrill Creek (Figure 42).

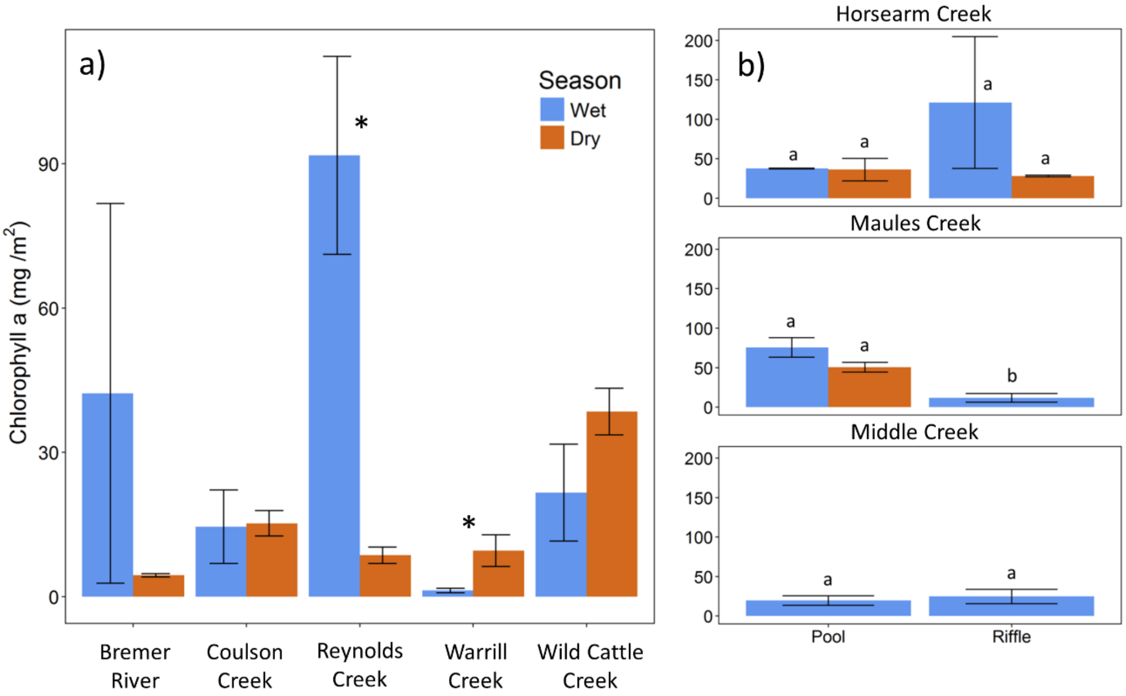

Figure 41. The average (± standard error) chlorophyll a concentration of periphyton in each habitat and season of the study sites in a) the upper Bremer River catchment, and b) the Maules Creek catchment. Dry conditions prevented dry-season sampling at Middle Creek and the riffle at Maules Creek. An asterisk indicates significant differences among a) seasons for each upper Bremer River catchment study site (one-way ANOVA) b) seasons and/or habitats for each Maules Creek catchment study site (one-way or two-way ANOVA).

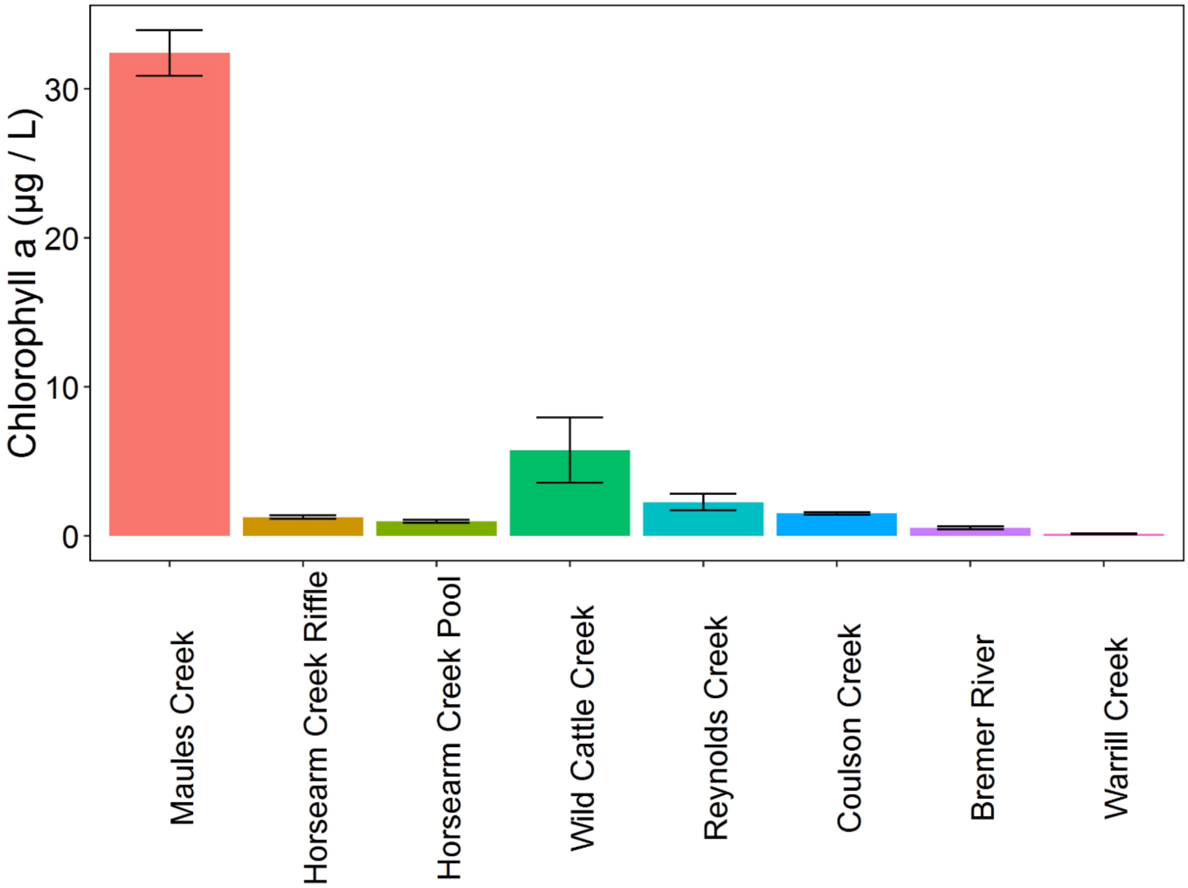

Figure 42. The average (± standard deviation) chlorophyll a concentration of seston (suspended in the water column) collected at each study site in the upper Bremer River catchment (Wild Cattle Creek, Reynolds Creek, Coulson Creek, Bremer River, Warrill Creek) and the Maules Creek catchment (Maules Creek, Horsearm Creek) during the drier season. Only dry-season sampling was undertaken for seston. Riffle and pool habitats were sampled only at Horsearm Creek.

3.5 Organic matter processing

3.5.1 Introduction

The decomposition of coarse particulate organic matter (e.g. leaves and bark) by physical and biological processes is a key ecosystem process in aquatic ecosystems because it forms the energetic base of many food webs. This is particularly the case in small, forested streams which receive proportionally more terrestrially-derived organic matter than larger, downstream reaches (Vannote *et al.*, 1980). Microorganisms (fungi, bacteria, algae) first colonise fallen litter in the form of microbial biofilms, and these biofilms subsequently consume this terrestrially-derived organic matter (Suberkropp and Klug, 1976; Gulis and Suberkropp, 2003). These microorganisms in turn become a food source for other bacteria (the microbial loop) and for grazing macroinvertebrates. Microbial colonisation and activity (Graça, 2001) as well as consumption of organic matter by invertebrate detritivores (Arsuffi and Suberkropp, 1989) are also influenced by the chemical composition of the organic matter, such as the carbon-to-nitrogen (C:N) ratio and concentrations of secondary compounds (e.g. tannins, phenolics). Such differences alter both palatability and nutritional ‘quality’ of different organic matter sources. The partial decomposition by microorganisms can make terrestrial organic matter more palatable for detritivores which, together with grazing macroinvertebrates, are then consumed by animals from higher trophic levels such as fish. Additionally, microorganisms that colonise terrestrial organic matter mediate many important biogeochemical processes (e.g. mineralisation and immobilisation) that control the uptake and export of dissolved and particulate carbon and nutrients to downstream aquatic and riparian ecosystems (Battin *et al.*, 2008). Physical processes, such as abrasion and leaching, can also break down coarse particulate organic matter into finer fragments that can be biologically consumed or exported downstream. Collectively, the decomposition of organic matter is an important process in streams that can contribute significantly to local and regional carbon cycling (Wallace *et al.*, 1997; Rosemond *et al.*, 2015).

Given the important ecological role of detrital processes in aquatic ecosystems, measurements of organic matter processing can provide important information on the functional integrity of streams, rivers, and the entire fluvial network (Wipfli *et al.*, 2007; Young *et al.*, 2008). It is increasingly recognised that functional assays, such as organic matter processing, often detect variation in, and controls on, specific ecosystem processes that cannot be inferred from physical (e.g. water quality, spatial extent of habitat) and structural (e.g. plant and animal community composition and abundance) data. Functional assays are particularly relevant for detecting human impacts in intermittent aquatic habitats where: a) the often harsh environmental conditions during the dry phase mean that the fauna is dominated by those same types of taxa that can also cope with the physio-chemical changes due to human impacts; and, b) high temporal variability means that defining an appropriate reference condition can be extremely difficult.

Cycles of wetting and drying govern variation in rates of organic matter breakdown in intermittent rivers. This is because those physical and biological processes mediating detrital processes, such as the presence of invertebrate detritivores, are largely dependent on the presence of water. Not surprisingly, comparative studies find that decomposition is generally slower in more intermittent than in perennial streams (Herbst and Reice, 1982; Pinna and Basset, 2004; Datry *et al.*, 2011). Antecedent hydrological conditions can also influence detrital processes, with reduced processing efficiency once flow resumes for leaves subject to preconditioning during dry periods (Dieter *et al.*, 2011). The processing of organic matter may also be lower in refuge aquatic habitats, with stagnant water associated with more acidic and oxygen-depleted conditions, reducing the abundance and activity of decomposers (Lake, 2003; Canhoto and Laranjeira, 2007).

Despite the pervasive role that surface hydrological conditions have in organic matter processing, little is known about the spatial and temporal patterns of detrital processing in hyporheic habitats of intermittent rivers. While reductions in rainfall may cause surface flowpaths to cease, saturated bank inflows and groundwater inputs often sustain subsurface flow in intermittent rivers. This groundwater-derived baseflow may play an important role in sustaining detrital processes in the hyporheic zone. It may therefore be expected that reductions in groundwater inputs, due to human activities, will lead to an increase in the spatial and temporal extent of drying which may impact on detrital processes in both surface and subsurface environments. This study investigates the patterns and controls of organic matter decomposition in surface and hyporheic habitats of groundwater-influenced intermittent rivers. Ultimately, understanding the rates of and controls on the decomposition of coarse particulate organic matter in small streams and rivers will allow us to understand how potential changes in intermittency and baseflow can affect the broader biogeochemistry and biological functioning of rivers systems.

3.5.2 Methods

3.5.2.1 Study design

Surface and hyporheic deployments of replicate cotton strips (for determining cellulose decomposition potential) and fine- and coarse-mesh leaf litter bags (Figure 43) were undertaken in pool, riffle, and dry-gravel bar habitats at the upper Bremer River catchment (and Maules Creek catchment sites – See Component 3) sites spanning seasonal changes in stream flow. Pool, riffle, and dry-gravel bar habitats were investigated as they are dominant in-stream habitats and represent a decreasing likelihood of hydrological permanency. Leaf litter bags were deployed twice (September to November 2015 and February to April 2016) and cotton strips four times (November 2015, January/February 2016, February/March 2016, and April/May 2016). Investigating how rates of organic matter processing differ with seasonal changes in flow, as well as among wetter and drier habitats, allows us to test the influence of flow variability on detrital processes at multiple scales.

The study design for organic matter processing experiments is summarised in Figure 44. In each stream, five replicate patches of each surface habitat type were randomly chosen (using a random number table based on habitat dimensions) and used for the placement of all subsequent organic matter processing experiments (Figure 44). At each patch, a steel bar was hammered into the substrate to attach both leaf litter bags and cotton strips with cable ties during experimental incubations (Figure 44). In order to explore differences in organic matter decomposition among surface and hyporheic environments, paired surface and hyporheic deployments of cotton strips and leaf-litter bags were undertaken in riffle habitats at all sites, and in all habitats (gravel bar, pool, riffle) within one site, Warrill Creek. Each hyporheic deployment occurred approximately 1m downstream of the paired surface habitat patch at a depth of 30 ± 5cm below the surface (Figure 44). Leaf-litter bags and cotton strips were buried following the procedure of Boulton and Foster (1998).

Figure 43. An example of a) replicate cotton strips used for cotton strip assays, b) coarse-mesh leaf litter bag, and c) fine-mesh leaf litter bag.

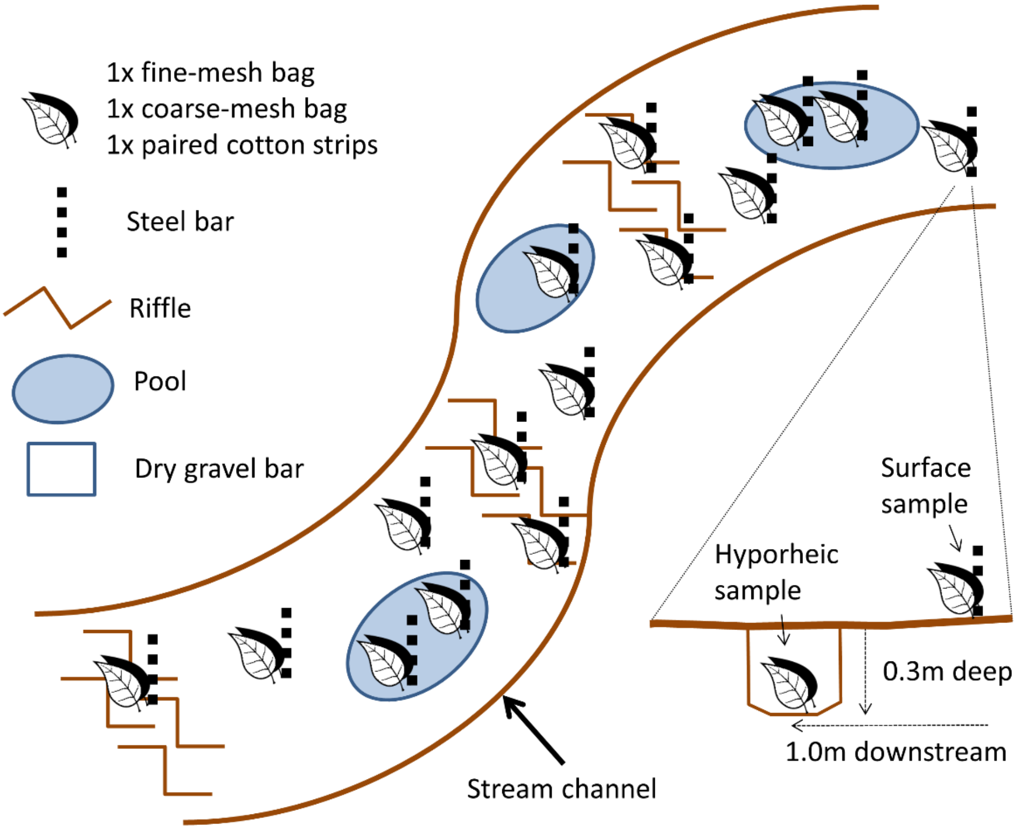

Figure 44. A schematic representation of the experimental study design for leaf litter (fine and coarse-mesh bags) and cotton strip assays. Inset plot shows longitudinal view of streambed with positioning of surface and hyporheic samples.

3.5.2.2 Cellulose decomposition potential

Cellulose decomposition potential of sediment was assessed with cotton strip assays (Boulton and Quinn, 2000). Cotton strip assays provide a standardised measure of organic matter processing because breakdown rates are not affected by variation in chemical composition, as can be the case when assessing decomposition with leaves whose chemical composition can vary widely within and among species (Boulton and Quinn, 2000). Unbleached and unprimed cotton fabric was used for assays in all streams. Ten replicate cotton strips (35mm by 60mm), affixed to rulers in pairs (i.e. 5 rulers in total) with rubber bands and cable ties, were placed cotton side up on the bottom of each surface or excavated hyporheic location at the beginning of each incubation period (Figure 44). Rulers were secured in place by being attached with cable ties to steel bars hammered into the stream. After approximately 28 days the rulers and strips were collected and rinsed, if necessary, to remove deposited sediment. Within 5 hours of collection the cotton strips were removed from the rulers and placed on paper to air-dry.

Once strips were dry, the tensile strength of each was measured with a Digital Force Gauges Series 7 tensiometer (Mark-101, New York, U.S.A.) at the Water Research Laboratory, University of New South Wales. Cotton tensile strength (kg) was recorded as the initial breaking point of each strip. The data were represented as the cotton tensile force ratio (CTFR) by dividing the cotton tensile strength of incubated strips with that of procedural controls as done previously in Burrows et al. (2014) and

Clapcott and Barmuta (2010a, 2010b). Force ratio values closer to one represent less cellulose decomposition than values closer to zero.

3.5.2.3 Leaf litter processing

Freshly-senesced *Eucalyptus tereticornis* leaves with minimal evidence of disease or blemishes were collected from riparian zones within the five study sites in the upper Bremer River catchment. *E. tereticornis* is the dominant eucalypt species in the study region and extends over much of eastern Australia. Leaves were transported to the laboratory in cotton fabric bags and dried at 60°C for 48 hours. Approximately 3g (±0.05) of leaves with stalks removed were placed into fine-mesh (0.5mm diameter, width=15cm, length=15cm) and coarse-mesh (5mm diameter, width=12cm, length=17cm) bags (Figure 43). Variation in leaf litter processing between fine- and coarse-mesh bags is commonly used to differentiate microbial (bacteria and fungi) leaf decomposition from macroinvertebrate leaf consumption because fine-mesh bags exclude macroinvertebrates. Leaf packs were deployed in surface habitats and the hyporheic zone for approximately 60 days. Upon collection samples were placed into labelled sealed bags and frozen within 24 hours. In the laboratory, samples were defrosted, rinsed, and dried at 60°C for 48 hours. The dry weight was recorded and samples were then combusted at 550°C for 40 minutes to obtain ash weight. The remaining litter ash-free dry mass (AFDM) was then calculated as the difference between dry and ashed weight remaining. Similarly, control leaves (dried, stored in the laboratory) and leaves leached for 24 hours (to account for mass loss due to leaching) were processed as above. The control leaves were used to calculate the initial litter AFDM by subtracting the initial dry weight from initial ashed weight.

The percent leaf mass remaining was calculated by the following equation:

$$\%\, Mass_{remaining} = \ AFDM_{remaining} / \ AFDM_{initial} \times 100$$

Decay rates (*k* coefficients) were calculated with a negative exponential decay model:

$$k = ln\left(\frac{M_t}{M_o}\right) \div t$$

where M_t is the AFDM at time *t* (i.e. AFDM remaining) and M_o is the initial AFDM. This equation assumes that the mass loss rates follow an exponential pattern.

3.5.2.4 Explanatory environmental variables

A categorical measure of substrate moisture status (dry, moist, or saturated) was visually estimated for each replicate surface and hyporheic habitat patch during the deployment and collection of leaf-litter bags and cotton strips. A numerical variable was constructed with combinations of the deployment-collection categorical measures to give a simple, but representative, measure of the relative degree of substrate saturation over the entire incubation period. Patches with higher values were assumed to be saturated for a longer period than those with lower values: Dry-Dry = 1, Dry-Moist = 2, Moist-Dry = 2, Dry-Saturated = 3, Saturated-Dry = 3, Moist-Moist = 4, Moist-Saturated = 5, Saturated-Moist = 5, and Saturated-Saturated = 6.

Mean daily pool water height over each leaf litter and cotton incubation period was calculated from 15 minute measurements and used as a proxy for variation in stream discharge among study sites and dates. Mean daily pool water temperature was also calculated from a temperature logger contained within the water height logger.

3.5.2.5 Statistical analyses

We assessed differences in the % leaf litter mass remaining among fine- and coarse-mesh bags, using linear mixed-effects models (LMMs). Separate LMMs were performed for each experiment (one or two) and depth (surface or hyporheic), and mesh size and habitat were treated as a fixed factors, with habitat nested within each study site (random variable). LMMs were also used to assess differences in the % leaf litter mass remaining and cellulose decomposition potential (i.e. cotton tensile force ratio) among substrate moisture status categories, with site considered a random variable in separate models for each experimental period. Variation in the % leaf litter mass remaining and cellulose decomposition potential among paired surface and hyporheic pool and dry-gravel bar habitats was assessed with two-factor analysis of variance, with experimental period and depth treatment as fixed factors. Because we conducted paired surface and hyporheic experiments in riffle habitats at all sites, we investigated variation in riffle habitat % leaf litter mass remaining and cellulose decomposition with LMMs: experimental period and depth treatment were fixed factors and site was a random variable. If interactions among factors were significant, we identified those factor levels responsible for this difference using Tukey's honest significance difference (HSD) test. We considered results significant if $p \leq 0.05$. LMMs were performed with the “lme4” R package and were, along with all other analyses, conducted in R (R Development Core Team 2011).

3.5.3 Results

3.5.3.1 Leaf litter decay rates

Leaf litter decay rates at the upper Bremer River catchment sites were, on average, much higher than comparative leaf litter studies in both surface and hyporheic habitats of perennial and intermittent streams in Australia and elsewhere (Table 12). Decay rates were consistently greater in the hyporheic zone than in all surface habitats (Table 12). Furthermore, decay rates were less variable in the hyporheic zone (coefficient of variation: CV = 4.9) than in gravel bars (CV = 15.2), riffles (CV = 19.5), and pools (CV = 8.3).

3.5.3.2 Microbial versus macroinvertebrate and/or physical litter processing

Leaf litter processing was predominantly mediated by microbial organisms in surface habitats, with the percent mass remaining of leaf litter generally similar in both fine (excludes macroinvertebrates) and coarse-mesh litter-bag treatments (Figure 44). However, leaf litter decomposition was 8% greater (by mass) in coarse-mesh compared to fine-mesh litter bags within pool habitats in the second (wetter and warmer) sampling period (Figure 45b; test=LMM), indicating that macroinvertebrate feeding may play a role in accelerating decomposition rates in certain circumstances. In the hyporheic zone, leaf litter decomposition was similar in fine-mesh compared to coarse-mesh litter bags during the first (Figure 45c; LMM output, t = -1.7, p = 0.09) and second (Figure 45d; LMM output, t = -0.3, p = 0.8) sampling period.

3.5.3.3 Influence of flow regime and substrate saturation in surface habitats

Leaf litter and cotton strip processing was greatest in surface habitats subject to more saturated conditions (Figure 45, Figure 46, Figure 47). Compared to dry-gravel bar habitat, 18% more leaf mass was consumed in pool habitats (LMM output: t = -14.9, p <0.0001) and 10% more consumed in riffle habitats (LMM output: t = -7.69, p <0.0001), pooling mesh-size treatments. Similarly, cotton tensile force ratio (CTFR) was 30% lower (i.e. greater tensile strength lost) in pool habitats (LMM output: t = -9.83, p <0.0001) and 25% lower in riffle habitats (LMM output: t = -8.40, p <0.0001) than in dry gravel bars (Figure 47).

T; Figure 48). Cotton degradation did not vary with pool water height (proxy for stream flow) in dry gravel bar habitat (LMM output, $t = -0.2$, $p = 0.8$; Figure 48). Average leaf litter decomposition in all habitats was also greater (lower % mass remaining) in the incubation period (experiment two) characterised by higher mean pool water height (LMM output, $t = 9.4$, $p < 0.001$) and this difference was greater for riffle habitat (LMM output, $t = -4.2$, $p < 0.001$; Figure 46) – average water temperature was, however, also greater in the second incubation period than in the first (mean increase across study sites = 3.3°C).

Organic matter processing was positively related with patch-scale assessments of substrate moisture status (Figure 49, Figure 50). For cotton strips, CTFR generally decreased (i.e. greater cellulose decomposition) along a gradient of substrate moisture status (LMM output, $t = -9.7$, $p < 0.001$), with 40% more cotton decomposition, on average among sites, in the wettest moisture category (category 6) than in the driest moisture category (category 1). Similarly, the % mass remaining of leaf litter was generally lower in the wetter substrate moisture categories in both incubation periods (Figure 50). In the first incubation period, the % mass remaining of leaf litter was only significantly lower in the wettest (category 6) substrate moisture category (LMM output, $t = -15.5$, $p < 0.001$). However, in both incubation periods, the percent mass remaining of leaf litter was 18% lower, on average, in the wettest substrate moisture category (category 6) than in completely dry substrate (LMM output, $t = -15.5$, $p < 0.001$).

3.5.3.3.1 Surface versus hyporheic organic matter processing

Leaf litter and cotton strip processing was greater in hyporheic than in surface environments (Figure 46 and Figure 47; Appendix 3), with variation in this pattern, particularly for surface habitats, consistent with changes in hydrological conditions. For instance, hyporheic organic matter processing in pool and riffle habitats was significantly greater than in the corresponding surface deployments only during the driest experimental periods (experiment one in Figure 46 and experiment four in Figure 47). On all sampling occasions, organic matter processing in gravel bar habitat was greater (lower % leaf mass remaining and CTFR) in the hyporheic zone than at the surface (Figure 45, Figure 46, Figure 47). Not surprisingly, the substrate moisture status of gravel bar habitats was significantly greater in hyporheic (mean ranking = 2.25) than in surface (mean ranking = 3.9) deployments ($p < 0.0001$).

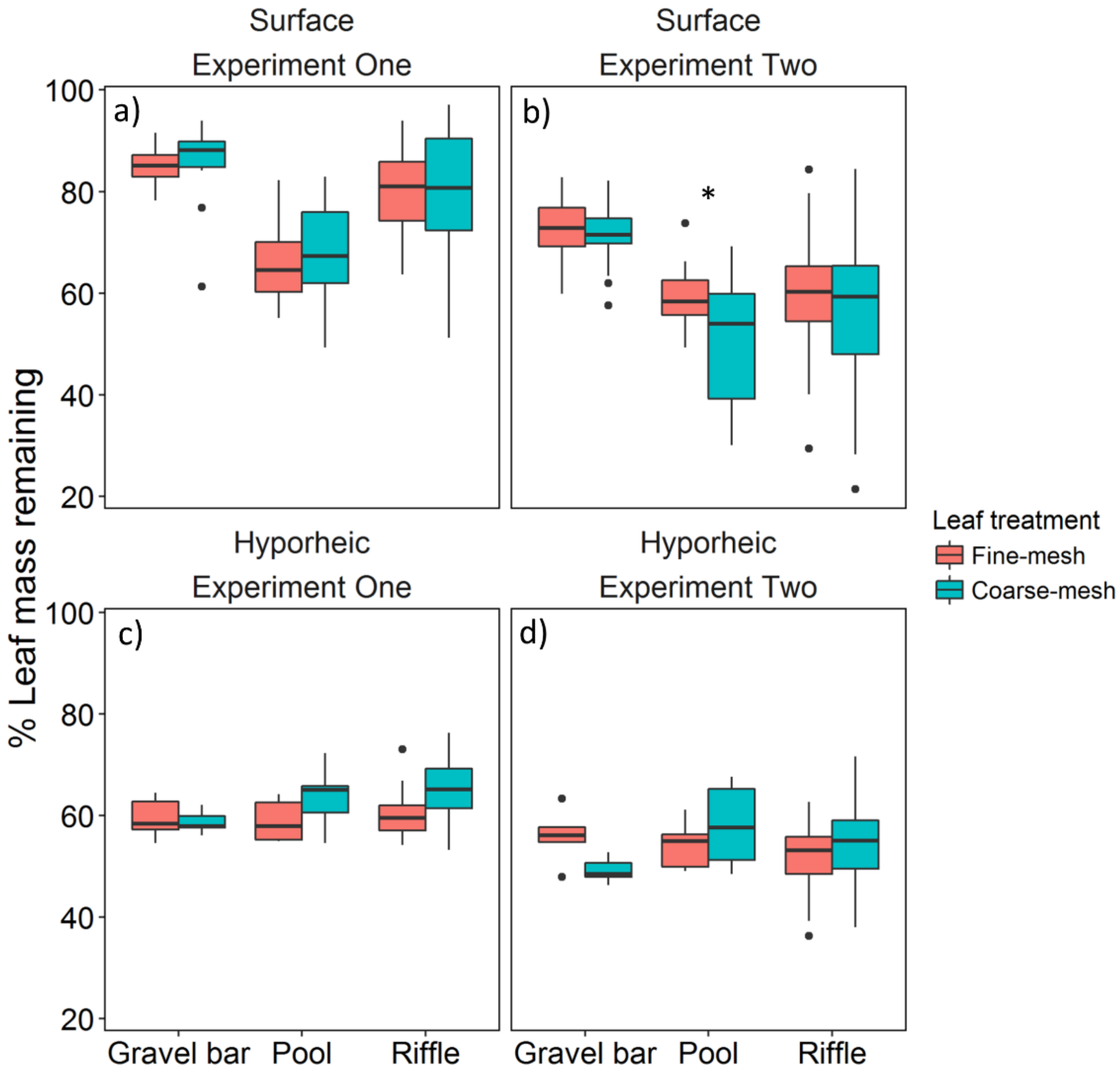

Figure 45. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the percent mass remaining of leaf litter in fine- and coarse-mesh litter bags that were deployed in the upper Bremer River catchment study sites in gravel bar, pool, and riffle habitat in surface (ab) and hyporheic (cd) environments during two experimental incubation periods (experiment one = 1st-4th September 2015 – 2nd-4th November; experiment two = 8th-11th February 2016 – 11th-12th April). An asterisk indicates significant differences among mesh-size treatments in each habitat, depth, and experiment combination. Less leaf mass remains (i.e. greater decomposition) in hyporheic than surface treatments, and there is generally no difference in leaf decomposition between fine- and coarse-mesh leaf litter bags.

Table 12. The mean (± standard deviation, where available) and range (minimum – maximum) in leaf litter decay rates (k) from this study (upper Bremer River catchment sites) and other comparative studies investigating leaf litter decay rates in intermittent and perennial stream habitats. Information on flow regime, climate, dominant habitat, mesh size of litter bags, and the species of leaf are also listed for each study. Rates of litter decay are generally greater in our study, especially in the hyporheic zone, compared to other investigations.

Study	**Flow regime**	**Climate**	**Habitat**	**Mesh size**	**Species**	***k* - mean (±sd)**	***k* - range**
Present	Intermittent	Sub-tropical	Gravel bar	Fine and coarse	*Eucalyptus tereticornis*	0.048 (0.007)	0.029-0.061
Present	Intermittent	Sub-tropical	Riffle	Fine and coarse	*Eucalyptus tereticornis*	0.053 (0.01)	0.018-0.07
Present	Intermittent	Sub-tropical	Pool	Fine and coarse	*Eucalyptus tereticornis*	0.059 (0.005)	0.046-0.07
Present	Intermittent	Sub-tropical	Hyporheic	Fine and coarse	*Eucalyptus tereticornis*	0.060 (0.003)	0.051-0.067
Datry et al.(2011)	Intermittent	Temperate	Riffle	Coarse (6mm)	*Alnus glutinosa*	0.0036 (0.004)	0.0005-0.0115
Abril *et al.* (2016)	Intermittent	Mediterranean	Isolated pools	Coarse (5mm)	*Populus nigra*	0.044 (0.005)	NA
Abril *et al.* (2016)	Intermittent	Mediterranean	Moist sediment	Coarse (5mm)	*Populus nigra*	0.013 (0.001)	NA
Abril *et al.* (2016)	Intermittent	Mediterranean	Dry sediment	Coarse (5mm)	*Populus nigra*	0.009 (0.002)	NA
Abril *et al.* (2016)	Perennial	Mediterranean	Running water	Coarse (5mm)	*Populus nigra*	0.053 (0.003)	NA
Bunn (1988)	Perennial	Mediterranean	Running water	Fine (0.01mm)	*Eucalyptus marginata*	0.0012-0.0015	NA
Bunn (1988)	Perennial	Mediterranean	Running water	Coarse (3.4mm)	*Eucalyptus marginata*	0.0014-0.0020	NA
Cornut *et al.* (2010)	Perennial	Mountain climate	Riffle	Coarse (5mm)	*Alnus glutinosa*	0.0292	NA
Cornut *et al.* (2010)	Perennial	Mountain climate	Hyporheic	Coarse (5mm)	*Alnus glutinosa*	0.0103	NA

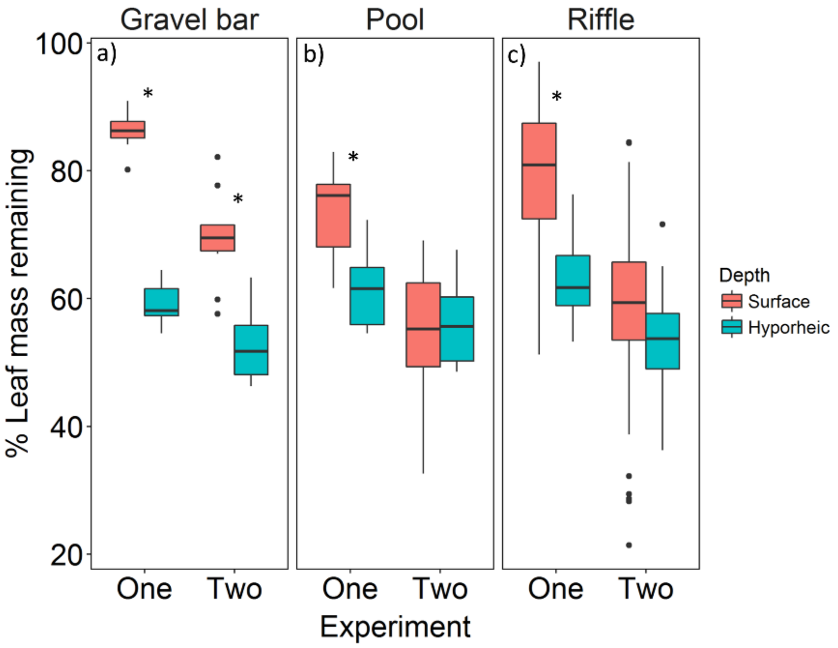

Figure 46. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the percent mass remaining of leaf litter bags (pooling fine- and coarse-mesh litter bags) that were deployed in the upper Bremer River catchment study sites in a) gravel bar, b) pool, and c) riffle habitat in surface and hyporheic environments during two experimental incubation periods (experiment one = 1st-4th September 2015 – 2nd-4th November; experiment two = 8th-11th February 2016 – 11th-12th April). An asterisk indicates significant differences among depth treatments in each habitat and experiment combination from pairwise comparisons. Less leaf mass remains in the hyporheic zone than in paired surface environment; however, this difference is less pronounced in wetter surface environments (i.e. pools and riffles).

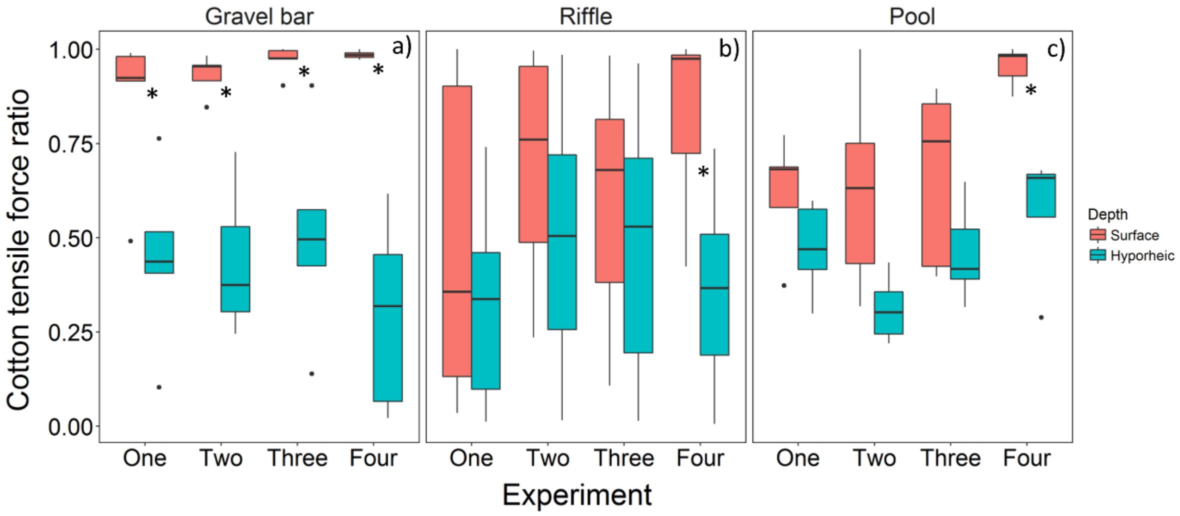

Figure 47. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the cotton tensile force ratio of cotton strips that were deployed in the upper Bremer River catchment study sites in a) gravel bar, b) riffle, and c) pool habitat in surface and hyporheic environments during four experimental incubation periods (experiment one, 3rd-4th November 2015 – 2nd-4th December; experiment two, 12th-14th January 2016 – 8th-11th February; experiment three, 8th-11th February – 7th-10th March 2016; experiment four, 11th-12th April – 9th-12th May 2016). Force ratio values closer to one represent less cellulose decomposition than values closer to zero. An asterisk indicates significant differences among depth treatments in each habitat and experiment combination from pairwise comparisons. There is more cellulose decomposition (i.e. lower cotton tensile force ratio) in the hyporheic zone compared to paired surface environment; however, this difference is less pronounced in wetter surface environments (i.e. pools and riffles).

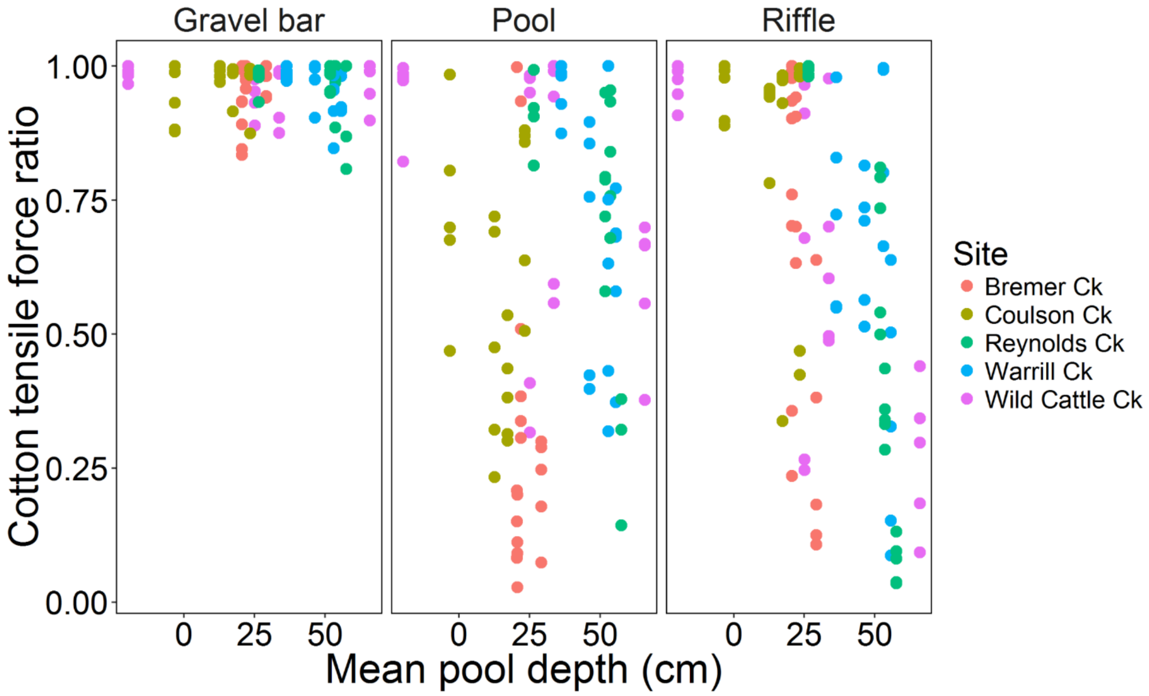

Figure 48. The mean cotton tensile force ratio of replicate cotton strips in each habitat and site plotted against mean pool depth at each site. Negative depths occur because the base of each water height logger was lower than the pool bottom. Cellulose decomposition is greater (i.e. lower cotton tensile force ratio) when mean pool depth at each site is greater.

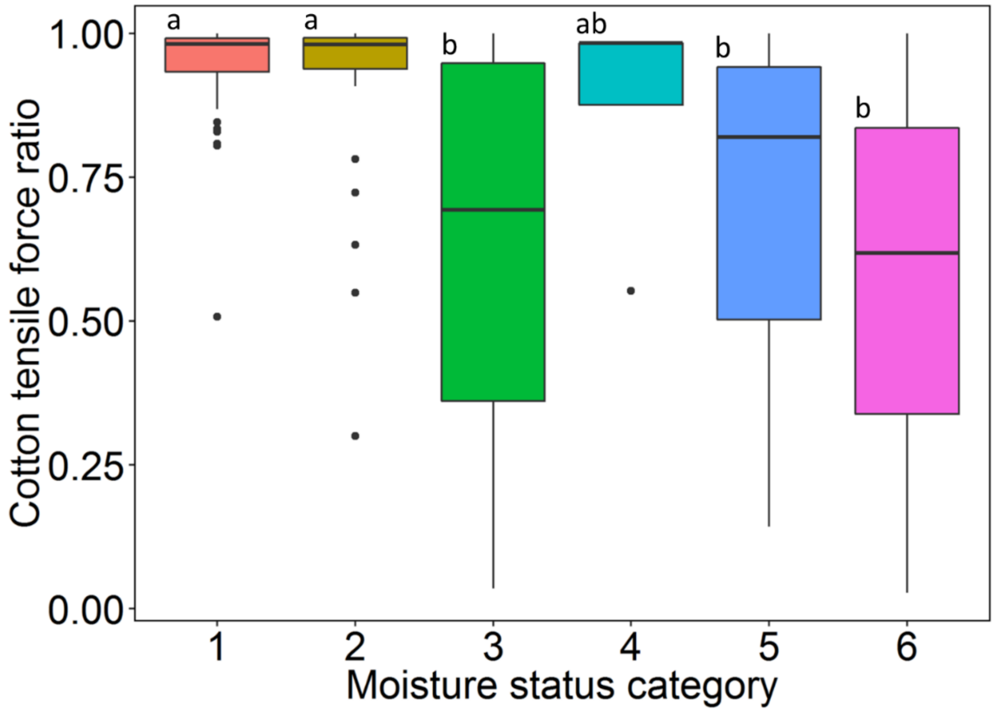

Figure 49. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the cotton tensile force ratio of cotton strips in six categories of substrate moisture status. Moisture status categories: dry-dry = 1; dry-moist = 2; moist-dry = 2; dry-saturated = 3; saturated-dry = 3; moist-moist = 4; moist-wet =5; wet-moist = 5; wet-wet = 6. Letters denote significant differences among moisture status categories (i.e. bars with the same letter are not significantly different). Force ratio values closer to one represent less cellulose decomposition than values closer to zero. Cellulose decomposition is greater (i.e. lower cotton tensile force ratio) when the substrate is wetter over the incubation period.

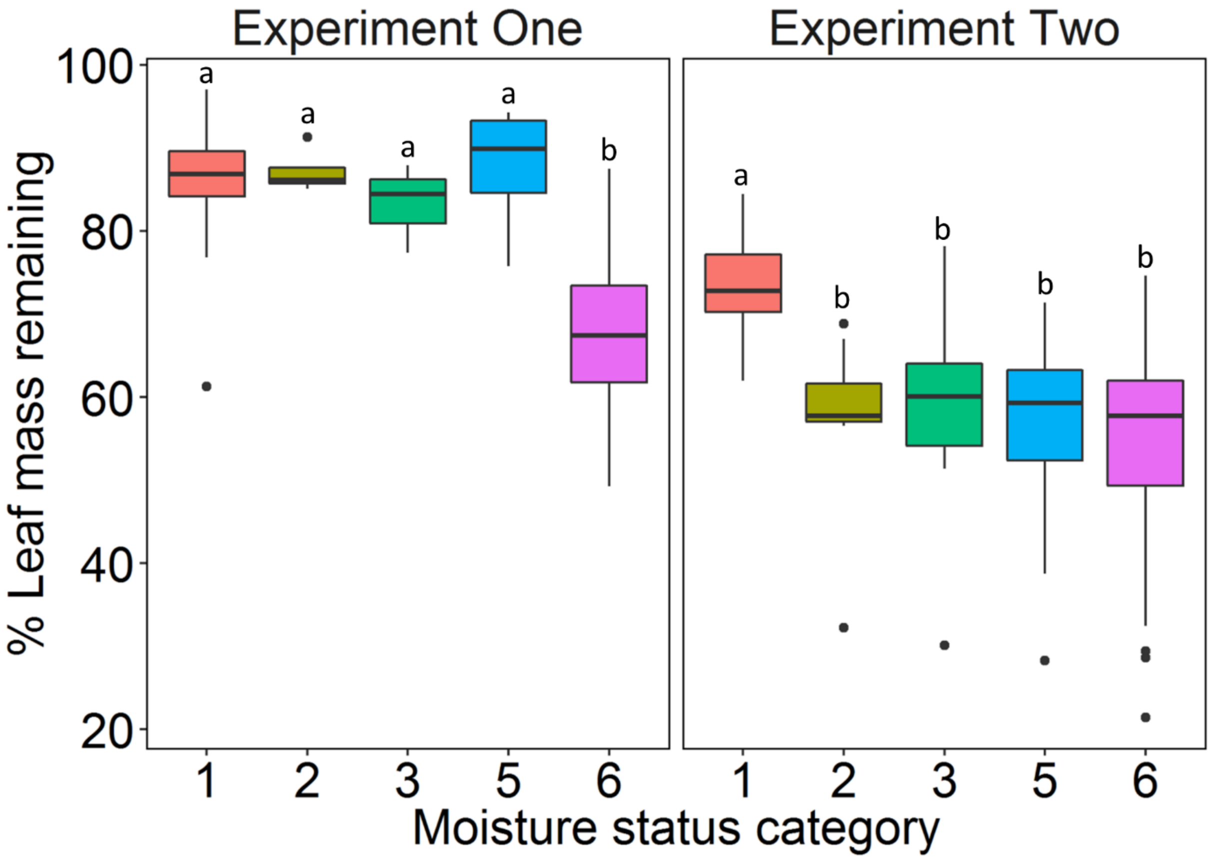

Figure 50. Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the % leaf mass remaining in fine- and coarse-mesh litter bags (pooled for analysis) in six categories of substrate moisture status and in two experimental periods. Moisture status categories: dry-dry = 1; dry-moist = 2; moist-dry = 2; dry-saturated = 3; saturated-dry = 3; moist-moist = 4; moist-wet =5; wet-moist = 5; wet-wet = 6. Letters denote significant differences among moisture status categories. Leaf decomposition is greater (i.e. lower % leaf mass remaining) when the substrate is wetter over the incubation period.

3.5.4 Discussion

The breakdown of particulate organic matter is a fundamental ecosystem process in streams and rivers and its measurement can provide important information on the functional integrity of aquatic ecosystems. Despite the high intermittency of our study streams, rates of leaf litter processing were sometimes an order of magnitude greater than in studies elsewhere that assessed breakdown rates in both perennial and intermittent streams (Table 12). Using two complementary measures of organic matter processing (leaf litter breakdown and cotton degradation), measured at multiple scales, we showed that detrital processes are largely driven by the degree of substrate saturation. Furthermore, both leaf litter processing and cotton degradation were greater in the hyporheic zone than in wetted and dry surface habitats. Overall, our results highlight that intermittent river systems are important locations for detrital processes and that the hyporheic zone sustains critical ecosystem processes even when surface habitats cease to flow.

3.5.4.1 Leaf litter decay

Leaf litter decay rates were, on average, much higher than in comparable leaf litter studies in both surface and hyporheic habitats of perennial and intermittent streams (Table 12). This finding was somewhat unexpected given that eucalypt leaves are generally considered to be a poor-quality food resource due to their high carbon-to-nitrogen ratio and high concentrations of polyphenols (Bunn, 1988; Boulton, 1991; Pozo *et al.*, 1998). We hypothesise that sustained subsurface baseflow during drier periods, due to groundwater input and/or saturated bank seepage, may have prevented the deterioration of physicochemical conditions that are usually associated with residual wetted habitats during dry conditions (Boulton and Lake, 1990; McHugh *et al.*, 2015). Consequently, microbial activity and rates of organic matter breakdown in the hyporheic zone were not affected by surface drying.

3.5.4.2 Role of substrate saturation in driving surface detrital processes

Surface detrital processing was primarily controlled by water availability, with both measures of organic matter processing strongly related to spatial (habitat- and patch-scale) and temporal (reach-scale) patterns of streambed saturation. In particular, cellulose decomposition was 40% greater in the wettest substrate moisture category compared to the driest moisture category, and an 18cm increase in mean pool water height (proxy for reach-scale hydrological variability) was associated with a 40% increase in mean leaf litter decomposition, pooling all sites and habitats. The degree of immersion has previously been documented to be a major factor controlling detrital processes in intermittent river habitats (Boulton, 1991; Maamri *et al.*, 1997; Pinna and Basset, 2004). For example, a decrease in flow permanence from 100 to 85% led to a four-fold decrease in leaf litter breakdown in a temporary stream in south-west France (Datry *et al.*, 2011). Additionally, Corti et al. (2011) found that leaf (*Populus* sp.) breakdown rates decreased with increasing cumulative emersed duration (i.e. total number of day of emersion during the experiment). Our findings, and the previous research, indicate that small reductions in flow permanence may have large impacts on detrital processes in surface habitats. While variation in the degree of substrate saturation among experimental incubations is likely the primary factor mediating organic matter decomposition, with several lines of evidence supporting this notion, we cannot rule out the co-influence of temperature: pool water height was often highest during the summer experimental incubations, making it difficult to investigate the influences of pool height and temperature separately.

3.5.4.3 Role of microbes for detrital processes

The processing of leaf litter (*E. tereticornis*) was dominated by microbial decomposition in both surface and hyporheic environments, with very little evidence of macroinvertebrate consumption or mass loss due to physical processes (i.e. fragmentation; Figure 45). While microbial decomposition dominated overall leaf litter processing, 8% more leaf processing was evident in the presence of macroinvertebrates in pool habitats during the wetter incubation period. Pool habitats act as a biological refuge in intermittent streams due to their lower geomorphic position and greater hydrological permanency than other surface habitats (Labbe and Fausch, 2000). This hydrological permanency likely leads to greater leaf palatability via greater leaching and pre-conditioning by microbes (Bunn, 1988) and, together with greater abundances of shredding macroinvertebrates, may increase the consumption of leaf material. Although macroinvertebrates appear to play an important role in mediating detrital processes in more saturated habitats during wetter periods, microbial decomposition was still the most important driver of detrital processes in our intermittent study steams.

3.5.4.4 Important role of the hyporheic zone

The hyporheic zone emerged as an important location of detrital processes in our intermittent study streams, with rates of leaf litter processing and cellulose breakdown consistently greater in hyporheic than in paired surface habitats. The strong patterns revealed in this study contrast with most previous research that found lower leaf decomposition in buried than in surface treatments (Metzler and Smock, 1990; Naamane *et al.*, 1999; Cornut *et al.*, 2010). Although we can only speculate as to what mechanisms lead to greater organic matter processing in the hyporheic zone, we know that: a) the hyporheic zone was always as wet as, or wetter than, corresponding surface habitat; and, b) differences between surface and hyporheic treatments were only significant during dry incubation periods and in drier habitats (i.e. gravel bar). Together, these results indicate that substrate moisture status was a key driver of the greater hyporheic organic matter processing. Other reasons may include a greater surface area of leaf and cotton in contact with sediment microorganisms, and more stable habitat conditions, in the hyporheic habitats in our study than in others due to the prevalence of groundwater contributions to baseflow.

3.5.4.5 Summary

Overall, our two complementary measures of organic matter processing indicate that rates of organic matter processing are principally linked to the degree of substrate saturation at multiple scales. At the patch scale, organic matter processing in surface habitats was greater when the benthic substrate was more saturated. At the habitat scale, organic matter processing was greater in those habitats that remain saturated for longer (pool > riffle > dry gravel bar). At the reach scale, organic matter processing within pool and riffle habitats was greater in incubation periods with greater hydrological connection. Overall, this study indicates that a reduction in baseflow, via groundwater extraction, will lead to a greater proportion of dry streambed, greater duration of flow intermittency, and may ultimately lower rates of particulate organic matter processing in surface habitats. Therefore our results support the hypothesised response of organic matter processing to a reduction in baseflow for surface habitats. A phenomenon common to all study streams was the greater rates of organic matter processing in the hyporheic zone compared to surface habitats. Hence, the hyporheic zone sustains critical ecosystem processes even when surface habitats cease to flow.

3.6 Basal resource use and food web dynamics

3.6.1 Introduction

Streamflow reductions are often associated with declines in the availability and quality of aquatic habitats (Rolls *et al.*, 2012), and may also lead to changes in the composition and size structure of aquatic assemblages and as well as interactions among species. Food webs reflect connections between organisms and basal resources (primary producers and terrestrial organic matter inputs) and are thus a powerful way of evaluating the ecological effects of altered stream flow (Thompson *et al.*, 2012). We used food webs and trophic interactions in this study to assess how reductions in stream flow may affect intermittent river ecosystems.

Understanding the extent of spatio-temporal variation in, and controls on, the contribution of terrestrial energy sources (i.e. allochthonous) versus photosynthetically-fixed sources (i.e. autochthonous) to food webs is fundamental when assessing ecological patterns and processes in streams and rivers (Hall and Meyer, 1998). Although terrestrial organic matter has long been recognised as an important basal energy source for higher consumers (Hall *et al.*, 2000; Reid *et al.*, 2008), research from more arid and semi-arid systems points to the prevalence of food webs dependent on algal production (Balcombe *et al.*, 2005; Bunn *et al.*, 2006; Hadwen *et al.*, 2010). Importantly, shifts between autotrophy and heterotrophy in some regions appear to be related to seasonal changes in hydrology, with autotrophy becoming an important basal resource during periods of low flow or hydrological disconnection (Finlay *et al.*, 2002; Bunn *et al.*, 2003). Quantifying the availability of basal resources, and relative energetic contribution to consumers, in periods of varying flow conditions can help evaluate the potential effects of reduced baseflow for aquatic food webs (Bunn *et al.*, 2003).

Along with shifts in the basal energy sources, drying can also affect the overall food web structure as well as interactions among organisms higher in the food web. For example, Sabo *et al.* (2010) found that food chain length increased in streams with higher baseflow and lower flow variability, suggesting that flow intermittency had a destabilising effect on food webs, primarily by reducing food-chain length through the elimination of top predators. Similarly, recent research of drying streams in New Zealand found that food webs became smaller and shorter along a gradient from larger to smaller wetted habitats, with habitat size negatively associated with flow intermittency (McHugh *et al.*, 2015).

In this study, we ask how a reduction in stream flow affects basal resource use and food-web structure in intermittent rivers. We answer this question by sampling basal resources and the tissue of consumers to examine the ratio of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). We collected samples from intermittent river catchments in two regions of eastern Australia. In addition, we sought to determine whether changes in surface-water food webs due to flow reduction would be reflected by similar changes in stable isotope values of hyporheic basal resources and consumers.

3.6.2 Methods

3.6.2.1 Collection of basal sources and consumers

Triplicate samples of basal food sources and consumers were collected from each study site in the upper Bremer River catchment and Maules Creek catchment for stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). $\delta^{15}N$ values are traditionally used to estimate trophic position and $\delta^{13}C$ is useful for determining food source pathways of higher consumers (i.e. resource coupling). In the upper Bremer River catchment sampling was conducted on 3-7th September 2015 (dry season sampling) and 7-10th March 2016 (wet season sampling). In the Maules Creek catchment sampling was conducted on 21st-23rd July 2015 (wet season sampling) and 5-6th April 2016 (dry season sampling). Sources included riparian grasses, riparian tree leaves, fine-particulate organic matter (hyporheic zone only), coarse-particulate organic matter, submerged and emergent macrophytes, suspended particulate organic matter (seston), and periphyton attached to rocks (epilithon), woody debris (epixylon), and large macrophytes. Isotope values for coarse-particulate organic matter and riparian tree leaves were combined and called ‘plants’ for statistics and figures because values were very similar. Additional sampling was undertaken on two separate occasions in sites within the upper Bremer River catchment to assess epilithon growing on the underside of rocks (referred to as ‘epilithon bottom’). This sampling occurred on the 12th May 2016 (wet period) and 13th July 2016 (dry period). Detailed methods for how these sources were sampled are available in Jardine et al. (2013). Benthic macroinvertebrates including gastropods and decapods were collected from a combination of hand picking and 1-2 m dip net sweeps of benthic surfaces and areas of littoral vegetation. Fish were occasionally sampled in the dip net sweeps but were also sampled using an electro-fisher in the Bremer River catchment. For each fish (maximum of five individuals per species per site and sampling occasion), a non-lethal fin clip was taken for stable isotope analysis, which is a reliable surrogate for muscle tissue in Australian freshwater fishes (Jardine *et al.*, 2011). Fish were not sampled in the Maules Creek catchment. Primary consumers included Ephemeroptera (Baetidae, Caenidae, and Leptophlebiidae) and Trichoptera (Calamoceratidae, Hydropsychidae, and Leptoceridae). Secondary consumers or predatory macroinvertebrates included Ameletopsidae, Belostomatidae, Coenagrionidae, Corydalidae, Dytiscidae, Ecnomidae, Gelastocoridae, Gerridae, Gomphidae, Gyrinidae, Hydrometridae, Isostictidae, Libellulidae, Megapodagrionidae, Notonectidae, Osmylidae, Pleidae, and Velidae.

3.6.2.2 Laboratory sample preparation and analysis

All submerged plants and invertebrate collections were rinsed with distilled water and any residual organic matter was removed under a dissecting microscope. All specimens were classified to family, genus, or species where possible. Muscle tissue was extracted from decapods and fish using a scalpel. Samples were then dried at 60°C for 24 hours and then ground and homogenised in a ball mill grinder. Plant samples were weighed to approximately 5 mg and animal tissue samples to 2 mg and then combusted in an EA 3000 elemental analyser (Eurovector, Milan, Italy). Sample gases were delivered to a Sercon isotope mass spectrometer (Sercon Limited, Crewe, UK) for isotope analysis of C and N. Working standards were liquids calibrated against IAEA CH6, CH7, N1 and N2 and had elemental composition that matched the samples (44% C and 11% N for animal tissues, 41% C and 2% N for plant tissues).

3.6.3 Data analysis

A gradient approach was used to assess the strength of consumer-resource coupling (Rasmussen, 2010; Jardine *et al.*, 2012) among sites in the Bremer River catchment in the wetter and drier sampling trips. Consumer-resource coupling could not be assessed statistically at sites within the Maules Creek catchment due to the very distinct flow regimes at each site and a lack of site replication. In this approach, the slope of the regression of consumer $\delta^{13}C$ versus source $\delta^{13}C$ is used as an indicator of the reliance of consumers to particular resources (Rasmussen, 2010). This approach is particularly useful for determining consumer reliance on particular algal C sources because the $\delta^{13}C$ value of algae varies considerably due to environmental factors such as flow, water quality, riparian cover, and productivity (Hadwen *et al.*, 2010; Jardine *et al.*, 2014). For each regression of a consumer versus a source, the 95% confidence intervals of regression slopes are determined to see whether they differ from zero or one (significant if $p \leq 0.05$). Four different consumer-resource coupling scenarios may result: local source dominant, slope different from zero but not from one; mixed source, slope different from one and zero; alternative source, slope different from one but not zero; unresolved source, slope similar to one and zero. In instances where a key basal resource was not sampled, we estimated a region on the $\delta^{13}C$ and $\delta^{15}N$ biplots where this basal resource should be by 'back applying' the known trophic enrichment gradient of $\delta^{13}C$ and $\delta^{15}N$ values. This approach is possible because increases in trophic levels (plant to grazer or grazer to predator) involve predictable increases in $\delta^{13}C$ (0.5 to 1‰) and $\delta^{15}N$ (2.2 to 2.4‰) values in the consumer versus its diet (Fry, 2006).

The difference in $\delta^{13}C$ values of dominant sources and consumers among the wetter (2015) and drier (2016) sampling trips in the Bremer River catchments were plotted in order to visualise changes in $\delta^{13}C$ due to reductions in stream flow. In this plot, lower $\delta^{13}C$ values indicate samples that are relatively more enriched in ^{13}C, and higher $\delta^{13}C$ values indicate samples more depleted in ^{13}C, in the drier than wetter sampling period. Differences in the C:N ratio of sources and consumers were assessed using one-way analysis of variance (ANOVA). All analyses were conducted in R (R Development Core Team, 2011).

3.6.4 Results

A strong food web resolution (distinct $\delta^{13}C$ and $\delta^{15}N$ values) was observed for sources and consumers within study sites in the upper Bremer River and Maules Creek catchments (Figure 51 & Figure 52). The sources in both catchments were isotopically distinct, with a large separation of algal sources visible in the $\delta^{15}N$ - $\delta^{13}C$ bi-plots (Figure 51 & Figure 52) Consumers generally had more enriched $\delta^{15}N$ values than sources (Figure 51 & Figure 52), with fish and decapods (shrimps, prawns and crayfish) having the most enriched values. In the Bremer catchment, mean source $\delta^{15}N$ values varied from -6.3 to 5.4‰ and consumer $\delta^{15}N$ values from -1.8 to 7.4‰. Mean source (varying from -1.2 to 11.1‰) and consumer (varying from 1.5 to 10.2‰) $\delta^{15}N$ values were enriched in the Maules catchment compared to the Bremer study sites. Neither catchment displayed the classic isotopic enrichment gradient for freshwater food webs, with $\delta^{13}C$ values for sources and consumers much more depleted than expected (typical algal enrichment gradient represented with a dashed line; Figure 51 & Figure 52).

3.6.4.1 Autochthonous versus allochthonous carbon-sources

3.6.4.1.1.1 Bremer River catchment

Primary, secondary, and higher-order consumers in the Bremer River catchment relied on algal-based carbon sources during the wetter season, with variation among sites in $\delta^{13}C$ values of autochthonous basal resources (i.e. periphyton) significantly related to variation in the $\delta^{13}C$ values of grazing macroinvertebrates (Ephemeroptera, but only partially), predatory macroinvertebrates, and decapods (Table 13). Although we could not resolve the contribution of periphyton to fish diets (possibly due to an omnivorous feeding strategy), variation in the $\delta^{13}C$ values of fish during the wetter season was correlated with that of decapods, which were in turn reliant on periphyton. This reliance on algal-based C was, however, not evident in the drier sampling period, with unresolved energy source(s) for consumers (Table 13).

While the mean $\delta^{13}C$ values for Ephemeroptera were significantly related to those of periphyton, the slope of this relationship was significantly different from 1, indicating that this taxon is also reliant on one or more other C sources. Although collected outside the two sampling occasions (i.e. wet and dry period), $\delta^{13}C$ values for epilithon growing on the underside of rocks (labelled 'Epilithon bottom' in figures) were close to average $\delta^{13}C$ values for Ephemeroptera (an algivore) – indicating that there may be an alternative food source.

Differences in $\delta^{13}C$ values between the wet and dry sampling periods were greatest for periphyton, with more depleted ^{13}C values in the drier season compared to the wetter season (Figure 53). Alongside changes in $\delta^{13}C$ values, periphyton was more nutritious (i.e. lower C:N ratio) in the wetter (9.8 ±0.7) sampling period than the drier (11.6 ±1.0) period (one-way ANOVA; $f = 4.9$, $p = 0.04$). Periphyton was an important energy source for many consumer groups and the ^{13}C values of these consumer groups were also more depleted in the drier season (Figure 53). Plants, seston, macrophytes, and fish $\delta^{13}C$ values were relatively similar between the two sampling periods. $\delta^{13}C$ values of epilithon collected from the underside of rocks showed the opposite pattern to periphyton, with enriched δ^{13}C values in the drier compared to the wetter sampling period (Figure 53).

3.6.4.1.1.2 Maules Creek catchment

Higher order consumers (decapods) were absent from the intermittent study site but present within the perennial gaining and losing study sites (Figure 52); fish, however, were not sampled. Due to the study design (three distinct flow regimes across the three sites), a gradient approach comparing the primary producer source contribution to consumers could not be applied to the study sites at Maules Creek. However, 'back applying' a typical trophic enrichment gradient to the dominant algivore at the perennial gaining and losing study sites (Ephemeroptera) indicates that a key algal source, with a δ^{13}C value between -35 and -40, was not collected.

3.6.4.2 Hyporheic food web

Analysis of the hyporheic food web was made difficult by the fact that only a small number of animals were collected in sufficient abundance to enable isotopic ratios to be determined. Nonetheless there was a diversity of taxa (see Component 3 for taxa collected), a number of which may be dependent on fine particulate organic matter (soil, small fragments of leaf litter, decomposing algae and faeces among other sources) due to their position above FPOM in the isotope biplots (Figure 54). These patterns were broadly similar across both regions, and both sites, with enriched δ^{13}C values compared to values for surface habitats (Figure 54).

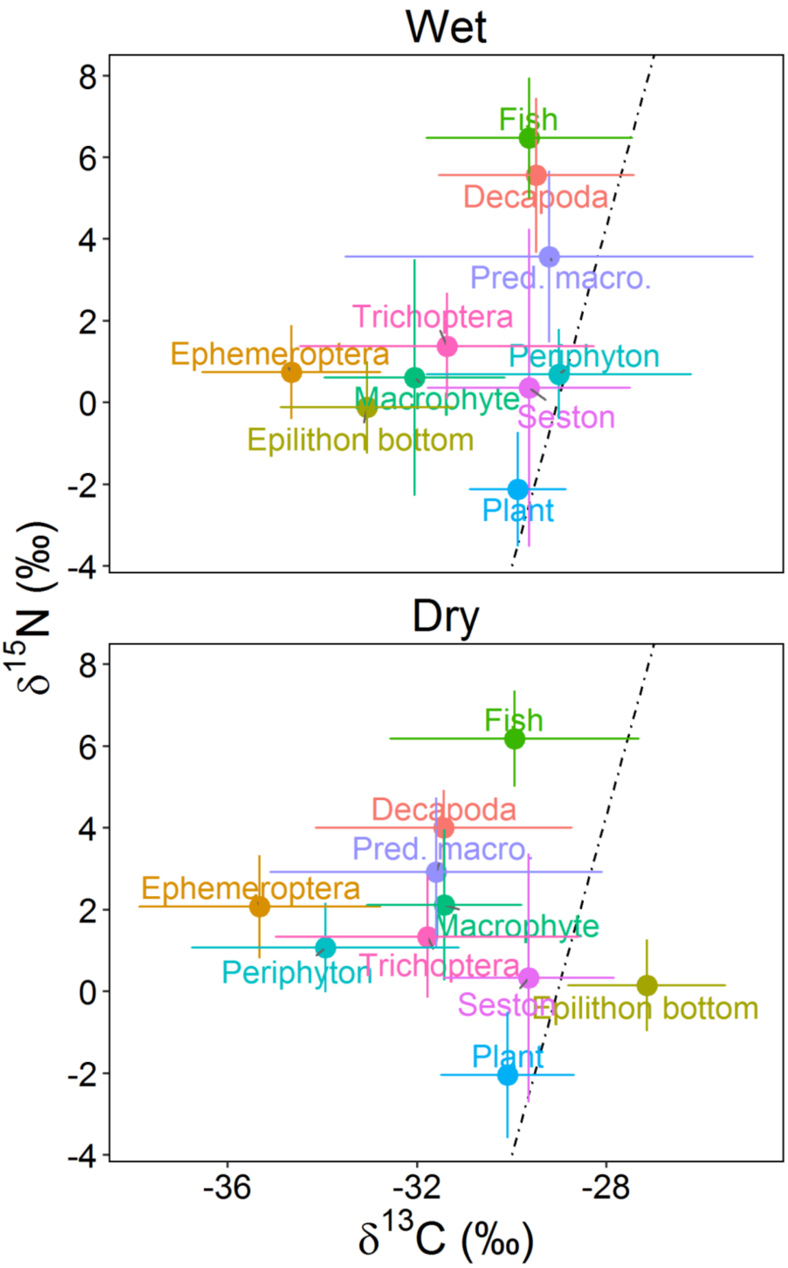

Figure 51. The mean ± standard deviation of the $\delta^{13}C$ and $\delta^{15}N$ values for sources and consumers of the five sites within the upper Bremer River catchment during the wet and dry sampling periods. The dashed line represents the intercept and gradient associated with a typical algal-dependent food web. Plant represents the combined isotope values for riparian vegetation and coarse-particulate organic matter. All food web components, including higher order consumers, were present in both the wet and dry sampling periods.

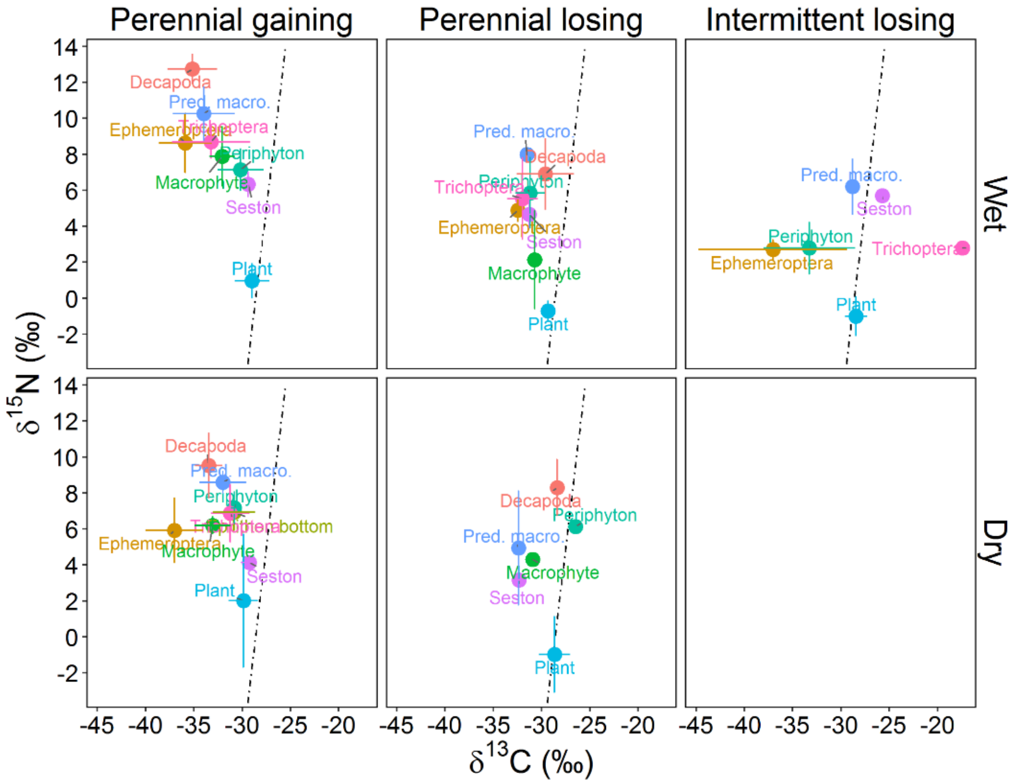

Figure 52. The mean ± standard deviation of the $\delta^{13}C$ and $\delta^{15}N$ values for sources and consumers in surface habitats of three study sites at Maules Creek catchment during the wet and dry sampling period. Perennial gaining, Horsearm Creek; Perennial losing, Maules Creek; Intermittent losing, Middle Creek. Plant represents the combined isotope values for riparian vegetation and coarse-particulate organic matter.

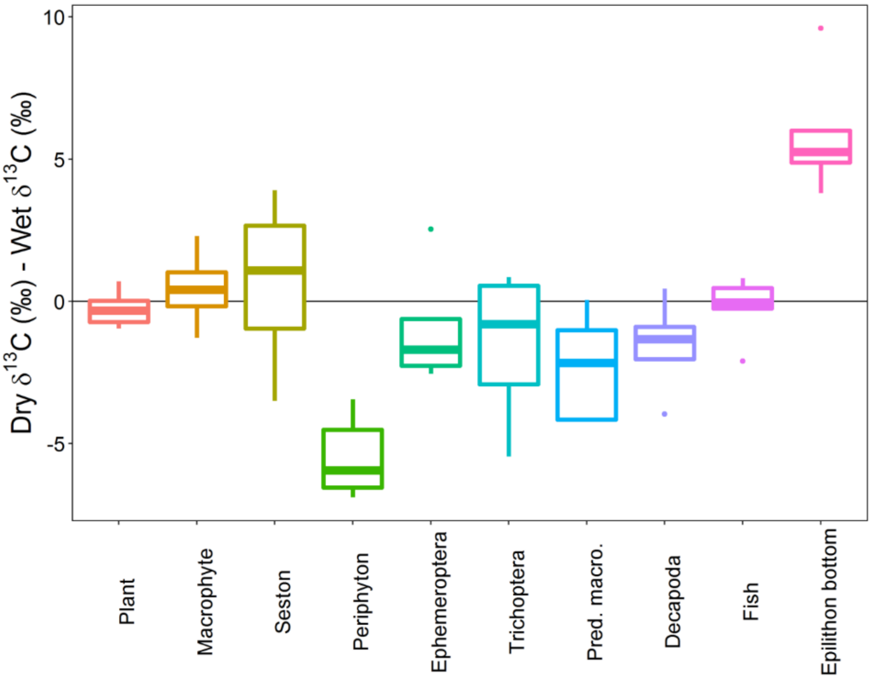

Figure 53. Boxplots of the differences in $\delta^{13}C$ values between dry and wet sampling periods for basal resources and consumers in the upper Bremer River catchment study sites. $\delta^{13}C$ values below 0 indicate a depleted $\delta^{13}C$ value in the dry season compared to the wet. Basal resources were grouped into plants, macrophytes, seston, periphyton, and epilithon bottom (sampled from underside of rocks). Consumers were grouped into Ephemeroptera, Trichoptera, predatory macroinvertebrate consumers (abbreviated to 'Pred. macro.'), Decapoda, and fish. Plant represents the combined isotope values for riparian vegetation and coarse-particulate organic matter. The $\delta^{13}C$ values of periphyton (including epilithon on the bottom of rocks) are more variable between seasons than other food web components.

Table 13. The consumer and source assessed, season, slope, R^2, p-value for testing confidence interval slopes that include 0 and 1, and the interpretation of each regression for consumer-resource coupling. All regression outputs are given for periphyton as a source, and only significant regression outputs are given for other consumer-resource relationships (i.e. fish and decapoda). Many consumers in the wetter sampling period show some reliance on periphyton as a carbon (i.e. energy) source.

Consumer	**Source**	**Season**	**Slope**	R^2	**Slope=1 *p*-value**	**Slope=0 *p*-value**	**Interpretation**
Primary consumer (Ephemeroptera)	Periphyton	Wet	0.64	0.95	0.03	0.006	Reliance on periphyton, but other sources also contribute
Primary consumer (Trichoptera)	Periphyton	Wet	0.29	0.07	NA	NA	Unresolved
Secondary consumer (Predatory macroinvertebrates)	Periphyton	Wet	0.72	0.83	0.2	0.03	Strong reliance on periphyton
Decapoda	Periphyton	Wet	0.40	0.91	0.4	0.03	Strong reliance on periphyton
Fish	Periphyton	Wet	0.54	0.69	0.1	0.08	Unresolved
Fish	Decapoda	Wet	1.4	0.82	0.4	0.03	Strong reliance on decapods
Primary consumer (Ephemeroptera)	Periphyton	Dry	0.05	0.002	NA	NA	Unresolved
Primary consumer (Trichoptera)	Periphyton	Dry	1.0	0.72	NA	NA	Unresolved
Secondary consumer (Predatory macroinvertebrates)	Periphyton	Dry	0.19	0.07	NA	NA	Unresolved
Decapoda	Periphyton	Dry	0.04	0.004	NA	NA	Unresolved
Fish	Periphyton	Dry	0.06	0.01	NA	NA	Unresolved

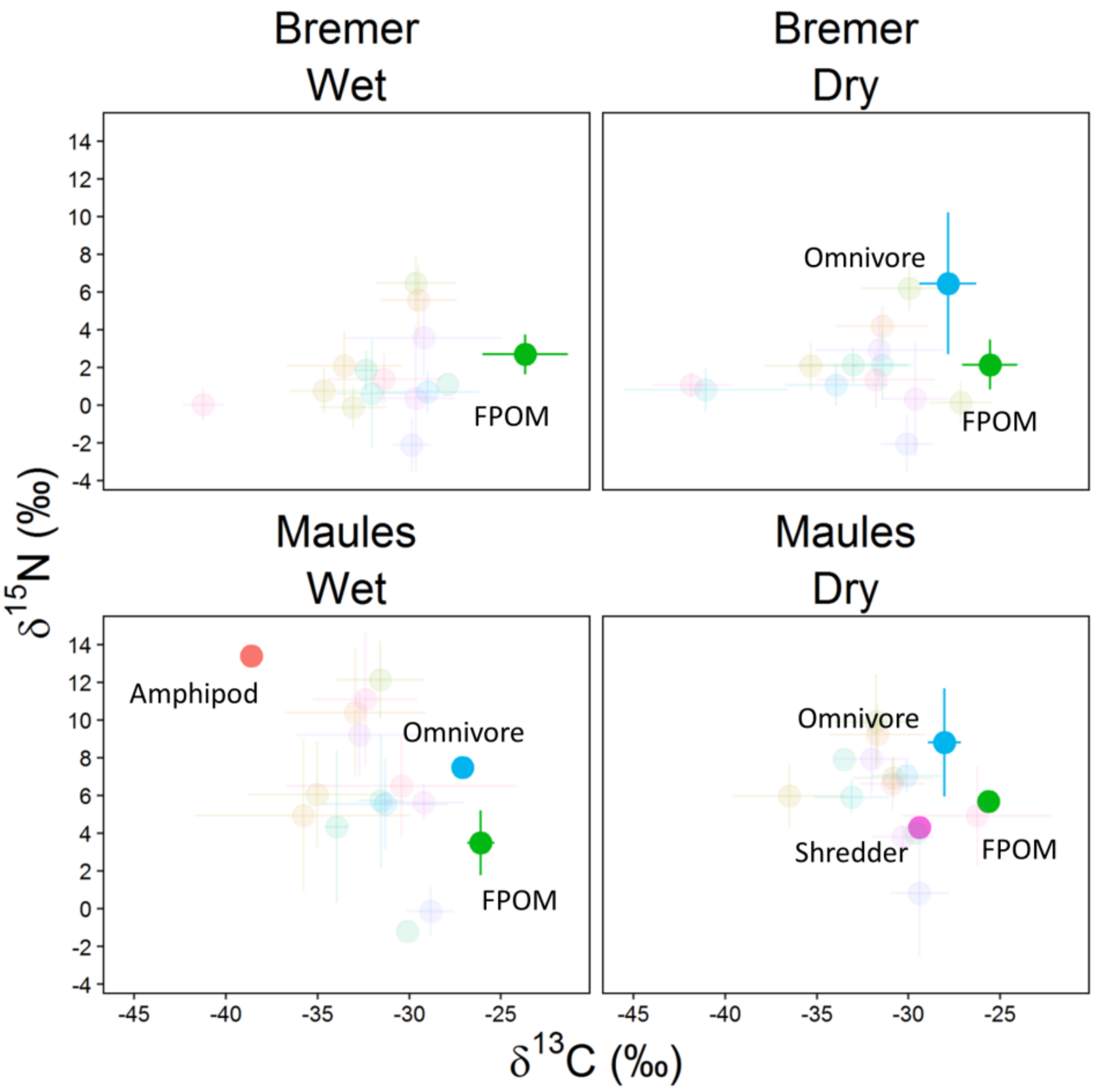

Figure 54. The mean ± standard deviation of the δ^{13}C and δ^{15}N values for sources and consumers (labelled by a-priori assigned functional feeding group) in the hyporheic zone at all sites in the upper Bremer River catchment and the Maules Creek catchment during the wet and dry sampling periods. The mean ± standard deviation of the δ^{13}C and δ^{15}N values for sources and consumers in surface habitats are also displayed in the background.

3.6.5 Discussion

Spatio-temporal patterns of stable isotope values in both study regions indicate that reductions in stream flow may affect the isotopic values and use of basal resources by consumers, as well as food-web structure in intermittent rivers. In the upper Bremer River catchment study sites, autochthonous C sources were important in the wetter sampling period, with no evidence of reliance on sampled basal resources in the drier period (see below for possible reasons for this result). Despite changes in the isotopic values of autochthonous C sources, and evidence of variation in its consumption by consumers, there were no changes in the food web structure of streams within the upper Bremer River catchment. Furthermore, there was a large variation in algal C sources among wet and dry sampling periods. At Maules Creek, the most ephemeral study site lacked higher order consumers (decapods), although it is possible that our sampling couldn't detect them. Together, these findings indicate that although flow variation can alter the isotopic values of important basal resources (algae), and possibly the consumption of these resources by consumers, the food web structure can remain unaffected. The persistence of residual wetted habitats due to baseflow is likely a major factor maintaining the food web structure through wet and dry periods.

3.6.5.1 Basal energy sources and food-web structure

A gradient approach (Rasmussen, 2010; Jardine *et al.*, 2012) comparing the primary producer source contributions to consumers revealed that, for the upper Bremer River catchment study sites, many consumers relied on autotrophy (reliance on autotrophic organisms) rather than allochthony (reliance on terrestrial organic matter). This reliance on autochthonous C was, however, evident only in the wetter sampling period, with no definitive C source during the drier sampling period. This trend contradicts previous research that indicates the dominance of allochthony during wet periods, with autotrophy becoming more important during drying as reduced terrestrial-aquatic hydrological connections transport less organic matter into recipient habitats (Reid *et al.*, 2008). However, research in sub-tropical and arid ecosystems has found that even with large terrestrial organic matter subsidies, algae can still be the major C source for aquatic food webs (Bunn *et al.*, 2003; Hadwen *et al.*, 2010). The relatively unimportant role of terrestrial organic matter as a C source for food webs aligns with the findings from organic matter processing experiments (see 'Organic matter processing' section), which found virtually no detritivore-mediated consumption of terrestrial leaf litter (eucalypt leaves). Despite this finding, we cannot rule out contributions from allochthonous C resources to food webs. The primary energy source fuelling food webs at Maules Creek could not be assessed due to the lack of replicates in each flow regime category.

The exact reasons for the inability to resolve the important basal resource in the upper Bremer River catchment study sites during the dry season remain unknown, but may be related to the seasonal starvation of consumers, selective or omnivorous feeding, large temporal variability in algal $\delta^{13}C$ values (see Hadwen *et al.*, 2010), and our inability to sample the important basal C source (see section below). Higher consumers may face periods of starvation during drier periods because their prey, such as macroinvertebrates and decapods, can avoid predation by taking refuge in the hyporheic zone during unfavourable surface conditions (Boulton, 2003; James, Dewson, & Death, 2008). Furthermore, given the decline in periphyton quality (C:N ratio) we observed in the drier sampling period, the change in their $\delta^{13}C$ values, and the large differences in $\delta^{13}C$ values of the different periphyton we sampled (i.e. epilithon underneath rocks), it is likely that primary consumers are selectively feeding on higher-quality algal sources in the drier period (Cruz-Rivera and Hay, 2000; Tall *et al.*, 2006).

Despite this potential change in dominant basal resources between seasons, there was no concurrent change in the overall food web structure in the upper Bremer River catchment study sites, with higher order consumers present in both the wet and dry sampling periods. Groundwater contributed to baseflow at these sites (Table 11), which likely sustains residual aquatic habitats (i.e. pools) for longer periods of time than in non-groundwater influenced intermittent rivers. We therefore hypothesise that groundwater has a stabilising influence on food web structure within intermittent rivers by reducing periods when the streambed is completely dry, and thus enabling higher order consumers to persist in refuge habitats. In contrast, the intermittent study site at Maules Creek (Middle Creek) can cease to flow for long periods of time (see Component 3: Results – Physical Hydrology) and residual pools can dry, as was observed during dry-season sampling. The lack of residual aquatic habitats during dry periods may likely be a major factor explaining the lack of higher order consumers at this site. Increased flow intermittency has been demonstrated to decrease food-chain length and eliminate top predators (Sabo *et al.*, 2010; McHugh *et al.*, 2015). Therefore, variation in the food web structure among sites in the upper Bremer River catchment and Maules Creek catchment offers a glimpse as to the effect of increased flow intermittency and/or reduced groundwater contributions to flow.

3.6.5.2 The $\delta^{13}C$ – depleted nature of food webs

Food webs in both regions were much more depleted in $\delta^{13}C$ than is normally expected from algal- and detrital-based food webs, which typically cluster around -30 to -28‰ for $\delta^{13}C$ (Reid *et al.*, 2008; Pettit *et al.*, 2016). We hypothesise that this is the result of substantial incorporation by algae of ^{13}C-depleted carbon dioxide (CO_2) arising from either soil microbial respiration or microbial respiration in groundwater. Highly ^{13}C-depleted values of algae (-46.3‰) in a New Zealand mountain spring were also attributed to the incorporation of ^{13}C-depleted CO_2 arising from soil community respiration (Rounick and James, 1984). The δ^{13}C of dissolved inorganic carbon (DIC) in freshwaters ranges from -26 to 0‰ and fractionation due to photosynthesis depletes δ^{13}C by 20‰ (Fry, 2006). Therefore, an unmeasured algal food source, with a δ^{13}C ranging from 40 to -38‰, is likely contributing to food webs in both regions. Water samples from surface, hyporheic, and groundwater habitats will need to be analysed for ^{13}C values of DIC and dissolved organic C in order to resolve this issue (this sampling is occurring). Regardless, the highly ^{13}C-depleted values of consumers indicate that food webs in both catchments obtain a large contribution of energy from autochthonous sources.

Aquatic food webs can also be depleted if methane-oxidising bacteria (MOB) form an important energy source for consumers (Grey, 2016). MOB can convert substantial amounts of biogenic or thermogenic methane into microbial biomass. If this forms an important energy source for consumers, the entire food web may become depleted in δ^{13}C values because methane-derived carbon can be as low as −110 to −50‰ depending on formation pathways (Grey, 2016). Research on energy reliance on methane-oxidising bacteria, however, reports δ^{13}C values for consumers less than -40‰ (Grey, 2016). Since no surface consumers in our study had values this deleted, it is unlikely that MOB are responsible for the depleted δ^{13}C food web values in this study.

3.6.5.3 Hyporheic food webs

Despite the difficulty in collecting enough specimens of hyporheic fauna for stable isotope analysis, several trends are evident among the two study regions. First, hyporheic fauna generally had more enriched δ^{13}C values than that of surface habitats, suggesting reliance on a different basal energy source. Given the lack of autotrophic activity in subsurface environments, this energy source is likely to be dissolved organic carbon (DOC), fine-particulate organic matter (FPOM), and/or coarse-particulate organic matter (CPOM) that has accumulated in the hyporheic zone, or heterotrophic microbes (bacteria) that have previously assimilated one of these carbon sources. Second, values for both omnivorous taxa and FPOM were similar among catchments and sampling periods, indicating that the hyporheic zone may represent a stable environment, at least compared to surface habitats. Lastly, the relatively δ^{13}C depleted omnivore collected in the Maules Creek catchment may suggest that despite this comparatively stable environment (relative to surface habitats), there may be spatially or temporally patchy influences of multiple energetic pathways and/or basal resources contributing to the maintenance of higher order food webs.

3.7 Fish and macroinvertebrate diversity and abundance

3.7.1 Introduction

Both fish and benthic macroinvertebrate communities can be strongly influenced by flow intermittency and they can therefore be useful indicators of hydrologic changes that cause a loss of perennial flow or relative increases in the frequency and duration of cease-to-flow spells (Bond *et al.*, 2008; Bond *et al.*, 2010; Clarke *et al.*, 2010). However, in naturally intermittent and ephemeral streams the use of fish and invertebrates as indicators of human impacts has proven challenging (Sheldon, 2005). The principal issues are that: a) the often harsh environmental conditions during the dry phase in intermittent streams can mean that the fauna is typically dominated by those same types of taxa that can also cope with the physio-chemical changes often caused by human impacts; and, b) the high temporal variability of intermittent and ephemeral streams means that defining an appropriate reference condition can be extremely difficult.

A number of studies have evaluated the ability of standard invertebrate biomonitoring approaches applied to intermittent streams, and found them to be inadequate to differentiate impaired sites (e.g. Chessman *et al.*, 2006; Chessman *et al.*, 2010). Nevertheless, the aim of this component of the current study was to compare patterns of species richness and abundance (and other indicators) based on fish and invertebrate surveys across the range of sites being sampled as part of the broader project. The objective is to provide further advice as to the relevance and/or utility of fish and invertebrate sampling to provide relevant data for assessing CMCSG impacts in intermittent streams based on traditional biomonitoring sampling approaches.

3.7.2 Methods

3.7.2.1 Fish surveys and macroinvertebrate collections

Fish surveys were conducted twice at each site in the Bremer River catchment, incorporating a dry and wet period of flow. A 100m reach was selected at each site that incorporated three main mesohabitat units: riffle, pools, and runs. We avoided sampling after high flows. Fish assemblages were intensively sampled by multiple-pass electrofishing with a Smith-Root model 12B Backpack Electrofisher (Smith-Root Incorporated, Vancouver, Canada). A complete description and evaluation of the sampling protocol can be obtained from Kennard et al. (2011). Briefly, up to 9 (depending on the extent of surface water habitat) electrofishing non-linear transects or 'shots' were conducted within each study reach. All collected fish were identified to species and counted. From the fish data, we calculated total abundance of each species, species richness, and the catch per unit effort for each species (total number of fish per electro-shot) in each survey. The average wetted width, depth, and velocity were calculated from five random point measurements within each shot. The percent mesohabitat type (e.g. riffle, run, pool), percent substrate coverage (e.g. mud, sand, fine gravel, cobble), and percent microhabitat type (e.g. aquatic macrophytes, leaf litter, emergent vegetation) were also estimated within each shot. Reach and catchment scale environmental variables (e.g. catchment area, mean annual runoff, pool water height, and catchment vegetation cover) were extracted from various sources, including geographical information systems and rainfall records, and used as predictor variables in subsequent data analyses.

Macroinvertebrate surveys were conducted twice at each site in the Bremer and Maules Creek catchments. Not all sites had surface water present during both surveys and no samples were collected from dry sites. Kick samples were collected following standard protocols consistent with those employed as part of the AusRivAS river health assessment program (Conrick and Cockayne, 2001). Briefly, a D-shaped 250μm mesh net held downstream of the operator was used to collect material disturbed from the bed using a kicking motion (kick-samples). Three replicate samples were collected from pool and riffle habitats respectively, but were combined in the field to reduce processing time in the laboratory. Samples were preserved using 70% ethanol and returned to the laboratory where animals were enumerated and identified to genera, or where this was not possible, to the lowest recognisable taxonomic unit.

3.7.2.2 Data analysis

In the case of the fish surveys, differences in assemblage structure, total richness, and total abundance among sites and sampling periods were assessed using permutational multivariate analysis of variance (PERMANOVA) using a nested design (Crossed fixed factors = Site and Season; Random factor = shot number nested within Site). Tests were performed on the resemblance matrix based on Euclidean distance (for abundance and richness) or Bray-Curtis similarity (for assemblage composition) of transformed ($ln(x+1)$) data. Pair-wise tests were undertaken to identify levels of factors contributing to any significant differences in PERMANOVAs (Anderson, 2006). Following the PERMANOVA, a permutational analysis of multivariate dispersions (PERMDISP) was conducted on the resemblance matrix to determine whether any separation between sites and seasons could be accounted for by differences in multivariate dispersions (Anderson, 2006). The differences in fish assemblage structure between sites and seasons were visualised using a non-metric multidimensional scaling (nMDS) ordination, and the fish species and environmental variables contributing to any differences in multivariate space were explored with Pearson correlation (considered influential if $r_p>0.4$). The correlation of environmental variables with fish assemblage structure, and the average dissimilarity of fish diversity and abundance between sites and sampling periods, were similar – we therefore chose to report only data for fish assemblage structure. Similar multivariate approaches (e.g. nMDS) were used to examine relationships between macroinvertebrate samples and environmental variables, but PERMANOVA was not conducted due to small sample sizes (this was a function of the decision to pool samples in the field, which itself reflected budget constraints). Univariate plots of commonly used indicators (e.g. species richness, abundance, EPT Richness and SIGNAL Score (Chessman, 2003)) were also used to provide a visual comparison of differences among sites.

3.7.3 Results

3.7.3.1 Fish assemblages

In total, 998 fish from 9 Australian native species were collected. Represented as catch per unit effort, over 2 times more fish were collected in the wet than in the dry sampling period (Figure 55). The collections were numerically dominated by two species: *Mogurnda adspersa* and *Melanotaenia duboulayi* represented over 91% of the total number of fish collected (Figure 55). The next most abundant fish was *Hypseleotris galii*, which represented 3% of the total fish collected (Figure 55).

Fish assemblage structure varied significantly among the two sampling times, but this seasonal effect was site-dependent (significant PERMANOVA interaction term, Table 14; Figure 56). Pairwise tests showed that fish assemblage structure was significantly different among sampling periods for Bremer River (t = 1.8, p = 0.04), Reynolds Creek (t = 2.4, p = 0.03), and Wild Cattle Creek (t = 4.7, p = 0.001), but not for Coulson Creek (t = 2.5, p = 0.11) and Warrill Creek (t = 1.5, p = 0.18). Despite the significance of sampling period for some sites, we cannot rule out that differences in multivariate dispersions are driving this finding ($\text{PERMDISP}_{\text{Sampling trip}}$; F = 11.8, p = 0.01). *Mogurnda adspersa* was the only species recorded at Coulson Creek, and Warrill Creek had the lowest catch per unit effort on both sampling occasions (Figure 56). The presence of relatively rare (*Craterocephalus marjoriae* and *H. galii*) and abundant species (*M. adspersa*) contributed to the average dissimilarity among sites and sampling periods (Figure 57). In particular, *M. adspersa* was responsible for much of the dissimilarity among sites, with only one recorded specimen at Warrill Creek in the second sampling period and greater abundances at Reynolds Creek, Wild Cattle Creek, and Coulson Creek (Figure 57). Sampling sites and occasions with more *M. adspersa* were also associated with greater mean annual discharge, more filamentous algae, higher pool water temperatures, lower concentrations of total and soluble reactive phosphorus, and lower coverages of fine gravel (Figure 57). Conversely, sampling sites and occasions characterised by fine-grained substrates and high phosphorus concentrations contained relatively high abundances of *C. marjoriae* and *H. galii,* and *Mel. duboulayi* was more common in deeper sites (Figure 57). The various parameters characterising flow at, or near, the time of sampling (daily and antecedent pool water height, monthly discharge, and wetted area) were not correlated with the dissimilarity in fish assemblage structure among sites and seasons (Table 14).

Total fish abundance per electro-shot did significantly vary among the two sampling times (Table 14), but pairwise tests revealed that this seasonal effect was only evident at Wild Cattle Creek (t = 9.2, p = 0.001). Furthermore, we cannot rule out that differences in multivariate dispersions among the two sampling periods are driving this finding ($\text{PERMDISP}_{\text{Sampling trip}}$; F = 6.6, p = 0.02). At Wild Cattle Creek, 35 individuals were collected in the dry season sampling and 289 individuals in the wet season sampling. Fish total richness varied among study sites but did not vary among seasons (Table 14). Differences in total abundance $\text{PERMDISP}_{\text{Sampling trip}}$; F = 4.5, p = 0.01) and richness $\text{PERMDISP}_{\text{Sampling trip}}$; F = 3.4, p = 0.02) among sites could have been due to differences in multivariate dispersions.

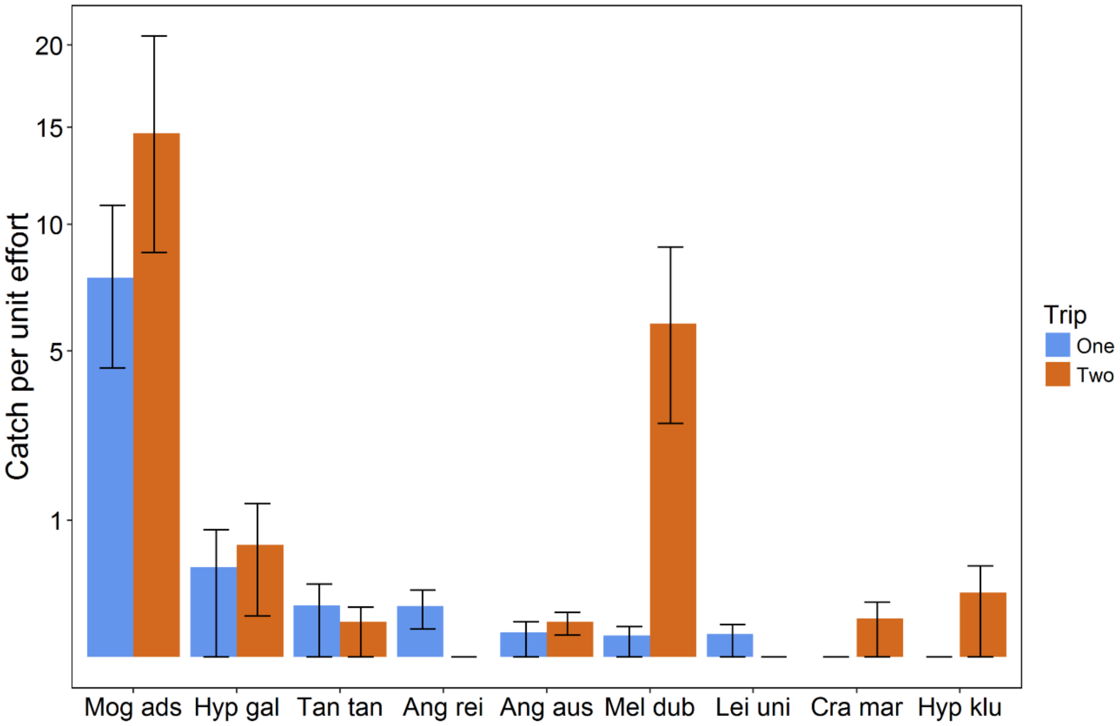

Figure 55. Fish catch per unit effort (total number of fish per electro-shot) of wetted surface habitat in the two sampling periods at sites within the upper Bremer River catchment. The y-axis has been square-root transformed. Mog ads, Mogurnda adspersa; Hyp gal, Hypseleotris galii; Tan tan, Tandanus tandanus; Ang rei, Anguilla reinhardtii; Ang aus, A. australis; Mel dub, Melanotaenia duboulayi; Lei uni, Leiopotherapon unicolor; Car mar, Craterocephalus marjoriae; Hyp klu, Hypseleotris klunzingeri. Mogurnda adspersa was the most abundant fish species.

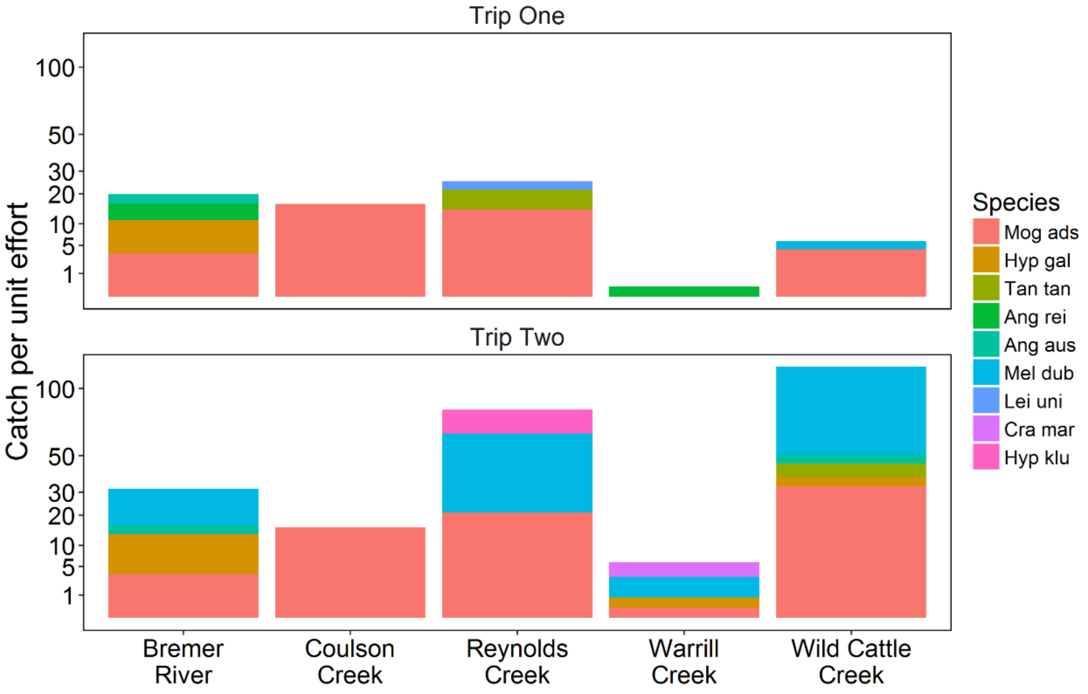

Figure 56. Fish catch per unit effort (total number of fish per electro-shot) of wetted surface habitat in the two sampling periods of each study site in the upper Bremer River catchment. The y-axis has been square-root transformed. Mog ads, Mogurnda adspersa; Hyp gal, Hypseleotris galii; Tan tan, Tandanus tandanus; Ang rei, Anguilla reinhardtii; Ang aus, A. australis; Mel dub, Melanotaenia duboulayi; Lei uni, Leiopotherapon unicolor; Car mar, Craterocephalus marjoriae; Hyp klu, Hypseleotris klunzingeri.

Table 14. Output of three permutational multivariate analysis of variance (PERMANOVA) tests performed on fish total richness, total abundance, and assemblage structure.

PERMANOVA results		**Total richness**		**Total abundance**		**Assemblage structure**	
Source	df	Pseudo-F	P(MC)	Pseudo-F	P(MC)	Pseudo-F	P(MC)
Site	4	5.4	0.002	8.0	0.001	9.0	0.001
Season	1	3.1	0.1	0.1	0.68	8.7	0.002
Shot (Site)	29	1.1	0.4	2.7	0.006	1.8	0.013
Site x Season	4	1.1	0.4	10.0	0.001	4.8	0.001
Residual	23						
Total	61						

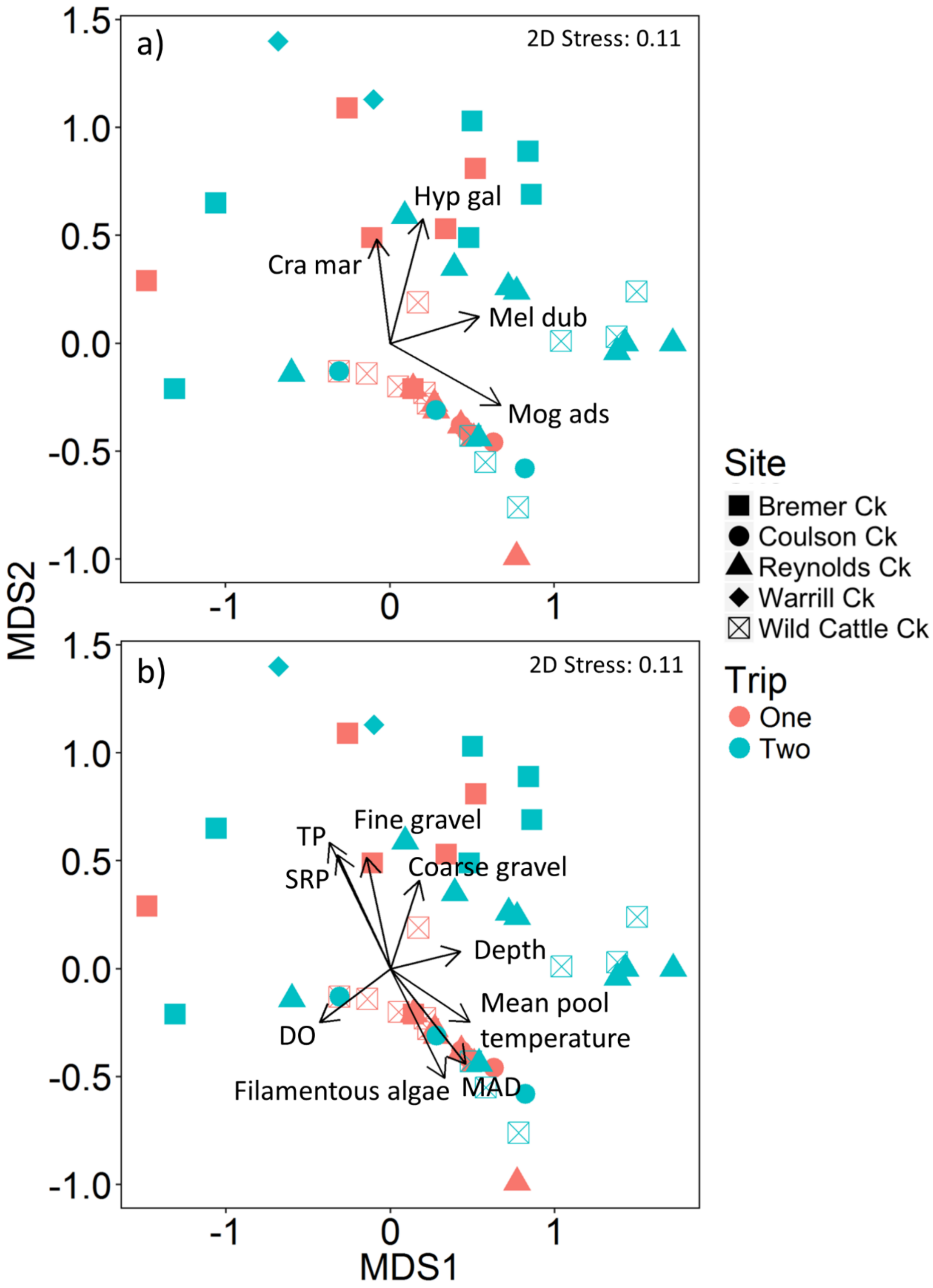

Figure 57. A non-metric multidimensional scaling (nMDS) representing the Bray Curtis similarity among fish assemblage structure of five streams in the upper Bremer River catchment and two sampling periods based on a resemblance matrix. The a) fish species and b) environmental variables that correlated (r_ρ>0.4) most to the average dissimilarity between sites and seasons in multidimensional space are overlain. MAD, mean annual discharge; DO, dissolved oxygen; SRP, soluble reactive phosphorus; TP, total phosphorus. The abundance of Mogurnda adspersa *is positively related to mean annual discharge, filamentous algae, and mean pool temperature.*

3.7.3.2 Macroinvertebrates

In total, 50,720 individual macroinvertebrates were collected from 57 distinct taxa (including 39 identifiable genera from 47 distinct families; Table 15). More taxa were collected at sites in the Bremer catchment than in the Maules Creek catchment (45 vs 36, respectively), but total abundances were similar (21,600 vs 29,120, respectively). At both sites, taxa typically associated with non-flowing habitats were common, including Chironominae, Podocopida and Cyclopoida (Table 15). A few taxa normally associated with flowing water were abundant in the upper Bremer River catchment (e.g. Baetidae, Elmidae), even during periods without surface flow (Table 15).

There were large differences between sites and trips in most of the univariate indices that were examined. In the Bremer catchment the most notable pattern between surveys was a much lower richness of invertebrates on the second sampling trip (Figure 58). Most sites had similar richness and abundance patterns with the exception of Coulson Creek and Wild Cattle Creek, which had lower numbers of taxa, including those from the Orders Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) which are known to be sensitive to water quality. SIGNAL scores ranged between 2 and 4, which if adopted as a measure of condition would indicate sites were in poor to moderate condition (SIGNAL ranges over 1-10). At the Maules Creek sites, only the perennial gaining reach (Horse Arm) could be sampled on both trips (Figure 59). Although there were differences between reaches in terms of abundance, richness patterns and SIGNAL score, the differences are difficult to interpret given the limited temporal replication and lack of spatial replication.

Multivariate analysis of the macroinvertebrate data failed to reveal any particularly strong spatial and/or temporal patterns for the upper Bremer River catchment sites (Figure 60), and there was only one variable, soluble reactive phosphorus (SRP) that was correlated with patterns in the community ordination (see bottom panel of Figure 60). Similarly, for the Maules Creek catchment sites, there were few significant correlations between the ordination space and either taxonomic or physio-chemical variation (in the latter case there were no correlated variables).

Table 15. Summary table showing the five most abundant invertebrate taxa collected on each sampling trip in the upper Bremer River and Maules Creek catchments.

Catchment	Year	Taxa	Total Abundance
Bremer River catchment	2015	Cyclopoida	2360
		Chironominae	2210
		Caenidae	1970
		Chydoridae	1710
		Leptophlebiidae	1320
	2016	Elmidae	1950
		Chironominae	1350
		Leptophlebiidae	1260
		Baetidae	960
		Cyclopoida	740
Maules Creek catchment	2015	Chironominae	9350
		Podocopida	6160
		Oligochaeta	4220
		Caenidae	2730
		Cyclopoida	1090
	2016	Chironominae	1030
		Podocopida	440
		Caenidae	160
		Cyclopoida	150
		Corixidae	100

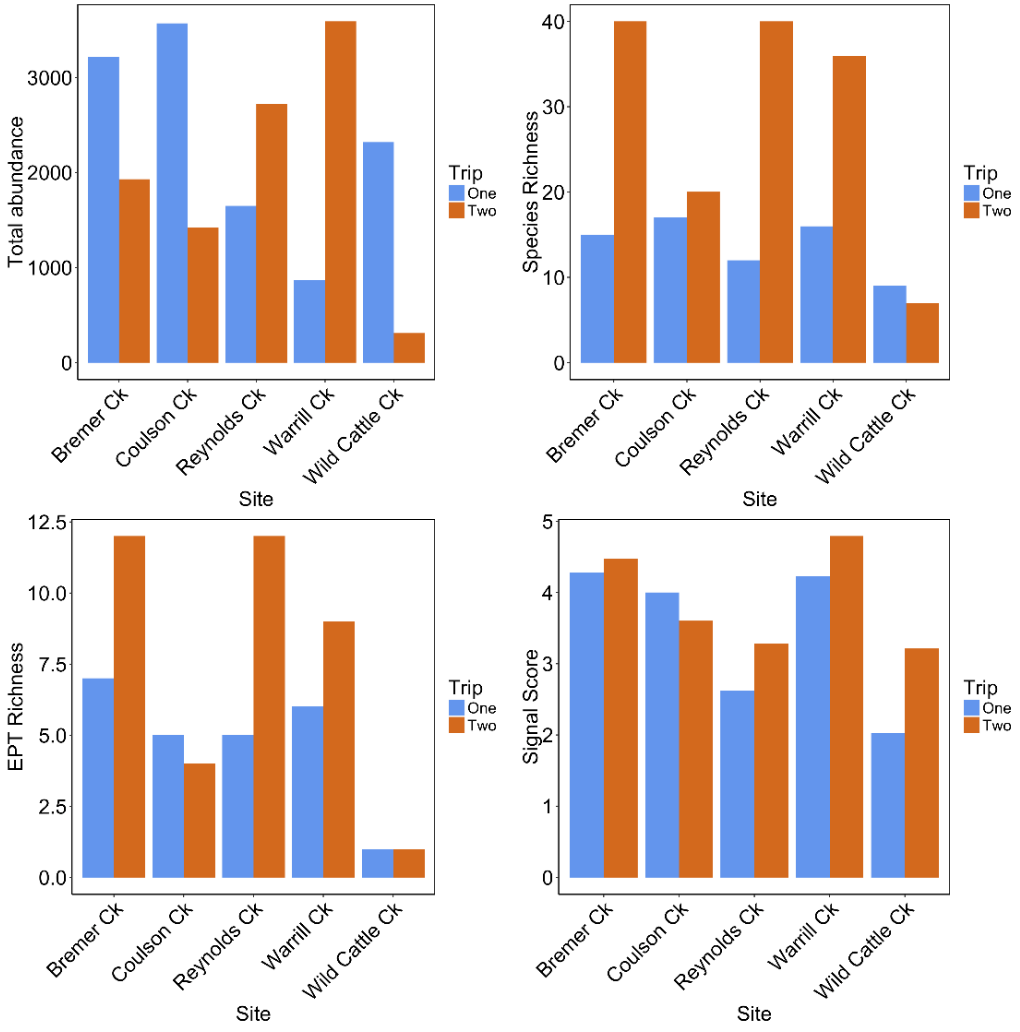

Figure 58. Plots showing macroinvertebrate assemblage characteristics at sites within the upper Bremer River catchment.

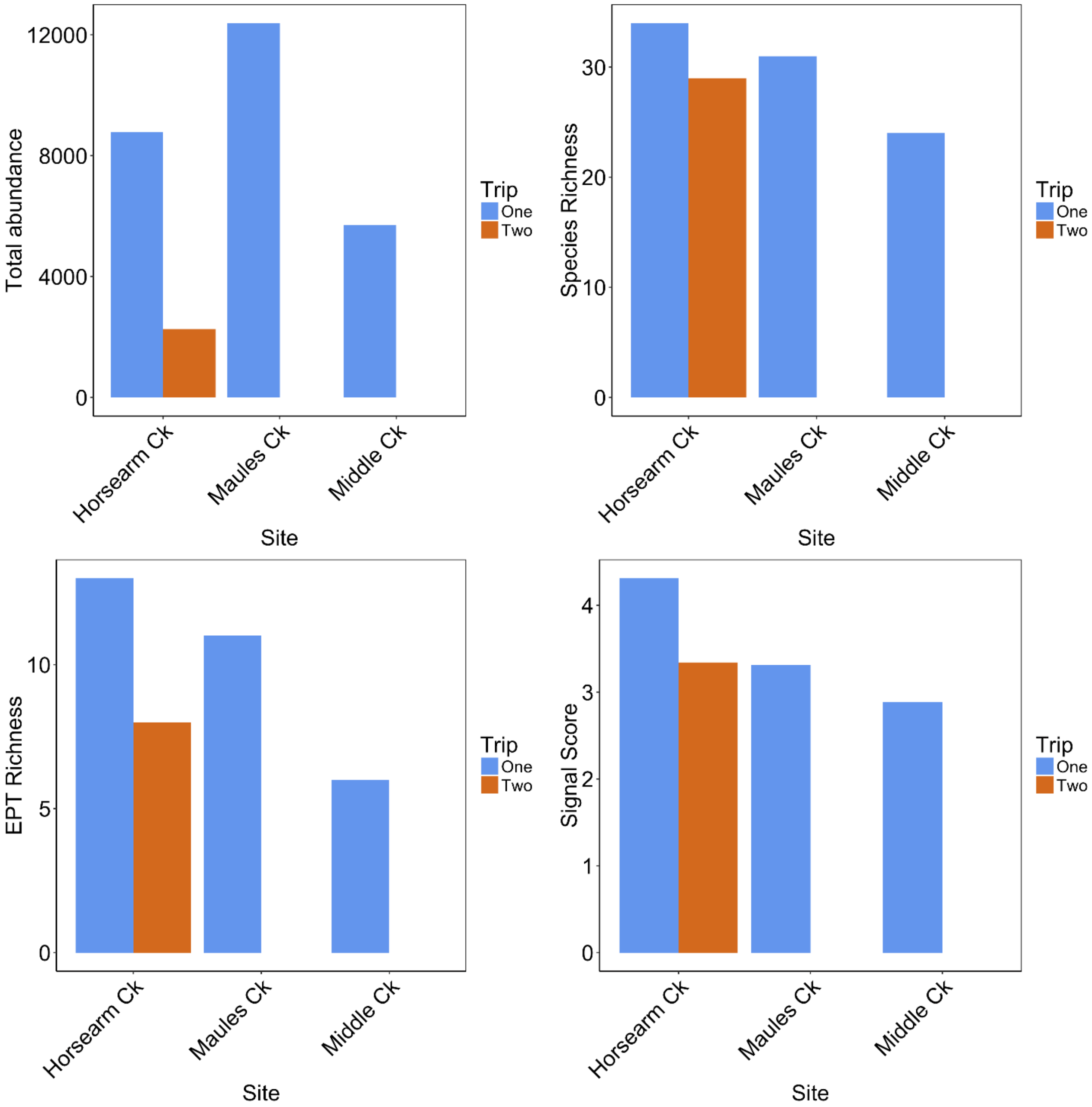

Figure 59. Plots showing macroinvertebrate assemblage characteristics at sites within the Maules Creek catchment.

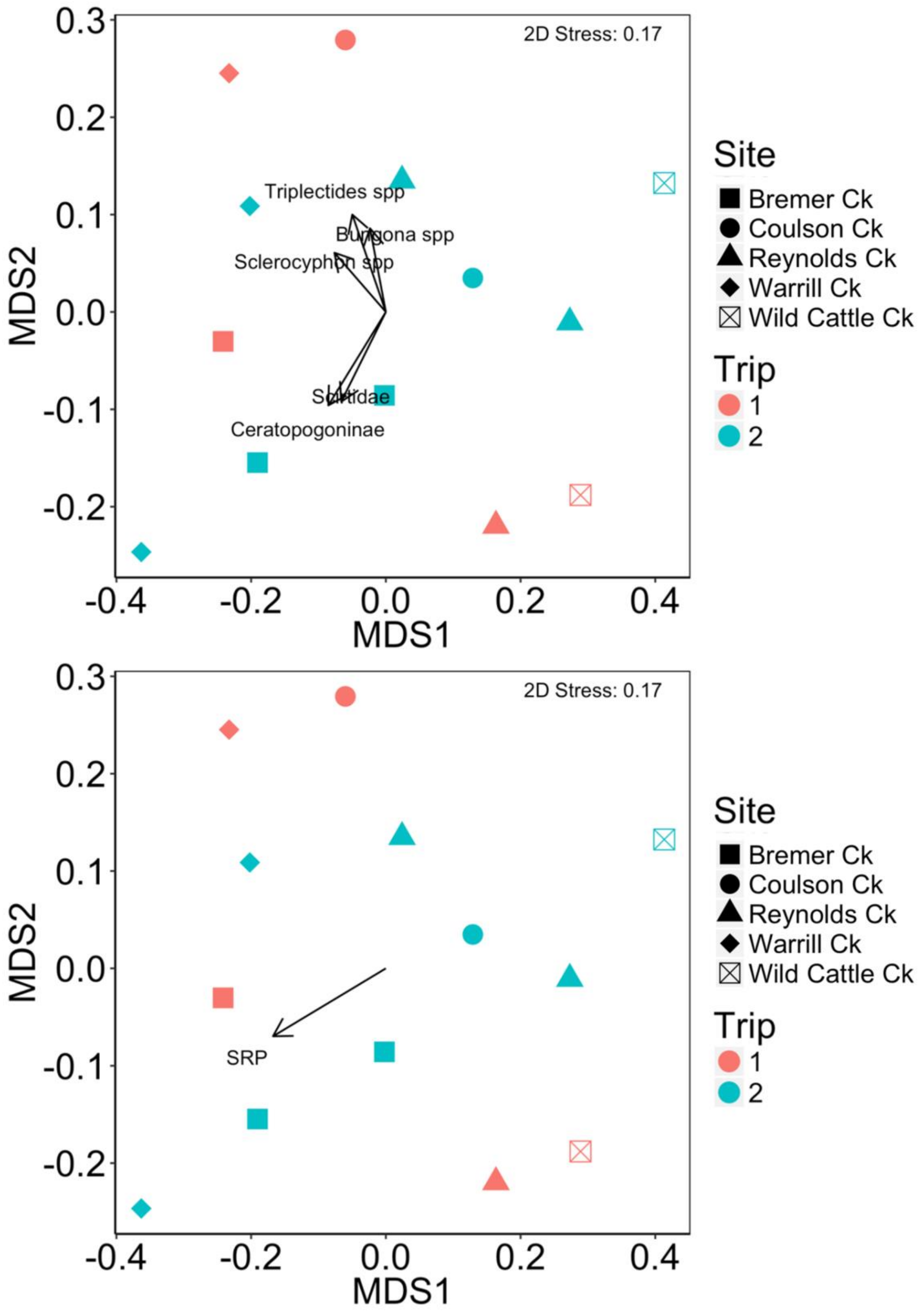

Figure 60. Plots showing results from non-metric multidimensional scaling applied to invertebrate data within the upper Bremer River catchment. The two plots show the same sites with arrows indicating taxa (top plot) and environmental variables (bottom plot) discriminating among sites. Variables with a correlation >0.3 are shown. SRP (soluble reactive phosphorus).

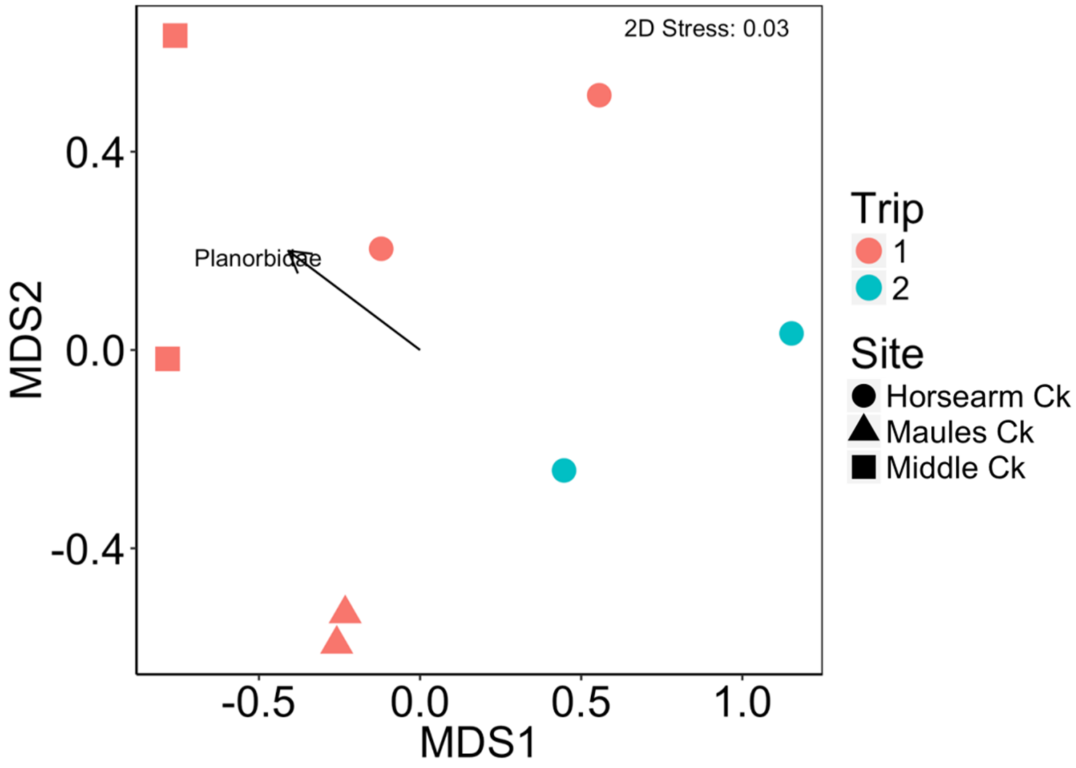

Figure 61. Plots showing results from non-metric multidimensional scaling applied to invertebrate data within the Maules Creek catchment. No physical variables showed strong discriminating power (r>0.3) so only the plot showing the single (snail) family (Planorbidae) significantly correlated with trends in ordination space is shown.

3.7.4 Discussion

3.7.4.1 Fish assemblages

Total fish species richness did not differ among the wet and dry sampling periods, and the total abundance was significantly greater in the dry sampling period at only one study site (Wild Cattle Creek). Therefore, it is difficult to support or reject the hypothesised response of changes in fish assemblage composition and abundances in response to flow variability. Fish assemblage structure was significantly different among wet and dry sampling periods in some sites, and these differences were, in part, correlated with long-term flow-regime characteristics (mean annual discharge). This variable was the only hydrological variable correlated with differences in fish assemblages among sites and sampling times. Driving this relationship was the fact that *M. adspersa* was more abundant in streams with a greater mean annual discharge. Local-scale spatial and temporal hydrological variables were not related to patterns in fish assemblages. Therefore, in our study sites, the characteristics of the long-term flow regime may be an important predictor of fish species composition and assemblage structure. While we had a small sample size (five sites in two seasons), our findings support recent ecological theory on temporal hierarchy in rivers that proposes an overarching influence of longer-term temporal patterns in flow variability on ecosystem structure and function (Biggs *et al.*, 2005).

Kennard et al. (2007) also found that long-term flow regime was an important predictor of fish assemblage structure. Those authors argue that this was because long-term flow regime governs the spatial and temporal availability and longitudinal connectivity of suitable habitat for fish species, which in turn influences local colonisation and extinction events (Magoulick and Kobza, 2003; Matthews and Marsh-Matthews, 2003). Supporting this notion is the fact that the two streams (Warrill and Coulson Creek) which had the lowest fish abundance and diversity, and for which fish assemblage structure did not change among wet and dry periods, are more intermittent than the Bremer River and Reynolds Creek sites (personal observation). Although Wild Cattle Creek is also classified as intermittent, deep residual clay-lined pools at this site (personal observation) may buffer against the negative effects associated with regular flow cessation. These deep residual clay-lined pools allowed the accumulation of filamentous algae, which was positively associated with the abundance of *M. adspersa* and mean annual runoff (Figure 57). The high intermittency at Wild Cattle Creek likely contributed to the dry-season crowding observed, with the lack of longitudinal flow connectivity hampering the ability of fish to disperse to more perennial stream reaches.

Ultimately, streams with a lower mean annual discharge will likely be more intermittent, and have low wetted habitat persistence and longitudinal connectivity, which may act as a broad-scale filter in determining fish assemblage structure. Groundwater contributions to baseflow may also play an important role in sustaining fish populations by buffering against periods of flow cessation by maintaining residual wetted habitats.

3.7.4.2 Macroinvertebrates

Overall the patterns of variation in the invertebrate assemblages were difficult to relate convincingly to spatial and/or temporal variation in hydrology or other environmental gradients. This finding clearly, in part, relates to the very limited replication. However, even with more replicates in space, experience suggests that many of the summary variables derived here would show considerable random variation associated with explanatory variables not explicitly considered in the study, or have shown values that could be (mis)-construed as being indicative of sites in poor condition. As noted by Chessman (2006; 2010) and others indicators such as SIGNAL typically show values indicative of impairment when calculated for intermittent streams. One of the more surprising findings was the relative abundance of a number of taxa such as Leptophlebiidae, which are typically associated with well oxygenated flowing habitats, at times when there was a lack of continuous surface flow. This finding likely reflects the presence of subsurface flows contributing sufficient water movement to maintain oxygen concentrations in the water column that would be less likely to occur in the complete absence of flow. Another challenge in interpreting the macroinvertebrate data was the incomplete picture of the recent flow history for each site. While sites are often classified on either the flow conditions at the time of sampling or the long-term hydrologic regime, it is conditions in the weeks and months prior to sampling that may have a very strong bearing on the presence/absence patterns of aquatic invertebrates (Bond *et al.*, 2012). One of the observations that was made during this study is that the often complex patterns of surface and subsurface flow within each site made it difficult to characterise surface hydrology even with depth loggers installed at each site. Further work is being done to try to calibrate these data to provide a clearer picture of antecedent pool depth, but this step in the analysis has proven difficult. Nonetheless, while antecedent 28-day pool depth (DEPTH) was not an influential variable in the analysis of invertebrate assemblage patterns, it was in the case of fish assemblages. Whether this is because fish are more sensitive to depth changes per se (rather than just the presence or absence of surface flow) remains to be explored further. A final comment on the invertebrate sampling is that the cost of sample collection is relatively low, but the costs of sample processing can be high. This was particularly the case for sites with large amounts of filamentous algae as the sorting time for such samples is greatly increased.

Given some of the prior observations about how invertebrates respond to hydrologic change when examined in terms of presence-absence patterns across large numbers of sites, an alternative approach to using them in assessment of impacts would be as a planning tool. For example, the patterns of presence and absence of both fish and invertebrates tend to show often clear trends in relation to the degree of flow permanence (Bond *et al.*, 2011; Bond *et al.*, 2012). Rather than seeking to test for impacts from altered hydrology, an alternative approach would be to use models built using existing large-scale datasets to set limits to hydrologic alteration, and to focus investment on monitoring and modelling efforts to better understand the likely hydrologic impacts that may arise from CMCSG extraction. Such approaches have been advocated for setting environmental flow targets for regulated rivers (e.g. Poff *et al.*, 2010) but in fact may be more appropriate for unregulated rivers subjected to broad-scale water withdrawals where the patterns of flow change may be far less complex than in regulated systems where flows can be manipulated via reservoir releases.

3.8 Conclusions and key findings

This research investigated the ecology of intermittent rivers in eastern Australia with the aim of identifying the ecological consequences of reduced baseflows. The majority of Australian rivers are intermittent (Kennard et al., 2010), and there is a pressing need to better understand the role of baseflows in supporting ecosystem patterns and processes within intermittent as well as perennial systems, especially within areas subjected to potential groundwater drawdowns.

Detrital processes were principally linked to the degree of substrate saturation at multiple scales, with drier conditions leading to reduced rates of organic matter processing in surface habitats. Detrital processing is a key ecosystem function in aquatic ecosystems because it forms the energetic base of many food webs. A reduction in baseflow will lead to a greater proportion of dry streambed, greater duration of flow intermittency, and will ultimately lower rates of particulate organic matter processing in surface habitats. Despite the negative impact on surface detrital processes, it’s important to note that organic matter processing in the hyporheic zone was largely unaffected by reductions in flow. Both leaf litter processing and cotton degradation were greater in the hyporheic zone than in corresponding wet and dry surface areas. Overall, our results highlight that intermittent river channels are important locations for detrital processes and that the hyporheic zone sustains critical ecosystem processes even when surface flow-paths cease.

Short-term and long-term reductions in flow may affect the sources of energy for food webs and the persistence of higher order consumers. Autochthonous C sources (algae) were important energy sources for aquatic consumers in wetter flow periods, with no evidence of reliance on sampled basal sources in drier periods. Given the algae require wetted habitats, extended periods of reduced flow (and cease-to-flow events) may reduce the availability of preferred energy sources for consumers, leading to less productive food webs.

The intermittent streams studied contained a diverse biotic assemblage, challenging the commonly held assumption that intermittent rivers and ephemeral streams do not contribute to local and regional aquatic biodiversity. Furthermore, macroinvertebrate taxa typically associated with well oxygenated, flowing habitats (i.e. mayflies) were present at times when surface flow had ceased. This observation likely reflects the importance of subsurface flow for maintaining oxygen concentrations in residual aquatic habitats at levels that would be less likely to occur in the complete absence of flow. Clearly, extended periods of reduced flow (and cease to flow events) will impact directly on fish and macroinvertebrates if habitats dry out completely, but the presence of refuge habitats, which may be associated with surface pools maintained by hyporheic flow, can alter the patterns that might otherwise be expected. Channel heterogeneity within individual streams will strongly influence the role that local refuges may play in sustaining waterbody-dependent biota (Davis *et al.*, 2013b), but may also add additional challenges when trying to generalise the effects of flow reductions.

Overall, our key findings are listed below:

- Intermittent river systems are important locations for detrital processes and the hyporheic zone sustains critical ecosystem processes even when surface flow-paths cease.

- Short-term and long-term reductions in flow may affect the availability and nutritional quality of important sources of energy for aquatic food webs. Given that algae require wetted habitats, extended periods of reduced flow (and cease-to-flow events) may reduce the availability of preferred energy sources for consumers, leading to less productive food webs.

- The intermittent streams studied contained a diverse biotic assemblage, and continual subsurface baseflow appears to be instrumental in sustaining these assemblages. This finding challenges the commonly held assumption that intermittent rivers and ephemeral streams do not contribute to local and regional aquatic biodiversity.

- Longer-term patterns in flow variability are an important predictor of fish species composition and assemblage structure. Streams with a lower discharge will likely be more intermittent and have low wetted habitat persistence and longitudinal connectivity, which may act as a broad-scale filter in determining fish assemblages. However, groundwater contributions to baseflow may play an important role in sustaining fish populations by buffering against periods of flow cessation by maintaining residual wetted habitats.

3.9 Evaluation of hypotheses

- Variation in stream flow regime and the degree of substrate saturation will influence organic matter processing in surface and hyporheic environments, with greater decomposition in wetter experimental periods and areas.
    - Organic matter decomposition was lower in drier surface habitats and principally related to the degree of substrate saturation. Consequently, our findings support the notion that a loss of in-stream wetted area will slow organic matter decomposition in surface habitats. However, rates of organic matter decomposition were consistently greater in the hyporheic zone than in surface habitats, and were largely unaffected by surface drying.

- Reductions in the availability and quality of autochthonous and allochthonous carbon sources in low flow periods will alter basal resource use and food web structure in surface and hyporheic environments.
    - We found evidence of altered basal resource use and isotopic values among wet and dry periods in surface environments in the upper Bremer River sites, partly supporting our hypothesis. However, we did not find any evidence that food web structure was affected in these sites. We could not identify patterns in basal resource signatures or food web structure at sites within the Maules Creek catchment.

- Variation in surface flow, and changes in longitudinal connectivity and pool persistence over time, will influence fish assemblage composition and abundances.
    - We found only limited evidence for short-term effects of flow variation on fish diversity and abundance. However, we found evidence that longer-term flow characteristics affect fish assemblage structure, partially supporting our hypothesis.

- Variation in surface flow, and associated changes in wetted-area persistence over time, will influence macroinvertebrate richness and abundances.
    - Overall, macroinvertebrate richness and abundance showed ambiguous patterns across sampling trips and sites ranging from perennial to ephemeral. We could not support or reject the hypothesis. Clearly more information regarding recent flow history (e.g. time since flows commenced or ceased) is necessary. This has important implications for longer-term monitoring programs.

3.10 References – Component 2

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4 Component 3 – Hydrogeochemistry, biogeochemical processes and the hyporheos / stygofauna (Short title: Subsurface ecology)

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4.1 Acknowledgements

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4.2 Introduction

4.2.1 Aims and purpose

The aims of research Component 3 were to understand how reductions in surface flow and groundwater baseflow affect the ecological structure, processes and function of the hyporheic zone in intermittent and perennial streams. The ecological values and processes studied were the biogeochemical processing of nutrients and organic carbon, and the diversity of hyporheic invertebrates (hyporheos) including stygofauna. We examined the effects of reduced surface and baseflow permanence by sampling in wet and dry periods and among streams of varying degrees of intermittency and perenniality. Multidisciplinary suites of methods were employed spanning hydrology, hydrochemistry, stream and groundwater ecology. A main objective was to integrate and synthesize the multidisciplinary findings into conceptual models to aid understanding of ecological responses to groundwater drawdown in streams due to coal seam gas extraction and coal mining. The work was conducted in two field locations (upper Bremer River and Maules Creek catchments).

4.2.2 Background

Hydrologic exchange flows (HEFs) are broadly defined to include the lateral and vertical exchanges of flow between the main stream channel and off-channel riparian and floodplain areas including surface and subsurface (Harvey 2016). Key defining characteristics of HEFs are the bidirectional exchange of flow that originates from and returns to the stream with no net water loss or gain to the stream. HEFs are distinct from watershed and groundwater flows that discharge only once across the surface-subsurface interface and generally lead to net losses or gains to the stream or aquifer (Figure 62). Hyporheic exchange flows are a type of subsurface HEF that occur beneath and alongside stream channels and are driven by hydraulic gradients caused by variability in the stream geometry, streambed topography and permeability (Harvey and Bencala, 1993). It may be difficult to determine whether losing or gaining stream conditions are due to localized hyporheic upwelling or downwelling, or larger scale exchanges of regional groundwater, or a combination of both.

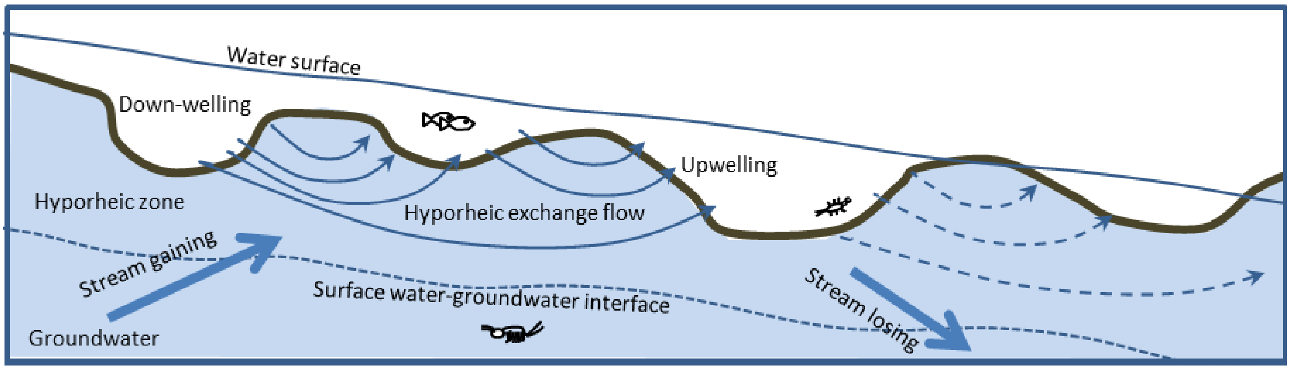


Figure 62: Schematic diagram showing hydrological relationships between streams, groundwater and hyporheic exchange flows. Hyporheic exchange flows are a type of subsurface hydrologic exchange flow (HEF) that are driven by hydraulic gradients caused by variability in the stream geometry, streambed topography and permeability. It may be difficult to determine whether losing or gaining stream conditions are due to localized hyporheic upwelling or downwelling, or larger scale exchanges of regional groundwater, or a combination of both.

A defining characteristic of hyporheic exchange flows is reduced flow velocity and increased contact time between water, sediments and organic matter (such as wood and leaves), thus facilitating many microbially mediated chemical reactions involving organic carbon, nutrients, metals and organics (Lautz and Fanelli 2008). Hyporheic zones tend to be focal areas for degradation of organic matter (Grimm and Fisher 1984: Mullholland et al. 1997) and they enhance the capacity of streams to retain and process carbon and nutrients (Boano et al. 2014). Upwelling flows tend to be enriched in nutrients that stimulate primary production by algae and aquatic macrophytes (Valett et al. 1994). Conversely, downwelling surface water supplies organic matter and dissolved oxygen (DO) to microorganisms and invertebrates (Boulton et al. 2010). Hyporheic zones provide habitat and other environmental requirements for rich assemblages of invertebrates, and in turn, their movement, feeding and excretion potentially stimulate microbial activity, enhance filtration and water quality and overall stream (and aquifer) ecosystem functioning (Fenwick et al. 2004; Boulton et al. 2008; Griebler et al. 2014). The hyporheic zone is a spatially and temporally dynamic ecotone where specialized obligate groundwater species (stygobites) interact with less-specialized species (stygophiles and stygoxenes) that occur in both surface water and groundwater (Gibert et al. 1994). The habitat may be thought of as a heterogenous three-dimensional mosaic of sediment sizes (e.g. cobbles, gravel, sand, silt) with buried patches of coarse particulate organic matter (wood and leaves) resulting in variable permeability and fluxes of water, dissolved oxygen, nutrients, DOC and fine particulate organic matter (FPOM) (Malard et al. 2002).

Hyporheic invertebrate assemblages (hyporheos) and aquifer invertebrate assemblages (stygofauna) comprise a mixture of taxa with varying ecological and evolutionary affinities to surface (epigean) and groundwater (hypogean) habitats respectively (Figure 63). Specialised stygofauna (stygophiles and stygobites) are taxa that, respectively, have an obligate, or facultative, dependence on groundwater. They are universally characterized by the possession of conservative and convergent morphological characters which are adaptive for subterranean life (termed stygomorphic characters), such as reduced pigment and eyes, and elongation of appendages. Stygoxenes are taxa that have specializations for life in surface waters, and they are typically pigmented and have functional eyes. Changes in conditions in subsurface habitats (e.g. through groundwater extraction, sedimentation) can alter the proportions of these categories, providing a potential indicator of subsurface community dynamics (Claret et al. 1999). While local and regional taxonomic differences limit comparisons of subsurface assemblages at generic or species level, classifications based on habitat affinities provide a 'common currency' that facilitates understanding of parallels and contrasts between different locations, as well as spatio-temporal changes within locations (Claret et al. 1999).

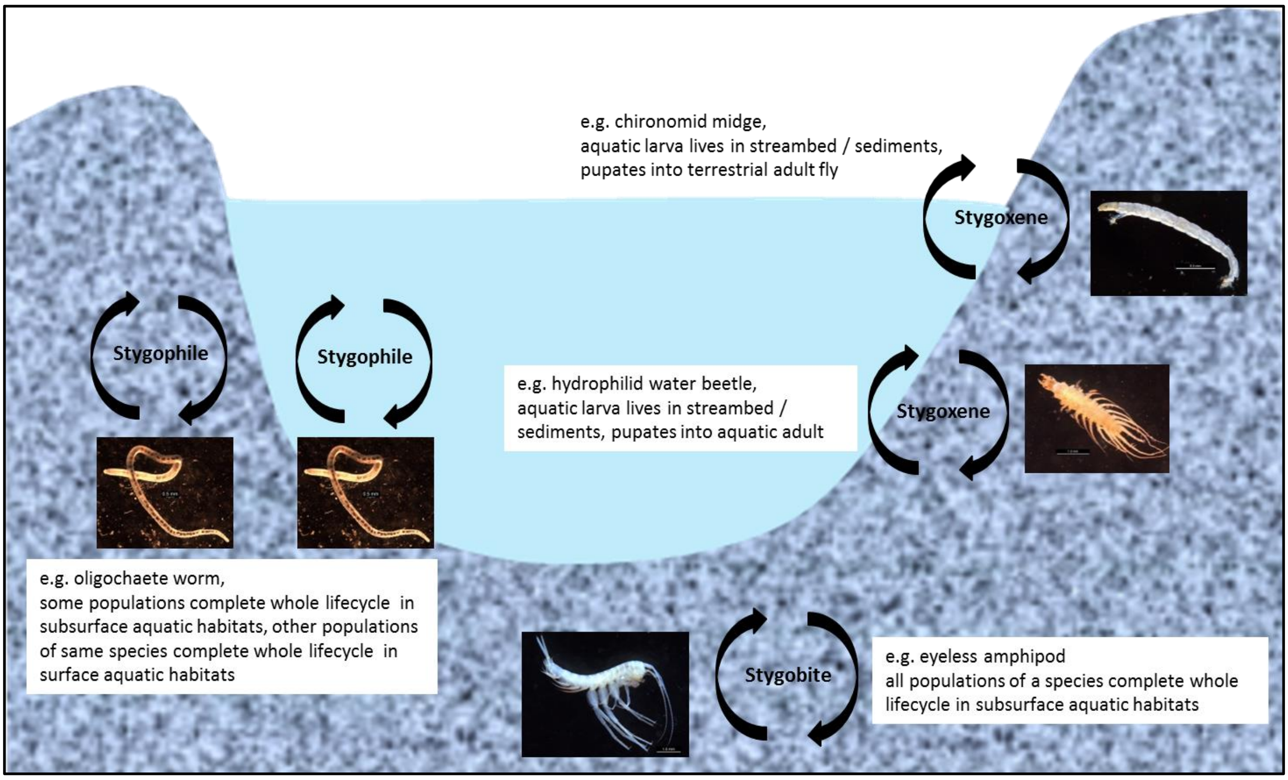


Figure 63: Classification of invertebrates found in hyporheic and aquifer habitats based on their ecological and evolutionary affinities to surface water and groundwater respectively. Specialised stygofauna (stygophiles and stygobites) are taxa that, respectively, have an obligate, or facultative, dependence on groundwater. Stygoxenes are taxa that predominantly live in surface waters and occur in groundwater incidentally or 'accidentally', although some populations or individuals may inhabit the subsurface during part of their life cycle, such as larval chironomids. Adapted and modified from Boulton et al. 2014.

While the taxonomic richness and functional diversity of hyporheic and aquifer ecosystems is globally well known (Gibert et al. 1994), and in the last two decades Australia has been revealed as a global hotspot of subterranean diversity (Humphreys 2008; Guzik et al. 2010), the functional aspects and ecosystem services contributed by this remarkable hidden biodiversity are poorly understood. Many studies have shown that a reduction in biodiversity has a negative effect on ecosystem function. 'Ecosystem function' is defined as the biological, geochemical and physical processes, and their rates, that take place or occur within an ecosystem. 'Ecosystem services' are the 'services' provided by natural (and semi-natural) ecosystems - 'ecosystem service providers' (ESPs) - that benefit, sustain and support the well-being of people. The links among biodiversity, ecosystem function, and ecosystem services in groundwater remain poorly known compared to links in surface ecosystems (Tomlinson and Boulton 2010).

While subterranean communities are characterized by many ancient lineages that have persisted over millennia and evidently have inherent resistance and resilience to environmental change, there is laboratory and field evidence that demonstrates they are sensitive and vulnerable to environmental stressors including changes in water quality (e.g. Sinton 1984) and water regime including drawdown (Eberhard 2004; Stump and Hose 2013). While stygofauna in aquifers are considered in current EIS policy and regulations in some Australian states (WA, NSW, Qld), hyporheic ecosystems have to date been ignored. In view of the intrinsic biodiversity conservation values and ecosystem services capacity of hyporheic ecosystems, there exists a strong case for improved understanding and integration of this largely 'invisible' ecological asset.

4.2.3 Hypothesised responses to lowering groundwater

The abstraction of groundwater is a global phenomenon that directly threatens groundwater ecosystems, but despite the global significance of this issue, the impact of groundwater abstraction and the lowering of groundwater tables on subsurface biota is poorly known (Stump and Hose 2013). Tomlinson and Boulton (2010) suggested that prolonged desiccation of sediments due to water table decline likely alters the balance between aerobic and anaerobic processes, changes the composition of microbial populations, and reduces the rate of anaerobic metabolism or switches it off altogether. Tomlinson and Boulton (2010) also hypothesised that disturbance of the groundwater regime such as lowering of the water table may change the rate and nature of subsurface ecological processes resulting in reduced availability of carbon, nitrogen and phosphorus which is likely to have flow-on effects for biodiversity and ecosystem services not only within the subsurface, but also in connected surface ecosystems such as rivers, riparian zones and estuaries.

Hyporheic exchange processes are important because aerobic metazoan aquatic invertebrates such as hyporheos and stygofauna require both dissolved oxygen (DO) and organic carbon (OC) for their respiration. Low OC fluxes could lead to a food-limited habitat, whereas high fluxes of reactive OC could deplete the DO content at relatively shallow depth in the streambed sediments and result in a DO-limited habitat. The balance and interplay between OC and DO is mediated by hyporheic exchange flows. Typically, an excess of labile OC over DO will lead to a sequence of reduction-oxidation (redox) reactions along hyporheic flow paths. Microbial communities performing denitrification, iron- and manganese-oxide reduction, sulphate reduction and methanogenesis will degrade OM along flow paths and cause a sequential change in water quality (Appelo and Postma, 2005). In the context of hyporheos and stygofauna these water quality changes can be described by the redox status of the pore water or groundwater. Importantly, whether the pore water is oxic (DO > 0.5 mg/L), suboxic (DO < 0.5 mg/L) or hypoxic – anoxic (DO < 0.1 mg/L), exerts a strong influence on groundwater invertebrates (Malard and Hervant 1999; Hahn 2006). We also postulate that the presence of reduced ionic species such as ferrous iron and managanese, which are among the products of the redox sequence as DO is consumed, may also influence, or at least indicative of, the habitat conditions for invertebrates. Changes in hyporheic flow paths and flow rates as a result of lowered groundwater levels may change the fluxes of DO and OM which can alter the redox status in parts of the streambed and increase or reduce the habitable zone for invertebrates. In hyporheic patches where the pore water becomes anoxic, we expect invertebrates will be absent and the patch will be dominated by anaerobic microbial activity.

Malard et al. (2002) synthesized previous work on how the arrangement, length and sediment characteristics of gravel bars affect hyporheic exchange, biogeochemical processes and invertebrate assemblages. Spatial variations in bed topography and sediment permeability result in a mosaic of patch types (e.g. gravel versus sandy patches) that differ in their hydrological exchange rate with the surface stream. Biogeochemical processes and invertebrate assemblages vary among patch types as a function of the flux of advected channel water that determines the supply of organic matter and terminal electron acceptors. Malard et al. (2002) concluded that the overall effect of surface–subsurface hydrological exchanges on nutrient cycling and biodiversity in streams depends not only on the proportion of the different patch types, but also on the frequency distribution of patch size and shape. Malard et al. (2002)

predicted that because nutrients are essentially produced or depleted at the downwelling end of hyporheic flow paths, reach-scale processing rates of nutrients should be greater in stretches with many small patches (e.g. short compact gravel bars) than in stretches with only a few large patches (e.g. large gravel bars). From our literature review we have adopted Boulton et al.'s (2014) conceptual model of the hydrochemical changes along a hyporheic flow path, and modified it to include our hypothesised responses in relation to reduced iron (Fe^{2+}) and hyporheic invertebrates (Figure 64).

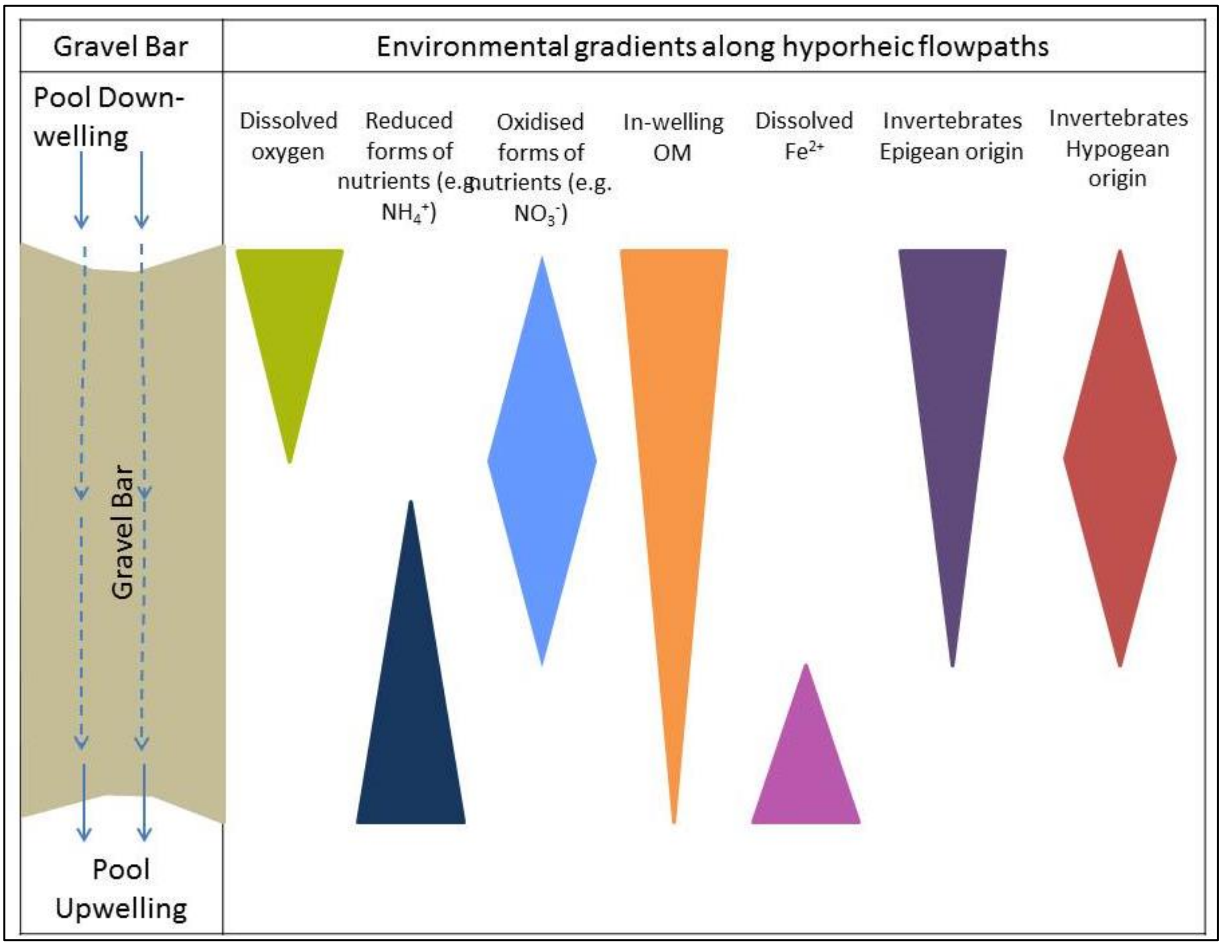


Figure 64: Conceptual schematic plan view of a gravel bar separating two pools (without surface flow between them) showing the relative gradients in biogeochemical processing that occur along the hyporheic flow path, and hypothetical responses of invertebrates assemblages in terms of the overall diversity (richness / abundance) of taxa with predominantly epigean affinities (stygoxenes) and taxa with strong hypogean habitat (groundwater) affinities (stygophiles and stygobites). Note that aerobic / anaerobic microbial processes have not been included here. Modified and expanded from Boulton et al. 2014.

Our hypotheses focus on three ecosystem responses:

1. Biogeochemical processing of nutrients and organic carbon
2. Organic matter decomposition rates
3. Diversity of hyporheic invertebrates (hyporheos) including stygofauna

Based on published literature and our preliminary conceptual models we anticipate the following responses to lowering stream and groundwater baseflow levels:

- variation in stream flow regime and the degree of substrate saturation will influence organic matter processing in surface and hyporheic environments, with greater decomposition in wetter experimental periods and areas (as per Component 2).

- variation in groundwater discharge (stream flow regime gaining or losing) and hyporheic exchange flow direction (upwelling vs. downwelling) and flow path length, will influence the biogeochemical processing of nutrients and organic carbon (OC), dissolved oxygen (DO) and redox hydrochemistry.
- variation in stream flow regime, hyporheic exchange flows, organic carbon (OC), dissolved oxygen (DO) and redox hydrochemistry will influence the richness, abundance and functional composition of hyporheic invertebrate assemblages.
- variation in stream baseflow - groundwater connectivity will influence the capacity of hyporheic ecosystems to withstand environmental changes that result in lowering groundwater levels, and also their capacity to recover when groundwater levels are restored.

4.3 Study sites

Figure 65 shows the Maules Creek catchment study sites, which are also depicted in a conceptual hydrogeological cross section aligned with the drainage of Maules, Horsearm and Middle Creeks (Figure 66), extending from the upstream intermittent site on Middle Creek via the perennial section of Horsearm Creek to the perennial losing downstream section of Maules Creek. Detailed maps of each study site are in the Appendix 3. Photographs of the Maules catchment study sites are shown in Figure 67, and Bremer catchment in Figure 68. Refer to component 2 section of this report for further details on the hydrology of the Bremer catchment study sites.

Figure 65: Maules Creek catchment study area indicating base-flow regimes in Maules Creek, Horsearm Creek and Middle Creek, and showing bore infrastructure.

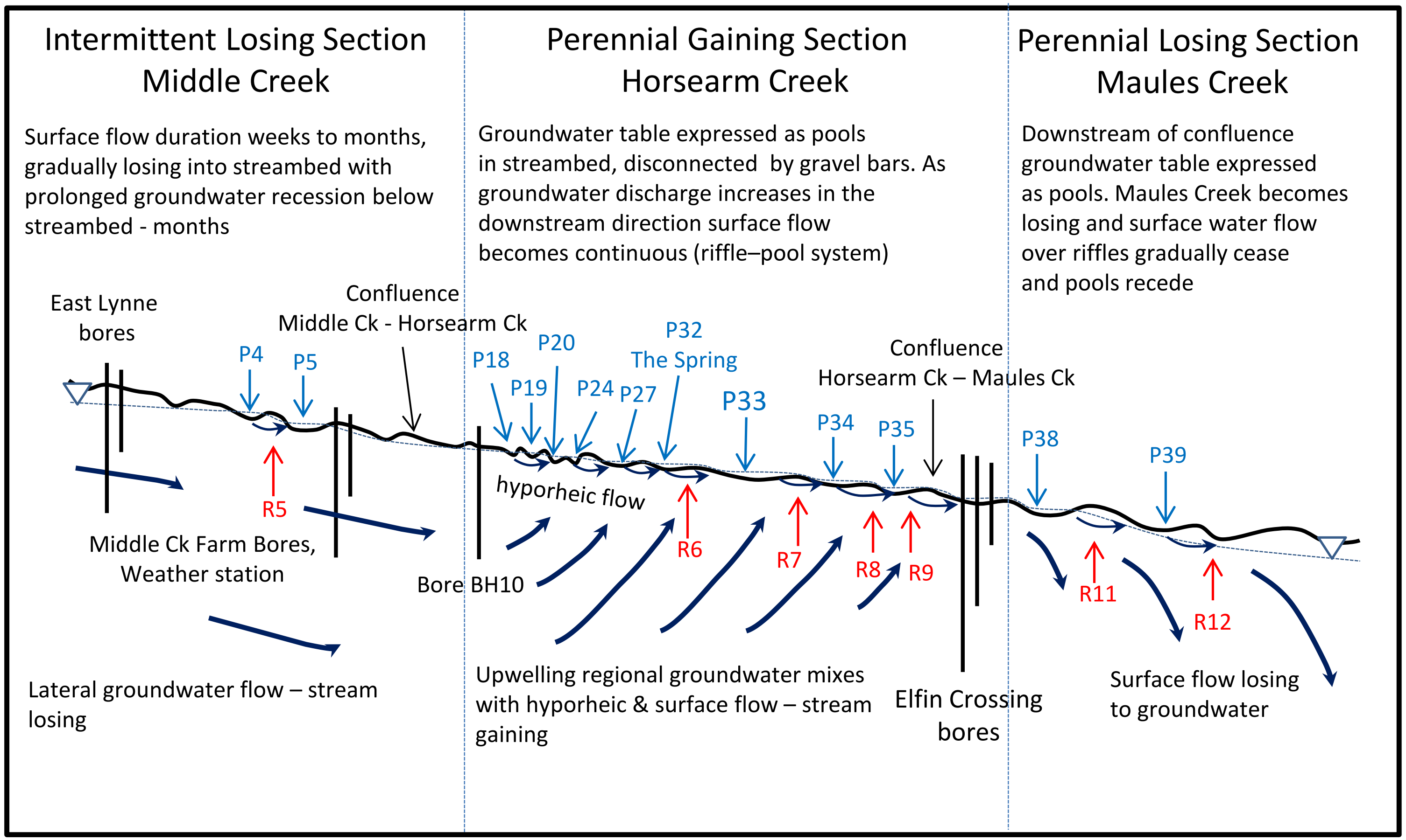


Figure 66: Conceptual longitudinal section from Middle Creek to Maules Creek showing the three hydrologic flow regimes with studied pools and riffles/bars numbered. Middle Creek is shown with losing conditions, however, it goes through 3 stages: (i) initial losing conditions during initial surface flow; (ii) a relatively long period of gaining conditions (or baseflow or return flow from bank storage); (iii) recession where the system becomes losing again. Note that not all pools and riffles are numbered and that the longitudinal distance is not to scale.

Figure 67: Maules catchment study sites: Middle Creek intermittent section, September 2015 (A), November 2015 (B), February 2016 (C); Horsearm Creek perennial gaining disconnected pools section, February 2015 (D), perennial gaining connected pools and riffle section (E); Maules Creek just upstream of Elfin Crossing (F); Maules Creek losing section, Riffle 11 July 2015 (G), October 2015 (H), Pool 39, February 2016 (I).

Figure 68: Bremer catchment study sites: Warrill Creek (A); Reynolds Creek (B); Bremer Creek (C); Coulson Creek (D); Wild Cattle Creek (E).

4.4 Methods

4.4.1 Hyporheic zone field campaigns

The main comparative sampling effort in the upper Bremer River catchment and Maules Creek catchment was undertaken in October 2015 (Round 1) and February 2016 (Round 2). These periods spanned the wet/dry seasons at Bremer, and the cool/hot seasons at Maules. The seasonal sampling campaigns were synchronised and occurred within the same two-week period at both locations. At the upper Bremer River catchment, five streams with intermittent flow regimes were sampled and at the Maules Creek catchment, three sites with intermittent losing, perennial gaining, and perennial losing regimes respectively. At Maules Creek catchment, additional sampling was undertaken at different sites and times to more fully characterise the spatial and temporal variability in each flow regime class and contrasting conditions of hyporheic exchange.

The overall survey effort at Maules Creek catchment comprised 159 samples of hyporheic fauna, most with concurrent field water quality measurements and comprehensive hydrochemical analysis (excluding the initial pilot study). These samples were spread across fourteen sub-sites (Appendix Figure 155-Figure 157) of which seven sub-sites were sampled on two occasions. The survey effort in the upper Bremer River catchment comprised 57 samples from five sub-sites, also with concurrent hydrochemistry sampling. The total field survey effort for Component 3 involved ten field trips undertaken between February 2015 and June 2016. Details of the survey effort for hyporheic invertebrates and matching hydrochemistry are available in the Appendix (Table 47 - Table 48).

4.4.2 Aquifer sampling field campaigns

In September 2015 and February 2016, fifteen existing groundwater bores were sampled at five locations alongside Middle Creek, Horsearm Creek, and Maules Creek. Most bores take in water from the superficial alluvial aquifer (hereafter referred to as the shallow aquifer) which comprises sands, silts and gravels. This aquifer is believed to be of Quaternary age. The bores are predominantly located adjacent to creeks covering all three hydrologic regimes encountered in the Maules Creek catchment from intermittent to perennial losing (Table 16, Figure 65). Three locations have multiple bores close to the creeks (5 m to 70 m). The bores have their bore- screens at different depths, and at different distances away from the creek bank, enabling a degree of horizontal and vertical stratification in sampling the aquifer. The screened interval was approximately one metre. At least two bores (BH12-4 and BH20-4) were screened in the deeper Maules Creek Formation (MCF), which is a Permian aged fractured-rock aquifer consisting of variable layers of claystone, siltstone, sandstone, conglomerate and coal. At Elfin Crossing (BH12-4) the MCF sits below a clay aquitard at approximately 25 m. At one bore (BH19-2) the age of the aquifer intersected at the screened depth of 23 m is unknown.

A qualitative estimation of the similarity between the water chemistry in bore and creek waters (sampled in May 2014 and Sept 2015) was made based on electrical conductivity (EC), alkalinity, Na and Cl. A high similarity was interpreted as high connectivity (i.e. where the creek is recharging the groundwater); however, dissimilarity does not necessarily mean a lack of connectivity (e.g. slow groundwater discharge to the creek could still have high connectivity). Aquifer permeability was qualitatively assessed during pump sampling and also from previous permeability tests undertaken by UNSW (unpublished data).

Table 16: Bore details including location, depth, aquifer, permeability, bore distance from creek, inferred aquifer-creek geochemical connectivity, creek flow regime, and sampled volumes in September 2016 and February 2016. Geochemical similarity to creek surface water (inferred connectivity) Similarity: +++ high; ++ intermediate; + low; - No similarity

Section	Bore No.	Northing (GDA 94)	Easting (GDA 94)	Mid-screen depth (m)	Aquifer	Permeability Hvorslev K (m/d)	Relative Permeability	Distance from creek (m)	Flow regime of creek adjacent to bore-hole (steady state)	Geochemical similarity to creek surface water (see Below)	Volume pumped (litres, purge + aquifer)
Middle Creek, East Lynne	BH20-2	6627763	228717.9	9.7	Shallow	0.17	Low	5	Intermittent Losing	+++	20+100 (Sept only)
	BH20-4	6627763	228717.9	40.3	Deep (MCF)	0.06	Low	5	Intermittent Losing	++	40+150
	BH21-1	6627765	228683.1	5.6	Shallow	n/a	Low	40	Intermittent Losing	n/a	0.1+0 (Feb only)
	BH21-2	6627765	228683.1	12.8	Shallow	0.15	Low	40	Intermittent Losing	+	30+150
Middle Creek Farm	BH18-2	6626170	227599.2	11.5	Shallow	n/a	Moderate-High	5	Intermittent Losing	+++	30+150
	BH18-4	6626170	227599.2	22.3	Shallow ?	7.64	High	5	Intermittent Losing	+++	30+150
	BH19-1	6626196	227555.3	9.3	Shallow	1.55	Moderate-High	60	Intermittent Losing	-	30+150
	BH19-2	6626196	227555.3	22.9	Unknown	0.97	Moderate-High	60	Intermittent Losing	-	30+150
Horsearm Creek	BH11-2	6623114	223779.2	23.5	Shallow	2.15	Moderate-High	5	Intermittent Losing	++	30+150
	BH10-2	6623459	221515.2	23.9	Shallow	2.29	Moderate-High	20	Perennial Gaining	+++	30+150
Maules Creek, Elfin Crossing (S bank)	EC21	6622616	220069	3.6	Shallow	n/a	Low	65	Perennial Gaining?	++	0+25
	BH9	6622612	220071.9	14.4	Shallow	n/a	Moderate-High	70	Perennial Gaining?	+	30+150
Maules Creek, Elfin Crossing (N bank)	BH 8	6622691	219985.4	6.5	Shallow	n/a	Moderate-High	25	Perennial Losing	+++	30+150
	BH7-2	6622696	219985	18.3	Shallow	n/a	Moderate-High	30	Perennial Losing	+++	30+150
	BH12-4	6622698	219988	40.7	Deep (MCF)	n/a	Low	30	Perennial Losing	-	40+150

4.4.3 Environmental factors and variables

The main environmental factors and variables measured and recorded are summarised in Table 17 below. To facilitate analysis and interpretation, factors and variables were grouped into ten broad types: site factors, sample factors, physical hydrology, sediments, physico-chemistry, nutrients, organics, major ions, minor ions, and stable water isotopes.

Table 17: Environmental variables, analytical methods and measurement scales.

Factor / Variable type	Factor / Variable	Type of data	Method used and scale
Site factors	Groundwater environment	Categorical	Quantitative categorical; hyporheos, aquifer
	Catchment + Subcatchment	Categorical	Available mapping
	Flow Regime	Quantitative categorical	Field observations
Sample factors	Sample Site		
	Sample Date (Trip No.)	DDMMYY, Trip No.	
	Subsamples per site date	n	
	Subsample depth (bgl)	cm (hyporheic), m (bores)	Field measurement
	Sample volume	l	Volumetric measurement
Physical hydrology	Connectivity aquifer-creek	Quantitative categorical	Geochemical data interpretation; High (3); intermediate (2); low (1); None (0)
	VHH (cm)	cm (+/-)	Field measurement
	VHH Cat.	Quantitative categorical	Histogram percentiles VHH (cm); Strongly DW (-1), near neutral (0), strongly UW (+1)
	VHH PLS Pred.	Quantitative categorical	PLS analysis
Sediments	Fine Sediments (ml)	ml	Laboratory measurement elutriated portion
	Fine Sediments Cat.	Quantitative categorical	Percentiles 33%; 1=little, 2=moderate, 3=lots
	Coarse Sediments Cat.	Quantitative categorical	Field estimation elutriated residue; 0=none, 1=little, 2=moderate, 3=lots
Physico-chemistry	Temperature	degrees C	Field loggers
	EC	µS/cm	Field measurement
	pH	pH units	Field measurement
	DO	mg/l	Field measurement
	DO threshold categories	Quantitative categorical	Data + literature
	Redox state	Quantitative categorical	Data interpretation
Nutrients	Nitrate, Ammonia	mg/l	Flow Injection Analysis
	P	µg/l	ICP-MS
	Phosphate	mg/l	Ion Chromatography
Organics	DOC	mg/l	
	Factor 1 – 'Microbial protein-like'	Intensity of fluorescent component	PARAFAC on fluorescence excitation-emission matrices (see Abbreviations and Glossary)
	Factor 2 – 'Unprocessed humic-like'	Intensity of fluorescent component	PARAFAC on fluorescence excitation-emission matrices
	Factor 3 – 'Processed humic-like'	Intensity of fluorescent component	PARAFAC on fluorescence excitation-emission matrices
	S(274-295nm)		Calculated from UV absorption spectrum

Factor / Variable type	Factor / Variable	Type of data	Method used and scale
Major Ions	Alkalinity	meq/l	Gran-method titration
	Na, Ca, Mg, K	mg/l	Inductively Coupled Plasma-Optical Emission Spectroscopy
	Cl, SO_4	mg/l	Ion Chromatography
Minor Ions	Si, Fe, Mn, Sr	mg/l	Inductively Coupled Plasma-Optical Emission Spectroscopy
	Al, Cu, Zn, Ba, Cr, Co, As	µg/l	Inductively Coupled Plasma-Mass Spectroscopy
	F, Br	mg/l	Ion Chromatography
Stable isotopes	delta 2H, delta 18O	‰	Los Gatos Isotope Analyser

4.4.4 *Physical Hydrology*

Time series of groundwater hydrographs, climate data including daily rainfall, and surface water gauging data were obtained for the entire study period for the Maules Creek catchment. Groundwater hydrographs with monitoring frequency of (15-30 min) are available from bores along Middle, Horsearm and Maules Creeks (see Figure 66 and Figure 65 for locations).

Within the stream channel auxiliary physicochemical data were obtained from eight vertical streambed temperature arrays (5 probes in each array, logging at 15 min intervals) providing time series of surface water and streambed temperature, pressure, and estimated streambed vertical flow velocities.

4.4.5 *Hydrochemistry*

Surface water samples covering the extent of the perennial gaining section of Horsearm Creek were collected during the pilot field trips. In addition, surface water samples from Pool 38 and 39 in the perennial losing section of Maules Creek were also collected.

For each fauna sampling location, hydrochemical sampling was also conducted from the temporary piezometer installed for the fauna sampling. Water was pumped (using a peristaltic pump) directly into a closed flow cell to measure dissolved oxygen (DO), pH, EC, and temperature. Water samples collected were filtered (0.45 µm) and stored at 5 °C for subsequent laboratory analysis (for list of analysed parameters and analysis method please see Table 17). For the round 2 main sampling, including the bore sampling at Maules Creek, additional samples were collected for liquid chromatography organic carbon detection (LC-OCD) that separates the organic matter into different fractions based on molecular weight.

At each sampling location, vertical hydraulic head difference (VHH) measurements were taken to allow each location to be classified as upwelling or downwelling. However, at some locations the results were ambiguous, potentially due to the temporary wells not having enough time to recover especially in low-permeability sediments. Therefore, Partial Least Squares (PLS) regression was applied to a subset of the dataset with reliable VHH measurements to determine if there was a chemical signature related to upwelling or downwelling which could be applied to the remaining dataset. The measured VHH, along with the results from PLS and hydrological conditions, were then used to classify each sample as either regional groundwater upwelling, hyporheic upwelling, or downwelling.

Using redox sensitive chemical parameters such as DO, nitrate, iron, manganese and sulfate, each sample was classified into a redox category following the method described in McMahon and Chapelle (2008) (see Table 18 for details).

Table 18: Threshold concentrations for determining redox category (from McMahon and Chapelle, 2008)

Redox Process	Water Quality Criteria (mg/L)					
	O_2	NO_3-N	Mn^{2+}	Fe^{2+}	SO_4^{2-}	Comments
Oxic						
O_2 reduction	≥0.5	-	<0.05	<0.1	-	-
Suboxic						
-	<0.5	<0.5	<0.5	<0.1	-	Further definition of redox proceses not possible
Anoxic						
NO_3^- reduction	<0.5	≥0.5	<0.05	<0.1	-	-
Mn(IV) reduction	<0.5	<0.5	≥0.05	<0.1	-	-
Fe(III)/SO_4^{2-} reduction	<0.5	<0.5	-	≥0.1	≥0.5	-
Methanogenesis	<0.5	<0.5	-	≥0.1	<0.5	-
Mixed						
-	-	-	-	-	-	Criteria for more than one redox process are met

4.4.5.1 Data Analysis

To explore the groupings present in the data and the physicochemical variables contributing to them, principal components analysis (PCA) was performed in the PLS toolbox (Eigenvector Research Incorporated). PCA was applied to the all physicochemical variables for Maules Creek and upper Bremer River catchments hyporheic samples separately. Prior to analysis the data were auto-scaled (in PLS toolbox) due to the different scales of the physicochemical variables.

4.4.6 Organic Matter (OM) Degradation Studies

Microbial activity and litter decomposition were assessed by cotton strip assays (assessing cellulose decomposition potential; CDP) and leaf litter incubations, the latter deployed in coarse mesh and fine mesh to distinguish the effect of macroinvertebrate shredders. The approach and study design were the same for components 2 and 3, except logistic constraints meant component 3 had two deployment periods for cotton strip assays whereas component 2 had four deployment periods. The design involved replicates deployed in riffle, pool and dry gravel-bar habitats, and under a range of flow regimes (intermittent, perennial gaining and perennial losing) and substrate saturation conditions (saturated, moist or dry). The first round of cotton strips and leaf packs was deployed in the Maules Creek catchment in September 2015, during a period of flow recession (see Figure 69), and the second round was deployed in March 2016, near the end of the hot season. For the organic matter incubations, three reaches were selected within the intermittent, perennial gaining and perennial losing sections of the Maules Creek catchment (Figure 65). Each reach was between 200 and 450 m in length and contained patches of riffle, pool and sub-aerially exposed dry gravel bar habitats (Appendix Table 49). Five patches within each type of habitat (15 patches per reach) were randomly selected (using a random number table). Each plot comprised a surface and a subsurface deployment, the latter buried 15 cm (cotton strip) or 30 cm (leaf packs) below the surface. Each surface or subsurface deployment comprised two replicate cotton strips attached to a ruler, one fine mesh and one coarse mesh leaf litter pack. This design resulted in a total of 90 cotton strips and 180 leaf packs per deployment period. The cotton strips were left in situ for six weeks, and the leaf packs for nine weeks, and then recovered for laboratory processing. The laboratory preparation and analysis of cotton strips and leaf litter are described in component 2.

Six temperature (subsurface) and nine temperature-light (surface) loggers were deployed with leaf packs in each section (see Appendix Table 49). Within each section, the loggers were placed to be broadly representative of the study section and habitat with respect to water depth and canopy cover (pools and riffles) and saturation (hyporheic).

To investigate the effect of substrate saturation on the microbial activity the moisture (saturated, moist or dry) at deployment and collection was recorded. The samples were then assigned to one of six sub-categories based on the various combinations observed: 1) dry-dry; 2) dry-moist 3) dry-saturated; 4) moist-moist; 5) moist-saturated and 6) saturated-saturated.

4.4.6.1 Data Analysis

To test for significant differences between the rounds a linear mixed model was created with round as a fixed effect and moisture status as a random effect, to account for the drier conditions in round 2. A linear model for each round for both the cotton strips and leaf litter packs was created to investigate the influence of moisture status, habitat, hydrological regime, depth (i.e. surface or hyporheic), and for the leaf packs size of mesh (i.e. fine or coarse). After the initial model was fitted drop1 function was used to select the most significant variables based on AIC values. All analysis was conducted in R (R version 3.3.1).

4.4.7 Invertebrate ecology

4.4.7.1 Hyporheic sampling

Comparison of hyporheic sampling results between different studies is complicated by differences in sampling protocols including, for example, replication, sample volume and taxonomic resolution (e.g. Boulton et al. 2004). To account for these variables we undertook pilot sampling at Maules Creek in February 2015 to investigate the effects of sampling volume, sample depth, and replication, on taxon capture rates. The pilot sampling was undertaken at one upwelling site (The Spring = Pool 32) and one downwelling site (The Confluence=Pool 35/Riffle 9). At each site this involved eight shallow (depth ca. -30cm) subsamples and eight deep (depth ca. -70cm) subsamples.

To examine the effect of sample volume on invertebrate capture rates in our study areas, 10 L was pumped from each piezometer; the first 6 L pumped was elutriated in a 10 L bucket, sieved through a 45 um mesh, and preserved in a labelled bottle (designated the “A” subsample). The next 4 L was then collected in the same manner and preserved separately (designated the “B” subsample). The taxon accumulation rates were then compared between the A sample (6 L pumped volume) and the A + B samples combined (10 L pumped volume) to assess any differences in efficiency of taxon recovery between the pumped volumes. After collection of the fauna samples, field physico-chemistry (temperature, pH, EC, DO) was collected from each piezometer by inserting a small PVC tube to the base of the piezometer and sucking out a water sample with a large syringe, taking care to avoid aeration of the sample. Vertical hydraulic head difference (VHH) at the sample depth was estimated (to +/- 0.5cm) using a clear plexiglass tube graduated in one centimetre intervals which was inserted to the base of the piezometer and then the piezometer was carefully removed and the difference in water levels between the surface and inside the tube were measured. Later in the study the hydraulic head difference was measured using a small electronic dip meter graduated in 1mm increments. At each site on this occasion a single “representative” water sample was collected from one shallow and one deep piezometer, along with a surface water sample, for laboratory analysis of major ions and other physicochemical parameters.

From the pilot studies, it was determined that subsequent sampling would be standardised to a 6 L volume per piezometer subsample with six piezometer subsamples (where possible, three shallow and three deep) per site. This arrangement was considered an acceptable trade-off between the desired aims of achieving statistical rigour with respect to in-site replication (time-consuming) and enabling adequate coverage of multiple sites (and times) across differing flow regimes (intermittent, perennial, losing, gaining) and directions of hyporheic exchange (upwelling, downwelling, neutral). Coupled field physico-chemistry and laboratory hydrochemistry samples were also collected from every piezometer immediately after collection of the fauna sample. Field physico-chemistry (pH, DO and EC) was measured in an inline flow cell (closed to the atmosphere) to ensure accuracy of readings, especially for DO.

4.4.7.2 Aquifer sampling

An inertia pump (Waterra Ltd.) was used to extract 180 to 190L of bore water, which was filtered through a 63 µm mesh sieve to collect invertebrates, following the methods of Boulton and Hancock (2009). Samples were preserved in 100% ethanol, stained with rose bengal, and processed later in the laboratory. The first 30 to 40L of bore water, representing the estimated bore volume to purge (allowing for water column depth and bore diameter), was preserved separately. The next 150 to 160L of pumped water represented the post-purge aquifer sample. Both purge and post-purge samples were sorted separately to evaluate the differences between bore and aquifer capture rates, and the data pooled for subsequent comparisons among sites. A few bores had smaller pumped volumes owing to smaller bore volumes, or difficulty in pumping. After sampling for stygofauna, the bore water was sampled for physico-chemistry (see Table 17). Bore depth and standing water level were measured with a dipper.

4.4.7.3 Invertebrate identification and functional group classification

A study of the effect of taxonomic resolution on assessment of hyporheic assemblage composition in the Rhone River, France (Boulton et al. 2004) found that identification to order instead of species level did not significantly change the general ordination patterns among sites. In our study taxa were identified to the level of order, family and provisional morpho-species or morpho-taxon, using published keys and available literature. Because many groundwater taxa are poorly described and exhibit conservative, convergent morphologies, identification to species level was not possible in many groups without specialist taxonomic expertise. The taxonomic resolution achieved in this study varied for different taxonomic groups and the true species-level richness was likely underestimated (Appendix 3: Figure 158 A,B,C, Figure 159 A,B,C). This unevenness in taxonomic resolution needs to be taken into consideration when interpreting the results.

Each morpho-species/taxon was assigned to one (or a combination where uncertain) of the conventional ecological categories used for classifying subterranean (hypogean) fauna based on their known or inferred ecological-evolutionary affinity with groundwater: stygobite (Sb), stygophile (Sp) or stygoxene (Sx) (sensu Gibert et al. 1994). A fourth major category was epigean taxa (Ep) (see Glossary for definitions). For some of the later analyses these categories were pooled into two main groups defined by their groundwater habitat affinity: (1) those taxa with an obligate or facultative dependence on groundwater (stygobites and stygophiles), and; (2) those taxa without such high dependence on groundwater (stygoxenes and epigean).

Ecological status remains uncertain for many species collected in this survey, and determining this status would require detailed morphological examination by taxonomic specialists and additional field studies beyond the scope of this project. For taxa of uncertain status, a conservative and mixed classification was adopted, e.g. Sp/Sb? for taxa that have a high probability of being stygophiles, and might actually be stygobites due to their regular occurrence in hyporheic samples, and/or their possession of apparent stygomorphies (e.g. depigmentation). Cyclopoid copepods are an example of a taxon that was assigned to this mixed classification. Additional ecological categories applied to the taxa collected in this study included predominantly epigean (Ep) forms such as Cladocera and various groups of Insecta (e.g. Odonata, Diptera: Simulidae) and parasites (P) including Branchiura and Nematomorpha. Another category applied was - Accidental / Terrestrial (Ac) – which were taxa known or suspected of being terrestrial in origin and captured as incidental ‘by-catch’ during sampling. Typical by-catch comprised Collembola and some Acarina. Finally, some taxa were classified as “unknown” ecological status, usually due to very poor specimen condition (damaged) and low taxonomic resolution, e.g. Arthropoda indeterminate. Terrestrial by-catch and poorly identified higher level taxa of unknown status were excluded from analysis.

4.4.7.4 Data analysis

Data were initially entered, manipulated and plotted in Microsoft Excel. Additional univariate data plots and ANOVA were performed in the R statistical software package. Because the number of sample dates varied among sites, as did the replication within them, it was decided to average the values for species richness and total abundance within each combination of date and site for the univariate analyses, and use these averaged values for subsequent analyses of variance. For the analyses of hyporheic exchange the design was regarded as a 2-way fixed factorial with hydraulic gradient (3 levels: “Strongly Downwelling”, “Neutral” and “Strongly Upwelling”) crossed with depth (2 levels: “Shallow” and “Deep”). Assignment of hydraulic gradient levels was based on field measurements of vertical hydraulic head (cm): “Strongly Downwelling” (VHH < -1.0cm), “Strongly upwelling” (VHH > 1.0cm), and “Neutral”, which encompassed neutral and weakly upwelling/downwelling sites ($-0.9 <$ VHH $< +0.9$cm). A priori treatment contrasts using “Strongly Downwelling” as the reference condition were used to assess differences between levels of hydraulic gradient. For the flow regime analyses, a priori treatment contrasts used ‘Intermittent’ as the baseline. Diagnostics showed no transformation was required for species richness, but abundance was $\log_{10}(y+1)$ transformed to avoid heteroscedasticity. Associations between univariate biotic indices (richness and abundance) and hydrochemistry variables were examined with Kendall’s tau correlations (considered influential if Rho > 0.3).

Multivariate analyses were conducted using EPrimer v7 + PERMANOVA. In EPrimer v7, fauna assemblage data were examined using the shade plots routine to evaluate the effect of dispersion weighting and transformations (square root, fourth root, log(x+1), presence-absence). Taxon abundance data were square root transformed because the resultant shade plots appeared to adequately account for the highly clumped dispersion of a few taxa. Resemblances were calculated using the Bray-Curtis similarity with a dummy variable with a value of 1 added to all samples to facilitate inclusion of otherwise empty (0 abundance) samples. Quantitative environmental variables were examined for skewness and correlation using the histograms and draftsman plot routines in EPrimer. Right skewed variables were transformed by square root or fourth root as required to approximate normality. Left skewed variables were transformed by log (+Max –V). No variables were strongly correlated ($r > 0.95$) and so none were removed from the dataset. All quantitative variables were normalised (after transformation) prior to multivariate analysis. Environmental resemblances were calculated based on Euclidean distances.

Associations between multivariate fauna assemblages and environmental variables (transformed, normalised) were examined with Pearson correlations (considered influential if $r_p > 0.4$) in EPrimer. Sampling adequacy between study areas, and between groundwater habitat affinity groups (stygobites/stygophiles and stygoxenes/epigean taxa), was assessed with taxon accumulation curves, and sampling completeness was evaluated using the standard estimators (e.g. Colwell 1984) available in EPrimer v7 (Appendix 2: Figure 158 Figure 159). Differences in assemblage structure among sites, sample dates, sample depth, flow regime, DO and hyporheic exchange expressed as vertical hydraulic head (VHH, cm) were assessed using PERMANOVA using a nested design (crossed fixed factors = regime and depth; nested factors = site nested within regime and date nested within site; continuous co-variables = DO and VHH).

4.5 Results

4.5.1 Physical Hydrology

A representative groundwater hydrograph near the streams (< 50 m) at each of three regions within the Maules Creek catchment is shown in Figure 69 (with times for our sampling campaigns marked). The hydrograph from borehole BH18-4 at Middle Creek Farm (Figure 69-a) shows a receding groundwater table until around the 16th of June when a flow event in Middle Creek recharged the aquifer and raised the water table by about 3 m. The groundwater hydrograph at BH10-2 (Figure 69-b) on Horsearm Creek, further downstream in the system (about 500 m upstream from the Perennial gaining section) shows the same pattern but much subdued (less than 1 m rise) and lagged in time by about six months. The flow event in June 2015 did not reach as far as BH10 as surface water flow. The groundwater hydrograph at BH7 on Maules Creek at Elfin Crossing shows an overall decline in the groundwater level (approximately one metre over the course of this study); however, the stream level remains constant over the entire study period.

Temporal variation in subsurface (from logger 1m below benthic surface) water temperature and water level at various locations within the Maules Creek catchment is shown in Figure 70. Over the course of this study the temperature remained stable at the ‘Spring’ site with minor seasonal changes but no diel variations. The lack of diel temperature variations indicates strong upward flow of constant-temperature groundwater flushing out the temperature variations (Rau et al., 2010). Due to the lack of daily variations, the water flux cannot be quantified using the temperature data. At the other sites, there were more pronounced seasonal variations with warming towards summer and cooling in the winter months. At the ‘Confluence’ site, the streambed temperatures show strong diel variations, even at 1 m below the streambed. These indicate strong downward flow (Rau et al. 2010). The ‘Spring’ site and the downstream sites, Pool 34 and the ‘Confluence’ had a stable water level over the course of the study relative to the intermittent and losing stream reaches. The other sites further downstream in the perennial losing section showed an overall decrease in the water level until June 2016 when the water level started to increase.

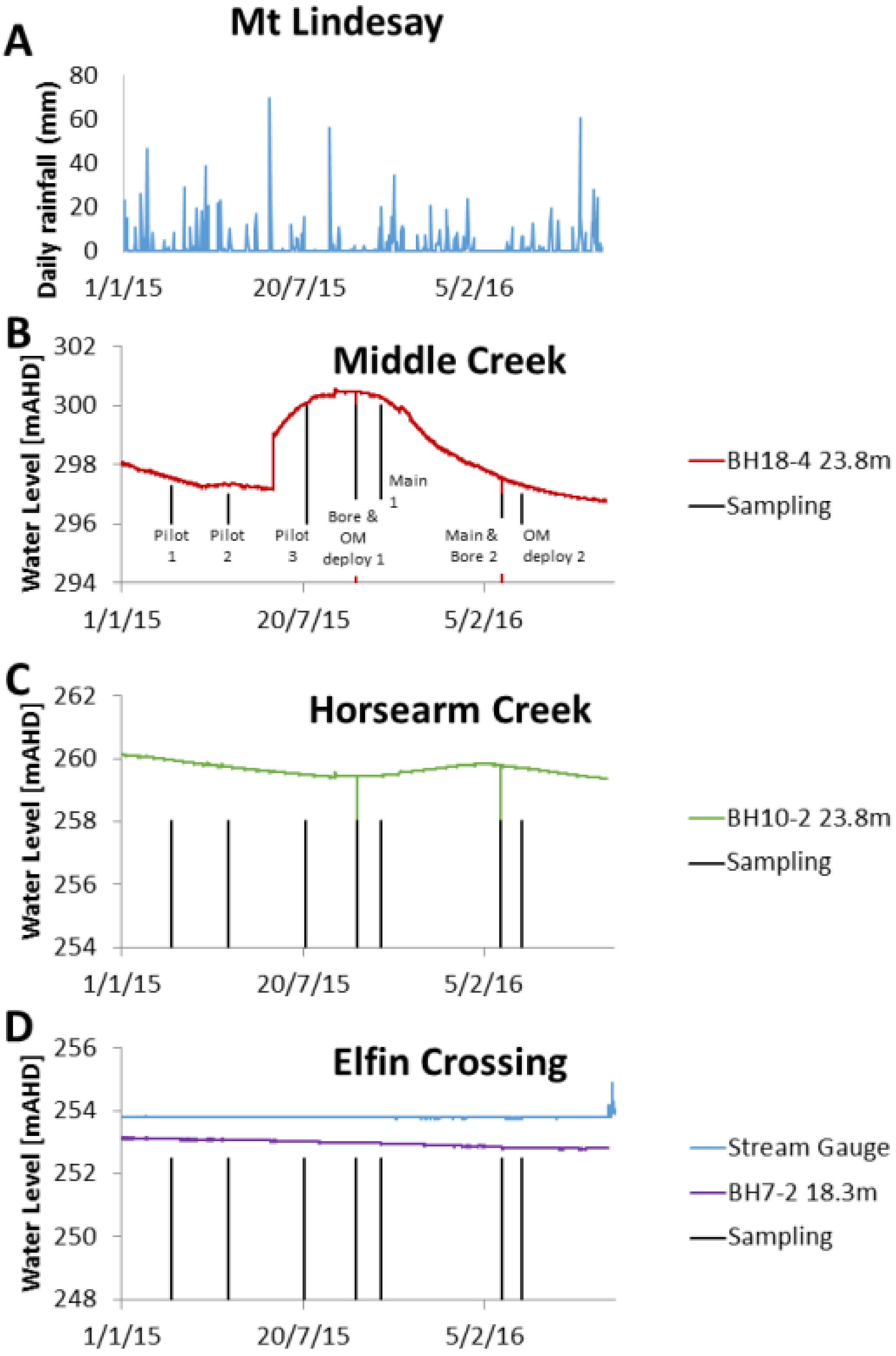


Figure 69: Rainfall and groundwater hydrographs for the study period. a) Daily rainfall from Mt Lindesay in the Nandewar range near Mt Kaputar at the top of the Maules Creek catchment, b) Borehole BH18-4 Middle Creek Farm, c) Borehole BH10-2 on Horsearm Creek (upstream of perennial gaining section) and d) Borehole BH7-2 on Maules Creek at Elfin Crossing (perennial losing section).

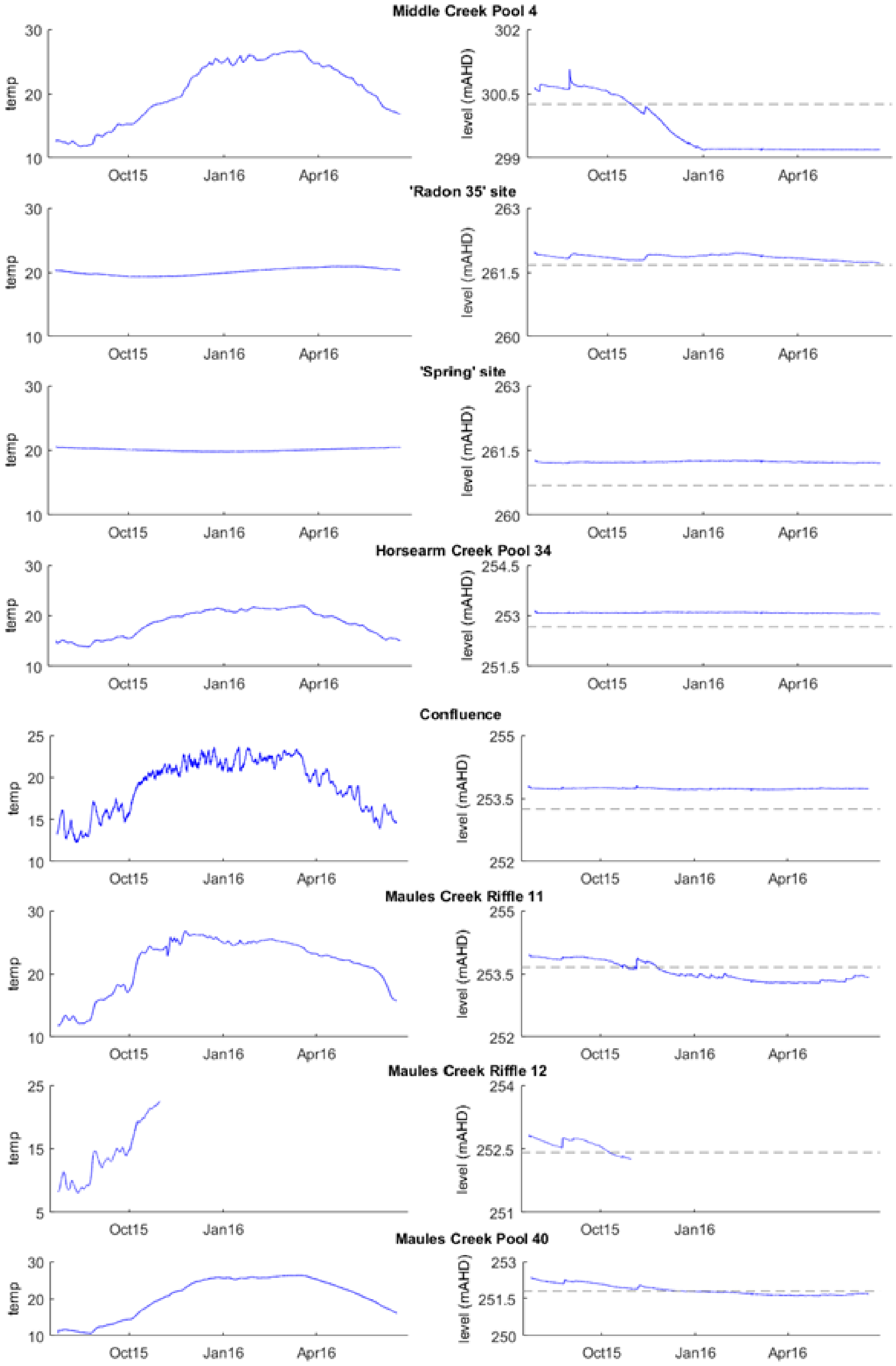


Figure 70: Temporal variation in water temperature (° C) and water level data (m AHD – meters above Australian Height Datum) from a logger deployed 1 m below the streambed for various sites in the Maules Creek catchment. The streambed level is shown as a grey dashed line.

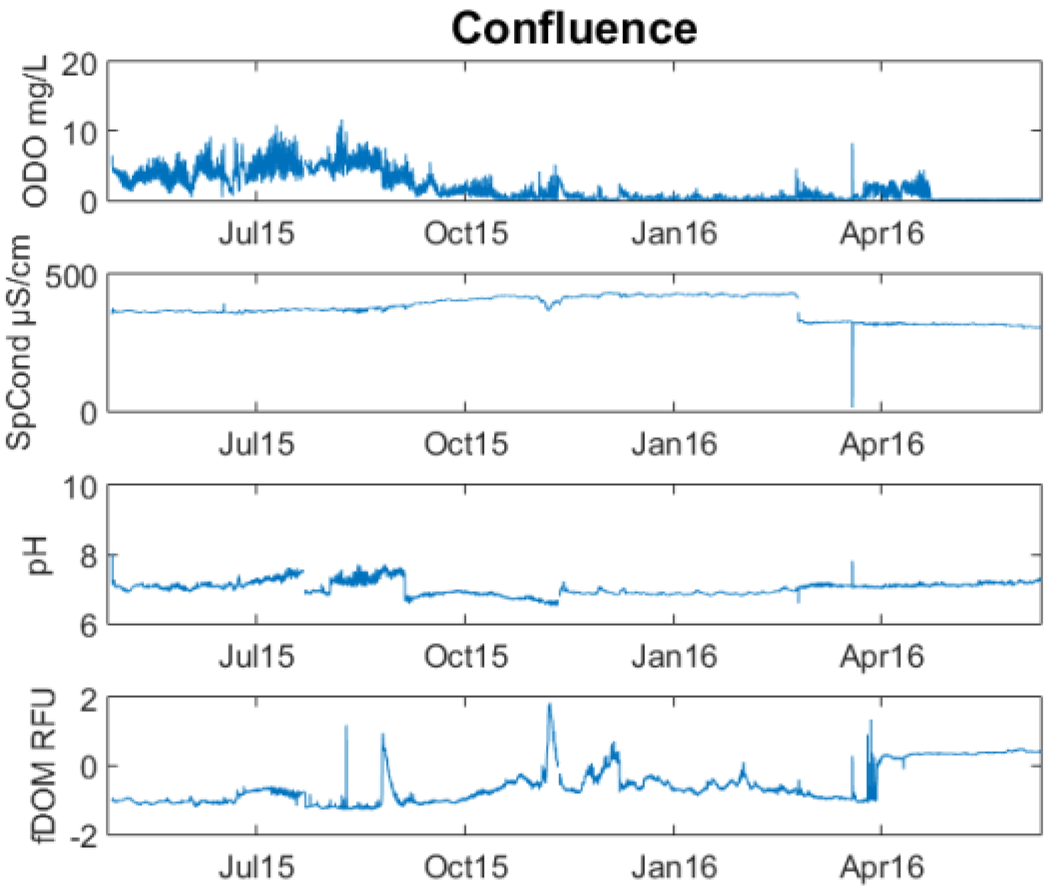


Figure 71: EX02 probe water quality data from the confluence of Horsearm and Maules Creek. Optical dissolved oxygen, ODO; specific conductivity, SpCond; fluorescent dissolved organic matter, fDOM.

Data from the EXO2 probe deployed at the confluence site is shown in Figure 71. DO is the highest from start of monitoring until October 2015, and becomes anoxic in April 2016, which corresponds with an increase in the fDOM. However, overall the measured fluorescence values of fDOM correspond with low values of DOC (< 1mg/L).

4.5.2 Hydrochemistry

4.5.2.1 Maules Creek catchment

The major ion chemistry of hyporheic water, groundwater, and selected surface water samples from the Maules Creek catchment is summarised in the Piper diagram in Figure 72. The surface water and hyporheic samples in the catchment are similar and evolve from a Ca-Mg-HCO_3 water type (with a high proportion of Na ~35%) in the upper catchment at Middle Creek towards a mixed Ca-Mg-Cl-HCO_3 water type in Horsearm and Maules creeks. This change in water type is also associated with a general increase in dissolved solids (here expressed as the EC) along a hydrological continuum, with EC of 86-138 µS/cm at Middle Creek; 315-490 µS/cm at Horsearm Creek and 364-488 µS/cm at Maules Creek. The groundwater samples in the upper catchment along Middle and Horsearm creeks are more variable in their major ion composition and many samples have a distinct Na-HCO_3 composition (with 35-65% Na). The EC of the groundwater varied from 96 to 648 µS/cm, with values increasing with lateral distance from the channel and with depth. Shallow groundwater samples close to the creeks are similar to the surface and hyporheic water samples. At Maules Creek the groundwater composition was also comparable to the surface and hyporheic water samples, with the notable difference of three deep (~40 m) samples from a confined aquifer, which has a Na-HCO_3 composition similar to the groundwater samples in the upper catchment. The Na-HCO_3 signature is caused by ion exchange of Ca for Na, which is typical for aquifers undergoing flushing of saline or brackish groundwater (Appelo and Postma 2005).

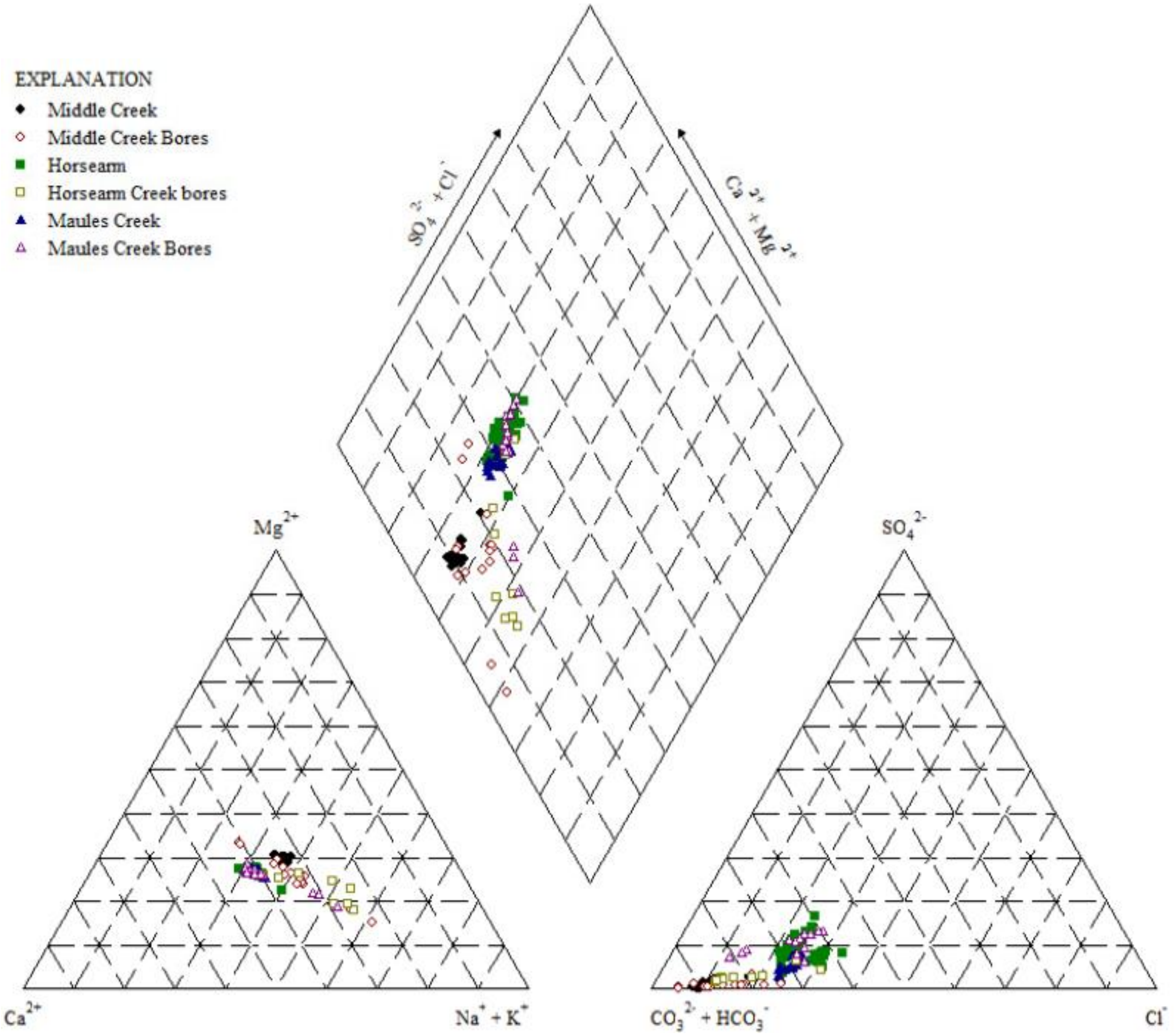


Figure 72: Piper plot of surface, hyporheic and groundwater samples from the Maules Creek catchment.

The surface water profiles along Horsearm Creek of selected physicochemical parameters are shown in Figure 72. Over the course of this project the chemistry of surface water samples collected from the same location remained consistent, especially along the perennial section of Horsearm Creek. The surface water from two of the identified sites of upwelling regional groundwater (labelled the ‘Spring’ and ‘Radon 35’) had lower EC (340 µS/cm) and DOC (0.5 mg/l), and higher DO (5.45 mg/l) and nitrate (0.82 mg/l) which are similar to value for the regional groundwater. Downstream of the ‘Spring’ there is a large decrease in nitrate concentrations in the surface water, which may be due to dilution by discharge of low-nitrate groundwater, but more likely nitrate uptake by primary producers.

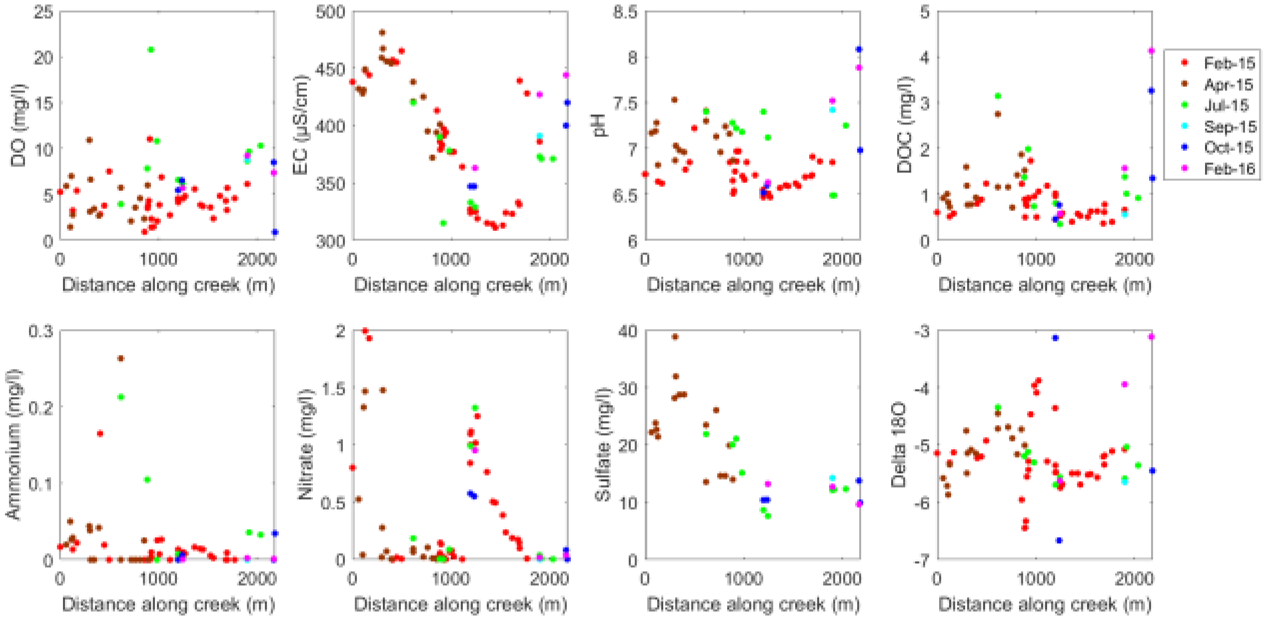


Figure 73: Surface water profiles for selected physicochemical variables along Horsearm Creek from the various field trips as part of this study. The 'Spring' site is located at 1196 m.

The classification of upwelling and downwelling for each sample site is shown in Table 19 . Sites at Middle Creek show temporal differences in their classification as Middle Creek switched from gaining to losing (see Figure 69).

Table 19: UW and DW classifications for sites in Maules Creek catchment

Hydrological Regime	Location	Trip	Classification
Intermittent	East Lynne	Sep-15	Upwelling groundwater
	Middle Creek	Jul-15	Upwelling groundwater
		Sep-15	Upwelling groundwater
		Oct-15	Downwelling
Perennial gaining	Pool 18	Apr-15	Downwelling
	Pool 19	Apr-15	Hyporheic upwelling
		Jul-15	Hyporheic upwelling
	Pool 20	Apr-15	Hyporheic upwelling
		Jul-15	Hyporheic upwelling
	Pool 24	Apr-15	Hyporheic upwelling
	Bar 6	Oct-15	Upwelling groundwater
		Feb-16	Upwelling groundwater
Perennial losing	Elfin Crossing	Sep-15	Downwelling
		Feb-16	Downwelling
	Riffle 10	Jul-15	Downwelling
	Pool 38	Feb-16	Downwelling
	Riffle 11	Jul-15	Downwelling
		Oct-15	Downwelling

Figure 74 -Figure 77 show selected hydrochemical variables at the sites of hyporheic sampling separated by flow regime. In general, the upwelling regional groundwater samples were more oxic than the hyporheic flow paths and downwelling samples. DO decreased from deep to shallow depths at the upwelling sites. Other oxidised species, such as nitrate, were higher for upwelling samples and reduced species, such as iron and ammonium, were higher for downwelling hyporheic than upwelling regional groundwater samples. In general, reduced species concentrations increased from downwelling shallow to deep sites. The identified upwelling hyporheic sites also were affected by depth, with the reduced species (Fe, Mn and NH_4) increasing from deep to shallow.

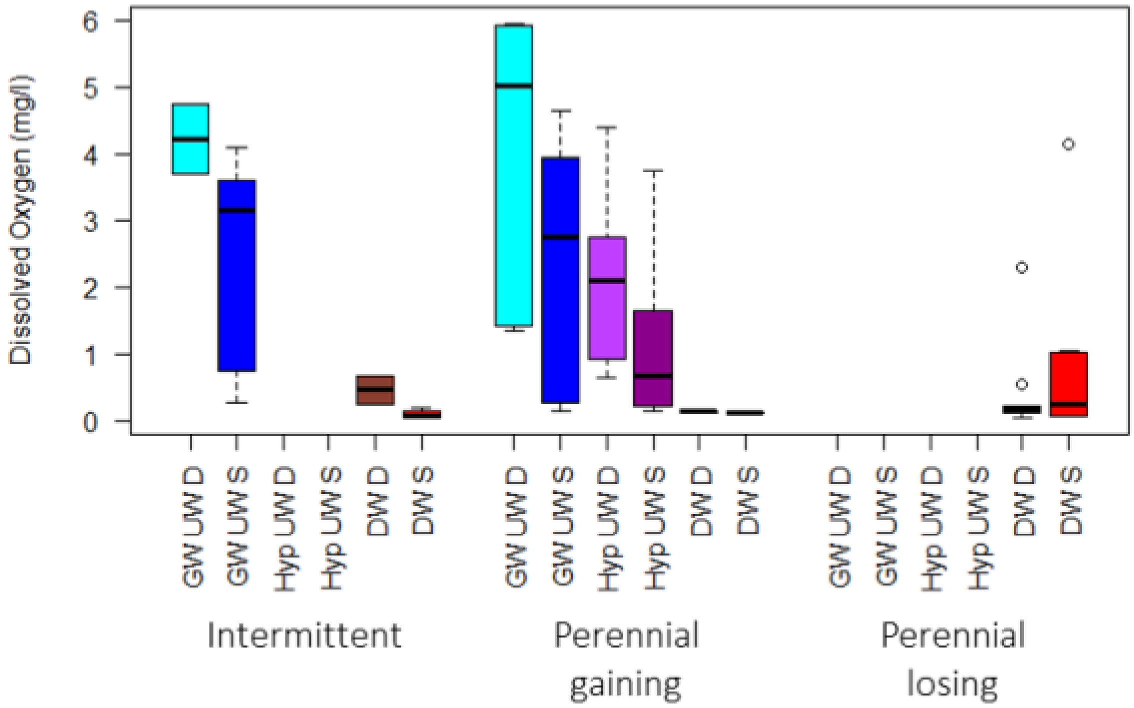


Figure 74: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of dissolved oxygen for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

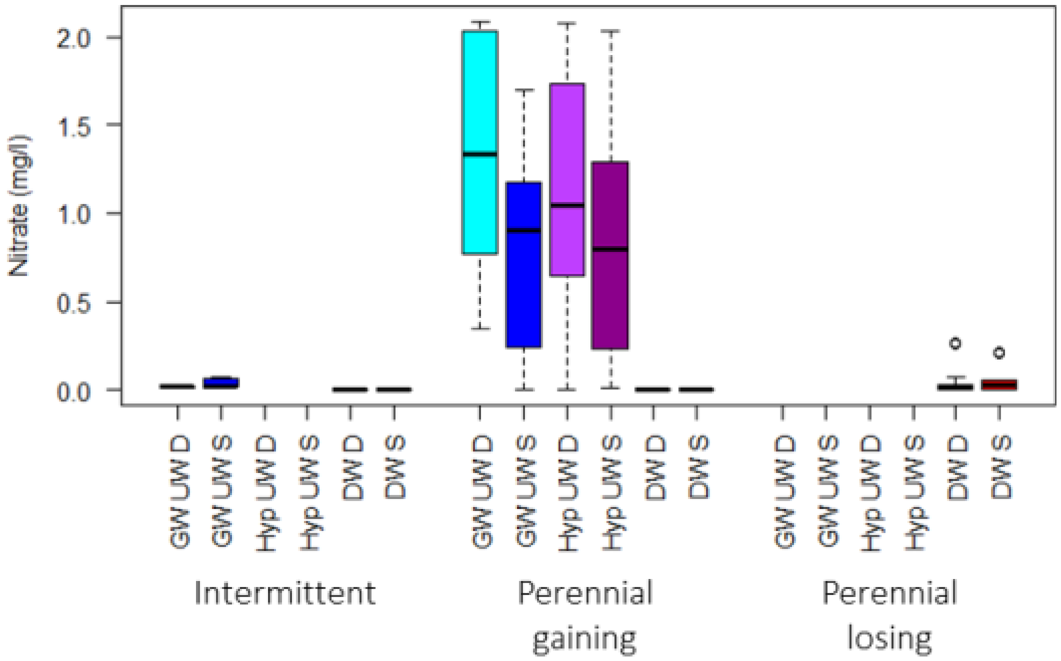


Figure 75: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of nitrate (NO_3^-) for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

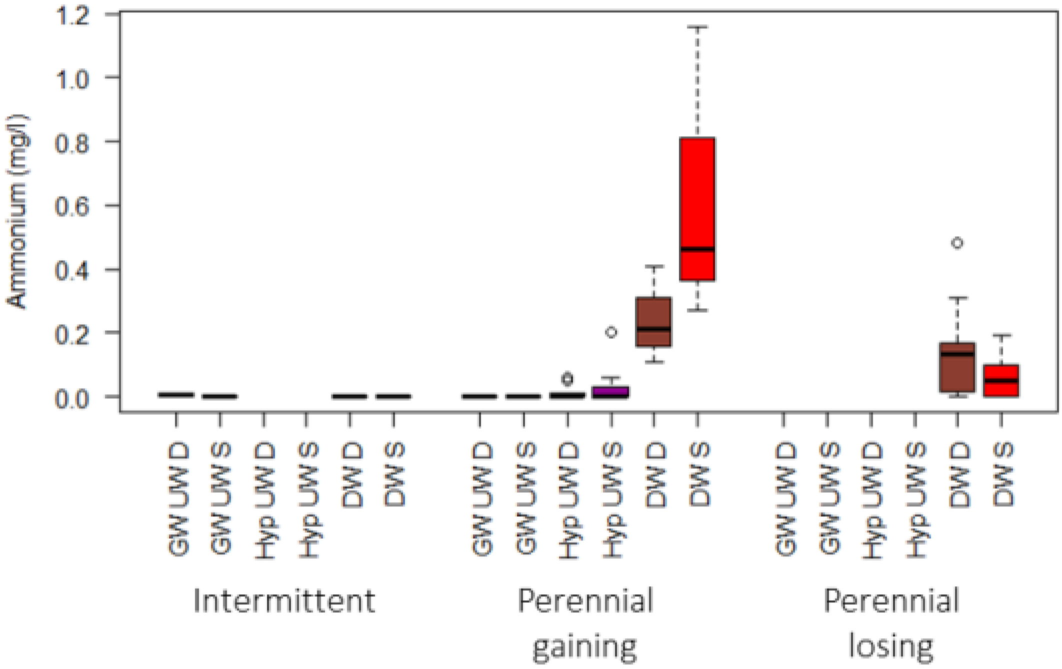


Figure 76: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of ammonium (NH_4^+) for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

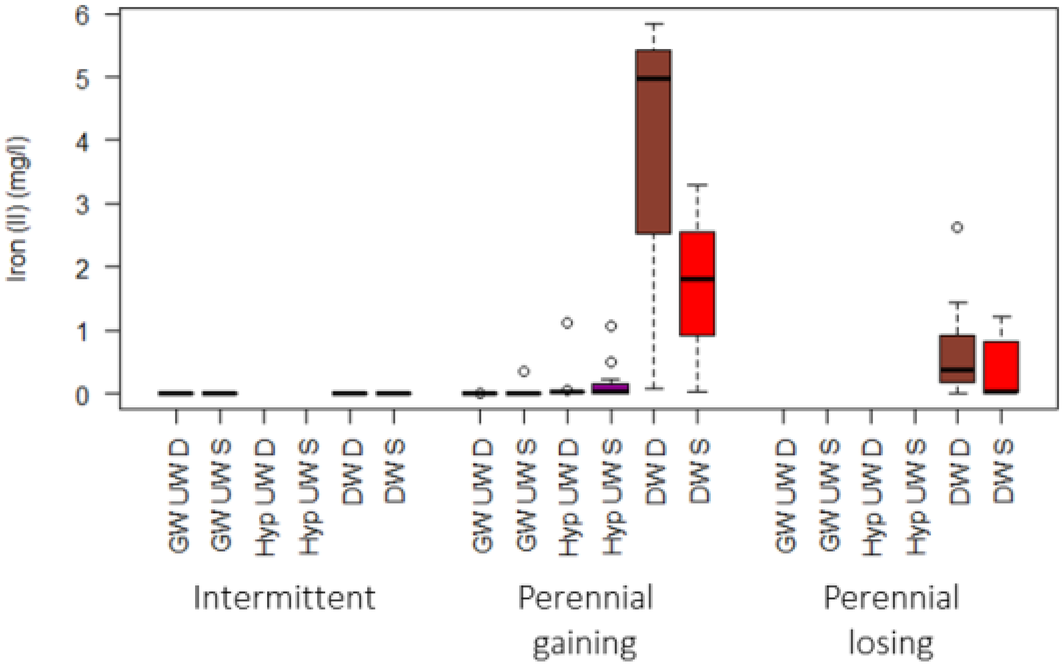


Figure 77: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of iron (II) for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

The DOC concentrations of the hyporheic samples separated by flow regime are shown in Figure 78. Samples collected in the intermittent section had the highest concentrations of DOC. In the perennial sections, the downwelling sites tended to contain higher DOC concentrations than the upwelling sites. In general, there was a trend with depth, with the shallow upwelling sites having greater DOC concentrations than the corresponding deep sites, and conversely the deep downwelling sites containing greater DOC concentrations than the corresponding shallow sites. In general, there was therefore an increase in DOC concentrations along flow paths regardless of whether the flow was up- or downwelling.

The ratio of the processed to unprocessed humic-like DOM fractions from PARAFAC is shown in Figure 79. The intermittent section had the highest ratio of processed to unprocessed material. The downwelling samples tended to higher ratios than upwelling samples. The shallow upwelling samples tended to have relatively higher processed humic-like content than the corresponding deep samples, and the deep downwelling samples had higher processed humic-like content than the corresponding shallow sites.

The spectral slope for the hyporheic samples is shown in Figure 80. The spectral slope is an index calculated from the ultra violet absorption spectrum and has been shown to be negatively correlated with the molecular weight of DOC (Helms, 2008). The downwelling samples had higher spectral slope values and therefore lower molecular weight of DOM than the upwelling samples. There was also a trend with depth, with shallow upwelling samples tending to have lower molecular weight than the corresponding deeper samples. Further, the deep downwelling samples had a lower molecular weight than the corresponding shallow sites. Over the course of this study the molecular weight of DOM decreased at Riffle 11 as this area dried.

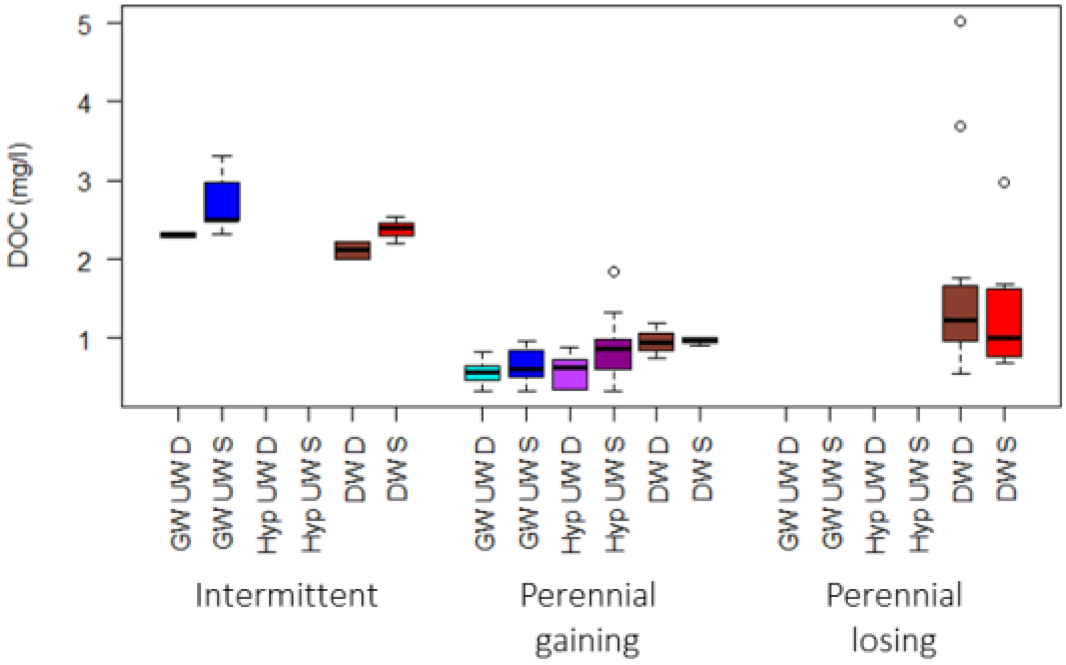


Figure 78: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of dissolved organic carbon (DOC) for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

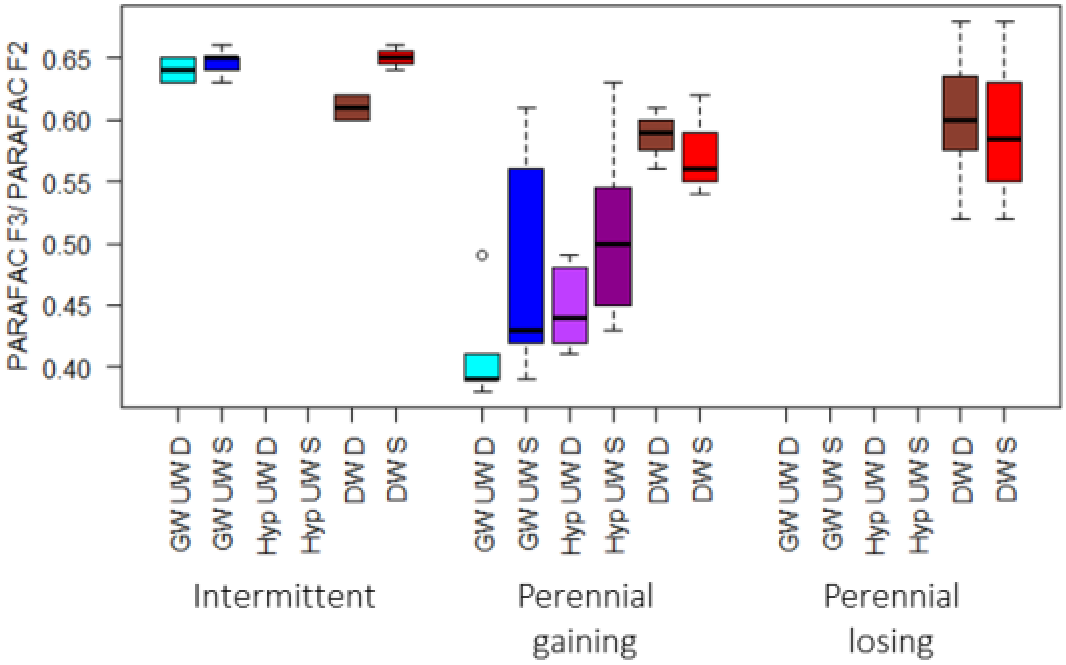


Figure 79: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of ratio of processed versus unprocessed (i.e. more fresh) humic-like content of DOM determined from PARAFAC analysis for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; DW D, downwelling deep; and DW S, downwelling shallow for each hydrological regime in Maules Creek catchment.

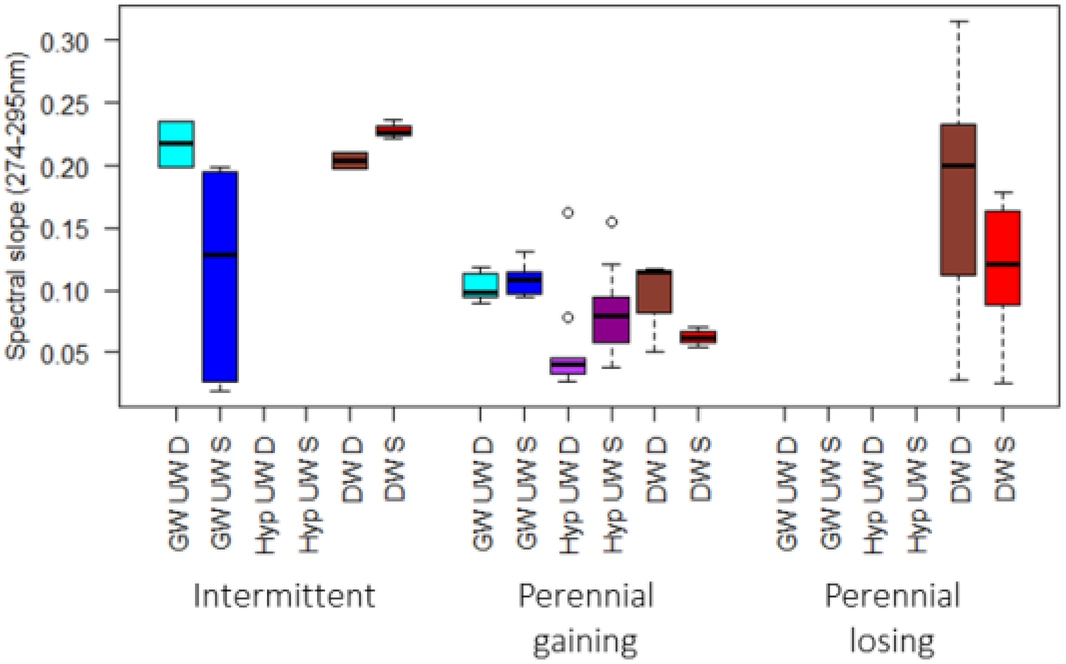


Figure 80: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of spectral slope of the ultra violet absorption at wavelengths from 275 to 295 nm for GW UW D, regional groundwater upwelling deep; GW UW S, regional groundwater upwelling shallow; Hyp UW D, hyporheic flow upwelling deep; Hyp UW S, hyporheic flow upwelling shallow; and DW D, downwelling deep; DW S, downwelling shallow for each hydrological regime in Maules Creek catchment. This index represents the molecular weight of DOC (i.e. high spectral slope, low molecular weight).

The results from LC-OCD analysis of the subsamples collected from the February 2016 sampling trip are shown in Figure 81 and Figure 82. The intermittent subsamples had the lowest proportion of low molecular weight DOC and the highest aromaticity. Conversely, the regional groundwater samples (from bores that have previously shown to be representative of the regional groundwater) had the highest proportion of low molecular weight DOC and the lowest aromaticity. The perennial losing and surface subsamples had similar aromaticity as the intermittent subsamples. The percent of low molecular weight DOC was higher with the subsamples from the perennial gaining section than with subsamples from the perennial losing section.

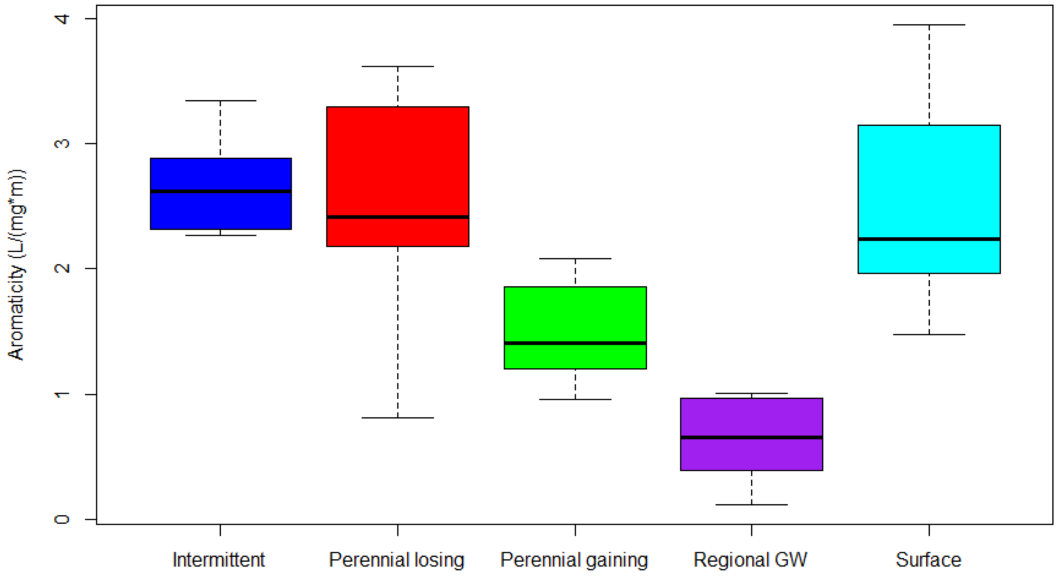


Figure 81: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values of aromaticity calculated from LC-OCD for each hydrological regime including regional groundwater sampled from bores in Maules Creek catchment.

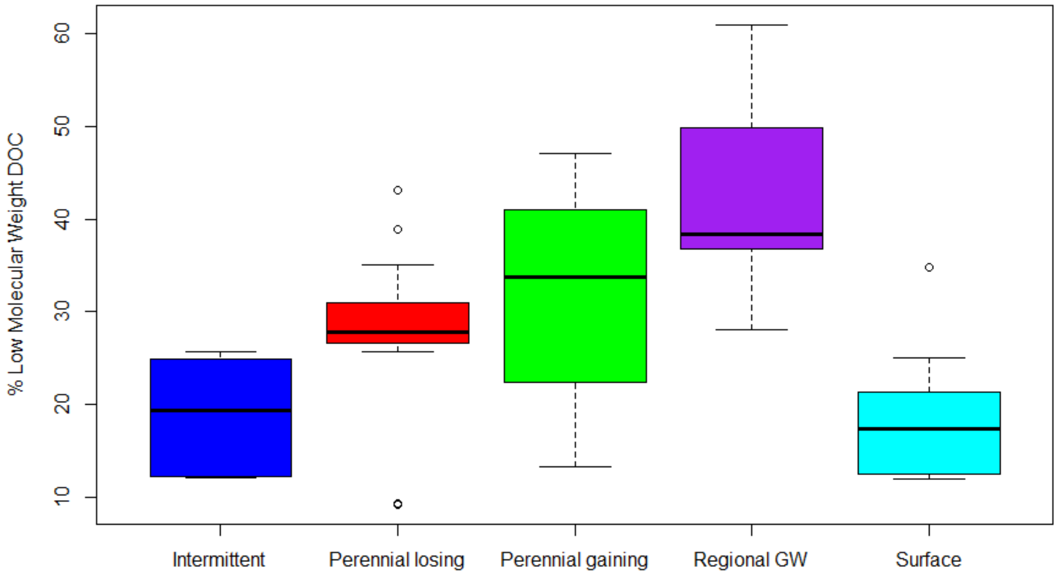


Figure 82: Box-whisker plot displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of spectral slope of % low molecular weight DOC from LC-OCD for each hydrological regime including regional groundwater sampled from bores in Maules Creek catchment.

4.5.2.2 Upper Bremer River catchment hydrochemistry

Major ion chemistry of surface and hyporheic water samples from the upper Bremer River catchment is summarised in the Piper diagram in Figure 83 (no bores were available for groundwater sampling in the upper Bremer River catchment). The surface water and hyporheic samples from the Bremer and Warrill Creeks had a Ca-Mg-HCO_3 composition typical of carbonate rock dissolution. The samples from Wild Cattle, Coulson and Reynolds creeks had a more mixed Ca-Mg-Na-Cl-HCO_3 water composition. The content of total dissolved solids (here represented by EC) increased in order from Warrill Creek (379-443 µS/cm) to Wild Cattle Creek (494-593 µS/cm), Coulson Creek (503-617 µS/cm), Bremer River (570-607 µS/cm) and Reynolds Creek (566-721 µS/cm). The EC (and in some cases the major ion composition – see Piper diagram) changed from sampling in October 2015 to February 2016. However, the change in EC was not consistent amongst the five upper Bremer River catchment sites, with increasing EC at Coulson, Warrill and Wild Cattle creeks and decreasing EC for Bremer River and Reynolds Creek.

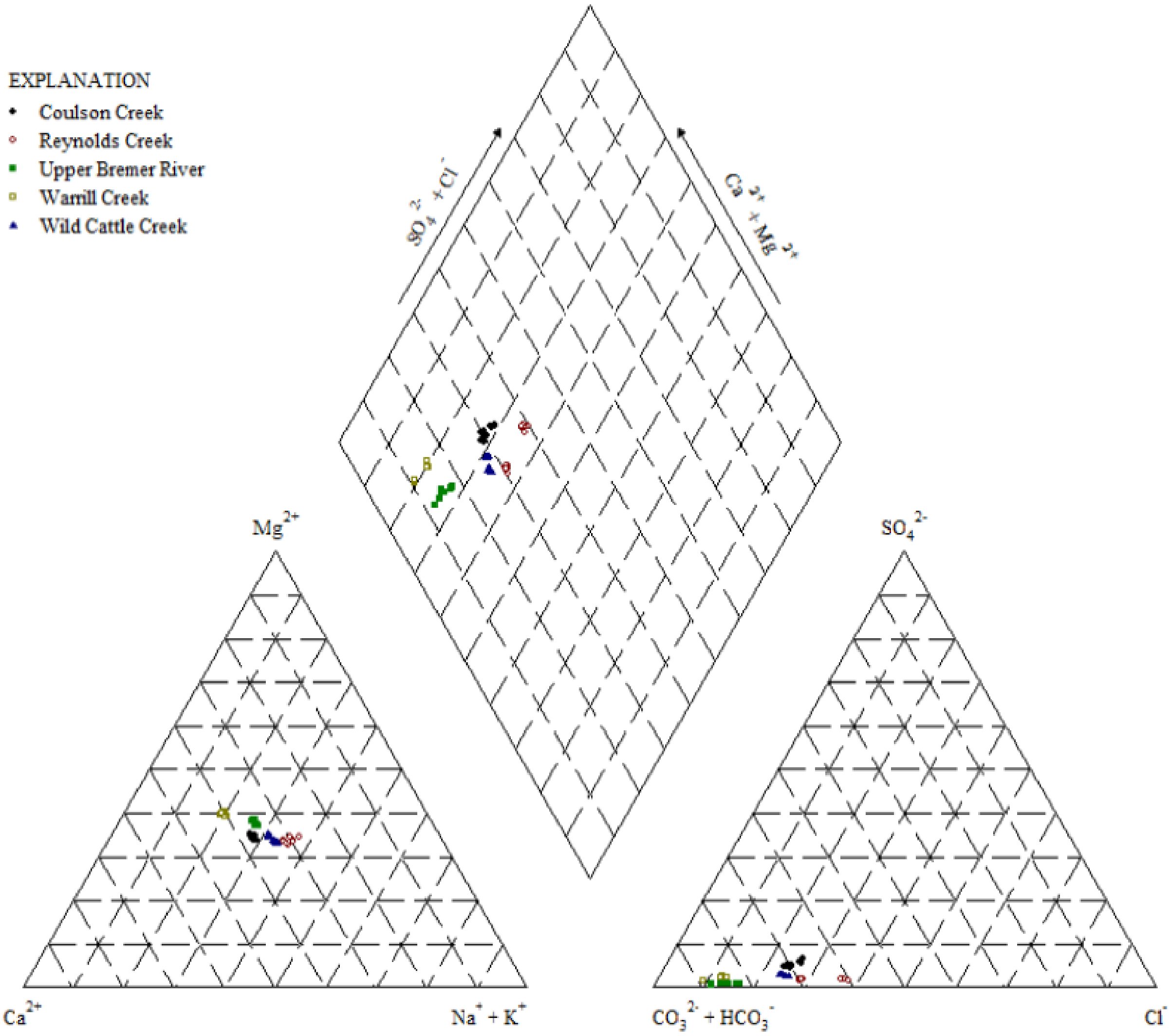


Figure 83: Piper plot of surface, hyporheic and groundwater samples from the upper Bremer River catchment.

The collected hyporheic samples from upper Bremer River catchment tended to be oxic as shown in Figure 84. Nitrate is shown in Figure 85 and the reduced species ammonia and iron are shown in Figure 86 and Figure 87, respectively. The oxic conditions in the upper Bremer River catchment resulted in higher levels of oxidised species compared to reduced species.

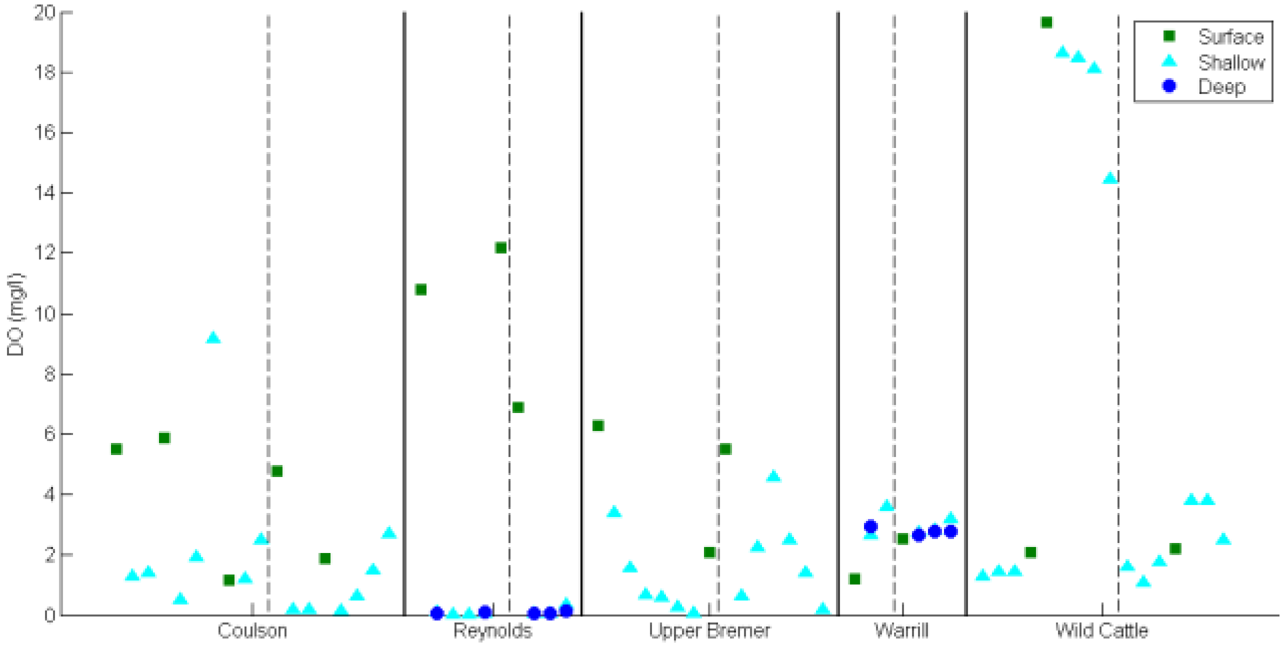


Figure 84: Dissolved oxygen (DO) concentration of hyporheic and surface water samples collected in the upper Bremer River catchment. The x-axis represents location along the channel in each study site (separated by vertical solid lines) and the vertical dotted line separates the two different sampling times.

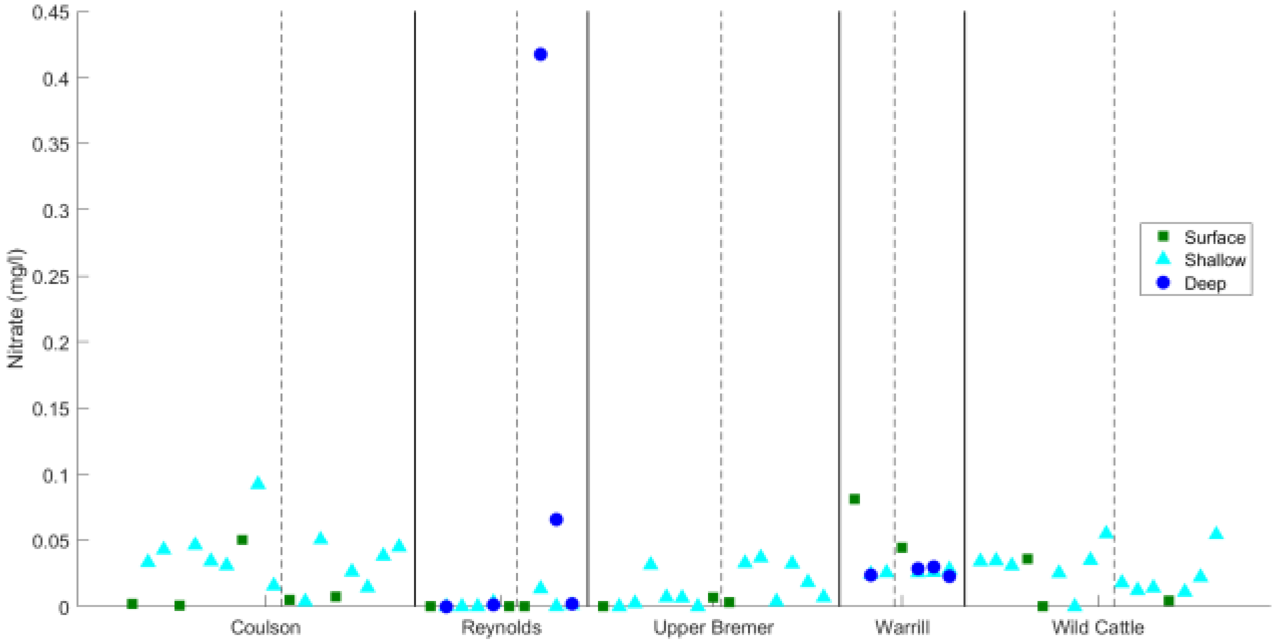


Figure 85: Nitrate (NO_3^-) concentrations of hyporheic and surface water samples collected in the upper Bremer River catchment. The x-axis represents location along the channel in each study site (separated by vertical solid lines) and the vertical dotted line separates the two different sampling times.

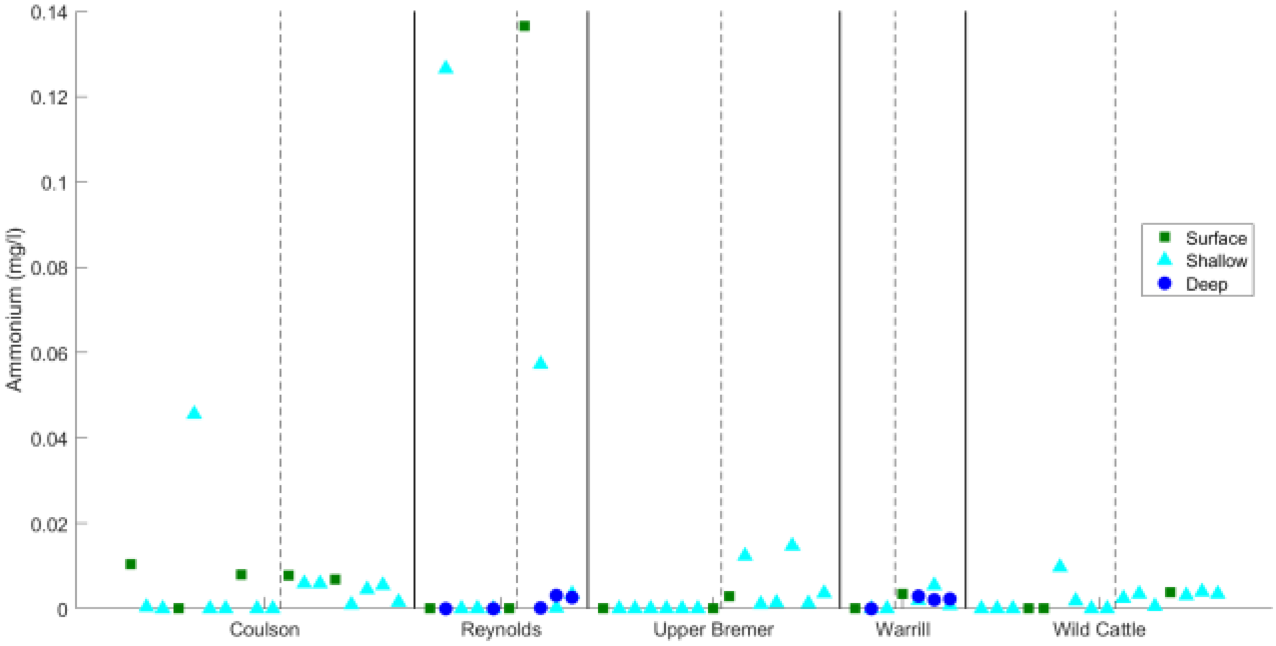


Figure 86: Ammonium (NH_4^+) concentrations of hyporheic and surface water samples collected in upper Bremer River catchment. The x-axis represents location along the channel in each study site (separated by vertical solid lines) and the vertical dotted line separates the two different sampling times.

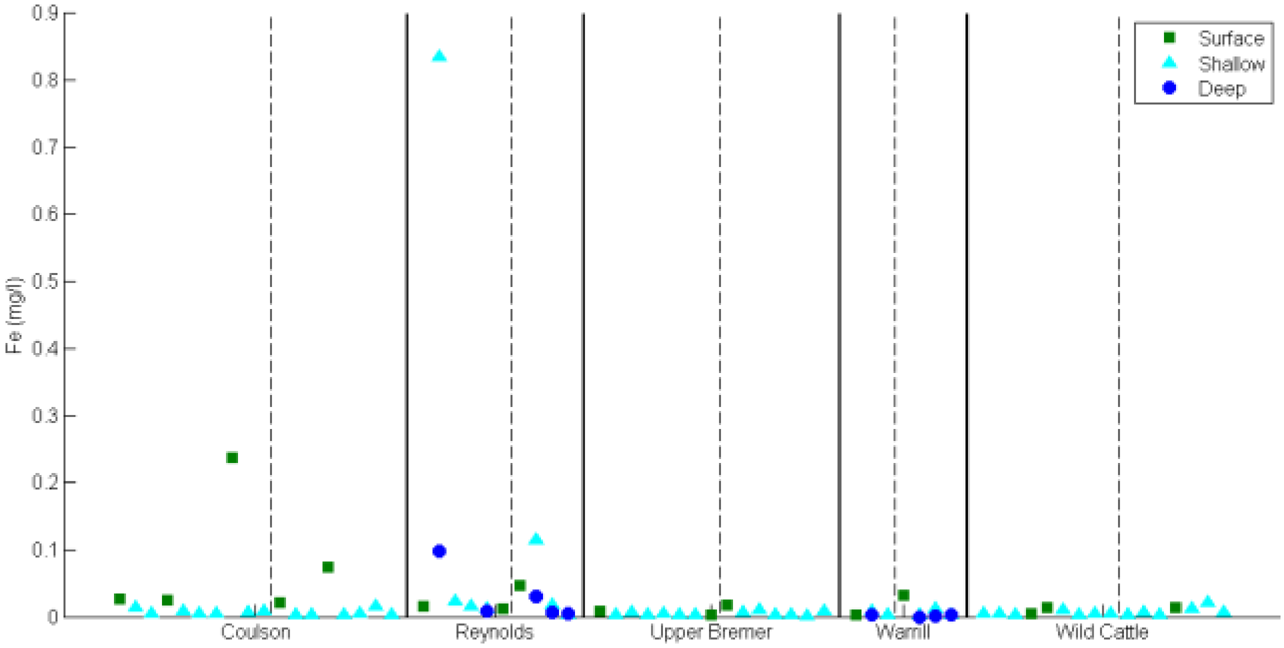


Figure 87: Fe(II) of hyporheic and surface water samples collected in upper Bremer River catchment. The x-axis represents location along the channel in each study site (separated by vertical solid lines) and the vertical dotted line separates the two different sampling times.

4.5.2.2.1.1 Data visualisation and statistical analysis

A plot of the principal component (PC) axes PC1 versus PC2 for the hyporheic Maules Creek catchment samples is shown in Figure 88 and for the hyporheic upper Bremer River catchment samples is shown in Figure 89. For both datasets, PC1 and PC2 split the samples based on location, with both axes explaining 41.5% and 49% of variation for the upper Bremer River catchment and Maules Creek catchment datasets, respectively. While the Maules Creek catchment samples were consistent over time, the upper Bremer

River catchment samples showed within site differences between the October 2015 and March 2016 sampling.

In the Maules Creek catchment analysis (Figure 88) the samples collected from just below the spring site (Bar 6) are separated from the other samples on their higher DO and nitrate concentrations characteristic of this regional groundwater. This analysis confirms that this site is near an area of regional groundwater discharge. The samples collected from the perennial losing section, i.e. Riffle 11 in July and October 2015 and Pool 38 February 2016, show an increase in DOC, humic-like organic matter, and reduced species (P, Mn and As) over time as there was a decrease in the surface water present.

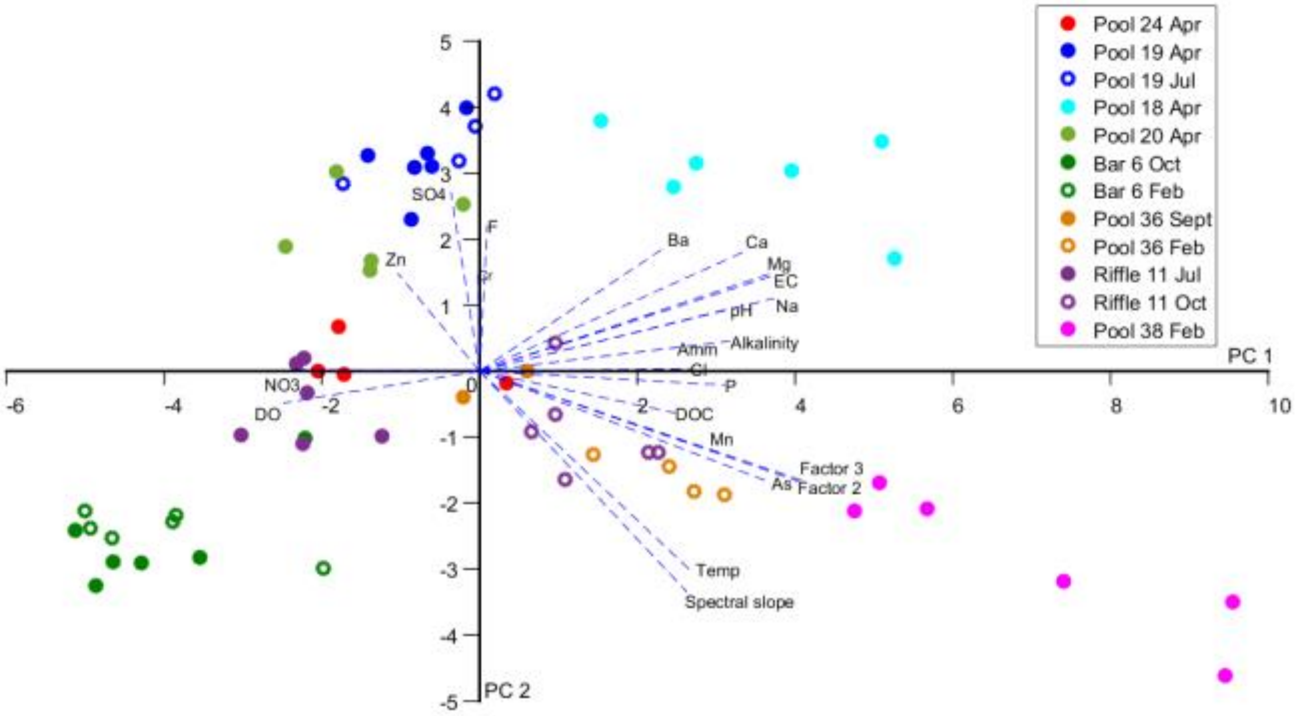


Figure 88: Maules Creek catchment hyporheic sites global principal components (PC) analysis of the physicochemical variables of the hyporheic samples showing axes PC1 and PC2 (excluding intermittent losing Middle Creek and East Lynne sites). The variables associated with each component are shown by dashed lines with the length of each line indicating the relative strength of its contribution.

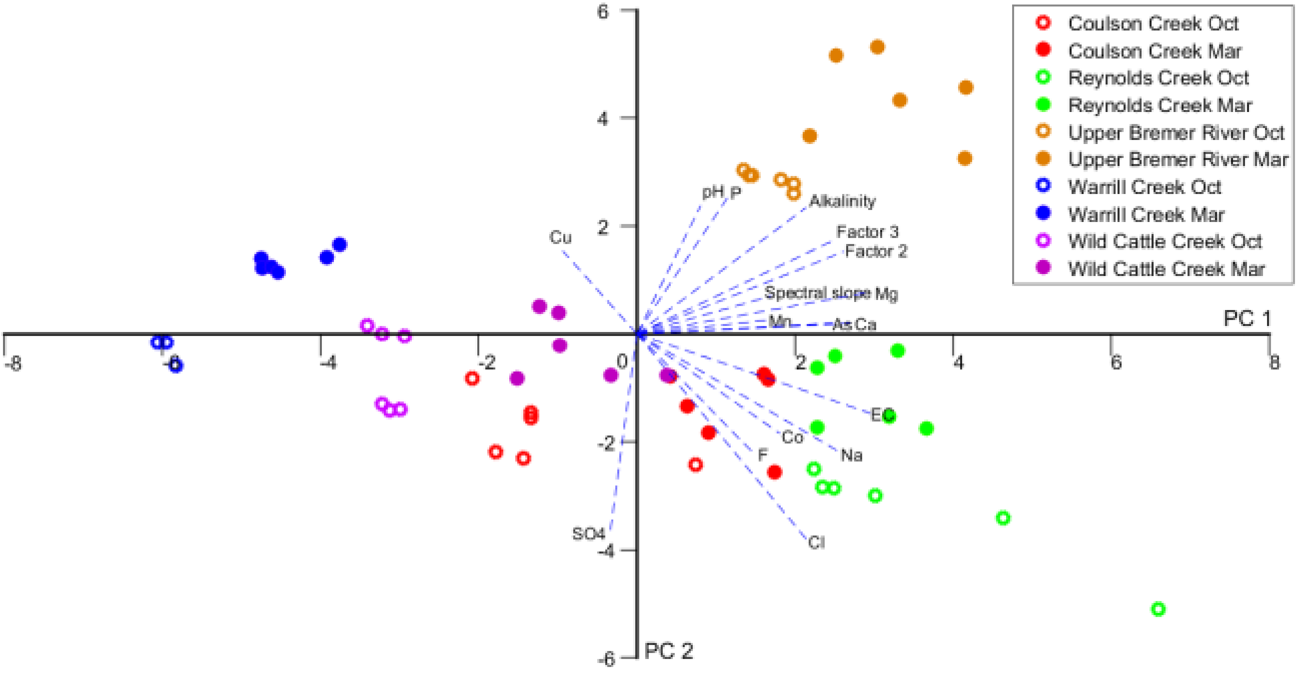


Figure 89: Upper Bremer catchment hyporheic sites global principal components (PC) analysis of the physicochemical parameters of the hyporheic samples showing axes PC1 and PC2. The variables associated with each component are shown by dashed lines with the length of each line indicating the relative strength of its contribution.

4.5.3 Organic Matter Degradation Studies

Results for the cellulose decomposition potential (CDP) experiments at Maules Creek catchment are shown in Figure 90. Overall, Round 2 (March to April 2016) had higher cotton tensile force ratios, that is less degradation than Round 1 (September to October 2015). In general, hyporheic samples showed a greater loss of tensile strength than the corresponding surface samples. Within each round, differences in the tensile strength were related to both the environment (e.g. pool, riffle or dry gravel bar and surface or subsurface) for the cotton strip placement and hydrological condition (e.g. saturated, moist or dry). The gravel bar samples tended to show the smallest decrease in tensile strength (i.e. higher cotton tensile force ratio) than the pool and riffle samples. The samples for the perennial gaining section had the smallest variation for the pool and riffle samples.

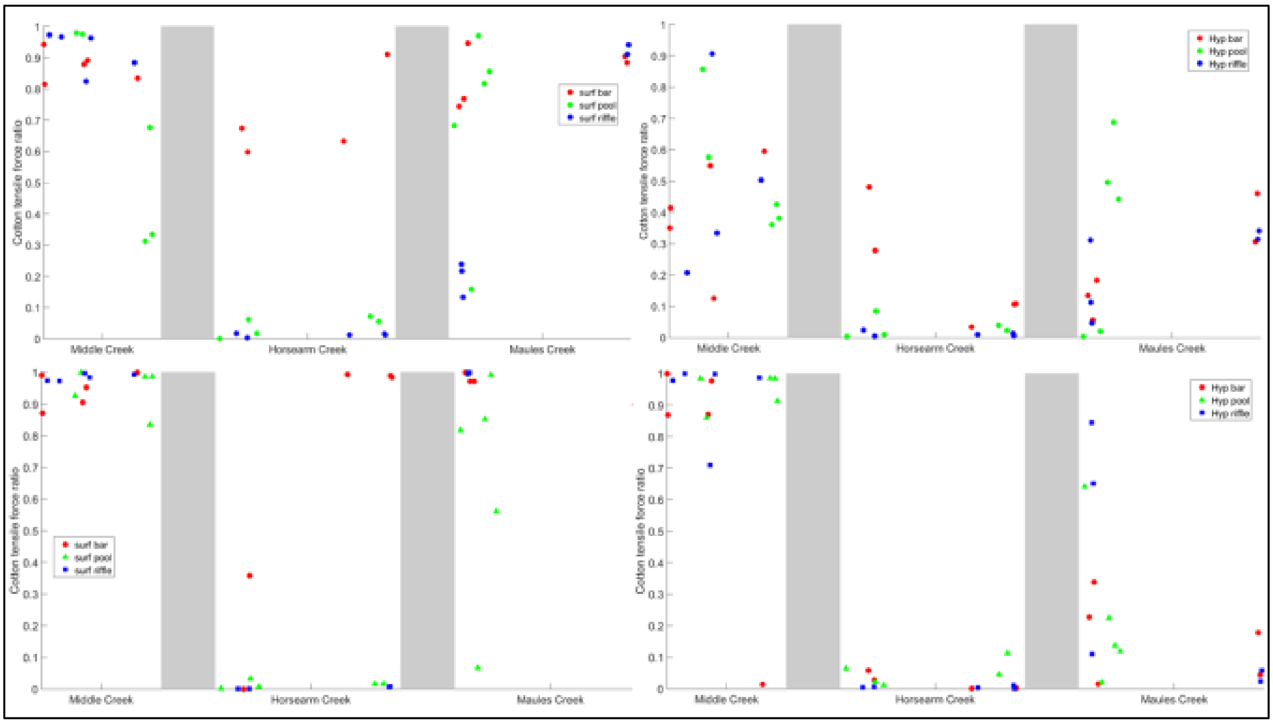


Figure 90: Cotton strip tensile force ratio results for Maules Creek catchment – Round 1 upper panels, Round 2 lower panels, surface environments left panels and hyporheic environments right panels. Force ratio values closer to one represent less cellulose decomposition than values closer to zero. The x-axis represents longitudinal distance (not to scale) along the channel (left upstream, right more downstream) in each study reach and the grey bars separate the different study sites.

The effect of moisture status on cotton strip degradation is shown in Figure 91. The cotton strips that remained dry or started saturated and became dry showed the least degradation (higher median cotton tensile force ratio) while the strips that remained saturated during the deployment showed the greatest degradation. The mixed categories (especially the moist-dry and saturated-moist) tended to display the largest variation, which could be related to the variable times for the transition in moisture status across the deployments.

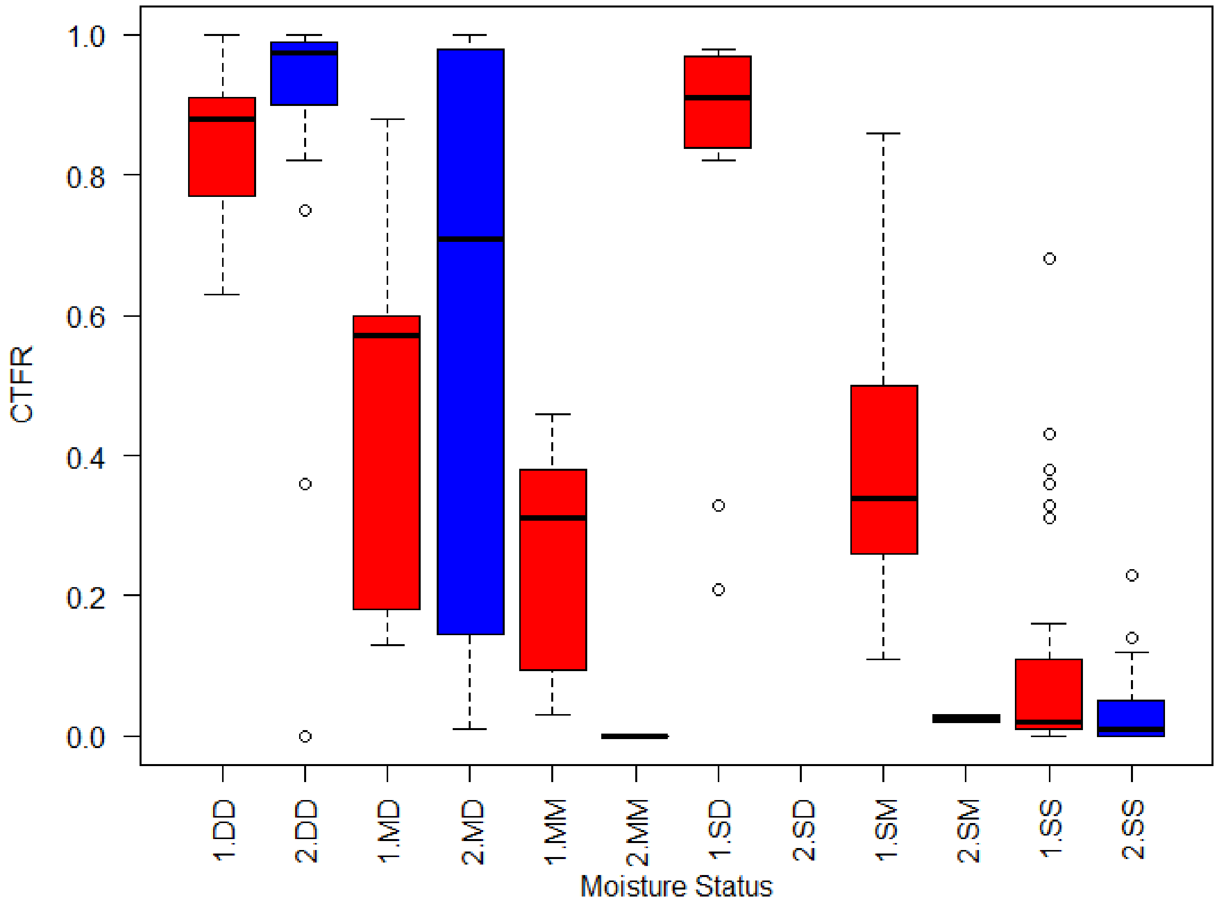


Figure 91: Median cotton tensile force ratio (CTFR) based on moisture status at the beginning and end of deployment for each Round with potential outliers shown as black hollow circles. (DD – dry; MD – moist to dry or vice versa; SD – saturated to dry; MM – moist; SM – saturated to moist; SS – saturated).

The results for leaf packs are shown in Figure 92. Within individual plots, most leaf packs in coarse mesh bags showed a slightly greater mass loss than their corresponding leaf packs in fine mesh bags. The surface samples display a larger overall range of mass loss percentages than the hyporheic samples. With the surface deployments, the dry gravel bar samples consistently had the lowest mass loss. In general, the riffle samples had the highest mass loss; however, for the intermittent site (Middle Creek) the pool samples had higher mass losses than the riffle samples. With the hyporheic deployments, the range in % mass loss was narrower and is much more consistent across the treatments. In general, the hyporheic mass loss is greater than loss in the corresponding surface habitat with the exception of the riffle deployments in the perennial gaining section.

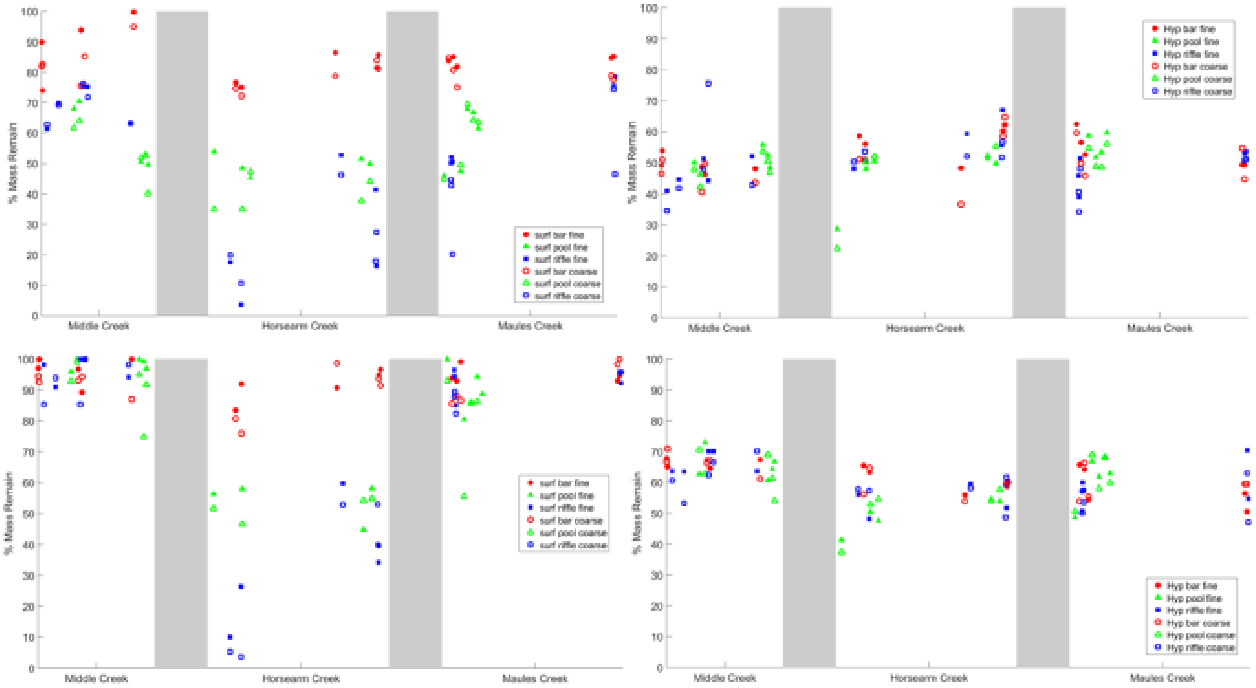


Figure 92: Leaf litter degradation results for the Maules Creek catchment - Round 1 upper panels, Round 2 lower panels, surface environments left panels and hyporheic environments right panels. The x-axis represents approximate longitudinal distance along the channel in each study site and the grey bars separate the different study sites. Fine and coarse in legend refers to the mesh size of the leaf litter bags.

The average leaf litter mass remaining for each habitat and for each hydrological regime is shown in Table 20. Riffle samples generally had the highest leaf litter processing; however, for the intermittent site (Middle Creek) the pool samples had higher leaf litter processing than the riffle samples. This is likely because as surface flow in this stream section ceased during the course of the deployment, the riffle sections dried first while the pools persisted. With the hyporheic deployments, the range of leaf litter processing was narrower and much more consistent across treatments. In most cases, hyporheic leaf litter processing was greater than processing in the corresponding surface habitat with the notable exception of the riffle deployments in the perennial gaining section.

Table 20: Percentage leaf litter mass remaining at each habitat for each hydrological regime in each leaf pack deployment. Round 1 = September – October 2015; Round 2 = March – April 2016.

	Riffle		Pool		Bar	
Round 1	Surface	Hyporheic	Surface	Hyporheic	Surface	Hyporheic
Intermittent	68.83	47.57	56.16	49.38	87.77	47.70
Perennial Gaining	25.35	54.58	44.79	46.03	79.56	54.69
Perennial Losing	53.53	46.74	58.11	53.95	81.71	52.48
Round 2						
Intermittent	94.57	64.39	94.53	64.48	94.42	66.42
Perennial Gaining	32.48	55.84	53.00	50.43	89.78	59.84
Perennial Losing	91.42	56.44	86.95	61.34	93.17	58.57

The effect of moisture status on the leaf litter processing is shown in Figure 93 (coarse and fine mesh combined). Overall processing was more consistent within each moisture status category for leaf litter than for the cotton strips. As with the results from the cotton strips, the greatest leaf-litter processing was for samples that remained saturated, and the samples that remained dry displayed the least leaf-litter processing, indicating that the greatest leaf degradation (by microbial activity, invertebrates and physical processes) was in saturated substrates. For most categories, with the exception of Moist-Dry, Round 1 showed greater mass loss than Round 2.

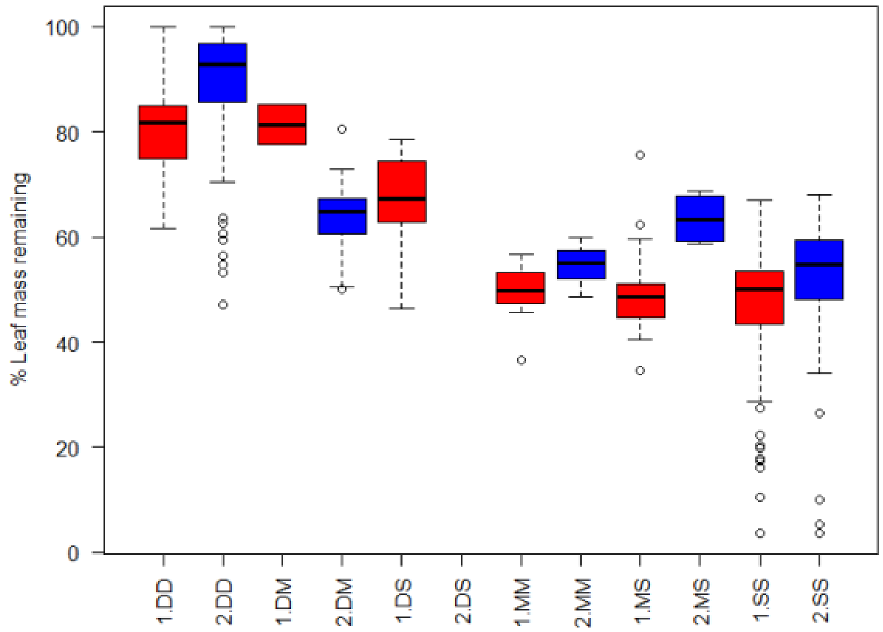


*Figure 93**:** Box-whisker plots displaying the median, lower and upper quartiles (25% and 75%), and minimum and maximum values excluding outliers (dots) of the percent leaf mass remaining in each moisture status category at the beginning and end of deployment for each round. (DD – dry; MD – moist to dry or vice versa; SD – saturated to dry; MM – moist; SM – saturated to moist; SS – saturated).*

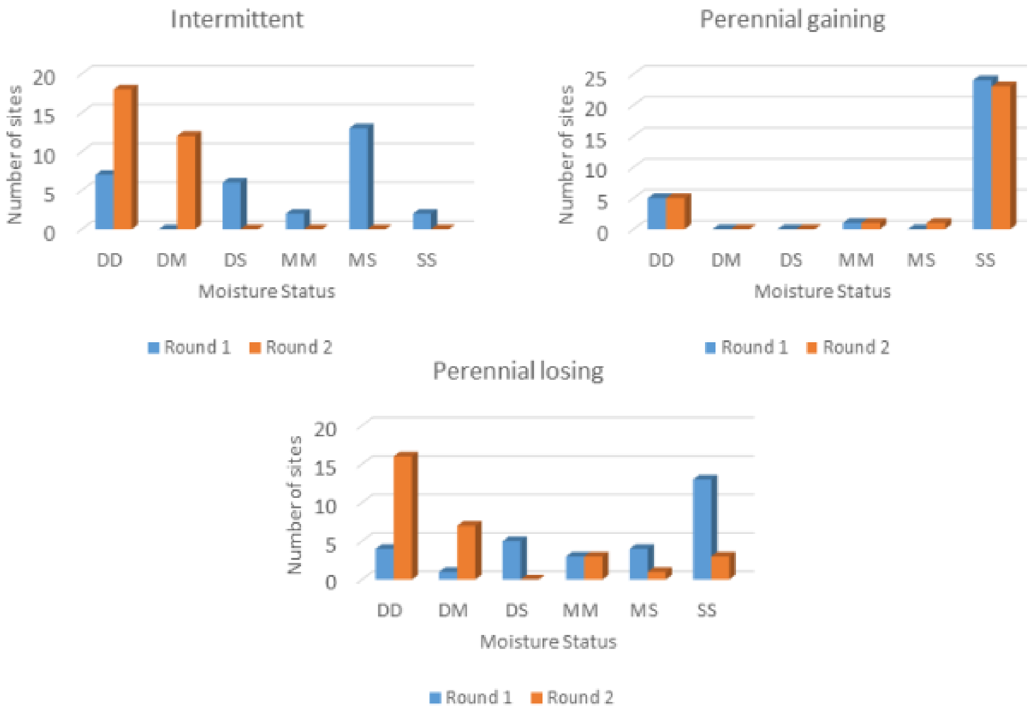


Figure 94: Comparison between deployments of moisture status for each hydrological regime at Maules Creek catchment. (DD – dry; DM – moist to dry or vice versa; DS – saturated to dry; MM – moist; MS – saturated to moist; SS – saturated).

The proportion of sites in each moisture status category in rounds 1 and 2 is shown in Figure 94. The groundwater decline observed in the intermittent (Middle Creek) and the perennial losing (Maules Creek) sections was reflected in an increase of dry (including dry-moist) sites. The proportion of sites in each moisture status was relatively consistent for the perennial gaining section.

Figure 95 shows the measured temperature for the surface and hypoheric site at Plot 13 in Pool 33, the perennial gaining section of Horsearm Creek. While both sites show diurnal fluctuations, the magnitude in these fluctuations and overall change is larger at the surface than in the hyporheic zone (~30 cm into the streambed).

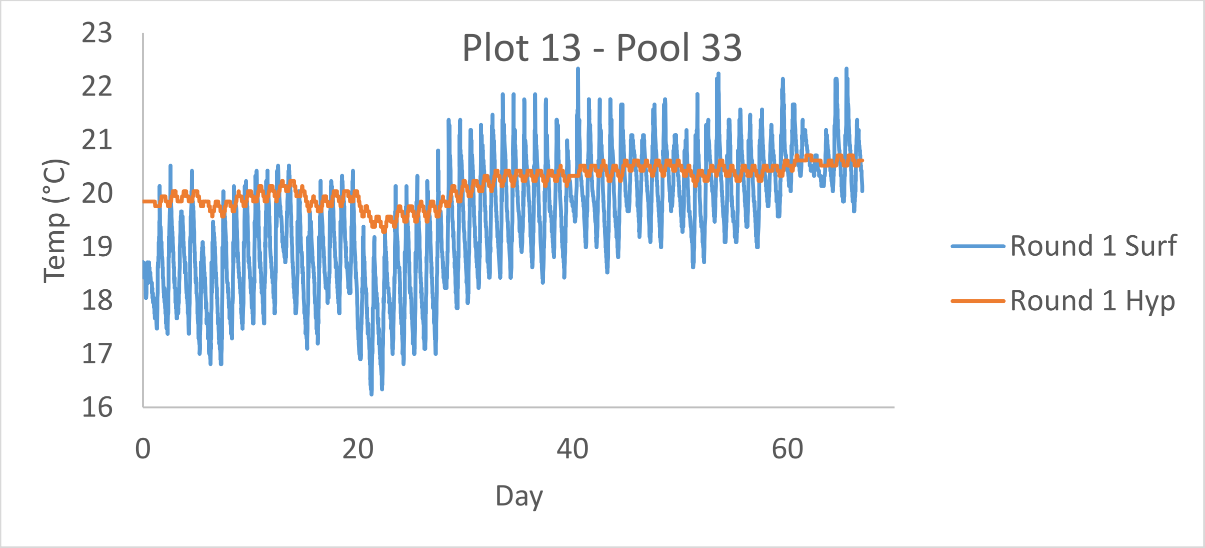


Figure 95: Comparison of surface and subsurface temperature from a HOBO logger deployed at Plot 13 in Pool 33 in the perennial gaining section of Horsearm Creek.

The amount of leaf litter degradation was significantly different between the deployment rounds ($p < 0.001$) but the amount of cotton strip degradation was not when changes in the moisture status were accounted for in a linear model. The results of the linear models for each round are summarised in Table 21. For each round, moisture status had the most significant impact on the mass loss of the leaf packs and degradation of the cotton strips. Depth (surface/hyporheic) also had a significant impact with Round 1 leaf packs. With the cotton strips, hydrological regime also had a significant impact for both rounds.

Table 21: Results of the linear models for the leaf packs and cotton strips

	Leaf Packs			Cotton strips		
	Variable	F value	p value	Variable	F value	p value
Round 1	Moisture	74.121	<0.001	Moisture	53.106	<0.001
	Depth	29.646	<0.001	Hydrological regime	8.2656	<0.001
Round 2	Moisture	84.69	<0.001	Moisture	61.354	<0.001
				Hydrological regime	16.902	<0.001

4.5.4 *Invertebrate ecology*

4.5.4.1 Identification and classification of taxa

Aquatic invertebrates were detected in most samples collected at Bremer River (91% of samples) and Maules Creek (81% hyporheos samples, 88% aquifer samples). More than 4,970 invertebrates, representing at least 53 provisional morpho-species / morpho-taxa belonging to 43 aquatic invertebrate families, were identified from the Bremer River and Maules Creek catchments combined (Table 22). Taxonomic resolution and recorded diversity were highest in crustaceans (14 families, 23 spp.) and insects (13 families, 12 spp.) followed by water mites (7 families, 7 spp.) with most other groups not identified below order level (Oligochaeta, Nematoda, Nematomorpha, Turbellaria, Gastropoda, Bivalva, Tardigrada, Rotifera, Hydrozoa). Total species richness is undoubtedly greater, especially in the typically diverse and taxonomically challenging groups of Oligochaeta, Acarina, Copepoda and Ostracoda. Images of hyporheic and aquifer taxa are shown in Figure 96 and Figure 97.

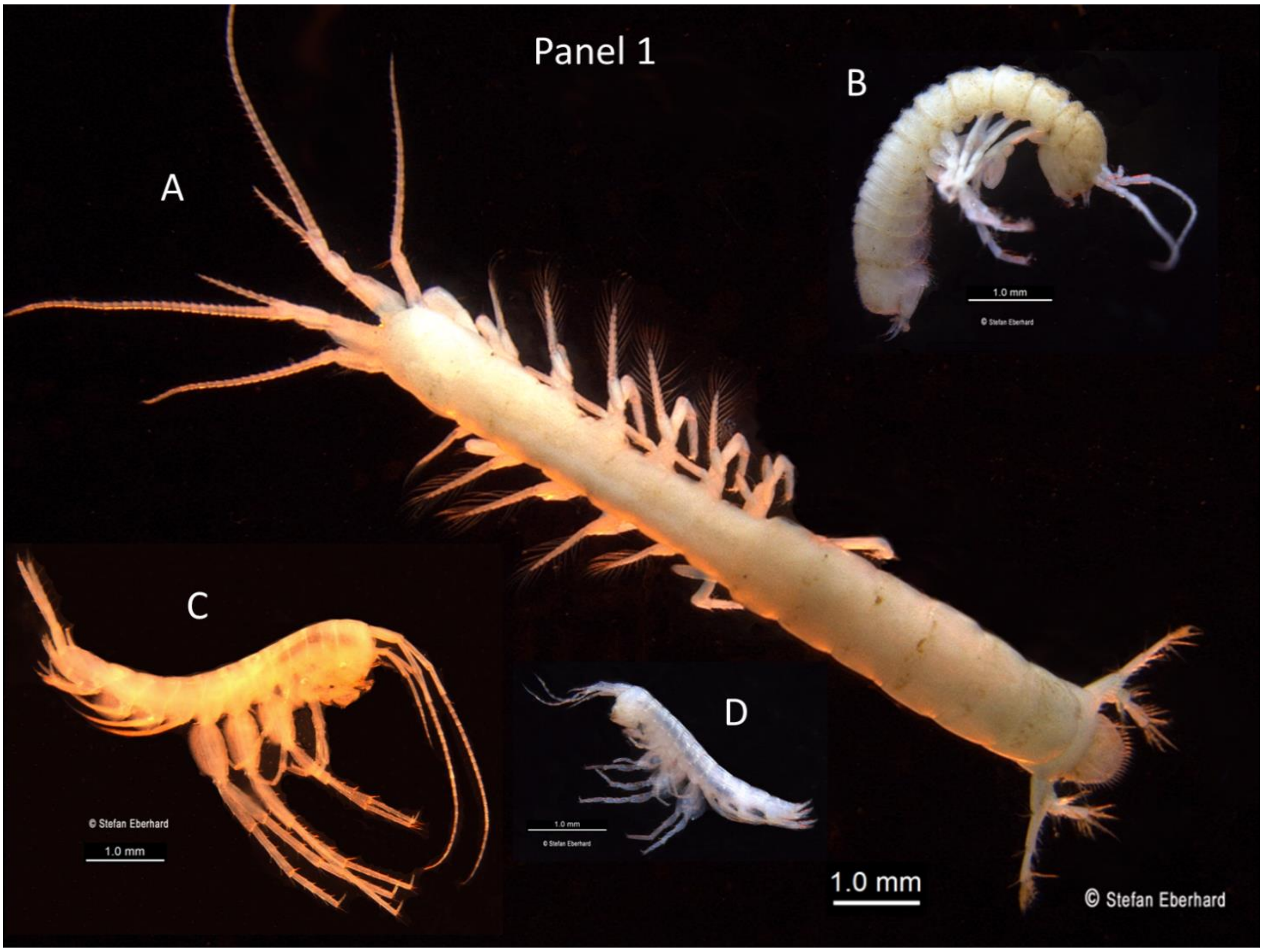


Figure 96: Hyporheic and aquifer invertebrates from Bremer (BR) and Maules (MC) catchments: Panel 1, Stygobite crustaceans; A, Psammaspididae (MC); B, Phreatoicidae (MC); C, Amphipoda (MC); D, Amphipoda (BR).

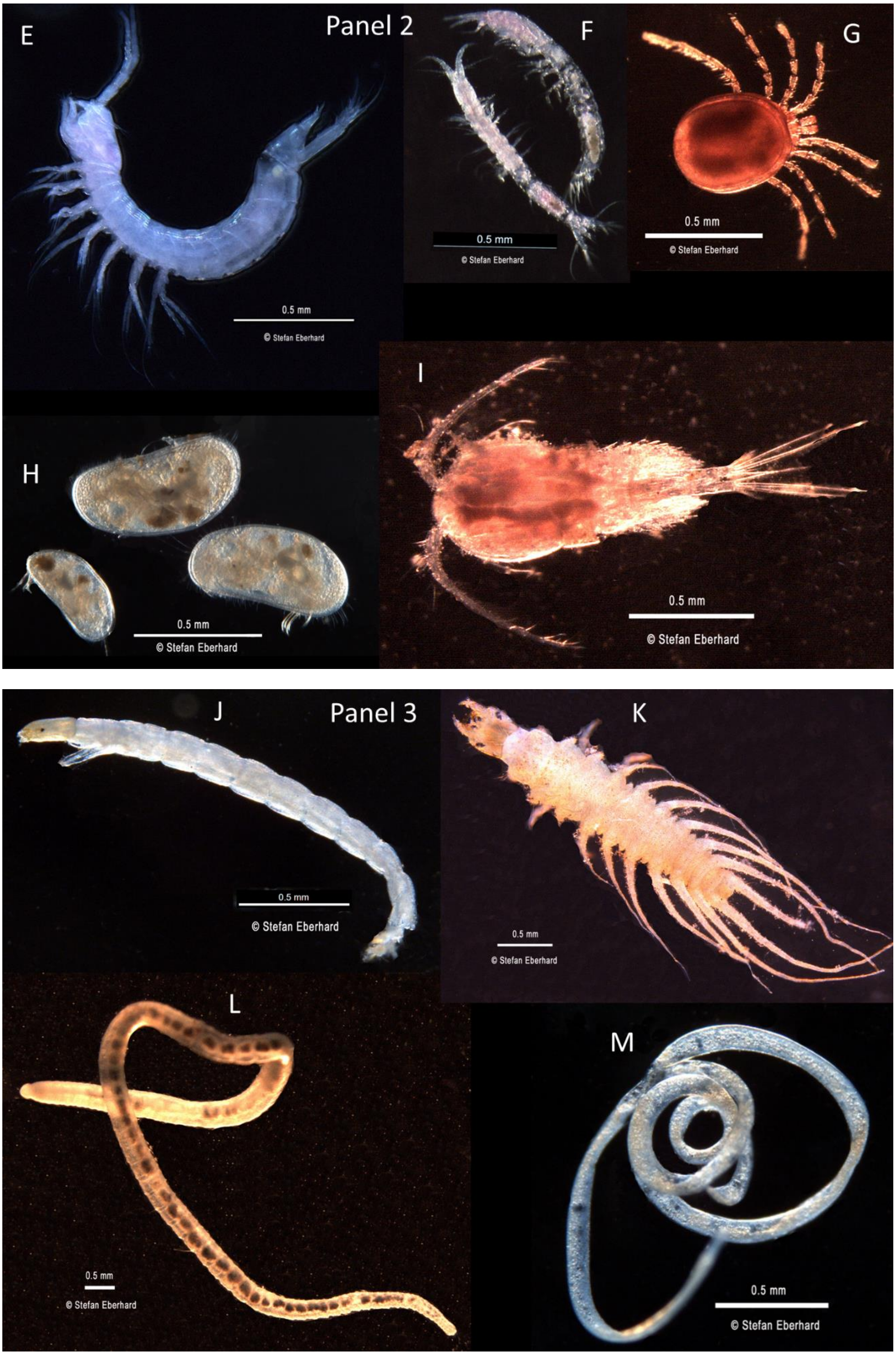


Figure 97 (Continued): Hyporheic and aquifer invertebrates from Bremer (BR) and Maules (MC) catchments: Panel 2, Stygobite crustaceans and water mite; E, Parabathynellidae (MC); F, Bathynellidae (BR); G, water mite (MC); H, Ostracoda (MC); I, Cyclopoida (BR); Panel 3, Stygoxene insects (J,K) and stygophile worms (L,M); J, Chironomidae (MC); K, Hydrophilidae (BR); L, Oligochaeta (BR); M, Nematoda (MC).

Comparing study areas, Maules Creek hyporheos comprised, minimally, 39 morpho-species in 33 families, and Bremer River hyporheos comprised 31 morpho-species belonging to 31 families (Table 22). Aquatic mites were notably more diverse. In the Maules Creek hyporheos, crustaceans (12 families) and water mites (five families) were notably more diverse compared with the Bremer River (eight and two families respectively). Comparing groundwater environments at Maules Creek, the aquifer recorded 24 morpho-species in 21 families, or approximately two-thirds of the richness recorded in the hyporheic zone (Table 22). The aquifer assemblage strongly overlapped with the hyporheic assemblage, with nearly all morpho-species collected in the aquifer also collected in the hyporheic zone. The only stygobite taxon not detected in the aquifer was Phreatoicidae, but this taxon was very rare in hyporheic samples and the limited sampling to date does not preclude its presence in the aquifer as well. Conversely, three morpho-species collected in the aquifer (one harpacticoid, one water mite, and one turbellarian) were not detected in the hyporheos, but may occur there considering the degree of hydrologic connectivity and overlap in invertebrate assemblages between the two environments.

Table 22: List of identified taxa (arranged systematically by higher taxonomic rank) with their inferred groundwater habitat affinity, and collection records (p = confirmed presence) from the Bremer catchment hyporheic (BCH), Maules catchment hyporheic (MCH) and aquifer (MCA). Taxon names in bold not counted in richness totals where they may represent synonymies. Abbreviations for groundwater habitat affinity (GHA) categories at bottom.*

Higher taxon rank 1	Taxon rank 2	Taxon rank 3 (family)	Lowest identified taxon	GHA*	BRH	MCH	MCA
Crustacea	Amphipoda	Amphipoda_Fam._indet._Ep	Amphipoda_Fam._indet._Ep	Ep		P	
		Neoniphargidae	Neoniphargidae_MC_Sb	Sb		P	P
		Neoniphargidae	Neoniphargidae_QLD_Sb	Sb	P		
	Syncarida	Bathynellidae	Bathynellidae_sp 1	Sb		P	P
		Bathynellidae	Bathynellidae_sp 2	Sb	P		
		Bathynellidae	Bathynellidae_sp 3	Sb	P		
		Bathynellidae	**Bathynellidae_indet.**	Sb		P	P
		Parabathynellidae	Parabathynellidae_sp 1	Sb		P	P
		Parabathynellidae	Parabathynellidae_sp 3	Sb	P		
		Parabathynellidae	Parabathynellidae_sp 4	Sb		P	P
		Parabathynellidae	**Parabathynellidae_indet.**	Sb			P
		Psammaspididae	Psammaspididae_indet.	Sb		P	P
	Isopoda	Janiridae	Janiridae_indet. NSW Sb	Sb		P	P
		Phreatoicidae	Phreatoicidae_indet. Sb	Sb		P	
	Copepoda	Cyclopoida_Fam. indet.	Cyclopoida_indet.	Sp/Sb?	P	P	P
		Harpacticoida_Fam. Indet	Harpacticoida_indet_sp 2	Sb	P		P
			Harpacticoida_indet_sp 3	Sb		P	P
			Harpacticoida_Fam. Indet	Sb		P	P
	Ostracoda	Candonidae?	Candonidae?_indet._cf sp1B QLD	Sp/Sb?	P		
			Candonidae?_indet._sp1/1B NSW	Sp/Sb?		P	P
			Candonidae?_indet._sp2 NSW	Sp/Sb?		P	P
			Candonidae?_indet._sp4 NSW	Sp/Sb?		P	
			Candonidae?_indet.	Sp/Sb?	P	P	P
		Limnocytheridae	Gomphodella_sp.	Sx/Sp?		P	P
	Branchiura	Branchiura_Fam. indet	Branchiura_indet.	Ep/P	P		
	Cladocera	Chydoridae	Chydoridae_indet	Ep	P		
	Cladocera	Cladocera_Fam. indet.	Cladocera_indet.	Ep		P	
Arachnida	Acarina (water mites)	Acarina_Fam. indet.	Acarina_sp1_shield	Sp?		P	P
		Aturidae	Aturidae_indet.	Sp?	P		
		Hygrobatidae	Hygrobatidae_indet.	Sp?			P
		Oribatida	Oribatida_indet.	Sp?	P	P	P
		Oxidae?	Oxidae?_indet.	Sp?		P	
		Pezidae	Pezidae_indet	Sb		P	

Higher taxon rank 1	Taxon rank 2	Taxon rank 3 (family)	Lowest identified taxon	GHA*	BR H	MC H	MC A
		Unionicolidae?	Unionicolidae?_indet.	Sp?		P	P
		Acarina_Fam. indet.	**Acarina_Fam. indet.**	Sp?/Ac?	P	P	P
Insecta	Coleoptera	Elmidae	Elmidae_indet.	Sx?	P	P	
		Hydraenidae	Hydraenidae_indet	Sx?	P		
		Hydrophilidae	Hydrophilidae_indet	Sx?	P		
		Coleoptera_Fam. indet.	**Coleoptera_Fam. indet.**	Sx?	P		
	Diptera	Ceratopogonidae	Ceratopogonidae_indet	Sx	P	P	
		Chironomidae	Chironomidae_indet.	Sx	P	P	
		Psychodidae	Psychodidae_indet	Sx	P	P	
		Simulidae	Simuliidae_indet.	Ep		P	
		Tipulidae	Tipulidae_indet	Sx	P		
		Diptera_Fam. indet.	**Diptera_Fam. indet.**	Sx?	P	P	P
	Ephemeroptera	Ephemeroptera_Fam. indet.	Ephemeroptera_indet.	Sx	P	P	
	Odonata	Odonata Fam. indet.	Odonata_indet.	Ep		P	
	Trichoptera	Ecnomidae	Ecnomidae_indet	Ep	P	P	
		Trichoptera_Fam. indet.	Trichoptera_indet.	Ep	P	P	
	Insecta indet.	Insecta_Fam. indet.	**Insecta_Fam. indet.**	Sx?	P	P	
Tardigrada	Tardigrada	Tardigrada	Tardigrada	Ep	P	P	
Mollusca	Bivalva	Sphaeriidae	Sphaeriidae indet.	Ep		P	
	Gastropoda	Physidae	Physa acuta	Ep	P		
		Gastropoda_Fam. Indet	Gastropoda_indet	Ep		P	
Rotifera	Rotifera	Rotifera_Fam. indet.	Rotifer_indet.	Ep	P	P	P
Hydrozoa	Hydrozoa	Hydridae	Hydridae_indet	Ep	P		
Nematomorpha (Gordian worms)	Nematomorpha	Nematomorpha	Nematomorpha_indet	P		P	
Platyhelminthes (flat worms)	Turbellaria	Turbellaria_Fam. Indet	Turbellaria_indet.	Sp/Sb?	P	P	P
			Turbellaria_indet. (eyed)	Ep			P
Nematoda	Nematoda (round worms)	Nematoda indet.	Nematoda indet.	Sp	P	P	P
Annelida	Oligochaeta (segmented worms)	Oligochaeta_Fam. indet.	Oligochaeta_indet.	Sp	P	P	P

Total morpho-taxa	31	39	24
Total families	31	33	21

***Key to groundwater habitat affinity (GHA) categories:**

Sb	Stygobite
Sp	Stygophile
Sp?	Stygophile (uncertain/inferred)
Sp/Sb?	Stygophile/possible Stygobite
Sx	Stygoxene
Sx?	Stygoxene (uncertain/inferred)
Sx/Sp?	Stygoxene/possible Stygophile
Ep	Epigean
P	Parasite
Ac	Accidental / Terrestrial
Unknown	

At higher taxonomic levels the hyporheic invertebrate assemblages were very similar between Bremer River and Maules Creek, with all groups frequently represented in groundwater (Crustacea, Acarina, Oligochaeta, Nematoda, Turbellaria), and numerous groups characteristic of stream benthos and occasional hyporheos (Figure 98 A, B). Copepoda were the numerically dominant taxon at most sites. Few of the groups characteristic of stream benthos and occasional hyporheos were detected in the Maules Creek aquifer, which as expected was dominated by typical groundwater groups (Figure 98 C).

In terms of the classification of morpho-species according to their groundwater habitat affinity, both Bremer River and Maules Creek hyporheos assemblages recorded similar proportions of taxa in each category (Figure 99 A, B). Taxa with high dependence on groundwater (stygobites + stygophiles) represented 43% and 54% of the invertebrate assemblages at Bremer and Maules respectively. In contrast to the hyporheic zone, the aquifer was dominated almost exclusively by stygobitic and stygophilic taxa (86%), with few stygoxenic / epigean taxa as expected (Figure 99 C). The higher-level taxonomic composition of stygobitic and stygoxenic groups was similar at both areas, but three stygobitic families (Psammaspididae, Janiridae, Phreatoicidae) collected at Maules were not detected in the Bremer, although this does not preclude their possible occurrence (Table 22).

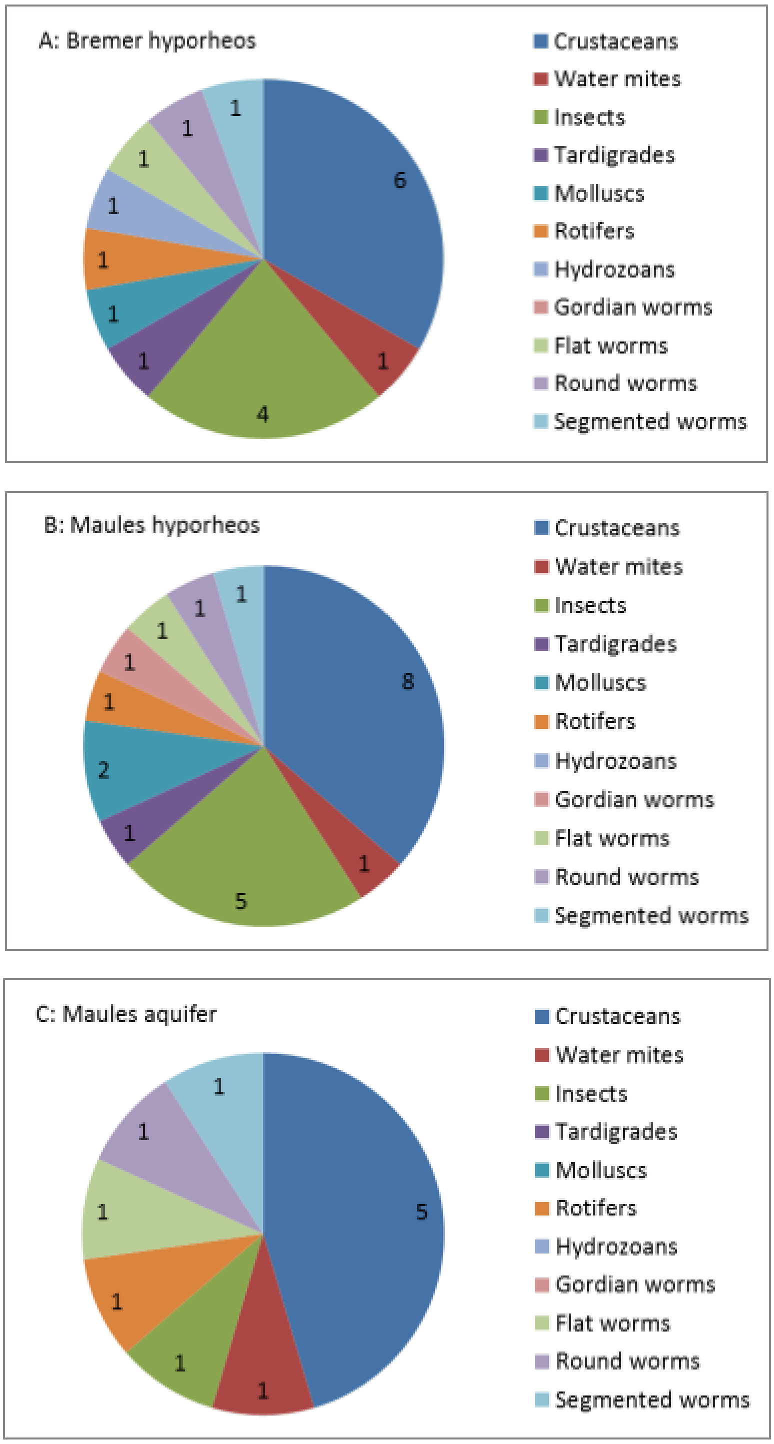


Figure 98: Comparison of the higher rank taxonomic composition in hyporheic and aquifer invertebrates identified in the Bremer River hyporheic zone (A), Maules Creek hyporheic zone (B), and Maules Creek aquifer (C). The number of nominal "orders" identified within each taxonomic group is labelled. At higher taxonomic levels the hyporheic invertebrate assemblages were very similar between Bremer River and Maules Creek, with all groups frequently represented in groundwater (Crustacea, Acarina, Oligochaeta, Nematoda, Turbellaria), and numerous groups characteristic of stream benthos and occasional hyporheos.

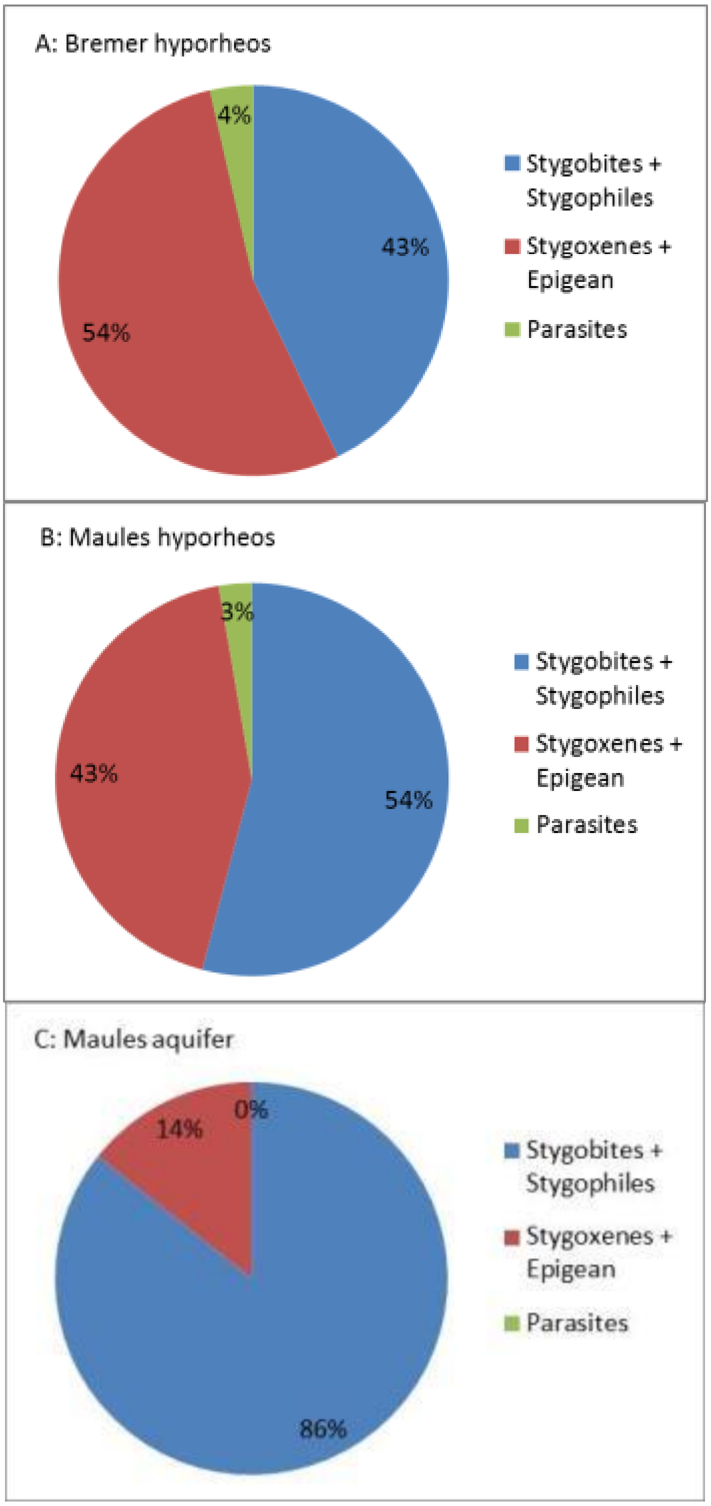


Figure 99: Proportions of morpho-taxa grouped according to their groundwater habitat affinity (GHA), collected in the Bremer River hyporheic zone (A), Maules Creek hyporheic zone (B), and Maules Creek aquifer (C). Bremer River and Maules Creek hyporheos assemblages recorded similar proportions of taxa in each GHA category, while the aquifer assemblage was dominated almost exclusively by stygobitic and stygophilic taxa, with few stygoxenic / epigean taxa.

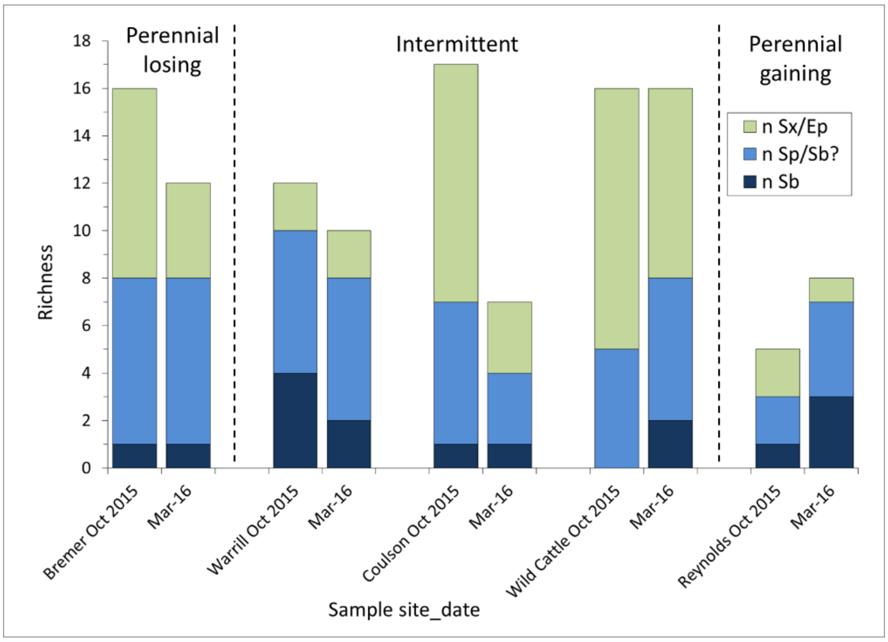
The contrast between the aquifer and hyporheic environments extends beyond the lower overall richness associated with the virtual absence of stygoxenic and epigean taxa in the aquifer. In terms of the number of animals recovered per unit volume of water pumped from the aquifer and hyporheic zone, the average number of animals extracted from the aquifer (excluding the purge volume samples) was an order of magnitude lower (average 1.1 animals / 10 litres pumped) than for the hyporheic zones at both Maules (34.1) and Bremer (47.3) (Table 23). Similarly, the average density of animals captured from inside the bore casing (=purge volume samples) was nearly eight times (8.0 animals / 10 litres) as high as in the aquifer matrix surrounding the bores (= post-purge samples).

Table 23: Overall sampling effort in Bremer and Maules catchments (hyporheic, bore, aquifer), showing the total number of samples, pumped volumes and the capture rate of animals expressed as: (1) average number of individuals per sample (range in brackets); (2) individuals per unit pumped volume, standardised to individuals per 10 litres for comparison between hyporheic and aquifer habitats, and, comparison of capture rates inside the bore and the surrounding aquifer. In terms of the number of animals recovered per unit volume of water pumped from the aquifer and hyporheic zone, the average number of animals extracted from the aquifer (excluding the purge volume samples) was an order of magnitude lower than for the hyporheic zones at both Maules and Bremer.

Location + environment	n samples	Total pumped volume (l)	Total individuals	Average individuals / sample (range)	Standardised individuals / 10 l pumped
Bremer hyporheic	57	342	1619	28.4 (0 - 318)	47.3
Maules hyporheic	100	600	2046	20.5 (0 - 222)	34.1
Maules bores purge volume	28	855	681	24 (0 - 196)	8.0
Maules aquifer post-purge	as above	3700	418	14.9 (0 - 80)	1.1

4.5.4.2 Bremer River hyporheos

Hyporheic invertebrate taxon richness and abundance recorded at the Bremer catchment sites are shown in Figure 100. The number of taxa recorded per site per sample period ranged from five to seventeen. Coulson Creek, Wild Cattle Creek and Bremer River were the richest sites. Warrill Creek was moderately rich, and Reynolds Creek recorded the lowest number of taxa. The abundance of taxa recorded per site per sample period was highly variable, both within and among sites, and ranged from only six individuals (Reynolds Creek in October 2015) to 523 individuals (Wild Cattle Creek, March 2016). Within sites, the number of taxa collected in each 6 L subsample (n=6) ranged from zero to thirteen (across all sites), and the number of individuals ranged from zero to >300. Comparing sample periods within each site, Bremer River, Warrill and Coulson Creeks recorded fewer taxa and individuals in the second round than in the first round, whereas Wild Cattle and Reynolds Creeks displayed the opposite trend, except that taxon richness at Wild Cattle Creek remained steady. In terms of groundwater habitat affinity, stygobitic and stygophilic taxa were identified at all five upper Bremer catchment creeks, and their abundance ranged from 57 to 96% of collected individuals within creeks (Figure 100). The hyporheos assemblages also included numerous families of stygoxenic/ epigean aquatic insects, which, while contributing to site richness (13 to 69% of taxa across sites) were of low abundance (mean 23% of individuals across sites; range 4 to 43%).



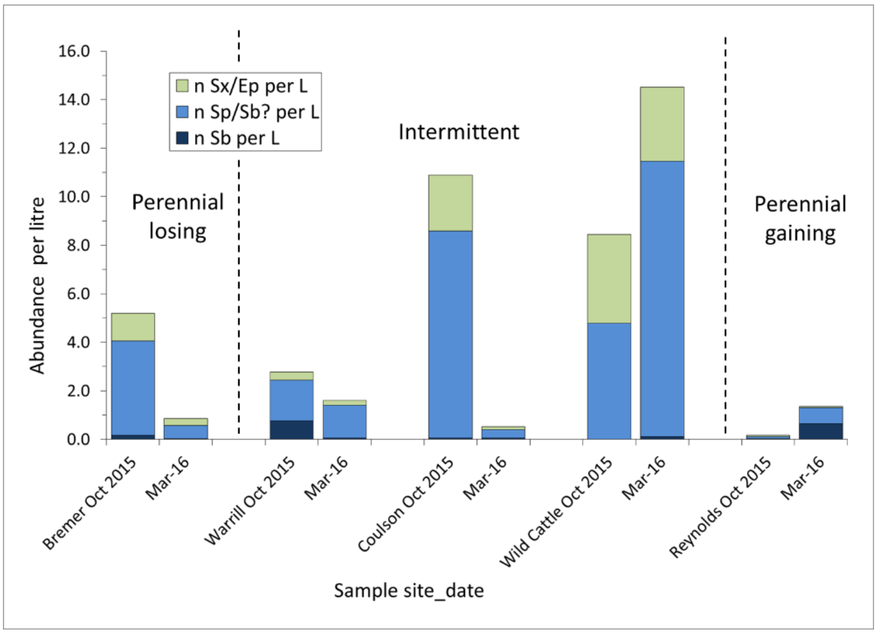


Figure 100: Bremer catchment hyporheic site richness (upper) and abundance (lower) recorded in October 2015 and February-March 2016 showing proportions of groundwater habitat affinity groups: Sb, stygobites; Sp, stygophiles; Sx, stygoxenes; Ep, epigean. Flow regimes were interpreted to be intermittent, except for Bremer River (perennial losing) and Reynolds Creek (perennial gaining). Stygobitic and stygophilic taxa were identified at all five upper Bremer catchment creeks, and the hyporheos assemblages also included numerous families of stygoxenic/ epigean aquatic insects, which, while contributing to site richness were of low abundance.The abundance of taxa recorded per site per sample period was highly variable, both within and among sites.

Multivariate analysis revealed differences in assemblages between sites and sampling dates, and no obvious patterns in relation to flow regime (Figure 101). Nine taxa were influential (Pearson r > 0.4) in the ordination structure, and included representatives of all groundwater habitat affinity groups (Appendix 3 Table 50). The most influential taxa were Nematoda (stygophile), Chironomidae (stygoxene) and Cyclopoida (stygophile/potential stygobite), and one stygobite ‘indicator’ (of connectivity to permanent groundwater habitat) taxon, Bathynellidae, was influential. Ordination assemblage patterns appeared to be strongly influenced by both groundwater habitat affinity groups - stygoxenes/epigean richness and stygobite/stygophile richness – as indicated by univariate biotic indices vectors (Figure 102). In this respect, Bremer catchment contrasted with the Maules catchment where ordination assemblage patterns appeared to be predominantly influenced by one groundwater habitat affinity group, namely stygobite/stygophile richness and abundance (see later below). A second contrast was that 'indicator' taxa (of connectivity to permanent groundwater habitat) – Bathynellidae, Parabathynellidae and Neoniphargidae - although present, were not influential in the assemblage ordination structure. Nine hydrochemistry variables correlated (Pearson r > 0.4) with the invertebrate assemblage structure (Figure 101). The most influential variable positively correlated with richness and abundance was DO, and the most influential inversely correlated variable was Bromide (Appendix 3 Table 50). Similarly to the Maules catchment, EC, Na, Mg, Ca, As and chloride were influential, however reduced ionic species, nutrients and organics were weakly correlated. There was weak evidence for an association with the volume of fine sediment, and very weak evidence for an association with hydraulic gradient (VHH) (Appendix 3 Table 50).

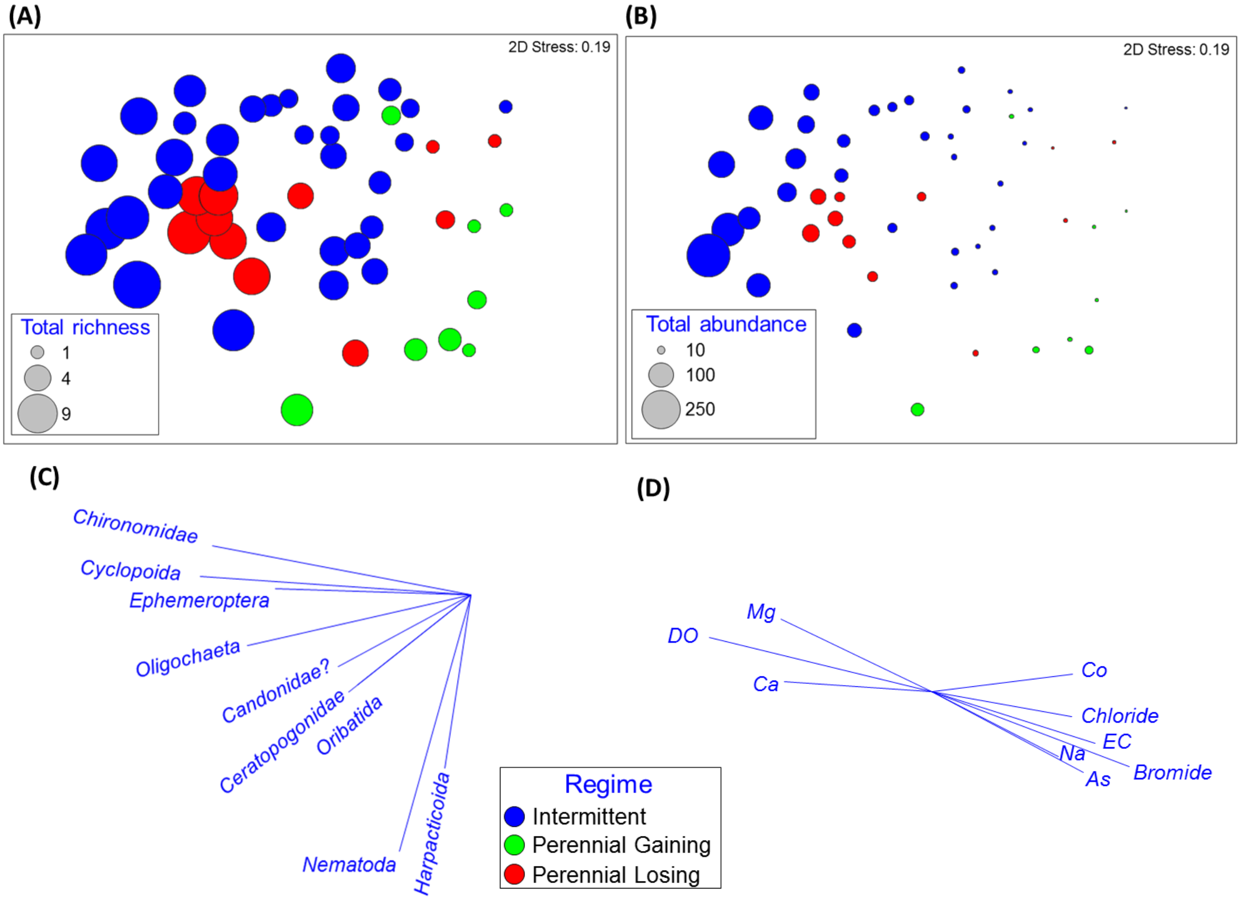


Figure 101: Multivariate analysis revealed differences in assemblages between sites and sampling dates, and no obvious patterns in relation to flow regime. Non-metric multidimensional scaling (nMDS) ordination bubble plots of hyporheos assemblages depicting richness (A) and abundance (B) for all samples in the Bremer catchment. The vectors represent influential invertebrate taxa (C) and hydrochemistry variables (D) that were most correlated (Pearson r > 0.4) to the average dissimilarity between hyporheos samples. Samples are coloured by the observed creek flow regime at time of sampling: intermittent (blue), perennial gaining (green), perennial losing (red). Invertebrate abundance data square root transformed (+dummy variable). Environmental variables with skewed distributions were transformed to approximate normality and then all variables were normalised.

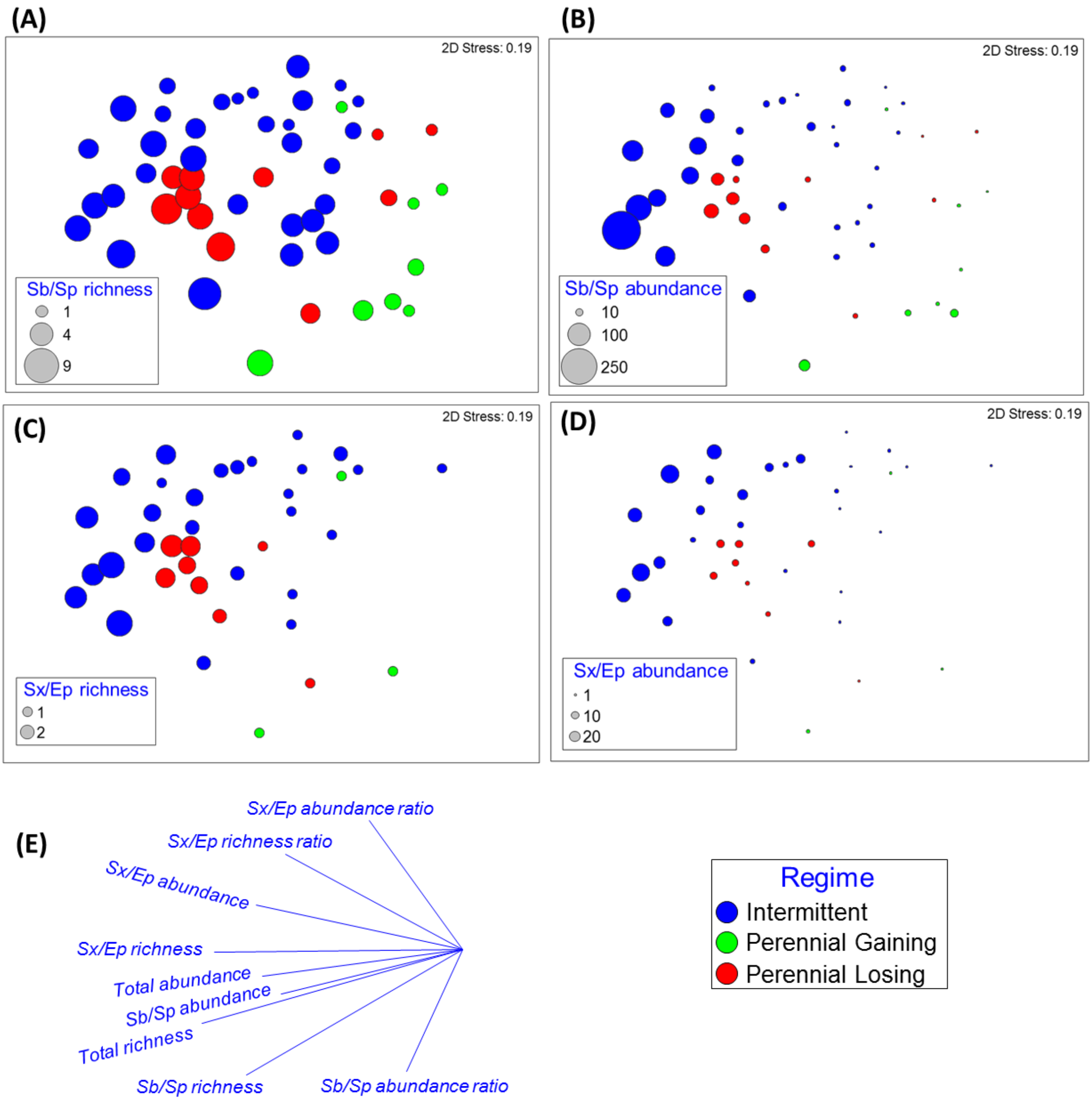


Figure 102: Ordination assemblage patterns appeared to be strongly influenced by both groundwater habitat affinity groups - stygoxenes/epigean richness and stygobite/stygophile richness (compare this with Maules Creek which appeared to be influenced, predominantly, by one groundwater habitat affinity group - stygobite/stygophile, richness and abundance). Non-metric multidimensional scaling (nMDS) ordination bubble plots of hyporheos assemblages for all samples in the Bremer catchment; bubble plots show differences between functional group: (A) Stygobite/stygophile (Sb/Sp) richness; (B) Sb/Sp abundance; (C) Stygoxene/epigean (Sx/Ep) richness; (D) Sx/Ep abundance. The vectors (E) represent influential univariate biotic indices that were most correlated (Pearson r > 0.4) to the average dissimilarity between hyporheos samples. Samples are coloured by the observed creek flow regime at time of sampling: intermittent (blue), perennial gaining (green), perennial losing (red). Invertebrate abundance data square root transformed (+dummy variable). Environmental variables with skewed distributions were transformed to approximate normality and then all variables were normalised.

After excluding sites/dates with few samples (n<3) and a few deep samples (most Bremer samples were limited to shallow depth due to thin alluvium), PERMANOVA tests of the null hypotheses of no differences in hyporheos assemblages based on flow regime, sample site and sample date, suggested that sample date was highly significant (p=0.0001). There was no support for an effect due to sample site, flow regime or VHH, however there was strong support for effects due to DO as a continuous co-variable (Table 24).

Table 24: PERMANOVA test of the null hypothesis of no differences in hyporheos assemblages based on flow regime, sample site and sample date, suggested that sample date was highly significant (p=0.0001) while there was no support for an effect due to sample site, flow regime or vertical hydraulic head (VHH), however there was strong support (p=0.0015) for effects due to dissolved oxygen (DO) as a continuous co-variable.

Source	df	Pseudo-F	P(perm)
VHH (cm)	1	0.72	0.6726
DO (mg/L)	1	3.55	**0.0015**
Regime	2	1.62	0.2791
Site(Regime)	2	0.76	0.6305
Date X (Site(Regime))	4	3.83	**0.0001**
Residuals	35		

4.5.4.3 Maules Creek hyporheos

Maules catchment exhibited similar high sample variability as found in the upper Bremer catchment. Within the Maules catchment, the number of taxa collected in each 6 L subsample ranged from zero to twelve (across all sites), and the number of individuals ranged from zero to >200 (Figure 103). The number of taxa recorded per site per sample period ranged from one to nineteen. In terms of groundwater habitat affinity groups, stygophilic taxa were recorded at all sites, and stygoxenic / epigean taxa at most sites. Stygobites were represented at most intermittent and perennial gaining sites, but ‘indicator’ taxa (of connectivity to permanent groundwater habitat) - Syncarida, Amphipoda and Isopoda - were not detected in the perennial losing section. In terms of abundance, stygophiles and stygobites were the numerically dominant categories at nearly all intermittent and perennial gaining sites (mean 89% of individuals across sites; range 41 to 100%). In the losing section, stygophiles made up most of the individuals. As observed at Bremer River, stygoxenes and epigean taxa comprised a relatively small proportion of sampled abundance, with one exception, Pool 4 in Middle Creek which was dominated by rotifers on one sampling occasion.

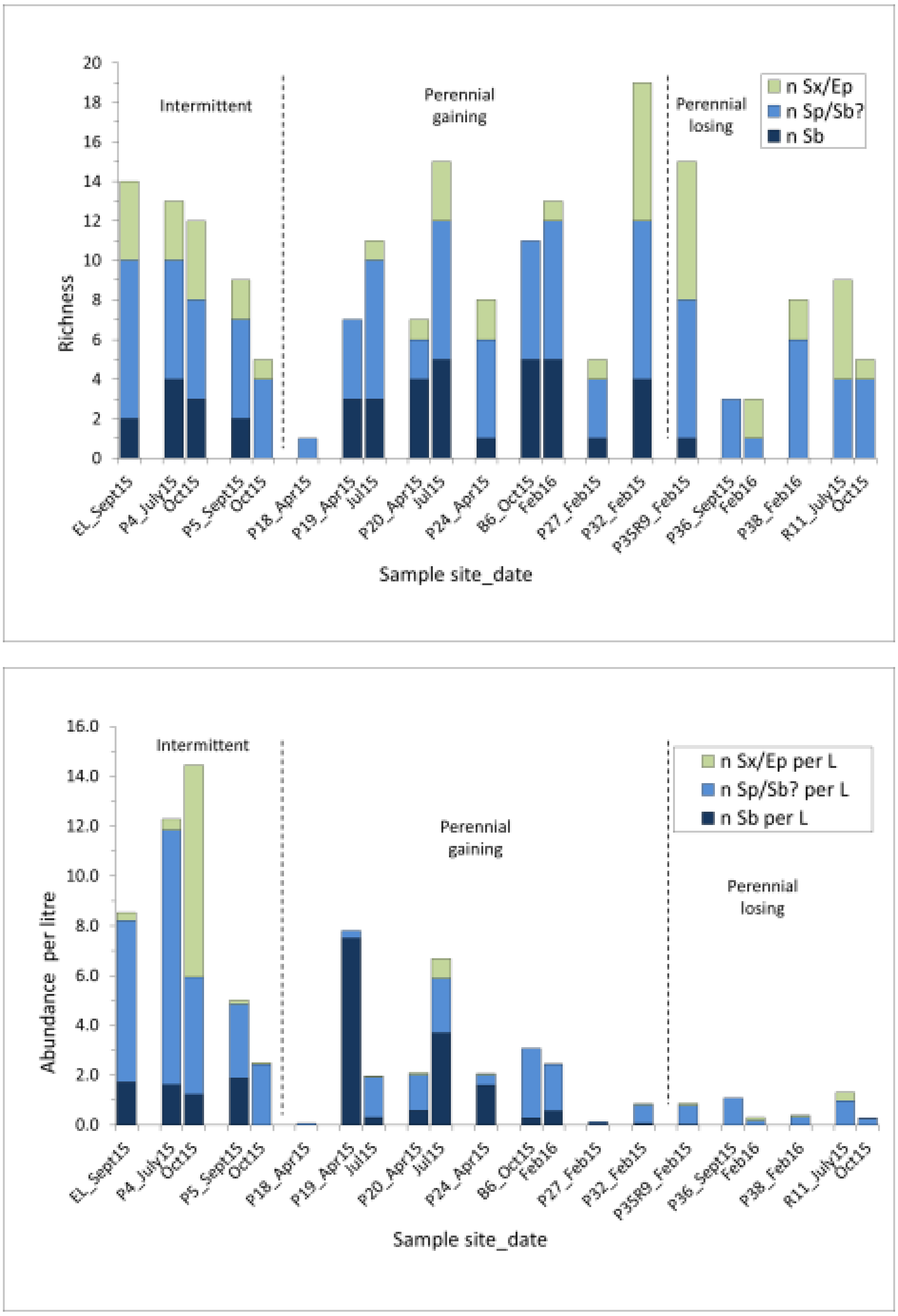


Figure 103: Maules catchment hyporheic site richness (upper) and abundance (lower) recorded at various sites and dates between February 2015 and February 2016, showing proportions of groundwater habitat affinity groups: Sb, stygobites; Sp, stygophiles; Sx, stygoxenes; Ep, epigean. Sites are grouped by flow regime and in order from upstream (left) to downstream. Stygobites were represented at most intermittent and perennial gaining sites, but were not detected in the perennial losing section. Stygophiles and stygobites were the numerically dominant categories at nearly all intermittent and perennial gaining sites, and as observed at Bremer River, stygoxenes and epigean taxa comprised a relatively small proportion of sampled abundance, with one exception, Pool 4 in Middle Creek. Maules catchment exhibited similar high sample variability as found in the Bremer catchment.

Multivariate analysis revealed differences in assemblages between sites and sampling dates, and possibly some structure in the ordination plot in relation to flow regime (Figure 104). Nine taxa, all stygobites or stygophiles, were influential (Pearson r > 0.4) in the ordination structure (Appendix 3: Table 56). There were no influential stygoxene taxa, unlike the Bremer catchment. The most influential taxon was

Cyclopoida, followed by Oligochaeta (stygophile/potential stygobite), Harpacticoida and Nematoda. Three (possibly four) ‘indicator’ taxa (of connectivity to permanent groundwater habitat) were influential: Bathynellidae, Psammaspididae, Neoniphargidae (and Harpacticoida). Ordination assemblage patterns appeared to be predominantly influenced by one groundwater habitat affinity group, namely stygobite/stygophile richness and abundance – as indicated by univariate biotic indices vectors (Figure 105). In this respect, Maules catchment contrasted with the Bremer catchment where ordination assemblage patterns appeared to be strongly influenced by both functional groups - stygoxenes/epigean richness and stygobite/stygophile richness (see above).

Twenty-five (25) hydrochemistry variables correlated (Pearson r > 0.4) with the invertebrate assemblage structure (Figure 104, Appendix 3 Table 56). The most influential were EC, Ca, Mg, Na, Sr, chloride, and humics (processed and unprocessed), for which all vectors were associated with lower richness and abundance. Vectors for influential reduced ionic species, Fe, Mn, ammonia, and the nutrient P, were also inversely associated with richness and abundance. In contrast, vectors for DO, nitrate, NOx and Cu were associated with higher richness and abundance. There was weak evidence for an association with hydraulic gradient (VHH), and very weak evidence for an association with the volume of fine sediment (Appendix 3 Table 56).

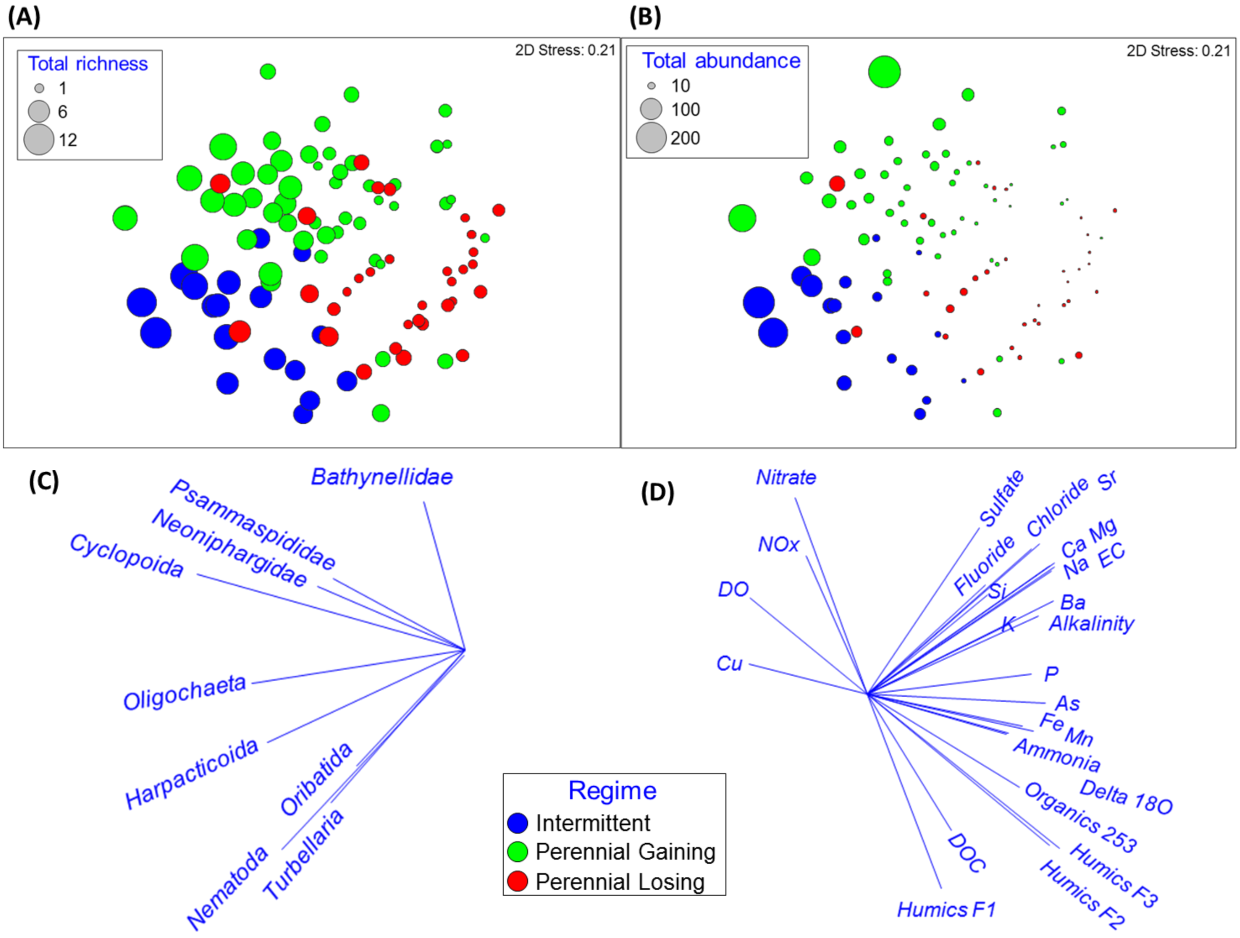


Figure 104: Multivariate analysis revealed differences in assemblages between sites and sampling dates, and possibly some structure in the ordination plot in relation to flow regime. Non-metric multidimensional scaling (nMDS) ordination bubble plots of hyporheos assemblages depicting richness (A) and abundance (B) for all samples in the Maules catchment. The vectors represent influential invertebrate taxa (C) and hydrochemistry variables (D) that were most correlated (Pearson r > 0.4) to the average dissimilarity between hyporheos samples. Samples are coloured by the observed creek flow regime at time of sampling: intermittent (blue), perennial gaining (green), perennial losing (red). Invertebrate abundance data square root transformed (+dummy variable). Environmental variables with skewed distributions were transformed to approximate normality and then all variables were normalised.

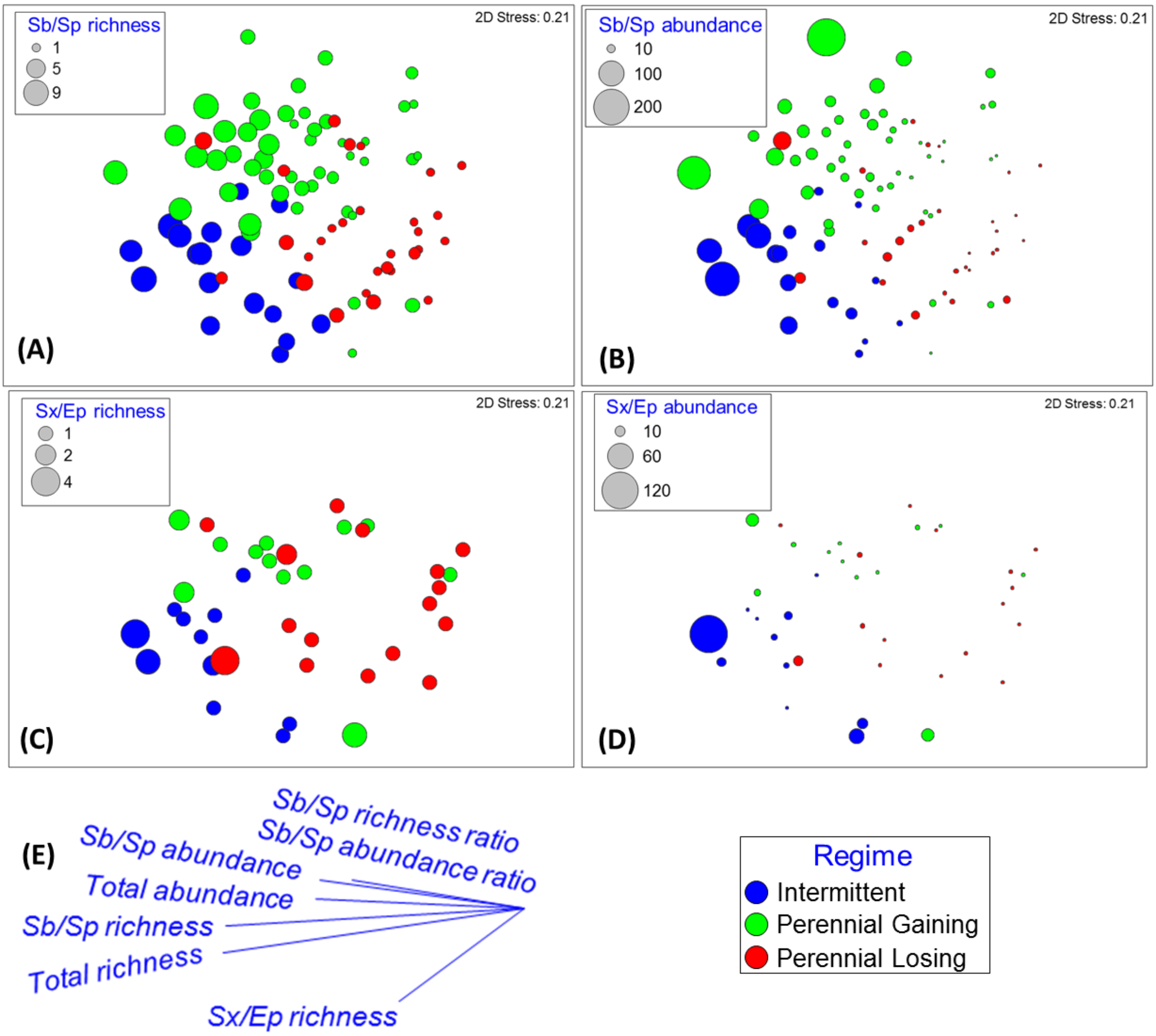


Figure 105: Ordination assemblage patterns appeared to be predominantly influenced by one groundwater habitat affinity group, namely stygobite/stygophile richness and abundance (compare this with Bremer catchment which appeared to be influenced by both groundwater habitat affinity groups - - stygoxenes/epigean richness and stygobite/stygophile richness). Non-metric multidimensional scaling (nMDS) ordination bubble plots of hyporheos assemblages for all hyporheos samples in the Maules catchment; bubble plots show differences between functional group: (A) Stygobite/stygophile (Sb/Sp) richness; (B) Sb/Sp abundance; (C) Stygoxene/epigean (Sx/Ep) richness; (D) Sx/Ep abundance. The vectors (E) represent influential univariate biotic indices that were most correlated (Pearson r > 0.4) to the average dissimilarity between hyporheos samples. Samples are coloured by the observed creek flow regime at time of sampling: intermittent (blue), perennial gaining (green), perennial losing (red). Invertebrate abundance data square root transformed (+dummy variable). Environmental variables with skewed distributions were transformed to approximate normality and then all variables were normalised.

MDS ordination suggested differences between samples and sites (plot not shown) based on their flow regime class (Figure 104). Also of interest were the possible influences of VHH and DO as continuous co-variables (non-interacting) (Figure 107, Figure 112). After excluding sites/dates with few samples, missing co-variables or only one depth stratum, PERMANOVA tests of the null hypotheses of no differences in hyporheos assemblages based on flow regime, sample site, sample date and sample depth, suggested that sample depth was significant (p=0.004), and that depth and regime interacted (p=0.03) (Table 24) . The co-variables VHH and DO were significant (p=0.02 and p=0.03 respectively) and these site-proximal variables strongly influenced the PERMANOVA model and reduced the reach-catchment scale effect of regime which otherwise appeared significant when these co-variables were excluded (analyses not shown).

Table 24: PERMANOVA test of the null hypothesis of no differences in hyporheos assemblages based on flow regime, sample site, sample date and sample depth, suggested that sample depth was significant (p=0.004), and that depth and regime interacted significantly (p=0.03). The continuous co-variables, vertical hydraulic head (VHH) and dissolved oxygen (DO), were also significant.

Source	df	Pseudo-F	P(perm)
VHH	1	2.72	**0.02**
DO	1	1.95	**0.03**
Regime	2	1.71	0.19
Depth	1	4.88	**0.004**
Site(Regime)	7	2.18	0.11
RegimexDepth	2	2.56	**0.03**
Date(Site(Regime))	3	1.23	0.23
Site(Regime)xDepth	7	1.22	0.40
Date(Site(Regime))xDepth	3	0.61	0.87
Residuals	56		

4.5.4.4 Maules Creek aquifer

Stygofauna were detected in all, except two, of the 15 sampled bores tapping the regional aquifers alongside Maules Creek and Middle Creek. The sampling did not detect stygofauna in bores BH21-1 and BH21-2, both of which were contaminated with oil when sampled. Overall richness and abundance in the bores containing fauna ranged from one to nine taxa, and up to 260 individuals, although most samples contained < 100 individuals (Figure 106).

Interestingly, stygofauna were detected in the two bores (BH20-4, BH12-4) screened in the deeper Maules Creek Formation aquifer. Stygofauna taxon richness and abundance in the deep bores were lower than in most other bores in the shallow aquifer, and comprised nematodes, oligochaetes, rotifers, copepods, ostracods and possibly water mites (identification unconfirmed). There was no obvious association between invertebrate richness or abundance and the inferred degree of hydrologic connectivity (high, medium, low, none) between the aquifer and adjacent creek based on interpretation of the geochemical similarity of bore water to creek water (refer to Table 24). For example, Bores 19-1 and BH19-2 had very low geochemical similarity to creek water but moderate to high invertebrate richness and abundances.

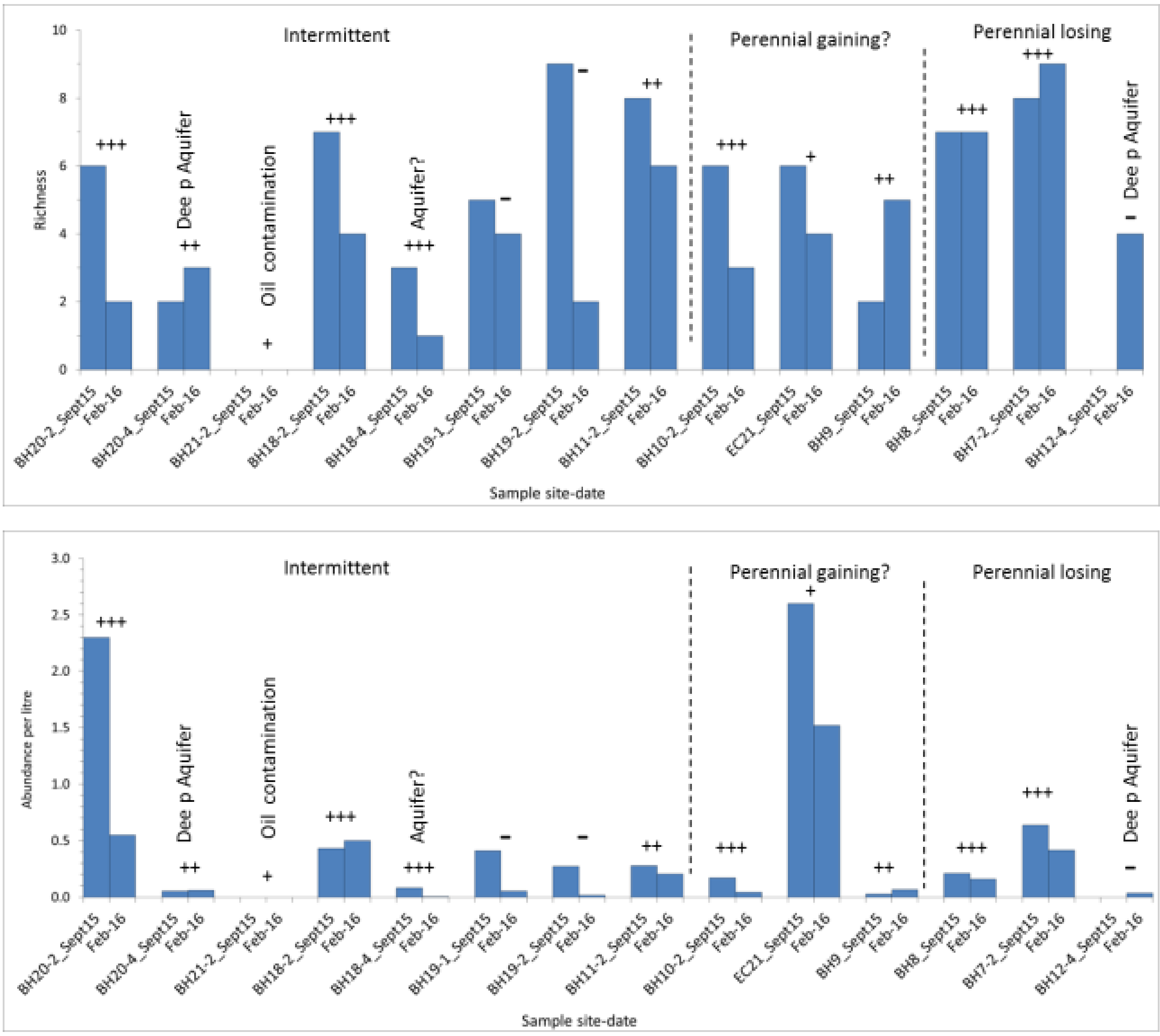


Figure 106: Maules catchment aquifer bores sampled in September 2015 and February 2016: richness (upper) and abundance (lower). Sites are grouped by flow regime of adjacent creek, arranged in order from upstream (left) to downstream. Groundwater habitat affinity groups are not distinguished because the aquifer assemblage was dominated by stygobites and stygophiles. Stygofauna were detected in all, except two, of the 15 sampled bores tapping the regional aquifers alongside Maules Creek and Middle Creek, and overall richness and abundance ranged from one to nine taxa, and up to 260 individuals, although most samples contained < 100 individuals. There was no obvious association between invertebrate richness or abundance and the inferred degree of hydrologic connectivity between the aquifer and adjacent creek based on interpretation of the geochemical similarity of bore water to creek water: high '+++'; medium '++'; low '+'; none '-'.

Multivariate analysis revealed differences in assemblages between sites and sampling dates, but no obvious patterns in relation to the flow regime of the creek adjacent to the bore, or the degree of hydrochemical similarity of aquifer and adjacent creek waters (Figure 107). Fourteen taxa were influential (Pearson r > 0.4) in the ordination structure; unsurprisingly most of these taxa were stygobites and stygophiles (Appendix 3: Table 56). The most influential taxa were Cyclopoida, Acarina and one 'indicator' taxon (of connectivity to permanent groundwater habitat), Parabathynellidae. Other less influential taxa included Bathynellidae, Harpacticoida and Candonidae.

Four hydrochemistry variables correlated (Pearson r > 0.4) with the invertebrate assemblage structure (Figure 107, G): pH, Ba, humics (Absorbance at 253 nm) and oxygen isotope ratio (delta 18O). DO was weakly correlated, and of the reduced ionic species, Fe^{2+} was the most strongly, and negatively, correlated

(0.37 MDS1). Nutrients, DOC and other organics were predominantly negatively correlated (Appendix 3: Table 56).

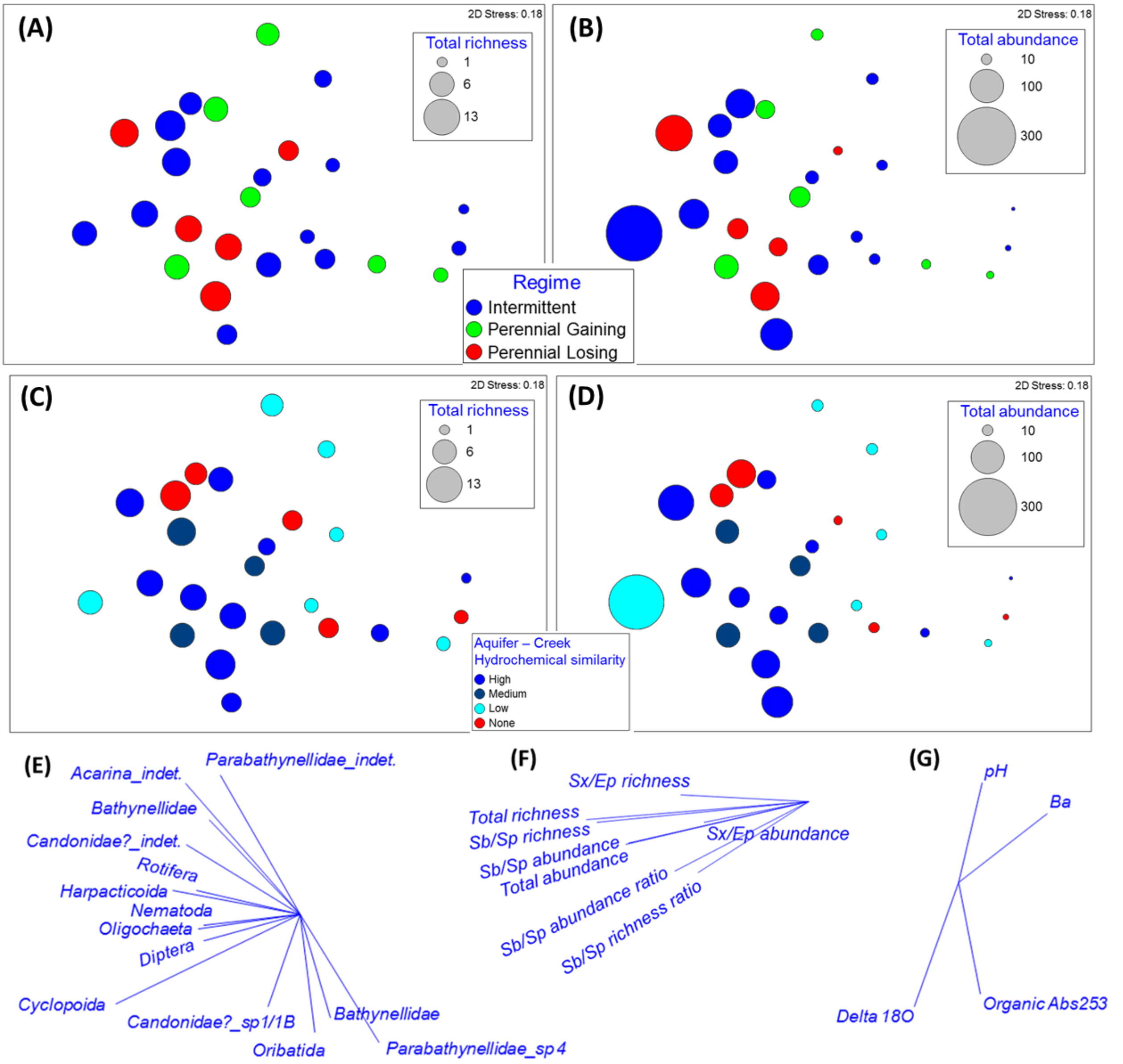


Figure 107: Multivariate analysis revealed differences in assemblages between sites and sampling dates, but no obvious patterns in relation to the flow regime of the creek adjacent to the bore, or the degree of hydrochemical similarity of aquifer and adjacent creek waters. Non-metric multidimensional scaling (nMDS) ordination bubble plots of Maules catchment aquifer assemblages depicting richness and abundance for all samples coloured by: Flow regime of adjacent creek (A,B); hydrochemical similarity of aquifer and adjacent creek waters (C,D). The vectors represent influential invertebrate taxa (E), univariate biotic indices (F) and hydrochemistry variables (G) that were most correlated (Pearson r > 0.4) to the average dissimilarity between invertebrate samples. Invertebrate abundance data square root transformed (+dummy variable). Environmental variables with skewed distributions were transformed to approximate normality and then all variables were normalised.

MDS ordination suggested there may be differences in assemblages between sites (Figure 108). Also of interest was the possible influence of DO as a covariable, in addition to the possible effect of creek flow regime considering its effect on adjacent hyporheic assemblages, and also, the degree of aquifer-creek connectivity inferred from hydrochemical similarity of aquifer and adjacent creek waters. After excluding the contaminated bore (BH21-4) and three samples missing DO measurements (BH20-2, BH20-4, BH21-2, all February 2016) PERMANOVA tests failed to reject the null hypotheses of no difference in aquifer assemblages based on either, the flow regime of the creek adjacent to the sampled bore, or, the inferred degree of aquifer-creek connectivity, while taking account of aquifer DO (Table 25).

Table 25: PERMANOVA tests failed to reject the null hypotheses of no difference in aquifer assemblages based on either: (Test A) flow regime of creek adjacent to aquifer; or (Test B) the degree of aquifer-creek connectivity inferred from hydrochemical similarity of aquifer and adjacent creek waters, while taking account of aquifer dissolved oxygen (DO) as a continuous co-variable. Refer Figure 109 for flow regime classes and connectivity categories.

Test	Source	df	Pseudo-F	P (perm)
A	DO mg/L	1	0.90	0.44
	Regime	2	0.60	0.81
	Site(Regime)	9	1.20	0.26
	Residuals	10		
B	DO mg/L	1	1.11	0.34
	Connectivity	3	1.44	0.18
	Site(Connectivity)	8	0.97	0.52
	Residuals	10		

Influence of dissolved oxygen and redox state

Both Bremer and Maules catchments showed positive correlations with dissolved oxygen (DO) in relation to richness and abundance (Table 27). Hyporheic and aquifer invertebrates were tolerant of suboxic conditions (DO < 0.5 mg/L) and intolerant of hypoxic-anoxic conditions (DO < 0.01 mg/L), and exhibited a trend of increasing abundance in oxic conditions (DO > 0.5 mg/L) (Figure 112, regression r2 = 0.10). With declining DO conditions and associated shifts in redox chemistry, fauna richness and abundance in the Maules catchment showed strong negative correlations with four reduced ionic species: ammonia, iron, phosphorus and arsenic. We found that when Fe2+ concentrations exceed about 0.1 mg/L, fauna abundance and richness are typically very low (Figure 113). The significant negative association between invertebrates and reduced ionic species was not evident in the Bremer catchment, possibly because fewer of the Bremer samples were anoxic, except for Reynolds Creek, which had the most reduced conditions (Figure 110) and recorded low abundances (Figure 111).

Table 26: Significant (rho > 0.3) Kendall's tau correlation coefficients between hydrochemical variables and taxon richness and abundance (in hyporheic subsamples). Both Bremer and Maules catchments showed positive correlations with dissolved oxygen (DO), and in the Maules catchment, richness and abundance showed strong negative correlations with the reduced ionic species: ammonium, iron (Fe), phosphorus (P) and arsenic (As).

Maules Creek catchment		
	Kendall's tau correlation	
	Richness	Abundance
DO	0.41	0.41
Nitrate	0.34	0.38
Ammonium		-0.31
pH	-0.33	-0.30
Alkalinity	-0.32	-0.32
Unprocessed humics (PARAFAC)	-0.38	-0.43
Processed humics (PARAFAC)	-0.57	-0.54
Na	-0.33	-0.33
Fe	-0.33	-0.32
P		-0.32
As	-0.31	-0.40

Bremer River catchment		
	Kendall's tau correlation	
	Richness	Abundance
DO	0.42	0.42
EC	-0.31	-0.31
Ca		-0.30
Br	-0.42	-0.42

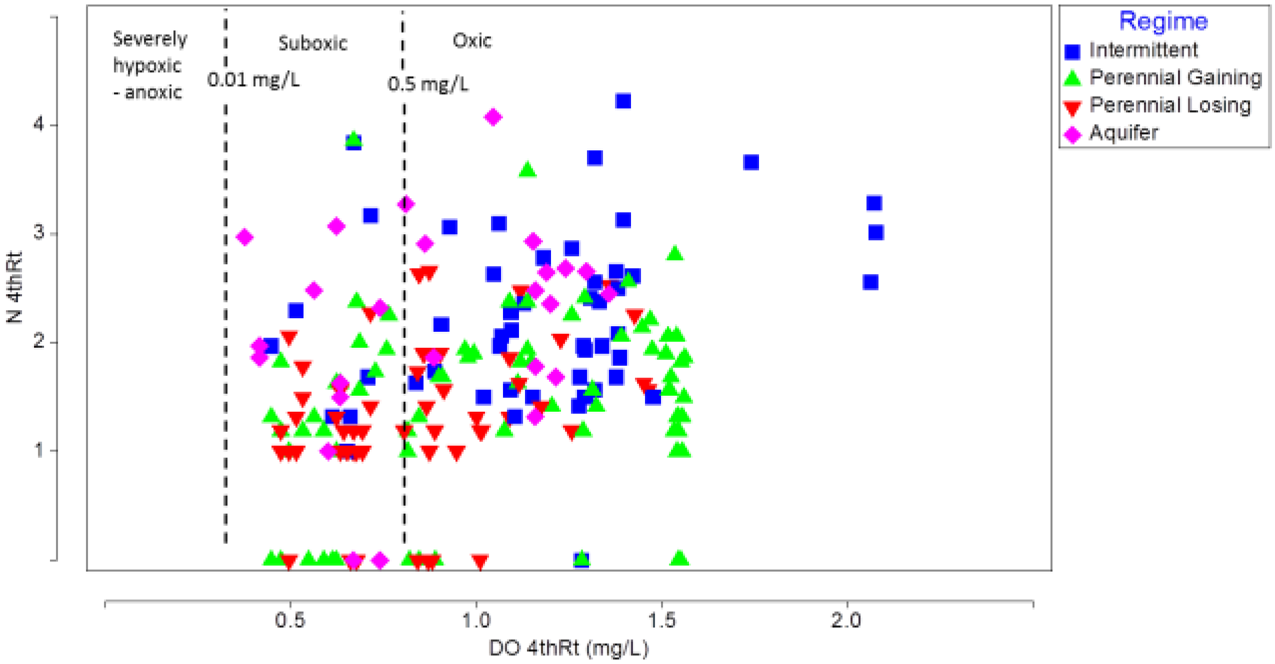


Figure 112: Hyporheic and aquifer invertebrates were tolerant of suboxic conditions (DO < 0.5 mg/L) and intolerant of hypoxic-anoxic conditions (DO < 0.01 mg/L), and exhibited a trend of increasing abundance in oxic conditions (DO > 0.5 mg/L). Bremer and Maules catchments sample abundance (4th root transformed) plotted against sample DO (4th root transformed) labelled by flow regime and groundwater environment (aquifer, stream hyporheic). Approximate DO tolerance thresholds (mg/L untransformed) and categories (oxic, suboxic, hypoxic-anoxic) for groundwater invertebrates based on Hahn (2006); Malard and Hervant (1994).

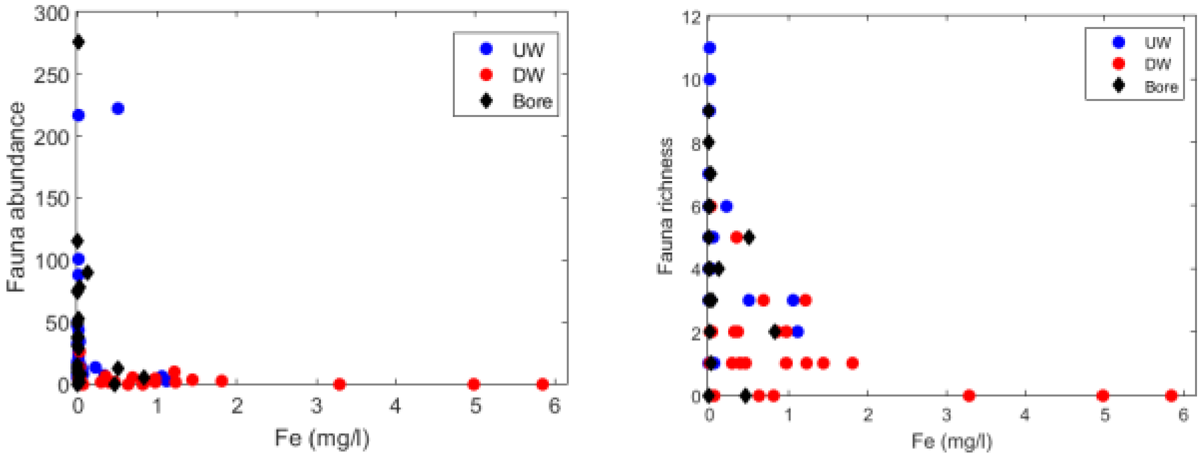


Figure 113: When iron Fe^{2+} concentrations exceeded approximately 0.1 mg/L, fauna abundance and richness were observed to decline. Maules catchment bivariate plots of Fe^{2+} versus fauna abundance and richness, untransformed data. Bore aquifer samples (black) are distinguished from hyporheic samples which are assigned to upwelling type (UW, blue) and downwelling (DW, red) type based on redox chemistry.

Patterns in relation to hyporheic exchange flows

Upwelling and downwelling vertical hydraulic head (VHH) gradients were stronger at Maules catchment sites (range -18.0 to +13.3cm) than at Bremer catchment sites (range -5.3 to +3.8cm). In both catchments, higher richness and abundance was generally associated with upwelling or weakly downwelling zones, and this association was most notable at the sites with an intermittent flow regime (Figure 114).

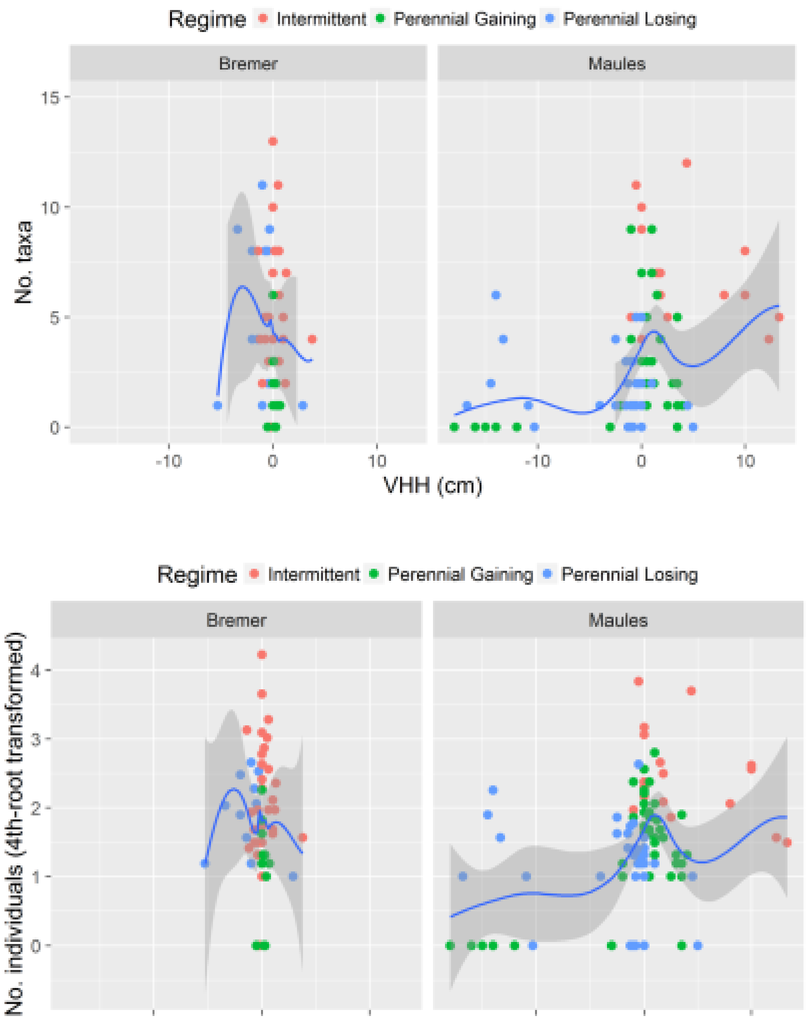


Figure 114: Plot showing the difference in strength of vertical hydraulic head difference (VHH) measured in the Bremer and Maules catchments and associations with invertebrate richness (upper plot) and abundance (lower). Sites are distinguished by their flow regime class. Smoothing line and 95% confidence interval shown. Intermittent samples in Maules catchment (Middle Creek) were limited to creek gaining conditions with no samples collected during losing conditions. Upwelling and downwelling vertical hydraulic head (VHH) gradients were stronger at Maules catchment sites (range -18.0 to +13.3cm) than at Bremer catchment sites (range -5.3 to +3.8cm). In both catchments, higher richness and abundance was generally associated with upwelling or weakly downwelling zones, and this association was most notable at the sites with an intermittent flow regime.

For the Bremer catchment shallow samples (only a few deep samples, so these were excluded), there was weak evidence for lower taxon richness in strongly upwelling sites than in strongly downwelling (3.88 taxa less, $t_{(13)} = 2.08$, $p = 0.058$). Neutral sites also had lower richness, but not significantly so (2.52 fewer taxa, $t_{(13)} = 1.72$, $p = 0.11$) (Figure 115). For total abundance, there was no evidence for any significant difference between VHH categories (both $p > 0.3$) (Figure 115).

For Maules taxon richness, there was no significant interaction between depth and VHH ($F_{(2,45)} = 1.24$, $p = 0.3$) nor any difference between depths ($F_{(1,45)} = 2.31$, $p = 0.14$). Across the levels of VHH there was moderate evidence that strongly upwelling sites had, on average, 1.48 more taxa than strongly downwelling sites ($t_{(48)} = 2.10$, $p = 0.04$) and weaker evidence for a similar increase in neutral sites (mean increase in species richness = 1.40, $t_{(48)} = 1.76$, $p = 0.086$) (Figure 115). For Maules log-transformed total abundance, there was no evidence for any interaction between depth and VHH ($F_{(2,45)} = 1.78$, $p = 0.20$), and weak evidence for the log abundance of shallow samples to be 1.8 × greater than that of deep sites ($F_{(1,45)}$ =

3.53, p = 0.067). As with taxon richness, strongly upwelling sites had more individuals than strongly downwelling (2.19 × as many individuals, ($t_{(47)} = 2.17$, p = 0.03), but there was less evidence for neutral sites to have more individuals (2.10 × as many individuals, $t_{(47)} = 1.82$, p = 0.08) (Figure 115).

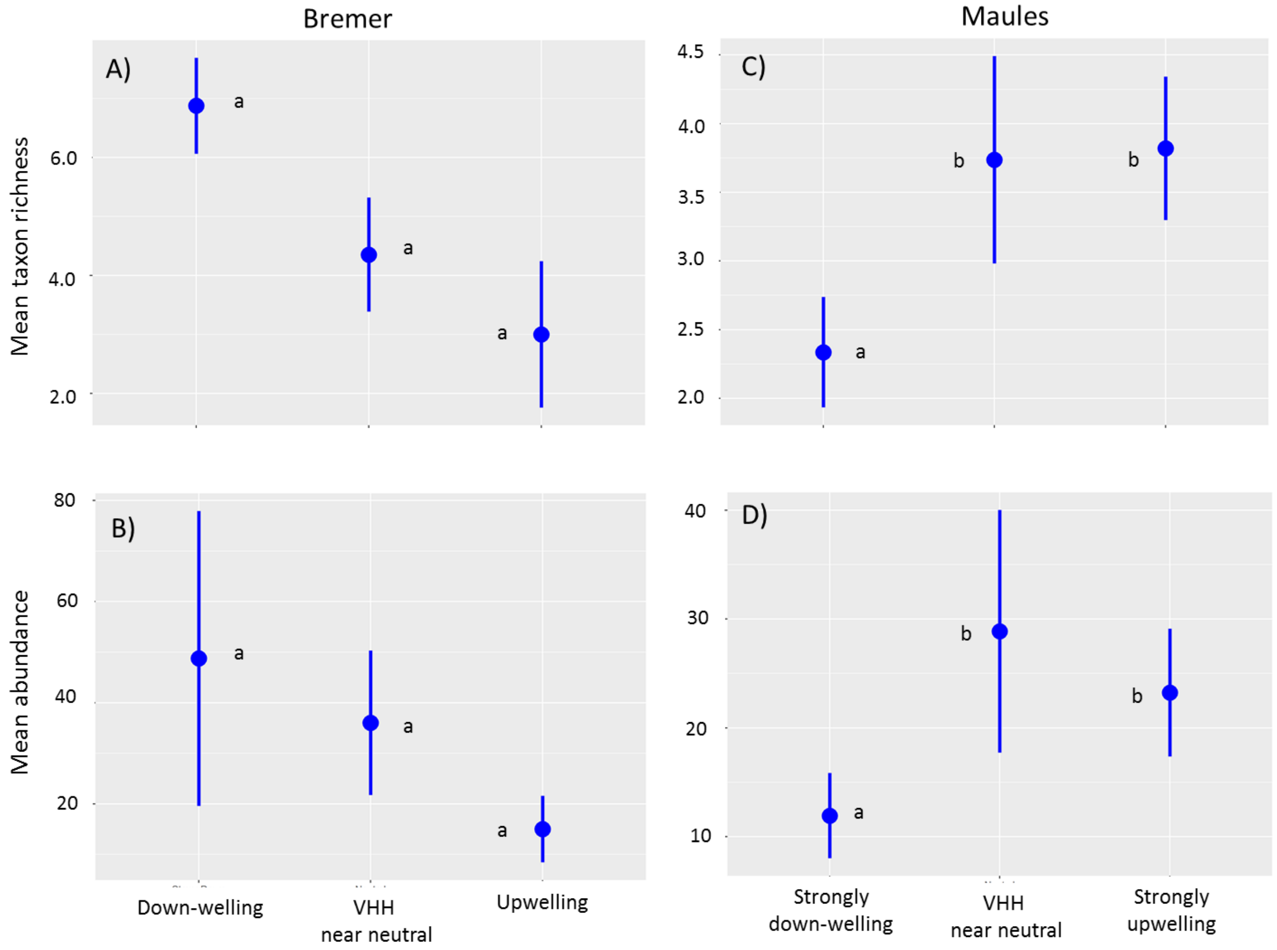


Figure 115: Plots of mean taxon richness and mean abundance (log transformed) ± 1 SE in relation to hyporheic exchange flows in the Bremer (plots A, B) and Maules (plots C, D) catchments. Hydraulic head difference categories based on field measurements of vertical hydraulic head (VHH, cm): "Strongly downwelling" (VHH < -1.0cm); "Strongly upwelling" (VHH > 1.0cm); "Neutral" which encompassed neutral, weakly upwelling/downwelling sites (-0.9 < VHH <+0.9cm). Note that VHH's in the Bremer catchment were not as strong as in the Maules catchment (Figure 116). Means with the same letter are not significantly different (p > 0.05). Maules catchment downwelling samples had significantly lower mean taxon richness and mean abundance than upwelling and VHH near neutral samples. Bremer catchment samples were not significantly different.

Patterns in relation to flow regime

For the Bremer catchment species richness, there was no significant difference by flow regime (both comparisons with intermittent, p > 0.1) (*Figure 117*). Similarly, for log total abundance, we found high within-regime variation and no evidence for any difference between flow regime classes (both p > 0.35) (*Figure 117*).

For the Maules catchment species richness there was no evidence for an interaction between regime and depth ($F_{(2, 32)} = 1.44$, p = 0.25) nor any differences by depth ($F_{(1, 32)} = 2.69$, p = 0.11). Both perennial gaining and perennial losing sites had substantially lower mean species richness than intermittent sites (3. 56 and 4.93 fewer species respectively, both $p \ll 0.001$) (*Figure 117*). For Maules log-transformed total abundance, there was no evidence of a regime × depth interaction ($F_{(2, 32)} = 0.56$, p = 0.58), and a weak effect of depth

($F_{(1, 32)} = 3.58$, $p = 0.068$, 1.81 × as many individuals in shallow samples than deep samples). Perennial gaining and perennial losing regime samples had strongly, significantly reduced abundances than intermittent regime samples (0.27 × and 0.14 × fewer individuals respectively, both $p < 0.005$).

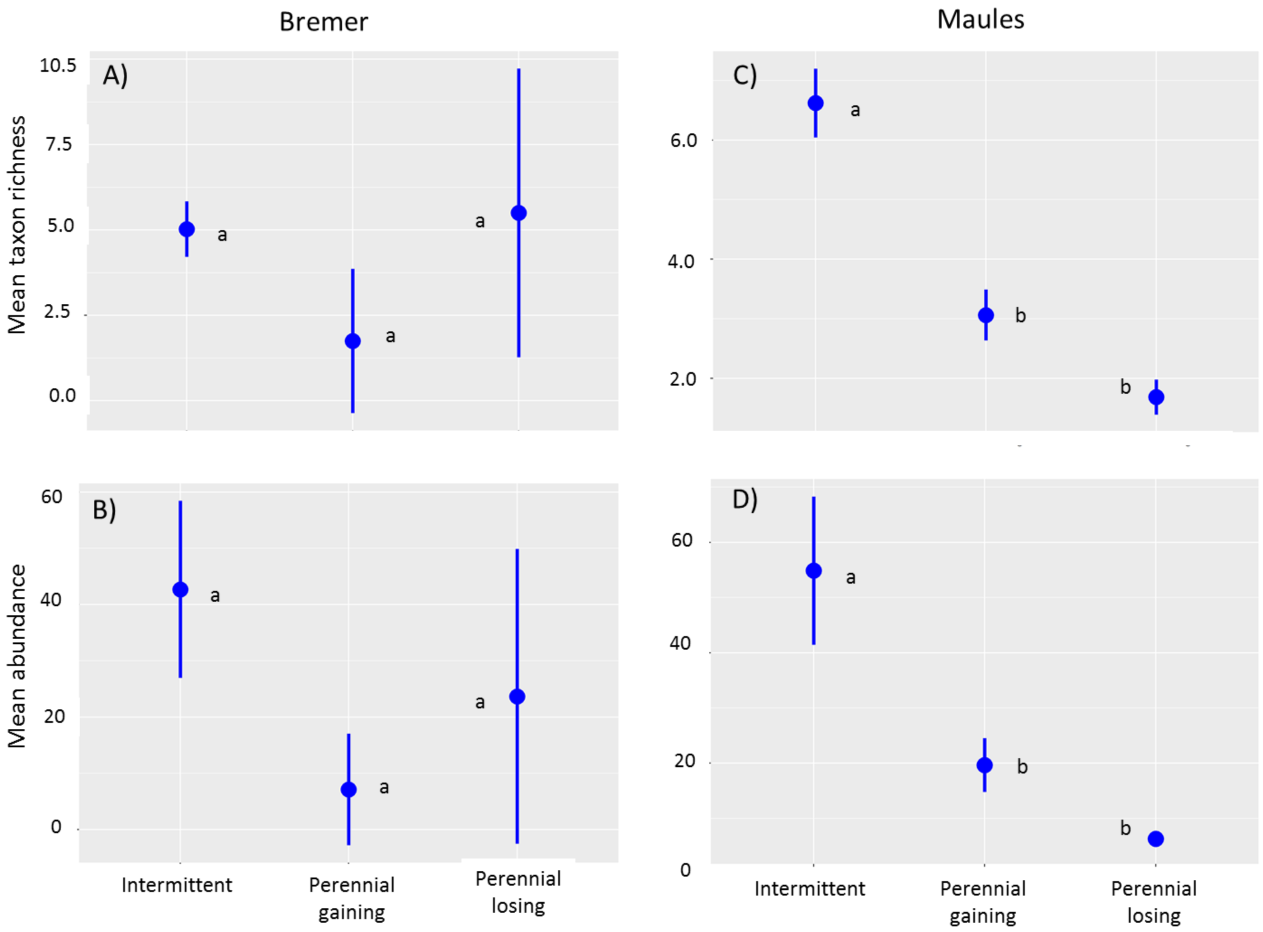


Figure 117: *Plots of mean taxon richness and mean abundance (log transformed) ± 1 SE in relation to flow regime class (intermittent, perennial gaining, and perennial losing) in the Bremer (plots A, B) and Maules (plots C, D) catchments. Means with the same letter are not significantly different (p > 0.05). Maules catchment intermittent flow regime samples had significantly greater mean taxon richness and mean abundance than the perennial gaining and perennial losing flow regime samples. Bremer catchment samples were not significantly different.*

Baseflow recession in Middle Creek and Maules Creek

The progressive baseflow recession recorded during the study period in the intermittent and perennial losing sections afforded the opportunity to explore the ecohydrological responses of hyporheic assemblages and abiotic variables during groundwater drawdown. Figure 118 shows an MDS ordination of the assemblages at Middle Creek intermittent sites and Maules Creek perennial losing sites, and their trajectories through time as the water table progressively receded and richness / abundance declined. At four sites, the hyporheic assemblages had congruent trajectories aligned with declining richness and abundance that converged at a single point in February 2016 when the hyporheic zone at these sites was desaturated to > 0.9 m depth below streambed level and no aquatic taxa could be collected.

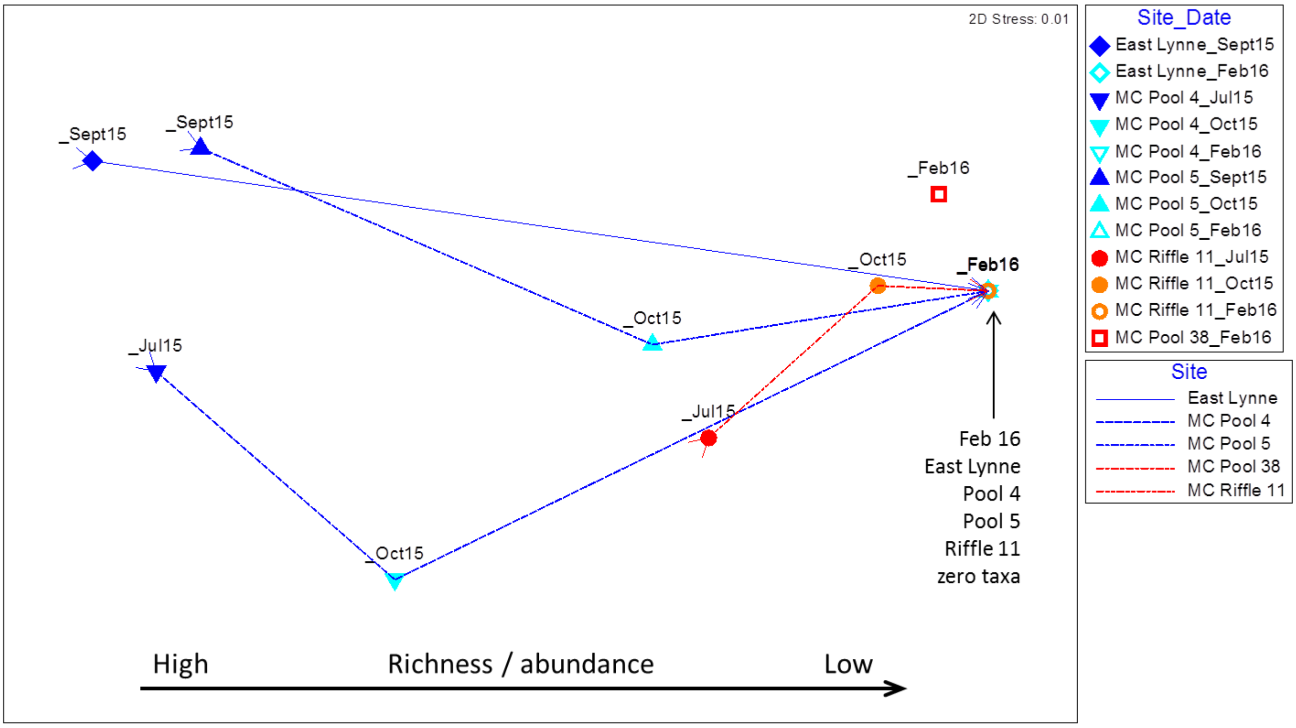


Figure 118: MDS ordination of site averages, intermittent and perennial losing sites, showing site trajectories through time as richness / abundance declined towards zero as the water table receded and the hyporheic zone became desaturated. Sites are coloured by their flow regime class: intermittent (blue), perennial losing (red, orange). Dispersion weighted, Bray-Curtis similarity (+dummy variable) (stress 0.01).

4.6 Discussion

4.6.1 Physical Hydrology

The hydrologic observations (stream flow and surface water and groundwater hydrographs) during the study period confirmed the characterisation of the Maules Creek stream research catchment based on previous studies (Andersen and Acworth, 2009, Rau et al., 2010, Cuthbert et al., 2016). The upper part of the system along the intermittent Middle Creek was dry 78% of the time, with one major flow event starting on June 16th and lasting for 122 days. Despite this flow event never making it as surface water flow all the way to the perennial section at Horsearm Creek, the perennial section of the system had continuous surface water flow throughout the study period supported by regional groundwater discharge. The very stable temperature data from the streambed temperature array at the 'Spring' site and the lack of diel temperature variations verified the gaining conditions (Rau et al. 2010) and the discharge of regional groundwater at this site. Larger-scale regional diffuse groundwater discharge along the perennial section of Horsearm Creek also contributes to maintaining a stable water level further downstream in Maules Creek at Elfin Crossing. The most downstream sections of the system (on Maules Creek) were losing for the entire study period as confirmed by an observed one metre decline in groundwater level in a bore adjacent to Elfin Crossing (see Figure 69d). At the upper end of this section the streambed temperature arrays showed strong diel variations confirming the losing conditions. The section of the system downstream of Elfin Crossing also had extended drying and shrinking of surface water expression from October 2015.

These hydrologic observations confirm the initially defined hydrological regimes assigned to the different stream reaches. The observations support the proposed framework of using these sites as a proxy for the effects of groundwater drawdown in the interpretation of the hydrochemical and ecological data. And finally it verifies the validity of the study design in regard to the selection of sampling sites.

4.6.1.1 Hydrochemistry

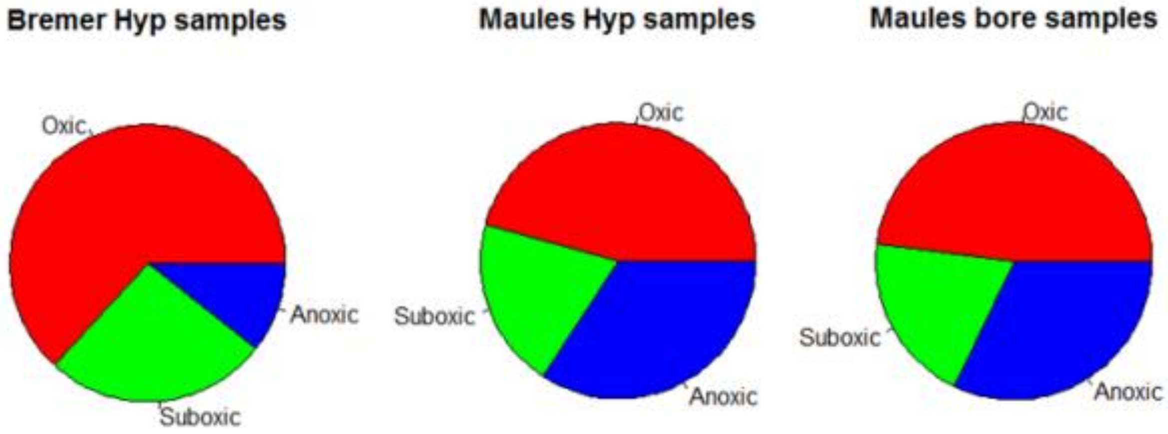


Figure 119: Redox categories of the hyporheic samples from upper Bremer River and Maules Creek catchments and bore samples from Maules Creek catchment. Approximate thresholds for DO categories: "Oxic" DO > 0.5 mg/L; "Suboxic" 0.49> DO >0.21 mg/L, "Anoxic" DO < 0.2 mg/L.

The proportion of oxic, suboxic and anoxic samples are shown in Figure 119 for both upper Bremer River and Maules Creek catchments. There is a higher proportion of oxic samples from the Bremer catchment than the Maules Creek catchment, and conversely a higher proportion of anoxic samples from Maules Creek catchment than upper Bremer River catchment. The proportion of suboxic samples is similar. The proportions in hyporheic and bore samples from Maules Creek catchment are similar.

For the Maules Creek catchment, the general similarity of surface and hyporheic water chemical compositions to the groundwater compositions near the creeks indicates significant surface water-groundwater interaction. Along the intermittent Middle Creek the interaction is mainly exchange and mixing of surface water with the groundwater during flow events. This mixing dilutes the groundwater near the creek (lower TDS than the aquifer in general) and in turn increases the amount of dissolved solutes in the surface water both with time over an event and as the flow event moves downstream. At the perennial gaining Horsearm Creek the interaction is dominated by the regional groundwater discharge and some shallow hyporheic exchange controlled by the streambed topography. In the downstream losing section along Maules Creek the shallow groundwater of the upper aquifer is receiving recharge from the creek. Due to the lack of groundwater samples in the upper Bremer River catchment it is not possible to make a similar analysis for this catchment. However, the observation that the major ion chemistry varied between the October 2015 and February 2016 sampling for the upper Bremer River catchment sites in contrast to the stable composition for the perennially gaining sites (on Horsearm Creek) in the Maules Creek catchment could indicate that the upper Bremer River catchment sites are hydrologically more transient (i.e groundwater contributions to surface water flows were lacking for considerable durations in the study period). This hypothesis is supported by the temporal variations in flow and surface water levels for the two catchments.

In the Maules Creek catchment, over the course of this project, the surface water chemistry of samples collected from the same locations has remained consistent, especially along the perennial section of Horsearm Creek (see Figure 73). This consistency is further evidence that regional groundwater discharge of constant composition maintains the flow in this reach. It was also evident from the PCA on the Maules Creek catchment hyporheic samples where the samples collected within the perennial gaining section of Horsearm Creek did not show any temporal separation (Figure 88). Conversely, the creeks in upper Bremer River catchment showed seasonal differences in both flow and chemistry, which suggest that the groundwater contribution for these creeks is transient and perhaps more localised.

Downwelling sites or upwelling hyporheic sites tended to be more reduced than the regional upwelling sites. This observation is supported by measured DO, manganese and iron concentrations (see Figure 120 - Figure 121) and measured nitrate (oxidised species) and ammonia (reduced species) concentrations (see Figure 122). The upwelling sites tended to contain only DO and nitrate while the downwelling sites contained only iron and ammonium. In general, the deep upwelling sites had higher amounts of nitrate while the deep downwelling sites had higher amounts of ammonia. Therefore, at downwelling sites, the surface water evolves along the redox sequence as it travels through the subsurface, resulting in more reduced conditions at depth (conceptually shown in Figure 123). At sites with upwelling of regional groundwater, as the water moves through the streambed to the surface it likewise progresses along the redox sequence due to sedimentary organic matter and its processing in the streambed, and consequently produces a pattern of increasingly anoxic conditions from deep to shallow (see Figure 123). It is reasonable to hypothesise that groundwater drawdown would cause a reduction in the regional groundwater discharge and increase in the number of downwelling sites. According to current conceptual models this would lead to more hypoxic/anoxic conditions and an anaerobic microbially dominated environment in the streambed. One example of the hypothesised changes due to groundwater drawdown is the generally reduced streambed chemistry at the perennially losing section on Maules Creek. Another example of this temporal change was observed in the intermittent Middle Creek where the hyporheic samples collected while Middle Creek was a gaining system (June – September) tended to be oxic while the samples collected after it had switched to a losing system were suboxic.

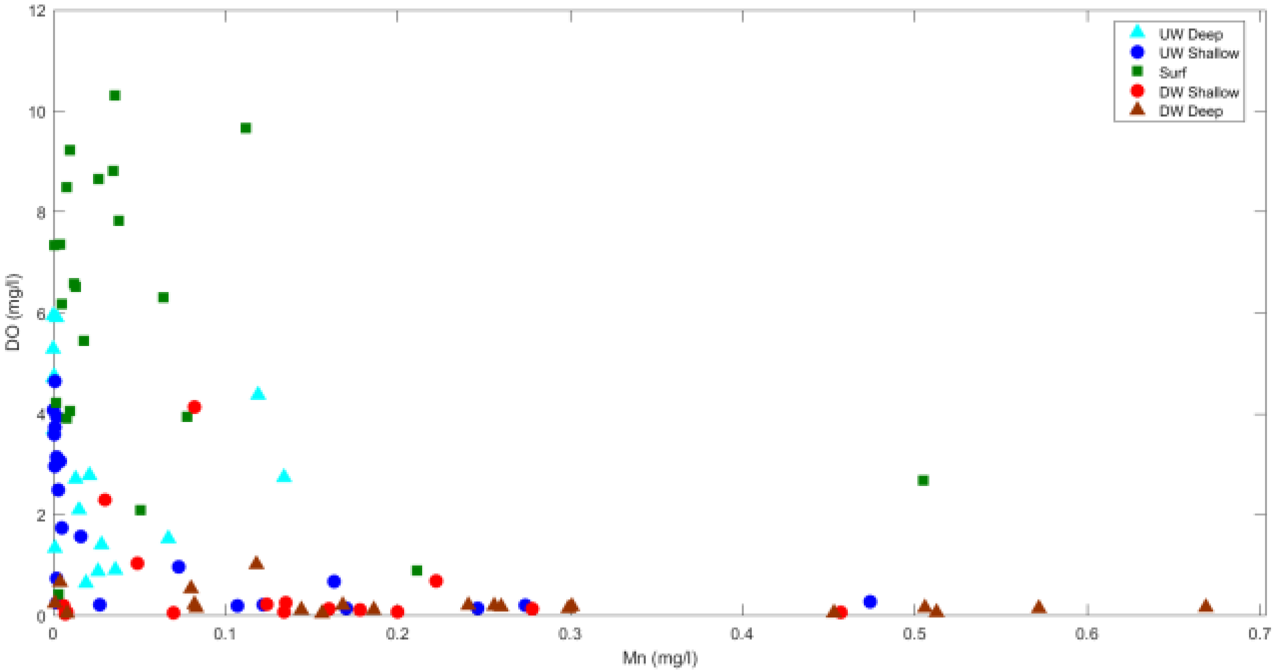


Figure 120: DO vs Mn for hyporheic and associated surface water samples in the Maules Creek catchment.

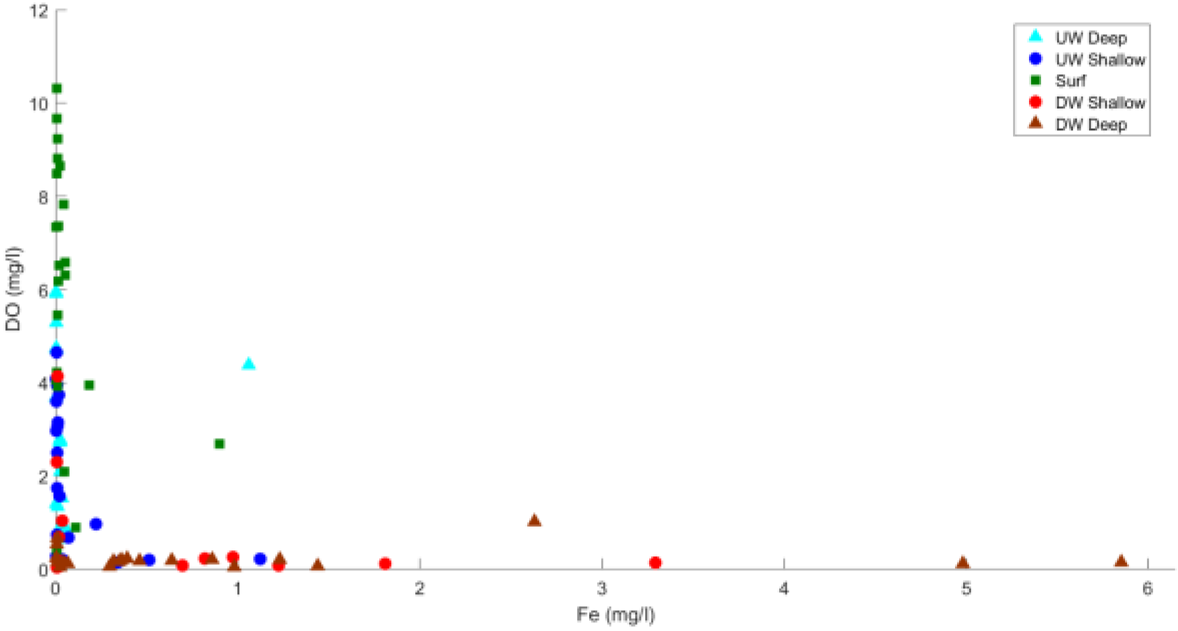


Figure 121: DO vs iron for hyporheic and associated surface water samples in the Maules Creek catchment.

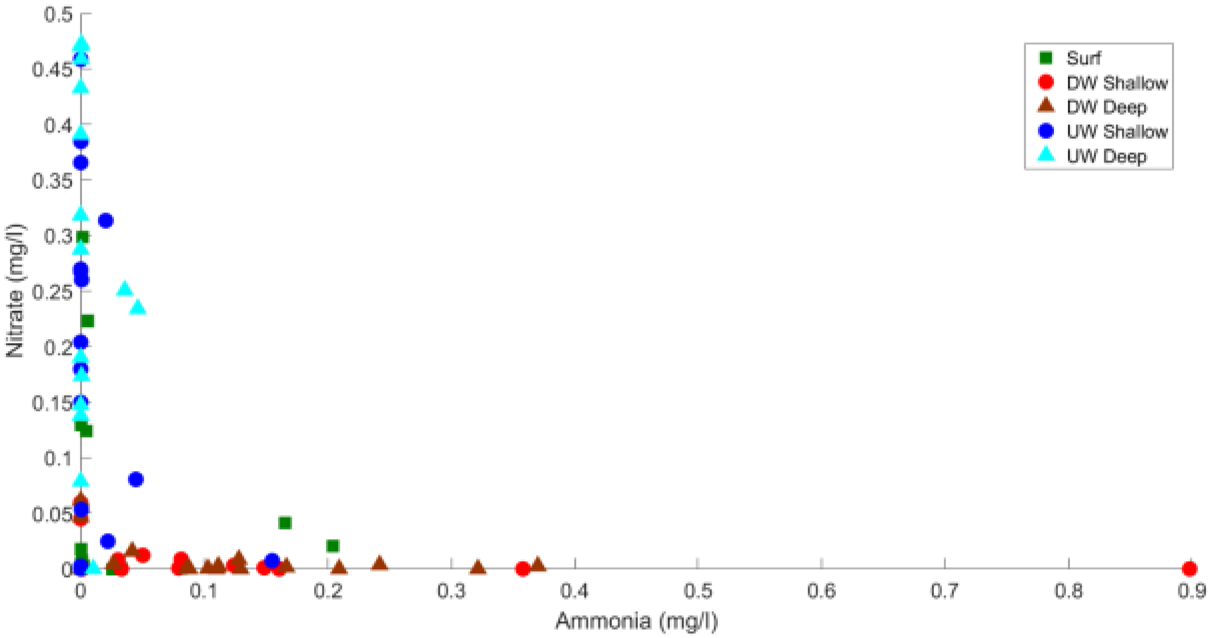


Figure 122: Nitrate vs ammonia for hyporheic and associated surface water samples along Horsearm and Maules Creek

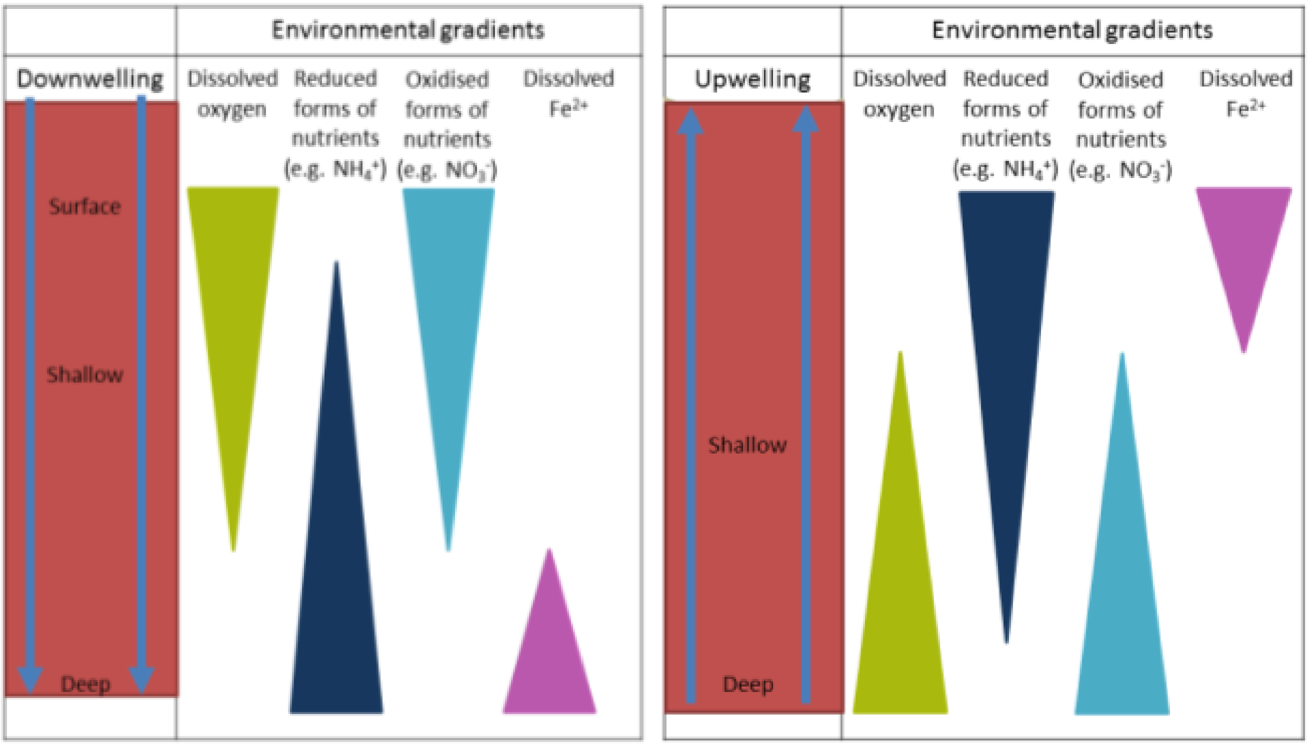


Figure 123: Conceptual models of redox chemistry at downwelling and upwelling sites of regional groundwater at Maules Creek catchment. Adapted and modified from Boulton et al. (2010).

Both identified sites of major regional groundwater discharge (the ‘Spring’ and ‘Radon 35’) are a source of nitrate. However, at Radon 35, the nitrate is very localised, potentially because this section of the creek is a series of disconnected pools. In the perennial gaining section of Horsearm Creek (including the ‘Spring’ site) with free surface flow nitrate is present along the reach as evidenced from the surface water nitrate profile along this section (see Figure 73). Initially, groundwater drawdown could cause the continuous riffle-pool system in this section to become a series of disconnected pools, similar to the upper reach of Horsearm Creek (‘Radon 35’ site) and hence areas of measurable nitrate concentrations would be localised to remaining areas of regional groundwater discharge. Further groundwater drawdown would potentially remove upwelling zones of regional groundwater altogether, such as the 'Spring', and hence remove the regional source of nitrate to the creek. In addition, a switch from gaining to losing conditions would lead to the hyporheic zone becoming a sink of surface nitrate (via denitrification) causing an overall decrease of available nitrogen in the system (Trauth et al. 2014). The mechanism of denitrification in losing reaches could potentially explain the decrease in nitrate in the perennial losing section compared to the perennial gaining section, but nitrate uptake by microbial biofilms (autotrophs and heterotrophs) undoubtedly plays a role as well (Peterson et al. 2001).

Zarnetske et al. (2011) showed that inorganic nitrogen release from mineralisation of OM and its subsequent nitrification and removal (denitrification) is related to location along the flow paths and the residence time in the hyporheic zone. As water flows through the hyporheic zone, initially there is a zone of net nitrate production as DO in the oxic water is both oxidising OM and nitrifying inorganic N released from the OM mineralisation. As the water becomes anoxic, denitrification will become dominant, consuming nitrate. OM mineralisation will continue, however, with ammonium being the inorganic form of N released. Eventually the rate of denitrification decreases due to lack of nitrate even though the redox conditions are favourable for denitrification. When iron reduction starts occurring phosphorus that is bound to iron hydroxides will be released. The combined processes of denitrification, iron-oxide reduction and phosphorous release result in the nitrate, ammonium and phosphate profiles shown in Figure 124, which can be summarised as decreasing NO_3/NH_4 and decreasing total inorganic N/P ratios along flow paths. This conceptual model is supported by the data collected as part of this study. An increase in ammonium as a function of hyporheic flow path length can be observed in the perennial losing section of the Maules Creek catchment at Pool 38 where ammonium increased along the drying streambed from October 2015. Also, samples that had high phosphate tended to be samples that were very reduced and classified in the redox scheme as iron reduction.

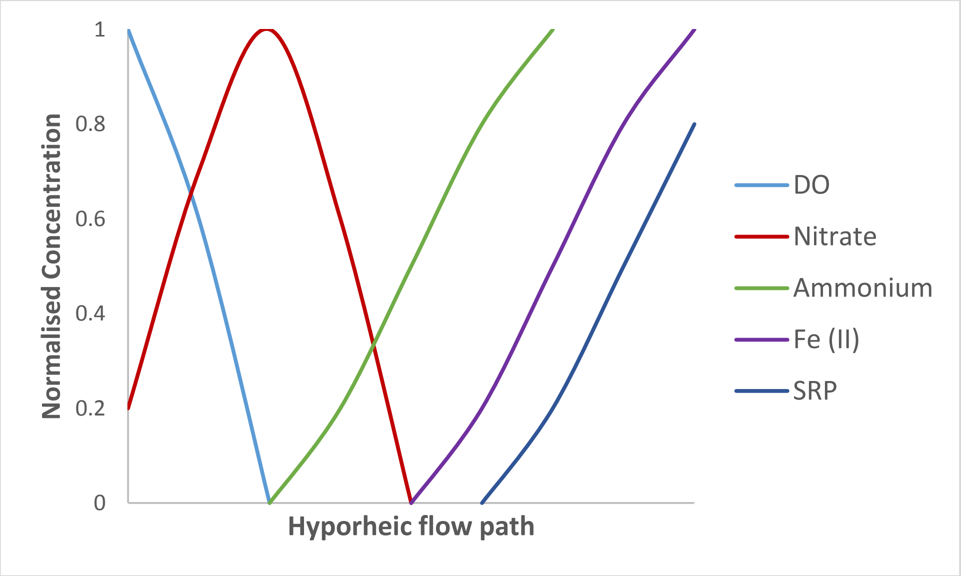


Figure 124: Conceptual model of the evolution of dissolved oxygen, nitrate (NO_3^-), ammonium (NH_4^+), ferrous iron (Fe^{2+}) and SRP (soluble reactive phosphorus) as a function of hyporheic flow-path length. Conceptually, any location along the x-axis then represents the relative upwelling hyporheic water chemistry at that location.

In the perennial sections, as water flows through the subsurface the total concentration of DOC increases (see Figure 78). PARAFAC analysis of the fluorescence signatures indicated that the relative processed humics content increases while the unprocessed humics content decreases and the UV absorption indicates the molecular weight decreases (conceptual model shown in Figure 125). These observations are suggestive of the hyporheic zone being a biogeochemical processing zone where sedimentary labile OM is mobilised. That sedimentary OM must play a role is deduced from the overall increase in DOC concentrations (i.e. another source apart from surface-water DOC).

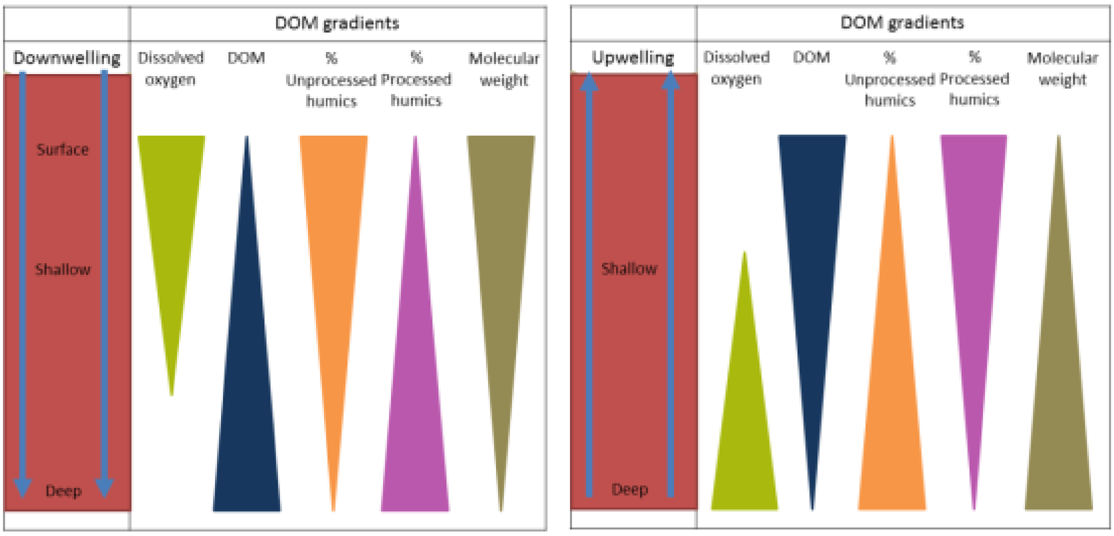


Figure 125: Conceptual models of organic matter at downwelling and upwelling sites of regional groundwater at Maules Creek catchment.

In relation to our initial research question (8): “How does stream flow regime, hyporheic exchange and degree of substrate saturation (as an analogue for groundwater drawdown) affect nutrient (N) biogeochemical processes and organic matter carbon (C) degradation rates in the hyporheic zone?”, the effects of groundwater drawdown on nutrient biogeochemical processes and organic matter degradation are complex and depend on a range of factors including hyporheic flow conditions and the degree of drying. With minor or moderate groundwater drawdown that maintains saturated conditions and hyporheic exchange (i.e. up- and downwelling), flow paths between wetted surface habitats would be expected to lengthen along with an increase in the residence time of water flowing through these flow paths. For locations where flow paths were initially very short (too short to become DO depleted), an increase in these flow paths would likely result in an increase in nitrate in the upwelling zone due to increased net nitrate production via additional OM mineralisation and nitrification. For slightly longer flow paths (where DO becomes depleted), increases in the flow path due to lowered groundwater levels would likely result in a decrease in nitrate, as denitrification becomes dominant but an increase in ammonium as OM mineralisation continues under reducing conditions. For longer flow paths, as the residence time of water increases in the hyporheic zone, conditions would become more reduced leading to reductive iron-oxide dissolution. This would lead to an increase in dissolved phosphorus as it is released from the dissolving iron-oxide surfaces. In summary, along a conceptual flow path, nitrate/ammonium ratios would increase initially and then decrease as denitrification sets in. As phosphorus starts to be released N/P ratios would decrease. Assuming that hyporheic flow is maintained these changes in N-speciation and changes in N/P ratios may have implications for stoichiometric requirements of primary and secondary production within residual wetted surface habitats. Finally, for more severe groundwater drawdown, hyporheic flow would cease altogether and remineralised nutrients would not discharge to remaining surface waters.

Groundwater drawdown that causes perennial reaches to become intermittent may lead to a decrease in the relative quantity of labile (i.e. biologically available) dissolved organic matter (DOM), compared to perennial systems. For instance, although DOC concentrations were greater in intermittent sites, various spectrometric indices indicated that this DOC was more processed (less fresh, with a high proportion of humic-like compounds) than at the perennial site. Ultimately, greater intermittency may increase the relative quantity of non-labile DOM compounds, reducing the amount of energy and nutrients incorporated in local food webs, and potentially increase in the export of biologically refractory DOM to downstream ecosystems.

4.6.2 OM Degradation Studies

In Round 1, at the intermittent site (Middle Creek) the pool samples had higher leaf litter processing than the riffle samples, as opposed to the perennial gaining sites where the riffle samples had the greater leaf litter processing. Probably, as surface flow in the intermittent section ceased during the course of the deployment, the riffle sections dried up first while the pools persisted.

With the hyporheic deployments, the range in % leaf litter mass remaining was narrower and much more consistent across the treatments. In general, leaf litter processing was greater in the hyporheic zone than in the corresponding surface habitat with the exception of the riffle deployments in the perennial gaining section. The narrow range of hyporheic leaf litter processing may be related to the more stable subsurface environmental conditions, especially temperature (see Figure 95 for an example) and moisture.

For both the cotton strips and the leaf packs, moisture made the highest contribution to the amount of degradation. Groundwater drawdown would lead to an overall decrease in the degree of saturation of surface and hyporheic habitats and hence a likely decrease in microbial activity and leaf litter degradation rates. This prediction is supported by comparing the results from Round 2 to Round 1 (see Table 20). The groundwater water levels declined at the intermittent and perennial losing sites from Round 1 to Round 2, causing an increase in dry sites in these locations (see Figure 94). Correspondingly there were significant increases in the percentage mass remaining for samples at these sites and hence decreased leaf degradation rates.

In relation to our initial research question (4): “How do stream flow regime, hyporheic exchange and degree of substrate saturation (as an analogue for groundwater drawdown) affect microbial activity inferred from organic matter degradation rates (in riffle, pool and hyporheic habitats)?”, our results indicate that for perennial gaining streams, microbial activity, inferred by cotton and leaf litter degradation, is generally high and consistent (as opposed to variable). In the context of groundwater drawdown and a general drying of the system we observed a loss of surface flow and pools becoming discontinuous (i.e. pools only connected by subsurface flow). Microbial activity could still be high locally, but had become more variable. Surface sites that were no longer saturated now had low microbial activity, but subsurface (hyporheic) sites could maintain high microbial activity as long as they were saturated or moist. As the system dried out further (more severe groundwater drawdown), microbial activity was reduced proportionally to the relative increase in dry streambed. This observation means that losses in wetted surface area lead to a proportional decrease in surface microbial activity. A subsequent drying of the streambed sediments will lead to further loss in subsurface microbial activity.

4.6.3 Invertebrate ecology

The sampling of hyporheic invertebrates revealed rich assemblages in both the Bremer River (31 taxa) and Maules Creek catchments (39 taxa), and in the Maules aquifer (24 taxa). Overall, 43 aquatic invertebrate families were identified, representing crustaceans, insects, water mites, various types of worms, snails, tardigrades, rotifers and hydrozoans. The taxonomic composition of assemblages was typical for groundwater invertebrates in alluvial aquifers and streams in eastern Australia (Tomlinson and Boulton 2010), and included all groundwater habitat affinity groups. Stygobites and stygophiles comprised around one-half of the invertebrate abundances in hyporheic habitats, and these groups dominated the aquifer communities, but total invertebrate densities in the hyporheic zone were an order of magnitude greater than in the aquifer, thus confirming the comparative richness of the hyporheos. Comparatively few studies of hyporheos have been conducted in Australia, thus limiting assessment of the regional significance of the Maules and Bremer assemblages. The richness of stygobite taxa at Maules Creek suggests that it might qualify as a local or regional 'hotspot' of subsurface biodiversity, but comparative regional studies are needed to confirm or refute such an assertion. One of the few other Australian studies, and likely the most intensive to date, was conducted over five years in the Never Never River in northern New South Wales (Boulton and Foster 1998; Boulton and Harvey 2003; Boulton et al. 2004). This study identified 30 major taxa including a remarkable diversity of water mites (46 species). Water mites were also a diverse faunal element in the hyporheos at Maules Creek (7 families), and eleven water mite taxa were identified from the Gwydir River alluvial aquifer (Korbel and Hose 2015).

The streams with intermittent flow regimes at both Bremer River and Maules Creek yielded an unexpectedly rich and abundant hyporheic fauna, and demonstrated that the hyporheic zone in such streams is potentially important for overall stream ecosystem function. Not unexpectedly, the perennial sections of our study streams also harboured a rich fauna although in the Maules catchment abundances were somewhat lower in the intermittent study section. The apparent association between hyporheos diversity and stream flow regime at Maules Creek most likely represents the integration of multiple factors that characterize the way that the sampled sites connect with baseflow from the regional aquifer.

Superimposed on the broad-scale flow regime settings in Maules Creek and Bremer River are the smaller-scale (reach and patch scale) effects of bidirectional hyporheic exchange and associated gradients in temperature, DO, organic matter and other abiotic variables. Consistent with other hyporheic ecology studies (e.g. Malard and Hervant 1999; Hahn 2006), we found that invertebrate assemblages also responded strongly to the small-scale habitat patchiness and variations in hyporheic exchange processes. Therefore, good management of stream hyporheic water regimes needs to consider maintenance of this small-scale habitat patchiness and spatio-temporal dynamics in hyporheic exchange processes.

In our study sites, oxic conditions, whether flows were upwelling, downwelling or lateral, supported the existence of hyporheos, including stygobites, stygophiles and stygoxenes. At Maules Creek, locally strong upwelling of oxic regional groundwater sustained rich hyporheos assemblages. Suboxic conditions were associated with depletion of invertebrate richness and abundance, and exclusion of taxa that are presumably intolerant of low DO. As expected, we found that suboxic conditions, progressing towards anoxic, were more typical in a losing stream flow regime, or other regimes (intermittent, perennial gaining) where downwelling occurred, or along extended lateral flow paths. Invertebrates, of course, cannot survive for extended periods in completely anoxic conditions, and as expected these zones were exclusively inhabited by iron metabolizing microorganisms, which were frequently observed at surface upwelling sites as yellow-brown deposits. These condition indicators increased their expression as baseflow receded and the water table declined.

In scenarios where progressive recession of surface flow and the groundwater table occurs, either naturally or anthropogenically, invertebrate assemblages are likely to become depleted as the flux and magnitude of oxic upwelling hyporheic exchange water, or oxic discharging groundwater, declines. Aquatic invertebrates require saturated substrates to survive, so desaturation of the hyporheic zone will result in local extirpation of the hyporheos. The progressive groundwater recession recorded during the study period in the intermittent and perennial losing sections was associated with the ecohydrological responses of hyporheic assemblages and abiotic variables during groundwater drawdown.

In relation to our initial research question (9): "How do stream flow regime, hyporheic exchange and degree of substrate saturation (as analogue for groundwater drawdown) affect invertebrate assemblages, and their associations with hydrochemistry and redox processes in the hyporheic zone?", the influences of stream flow regime and hyporheic exchange-flow gradients were equivocal in our study sites. In the Maules catchment, where locally strong discharge of oxic regional groundwater occurs in combination with hyporheic upwelling, richness and abundance were significantly higher than in habitat patches where the hydraulic gradient was weakly upwelling, neutral, or downwelling. At a broader spatial scale within the Maules catchment, we found that the hyporheos was significantly more diverse in the stream section with an intermittent flow regime (Middle Creek) than in the sections with perennial gaining and losing regimes. In the Bremer catchment, where groundwater discharge and hyporheic exchange flow gradients are more muted, we found no statistically significant ($p< 0.05$) differences related to stream flow regime or hyporheic exchange. The equivocal evidence for influence of stream flow regime on hyporheos assemblages, and the unexpected diversity found in the intermittent section in the Maules catchment, suggest that the surface expression of flow regime per se is of less consequence in shaping hyporheos patterns than stream baseflow - groundwater connectivity.

In relation to our initial research question (10): "What is the degree of hydrologic connectivity, hydro chemical and invertebrate assemblage similarities, between the hyporheic zone and regional aquifer at Maules Creek, and what are the implications of this with respect to understanding and managing groundwater drawdown impacts on hyporheic ecosystems?", our study confirmed the fundamental relevance of the hydrological interconnectivity between streams and groundwater. The aquifer bore sampling findings supported our hypothesis that the hyporheic zone at Maules Creek shares strong hydrologic connectivity and faunal similarities with the regional superficial aquifer. The implications of this strong hydrologic connectivity are are that the regional aquifer functions as a retreat or 'refuge' for hyporheic stygobites and stygophiles when the hyporheic zone becomes desaturated, for example during drought or groundwater drawdown from pumping. Likewise, the aquifer may also function as the source for stygobite and stygophile recolonization of the hyporheic zone when it becomes re-saturated.

Groundwater (baseflow) connectivity may confer resistance and resilience in stream and hyporheic ecosystems, but the nature, context and regime of stream, baseflow and aquifer connectivity are important. High stream-aquifer connectivity (permeability and exchange) confers "rapid" and strongly coupled hydroecological responses to drawdown, and conversely, suggests potential for recovery of hyporheos when conditions are restored, providing reservoir populations of groundwater fauna are sustained in connected aquifers. This scenario was exemplified at Maules Creek. In contrast, streams with thin alluvial sediments covering relatively impermeable bedrock such as Bremer River, and/or which have low connectivity to adjacent aquifers, may be less resilient to groundwater drawdown impacts and have lower capacity to recover. From our findings, it is inferred that hyporheic ecosystems, or at least those with strong hydrologic connectivity to oxic baseflow / aquifers, may be resilient and able to recover from temporary groundwater drawdown as may be experienced during extended droughts or pumping for water supply, irrigation, mine dewatering or CSG extraction for example. Conversely, it might be expected that hyporheic ecosystems that have weaker or disconnected hydrologic connectivity, or where the regional groundwater is suboxic/anoxic, will be less resilient and more vulnerable to the effects of drawdown. In all scenarios, the cumulative effects of short to medium-term human activities such as pumping, superimposed on medium to long term climatic variability including droughts and climate change, need to be considered.

4.6.3.1 Sampling considerations

We found that hyporheic invertebrate assemblages were locally highly variable across small vertical and lateral spatial scales. At sub-metre habitat patch scales, taxonomic richness may vary by one order of magnitude, and overall abundance by two orders, and the dispersion of individual taxa may be extremely "clumped" between neighbouring patches. This high localized variability in assemblages is associated with inherent habitat patchiness, the enormous 'invisible' heterogeneity that challenges groundwater ecologists worldwide (Griebler et al. 2014). The implications of this heterogeneity for surveys and monitoring generally are the need for adequate spatio-temporal replication within and among sites, supported by pilot studies and progressive, iterative evaluation of results along the way. Additionally, the general paucity of baseline hyporheic ecology studies in Australia means there is little existing benchmark context against which to make comparisons and assessments. Notwithstanding these limitations, because biodiversity is typically positively correlated with rates of ecosystem function (e.g. Boulton et al. 2008), hyporheic invertebrate diversity indices such as richness, abundance, assemblage composition and habitat affinities ought to be useful tools for assessing and monitoring stream and groundwater ecosystem health and function. While local and regional taxonomic differences limit comparisons of subsurface assemblages at generic or species level, classifications based on habitat affinities provide a 'common currency' that facilitates understanding of parallels and contrasts among different locations, as well as spatio-temporal changes within locations (Claret et al. 1999). Changes in conditions in subsurface habitats (e.g. through groundwater extraction and sedimentation) may alter the proportions of these categories, providing a potential indicator of subsurface community dynamics.

In the Maules Creek catchment it was observed that invertebrate abundance and richness decreased dramatically as conditions became more hypoxic, and invertebrates almost disappeared completely when streambed redox hydrochemistry suggested anoxic conditions. Anoxic conditions were reliably predicted by an increase in dissolved reduced iron (ferrous iron: Fe^{2+}). Because specialised groundwater invertebrates are tolerant of low DO concentrations (DO < 0.5 mg/L) (Malard and Hervant 1999, Hahn 2006) including concentrations below most field instrument detection limits (ca. < 0.2 mg/L), we suggest that ferrous iron may be a reliable indicator of completely anoxic conditions and therefore unsuitable habitat for invertebrates. As redox-sequences are well described globally, for a range of saturated subsurface aquatic environments (streams, estuaries, lakebeds – Appelo and Postma 2005), we expect that our observations in relation to ferrous iron and anoxia have general transferability. Nonetheless we recommend further field studies to test the wider applicability of our observed associations between ferrous iron and groundwater invertebrate richness and abundance.

While the differences between aquifer and hyporheic invertebrate capture rates, the latter of which were one to two orders of magnitude greater, were inevitably influenced by the different pumping methods and pumping rates, site matrix characteristics and different behavioural responses of taxa to pumping, our results nonetheless suggest that invertebrate population densities and biomass in the hyporheic zone at Maules Creek were substantially greater than in the adjacent aquifer (when measured as per unit volume pumped). Considering only the bore aquifer sampling, the unnaturally high abundances inside bores are generally attributed to the attraction of stygofauna into the bore because of the opening to the surface, which allows additional inputs of food sources such as terrestrial invertebrates (and small vertebrates such as lizards) that accidentally fall in (e.g. Eberhard et al. 2009). For this reason, sampling protocols that use pumping and account for purge and post-purge sampling artefacts are recommended good practice (Hancock and Boulton 2009), although pumping methods are more time consuming than net haul methods, which only sample the animals inside the bore (Eberhard et al. 2004).

For future sampling in other locations, we advocate a flexible and adaptive approach that is responsive to site-specific conditions and context, rather than a prescribed survey effort. Good practice would require that each location is assessed on a case-by-case basis, and initial desktop studies are validated by field study. For data analysis, non-parametric permutational methods applied to multivariate data are appropriate for typically highly variable and patchy invertebrate abundance and distribution patterns, and complement parametric treatment of univariate indices such as richness and abundance. Evaluation of sampling adequacy and completeness during surveys and monitoring is effectively undertaken using taxon accumulation curves, and the various commonly used estimators of total species richness (e.g. Colwell et al. 2004).

4.6.4 Integration – site specific models

The following section integrates the physical hydrology, hydrochemistry, organic matter degradation and invertebrate ecology results to develop conceptual models of the ecohydrological functioning and responses spanning the study period for each of the study sites in Maules Creek and Bremer River catchments. In the Discussion section following these site-specific models are further developed and generalised for application in broader contexts and other regions where CSGLCM occur.

4.6.5 Middle Creek Farm (intermittent)

The conceptual model of the intermittent study site at Middle Creek Farm is shown in Figure 126, and the ecohydrological cycle observed over 12 months is depicted in time-series “snap-shots” (Figure 127), with supporting information in Table 27. In summary, the conceptual models for the intermittent flow regime section in Middle Creek Farm highlight the following:

(1) Lithology, hydrology. The coarse alluvium confers high hydraulic permeability and aquifer-creek connectivity, facilitating transport and bi-directional exchange between creek and aquifer of DO, nutrients, POM, DOM and other solutes, as well as microorganisms and invertebrates. The high permeability and connectivity also induce rapid and weakly buffered ecohydrological responses of the hyporheic zone to changes such as groundwater recession / drawdown occurring in the adjacent aquifer.

(2) Hydrogeology. High aquifer-creek connectivity combined with a relatively thick depth of alluvium (>20m thick) and shallow water table (generally < 5m below ground level) confers buffering capacity (resilience) in terms of groundwater habitat availability and persistence during episodes of groundwater recession / drawdown. This combination of aquifer properties and strong hydrologic connectivity also confers a potential capacity for recovery if the aquifer functions as a refuge for the stygobite and stygophile functional components of the hyporheos during episodes of hyporheic desaturation / DO and redox shifts, and a source for recolonization of the hyporheic zone if conditions become suitable.

(3) Flow regime. The intermittent flow regime confers a dynamic and alternating gaining/losing regime with superimposed upwelling/downwelling VHH/HEF paths, both driven by the interplay between recharge and discharge processes in the catchment. The input of oxic regional groundwater maintains suitable habitat for aerobic invertebrates when the hyporheic zone is saturated.

(4) Aerobic microbial metabolism dominates biogeochemical processes. While it is a gaining system oxic conditions (DO > 0.5 mg/L) are prevalent, but after the switch to losing conditions the hyporheic zone becomes progressively suboxic (DO < 0.5 mg/L) to anoxic. OM degradation is highly dependent on substrate moisture conditions, with high leaf decay rates when saturated and a decrease as the system becomes losing and eventually surface flow ceases.

(5) Groundwater recession/drawdown shifts perennial gaining flow regimes and/or upwelling HEFs towards losing and/or downwelling conditions. Invertebrate metabolism in the hyporheic zone may be reduced, but continuing input of oxic regional groundwater maintains a suitable habitat for invertebrates. As the hyporheic zone becomes desaturated, stygobites and stygophiles retreat downwards with the falling water table, but non-specialised stygoxenes and epigean invertebrates are excluded. Below the hyporheic zone at depth in the aquifer, the oxic regional groundwater continues to provide suitable conditions for stygobites and stygophiles.

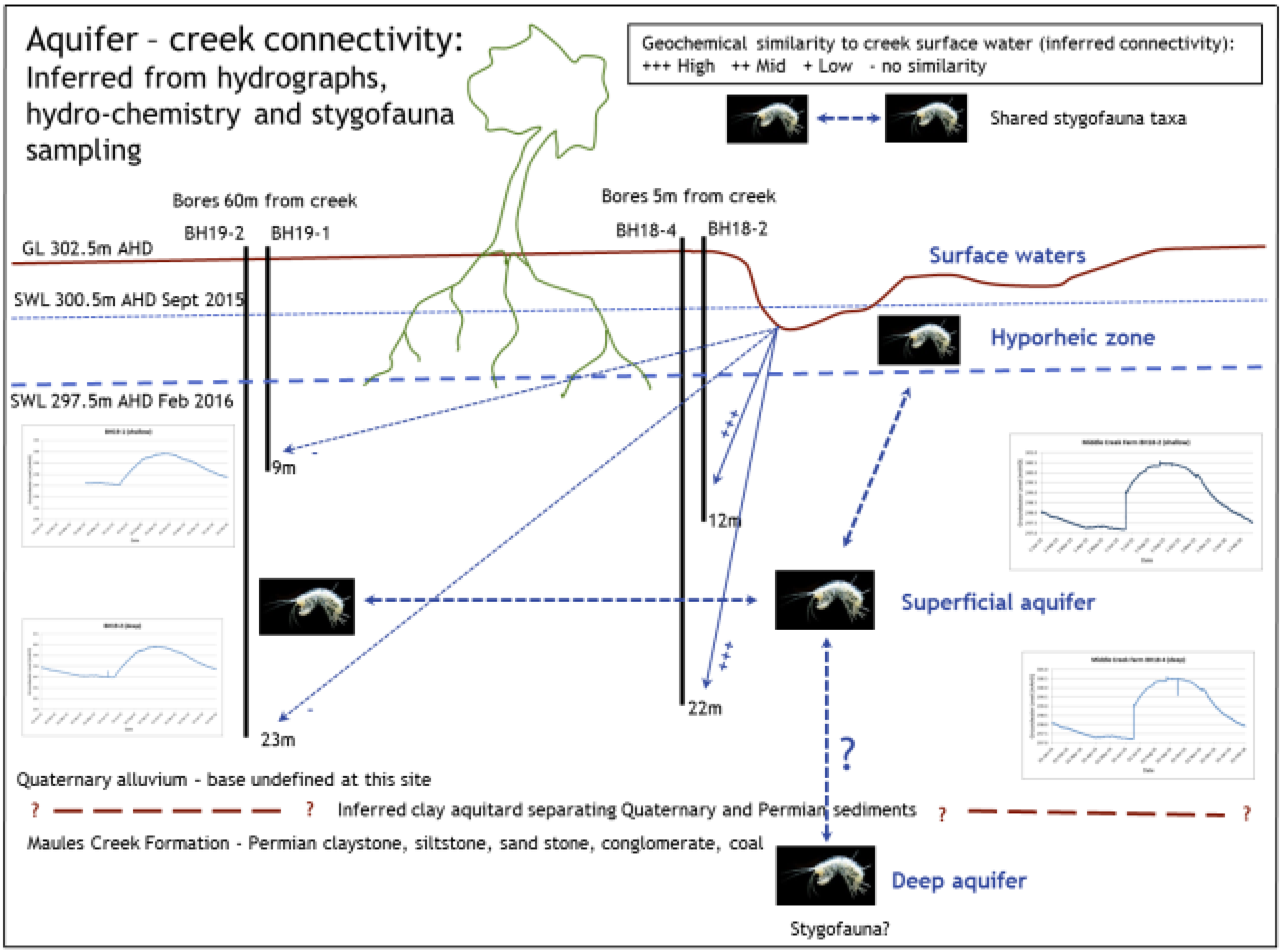
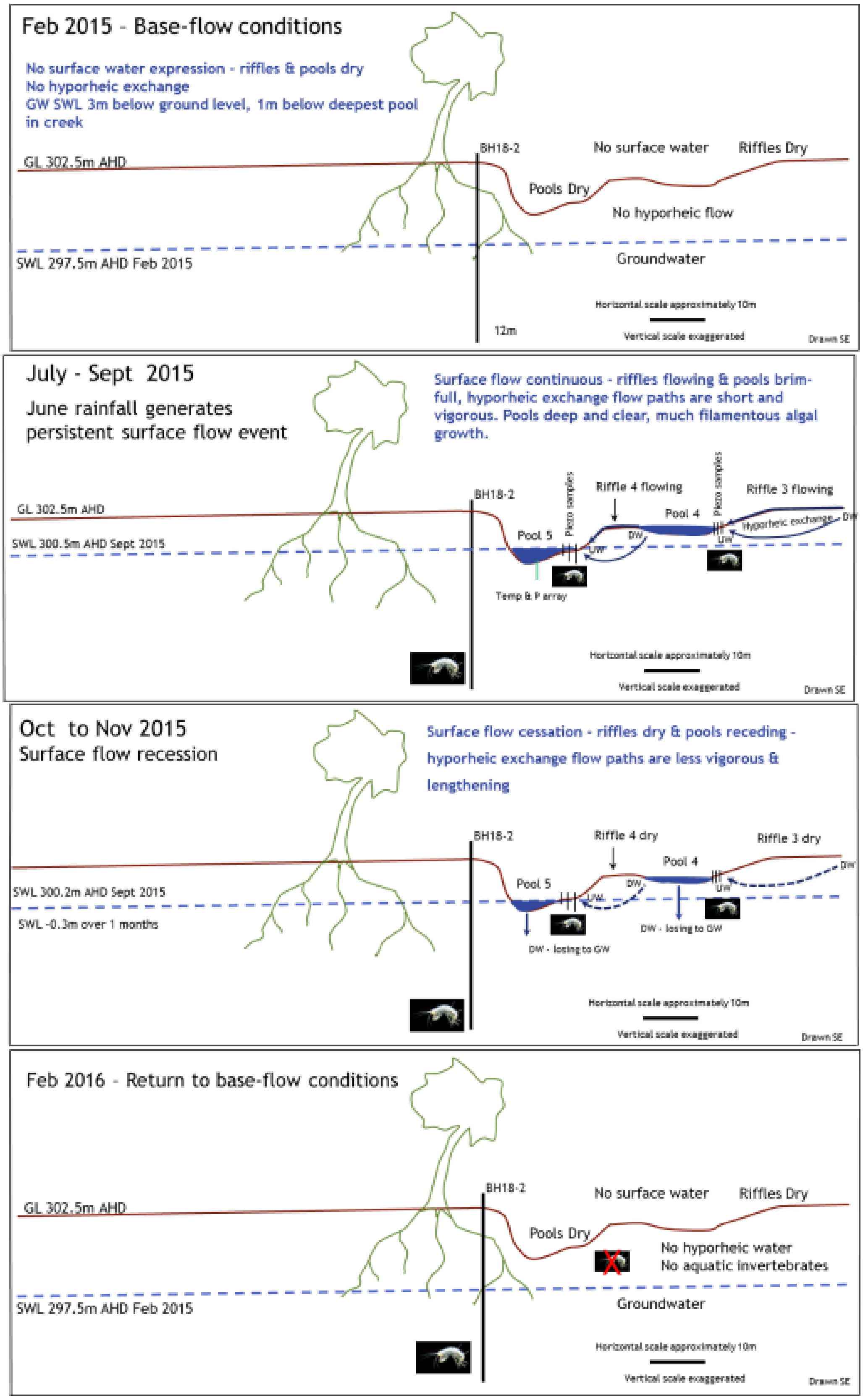


Figure 126: Middle Creek Farm conceptual diagram of aquifer – creek connectivity inferred from hydrographs, hydro-chemistry and stygofauna sampling.

Based on the bore water level monitoring, this model emphasises the strong connectivity between the aquifer and creek surface-hyporheic flow. The hydrographic responses of the shallow aquifer measured in the bores located 60m away from the creek were more muted but still coincident with those in the bores located 5m from the creek. Geochemical similarity between creek water and aquifer water is very high close to the creek bank but further away from the creek the aquifer water had little similarity to the creek water at the time of sampling. The similarity between aquifer water and creek water is assumed to be dynamic and variable, depending on the conditions of recharge and discharge between the two water bodies. The streambed and hyporheic zone are developed in the same geomorphic material as the shallow aquifer, Quaternary alluvium which exceeds 20m depth at this site. The coarse alluvium is moderately to highly permeable as demonstrated by bore pump tests. During recharge events creek surface flow recharges the aquifer. Following recharge of the aquifer by creek water there is a prolonged recession with reversal of flow direction as the aquifer discharges into the creek. The recession is expressed on the surface as gradual decline and loss of surface flow. The high permeability facilitates the transport and bi-directional exchange between creek and aquifer of DO, nutrients, POM, DOM and other solutes, as well as microorganisms and invertebrates. When the hyporheic zone is saturated aquifer invertebrates (stygobites and stygophiles) colonise this zone along with stygoxenes and epigean invertebrates. As the groundwater level recedes and the hyporheic zone becomes desaturated the stygobites and stygophiles retreat downwards and laterally into the aquifer. The stygoxenes and epigean invertebrates which are not groundwater specialists are limited to the shallow hyporheic and benthic zones, so local populations die out, or as in the case of many insects with aquatic larval and nymphal life cycle stages, metamorphose into terrestrial adult forms.

The ecohydrological cycle observed over 12 months in Middle Creek is depicted in time-series of "snap-shots" conceptual models with a supporting table (Figure 127, Table 27). At the beginning of the study in February 2015 the streambed in Middle Creek Farm was completely dry and owing to the depth to groundwater being > 2m below the streambed it was not possible to sample using temporary piezometers which are limited to < 1m depth. However, in July 2015 the stream was observed to be flowing and extensive growths of filamentous algae indicated that the flow had been persisting for some time (Appendix Figure 160). A significant rainfall event in the upper catchment area on 16th June 2015 was responsible for the initiation of a major surface flow event that persisted for some months (Appendix Figure 161). The gradual decline of surface water flow and the groundwater recession was recorded in the water level changes logged in monitoring bore BH18-4 (and three other nearby bores). In July 2015, the pools and riffles were connected by continuous surface flow. In September and October water levels had dropped slightly and the riffles were starting to become partly discontinuous, but hyporheic exchange beneath the riffle zones remained vigorous owing to the streambed topographic gradient. In November 2015, water levels had declined noticeably and all riffles had ceased flowing and the pools had contracted substantially (Figure 161). There was vigorous green algal growth in the standing pools which remained. By February-March 2016 all surface water was gone as the groundwater continued to recede. No hyporheic samples could be obtained during February 2016 because the water table had receded below the maximum depth (0.9 m) of the sampling equipment.



Feb 2015 - Base-flow conditions

No surface water expression - riffles & pools dry
No hyporheic exchange
GW SWL 3m below ground level, 1m below deepest pool in creek

July - Sept 2015

June rainfall generates persistent surface flow event

Surface flow continuous - riffles flowing & pools brim-full, hyporheic exchange flow paths are short and vigorous. Pools deep and clear, much filamentous algal growth.

Oct to Nov 2015
Surface flow recession

Surface flow cessation - riffles dry & pools receding - hyporheic exchange flow paths are less vigorous & lengthening

Feb 2016 - Return to base-flow conditions

Figure 127: Middle Creek Farm eco-hydrological conditions observed from riffle 3 to pool 5 indicating changes from February 2015 to February 2016. Note that for illustrative purposes the left side of diagram is a NW-SE cross section through the creek channel and the right side is a SW-NE longitudinal section along the channel. Refer to diagrams above for additional context.

Table 27: Middle Creek Farm supporting table for Figure 127: ecohydrological conditions, changes and responses observed/measured during stages of the recharge-recession cycle in this creek with an intermittent flow regime, February 2015 to April 2016.

Time stage	Hydrological state or change	Hydrochemistry response	OM microbial degradation response	Hyporheic invertebrate response
1. Feb 2015	Creek bed dry, hyporheic zone desaturated.			Hyporheos absent / dormant
2. June – Sept 2015	Major rainfall event, surface flow continuous, hyporheic zone saturated and HEF paths short steep gradients, aquifer recharge.	Oxic conditions		Moderate to high richness and abundance, all functional groups.
3. Oct – Nov 2015	Creek losing, surface flow discontinuous (riffles dry), HEF paths lengthening and gradient declining, aquifer watertable receding.	Shift towards suboxic conditions, increase in reduced species such as phosphate.	Surface rates 0.0582 /day Subsurface rates 0.0607 /day	Moderate to high richness and abundance, all functional groups.
4. Feb – Apr 2016	Return to stage 1 baseflow conditions.		Surface rates 0.0283 /day Subsurface rates 0.0526 /day	Hyporheos absent / dormant

4.6.6 Horsearm Creek (perennial gaining)

The conceptual model of the perennial gaining study site is shown in Figure 128 with supporting information in

Table 28. Initial pilot sampling was undertaken in The Spring commencing in February 2015, and the last samples in Bar 6 during February 2016 (Appendix 3: Figure 162). Baseflow responses to the June recharge event experienced upstream in Middle Creek were strongly muted and there was little change in stream water levels in the perennial gaining section during the course of the study.

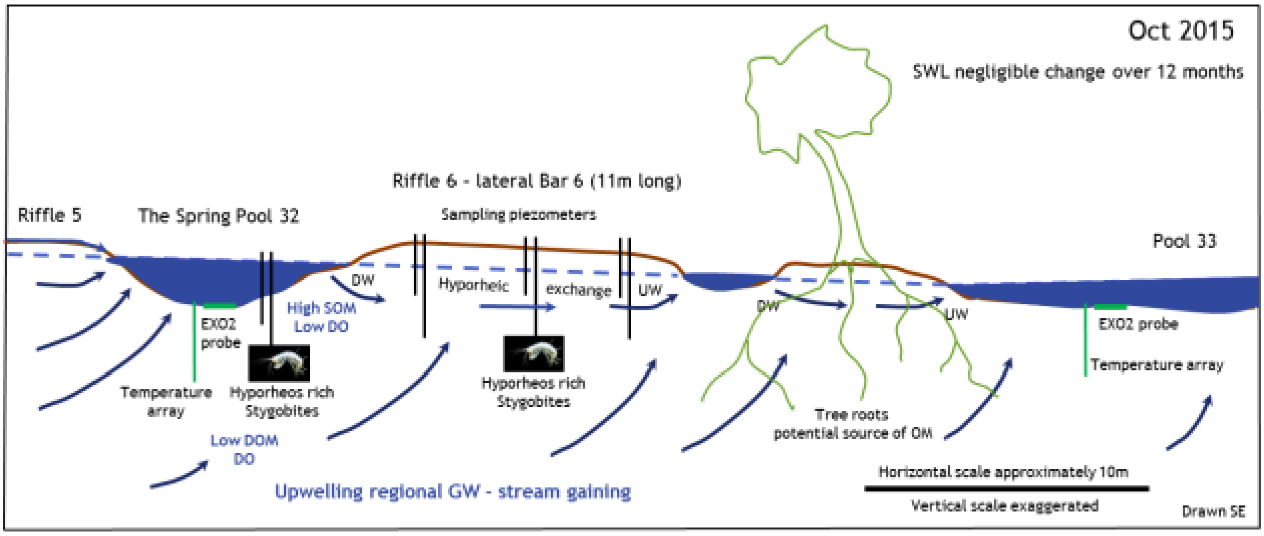


Figure 128: Horsearm Creek perennial gaining section study sites Pool 32 (The Spring) - Riffle / Bar 6 – Pool 33 from February 2015 to February 2016, showing negligible change in groundwater levels. Upwelling regional groundwater (GW) is oxygenated with low DOM and interfaces with high loads of sedimentary organic matter (SOM) in the shallow hyporheic zone. Stygobitic fauna appear to exploit this interface. Submerged tree roots represent another potential source of OM for invertebrates in the stream and hyporheic zones.

Table 28: Horsearm Creek perennial gaining section supporting table for Figure 128: ecohydrological conditions, changes and responses observed/measured during the study period in this section of creek with perennial gaining flow regime, February 2015 to April 2016.

Time stage	Hydrological state or change	Hydrochemistry response	OM microbial degradation response	Hyporheic invertebrate response
1. Feb 2015	Creek flow continuous, pools and riffles, hyporheic zone saturated.	Oxic conditions Upwelling GW a source of nitrate		High richness and abundance, all functional groups
2. Sept – Oct 2015	Little change.	Little change	Surface rates 0.0585/day Subsurface rates 0.0581/day	Not sampled, inferred similar to above based on other sites sampled in this section.
3. Feb – Apr 2016	Little change.	Little change	Surface rates 0.0553/day Subsurface rates 0.0562/day	High richness and abundance, all functional groups

4.6.6.1 Maules Creek (Elfin Crossing perennial gaining / losing)

An ecohydrological model was developed and based on the bore data, hydrochemistry and invertebrate sampling (Figure 129, Table 29). Elfin Crossing and the confluence of Horsearm Creek with Maules Creek coincide approximately with where the creek flow regime changes from perennial gaining (upstream) to losing (downstream) (Appendix 3: Figure 163). Bores on the north bank of the creek at Elfin Crossing are situated in a losing groundwater regime while bores on the south bank may possibly be situated in a gaining regime. Sampling at Elfin Crossing was primarily focused on the aquifer and bores in this vicinity.

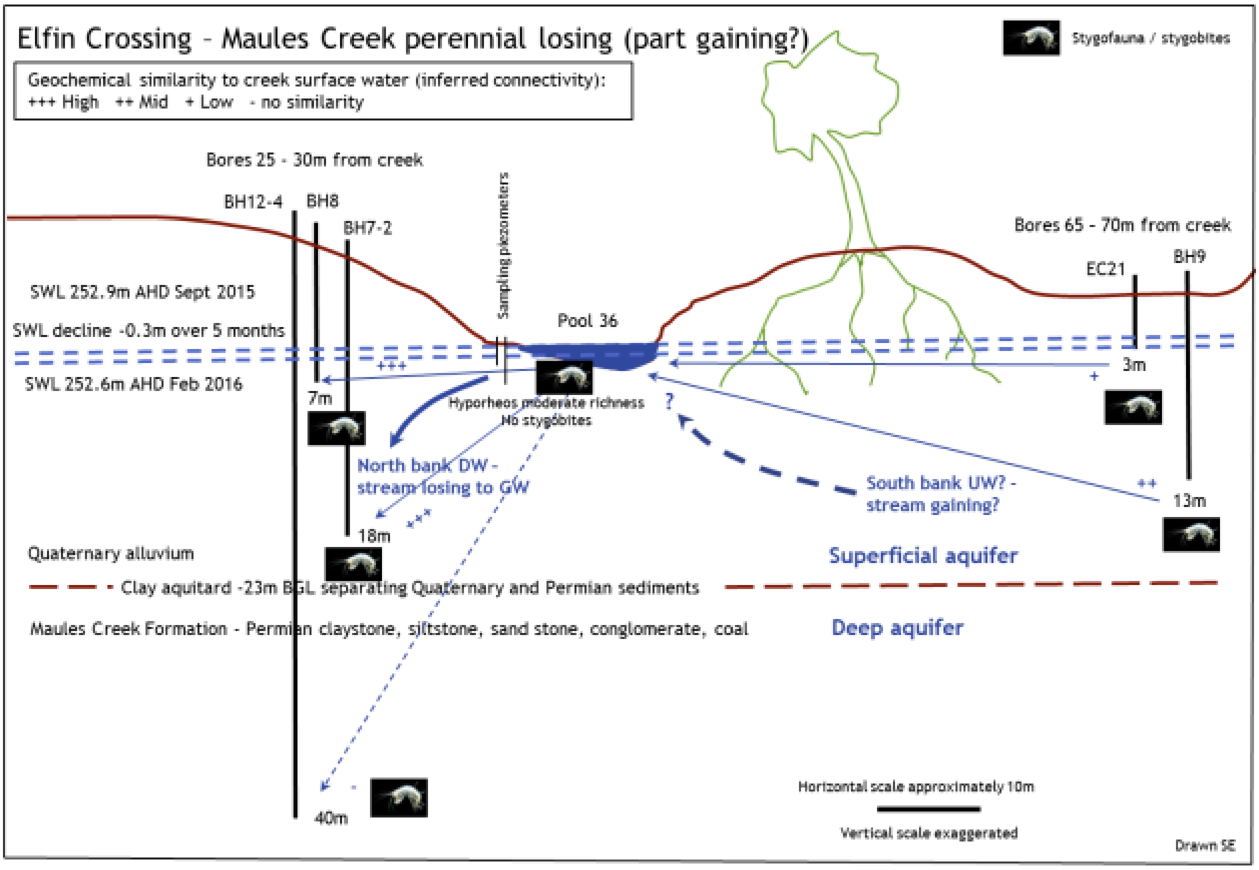


Figure 129: Maules Creek at Elfin Crossing showing study site Pool 36 and bores with inferred connectivity based on hydrochemistry. Note that stygofauna were collected in the deep aquifer here.

Table 29: Maules Creek (Elfin Crossing) supporting table for Figure 129: ecohydrological conditions, changes and responses observed/measured during the study period in this section of creek with perennial gaining / losing flow regime, February 2015 to February 2016.

Time stage	Hydrological state or change	Hydrochemistry response	OM microbial degradation response	Hyporheic invertebrate response
1. Feb 2015	Creek flow continuous, pools and riffles, hyporheic zone saturated.	Not sampled	Not sampled.	Not sampled.
2. Sept – Oct 2015	Little change in surface flows and HEF, aquifer receding.	Reduced conditions (reduced species such as ammonia, iron and phosphate present)	Inferred similar to Maules losing section prior to complete desaturation	Low richness, low abundance, stygobite functional group not detected.
3. Feb 2016	As above.	As above	As above	As above.

4.6.6.2 Maules Creek (perennial losing)

The conceptual model of the perennial losing section is shown in Figure 128 with supporting information in

Table 28. In summary, the conceptual model for the losing section of Maules Creek highlights the following:

(1) Lithology, hydrology. The coarse alluvium confers high hydraulic permeability and aquifer-creek connectivity, facilitating transport and bi-directional exchange between creek and aquifer of DO, nutrients, POM, DOM and other solutes, as well as microorganisms and invertebrates. The high

permeability and connectivity also induces rapid and weakly buffered ecohydrological responses of the hyporheic zone to changes such as groundwater recession / drawdown occurring in the adjacent aquifer.

(2) Hydrogeology. High aquifer-creek connectivity combined with a relatively thick depth of alluvium (>20m thick) and shallow water table (generally < 5m below ground level) confers buffering capacity (resilience) in terms of groundwater habitat availability and persistence during episodes of groundwater recession / drawdown. This combination of aquifer properties and strong hydrologic connectivity also confers a potential capacity for recovery if the aquifer functions as a refuge for the stygobite and stygophile functional components of the hyporheos during episodes of hyporheic desaturation / DO and redox shifts, and a source for recolonization of the hyporheic zone if conditions become suitable.

(3) Flow regime. The perennial losing flow regime confers predominantly downwelling VHH / HEF paths and a tendency in OM, DO and redox gradients towards suboxic – anoxic conditions producing uninhabitable conditions for invertebrates. In contrast to the other sites, there is no compensatory input of oxic regional groundwater. Anaerobic microbial metabolism dominates biogeochemical processes.

(4) Groundwater recession/drawdown shifts perennial gaining flow regimes and/or upwelling HEFs towards losing and/or downwelling conditions. Invertebrate metabolism and productivity in the hyporheic zone is reduced as conditions rapidly become anoxic. Below the hyporheic zone at depth in the aquifer, oxic regional groundwater provides suitable conditions for stygobites and stygophiles.

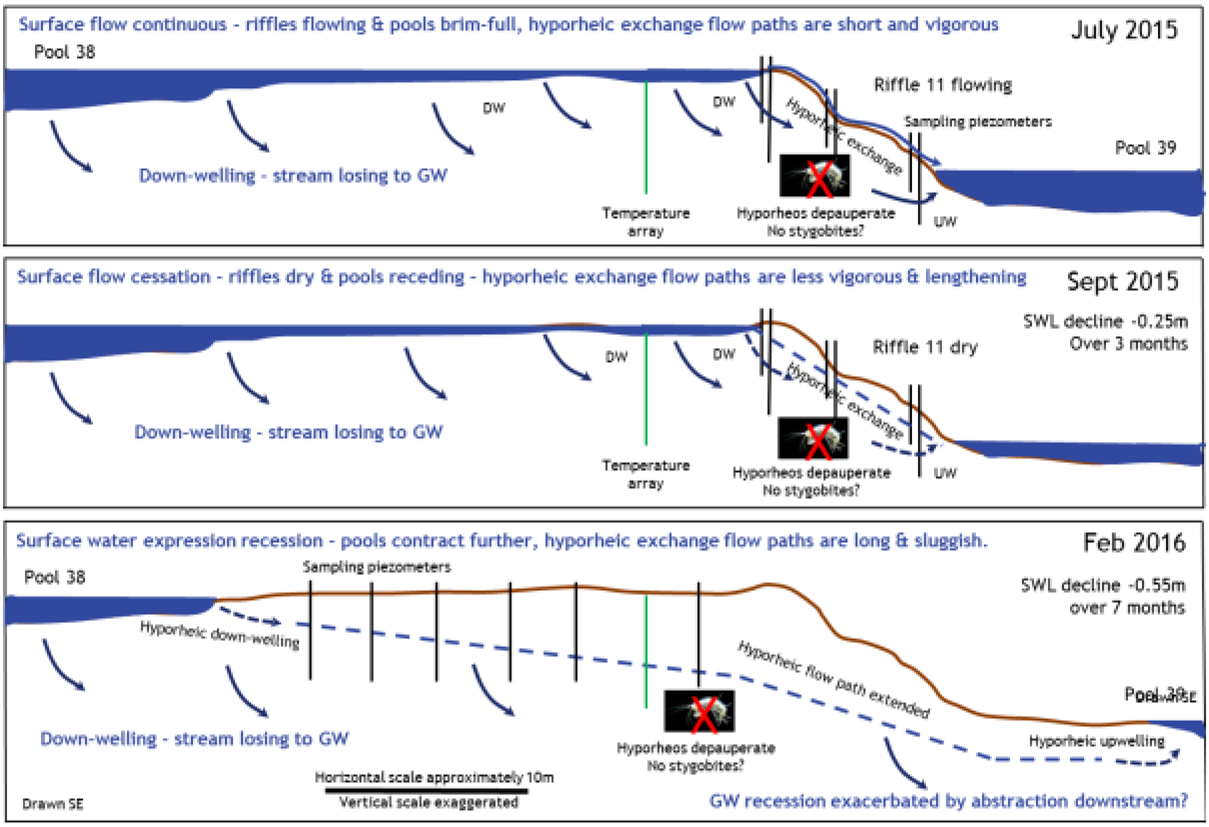


Figure 130: Maules Creek perennial losing section study sites Pool 38 - Riffle 11 – Pool 39 from July 2015 to February 2016, showing the cessation of riffle flow and gradual drying and contraction of pool 38.

Table 30: Maules Creek (perennial losing section) supporting table for Figure 130 ecohydrological conditions, changes and responses observed/measured during the study period in this section of creek with perennial losing flow regime, July 2015 to June 2016.

Time stage	Hydrological state or change	Hydrochemistry response	OM microbial degradation response	Hyporheic invertebrate response	Comments / Integration
1. July 2015	Creek losing, surface flow continuous, riffles and pools, hyporheic zone saturated and HEF paths short steep gradients, aquifer receding.	Reduced conditions		Moderate richness, low abundance, stygobite functional group not detected.	
2. Sept – Oct 2015	Creek losing, surface flow ceased, riffles dry, pools full, HEF paths less vigorous, aquifer watertable receding.	Shift towards suboxic conditions. Increased DOC	Surface rates 0.0562 /day Subsurface rates 0.0580 / day	Moderate richness, low abundance, stygobite functional group not detected.	
3. Feb – Apr 2016	Creek losing, surface water expression highly contracted to remnant pools, HEF path length greatly increased along low gradient, watertable receding.	Shift towards very reduced conditions (iron reduction). Increased DOC	Surface rates 0.0372 / day Subsurface rates 0.0520 / day	Moderate richness, low abundance, stygobite functional group not detected.	

At the beginning of the study in February 2015 this section had continuous surface flow characterised by long pools interspersed by short riffles (Appendix 3: Figure 134). Due to low rainfall and little surface recharge from upstream, the section had a gradual decline in flow and receding water levels over the following twelve months, initially with the cessation of riffle flow followed by contraction and drying of the pools (Figure 164). Between July and September 2015, the creek and groundwater level declined approximately 0.25m when surface flow through riffle 11 ceased. Between September 2015 and February 2016 water levels declined a further 0.30m and the length of pools 38 and 39 had shrunk to lengths of less than 40m (approximately). As the pools receded the length of the hyporheic flow path between pools 38 and 39 increased to more than 200m by February 2016. Upwelling water at the head of Pool 39 in February 2016 was hypoxic / anoxic and contained Fe^{2+}, which on contact with air became rapidly oxidised producing a yellow deposit (Appendix 3: Figure 165). Thus over the entire twelve month period water levels declined approximately 0.55m overall and the surface expression of water in pools 38 and 39 had contracted linearly by some 70 to 80%. Hyporheic sampling was focused on riffle 11 with a length of approximately eight metres and hydraulic head difference of more than 0.75m, which connected the flow from pool 38 (approximately 125m long) to pool 39 (approximately 210m long). In February 2016, the hyporheic flow beneath riffle 11 had receded below the maximum depth of the sampling piezometers (0.9m) so sampling was undertaken a short distance upstream in the dry bed of pool 38 (Appendix 3: Figure 164).

The losing conditions in Maules Creek below Elfin Crossing may be exacerbated by groundwater abstraction for cotton irrigation which occurs further downstream. If this is the case it may have the effect of causing the groundwater drawdown to migrate in the upstream direction and cause surface water following a rainfall derived flow event in Maules Creek to drain to groundwater sooner. The ‘switch point’ from gaining to losing conditions, currently in the vicinity of Elfin Crossing, might be expected to shift either upstream or downstream, or remain steady, depending on the balance of recharge to discharge as well as flow conditions in the creek.

4.7 Synthesis for Component 3

4.7.1 Key Findings

The key findings and implications for EIA integrate the individual case studies of components 2 and 3 and are generally recognised, although sometimes under-emphasised, in the published literature.

Key finding 1.

Baseflow is important. Groundwater is essential for sustaining baseflow, hydroecological processes and ecosystem structure and function in surface and subsurface (hyporheic) habitats in both intermittent and perennial streams.

Implication for EIA. Consider stream baseflow in intermittent (and perennial) streams.

Key finding 2.

Hyporheic exchange flows are important. The hyporheic zone plays an important role in hydroecological processes and stream ecosystem functioning in both intermittent and perennial streams.

Implication for EIA. Consider hyporheic exchange flow in intermittent (and perennial) streams.

Key finding 3.

Groundwater recession (drawdown) is a stressor. Groundwater recession/drawdown reduces baseflow and changes hyporheic exchange flows, which may deplete ecological values and the hydroecological functioning of streams and associated groundwater-dependent ecosystems (GDEs). The very narrow vertical range (generally < 1 to 2 m depth) of the hyporheic zone means that only small amounts of groundwater drawdown may have a significant impact on the ecological functioning and baseflow connectivity of streams.

Implication for EIA. Consider the effects of drawdown on baseflow and hyporheic exchange flow (in intermittent and perennial streams).

Key finding 4.

Ecosystem assets and services that are potentially depleted by groundwater drawdown include biodiversity, detrital processes, and important biogeochemical processes mediated by microbes such as carbon and nutrient transformations (see further details in case study sections following below).

Implication for EIA. Consider surface and subsurface biodiversity, detrital processes, and biogeochemical processes.

Key finding 5.

Conceptual models were useful for integrating our multidisciplinary findings (including hydrology, hydrogeology, hydrochemistry, stream and groundwater ecology) towards a holistic synthesis, to aid understanding of hydroecological responses in streams due to changes in groundwater regime.

Implication for EIA. Consider conceptual models as a useful tool for integrating information across disciplines, and as an aid for assessing potential hydroecological responses due to changes in groundwater regime.

The additional key findings below are context-specific to the case studies at Maules Creek and Bremer River, and are expanded and generalised, with precautionary inferences, where possible.

Key finding 6.

Intermittent streams are ecologically important. Intermittent streams with a groundwater baseflow component harbor a rich biodiversity in both surface and subsurface (hyporheic) habitats.

Implication for EIA. Consider surface and subsurface diversity in intermittent streams.

Key finding 7.

Even when surface flow has ceased, important ecosystem processes still occur in the subsurface (hyporheic) zone of intermittent streams.

Implication for EIA. Consider dry (and wet) phase hydrology, processes and diversity in intermittent streams.

Key finding 8.

Obligate groundwater invertebrates (stygobites) are an ecological group that are 'signature' indicators of baseflow connectivity between streams and permanent groundwater (aquifers), and they respond to changes in groundwater condition and quality (DO and redox state), hyporheic exchange and stream flow regime.

Implication for EIA. Consider the representation of ecological functional groups and 'signature' stygobite taxa.

Key finding 9.

Streams with only a thin alluvial cover and hence shallow hyporheic / groundwater habitat may harbor a rich hyporheos. At Bremer River, we found a rich hyporheos inhabiting thin veneers of saturated alluvium (< 1m) overlying relatively impermeable and uninhabitable strata (clay or tight bedrock). In contrast at Maules Creek, the hyporheic habitat is comparatively deep (> 10m) alluvium. The shallow hyporheic habitats are likely to be more sensitive and vulnerable to changes in groundwater levels.

Implication for EIA. Consider local context and habitat characteristics, assessed on a case by case basis, and validated through field studies as appropriate.

Key finding 10.

Groundwater (baseflow) connectivity confers resistance and resilience in stream ecosystems, but resistance and resilience depend on the nature, context and regime of stream, baseflow and aquifer connectivity. At Maules Creek, compared with Bremer River, enhanced resistance and resilience is conferred by the combination of deep alluvium and strong baseflow of oxic regional groundwater.

Implication for EIA. Similar to above, consider the local context and assess on a case by case basis, with field validation studies as required.

4.7.2 General conceptual models

Our project findings have been conceptualized in Figure 131 and Table 31 below.

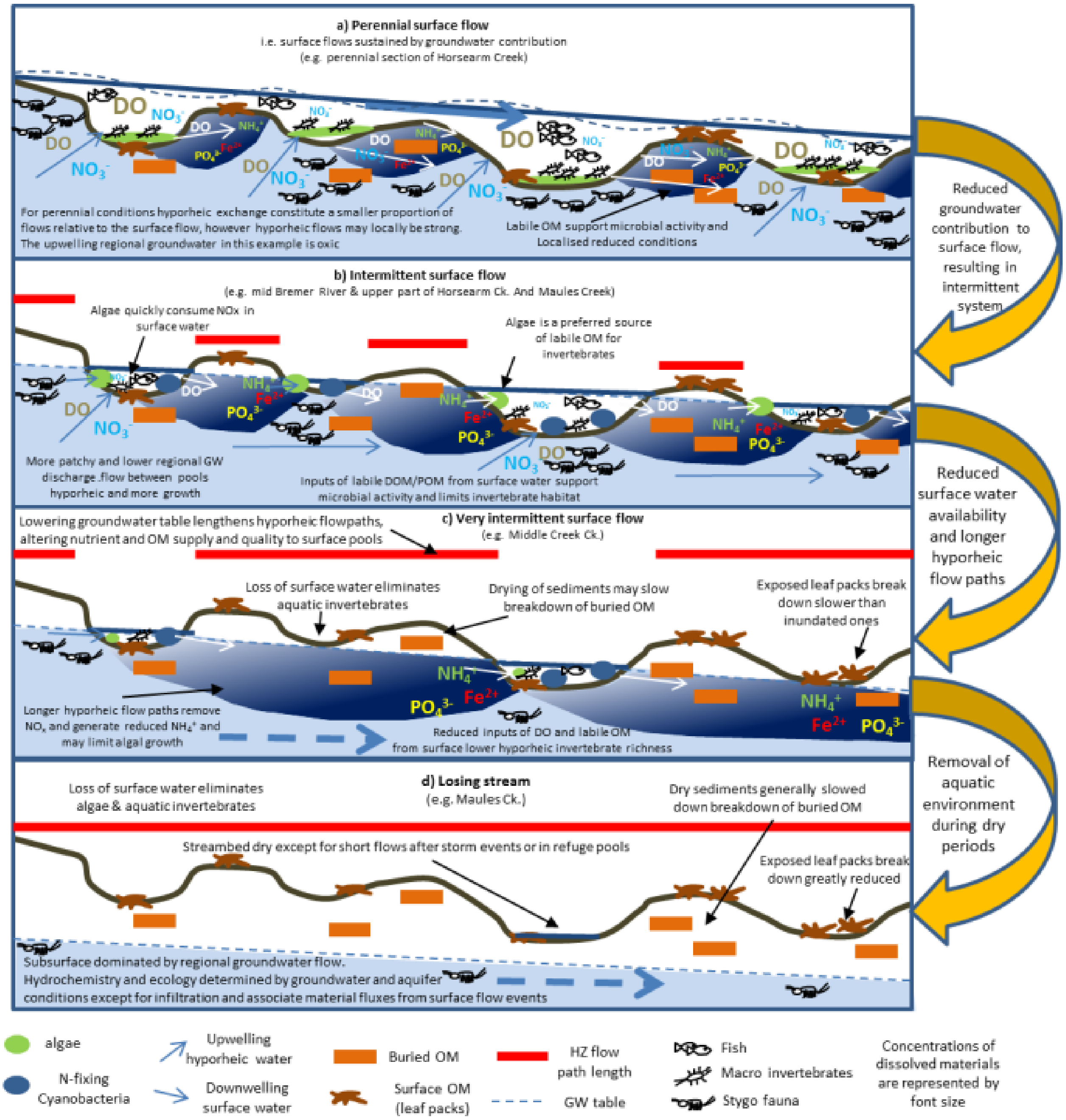
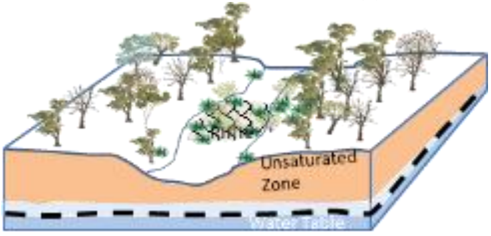
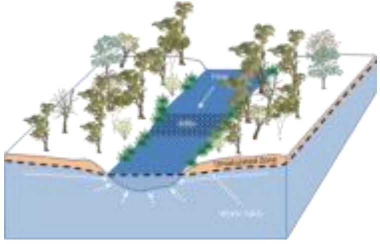
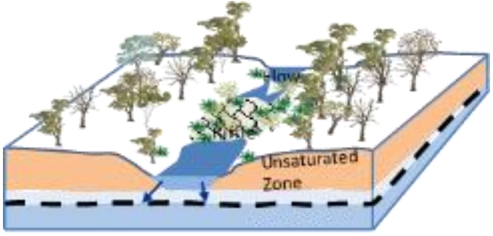
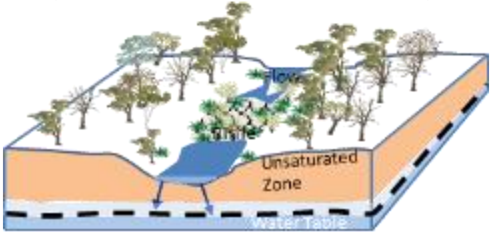
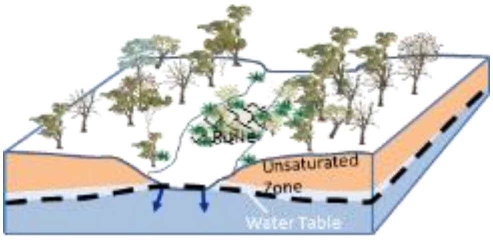
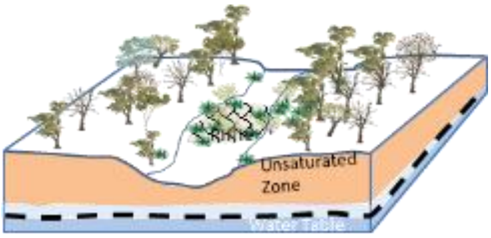


Figure 131: Conceptual longitudinal in-stream and hyporheic zone ecological and biogeochemical changes in response to increased groundwater drawdown. This model is mostly relevant for sites with oxic regional groundwater. From top to bottom: a) continuous surface water flow (perennial or intermittent during flow event) with considerable baseflow (regional groundwater discharge). Short hyporheic flow paths and oxic conditions favour rich and abundant hyporheos; b) discontinuous pools with some regional groundwater contribution maintaining pools, but with subsurface flow (hyporheic flow) between pools. The longer subsurface flow paths (and residence times) allow redox reactions to progress towards more reduced conditions with nitrate reduction and some manganese-oxide and iron-oxide reduction. As a consequences zones of hypoxia/anoxia are larger and invertebrate fauna habitat reduced; c) Further reduction in the groundwater level limits surface water expression and flow is now predominantly along hyporheic flow paths. The redox conditions are now further reduced with iron-oxide reduction, sulfate reduction and methanogenesis. The iron-oxide reduction leads to release of phosphate from the dissolving iron-oxide surfaces. Anoxic conditions make the habitat unsuitable for invertebrates, and much of the hyporheic flow path is dominated by anaerobic microbes. Hyporheic invertebrates are limited to smaller areas of oxic downwelling hyporheic flow, or the deeper oxic parts of the regional groundwater; and d) For further lowering of the water table surface expressions of groundwater is lost and streambed generally dry except for intermittent pools following flow events. Subsurface flows are dominated by regional groundwater flows, but with recharge during flow events. Stygofauna retreat to deeper oxic parts of the regional groundwater. For prolonged dry periods the subsurface hydrochemistry and ecology may resemble the regional conditions in the aquifer.

Table 31: Ecohydrological conditions, changes and responses observed/measured/inferred during stages of recharge, recession or groundwater drawdown cycle in a stream with an intermittent flow regime and groundwater baseflow component.

	Ecological response			
Time stage	**Hydrological state or change**	**Biogeochemical processes, Nutrient and organic C**	**Detrital processes**	**Hyporheos & stygofauna**
Pre- recession/drawdown intermittent dry phase 	No surface flow. HZ may be saturated, partially saturated, desaturated. HZ connected or disconnected.	Depending on oxic / suboxic / anoxic status of baseflow component, generally reduced conditions in saturated HZ. Fe^{2+}, Mn^{2+} and NH$_4^+$ present.	Surface rates low. Subsurface rates depend on sediment moisture status	Hyporheos present/absent, depending on degree of saturation / desaturation of HZ and connection/disconnection.
Pre-drawdown intermittent wet phase 	Pools and riffles connected by continuous surface flow, duration sustained via baseflow, hyporheic zone saturated and HEF paths short with steep gradients.	More oxic HZ conditions for streams connected to aquifers with oxic groundwater	Surface rates high. Subsurface rates high	Hyporheos rich, including epigean and hypogean groups (stygophiles and stygobites)
During recession / drawdown transition early stage 	Decreased or ceasing gaining conditions. Riffle flow ceases, surface pools sustained by reduced baseflow or contracting as water table declines, HEF paths lengthening and gradient declining.	Shift towards suboxic conditions with decreases in DO and NO$_3^-$ (denitrification) and anoxic conditions with increase in reduced species such as Fe^{2+}, Mn^{2+} and NH$_4^+$ and release of sediment bound phosphate.	Surface rates variable, depending on moisture status. Subsurface rates depend on moisture status	Hyporheos present, depending on DO and redox conditions, with epigean and hypogean groups (stygophiles and stygobites).
During recession / drawdown transition later stage 	Creek losing. Pools contracting, HZ partially desaturated and long flow HZ flow paths between remaining pools, capillary fringe connected	Minimal HEF returning to creek. Increase in reduced species such as Fe^{2+}, Mn^{2+} and NH$_4^+$ and release of sediment bound phosphate	Surface rates variable, depend on moisture status.	Hyporheos depleted, depending on DO and redox conditions.

	Ecological response			
Time stage	**Hydrological state or change**	**Biogeochemical processes, Nutrient and organic C**	**Detrital processes**	**Hyporheos & stygofauna**
Maximum drawdown – scenario **connected** 	Surface regime intermittency increased towards temporary / episodic, HZ partially desaturated, capillary fringe connected	Losing flow paths into aquifer. No reoxygenation as HEF does not return to the surface. Reduced species such as Fe^{2+}, Mn^{2+} and NH_4^+ and release of sediment bound phosphate	Surface rates variable, depending on moisture status. Subsurface rates depend on moisture status of sediments	Hyporheos depleted, depending on redox conditions and degree of desaturation of HZ.
Maximum drawdown - scenario **disconnected** 	Surface regime intermittency increased towards temporary / episodic, shortened baseflow periods following flows, HZ desaturated, capillary fringe disconnected a majority of the time	HZ generally desaturated and returning to oxic conditions in desaturated zone (but without flow).	Surface rates low Subsurface rates depend on moisture status of sediments	Hyporheos absent, stygoxenes / epigean groups locally extinct, stygobites and stygophiles retreat with declining water table.
Post drawdown / Recovery	Return to “typical” conditions as at start of cycle top panel.	Restored	Restored	Stygoxenes and epigean groups recolonize from adjacent surface populations. For hypogean groups, depends on connectivity to, and persistence of aquifer reservoir populations of stygobites and stygophiles.

4.7.3 Evaluation of hypotheses

- Variation in stream flow regime and the degree of substrate saturation will influence organic matter processing in surface and hyporheic environments, with greater decomposition in wetter experimental periods and areas (as per Component 2).
    - Organic matter decomposition was lower in drier surface habitats and principally related to the degree of substrate saturation. Consequently, our findings support the notion that a loss of in-stream wetted area will slow organic matter decomposition in surface habitats. However, rates of organic matter decomposition were consistently greater in the hyporheic zone than in surface habitats, and were largely unaffected by surface drying (as per Component 2).
- Variation in groundwater discharge (stream flow regime gaining or losing) and hyporheic exchange flow direction (upwelling vs. downwelling) and flow path length, will influence the biogeochemical processing of nutrients and organic carbon (OC), and redox hydrochemistry.
    - Biogeochemical processing of nutrients and organic carbon (OC), and redox hydrochemistry were principally related to the interplay between groundwater discharge and hyporheic exchange gradients. Hyporheic downwelling and stream losing conditions were characterised by chemically reducing conditions. Hyporheic upwelling and stream gaining hydrochemical conditions were dependent on the length and/or residence time along hyporheic flow paths, as well as the influence of regional groundwater discharge mixing with hyporheic exchange flows.

In the Maules catchment, localised discharge of oxic regional groundwater exerted a strong influence on hyporheic biogeochemical processes by sustaining aerobic biogeochemical processes to a greater degree than would be expected if groundwater discharge was anoxic.

  - Generally, we found that nitrate/ammonium ratios initially increased along hyporheic flow paths, presumably due to nitrification coupled to organic matter mineralisation, and then decreased as denitrification set in. As the length and/or residence time along hyporheic flow paths increased, hydrochemical conditions became more reduced and progressed from oxic to suboxic (DO < 0.5 mg/L) and then hypoxic-anoxic (DO < 0.2 mg/L), leading to reduced ionic species including ferrous iron and manganese. We found that the onset of iron-oxide reduction coincided with release of phosphorus and a reduction in nitrogen/ phosphorus ratios.

- Variation in stream flow regime, hyporheic exchange flows, organic carbon (OC), dissolved oxygen (DO) and redox hydrochemistry will influence the richness, abundance and functional composition of hyporheic invertebrate assemblages.

  - Hyporheic invertebrate assemblages, richness and abundance were highly variable over small spatial scales, and at different time periods of sampling. In our study sites, oxic conditions, irrespective of flow regime class and hyporheic exchange direction, supported the existence of hyporheos. In both Bremer and Maules catchments, suboxic conditions (DO < 0.5 mg/L) were associated with depletion of invertebrate richness and abundance, and invertebrate absence was correlated with severely hypoxic / anoxic conditions and reduced ionic species (especially iron).

  - The influence of stream flow regime and hyporheic exchange flow gradients was equivocal in our study sites. In the Maules catchment, where locally strong discharge of oxic regional groundwater occurs in combination with hyporheic upwelling, richness and abundance were significantly higher than in habitat patches where the hydraulic head difference was weakly upwelling, neutral, or downwelling. At a broader spatial scale within the Maules catchment, we found that the hyporheos was significantly more diverse in the stream section with an intermittent flow regime (Middle Creek) than in the sections with perennial gaining and losing regimes. In the Bremer catchment, where groundwater discharge and hyporheic exchange flow gradients are more muted, we found no statistically significant ($p< 0.05$) differences related to stream flow regime or hyporheic exchange.

  - We found limited evidence of associations between hyporheic diversity and measures of organic carbon, namely negative correlations of richness and abundance with processed and unprocessed humics (PARAFAC) in the Maules catchment only.

  - The functional composition of hyporheic invertebrate assemblages, as characterised by the representation of stygobites, stygophiles and stygoxenes, appeared to be coupled with hydrologic connectivity between the hyporheic zone and permanent regional groundwater (aquifers). Stygobites that were ‘indicator taxa’ of connectivity to permanent groundwater included the Bathynellidae, Parabathynellidae and Psammaspididae. Some evidence for changes in functional composition was suggested by the inability of sampling to detect stygobites in the losing section of Maules Creek, but this may be a consequence of the depauperate assemblages and limited sampling.

  - The equivocal evidence for influence of stream flow regime on hyporheos assemblages, and the unexpected diversity found in the intermittent section in Maules catchment, suggest that the surface expression of flow regime per se is limited consequence in shaping hyporheos patterns, which are more directly linked to stream baseflow - groundwater connectivity.

- Variation in stream baseflow - groundwater connectivity will influence the capacity of hyporheic ecosystems to withstand environmental changes that result in lowering groundwater levels, and also their capacity to recover when groundwater levels are restored.

- The aquifer bore sampling findings supported our hypothesis that the hyporheic zone at Maules Creek shared strong hydrologic connectivity and faunal similarities with the regional superficial aquifer. The implications of this similarity were that the regional aquifer functions as a retreat or 'refuge' for hyporheic stygobites and stygophiles when the hyporheic zone becomes desaturated, for example during drought or groundwater drawdown from pumping. Likewise, the aquifer may also function as the source for stygobite and stygophile recolonization of the hyporheic zone when the hyporheic zone becomes re-saturated.

- We found no evidence to contradict our hypothesis that groundwater (baseflow) connectivity likely confers resistance and resilience in stream and hyporheic ecosystems, but the nature, context and regime of stream, baseflow and aquifer connectivity are likely to be fundamentally important.

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5 Component 4 – Biodiversity and risk assessment of Great Artesian Basin spring wetlands

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5.2 Summary

This project component aims to conceptualise and quantify the environmental determinants of GAB spring wetland species composition and biodiversity patterns and how resident species respond to cumulative impacts of groundwater drawdown and other threats. Making use of best-available datasets and expert knowledge, the project undertakes a GAB-wide assessment of the current biodiversity and conservation values of spring wetlands, how characteristics of springs influence those values and how those values are currently at risk from the cumulative impacts of a range of threatening processes.

Key research questions are:

1) What is the current state of knowledge concerning freshwater biodiversity endemic to GAB springs, with respect to taxonomy, distribution, population status, population connectivity, ecology and threats and how does this knowledge vary among taxa?

2) What are the key environmental determinants of the distribution and biodiversity patterns (e.g. taxonomic and phylogenetic richness, diversity and endemism) of taxa endemic to GAB springs?

3) How well do existing conservation measures (i.e. representation within protected areas, inclusion in species' conservation status listings) align with our new assessment of the taxonomy and present-day distribution of GAB endemic taxa?

4) How does vulnerability to threats posed by groundwater drawdown and other stressors vary among endemic flora and fauna of GAB spring wetlands?

5) How does relative exposure to the different threats posed by groundwater drawdown and other stressors vary among GAB spring complexes?

6) Which GAB spring complexes are at the highest cumulative risk (i.e. contain high number of vulnerable species and are exposed to the highest threat intensities)?

7) What on-ground management and monitoring activities are appropriate for managing threats in GAB spring complexes identified as being at high risk?

The results of the study can be used to develop hypotheses concerning the mechanisms responsible for these hydro-ecological associations that can be tested and validated in future projects using field and/or lab studies. Collectively, this information will provide a foundation to understand, predict and monitor the responses of spring ecological communities to hydrological and physicochemical changes associated with coal seam gas and coal mining development and how these stressors interact with existing threatening processes. Improved understanding of these relationships will inform prediction, management and mitigation of impacts on GAB spring wetlands.

Key project outputs:

- New knowledge of environmental determinants of GAB spring species composition, biodiversity patterns and ecological responses to cumulative impacts of human activities and other stressors
- Improved understanding of the mechanisms responsible for hydro-ecological associations as a basis for further testing and validation in future projects using field and/or lab studies
- Comprehensive Endnote library (and pdfs) of literature for GAB spring flora and fauna
- Conceptual models (e-versions)
- Geodatabase of all data layers (species distributions, biodiversity attributes, environmental attributes and risk assessment results)

Key project outcomes:

- Foundation to understand, predict and monitor the responses of GAB spring ecological communities to hydrological and physicochemical changes associated with coal seam gas and coal mining development and other stressors
- Provides a compendium of information (literature, data, models) that can be used by OWS and IESC to assess development proposals
- Enables evaluation of relative ecological and biodiversity values of an area subject to proposed mining development compared with elsewhere
- Can inform future updates to conservation listing of GAB discharge spring ecological community (and individual species) by DoE Threatened Species Scientific Committee

5.3 Background

Spring wetlands are a characteristic groundwater-dependent feature of the Great Artesian Basin (GAB), a deep regional groundwater system that covers 22% of the Australian continent. GAB springs have great ecological, scientific, anthropological and economic significance (Mudd 2000). Due to prolonged isolation, many springs and spring complexes contain rare and endemic species of plants, fish, molluscs, isopods, amphipods and ostracods that have undergone significant genetic differentiation and speciation. The springs are also important as refuge areas for arid zone wildlife and habitat for migratory birds (Mudd 2000). Widespread and sustained development of the Great Artesian Basin over the past century for agricultural uses and resource extraction has seen an overall decline in the flows from springs in many parts of the GAB and recent mining and extraction of metals, coal and petroleum products have exacerbated this problem (Fensham et al. 2010).

Springs that originate from the GAB are often referred to as ‘discharge’ springs (Figure 132, Table 32). Rainwater recharge enters primarily along the uplifted eastern edge of the Great Dividing Range. It flows in a generally south-westerly direction where, it is forced to the surface along faults and weaknesses creating springs (Habermehl 1982). Springs are clustered at a range of spatial scales (Table 32). Numerous vents can feed each spring. Springs emerging in the same geological setting are clustered into ‘complexes’, which can contain between one and hundreds of springs that are usually less than 10km apart (Figure 132). Complexes are also grouped at a larger scale into 13 ‘supergroups’. Water in springs fed by the GAB generally has a low dissolved solids concentration and is hyper-alkaline and with stable temperatures. These features distinguish GAB springs from other forms of inland water body, including ‘tertiary’ springs from non-GAB sources (Fensham et al. 2004, 2011). The form a spring takes when reaching the surface is variable and contingent on groundwater pressure and the locality. Springs can range from small soaks less than 1m^2, to large limnocrenic wetlands exceeding 1000m^2 or mound springs formed by mud deposits or accumulating peat deposits.

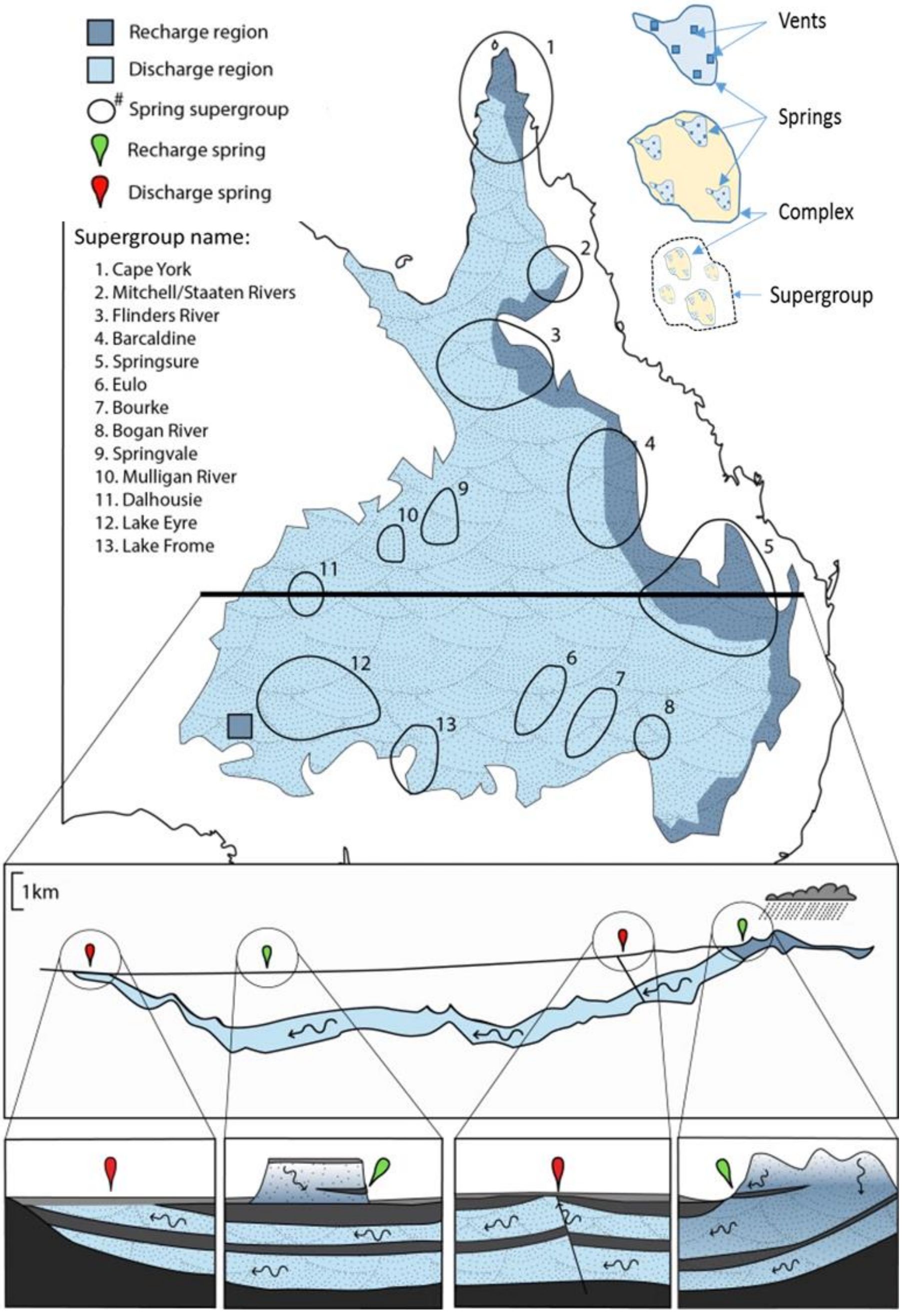
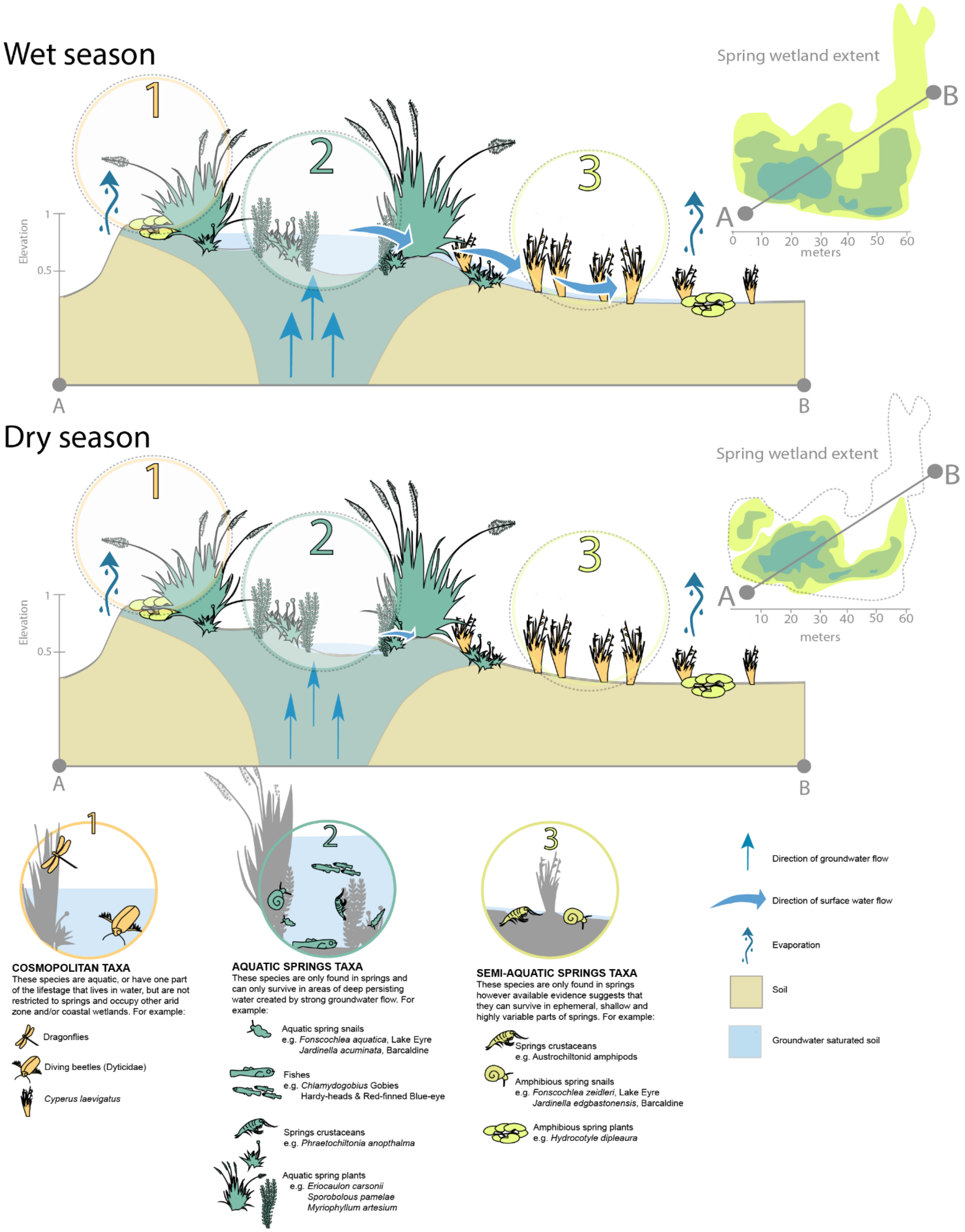


Figure 132: Conceptualisation of the Great Artesian Basin showing the location of spring supergroups, location of aquifer discharge and recharge zones, hydrogeological cross-section depicting water sources and flow paths to discharge and recharge springs, and the hierarchical nature of vents, springs, complexes and supergroups (Source: Renee Rossini).

Table 32: Definition of springs at a range of spatial scales (source: Fensham and Fairfax 2003, Fensham et al. 2011, Miles et al. 2012, Department of the Environment 2016).

Term	Definition
Vent	Point of natural groundwater discharge at the ground surface
Spring	A vent or vents where the natural groundwater outflow forms a single spring wetland
Discharge spring (also referred to as 'artesian' spring)	Groundwater outflow is from confined aquifers in which the water has had an extremely long residence time and where the water reaches the ground surface via faults in the strata overlying the aquifer or from thin or exposed parts of the aquifer
Recharge spring (also referred to as 'non-artesian' spring)	Groundwater outflow is overflow from unconfined aquifers (generally associated with outcropping sandstones), the water has a relatively short residence time compared with confined underground aquifers (except in western GAB) and water flow rates are affected by recent rainfall events
Tertiary spring	Spring emerging from non-GAB sources, primarily from tertiary aquifers that overlie the GAB
Spring group	Multiple springs all related to the same hydrogeological structure and probably the same aquifer (or groundwater source) and hydrochemistry (at the present time)
Spring complex	Collection of springs all related to a particular location and hydrogeological feature(s)
Supergroup	Major regional clusters of spring complexes with some consistent hydrogeological characteristics

In the Australian arid zone, most surface water bodies are ephemeral (e.g. billabong, rockholes) and are characterised by a 'boom and bust' cycle of water abundance and water scarcity. They are dominated by a cosmopolitan suite of animals and plants with adaptations that allow them to persist through times of water scarcity and disperse and reproduce at times of water abundance. These organisms are generally found across the arid zone or, in some cases, in coastal wetlands and watercourses (Fensham et al. 2011). In contrast, GAB springs are home to some of these cosmopolitan species (Figure 133) as well as a unique assemblage of plants and animals that cannot be found in any other freshwater environment (i.e. they are endemic to springs fed by the GAB) (Ponder 1995, Fensham et al. 2011). These endemic species are ecologically and taxonomically diverse, including animals that are fully aquatic and rely on permanent water in spring pools like fishes (Figure 133) and those that are semi-aquatic and live in the spring margins and outflow zones that change in wetness and spatial extent due to seasonal and interannual variations in rainfall and spring outflow (Figure 133; Ponder et al. 1989, Fensham et al. 2004, Rossini et al. in press). Depending on their position in the landscape, some springs may become periodically connected to other freshwater habitats via flowing stream networks and floodplain inundation during the wet season, while others may remain entirely isolated. Collectively, variation in these environmental characteristics of springs is likely a major determinant of flora and fauna composition and abundances.



COSMOPOLITAN TAXA
These species are aquatic, or have one part of the lifestage that lives in water, but are not restricted to springs and occupy other arid zone and/or coastal wetlands. For example:

- Dragonflies
- Diving beetles (Dyticidae)
- *Cyperus laevigatus*

AQUATIC SPRINGS TAXA
These species are only found in springs and can only survive in areas of deep persisting water created by strong groundwater flow. For example:

- Aquatic spring snails
  e.g. *Fonscochlea aquatica*, Lake Eyre
  Jardinella acuminata, Barcaldine
- Fishes
  e.g. *Chlamydogobius* Gobies
  Hardy-heads & Red-finned Blue-eye
- Springs crustaceans
  e.g. *Phraetochiltonia anopthalma*
- Aquatic spring plants
  e.g. *Eriocaulon carsonii*
  Sporobolous pamelae
  Myriophyllum artesium

SEMI-AQUATIC SPRINGS TAXA
These species are only found in springs however available evidence suggests that they can survive in ephemeral, shallow and highly variable parts of springs. For example:

- Springs crustaceans
  e.g. Austrochiltonid amphipods
- Amphibious spring snails
  e.g. *Fonscochlea zeidleri*, Lake Eyre
  Jardinella edgbastonensis, Barcaldine
- Amphibious spring plants
  e.g. *Hydrocotyle dipleaura*

Figure 133: Conceptual model of seasonal variation in a naturally functioning discharge spring and the cosmopolitan, aquatic, and semi-aquatic species it sustains.

The aquatic biodiversity restricted to GAB springs is highly threatened by a range of processes. Akin to arid zone springs globally, Australia's GAB springs have experienced a history of loss and physical disturbance (Fairfax and Fensham 2002, Fairfax and Fensham 2003). This legacy may continue as many GAB discharge springs occur in areas of current and potential future CSG and coal mining developments (Fensham et al. 2010). The ecological consequences of groundwater decline for GAB springs and the resident biota may be substantial, but difficult to detect due to time lags and long response times and potentially difficult to reverse (e.g. aquifer collapse, salinity intrusion, shifts in ecosystem type/function; Kath et al. 2014). Furthermore, impacts of groundwater drawdown could greatly increase the vulnerability of endemic spring flora and fauna to threatening processes associated with land use, livestock damage, invasive species and climate change (Fensham et al. 2010). Whilst previous risk assessments have predicted potential effects on springs due to groundwater drawdown, none have taken a basin-wide approach or considered the ecological results of the extinction of springs (e.g. they have predicted whether springs are lost, not the resulting biodiversity losses) or the potential for drawdown to interact with other threatening processes.

Quantifying the hydroecological processes that shape and sustain the distinctive biotic assemblages of discharge spring wetlands of the Great Artesian Basin (GAB) and establishing how species respond to threatening processes is a challenging goal. Recent research casts light on the evolution, ecology and history of springs but up-to-date compilations of this literature are scarce (the most recent being the 'recovery plan' for the community of native species reliant on GAB springs; Fensham et al. 2010) and renders assessments of biodiversity and threats at a basin-wide scale impossible. By understanding the environmental determinants of spring biodiversity and how resident species respond to threatening processes, we can inform prediction, management and mitigation of impacts on GAB spring wetlands.

5.4 Spatial assessment of biodiversity patterns and conservation values of discharge springs across the Great Artesian Basin

Renee Rossini, Mark Kennard, Rod Fensham, Travis Gotch, Doug Ward and Ben Stewart-Koster

5.4.1 Key objectives:

- Review the state of knowledge available regarding GAB spring endemic species and collate an updated list of all taxa within the plants, fishes, molluscs, amphipods and isopods with sufficient evidence to classify them as a GAB spring endemic, including sub-species and clades
- Review and update information regarding the basin-wide distribution of these taxa and their range extents
- Analyse spatial patterns in the composition, richness, diversity and endemism (taxonomic and phylogenetic) of GAB spring complexes
- Explore relationships between environmental characteristics and biodiversity/species composition of spring complexes
- Identify knowledge gaps for all taxa in the review
- Assess the alignment of conservation listings of all taxa in the review

5.4.2 Background

Wetlands supported by springs (Shepard 1993, Kreamer and Springer 2008, Unmack and Minckley 2008) provide habitat for cosmopolitan species as well as specialised taxa not known from other habitats (Ponder 1995, Myers and Resh 1999, Fensham et al. 2011, Cantonati et al. 2012). Despite their conservation value, springs across the globe share parallel stories of habitat loss (Fairfax and Fensham 2003, Stevens and Meretsky 2008, Unmack and Minckley 2008, Cantonati et al. 2016). The Great Artesian Basin (GAB) in Australia, one of the world's largest actively recharging aquifers, feeds thousands of springs spanning arid and semi-arid regions (Figure 132, Figure 134) (Habermehl 1982). Groundwater with a long residence time in the aquifers of the GAB has a distinct water chemistry (Habermehl 1982) creating spring ecosystems for plant and animal species, many of which cannot be found in other freshwater environments and have very small geographic ranges (Ponder 1995, Fensham et al. 2011).

Due to habitat specificity, small geographic range sizes, and exposure to a range of threatening processes including rapid habitat loss due to unrestricted extraction of water from their source aquifers (Fairfax and Fensham 2002, 2003), most endemic spring fauna and flora face significant extinction risk and are thus of considerable conservation interest. In 2001 these threats were acknowledged with the listing of the GAB springs and their "community" of flora and fauna under the federal Environmental Protection and Biodiversity Conservation (EPBC) Act 1999. The review conducted as part of the recovery plan for the listed community (Fensham et al. 2010) has not been updated despite considerable progress on further inventory, conventional taxonomy and molecular studies of the organisms. Attempts to rank springs in regard to their endemism and diversity have occurred (Fensham and Price 2004), but this ranking only included flora. There is still very little information on how taxa are distributed at a basin-wide scale or the physical features of spring localities that are associated with the diversity of endemic taxa. Without such information, it is impossible to assess biogeographic patterns of diversity in springs, to consider the effectiveness of current research or conservation programs or to consider how threatening processes may lead to biodiversity loss within the GAB spring system.

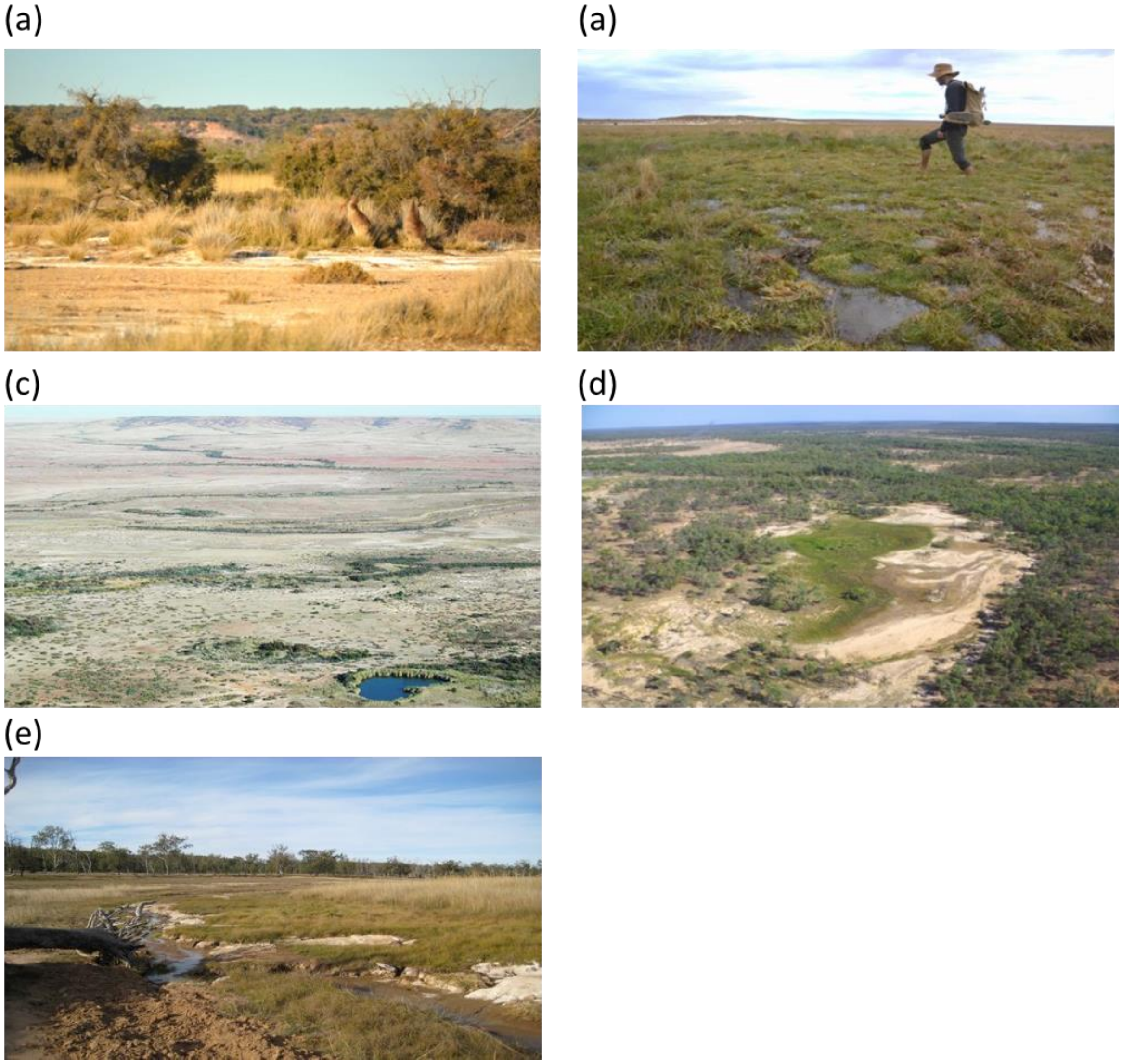


Figure 134. Examples of GAB springs. (a) small spring in the centre of the Edgbaston Reserve in the Pelican Creek complex, Barcaldine supergroup, Queensland (photograph: Renee Rossini), (b) main spring at the Elizabeth Springs complex, Springvale supergroup, Queensland (photograph: Renee Rossini), (c) aerial shot of a large spring in the foreground and the landscape surrounding it at the Dalhousie complex, South Australia (photograph: Robert Brandle DEH, taken from the Witjira National Park Management Plan 2009), (d) aerial shot of a large spring at Doongmabulla in the Moses complex, Barcaldine supergroup, Queensland (photograph: Roderick Fensham), (e) large spring in the Yowah complex, Eulo supergroup, Queensland (photograph: Roderick Fensham).

5.4.3 Approach

5.4.3.1 Data acquisition

Our review focussed on five groups of organisms (fishes, molluscs, amphipods, isopods and plants) as an initial review suggested the availability of data and taxonomic refinement in other taxonomic groups that potentially include endemic species (e.g. Acari, Platyhelminthes, Arachnida and Insecta) was not sufficient. Data used for this review came from a range of sources. Information regarding springs, their location and their current dormancy status was taken primarily from two publically available databases - the Lake Eyre Basin Springs Assessment (LEBSA) available through the Queensland Government and WaterConnect via the South Australian government. Information regarding endemic species and their distribution was acquired either from these databases, literature review and expert consultation (see below for method). The source for information from spatial layers (such as land-use types, locations of conservation reserves etc.) is provided below.

5.4.3.2 Location and environmental characteristics of spring complexes

We compiled a database of GAB springs with information sourced from the Lake Eyre Basin Springs Assessment and WaterConnect via the South Australian government. This data comprised spring data recorded as individual spring vents. Each spring vent has been allocated a spring wetland ID such that a collection of spring vents forms a spring wetland. “Non-GAB” springs and ‘recharge’ springs were removed prior to analysis and we focussed only on those identified as ‘discharge’ springs. Non-discharge springs were excluded from the analysis as they do not form part of the threatened ecological community listed under the EPBC, because they generally lack endemic taxa and because knowledge regarding their distribution is still rudimentary (for reviews of their characteristics compared to discharge springs, see Fensham et al. (2011) and Silcock et al. (2016)).

Individual spring wetlands can have multiple vents but the primary unit for analysis was the spring wetland, and thus springs with multiple vents were only included as a single location (total of 6,308 spring wetlands) which was determined as the centroid of a minimum-bounding polygon constructed around all vents in that spring. Spring complexes (i.e. collections of springs) were also delineated with a minimum-bounding polygon surrounding all springs attributed as belonging to each spring complex. These polygons were then used to calculate a complex centroid and an estimate of complex area as a surrogate for total wetland area (ideally we would have summed the wetted area for all spring wetlands within each complex, but these data were unavailable or inconsistently and infrequently estimated for many of the 6,308 springs). The final dataset contained 326 spring complexes.

Environmental characteristics of spring complexes included measures of: spring surface water persistence; relative spatial proximity and hydrologic connectivity, and climate, landscape topography and primary productivity (Table 33). Spatial proximity of complexes to one another was estimated as the euclidean distance between all 326 spring complex centroids (as the crow flies), and averaged for each complex. Two measures of hydrologic connectivity among complexes were developed: drainage line connectivity and surface water inundation connectivity. Drainage line connectivity (akin to the ‘landscape resistance distance’ measures employed by Morán-Ordóñez et al. 2015) utilized the DEM-derived drainage network (Stein et al. 2014) and the ArcGIS Network Analyst tool to construct a table of hydrologic distances between all 6,308 spring wetland polygons across all complexes. For each of the 326 complexes, the number of all other complexes that it was connected to via at least one hydrologic (stream network) connection between spring polygons within each respective complex, was summed. Similarly, surface water inundation connectivity utilized the ‘Water Observations from Space’ (WOfS) data set (Mueller et al. 2016) and the 6,308 spring wetland centroids which were intersected with the WOfS data set. As for drainage line connectivity, surface water connectivity for each of the 326 complexes was calculated as the number of all other complexes that it was connected to via at least one hydrologic (inundated area) connection between spring centroids within each respective complex. As well as the frequency of hydrologic connections among spring complexes, we also calculated the average hydrologic distance among connected complexes using the drainage line connectivity and surface water inundation connectivity datasets.

Table 33: Environmental attributes of spring complexes hypothesised to be ecologically important determinants of the distribution of endemic flora and fauna. Attributes were used as candidate predictor variables in explanatory models of biodiversity attributes and species distributions. An explanation and method of derivation for each attribute is given. The location, complex membership and physical characteristics of vent/springs are sourced from Lake Eyre Basin Springs Assessment datasets. Climate, topography and primary productivity data sourced from Stein et al. (2014). Water Observations from Space data set sourced from Mueller et al. (2016).

Environmental attribute	Unit	Explanation and method of derivation
Complex size		Represents potential likelihood of supporting greater number of species (based on species-area relationship theory)
Total number of springs per complex	count	Total number of springs per complex
Total complex area	ha	Total complex area estimated by calculating a minimum area polygon around spring centroids within each complex
Physical characteristics		Description of the surface composition and general morphology of spring vents (derived from field surveys of 170 Qld complexes only, equivalent information unavailable for SA)
Dominant surface composition of vents	% of vents per complex	Categorical description of the dominant surface composition of the vent as either Peat, Mud (exuded), Rocky seep (fractured), Sand/Silt, Carbonate (travertine) or Water/soak
General morphology of vents	% of vents per complex	Categorical description of the general morphology of the vent as either Mound, Flat, Closed depression (concave), Open depression (watercourse) bed, Open depression (watercourse) bank or Terraced.
Spatial proximity and hydrologic connectivity		Represent surrogates for biogeographic and ecological mechanisms that influence species distributions (e.g. via dispersal and recolonization processes)
Mean Euclidean distance	km	Average Euclidian distance between each complex and all other complexes (higher number = more isolated)
Count connected by drainage network	count	Count of the number of complexes that are connected to a given complex via the 9” DEM derived drainage network (lower number = more isolated)
Mean drainage network distance	km	Average of the drainage network distance between a given complex and all other complexes that are hydrologically connected (higher number = more isolated)
Count connected by surface water inundation areas	count	Count of the number of complexes that are connected to a given complex via surface water inundation areas (based on Water Observations from Space dataset) (lower number = more isolated)
Mean surface water inundation distance	km	Average of the surface water inundation distance between a given complex and all other complexes that are hydrologically connected (higher number = more isolated)
Climate, topography and primary productivity		Represent surrogates for critical environmental regimes (light, moisture, thermal) and habitat characteristics that influence species distributions. (calculated as the mean of spring centroid values within each complex)
Elevation	m	Estimated using 9” DEM
Mean annual solar radiation	MJ/m^2/day	Measure of the rate of solar energy arriving at the Earth's surface from the Sun's direct beam. Influences water temperature and evaporation rates.
Mean annual air temperature	^{o}C	Influences rates of aquatic chemical and biological processes and distributions of species (via thermal tolerances)
Mean annual rainfall	mm	Influences aquatic habitat availability and connectivity, soil moisture, rates of weathering of rock and hence their hydrogeological properties and the release and transport of solutes and materials
Mean annual rainfall erosivity	(MJ mm) / (ha hr yr)	Indicator of rainfall intensity, an important influence on processes of infiltration, runoff generation and erosion
Mean annual net terrestrial primary productivity	tC ha^{-1}	Primary productivity is the measure of carbon intake by plants during photosynthesis, and this measure is an important indicator for studying the health for plant communities. Net Primary Productivity (NPP) is the amount of carbon uptake after subtracting Plant Respiration (RES) from Gross Primary Productivity (GPP).

5.4.3.3 Literature review and re-evaluation of the endemic taxa list

The list of species assessed as being endemic to GAB springs was derived using a three-stage literature review consisting of: 1) construction of a list of candidate species, sub-species and populations; 2) literature review and summary of currently available published information pertaining to each species; and,

3) expert consultation regarding the review, assigned endemic status and data pertaining to the organisms' distributions.

An initial list of candidate organisms was constructed primarily from all taxa already listed under the EPBC "threatened community", either individually or within the blanket listing for "the community of native species dependent on natural discharge from the GAB". Species flagged during expert consultation were added. Any organism previously documented as a single species but now considered a complex of multiple species was expanded. Any species for which there was sufficient data regarding patterns of genetic differentiation across populations was split into clades or sub-species of the parent species. Any species that current research suggests is not endemic to GAB springs was excluded. Unfortunately the information available regarding some taxa currently included in the "threatened community" was insufficient for determining distribution across the GAB with confidence; these taxa were also excluded (Appendix 4:Table 59).

A systematic review of all literature pertaining to any organism included in the list of candidate species was conducted. Published literature for each species was identified from journal databases (Web of Science, Scopus), the Atlas of Living Australia, the Biodiversity Heritage Library and expert consultation. Candidate organisms were classified as endemic to the GAB, crenophile (i.e. would likely perish in the wild without GAB springs) or non-endemic, with all non-endemic taxa excluded from the final list.

Researchers and on-the-ground managers of spring wetlands and spring-dependent species were identified as experts in each of the five groups of organisms. Following the review of each group by the reviewers, the summaries and classifications regarding endemism were shared with experts for their opinion. A discussion was facilitated between all included parties until a final consensus list was agreed upon. In cases where a consensus was difficult to reach, a precautionary approach was taken and the more conservative list was accepted.

The final list of taxa was matched against the official list and lists of occupied complexes that form the definition of the "threatened community" or any species lists available through databases for each complex. In some cases, spring complexes had changed name or been expanded or divided, the distribution of taxa was unknown, or records were questionable. For some species with high intraspecific diversity, evidence suggested they should be split into clades, but not all complexes had been analysed. In each of these cases the authors of the LEBSA (primarily R.J. Fensham) and WaterConnect (primarily T. Gotch) were consulted regarding spring complexes and experts or researchers with the most experience with collecting each taxon were consulted to ascertain the final occupancy status of problematic taxa. Where a population's clade affinity was not directly ascertainable from the literature or experts, the geographical location and previous published literature regarding connectivity between complexes in similar taxa were consulted and a clade affinity assigned to the taxon. The final set of taxa for which we were confident of taxonomy and present-day distributions (presence-absence of 98 taxa across 326 spring complexes – see Results) represented the best available and most up-to-date information available for biodiversity analyses.

5.4.3.4 Patterns of endemic biodiversity

We quantified spatial variation in biodiversity values of spring complexes using a range of attributes including taxonomic and phylogenetic richness, diversity and endemism, and assemblage beta diversity (Table 34). The methods of Fensham and Price (2004) were also used to assign a conservation rank that simultaneously captured the number, endemic status and potential isolation from other populations for all GAB spring complexes containing one or more endemic taxa. In this system, complexes with the same taxonomic composition can receive different scores as it also takes into account isolated populations of more broadly distributed taxa.

Table 34: Method, rationale and key references for each of the biodiversity attributes used to characterise spring complexes.

Biodiversity attribute	Method, rationale and key reference
Taxonomic Richness (S)	Number of taxa in a spring complex (also referred to as alpha diversity)
Taxonomic Diversity (H')	Shannon Diversity. An index that incorporates the number of taxa and the evenness of the distribution of individuals across species (we used area of occupancy in spring complexes as our measure of abundance). The index can increase either by having additional unique taxa or by having greater taxon evenness (Shannon, 1948)
Taxonomic endemism (TE)	An index of endemism identifying areas where taxa with restricted ranges are concentrated. Based on the number of taxa within a spring complex weighted by the inverse of each taxon's distribution range (also known as weighted endemism). This index ranges from one, where all taxa in a complex have broad geographical ranges, to infinity, with large values indicating the presence of taxa with range-size rarity (i.e. areas with high endemism) (Rebelo & Siegfried, 1992).
Phylogenetic Diversity (PD)	A measure of diversity based on units of phylogenetic variation (instead of taxa) (Faith 1992, Faith et al., 2004). PD is calculated as the sum of those branch lengths of the phylogenetic tree representing the taxa occurring in a spring complex. Areas with high PD may represent centres of current speciation and may be important areas to protect for maintenance of evolutionary processes. High PD could arise by having a high number of closely related taxa or by having few taxa that are phylogenetically divergent from one another. PD incorporates complementarity in that the score contributed by a given taxon in a spring complex depends on how closely it is related to other taxa present. Complete molecular phylogenies are not available for most taxonomic groups and taxa considered in this study, so we used published phylogenies and assumed equal branch lengths (see Figure 135).
Phylogenetic Endemism (PE)	Phylogenetic endemism (PE) is a measure of the degree to which elements of evolutionary history are spatially restricted in space. PE combines the phylogenetic diversity (PD) and taxonomic endemism (TE) measures to identify areas where substantial components of phylogenetic diversity are restricted (Rouser et al., 2009). To estimate the degree of PE represented by the taxa in a given area, the range size of each branch of the phylogenetic tree (rather than the range of each taxon) is quantified. PE is therefore the sum of branch length ⁄ clade range for each branch on the tree (where a clade is a single branch on the tree consisting of an organism and all its descendants).
Assemblage Beta Diversity (BD)	Assemblage beta diversity is defined as the variability in species composition among sampling units for a given area and was measured as the average dissimilarity from individual observation units (spring complexes) to their group centroid (all spring complexes) in multivariate space, using the Bray-Curtis dissimilarity measure (Anderson et al. 2006)
Conservation Rank (CR)	The Fensham and Price (2004) Conservation Rank incorporates the number of species at a complex, their geographic isolation at a basin-wide scale and the isolation of each population in species with a distribution that spans multiple complexes

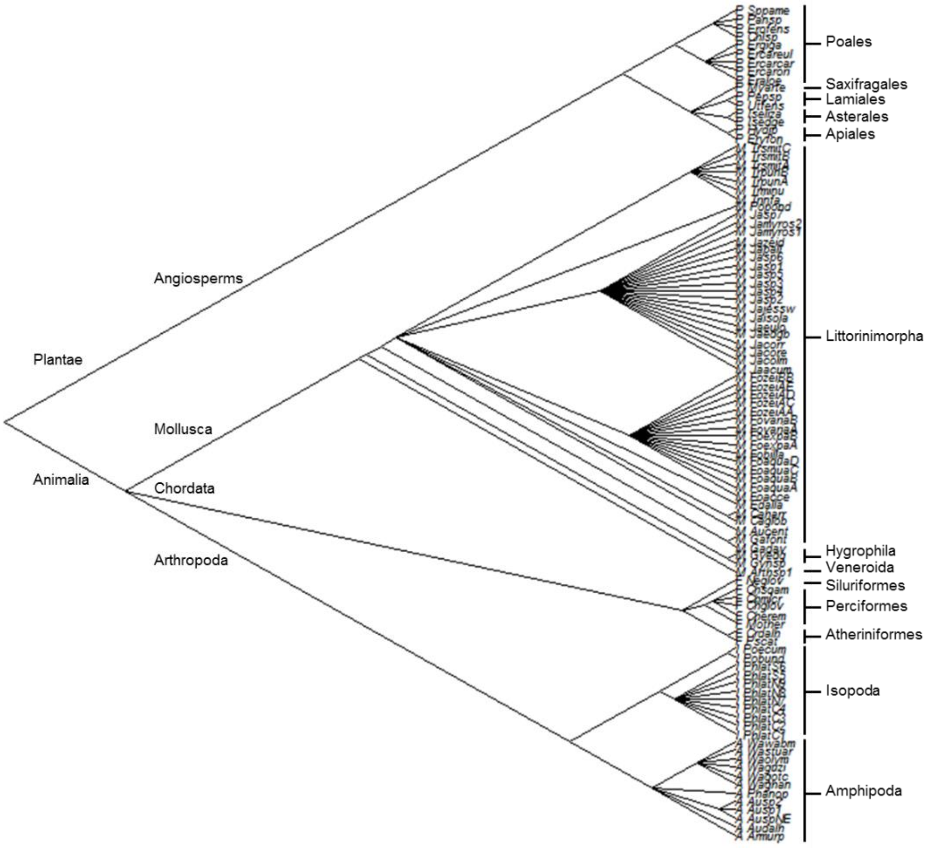


Figure 135: Phylogeny used for calculation of phylogenetic diversity (PD) and phylogenetic endemism (PE).

5.4.3.5 Characterising endemic assemblages

In this report, the word 'assemblage' is used to refer to a collection of endemic taxa within a spring complex that have been grouped together with other complexes of similar composition using heuristic guidance of statistical clustering techniques outlined in Legendre and Legendre (2012) and with the methodology provided in Borcard et al. (2011). A dissimilarity matrix for presence-absence data of endemic species in springs was constructed using the 'altGower' function within the Vegan package 'vegdist'. Clustering of assemblages was trialled with a range of methods, with the final method choice being the method that did not sink obviously distinct complexes into an assemblage (e.g. many methods sunk Dalhousie into other Lake Eyre complexes) and was most parsimonious with the original distance matrix (ascertained using Cophenetic correlation, Borcard et al. 2011). Both Ward's and the weighted pair-group method using arithmetic averages (WPGMA) returned the best results. The analysis continued with Ward's method as both methods generally grouped assemblages in the same way but WPGMA tended to favour multiple complexes as assemblages and large somewhat dissimilar groups. The final number of assemblages was ascertained by assessing optimal silhouette widths via the Mantel test (Borcard et al. 2011). Clustering of between 17 to 33 assemblages had relatively equal average silhouette widths so the silhouette plots for each proposed number of assemblages were assessed to ascertain the minimum number of groups with the best outcome for group membership (i.e. all schemes with <33 assemblages had groups of complexes with one or more misaligned group members and negative silhouette widths); a final number of 33 assemblages was chosen.

5.4.3.6 Modelling relationships of environmental characteristics of spring complexes with biodiversity attributes and taxonomic composition

To quantify relationships between environmental characteristics of spring complexes (Table 33) and spatial variation in each biodiversity attribute (Table 34), we used multiple regression (hurdle models). Hurdle models are two step models well suited for zero-inflated data (such as in this study), where the model fitting is separated into two steps (Cunningham and Lindenmeyer 2005). The first step is a binary component, where the zero and non-zero values of the response (biodiversity attribute in this case) is modelled, and the second component is where the non-zero component of the data is modelled for the subset of locations containing non-zero data (in our case, spring complexes containing one or more taxa) (Martin et al. 2005). We applied a zero-inflated Poisson (ZIP) regression model to the non-zero species richness observations (appropriate for count data) and log-normal regression models for the non-zero values of each of other biodiversity attributes (appropriate for continuous data). The binomial component of the ZIP model produced a predicted probability of occurrence of non-zero response data (i.e. contains one or more taxa) at each complex. We used the taxon prevalence across complexes containing one or more taxa (i.e. 76 of 326 complexes, prevalence = 0.233) as a threshold to convert these probabilities to a predicted presence or absence, which is appropriate when the objective is to derive unbiased estimates of prevalence (Liu et al. 2005; Freeman & Moisan, 2008). All models were fitted in the R statistical environment (R Core Team 2016).

Across the entire region, the majority of predictor variables were generally minimally correlated and represented potential surrogates for mechanistic drivers of species distributions and abundances (Table 33). The exception to this pattern was that the count and mean hydrologic distance of complexes connected by drainage network or by surface water inundation were correlated with one another ($r>0.8$) so only the count variables were used as predictors. All predictor variables were scaled (range-standardised) prior to analysis. We used Akaike's Information Criterion (AIC) to select the most probable model for each regression model, using the glmulti package (Calcagno and de Mazancourt 2010) to test all possible candidate models. We assessed the fit of the binomial component by calculating the correct classification rate of the model and also by calculating the area under the receiver operating characteristic (ROC) curve (AUC), a common metric for assessing sensitivity and specificity of binomial models (Fielding & Bell, 1997). The value of the AUC ranges between 0.5 (no agreement) to 1.0 (perfect agreement) with an AUC>0.7 usually considered as acceptable model performance. We estimated the ROC curve using the pROC package (Robin et al. 2011). The percentage variance explained (R^2) was used to assess model fits for the continuous response variables.

We also attempted to identify associations between environmental characteristics of spring complexes and spatial variation in taxonomic composition. We trialled two multi-response modelling methods (Multivariate Adaptive Regression Splines and Multivariate Classification and Regression Trees; Leathwick et al. 2005, De'ath 2002) as they can accommodate a variety of predictor variable data types, and are well suited to capturing non-linear relationships and interactions among predictors. Unfortunately, preliminary analyses yielded very poor models (little power to explain variation in taxon occurrences and very weak relationships with environmental variables). This was likely due to the very low frequency of occurrences of most taxa (often only 1 occurrence per taxon – see Results); such response data are notoriously difficult to model. These models are not discussed further in this report.

5.4.3.7 Categorising and reviewing literature gaps

During the construction of the taxa list, all literature pertaining to each taxon was collated and categorised to quantify the amount of published information available for each taxon. All publications that included reference to the taxon in question were assigned to one of six information types: taxonomy, distribution, abundance, population connectivity, ecology and threats. These categories were chosen as they form the key types of information required to understand or conserve these taxa. Once all literature had been reviewed, an ordinal score of relative information availability for each information type was estimated for each taxon. Scores ranged from 1 representing little or no information (i.e. data deficiency) to 4 representing very thorough information. The scoring criteria for each information type are detailed in Appendix 4: Table 60.

5.4.3.8 Review of conservation status and assessment of taxa representation within protected areas

Endemic taxa are protected via Australian threatened species legislation at federal or state levels. Threatened species legislation in Australia encompasses all species deemed as part of the “community of native species dependent on discharge of water from the GAB” which are protected as part of the threatened community listing under the Environmental Protection and Biodiversity Conservation Act (EPBC Act). In addition to this listing, some individual species are also listed under either the EPBC or relevant state conservation acts. Many species endemic to springs have also been assessed under the IUCN Red List. Their current status was accessed via the IUCN online database.

Species or populations of a species can be protected within National Parks, Conservation Reserves, Indigenous Protected Areas or private landholder conservation agreements. We quantified representation of endemic spring taxa within existing protected areas that meet the IUCN definition using the most recent available (2014) version of the Collaborative Australian Protected Area Database (CAPAD 2014). For each complex, we calculated the number of springs that intersected protected areas. For each taxon, we calculated the number of occurrences in complexes that intersected protected areas.

5.4.4 *Results*

5.4.4.1 Distribution of GAB springs and their current activity status

In total, 6,308 individual Great Artesian Basin springs (each of which could have one or multiple vents) were identified as existing at present or to have sufficient historical evidence to suggest they existed prior to the year 2000. Those springs have been divided into 326 complexes spread across the 13 supergroups. There is considerable variance in the number of springs associated with the Great Artesian Basin per complex and number of complexes per supergroup (Figure 136). Some supergroups are composed of a single small complex containing one spring (Mitchell/Staaten River, Bogan River) while others vary greatly in the number of complexes and springs per complex (Lake Eyre, Eulo, Flinders River), the largest being the Hermit Hill complex in the Lake Eyre supergroup with 429 springs (Figure 136).

Of the 6,308 springs, 5,412 remain active while 1,161 (18.4%) showed no evidence of flow in recent surveys and are assumed to be dormant. The current status of springs varies considerably across supergroups (Figure 136). Three supergroups show no evidence of dormant complexes (i.e. all complexes contain 100% active springs - Mitchell/Staaten, Dalhousie, Springsure and Lake Frome). In contrast, some supergroups show evidence of considerable spring dormancy and are currently composed of entirely dormant complexes (Bogan River) or contain fewer than 70% of the original number of springs (Flinders River – 19%, Eulo – 41%, and Bourke – 69%).

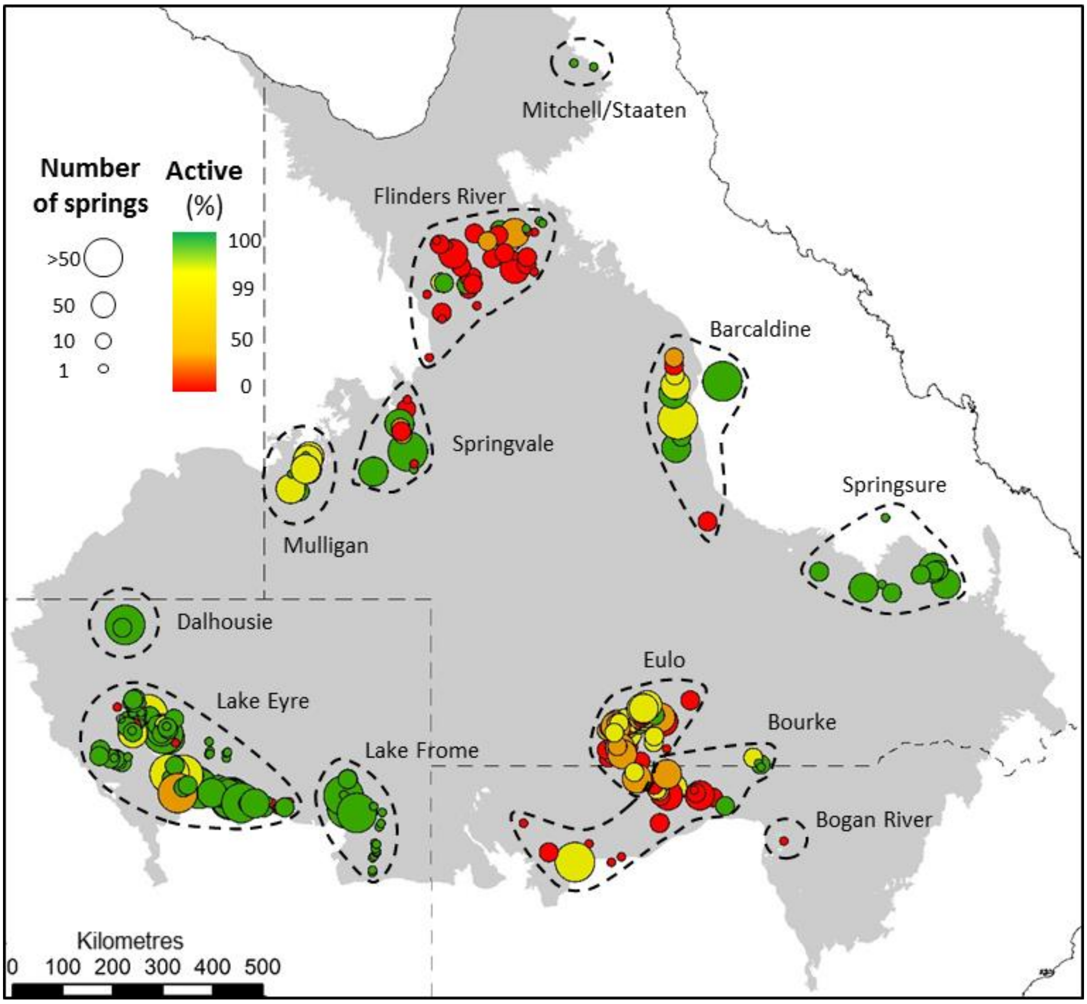


Figure 136: Map of GAB (shaded area) and spring complexes characterised by their status (% springs active). Spring complexes belonging to each supergroup are enclosed by dashed lines.

5.4.4.2 Endemic taxa in GAB springs

Many organisms were excluded from this review because insufficient data were available (Appendix 4: Table 59). The review incorporated 98 taxa of plant (superorders Monocotyledon and Dicotyledon), molluscs (classes Bivalvia and Gastropoda), fish (class Actinopterygii), amphipods and isopods (both within class Malacostraca) with sufficient evidence to suggest they are found only in, or would perish without, GAB discharge springs (Table 35). In the final list, 48 have full species status and 19 are undescribed but have sufficient expert support for their taxonomic distinction at the species level. Two species are represented by multiple described subspecies and there are 7 taxa that are currently described as a single species but have evidence to suggest that they should be considered as a set of distinct clades or sub species. When separated, they equate to a total of 27 taxa. The taxon distribution database used for subsequent analyses represents the best available knowledge concerning the presence-absence of all 98 taxa across all 326 complexes.

Table 35: List of all fish, mollusc, amphipod, isopod and plant taxa incorporated into this review of endemic species in Great Artesian Basin springs, including their higher taxonomic classification, species, any included sub-species and their status (D – described, U – undescribed, PS – putative subspecies, DS – described subspecies). Also shown for each taxon are their frequency of occurrence (number of spring complexes, FoC), area of occupancy (total area of complexes, AoO), percentage of occurrences within protected complexes (% Protected) and the species-level endemism type (indicted with boxes for multiple taxa within a given species) to which they were assigned (see text).

Kingdom	Phylum	Class	Order	Family	Taxon	Code	FoC	AoO	% protected	Taxon. status	Endemism type
Animalia	Chordata	Actinopterygii	Atheriniformes	Atherinidae	*Craterocephalus dalhousiensis*	F_Crdalh	1	56.8	100	D	Narrow
Animalia	Chordata	Actinopterygii	Atheriniformes	Pseduomugilidae	*Scaturiginichthys vermeilipinnis*	F_Pscat	1	29.7	100	D	Narrow
Animalia	Chordata	Actinopterygii	Siluriformes	Plotosidae	*Neosilurus gloveri*	F_Neglov	1	56.8	100	D	Narrow
Animalia	Chordata	Actinopterygii	Perciformes	Eleotridae	*Mogurnda thermophila*	F_Mother	1	56.8	100	D	Narrow
Animalia	Chordata	Actinopterygii	Perciformes	Gobiidae	*Chlamydogobius eremius*	F_Cherem	15	59.9	27	D	Broad supergroup
Animalia	Chordata	Actinopterygii	Perciformes	Gobiidae	*Chlamydogobius gloveri*	F_Chglov	1	56.8	100	D	Narrow
Animalia	Chordata	Actinopterygii	Perciformes	Gobiidae	*Chlamydogobius micropterus*	F_Chmicr	1	0.5	100	D	Narrow
Animalia	Chordata	Actinopterygii	Perciformes	Gobiidae	*Chlamydogobius squamigenus*	F_Chsqam	1	29.7	100	D	Narrow
Animalia	Mollusca	Bivalvia	Veneroida	Lasaeidae	*Arthritica* sp. AMS C.449156	M_Arthsp1	2	80.8	0	U	Narrow supergroup
Animalia	Mollusca	Gastropoda	Hygrophila	Planorbidae	*Glyptophysa* n.sp.	M_Gynsp	1	29.7	100	U	Narrow
Animalia	Mollusca	Gastropoda	Hygrophila	Planorbidae	*Gyraleus edgbastonensis*	M_Gyedg	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	*Gabbia davisi*	M_Gadav	1	19.0	0	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	*Gabbia fontana*	M_Gafont	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Austropyrgus centralia*	M_Aucent	1	56.8	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Caldicochlea globosa*	M_Caglob	1	56.8	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Caldicochlea harrisi*	M_Caharr	1	56.8	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Edgbastonia allanwilsi*	M_Edalla	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea accepta*	M_Foacce	13	4.4	0	D	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea aquatica* Clade A	M_FoaquaA	3	0.4	0	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea aquatica* Clade B	M_FoaquaB	7	13.2	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea aquatica* Clade C	M_FoaquaC	3	83.1	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea aquatica* Clade D	M_FoaquaD	11	8.7	82	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea billakalina*	M_Fobilla	4	83.1	0	D	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea expandolabra* Clade A	M_FoexpaA	2	0.4	0	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea expandolabra* Clade B	M_FoexpaB	6	13.2	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea variabilis* Clade A	M_FovariaA	3	2.8	33	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea variabilis* Clade B	M_FovariaB	12	8.0	58	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea zeidleri* Clade A	M_FozeiAA	1	45.2	0	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea zeidleri* Clade B	M_FozeiBB	12	14.1	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea zeidleri* Clade C	M_FozeiAC	2	37.8	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea zeidleri* Clade D	M_FozeiAD	12	8.7	83	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Fonscochlea zeidleri* Clade E	M_FozeiAE	9	4.4	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella acuminata*	M_Jaacum	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella colmani*	M_Jacolm	2	43.6	0	D	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella coreena*	M_Jacore	1	2.8	0	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella corrugata*	M_Jacorr	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella edgbastonensis*	M_Jaedgb	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella eulo*	M_Jaeulo	2	5.6	0	D	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella isolata*	M_Jaisola	2	0.5	50	D	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella jesswiseae*	M_Jajessw	1	29.7	100	D	Narrow

Kingdom	Phylum	Class	Order	Family	Taxon	Code	FoC	AoO	% protected	Taxon. status	Endemism type
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.410721	M_Jasp1	1	2.7	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.156780	M_Jasp2	1	0.7	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.400131/QMS04_1	M_Jasp3	1	4.4	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.400130/QMS05	M_Jasp4	1	4.4	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.400133/QMS04	M_Jasp5	1	4.4	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* n.sp. AMS C.400132/QMS04_2	M_Jasp6	1	4.4	0	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* sp. AMS C.447677	M_Jasp7	2	0.9	0	U	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella pallida*	M_Japall	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella zeiderorum*	M_Jazeid	1	29.7	100	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* sp. AMS C.415845_1	M_Jamyros1	1	29.7	100	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Jardinella* sp. AMS C.415845_2	M_Jamyros2	1	29.7	100	U	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Posticobia ponderi*	M_Popond	1	<0.1	0	D	Narrow
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia inflata*	M_Trinfa	2	0.4	0	D	Narrow supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia minuta*	M_Trminu	7	1.9	0	D	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia punicea* Clade A	M_TrpunA	10	8.7	100	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia punicea* Clade B	M_TrpunB	9	4.4	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia smithii* Clade A	M_TrsmitA	6	12.7	0	PS	Broad supergroup
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia smithii* Clade B	M_TrsmitB	7	83.6	0	PS	
Animalia	Mollusca	Gastropoda	Littorinimorpha	Tatediae	*Trochidrobia smithii* Clade C	M_TrsmitC	5	2.0	100	PS	
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Arabunnachiltonia murphyi*	A_Armurp	2	37.8	0	D	Narrow supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Austrochiltonia* n.sp. AMSP68165	A_Ausp1	2	34.0	50	U	Narrow supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Austrochiltonia* sp. AMS P68160	A_Ausp2	1	4.4	0	U	Narrow
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Austrochiltonia dalhousiensis* sub.sp. *dalhousiensis*	A_Audalh	1	56.8	100	DS	Narrow
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Austrochiltonia* n.sp. (North Eyre)	A_AuspNE	8	13.5	0	U	Broad supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Phraetochiltonia anopthalma*	A_Phanop	1	56.8	100	D	Narrow
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia ghania*	A_Waghan	5	2.0	0	D	Broad supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia gotchi*	A_Wagotc	5	45.7	0	D	Broad supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia guzikae*	A_Waguzi	1	0.9	0	D	Narrow
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia olympicdamia*	A_Waolym	4	1.1	0	D	Broad supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia stuarti*	A_Wastuar	4	7.2	100	D	Broad supergroup
Animalia	Arthropoda	Malacostraca	Amphipoda	Chiltoniidae	*Wangiannachiltonia wabmakdarbu*	A_Wawabm	6	1.5	83	D	Broad supergroup
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 1/C	I_PhlatC1	13	53.9	69	PS	Broad supergroup
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 2/C	I_PhlatC2	1	35.6	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 3/C	I_PhlatC3	2	0.4	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 4/C	I_PhlatC4	1	2.2	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 5/S	I_PhlatS5	3	1.4	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 6/S	I_PhlatS6	8	2.8	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 7/N	I_PhlatN7	2	0.5	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 8/N	I_PhlatN8	2	0.1	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Amphisopodidae	*Phraetomerus latipes* Clade 9/N	I_PhlatN9	5	12.6	0	PS	
Animalia	Arthropoda	Malacostraca	Isopoda	Ponderellidae	*Ponderiella bundoona*	I_Pobund	2	7.1	0	D	Narrow supergroup
Animalia	Arthropoda	Malacostraca	Isopoda	Ponderellidae	*Ponderiella ecomanufactia*	I_Poecum	2	7.1	0	D	Narrow supergroup
Plantae	Angiosperms	~Eudicots	Apiales	Apiaceae	*Eryngium fontanum*	P_Eryfon	2	35.0	100	D	Narrow supergroup
Plantae	Angiosperms	~Eudicots	Apiales	Araliaceae	*Hydrocotyle dipleura*	P_Hydip	8	80.6	38	D	Broad

Kingdom	Phylum	Class	Order	Family	Taxon	Code	FoC	AoO	% protected	Taxon. status	Endemism type
Plantae	Angiosperms	~Eudicots	Asterales	Campanulacea	*Isotoma* sp. (Edgbaston)	P_Isedge	3	39.4	67	U	Broad
Plantae	Angiosperms	~Eudicots	Asterales	Campanulacea	*Isotoma* sp. (Elizabeth)	P_Iseliza	1	0.5	100	U	Narrow
Plantae	Angiosperms	~Eudicots	Lamiales	Lentibulariaceae	*Utricularia fenshamii*	P_Utfens	5	38.6	60	D	Broad
Plantae	Angiosperms	~Eudicots	Lamiales	Phrymaceae	*Peplidium* sp.	P_Pepsp	2	35.0	100	U	Narrow supergroup
Plantae	Angiosperms	~Eudicots	Saxifragales	Haloragaceae	*Myriophyllum artesium*	P_Myarte	19	126.4	21	D	Broad
Plantae	Angiosperms	~Monocots	Poales	Eriocaulaceae	*Eriocaulon aloefolium*	P_Eraloe	1	29.7	100	D	Narrow
Plantae	Angiosperms	~Monocots	Poales	Eriocaulaceae	*Eriocaulon carsonii* sub.sp. *orientalis*	P_Ercarori	9	65.3	44	DS	Broad
Plantae	Angiosperms	~Monocots	Poales	Eriocaulaceae	*Eriocaulon carsonii* sub.sp. *carsonii*	P_Ercarcar	9	7.7	11	DS	Broad
Plantae	Angiosperms	~Monocots	Poales	Eriocaulaceae	*Eriocaulon carsonii* sub.sp. *euloense*	P_Ercareul	1	4.4	0	DS	Narrow
Plantae	Angiosperms	~Monocots	Poales	Eriocaulaceae	*Eriocaulon giganticum*	P_Ergiga	1	29.7	100	DS	Narrow
Plantae	Angiosperms	~Monocots	Poales	Poaceae	*Chloris* sp.	P_Chlsp	2	35.0	100	U	Narrow supergroup
Plantae	Angiosperms	~Monocots	Poales	Poaceae	*Erogrostis fenshamii*	P_Erofens	2	1.3	50	D	Narrow supergroup
Plantae	Angiosperms	~Monocots	Poales	Poaceae	*Panicum* n.sp.	P_Pansp	1	5.3	100	U	Narrow
Plantae	Angiosperms	~Monocots	Poales	Poaceae	*Sporobolus pamelae*	P_Sppame	8	83.3	38	D	Broad

5.4.4.3 Biodiversity of spring complexes - types of endemism, spatial patterns and relationships with environmental characteristics

There was a diverse range of distribution patterns across taxa that were distilled into four main types (Figure 137a, Table 35). The most frequent was 'narrow' endemism (44% of taxa), whereby a taxon is found in only one complex having on average a full extent of occurrence of ~61 ± 11km^2 (Figure 137a). This is followed by 'narrow supergroup' and 'broad supergroup' endemism whereby a taxon occupies few (≤5) or many complexes (>5), respectively, but is restricted to a single supergroup (Figure 137b and c, respectively). Finally, 'broad' endemism refers to widespread taxa occurring across many complexes and supergroups (including 6 plant taxa, Figure 137d). The only exception to these groups is one undescribed *Isotoma* (sp. Edgbaston), which is spread across two supergroups but occupies only three complexes.

Molluscs make up 52% of the listed taxa and the majority of these are narrow-range endemics (57%) (Figure 137e). The arthropods considered here collectively account for the next largest contribution to the overall count (23%) with amphipods and Isopods accounting for 11 and 12% respectively (Figure 137e). A third of amphipods and isopods have narrow range status, with the majority being narrow range supergroup endemics. The fishes contribute 8% of the taxa on the final list, with 7 of the 8 taxa having narrow ranges and one broadly distributed supergroup endemics (Figure 137e). Vascular plants make up 16% of the final list, but differ from the fauna in being composed of approximately equal parts of all types of endemism including broad range (Figure 137e).

Seventy-six of 326 complexes contained one or more endemic taxa. There was considerable spatial variation in taxon richness, diversity, endemism and beta diversity of spring complexes, but spatial patterns in biodiversity attributes were generally consistent and most attributes were highly correlated with one another (Pearson's r > 0.5, Table 36). Complexes within the Barcaldine, Dalhousie, Lake Eyre and Eulo supergroups had the highest taxonomic richness and diversity (Figure 138). Assemblage beta diversity was also relatively high in these supergroups, as well as in the Springvale supergroup. The Lake Eyre supergroup is distinct because complexes there are taxon-rich but have low phylogenetic richness and endemism (Figure 138). The Lake Eyre supergroup contrasts to complexes with high taxonomic richness, phylogenetic richness and endemism in the east (Barcaldine and Eulo supergroups) and Dalhousie (Figure 138). The highest taxonomic richness, taxonomic endemism, and overall phylogenetic diversity and endemism were recorded at the Pelican Creek complex (containing Edgbaston and Myross springs) in the Barcaldine supergroup (26 taxa) (Figure 138). This complex contains almost exclusively narrow range endemic species from most taxonomic classes considered here. The relative conservation rank of spring complexes (Fensham and Price 2004) was strongly correlated with all other biodiversity attributes (Pearson's r > 0.62, Table 36).

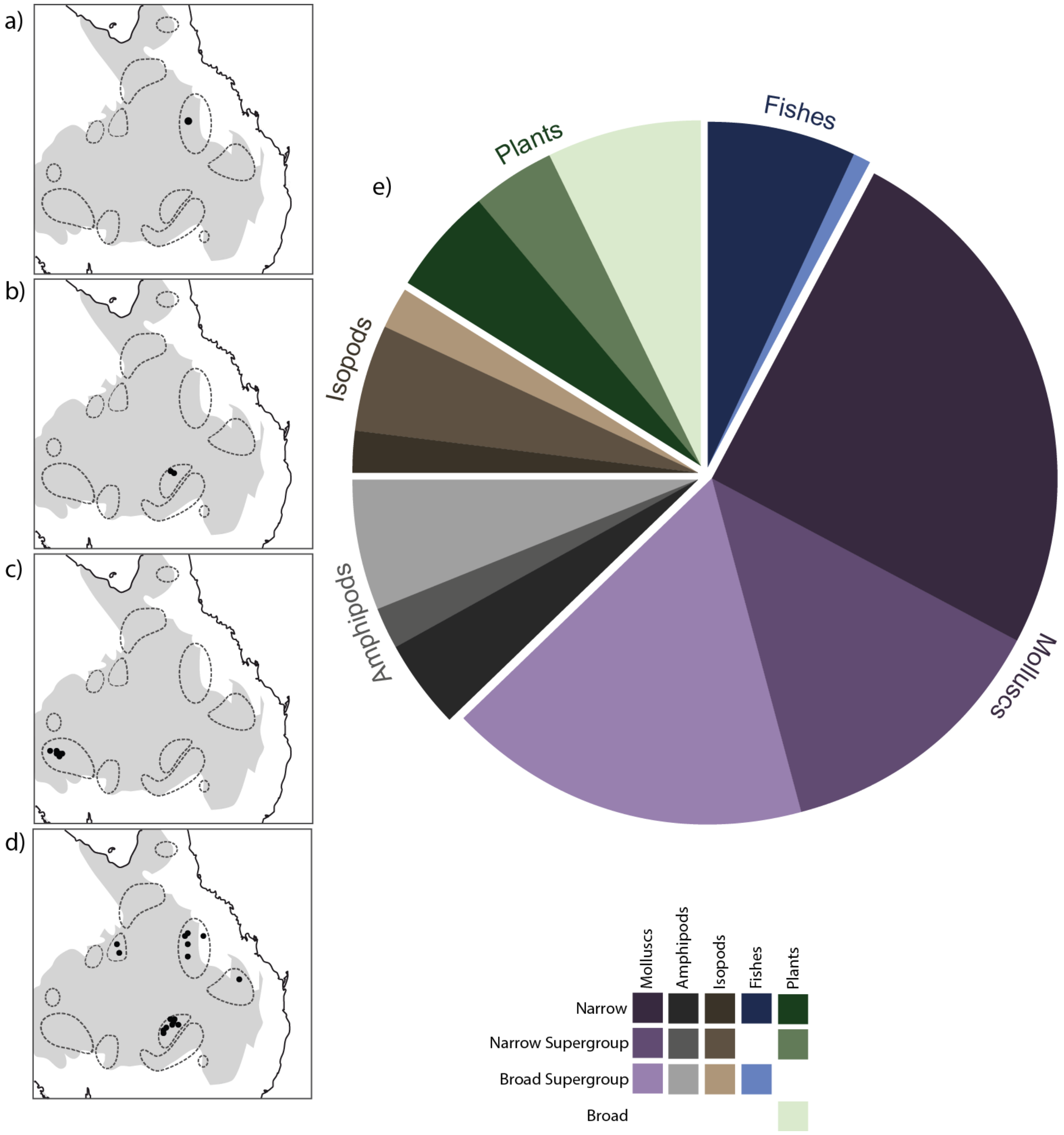


Figure 137: Examples of each of the four identified types of endemism as distribution maps of four representative taxa from each type: a) narrow, the fish Scaturiginichthys vermeillipinnis*, b) narrow supergroup, the isopod* Ponderiella bundoona*, c) broad supergroup, the mollusc* Trochidrobia minuta *and d) broad, the plant* Myriophyllum artesium *(the most broadly distributed taxon from this analysis). The Great Artesian Basin is indicated with grey shading, dashed lines indicate supergroup boundaries (for supergroup names see Figure 136) and black circles indicate complexes within those supergroups occupied by the species. e) The relative contributions to the list of 98 taxa within each group of organisms assessed here and the proportion of each endemism type within each organisms group (see legend for colour codes of each respective organism group and endemism type). Note that there are no examples of 'Broad' endemism type in any animal, and no examples of 'Broad supergroup' endemism in plants.*

Table 36: Correlation coefficients (Pearson's r) among biodiversity attributes of spring complexes (n=326).

Biodiversity attribute	S	H'	TE	PD	PE	BD
Taxonomic Richness (S)						
Taxonomic Diversity (H')	0.944					
Taxonomic Endemism (TE)	0.807	0.617				
Phylogenetic Diversity (PD)	0.965	0.978	0.676			
Phylogenetic Endemism (PE)	0.829	0.649	0.998	0.707		
Assemblage Beta Diversity (BD)	0.878	0.949	0.519	0.957	0.557	
Conservation Rank (CR)	0.877	0.714	0.989	0.764	0.993	0.618

Multiple regression modelling to explain spatial variation in biodiversity attributes of spring complexes according to their environmental characteristics revealed good model accuracy for predicting the presence or absence of endemic species (binomial component) among spring complexes, with the most probable model having a correct classification rate of 0.73, and an area under the ROC curve of 0.78. The most probable models for predicting variation in biodiversity attributes at the subset of 76 spring complexes containing one or more taxa (non-zero component), showed weak to moderate fits to the data, with pseudo-r^2 values ranging from 0.26 to 0.38 (Table 37). Environmental variables selected for predicting the presence or absence (binomial component) of biodiversity across spring complexes were the total number of springs per complex, spatial isolation of complexes, drainage network connectivity and mean annual rainfall. Environmental predictors of variation in biodiversity at complexes containing one more taxa (non-zero component), were broadly similar among biodiversity attributes and included measures of complex size, and mean annual temperature, which were selected in all models. The degree of spatial isolation, drainage network connectivity and rainfall erosivity were also included in the most probable models for all biodiversity attributes except those describing taxonomic endemism and phylogenetic endemism (Table 37). Biodiversity attributes were negatively associated with increasing geographic isolation and increasing hydrologic connectivity among complexes. In contrast, complex area and mean annual temperature were positively associated with biodiversity for most attributes. The broadly similar sets of environmental attributes selected for each model, and similarities in the direction of the environment-response relationships (as indicated by the sign of model parameter estimates, Table 37), are unsurprising given the generally high correlations among biodiversity attributes (Table 36).

Cluster analysis identified 33 distinct "assemblages" (Figure 139a). Few complexes possess the same set of taxa (Figure 139b) and seventeen assemblages occur at only one complex (Figure 139b). Many complexes from Lake Eyre contain the same assemblage or contain assemblages that are highly similar (Figure 139a, b; assemblages 1, 2, 3 and 4 or assemblages 31, 32 and 33). Complexes from the Eulo, Barcaldine and Dalhousie supergroups host assemblages that are generally dissimilar to all others (Figure 139a, b; assemblages 12 - 25), though a small number of equally distinctive examples exists in Lake Eyre (assemblages 8, 9, 10 and 13). One assemblage that is found at complexes from different supergroups (assemblage 19), contains a broadly distributed plant species (UFEN, *Utricularia fenshamii*) and little else (Figure 139c).

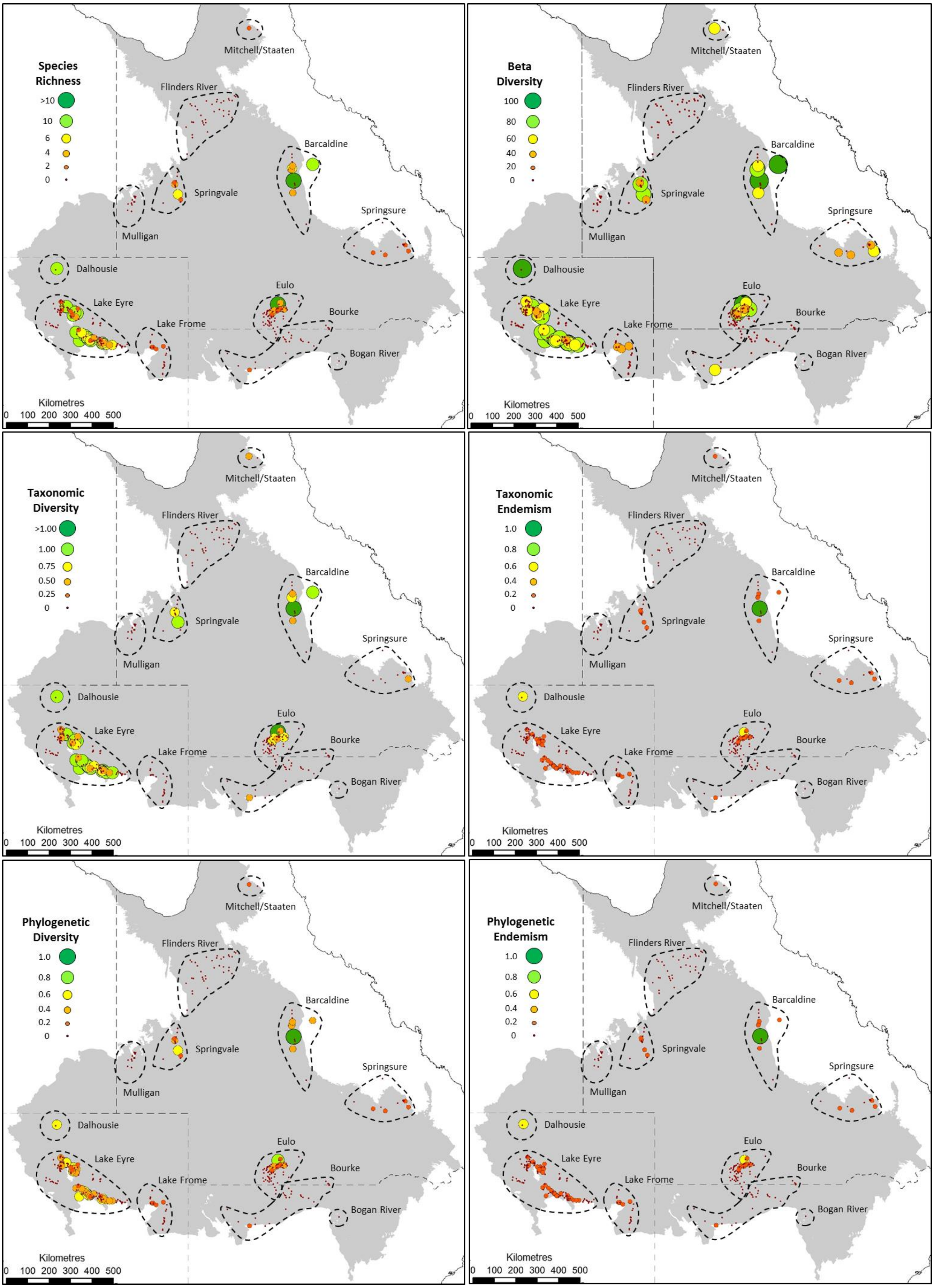


Figure 138: Spatial variation in biodiversity attributes of spring complexes. Spring complexes belonging to each supergroup are enclosed by dashed lines.

Table 37: Parameter estimates (± SE) and significance levels from the hurdle multiple regression models relating environmental variables to biodiversity attributes. Also shown are measures of model performance including the correct classification rate and AUC value for the binomial (0/1) component of the model, and the pseudo R^2 for the non-zero components (biodiversity attributes). (AUC = area under the receiver operating characteristic curve)

			non-zero component													
	0/1 component		**Taxonomic Richness (S)**		**Taxonomic Diversity (H')**		**Taxonomic Endemism (TE)**		**Phylogenetic Diversity (PD)**		**Phylogenetic Endemism (PE)**		**Assemblage Beta Diversity (BD)**		**Conservation Rank (CR)**	
Predictor variable	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**	**Estimate**	**P**
Number of springs per complex	0.03 ± 0.01	0.000					0.01 ± 0.00	0.10								
Complex area			0.33 ± 0.05	0.00	0.10 ± 0.03	0.000	0.37 ± 0.13	0.01	0.23 ± 0.06	0.00	0.49 ± 0.10	0.00	0.13 ± 0.03	0.00	0.54 ± 0.10	0.00
Mean Euclidean distance	-0.77 ± 0.44	0.080	-1.29 ± 0.32	0.00	-0.23 ± 0.07	0.002			-0.56 ± 0.17	0.00	-1.00 ± 0.51	0.05	-0.32 ± 0.10	0.00	-1.51 ± 0.46	0.00
Count connected by drainage network	-0.54 ± 0.20	0.007	-0.37 ± 0.10	0.00	-0.10 ± 0.03	0.000			-0.31 ± 0.06	0.00	-0.36 ± 0.16	0.03	-0.13 ± 0.04	0.00	-0.38 ± 0.11	0.00
Count connected by surface water inundation areas			0.10 ± 0.06	0.11					0.08 ± 0.06	0.17	0.22 ± 0.11	0.04			0.22 ± 0.10	0.04
Elevation			-0.44 ± 0.19	0.02												
Mean annual solar radiation					-0.32 ± 0.09	0.001			-0.72 ± 0.22	0.00			-0.44 ± 0.12	0.00	-0.61 ± 0.29	0.04
Mean annual temperature			0.52 ± 0.10	0.00	0.37 ± 0.09	0.000	0.58 ± 0.13	0.00	0.95 ± 0.23	0.00	0.51 ± 0.12	0.00	0.50 ± 0.13	0.00	1.26 ± 0.35	0.00
Mean annual rainfall	0.73 ± 0.47	0.118	2.12 ± 0.48	0.00							0.76 ± 0.51	0.14				
Rainfall erosivity			-0.81 ± 0.28	0.00	-0.15 ± 0.08	0.055	-0.41 ± 0.13	0.00	-0.40 ± 0.19	0.04			-0.20 ± 0.10	0.06		
Net terrestrial primary productivity															0.62 ± 0.31	0.05
Model performance																
Classification rate	0.73															
AUC	0.78															
Pseudo R^2			0.38		0.37		0.26		0.38		0.38		0.36		0.30	

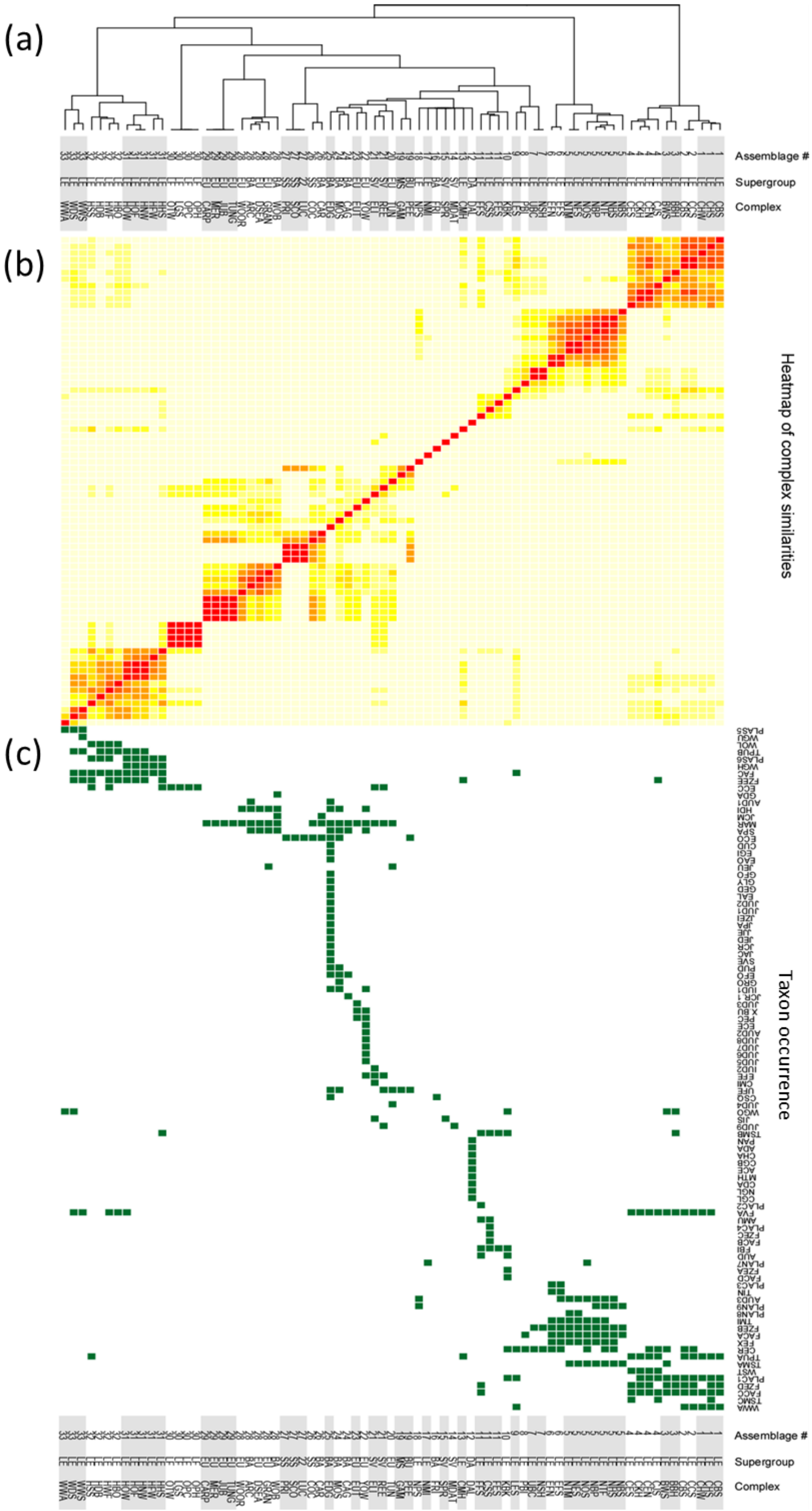


Figure 139: Assemblages of endemic organisms across complexes identified using a) clustering based on species presence/absence across complexes with each complex denoted by a 3 letter code (see Appendix 4: Table 61 for full name and location) and the identity assigned to the assemblage as a number, b) the similarities within and across these assemblages displayed as a heat-map (red= most similar – light yellow= most dissimilar), and c) taxon occurrences in each complex.

5.4.4.4 Current state of knowledge and key data deficiencies

Across all taxa there are differences in the types of data available (Figure 140). For 30% of taxa, a published taxonomic description is yet to be made. Relatively sound information is available regarding the presence or absence of taxa among spring complexes, but knowledge concerning taxon distributions at finer spatial scales (i.e. individual springs within complexes) is available for only ~70% of taxa. In addition, for >75% of organisms there are no published estimates of abundance anywhere within the range. Nor is there information regarding the connectivity between populations of most taxa. Literature regarding the basic ecology of >50% of taxa is completely absent and the vast majority of species have little information available regarding how they respond to threatening processes (Figure 140).

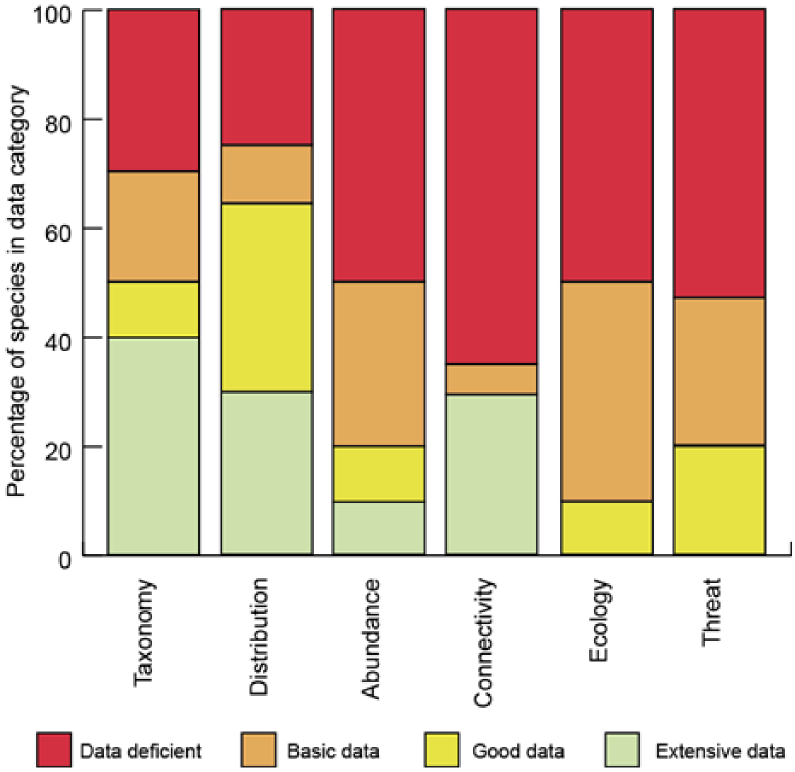


Figure 140: The varying levels of data deficiency for different information types (taxonomy, distribution, abundance, connectivity, ecology, threats) identified across all taxa (red = data deficient, orange = basic data, yellow = good data and green = extensive data).

The relative quantity and nature of available data differs considerably across taxonomic groups. Of all groups the fishes have the best scores overall (Figure 141), but there is considerable variation among taxa. For example, the red-finned blue-eye *Scaturiginichthys vermeilipinnis* scored the best of any taxon while the Dalhousie catfish *Neosilurus gloveri* remains little more than a morphological description. The molluscs have the broadest range of data scores (Figure 141), with equal numbers scoring the highest (e.g. *Fonscochlea* and *Trochidrobia*) and the lowest (e.g. *Glyptophysa* and *Gabbia*) (Figure 141). The low-scoring taxa tend to be within the less speciose families (e.g. the only species of bivalve scored lowest as well as a large swathe of undescribed *Jardinella* from the eastern supergroups). Both groups of Malacostraca considered here scored moderately in general (Figure 141). As with molluscs, low scoring taxa are from radiations outside of the popular Lake Eyre model system (both species of *Ponderiella* and an undescribed *Austrochiltonia* from Queensland). Most plant taxa fall within the moderate range of scores, though three species have low scores (*Isotoma*, *Chloris* and *Peplidium*) (Figure 141).

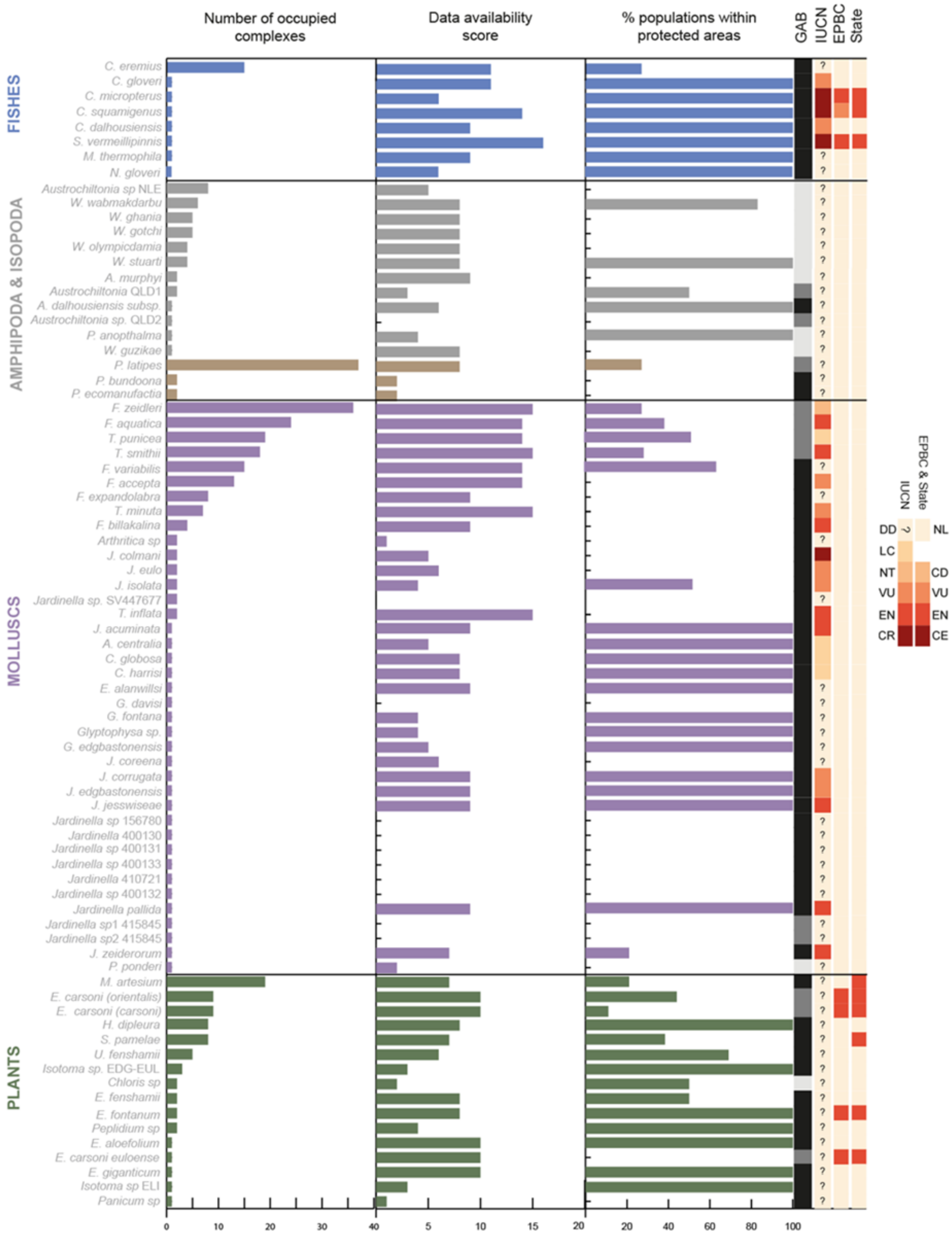


Figure 141: Composite figure showing information for each taxon at species level regarding the narrowness of its range, the amount of information currently available about it, the percentage of populations within a conservation area and the alignment of four different types of legislative conservation protection. GAB represents species named within the EPBC "threatened community", with light grey signifying that they are currently absent, dark grey signifying they are listed as a single species when there is evidence for numerous species and black that they are included. The other three (IUCN, EPBC and State) represent different types of conservation assessment with the representative levels of each in the legend. Codes are: NL - not listed, CD - conservation dependent, VU - vulnerable, EN - endangered, CE - critically endangered, DD - data deficient, LC - least concern, NT - near threatened, CR - critically endangered.

5.4.4.5 Representation within protected areas and current taxon conservation listings

Sixty-six of the 326 spring complexes contain at least one spring occurring within a designated protected area (Figure 142), although only 17 of the 66 partially-protected complexes contain any of the endemic taxa considered here. Of these, very few complexes are completely encompassed within conservation reserves (Figure 142). The Dalhousie complex (Dalhousie supergroup), Elizabeth Springs complex (Springvale supergroup), Scotts Creek complex (Springsure supergroup), Peery complex (Bourke supergroup) and ten complexes from Lake Eyre are completely encompassed in national parks or other protected area designations. Two other complexes within the Barcaldine supergroup have high biodiversity values and are partly within conservation reserves: part of the Pelican Creek complex (76% of springs) is conserved within the Edgbaston Reserve and part of the Moses complex (53% of springs) is conserved within the Doongmabulla Mound Springs Nature Refuge.

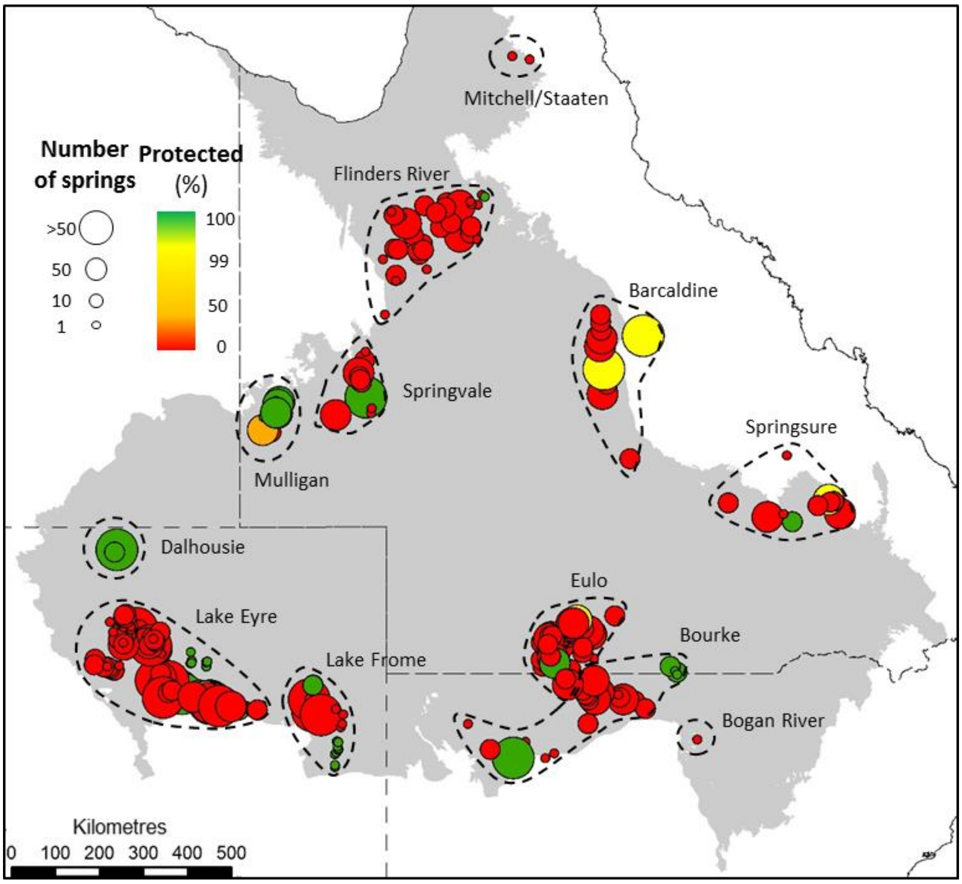


Figure 142: Map of GAB (shaded area) and spring complexes characterised by their level of protection (% springs within IUCN protected areas; source: CAPAD 2014). Spring complexes belonging to each supergroup are enclosed by dashed lines.

When considering the conservation of individual species, the conservation status assigned within the four types of conservation listing considered here did not align for each taxon and the listings are biased toward fish and plant taxa (Figure 141). Plants and fishes are relatively well assessed and represented in conservation listings (Figure 141). All taxa have all or at least some populations within protected areas except for *E. carsoni* sub sp. *euloense* (Figure 141, Figure 142). Three of the fishes are federally listed across varying levels (*S. vermeilipinnis, C. micropterus and C. squamigenus*), though none match the IUCN level (all critically endangered) (Figure 141). In many cases species remain unassessed and receive no listing under state or federal legislation (e.g. *N. gloveri and M. thermophila*) (Figure 141). In contrast, many endemic plants have better protection under national and state level listing than what is recommended under the IUCN (Figure 141). Many are listed as endangered under the EPBC (*Eriocaulon carsonii* excluding sub species, *Eryngium fontanum*), which aligns with their state listing, though state legislation in Queensland recognises two additional endangered species (*Myriophyllum artesium* and *Sporobolus pamelae*), one of which is not recognised in other states where it occurs (*M. artesium*) (Figure 141). However, newly discovered and undescribed species with very narrow distributions are currently not assessed or listed even though many of them have much narrower ranges than those that are listed (e.g. both species of undescribed *Isotoma*, the undescribed *Chloris* and *Eriocaulon aloefolium* and *E. giganticum*) (Figure 141).

In contrast, the invertebrates are poorly represented in any conservation listing (Figure 141). All invertebrate groups are composed primarily of taxa with no populations within a protected area. No taxa of endemic spring invertebrate are listed individually under the EPBC act or under any state-level legislation even though, according to the IUCN, a large number of gastropods are threatened (Figure 141). *Jardinella colmani* is considered critically endangered, and most species of described *Fonscochlea* and *Jardinella* are endangered or vulnerable (Figure 141). While the majority of species that are yet to be assessed or listed under any legislation are undescribed, many described species (primarily those within the Bythiniidae and Planorbidae) are yet to be evaluated. The species within a recent expansion of a complex of *Wangiannachiltonia* are yet to be assessed or added to the GAB "threatened community" list. Unfortunately, no arthropod has been assessed under the IUCN at the time of writing.

5.4.5 Discussion

Endemic organisms that inhabit GAB springs are varied and represent a broad range of taxa, the full diversity of which is yet to be discovered. Most have highly restricted distributions and much of the biodiversity is concentrated in particular parts of the basin. Whilst physical characteristics of spring complexes are useful for explaining the presence or absence of endemic species, they are not useful for predicting the locations of biodiversity 'hot spots'. We know very little about the majority of species in the system, particularly regarding their ecology and response to threats. These knowledge gaps are worrying as the species with the least available information are often those with narrow ranges that are not currently included within protected areas or national conservation legislation.

We expect that complexes with more springs in close proximity that are well connected to other complexes should foster higher diversity (Davis et al. 2013). We found that some environmental characteristics of spring complexes (e.g. spring surface water persistence; relative spatial proximity and hydrologic connectivity) were good predictors of the likelihood of spring complexes containing any endemic taxa (correct classification rate of 0.73). However, spatial variation in biodiversity at springs containing one or more endemic taxa was generally weakly related to environmental characteristics (pseudo-r^2 values ranging from 0.26 to 0.38). This data suggests that spring complexes with many well connected springs contain endemic species, however the environmental conditions of each complex cannot necessarily explain the biodiversity it holds. There are numerous potential reasons why this may be the case. There may be unmeasured elements of spring environments that are important determinants of species distributions, or different species respond to different mechanisms, masking overall patterns. Historical processes may be more important than contemporary ones (as seen in other spring systems, see for example Horsak et al. 2007). Geological and past hydrological processes are likely to have led to alternate phases of isolation and connection of springs (Bauzà-Ribot et al. 2011), which may explain the co-occurrence or disjunct distribution of some species that are now isolated (Fattorini et al. 2016). The ancestors of some species that are currently present in springs were once broadly distributed, but as aridity intensified they were left 'stranded' in springs (Ponder, 1986; Murphy et al. 2012). Some of these species have likely gone extinct, while others have diversified but not necessarily where they are currently found (e.g. Ponder and Colgan, 1989). Quantifying these historic connectivity pathways was beyond the scope of this study but has demonstrated the diverse evolutionary narratives of some endemic taxa (e.g. the amphipoda, see Murphy *et al.* 2009). Finally, supergroups with low diversity happen to be those that have experienced considerable loss of springs (e.g. Flinders River, Eulo and Bourke; Figure 136; Fairfax and Fensham 2002, Fensham and Fairfax 2003). Considering the prevalence of species with ranges restricted to a single complex, and that numerous whole complexes have disappeared since the late 1800s, the diversity we see now is unlikely to be that which persisted before European colonisation.

The history of species discovery in GAB springs is a recent one and will continue to be updated as new techniques are employed. Since the 1980s, there has been a rapid increase in the number of species recognised to be endemic to springs, but this is by no means representative of the full diversity of taxa that remain undescribed (Figure 143). For larger and more charismatic taxa such as fishes, the rate of description was relatively rapid and the species currently described or in collections awaiting description could be considered a fair estimate of the diversity within the system. For amphipods, recent molecular investigations have revealed that many populations once thought to be a single species are in fact a complex of morphologically similar species (e.g. the *Wangiannachiltonia* radiation; King 2009). Wide arrays of taxa still await basic investigation. The algae for example are completely overlooked even though they form a large component of endemic spring diversity in other systems (Cantonati et al. 2012a). The same can be said for Acari and Ostracoda, both of which are proposed to contain endemics (Ponder et al. 2010) and can be abundant in springs (Rossini et al. 2015). The sampling of seasonal fauna with ecologically distinct life-stages such as damselflies and caddis flies is also needed to gain a more robust idea of diversity within the Insecta, especially considering at least one dragonfly has been identified as a putative endemic (Ponder et al. 2010). Sampling for each of these diverse types of fauna will require specialised methods and targeted sampling to ensure the full diversity is captured (Cantonati et al. 2007, Rossini et al. 2015). It should also be noted that this review has made no account of the diverse flora and fauna that utilise springs but are not endemic to them (see Fensham et al. 2011). While the adaptations of these species mean they are not tied to springs, they would still be affected if springs failed to act as permanent water sources in an arid landscape. Thus, although our estimate of diversity is considerably larger than previous accounts (Figure 143), it is by no means a proper representation of the full diversity in the GAB system.

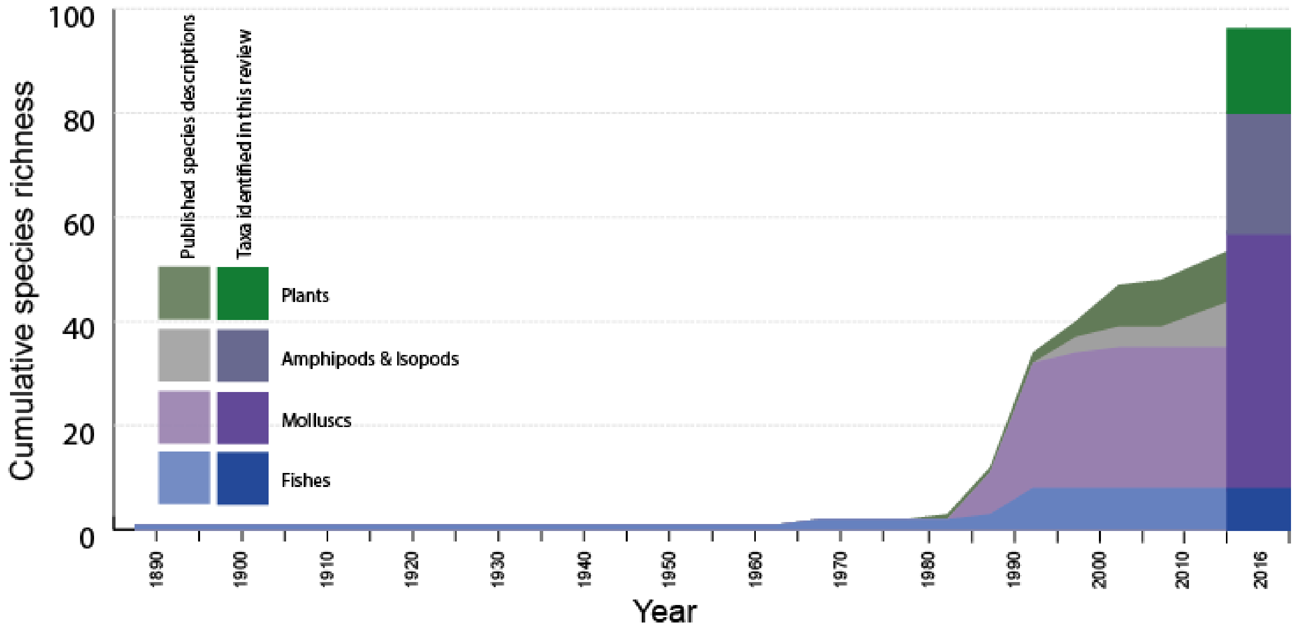


Figure 143: Graphical representation of the rapid rate at which species endemic to GAB springs have been described, broken up into broad taxonomic groups denoted by colour: fishes (blue), molluscs (purple), amphipods and isopods (grey) and plants (green). The estimates of taxa richness made by this review are added as a dark expanded column in 2016.

5.4.5.1 Conservation implications

If conservation of GAB springs is approached from a perspective focussed on spring complexes and the assemblages of species within them, this review demonstrates that the 'community' of species dependent on GAB springs is by no means spatially homogenous. This heterogeneity has implications for how the system is currently conserved. High diversity is concentrated in particular regions. Over 70% of GAB spring complexes host none of the endemic species included in this review, and those that do host endemics generally contain complex-specific assemblages. Therefore the protection of an arbitrary proportion of springs cannot guarantee the persistence of diversity in the 'community' as a whole. For example, large national parks and conservation reserves at Dalhousie or Edgbaston, whilst containing considerable numbers of species, do not guarantee the protection of the portion of the 'community' in the Yowah complex. Likewise, offsetting the conservation of one complex at the expense of another needs to be thoroughly reviewed, as very few complexes are analogous.

A basin-wide approach that acknowledges the distinctive biodiversity in each complex is needed and is likely to involve a broad range of stakeholders. Many endemic assemblages are found on private property. This does not necessarily hinder their preservation, however landholders need to be engaged and supported for good conservation behaviour. For example, property managers in the Barcaldine complex currently manage a recently discovered population of the Edgbaston Goby (*C. squamigenus*). Indigenous custodians play a vital role in actively managing springs within Indigenous Protected Areas, for example the Arabunna community manage Finniss Springs in the Lake Eyre region and the Lower Southern Arrernte people and Wangkangurru people participate in the eradication of invasive date palm in springs at Witjera National Park (T. Gotch, pers. comm.). In cases where very high biodiversity values exist however, springs need to be protected. In the past decade, strong actions have been taken to safeguard this diversity. For example, joint action of both the Federal Government and the not-for-profit conservation organisation 'Bush Heritage Australia' guaranteed the annexing of the most diverse complex in the GAB within a protected area (the Edgbaston portion of the Pelican Creek springs complex). The Elizabeth Springs complex (Springsure supergroup) and Witjira-Dalhousie spring (Dalhousie supergroup) were annexed for conservation in 2009. However, many spring supergroups still remain outside of protected areas.

On-ground actions undertaken in protected areas offer protection from terrestrial threats (e.g. disturbance by ungulate grazers, physical modification by property owners) but they are pointless if the groundwater resources that maintain springs are not also safeguarded. Groundwater drawdown is considered the primary driver of the loss of springs that has occurred since the 1890s (Fairfax and Fensham 2002, Fairfax and Fensham 2003). Drawdown is a long-term and broad-scale process and thus requires long-term commitment to basin-wide rehabilitation and management. Recent successes from basin-wide programs such as the GABSI (Great Artesian Basin Sustainability Initiative) have led to the revival of some previously extinct springs and an increase in basin pressure (Hassall & Associates Pty Ltd. 2003, Sinclair, Knight, Merz Pty Ltd. 2014). These results show that both the number of springs and their size and permanence are correlated with the presence of endemic taxa. In groups where assessments have been made, the majority of species rely on permanent deep groundwater-fed pools for persistence of fishes (e.g. Kodric-Brown and Brown, 1993) and snails from Lake Eyre (Ponder and Colgan, 1989). Groundwater resources in the GAB need to be safeguarded and sustainably managed to ensure that broad-scale reductions in groundwater pressure and resulting loss of springs does not continue.

Our research suggests that there is scope to update the list of taxa that form the 'community' listed under threatened species legislation. For example, at present no species within the *Wangiannachiltonia* complex delineated in 2015 are included in the description of the 'community'. This list could also incorporate genetic diversity within species (Murphy et al. 2015). Describing species requires time and support – for example, six years elapsed between the publication that delineated clades (Murphy et al. 2009) and the official description of the most recently described set of species (Murphy et al. 2015b). This provides one reason for incorporating knowledge regarding discrete units within species as they are published – as a pre-emption of species to be incorporated. Another reason to include taxonomic units below species level, either as sub-species or distinct clades within species, is to preserve the genetic diversity and adaptive potential within the system (Moritz 2002, Frankham 2005, Smith & Grether 2008). Regarding distinct clades or sub-species as evolutionary distinct units (ESU) in order to conceptualise the importance of preserving genetic diversity is highly applicable to GAB springs (Murphy et al. 2015a, b) and general species conservation (Fraser and Bernatchez 2001). This approach would involve incorporation of sub-species (e.g. *Eriocaulon carsoni* comprises 3 sub-species), taxa identified as putative species or sub-species yet to be described (e.g. *Phraetomerus latipes* comprises between 3 and 9 species (N. Murphy and B. Wilson, pers.

com), and clades within species known to be distinct (e.g. the five clades of *F. zeidleri*). Without protecting ESUs, the conservation of other processes associated with intraspecific diversity like ecological function (Hughes et al. 2008) and the potential for further speciation are not assured.

Our research highlights the lack of invertebrate fauna listed as threatened. The majority of endemic species in GAB springs are invertebrates. At present the only species of invertebrate afforded the same level of conservation protection as the fishes and plants in GAB springs is a terrestrial species of snail associated with tertiary springs (the Boggomoss snail, *Adclarkia dawsonensis*). Numerous invertebrates have been deemed of considerable conservation concern in IUCN assessments. There is evidence that some of these are currently at risk of extinction. For example, the freshwater snail *Jardinella colmani* is listed as critically endangered under the IUCN; it is a narrow range endemic for which 100% of its population remains outside of conservation reserves, is data deficient and recent surveys have failed to find any live individuals (Laffineur, pers coms). This means it is technically of equal or greater conservation concern than the GAB systems' most heavily protected species, the red-finned blue-eye (*Scaturiginichthys vermeilipinnis*). Many invertebrate taxa may be in a similar situation. Most remain unassessed under the IUCN or EPBC, are data deficient, have narrow ranges, and have no part of that range within conservation reserves. We note that a number of research studies have suggested that a greater priority be placed on conserving threatened invertebrates (e.g. Ponder 1994, Hutchings 2004, Strayer and Dudgeon 2010, Cardoso et al. 2011, Bland et al. 2012).

5.4.6 Key findings

To conclude this section, we present five areas, informed by our assessment, which could be the focus for future GAB springs research and conservation management.

1. Describing and revealing undocumented diversity will help to preserve its evolutionary potential

Taxonomy is key to conservation of GAB spring flora and fauna. Numerous species are collected and await description. Whole phyla remain unexplored. Understanding the distribution and ecology of endemic taxa cannot progress until species are described. For conservation of GAB springs to progress, efforts need to be made to ensure taxonomists are supported. Effective conservation also needs to embrace the fact that diversity is not fixed – species have and will continue to diverge in springs as geographical isolation and environmental change shape their evolution. Conserving biodiversity and understanding species will be complicated if cryptic species complexes are present but remain unidentified. Therefore, understanding species is not only about diversity across taxa, but also within them.

2. Update conservation listing

This review has highlighted that GAB-dependent species that are listed individually as well as being listed as part of the 'threatened community' are the focus of more dedicated conservation attention and efforts than those species that are not presently listed. Our study highlights additional taxa belonging to the 'community of native species dependent on natural discharge of groundwater from the GAB' (Figure 141). There is a scientific basis for an assessment and re-evaluation of all taxa (in addition to those considered here but excluding plants reviewed previously by Silcock et al. 2011) using EPBC and IUCN guidelines for consideration by the Threatened Species Scientific Committee. This assessment was beyond the scope of our study but should now be readily achievable for some taxon groups (particularly the invertebrates)

3. Broaden the focus to other organisms and increase the spatial resolution of the assessments

The data we have regarding endemic GAB species is focussed on particular groups of taxa and their complex-scale distributions. Finer-resolution distribution data (i.e. springs within complexes) and information concerning abundance for a broader range of taxon groups is required for effective conservation management of springs. There are also uneven levels of knowledge available on the ecology and conservation of taxon groups in different parts of the GAB. For example, five publications regarding the ecology and conservation of the red-finned blue-eye are available (Wager and Unmack 2004, Fensham et al. 2006, Fairfax et al. 2007, Kerezsy and Fensham 2013, Nicol et al. 2015) while we lack basic information for the narrow range snail *J. colmani*. A considerable body of work concerning the population genetics of species endemic to Lake Eyre forms the foundation of all of our understanding of dispersal and connectivity (Worthington-Wilmer and Wilcox 2007, Worthington-Wilmer et al. 2008, Murphy et al. 2010, Worthington-Wilmer et al. 2011, Robertson et al. 2014). Extensive surveys and reviews within the Tanteinae snails reveal patterns of diversity and divergence (Ponder et al. 1989, Ponder and Clark 1990, Ponder et al. 1995, Perez et al. 2005, Ponder et al. 2008), while despite evidence of considerable diversity, we still have no full account of endemic ostracods. This review has highlighted types of data we currently lack, and the locations and species for which this data is most urgently needed. It has also highlighted that such efforts need not be dispersed across the entire basin, but can be focussed upon biodiversity 'hot-spots'. Emphasis needs to be placed on prioritising research that fills these knowledge gaps.

4. Improve collaboration across research effort

For organisms or complexes where research programs currently exist, the types of questions being asked are contingent on the interests of the research group. In the Lake Eyre complex, molecular biologists are providing detailed information about species boundaries and population connectivity (Ponder et al. 1995, Murphy et al. 2010, Murphy et al. 2013) but there is little ecological data to complement these models. The opposite can be said for the northern complexes, where conservation, diversity and biogeography are a focus (Fensham et al. 2004, Fensham and Price 2004, Fensham et al. 2011, Silcock et al. 2011, Powell et al. 2015). This research divide extends beyond biology, with those that study the organisms that live in the surface manifestation of springs and those that study the hydrodynamics of the GAB. Likewise, those assessing potential impacts on springs are not necessarily engaging with taxonomists or ecologists with the expertise to identify potential new species, comment on sampling designs or provide comment on the ecological mechanisms behind biodiversity patterns. This lack of collaboration means surveys of spring complexes may be overlooking diversity. It also means we still cannot answer vital questions about the relationship between groundwater pressure within the GAB and the size of springs or the species that occupy them. Collaborations across on-ground managers, consultants and research groups with interest in springs need to be fostered and will facilitate identification of knowledge gaps and research priorities.

5. Focus on the ecology of species and their responses to threats

Information regarding the ecology of GAB spring endemic species, their potential responses to threats, and even variation in the nature and intensity of threats themselves, are the most deficient. Even basic information regarding abundance, environmental requirements (beyond the need for GAB fed springs), the effects of introduced species, or changes in spring flow are deficient for most species, even though these threats have been emphasised as major threats to the system since its conservation listing (Fensham et al. 2010). This lack of information limits our ability to prioritise species for conservation or manage species persistence. Endemic GAB spring taxa have restricted distributions, have very few populations within protected areas, and may have high exposure to threats meaning they may be at substantial risk from the cumulative impacts of a range of threats. The lack of data, the size of the GAB, and the diversity of species in question, has previously precluded efforts to systematically evaluate threat exposure to endemic species, understand species' vulnerability to threatening processes and prioritiese risks. These knowledge gaps are evaluated in the next section of this report.

5.5 Assessment of the cumulative risks of groundwater drawdown and other stressors on endemic flora and fauna of Great Artesian Basin springs

Mark Kennard, Renee Rossini, Rod Fensham, & Doug Ward

5.5.1 Key objectives:

- Identify key threats to endemic flora and fauna of GAB spring wetlands (including groundwater drawdown and other stressors)
- Assess taxa vulnerability to threats (incorporating measures of resistance, resilience and certainty) using literature, ecological theory, unpublished data and expert knowledge
- Conduct risk assessment to assess cumulative impacts of threats and evaluate:
    1. which complexes are exposed to the greatest number and intensity of threats,
    2. how vulnerability to threats varies among species and
    3. which complexes are at the highest risk (i.e. contain high number of vulnerable species and are exposed to the highest threat intensities)
- Synthesise on-ground management and monitoring of GAB springs in the context of risk assessment results

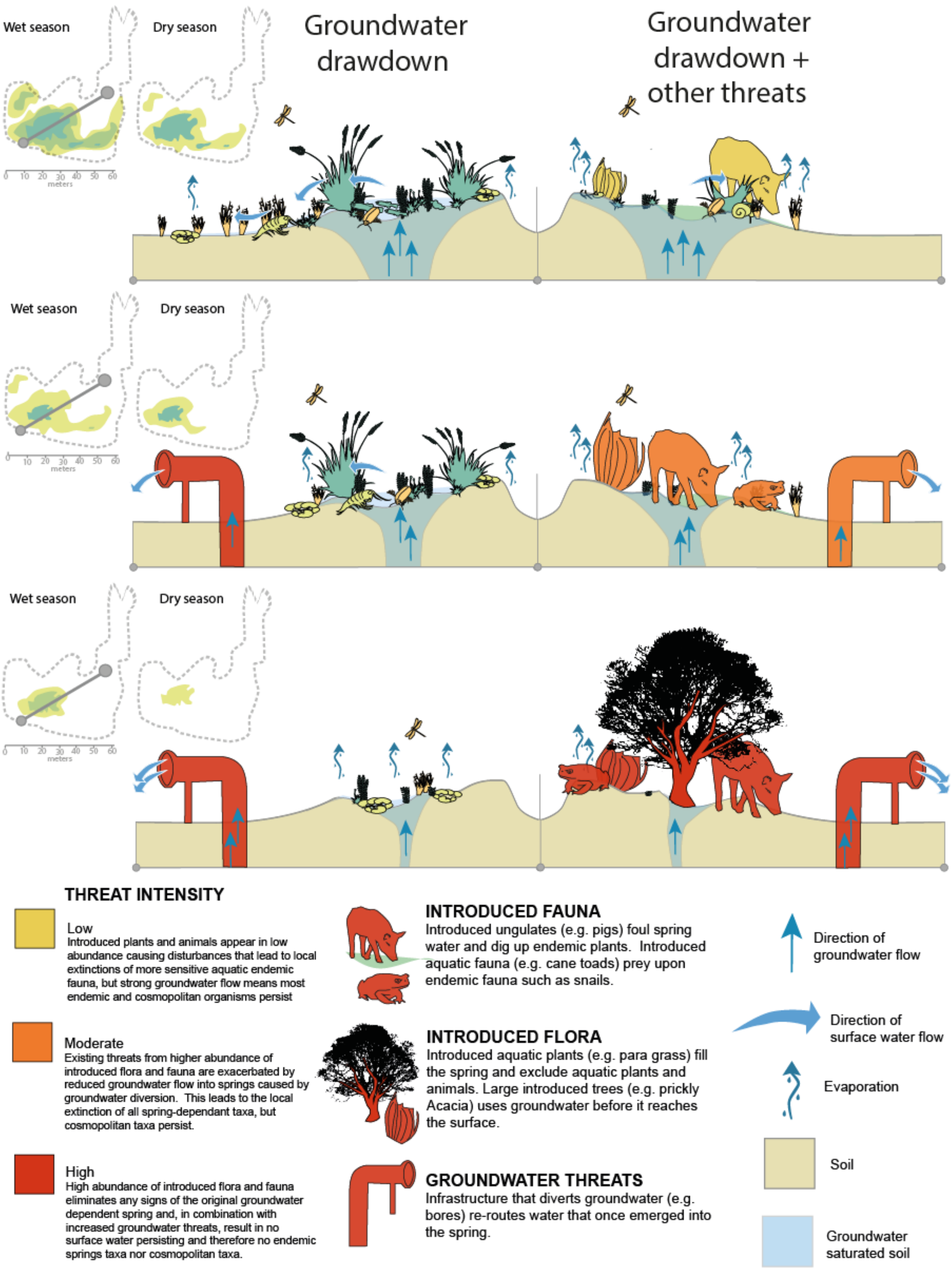
5.5.2 Background

Acknowledgement of the uniqueness of GAB springs and the severity of the threats they face has been a relatively recent phenomenon. Akin to arid-zone springs globally, the unique flora and fauna of the GAB have experienced considerable habitat loss and extinction due to the unrestricted extraction of water from their source aquifer and physical modification (Fensham et al. 2010). Spring flora and fauna continue to face threats from a range of processes (Figure 144, Table 38) including reduced habitat caused by the depletion of groundwater pressure (e.g. due to water extraction from artesian bores, mining operations, etc.) to physical alteration of the spring environment (i.e. construction of drains and slabs) to the introduction of invasive species, either to the spring directly (e.g. the ponded pasture, para grass (*Urochloa mutica*), date palms (*Phoenix dactylifera*), and introduced fish such as *Gambusia holbrooki*) or via disturbance associated with introduced ungulates (e.g. physical disturbance caused by cows, pigs and camels). Cumulative impacts from these potentially interacting threats are likely to be compounded under future climate change scenarios. For example, basin-wide hydrogeology (i.e. changed recharge regimes) and local processes (i.e. altered evapotranspiration rates) are likely to change the seasonal dynamics of springs while climatic shifts will impact on the distribution and abundance of endemic taxa and the invasive organisms that threaten them. Some threats may be more pertinent to particular taxa, or in particular regions, and poor taxonomic understanding or data deficiency regarding species distributions, may lead to underestimation of extinction risks (Bland et al. 2012).

The spatial distributions of threats are varied, but in many places little is known about which stressors are having the biggest impact on spring ecosystems or their cumulative effects (i.e. combined effects of multiple, potentially interacting threats). Mapping where threats occur is important for management, but does not explicitly account for differences in the extent and nature of species' responses to threats (Halpern et al. 2007, 2015). Understanding these differences in species' responses is critical to identifying which threats have the biggest impact on spring ecosystems as a whole (i.e. incorporates the cumulative effect of each threat on every species in the area) and how to best address them at different scales. Quantifying these differences allows threats to be ranked on the severity of their impact on species as well as allowing springs to be ranked on their overall risk (by combining threat exposure and relative species vulnerability). Such assessments in turn can inform biodiversity conservation, threat mitigation and spatial planning of decision-making.

Several formal risk assessments for GAB springs have been done in the past or are currently underway. Green et al. (2013) developed a risk assessment framework to evaluate risk factors associated with reductions in groundwater pressure in the GAB and applied the framework to a small number of GAB springs in South Australia. Miles et al. (2012) undertook a basin-wide assessment of spring supergroups at risk from modelled changes in climate and groundwater development. The Office of Groundwater Impact Assessment (2016a) assessed risks to springs in the Surat Cumulative Management Area from groundwater drawdown. Finally, Barrett et al. (2013) provided a broad overview of the risk assessment approach currently being undertaken for the Bioregional Assessment Program to evaluate risks associated with impacts from coal seam gas and coal mining development on water-dependent assets (including some GAB springs). The risk assessment approach being undertaken in this project contains elements common to these other risk assessments but complements and extends them because: it is whole of GAB scale, is species focussed, explicitly incorporates ecological response mechanisms and acknowledges the diversity of species and potential differences in their response to different threats, and does not use qualitative approaches to establish discretised risk ratings (instead, uses quantitative continuous data to estimate exposure and risk).

This section outlines the development and application of a cumulative risk assessment framework to estimate the cumulative impacts of current threats to the endemic flora and fauna of GAB spring wetlands. The term 'cumulative risk assessment' is defined as an analysis, characterization, and quantification of the combined (additive or interactive) risks to the environment from multiple anthropogenic threats over time (U.S. EPA 2003). Our approach follows most elements of this definition except that we do not assess the effects of interacting threats due to insufficient knowledge and we assess present-day, not future changes in threats. The assessment accommodates uncertainty and data paucity, integrates multiple threats, ecological and habitat processes, and considers the species-specific attributes that confer resistance and resilience to disturbance. The assessment uses best available data to quantify threat exposure, and uses literature, unpublished data, ecological theory and expert knowledge to estimate species vulnerability to threats.



THREAT INTENSITY

🟨 Low
Introduced plants and animals appear in low abundance causing disturbances that lead to local extinctions of more sensitive aquatic endemic fauna, but strong groundwater flow means most endemic and cosmopolitan organisms persist

🟧 Moderate
Existing threats from higher abundance of introduced flora and fauna are exacerbated by reduced groundwater flow into springs caused by groundwater diversion. This leads to the local extinction of all spring-dependant taxa, but cosmopolitan taxa persist.

🟥 High
High abundance of introduced flora and fauna eliminates any signs of the original groundwater dependent spring and, in combination with increased groundwater threats, result in no surface water persisting and therefore no endemic springs taxa nor cosmopolitan taxa.

INTRODUCED FAUNA
Introduced ungulates (e.g. pigs) foul spring water and dig up endemic plants. Introduced aquatic fauna (e.g. cane toads) prey upon endemic fauna such as snails.

INTRODUCED FLORA
Introduced aquatic plants (e.g. para grass) fill the spring and exclude aquatic plants and animals. Large introduced trees (e.g. prickly Acacia) uses groundwater before it reaches the surface.

GROUNDWATER THREATS
Infrastructure that diverts groundwater (e.g. bores) re-routes water that once emerged into the spring.

↑ Direction of groundwater flow

Direction of surface water flow

Evaporation

Soil

Groundwater saturated soil

Figure 144: Conceptual model of hypothesised responses of discharge springs to cumulative groundwater drawdown and interactions with other threats.

Table 38: Summary of mechanisms of impact of different threats to GAB spring wetlands and potential ecological consequences. (Sources: Noble et al. 1998, Fensham & Fairfax 2003, Box et al. 2008, Silcock 2009, Fensham et al. 2010, OGIA 2016b). The type of disturbance (pulse, press or ramp; after Lake 2000) for each threat is also indicated.

Primary threat and mechanisms of impact	Ecological consequences
Aquifer drawdown (*Ramp disturbance*)	
Reduction in groundwater discharge to springs causing reduction in seasonal persistence, extent, and depth of aquatic habitat	Loss of habitat for fully aquatic plants & animals Changes in number & connectivity of local aquatic habitat patches (vents) affecting local meta-population persistence (springs/complexes)
Reduction in soil moisture in marginal zones	Loss of habitat for rooted semi-aquatic plants
Changes in water chemistry (e.g. salinity, pH, dissolved ions) in pooled water due to reduced spring discharge and flushing; increased concentration of solutes	Exceedance of physiological tolerance limits for some plants & animals Reduction in dissolved calcium carbonate critical for shell formation in molluscs and exoskeleton development in crustaceans
Reduction in water temperature (in thermal springs)	Increased potential for colonisation by non-indigenous plant & animal species with lower thermal tolerances leading to negative impacts (e.g. competition, predation) on indigenous species
Increase in exposure of wetland soils around margins leading to changes in soil chemistry (e.g. increased salt accumulation; oxidization of peaty wetland soils and potential acid production)	Exceedance of physiological tolerance limits for some plants & animals Changes in nutrient availability for plants
Slumping of mound as the saturated organic rich wetland soil dries and shrinks. Oxidation of peaty wetland soils, alteration of soil structure. Potential increase in erosion of the former wetland soil due to reduced soil stability	Loss of semi-aquatic and terrestrial vegetation within the discharge tailwater zone. Degradation and ultimate collapse of large mounds composed of peat
Human modification of springs (*Press disturbance*)	
Excavation to increase size & permanence of waterbodies (i.e. for human / stock water use) and associated disturbance within and surrounding spring wetland	Increased seasonal persistence, extent, and depth of aquatic habitat, favouring a sub-set of species Direct removal/stranding of plants & animals Increase turbidity & other negative WQ changes
Construction of raised concrete structures that limit surface flow (i.e. box bores)	Increased depth & complete change in aquatic habitat structure Direct removal/stranding of plants & animals Increase turbidity & other negative WQ changes
Inundation/impoundment (damming)	Major transformation of spring habitat
Physical modifications (i.e. troughing, infilling)	Drying of aquatic habitat, reductions in soil moisture, change in mound structure, exposure/desiccation of aquatic fauna
Tourist access	Trampling, nutrient addition, physical removal (e.g. plant specimens), introduction of invasive flora, fauna and pathogens
Disturbance from livestock and feral animals (e.g. cattle, pigs, sheep, goats, camels) (*Pulse or Ramp disturbance*)	
Trampling, rooting, browsing	Suspension of sediments leading to elevated turbidity and reduced primary production Direct damage to nests and all life stages of aquatic fauna (eggs/juveniles/adults) Accelerated soil erosion on land and in riparian zone leading to increased sedimentation of aquatic habitats, reduced water depth and/or destruction of mound spring structure Direct damage to aquatic and riparian vegetation Reduced abundance of native *Phragmites* and hence favours increases in other plants (through reduced competition for light/space) Cascading negative consequences include major changes in spring structure/depth/extent Reductions in food resources for aquatic fauna via loss of aquatic and riparian vegetation that are substrates for algae and provide organic detritus Loss of habitat structure for aquatic fauna (i.e. refuge, foraging, growth, attachment substrate) via loss of aquatic and semi-aquatic vegetation
Defecating & Urinating	Addition of fine particulate organic matter leading to smothering of benthic aquatic habitat Nutrient enrichment and increased unpalatable algal growth (e.g. filamentous algae)
Introduced aquatic flora (para grass, *Hymenachne*, rubber vine, date palm, bamboo, athel pine) (*Press disturbance*)	

Primary threat and mechanisms of impact	Ecological consequences
Shading due to riparian infestation or overgrowth (e.g. rubbervine, date palm, prickly acacia, bamboo)	Reduced light transmission into water column, curtailing photosynthesis and primary production (algae and native macrophytes)
Proliferation across waterbody & margins (e.g. para grass, *Hymenachne*, prickly acacia, and most other large plants)	Dense thickets monopolise space, light and nutrients leading to increased competition with native flora and Habitat transformation (e.g. reduction in open water habitat) for aquatic fauna
Groundwater drawdown & surface dewatering by large deep-rooted trees	Loss of aquatic habitat for aquatic plants and fauna Reduction in soil moisture impact on semi-aquatic plants
Organic matter build-up from increased allochthonous inputs (e.g. leaves)	Declines in water quality & habitat availability for aquatic fauna
Introduced aquatic fauna (e.g. cane toad, red-claw, *Gambusia*) (*Press disturbance*)	
Predation	Direct predation on eggs, juveniles & adult aquatic fauna
Competition for food	*Gambusia* and/or toad tadpole consumption of organic detritus, algae *Gambusia* and/or toad adult consumption of aquatic and riparian animals (e.g. crustaceans, insects)
Poisoning	Predation by native fish and crustaceans on toad eggs & tadpoles

5.5.3 Categorising threats

Each of the threats described earlier constitutes an anthropogenic disturbance to GAB spring flora and fauna. Disturbances can be characterized by the temporal nature of the stressor and the ecological responses to the stressor (Lake 2000, 2003) and three types of disturbance have been recognised: pulse, press and ramp (Figure 145). Pulse disturbances are short-term and sharply delineated (Figure 145a) such as from occasional livestock, feral animal or tourist visitation. Press disturbances arise sharply and then maintain a relatively constant level (Figure 145b); for example, human modification of springs and invasion by introduced plants and aquatic animals. Ramp disturbances (Figure 145c), increase in strength (and often spatial extent) over time. Ramps may steadily increase in time without an endpoint, or reach an asymptote after an extended period. Examples of ramps include the effects of groundwater drawdown and climate changes. Ecological responses to these different types of disturbances can also have a pulse, press, or ramp trajectory. For example, a pulse disturbance can elicit a pulse or press response (Figure 145a), a press disturbance can yield a press response (Figure 145b) and a ramp disturbance can produce a ramp response (Figure 145c).

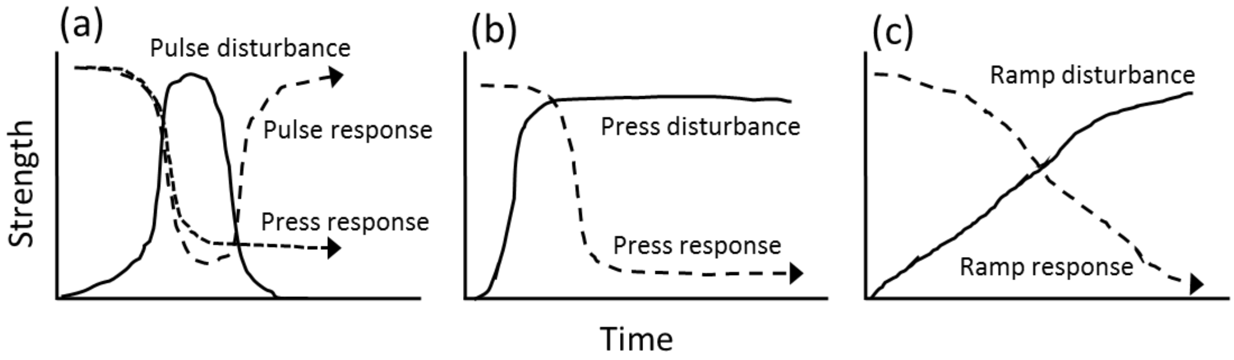


Figure 145: Pulse (a), press (b) and ramp (c) disturbances (solid lines), and potential ecological responses (dashed lines) (Source: adapted from Lake 2000, 2003).

The nature of the ecological response is linked with the capacity of the species to withstand a disturbance (resistance), and capacity of the species to recover from disturbance (resilience) (Lake 2000). Resistance and resilience of a species are governed by such factors as resource requirements, physiological tolerances, behavioural attributes and life history characteristics, which collectively determine the ability of populations of a given species to persist through and/or recover from disturbance (Table 39). Together, resistance and resilience summarise both the likely short- and longer-term impacts of disturbance on species' populations with resistant species likely to show only minor, or delayed responses, and resilient species likely to recover well after a disturbance even if they may be severely affected at the time (Lake 2003, Crook et al. 2010).

Table 39: Species' traits hypothesised to confer resistance and/or resilience to environmental stress for GAB spring endemic flora and fauna.

Species traits conferring resistance and/or resilience	Resistance	Resilience
Low resource specialisation (breadth of food resources consumed and habitat conditions occupied)	yes	
Growth form flexibility (plants)	yes	
High physiological tolerance (e.g. temperature, salinity, turbidity, water stress)	yes	
Low vulnerability to predation (e.g. ability to avoid or survive the effects of predation/browsing)	yes	
Low vulnerability to diseases and parasites	yes	
High competitive ability (ability to out-compete other species for food, habitat and other resources)	yes	
Low reproductive specialisation (i.e. range of environmental conditions over which reproduction and recruitment can occur)	yes	
High abundance/biomass/ area of occurrence	yes	yes
Physical/behavioural strategies to avoid/withstand disturbance (e.g. high mobility, desiccation resistance through dormancy, trampling resistance, etc.)	yes	yes
High dispersal/recolonisation ability		yes
High reproductive capacity (i.e. based on fecundity, frequency & duration of reproduction events, generation time, degree of parental care, reproductive mode (e.g. vegetative vs seeds), seed store persistence?)		yes
Longevity		yes

5.5.4 Risk assessment approach

The approach used to assess risks to GAB spring wetlands and their endemic flora and fauna from cumulative impacts of current threats (described in Table 38) is modified from Halpern et al. (2007, 2008, 2015). Cumulative impacts (I_C) are calculated for each spring complex as follows:

$$I_C = \sum_{i=1}^{n} \frac{1}{m} \sum_{j=1}^{m} T_i * S_j * \mu_{i,j}$$

where T_i is the log(x+1)-transformed normalised value (scaled between 0 and 1) of a threat at spring complex *i*, S_j is the presence or absence of species *j* (either 1 or 0, respectively), and μ_{ij} is the vulnerability weight for the threat *i* and species *j* (range 1 to 3, Table 40), given $n = 5$ threats and $m = 98$ species.

Vulnerability weights are calculated by combining expert scores for two vulnerability measures (resistance and resilience) and the certainty measure (Table 40) into a single weighted-average vulnerability score that represents (in relative terms) how vulnerable a given species is to a given threat. We used expert judgment to estimate the vulnerability weights (μ_{ij}) because empirical data on GAB endemic species' responses to threats are lacking. Practitioners often rely on expert knowledge is such situations (Burgman et al. 2005; Runge et al. 2011; Martin et al. 2012). However, expert knowledge is uncertain, as it is based on an expert's own observations and judgment (Regan et al. 2002). Management decisions that do not account for parameter uncertainty can lead to suboptimal outcomes, which might increase species risk of extinctions (McDonald-Madden et al. 2010).

Table 40: Ranking system for each vulnerability measure used to assess how threats affect GAB spring endemic flora and fauna (modified from Halpern et al. 2007).

Vulnerability measure	**Definition**
Resistance	Ability of a species to withstand a disturbance (i.e. resist changing its "natural" state in response to a threat)
No threat (0)	
High (1)	No significant change in abundance/biomass until extreme threat levels
Medium (2)	Moderate intensities or frequencies of a threat lead to change
Low (3)	Slightest occurrence of a threat causes a change, or all-or-nothing threats
Resilience	Ability of affected species to recover from disturbance (i.e. return to its pre-threat state following disturbance). For persistent threats, assumes removal of the threat.
No threat (0)	
High (1)	Short duration before species can recolonise or recover from threat
Medium (2)	Moderate duration before species can recolonise or recover from threat
Low (3)	Long duration before species can recover from threat
Certainty	Relative confidence that the assessment is true, given the state of data and knowledge available
Low (1)	Very little or no empirical work exists
Medium (2)	Some empirical work exists and/or expert has some personal experience
High (3)	Body of empirical work exists and/or the expert has direct personal experience

We used a quasi-Delphi method (Burgman 2005) to estimate species' vulnerability to threats by asking each expert to independently assess and score each taxon for which they felt they had sufficient ecological knowledge and expertise. Uncertainty in expert judgment regarding the true value of the responses was also estimated for each species-by-threat combination. The experts were then presented with all scores and asked to consider whether they wished to revise or query any scores. A final consensus on the most appropriate scores was reached by group discussion and email.

For each threat-and-species combination, each 1–3 rank is multiplied by the certainty value, and the sum of these weighted values for each vulnerability measure is divided by the sum of the certainty values. This weighted average gives greater importance to values with higher certainty (and presumably higher precision), but may down-weight scores for poorly understood threat-and-species combinations.

The cumulative impact of a particular threat (I_D) across all species is calculated as:

$$I_T = \sum_{j=1}^{m} T_i * S_j * \mu_{i,j}$$

and the cumulative impact of all threats on a particular species (I_S) is calculated as:

$$I_S = \sum_{i=1}^{n} T_i * S_j * \mu_{i,j}$$

Cumulative exposure to all threats (E_T) for each spring complex (unweighted by species' occurrence/vulnerability) is calculated as:

$$E_T = \sum_{i=1}^{n} T_i$$

By combining information on weighted species vulnerabilities to threats, species distributions, and relative threat intensities across spring complexes, the sum of these vulnerability-weighted threat-by-species combinations then represents the relative cumulative impact of threats on all species in a particular complex.

From these data, we can evaluate:

1) which complexes are exposed to the greatest number and intensity of threats
2) how vulnerability to threats varies among species and,
3) which complexes are at the highest risk (i.e. contain high number of vulnerable species and are exposed to the highest threat intensities)

The relative exposure of each complex to the five major current threats identified by Fensham et al. (2010), comprising aquifer (groundwater) drawdown, human modification of springs, disturbance from livestock and feral animals, introduced flora and introduced aquatic fauna, was calculated. This calculation was undertaken using a range of existing and readily available data layers (described in Table 41) and Arc GIS. Spring complex polygons or spring points (depending on the nature of the threat data) were intersected with each threat layer. For each major threat type, multiple individual threat layers (Table 41) were integrated into composite threat exposure indices by taking the average of the log(x+1) transformed and normalized (scaled between 0 and 1) individual threat layers and then renormalizing to range between 0 and 1. There are many ways to integrate different data layers; we chose a simple averaging method to integrate individual threat layers as this approach has been demonstrated to be the least prone to bias, missing values and other potential problems (Robinson & Kennard 2010). Examples of data layers used to characterise exposure associated with the threat of potential groundwater drawdown are shown in Figure 146.

Table 41: Data layers used to characterise threats.

Threat	Source data layer(s)
Aquifer (GW) drawdown	Includes direct measures & indirect surrogates
	Spring activity status from GAB springs database
	Bore density (bores/km^2) for those uncapped bores within 6km buffer surrounding each complex polygon. Source: National Groundwater Information System (BOM 2016). Used as a measure of potential for local and regional groundwater drawdown.
	Projected change in groundwater level under current climate and current groundwater development (Scenario A). Source: Great Artesian Basin Water Resource Assessment (Welsh et al. 2012).
	Existing coal and petroleum mining production activity (excludes Exploration & Tenement Licenses). Source: Energy & Resource Insights (2016)
Human modification of springs	Includes direct measures
	Lake Eyre Basin Springs Assessment database (Excavation damage) (data for 170 Qld complexes only)
Disturbance from livestock and feral animals (cattle, pigs, sheep, goats, camels)	Includes direct measures & indirect surrogates
	Australian Collaborative Land Use and Management Program (ACLUMP) (2014) Land Use of Australia Interim Version 5, 2010-11 (grazing native vegetation or modified pastures).
	Lake Eyre Basin Springs Assessment database (stock & pig damage) (data for 170 Qld complexes only)
European cattle (*Bos taurus*), feral pig (*Sus scrofa*), sheep (*Ovis aries*), goat (*Capra hircus*), camel (*Camelus dromedaries*)	Atlas of Living Australia occurrence records (individual records within a 1km buffer of spring complex polygons)
Introduced flora	Includes direct measures
Para grass (Urochloa mutica), olive Hymenachne (Hymenachne amplexicaulis), rubber vine (Cryptostegia grandiflora), date palm (Phoenix dactylifera), bamboo (Arundo donax), athel pine (Tamarix aphylla)	Atlas of Living Australia occurrence records (method described above).
Introduced aquatic fauna	Includes direct measures
Gambusia (Gambusia holbrooki), Red-claw crayfish (Cherax quadricarinatus), Cane toad (Rhinella marina)	*Gambusia holbrooki* - Atlas of Living Australia occurrence records (method described above) plus sampling records from the Lake Eyre Basin Springs Assessment database. All other taxa: Atlas of Living Australia occurrence records.

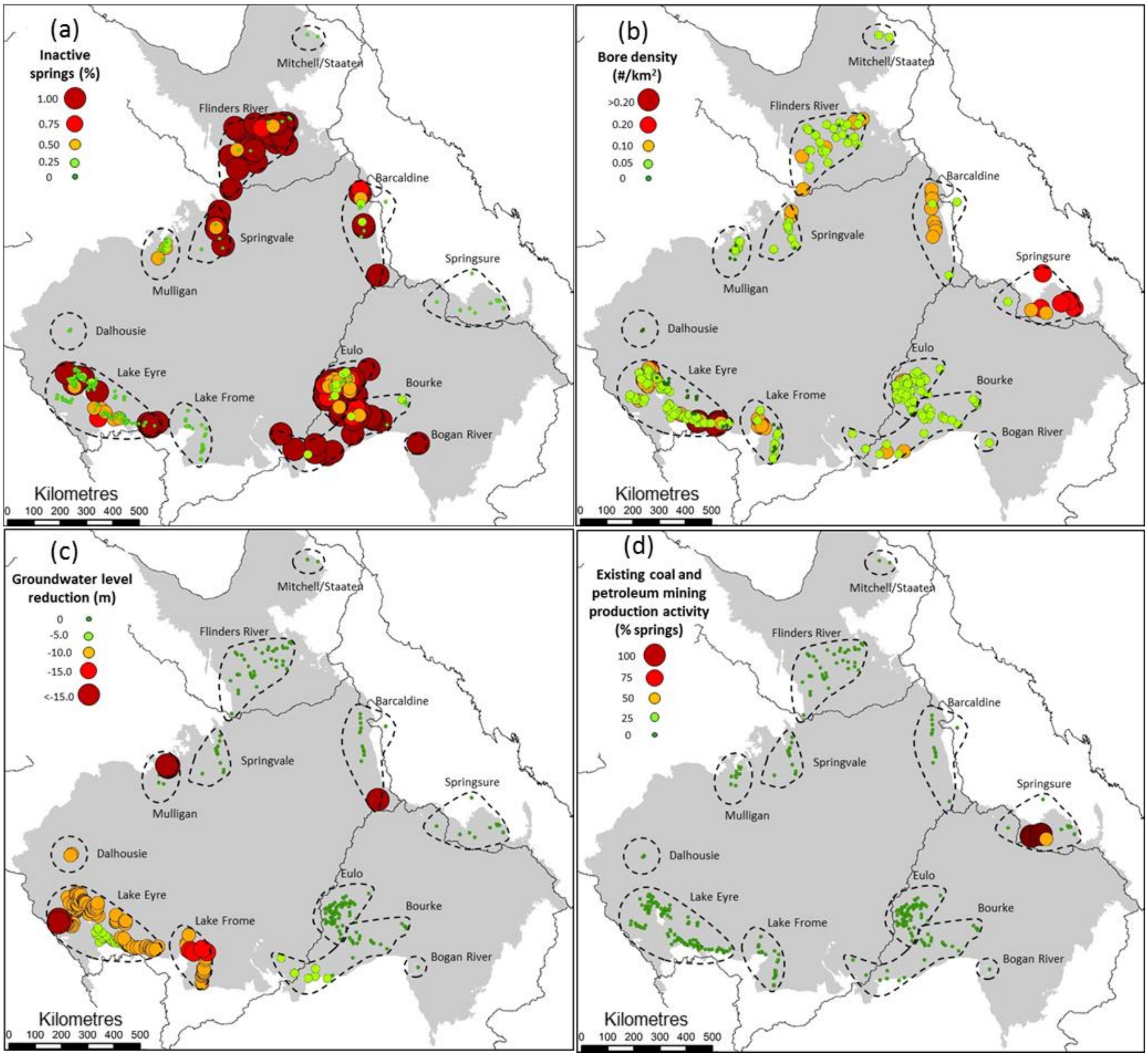


Figure 146: Map of GAB (shaded area) and spring complexes characterised by their relative threat exposure: (a) % inactive springs, (b) bore density, (c) groundwater level reduction and (d) existing coal and petroleum mining production activity (see Table 42 for data sources). Spring complexes belonging to each supergroup are enclosed by dashed lines.

5.5.5 Results

5.5.5.1 Threat exposure

All supergroups contained one or more spring complexes exposed to a high number and high intensity of threats (e.g. cumulative threat exposure scores > 1.5, Figure 147) but there was substantial variation in threat type and degree of exposure within and among supergroups (Figure 147, Figure 148). Threats posed by groundwater drawdown and animal disturbance were most widespread (occurring at 99.7% and 82.5% of complexes, respectively) whereas human modifications, introduced flora and introduced aquatic fauna were patchily distributed but often of high intensity (Figure 147, Figure 148).

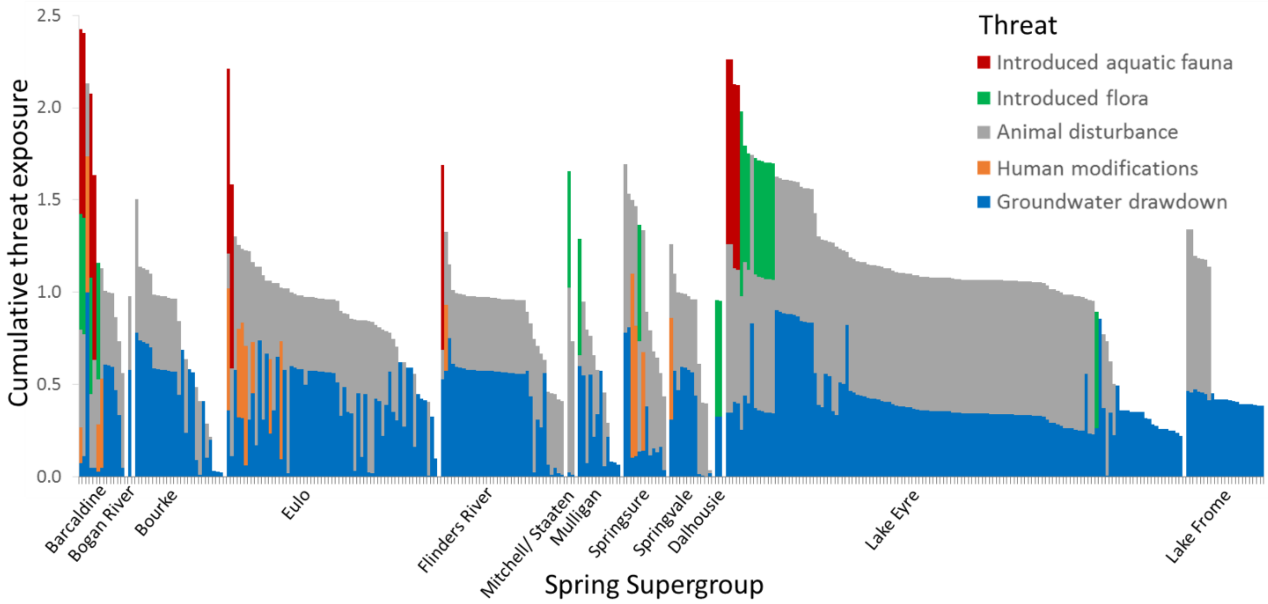


Figure 147: Cumulative exposure to each threat for all 326 wetland complexes (arranged by supergroup). Each threat can have a maximum exposure score of 1 at a given complex and the combined exposure to all threats can have a maximum score of 5 at a given complex.

5.5.5.2 Taxon vulnerability

Expert elicitation resulted in all fish, mollusc and crustacean taxa being estimated to be vulnerable to varying degrees to all threats, whereas plants, unsurprisingly, were not regarded as being vulnerable to introduced aquatic fauna but were vulnerable to all other threats (Figure 149a). Taxa with the highest overall vulnerability in each taxon group were the red-finned blue-eye (*Scaturiginichthys vermeilipinnis*), the mud snail from Lake Frome (*Posticobia ponderi*), an amphipod shrimp with stygobiotic origins endemic to the Dalhousie spring complex (*Phraetochiltonia anopthalma*) and a herbaceous perennial plant (*Eryngium fontanum*) (Figure 149a, Figure 150). Certainty in taxon vulnerability scores varied substantially among and within taxon groups, and among threat types (Figure 149b). Across all threats, certainty in vulnerability to threats was highest among molluscs (*Edgbastonia*, *Gyraleus*, *Glyptophysa*, *Gabbia* and 14 *Jardinella* taxa) and fish (*Scaturiginichthys*) and lowest among the crustacea and some molluscs (*Arthritica*, *Austropyrgus*, *Posticobia* and 10 *Jardinella* taxa). Across all taxa, certainty in taxon vulnerability to threats was highest for effects of groundwater drawdown, human disturbance and introduced aquatic fauna, and least certain for animal disturbance and introduced aquatic flora.

On average, fish were estimated to be most vulnerable to groundwater drawdown, slightly less vulnerable to human modifications, animal disturbance and introduced aquatic fauna, and were least vulnerable to introduced flora (Figure 151). These trends were generally similar for molluscs and crustaceans but vulnerability scores were lower overall for all threats in comparison to fish. Plants were most vulnerable to groundwater drawdown, human modifications and animal disturbance and had similar vulnerability to introduced fauna as for fish, molluscs and crustaceans (Figure 151).

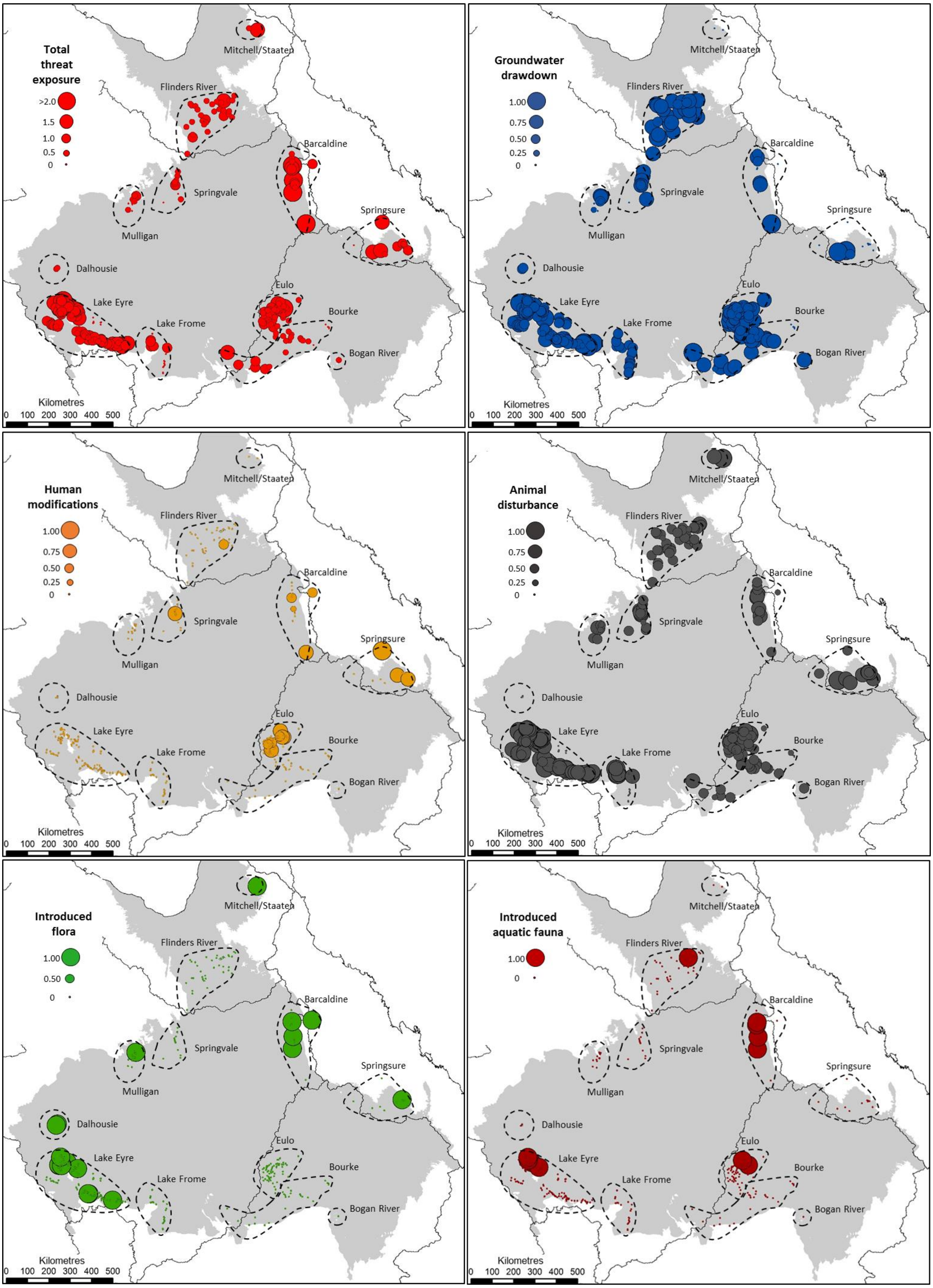


Figure 148: Spatial variation in cumulative threat exposure of spring complexes (upper left plot) and relative exposure to each threat. Spring complexes belonging to each supergroup are enclosed by dashed lines.

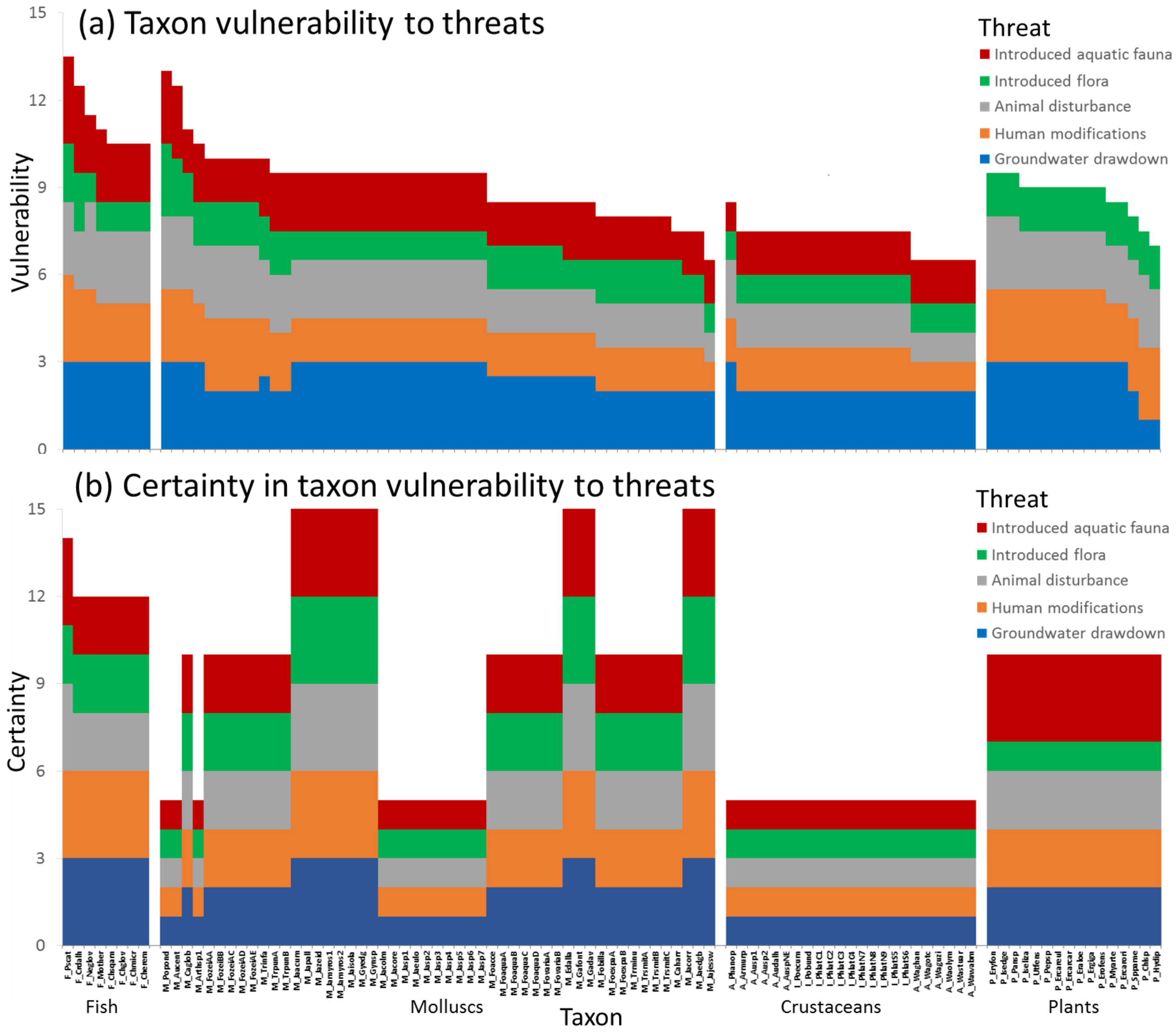


Figure 149: Expert elicited scores for (a) vulnerability to each threat for all 98 endemic taxa (arranged by taxonomic group) and (b) certainty in taxon vulnerability to threats. Vulnerability to each threat ranges from 0 (no threat) to 3 (high), respectively. Certainty ranges from 1 (low certainty) to 3 (high certainty). Each taxon can have a maximum potential vulnerability and certainty score of 15.

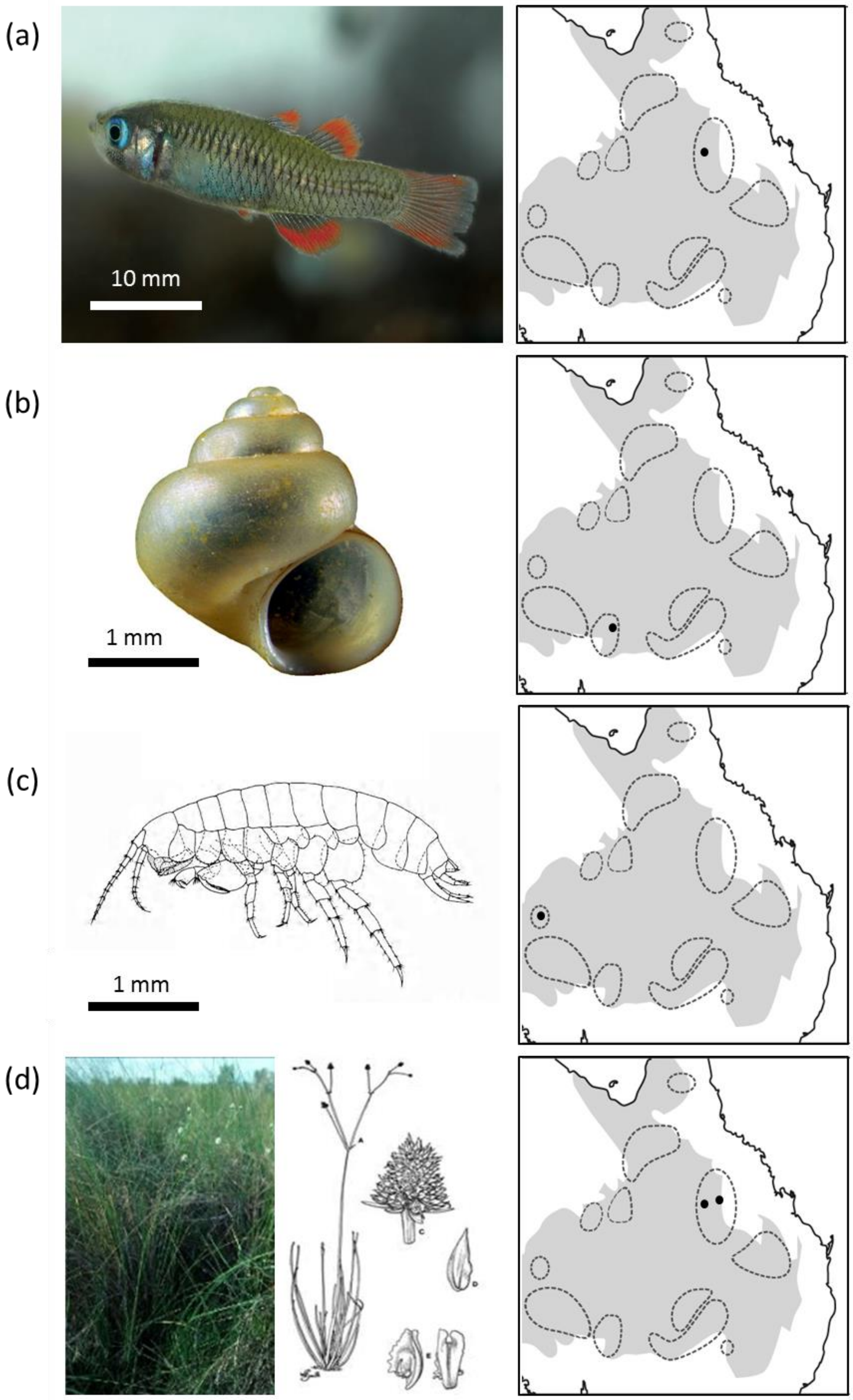


Figure 150. Images of taxa with the highest overall vulnerability in each taxon group (left panel) and their distribution among spring complexes (right panel). (a) red-finned blue-eye – Scaturiginichthys vermeilipinnis*, (source: Kerezsy and Fensham 2013), (b) the mud snail from Lake Frome –* Posticobia ponderi *(source Ponder et al. 2016), (c) an amphipod shrimp endemic to the Dalhousie spring complex –* Phraetochiltonia anopthalma *(source: Zeidler 1991), and (d) the herbaceous perennial plant –* Eryngium fontanum *(source: Queensland Herbarium).*

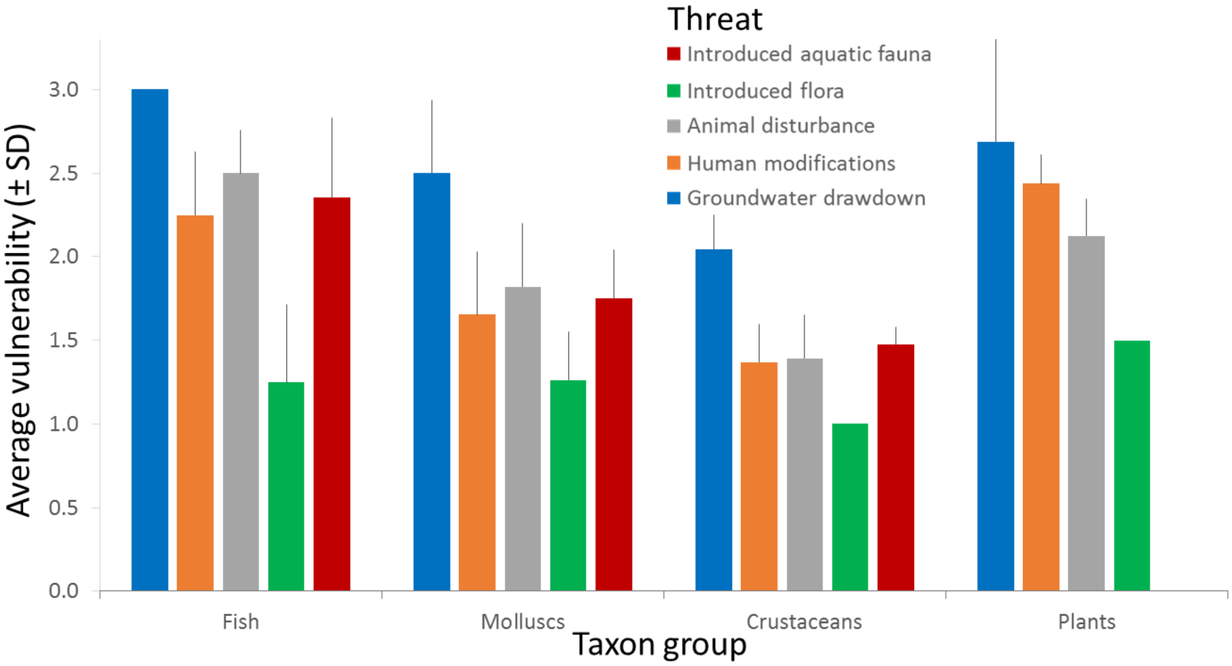


Figure 151: Certainty-weighted average vulnerability (± SD) to each threat for each taxon group. Vulnerability to each threat ranges from 0 (no threat) to 3 (high), respectively.

5.5.5.3 Cumulative risk

Cumulative risk assessment for the subset of 76 spring complexes containing one or more endemic taxa of fish, molluscs, crustaceans and plants, revealed that the Pelican Creek complex (containing Edgbaston and Myross Springs) in the Barcaldine supergroup in Queensland was at substantially higher risk from threats than all others (Figure 152, Figure 153, Appendix 4: Table 61). This complex is exposed to all five threats with the highest cumulative intensity (Figure 152a) and contains 26 species with representatives from all four taxon groups (Figure 152b) that were vulnerable to all five threats (Figure 152c). Thus, the Pelican Creek complex can be considered to be of extremely high conservation concern. Other complexes at relatively high risk (i.e. > upper 25th percentile of total cumulative risk scores) included Moses complex (Barcaldine supergroup), Yowah Mud complex (Eulo supergroup), Dalhousie springs complex (Dalhousie supergroup) and 15 complexes from the Lake Eyre supergroup (Figure 152, Figure 153). All 76 complexes were identified as being at risk from groundwater drawdown and 86% were at risk from animal disturbance (Figure 152). Human modifications, introduced flora and introduced aquatic fauna posed risks to endemic species at 10 or fewer complexes. Some spring complexes in the Lake Eyre and Lake Frome supergroups had a relatively high number of vulnerable species but were assessed as being at relatively low risk because they had comparatively low exposure to threats (Figure 152). No spring complexes from the Bogan River, Mulligan and Flinders River supergroups were identified as being at risk as they did not contain any endemic taxa (Figure 138), despite being exposed to a variety of threats (Figure 147).

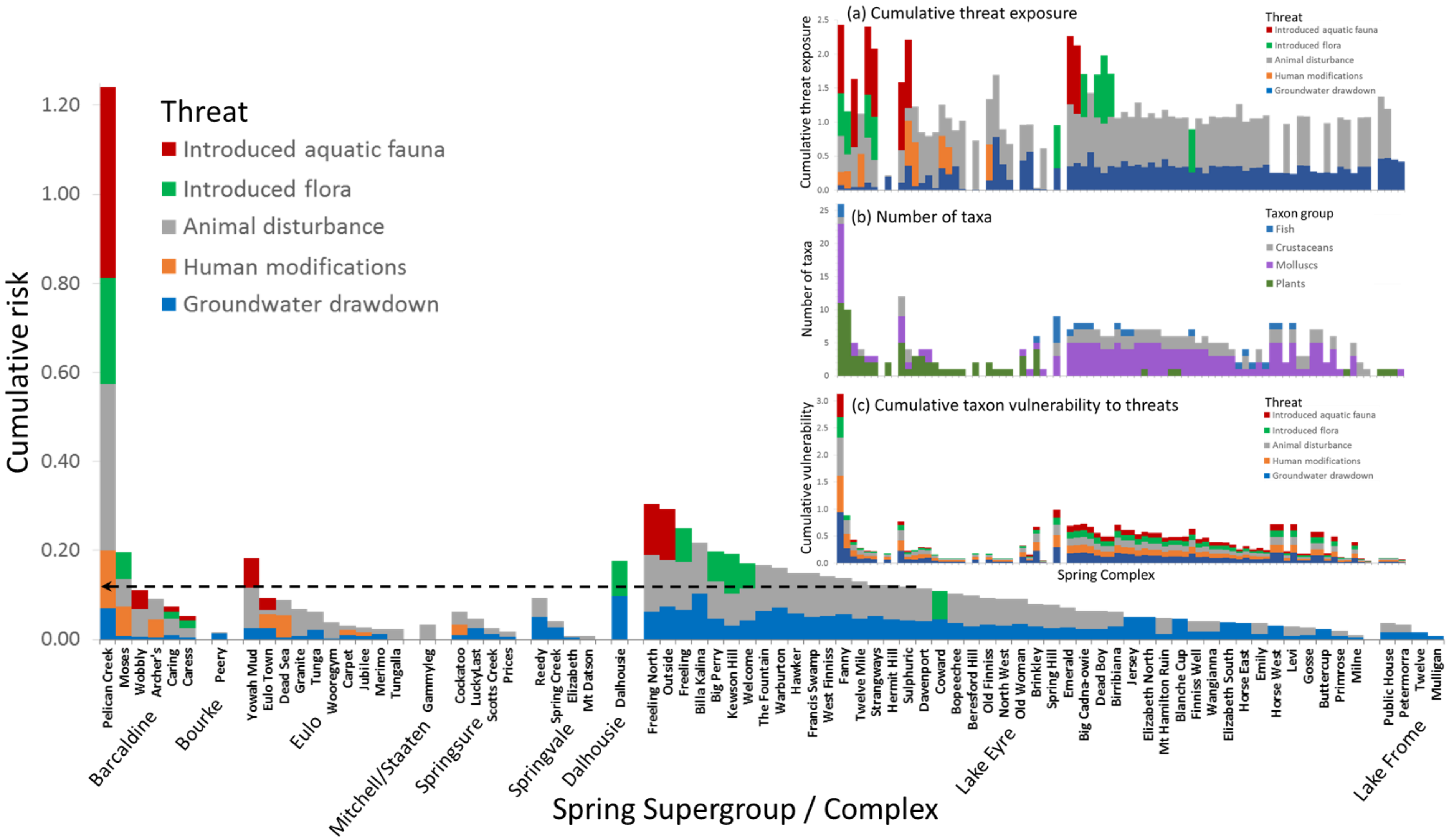


Figure 152: Cumulative risk scores for the 76 wetland complexes (arranged by supergroup) containing one or more endemic species. Risk combines threat exposure data (inset a) with taxon occurrences (inset b) and certainty-weighted taxon vulnerability to threats (inset c) for each complex (note – ordering of complexes in the inset charts is the same as for the main chart). A high risk score for a given complex could be attained by that complex having high exposure to many threats and containing a high number of highly vulnerable species estimated with high certainty (maximum possible risk score = 15). Dashed arrow indicates a notional 'high risk' threshold (corresponding to the upper 25th percentile of risk scores).

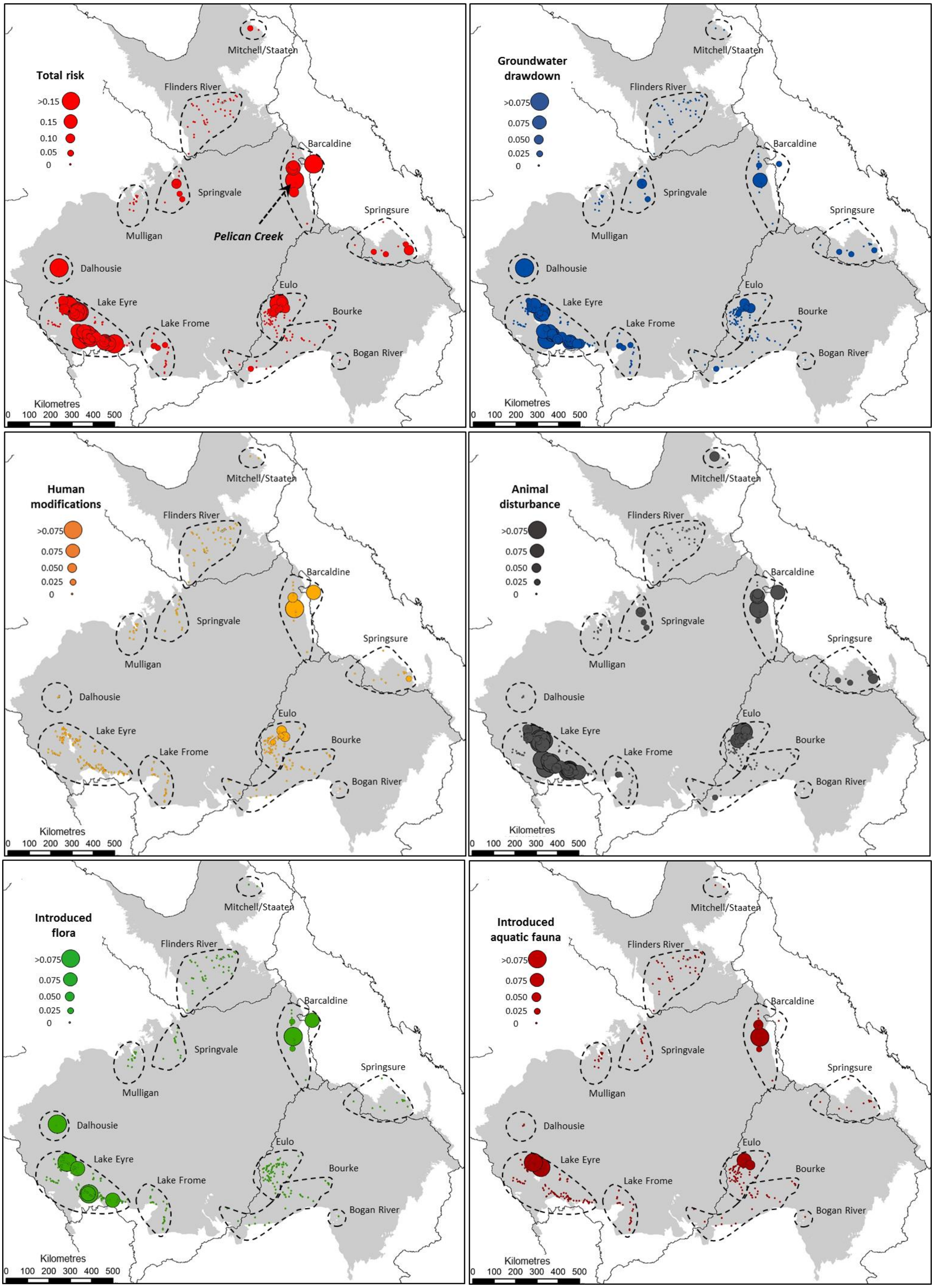


Figure 153: Spatial variation in cumulative risks of threats to spring complexes (upper left plot) and relative risks of each threat. Spring complexes belonging to each supergroup are enclosed by dashed lines. The spring complex with the highest cumulative risk (Pelican Creek) is indicated with an arrow.

5.5.6 Discussion

5.5.6.1 Risks to GAB spring wetlands and their endemic flora and fauna

Our quantitative assessment of threat exposure to GAB spring complexes revealed that all supergroups contained one or more complexes that were exposed to a high number and high intensity of threats, but spatial patterns in threat type and degree of exposure varied widely. Threats posed by groundwater drawdown and animal disturbance were most widespread (occurring in almost all complexes) whereas human modifications, introduced flora and introduced aquatic fauna were patchily distributed but often of high intensity. These findings complement and extend previous assessments of nature and distribution of threats to GAB springs and their resident flora and fauna (e.g. Fensham and Fairfax 2003, Fensham et al. 2010, Powell et al. 2015).

Our cumulative risk assessment (combining threat exposure, taxon occurrences and taxon vulnerability) for the subset of 76 spring complexes containing one or more endemic taxa revealed that the Pelican Creek complex (containing Edgbaston and Myross Springs) in the Barcaldine supergroup in Queensland was at substantially higher risk of threats than all others. This complex is exposed to all five threats with the highest cumulative intensity and contains 26 species with representatives from all four taxon groups that were vulnerable to all five threats. Thus, the Pelican Creek complex can be considered to be of extremely high conservation concern. Other complexes at relatively high risk included Moses complex (Barcaldine supergroup), Yowah Mud complex (Eulo supergroup), Dalhousie springs complex (Dalhousie supergroup) and 15 complexes from the Lake Eyre supergroup.

Groundwater draw down was one of the most pervasive basin-wide threats and is likely to amplify the effect of other threats already affecting endemic GAB species. For example, reduced groundwater pressure is likely to result in springs being more seasonally variable, is likely to decrease the amount of available spring pools potentially increasing competition or predation by invasive species (e.g. *Gambusia* have been shown to be more efficient predators and more aggressive competitors when water availability is low (Pyke, 2008)), or increasing the effect of trampling and disturbance by introduced ungulates as the spring becomes smaller (Figure 144). Many GAB spring wetlands occur in areas of current and potential future coal seam gas and coal mining developments (Fensham et al. 2010, Energy & Resource Insights 2016). The ecological consequences of groundwater decline for GAB springs and the resident biota may be substantial but difficult to detect (e.g. due to time lags and long response times) and potentially difficult to reverse (e.g. aquifer collapse, salinity intrusion, shifts in ecosystem type/function; Kath et al. 2014).

5.5.6.2 Management of threats and monitoring efficacy

Complexes and species with high cumulative risks are subject to varying degrees of active on-ground management – whilst the conservation attention afforded some locations and species matches their threat exposure (e.g. red-finned blue-eye) many locations and species receive little to no attention. Effective management of GAB springs will require a set of conservation management actions to be identified and implemented to effectively mitigate or prevent current and future threatening processes. Candidate management actions to ameliorate/mitigate threats to GAB springs are listed in Table 42. Some of these actions are currently being implemented on-ground to ameliorate or mitigate threats to GAB springs within complexes we identified as being at high risk. All of these actions vary in their ease of implementation, cost-effectiveness and likelihood of being applied. For those threats that are more difficult to control (e.g. the pervasive effects of groundwater drawdown and climate change), it may more feasible and realistic to control other threats in order to improve the resilience of GAB springs and their resident species. Even if such threats can be controlled, it may take years for some springs to regain their native species composition through natural recolonisation processes due to their extreme isolation, particularly for obligate freshwater taxa without strong dispersal abilities.

Monitoring to detect ecological responses is critical to effective and efficient on-ground management, but alone is not sufficient to understand threats. Monitoring allows evaluation of the effectiveness of management actions and facilitates modification if required (i.e. adaptive management). For some species, such monitoring, management and intervention are well developed and documented (e.g. red-finned blue-eye; Kerezsy and Fensham 2013). This is related to their threatened species listing and emphasises the importance of remedying such conservation legislation. For some species, long-term monitoring programs have detected extinctions (e.g. associated with Olympic Dam bore fields; see Fensham et al. 2010) or revealed long and short-term spatiotemporal changes in diversity (Worthington-Wilmer et al. 2011; Rossini et al. in press). This emphasises the utility of targeted monitoring programs, particularly given that research that directly documents the effect of threats on endemic species is rare (for some examples to date see Munro et al. 2009, Davies et al. 2010 and Nicol et al. 2015). Monitoring can be implemented to detect changes in the intensity of the key threatening processes listed in Table 42, particularly those that remain data deficient or in areas where the effects of threats remain under-studied (e.g. monitoring groundwater levels and spring discharge regimes in the context of historic regimes in areas at risk from new mining developments). However, such monitoring needs to be designed in a way that is able to detect changes in abundance and diversity over spatial and temporal scales of relevance – all factors that have been shown to be sensitive to the methodology used (Cantonati et al. 2007, Rossini et al. 2015, Rossini et al. in press). As threatening processes are compounded in particular areas and natural fluctuation in endemic species populations may remain undocumented, manipulative experiments are needed to disentangle the effects of different threats from natural spatiotemporal variability.

Table 42: Candidate management actions to ameliorate/mitigate threats to GAB springs. (Source: modified from Fensham et al. 2010, Firn et al. 2015a,b,c and OGIA 2016b)

Threat	Candidate management action
Aquifer drawdown	• Control flow from bores in strategic locations (including capping of bores in close proximity to high priority springs) • Control new groundwater allocations and unlicensed groundwater extraction
Disturbance from livestock and feral animals	• Implement and maintain appropriate grazing management; fencing of selected springs to exclude stock and feral animals • Control feral animal species (e.g. shooting, poisoning, trapping, biocontrol)
Human modification of springs	• Protect high conservation value discharge springs from excavation, and manage them through perpetual agreements • Prohibit the inundation of springs • Manage tourist access, and develop and implement visitor management plans for selected sites subject to tourism • Continue to raise awareness of issue with landholders/managers
Introduced aquatic flora	• Control invasive weed species (e.g. manual removal, herbicides, fire management) • Put in place secure, tenure-based agreements to prohibit the establishment of exotic ponded pasture species in discharge spring wetlands • Implement protocols to avoid transportation of organisms from one location to another • Buffer habitat areas from the impacts of activities like cultivation • Continue to raise awareness of issue with landholders/managers
Introduced aquatic fauna	• Direct eradication and preventing further spread of *Gambusia* and other exotic fauna, including through the control of bores to reduce stream flows in bore drains (which will greatly reduce the habitat for aquatic pests including *Gambusia*, and reduce their capacity to disperse into spring wetlands) • Implement protocols to avoid transportation of organisms from one location to another • Surveillance and biosecurity hotspots • Education • Chemical control (e.g. rotenone)
Climate change	• Reduce intensity of other threats (e.g. relocate stock water supply bores)

Different management actions will likely be needed to achieve the conservation goals (e.g. protection, threat mitigation, rehabilitation), and therefore monetary estimates of management cost should ideally be linked to decision-making concerning which management actions to implement in which places (e.g. Firn et al. 2015c). The incorporation of realistic and spatially explicit cost estimates for different management actions would allow cost–benefit trade-offs to identify the most efficient combination of actions and where they should be spatially prioritised to achieve the conservation goals (Carwardine et al. 2012). However, the cost of each management action must include an estimate of each action's efficacy, which usually relies on expert knowledge and information regarding the ecology of the species in question (e.g. Cattarino et al.

2016). More objectively derived estimates of conservation benefits that are gained through monitoring programs could help increase the efficacy of a management plan. Adaptive management plans, where information is gained through well-defined monitoring programs (see Section 6 Summary: application of the research and implications) in the early stages of the plan or from previous experiences, can be incorporated in the decision-making process and would greatly improve the cost-efficiency of conservation management. The basin-wide assessment presented here represents an opportunity to prioritise such monitoring at complexes where the diversity of vulnerable taxa is high and the cumulative exposure to threatening processes is high.

Biodiversity offsets have developed as a mitigation measure and economic instrument to compensate for biodiversity losses from development. The foundation of offsetting logic is that equivalent gains can be made elsewhere, once avoidance and mitigation strategies have been exhausted through the environmental impact assessment process (Gibbons 2012, Bull et al. 2013, Maron et al. 2016). However, we do not consider 'biodiversity offsets' as an effective risk management strategy for springs exposed to current or future threats. This is because many taxa endemic to GAB springs have extremely restricted distributions (i.e. occur in only a single spring – see Figure 141) and thus there are no other 'equivalent' springs that could be conserved or managed to protect lost species. In these cases, 'managed relocation' of species at high risk of exposure to threats may be considered a candidate management action to avoid species extinction. However this management approach is not without considerable challenges for conserving freshwater species (see Olden et al. 2011). In the GAB systems captive breeding had little success (e.g. failure to maintain captive bred populations of red-finned blue-eye; see Fairfax et al. 2007), and like managed relocations, will remain a risky measure to rely upon without better knowledge concerning the life-history, ecology and environmental requirements of threatened species.

5.5.6.3 Assumptions, challenges, and future directions

There are several limitations and assumptions of the cumulative risk assessment approach used here that are common to most spatially explicit cumulative risk assessments (reviewed in Halpern and Fujita 2013). The most relevant to our study include:

- *Threat layers are assumed to be of equal importance*. Decisions about which threats to include in the assessment, and how to combine or split related threats, can have important implications for how much of a potential impact any given threat or group of threats can contribute to overall cumulative risk. We used expert knowledge to combine individual threat layers into meaningful groups that directly corresponded to the major threatening processes identified in the literature as affecting spring endemic species.

- *Appropriate characterisation of threat exposure*. This is dependent on such factors as: the accuracy, currency and spatial grain size of the individual threat data layers, the appropriate choice of spatial extent of threats relative to springs/complexes for calculation of exposure (this necessarily varied with each threat layer – see Table 41), and the methods used for integration of individual data layers within each threat type and their subsequent transformation.

- *Accurate estimation of species distributions*. Our taxon distribution data represented the best available and most up-to-date information available on the presence-absence of taxa across spring complexes (see earlier section).

- *Transforming and normalizing threat layers*. This was required so that very different kinds of threat data layers with variable distributions and unique measurement units could be compared to each other. However, decisions about if or how to transform (which determines whether skew in data is preserved, minimized or removed altogether) and whether or not to normalize (resulting in every other intensity value for the stressor being rescaled to the maximum value) the data layers may have important but unevaluated consequences for the resulting risk assessment.

- *Linear response of species to threats*. Our cumulative risk assessment relied on assumptions of linear and additive responses of species to increasing intensity of threats. However, threshold or non-linear responses to intense or cumulative stress are also possible but are difficult to quantify.

- *Consistent species responses*. The development and application of the vulnerability weights that defined how each species responds to a particular threat requires a fundamental assumption that all individuals (within a taxon) respond the same way to a threat, and that any given individual responds in the same way at all points in time.

- *Vulnerability weights are sufficiently accurate*. Extremely limited available knowledge required that expert judgement was used to estimate taxon vulnerability to threats. We assumed our estimates to be representative and accurate and we explicitly represented the certainty of our estimates. However, the estimates be could be refined through surveying a broader pool of experts and improving the approach to estimating and representing uncertainty (e.g. see McBride et al. 2012).

- *Linear response of species to cumulative threats*. We assumed linear responses of species to increasing intensity of threats. However, threshold or non-linear responses to intense or cumulative stress are also possible (e.g. Poff et al. 2010) but are difficult to quantify due to lack of knowledge.

- *Additive model*. We assumed additive responses of species to increasing numbers of threats. We did not consider responses to interactions among threats (i.e. antagonistic or synergistic – see Brown et al. 2014) due to the rapidly escalating complexity and uncertainty in eliciting those responses for more than one threat at a time.

The assumptions described above were necessary due to the challenges arising from data limitations and knowledge uncertainties. Many additional challenges remain that are common to most cumulative risk assessment approaches. For example, characterising some of the major threatening processes was challenging in our study because of missing or imperfect data and some threats lacked direct measures so surrogates were used instead (e.g. data on surrounding bore density and existing coal and petroleum mining production activity near spring complexes were used as indirect measures of potential for local and regional groundwater drawdown). We also assumed that threats were fixed in space and time as we had little knowledge of how threats may propagate across the landscape, or how they may vary in intensity and distribution historically. Collectively, these factors may have had a bearing on our risk assessment results, given we used static present-day threat exposure data.

Notwithstanding these assumptions, our assessment of the cumulative risks posed by groundwater drawdown and other threats to GAB springs and their endemic species is the most up-to-date, comprehensive (in terms of spatial extent, number of threatening processes and number of taxa assessed), ecologically relevant (as it is species-focussed and explicitly incorporates ecological-response mechanisms with a solid grounding in ecological theory), quantitative (i.e. uses continuous data to estimate exposure and risk instead of qualitative and discretised risk ratings) and scientifically robust (incorporates uncertainty and uses a transparent, repeatable and well established method supported by recent high quality scientific literature), yet undertaken.

Our cumulative risk assessment of GAB endemic species could be extended and improved in a number of ways. The assessment was made for 96 taxa from five groups of organisms endemic to the GAB (fishes, molluscs, amphipods, isopods and plants). As more reliable data become available, the approach could be readily extended to include other non-endemic taxa for which GAB springs are nevertheless an important habitat and for other major groups of organisms (see previous section). It would also be relatively straightforward to assess species' vulnerability and risks to projected future environmental changes associated with climate change and groundwater development (e.g. using the approach outlined in Halpern et al. (2015) and the modelled threat scenario projections available from the Great Artesian Basin Water Resource Assessment (Welsh et al. 2012)).

5.5.7 Key findings

To conclude this section, we present three areas, informed by our assessment, which could be the focus for the future of GAB springs research and conservation management.

1. Some GAB springs and their endemic species are clearly at high risk – strategic, coordinated, inclusive and committed management is urgently required to address current and future threats

Evidence from past practices reveals that unrestricted and unmanaged access to groundwater (through bore extraction) and other threats led to a high proportion of springs to go extinct (Figure 136). Many of these historical impacts were concentrated in particular areas that still contain highly restricted endemic taxa (i.e. to a single spring), so it is extremely likely that undocumented biodiversity has already been driven to extinction. Our cumulative threat exposure analysis and risk assessment results further highlight the urgency of the problem, given the continued, widespread, and pervasive threats that springs are currently exposed and the likelihood of these threats being exacerbated by climate change and expansion of the coal seam gas and coal mining industry.

Threat management is required at different tiers with different stakeholders. For example, feral animals can be easily managed by on-ground practices like fencing, trapping and shooting. In contrast, groundwater drawdown is a basin-wide problem that needs national leadership and management to complement state-based and regional initiatives because losses in one place can lead to drawdown in another. Some localities stand as testament to the ability of multiple stake-holders to work together to preserve 'hot-spots' of biodiversity in this system (e.g. the Edgbaston portion of the Pelican Creek complex). However, this report emphasises that such activities need to be fostered elsewhere and provides a basin-wide perspective for facilitating this process.

On-ground action to mitigate threats cannot be effective without complementary efforts to protect from further losses of groundwater GAB pressure. A number of initiatives currently exist to facilitate more effective and strategic management, including the Great Artesian Basin Sustainability Initiative (GABSI), the Great Artesian Basin Strategic Management Planning process currently underway involving federal and state/territory governments and the Great Artesian Basin Coordinating Committee (GABCC).

2. Science is needed to establish ecological responses to threats and benefits of actions

This review has highlighted that quantitative data is lacking concerning population trends, responses to threats and benefits of management actions to mitigate those threats for most GAB endemic species. Targeted monitoring of population trends is needed in order to understand natural temporal variation within spring systems (e.g. Rossini et al. in press) and populations within springs act as metapopulations that shift across the landscape (Murphy et al. 2010). Targeted monitoring can in turn inform managers on how species are or are likely to be responding to environmental changes and highlight conservation concerns that would require management actions. Examples of ongoing monitoring programs exist in some South Australian springs and at Edgbaston springs. However these programs concern only two of 33 unique 'assemblages' of species that exist in GAB springs. Fostering a basin-wide approach to monitoring that ensures that biodiversity 'hot-spots' and localities with the most pertinent exposure to threats will help to expand on these past successes and fill knowledge gaps. Our expert elicitation revealed that most species are highly vulnerable to groundwater drawdown. However, we lack precise evidence of actual species responses and how cumulative interacting threats may exacerbate vulnerability. Some of these knowledge gaps are relative straightforward to address, at least for some species and threats (e.g. using experimental mesocosms to simulate water drawdown and recovery and monitoring ecological responses such as physiology and behaviour). These questions come hand-in-hand with research regarding the ecology of endemic GAB species – another literature gap at a basin-wide scale.

Monitoring programs are not only important for management; they provide knowledge that is useful to both academic science and community conservation. Long-term monitoring programs in Lake Eyre have facilitated cutting-edge research into the evolutionary origins (e.g. Murphy et al. 2012) and patterns of population connectivity at landscape scales (e.g. Worthington-Wilmer et al. 2008). They have allowed ecologists and taxonomists to officially describe over 50 species that prior to the 1980's were unknown to science, and to continue to identify at least 50 more. These species are ecologically and taxonomically diverse – some have transitioned from rainforest creek-dwelling species to arid-zone spring specialists (e.g. Ponder et al. 1989; Perez et al. 2005) others have colonised the surface after persisting within underground aquifers (e.g. Murphy et al. 2009). By understanding them, researchers have been able to document the quaternary changes in Australia's climate (Ponder and Colgan 1990; Murphy et al. 2012), document speciation (e.g. Murphy et al. 2015b) and document the adaptations needed to persist in the Australian arid-zone. This in turn provides information and understanding about species that increases the concern of the general public for their conservation (e.g. 'friends of the mound springs' are a voluntary organisation that conserve South Australian mound springs, ~20 individuals volunteer every year to aid in conserving the red-finned blue-eye, and numerous property owners manage populations of threatened species or have entered into voluntary conservation agreements). Greater understanding and awareness also facilitates community learning and cultural well-being (e.g. undergraduate science students from the University of Queensland facilitate annual invertebrate sampling at Edgbaston and Indigenous Custodians are strongly involved in the management of Witjera and Finniss Springs in South Australia).

3. Many on-ground management actions to mitigate threats can be implemented now

The high concentration of endemic species in relatively small areas means that it should be feasible to identify appropriate threat management strategies and successfully implement them (notwithstanding the challenges identified above). Our research identified a small set of highest risk spring complexes that could be prioritised for the establishment of monitoring programs and targeted management in the short term (see Appendix 4: Table 61). So many springs of conservation importance are on private property (freehold and leasehold) and many threats are localised and can be relatively easily managed at a local scale. Engaging and incentivising private property owners about values of their own springs and the need to protect and monitor them would be a major leap forward in this regard.

5.6 References – Component 4

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6 Synthesis: application of the research, key findings and project outcomes

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6.1 Background

This project set out to quantify ecological responses to changes in surface water and groundwater hydrology in a range of regions and environmental settings. We anticipate the methods and key findings from our research will be valuable for OWS, IESC, proponents of coal seam gas (CSG) or large coal mining development projects, the broader scientific community and other stakeholders. This section outlines key considerations when applying this knowledge (i.e. how transferable our findings are to other situations and scales). We detail specific issues associated with transferability and scaling for each of the key ecological response themes examined in the project (relating to biogeochemistry, ecosystem processes, groundwater dependent vegetation, hyporheic fauna, macroinvertebrates and fish). We also outline how our methods and research findings can inform the development and implementation of monitoring programs aimed at assessing performance of proposed measures to mitigate potential impacts of CSG or large coal mining development. In particular, we highlight ways in which some of our findings and research methods offer potential for monitoring impacts of water-related threats associated with coal seam gas extraction and coal mining. We conclude with a synthesis of key project findings and describe expected outcomes of the project.

6.2 Inferential strength, transfer and scaling of ecological response relationships

This section concerns the inferential strength and potential broader application of the research concerning ecological responses to changes in surface water and groundwater hydrology (hereafter termed ‘ecological response relationships’). It is intended to provide general background and key considerations about how our key findings can be applied in new contexts. Inferential strength and transferability of research is especially relevant to environmental assessments for new coal seam gas (CSG) or large coal mining development proposals (either greenfield or brownfield). These assessments usually draw on past research conducted in different areas to conceptualise ecological responses, to predict potential impacts of the development and to design monitoring programs to detect those impacts as a condition of project approval (IESC 2015). Our research findings, if appropriately applied, can strengthen the scientific basis for these assessment and monitoring designs.

6.2.1 Inferential strength of ecological response relationships and issues of scope and scale

The inferential strength of an ecological response relationship reflects the quantity and quality of ‘evidence’ available that supports that relationship. In other words, how true is the relationship (i.e. is there demonstrated cause and effect) and where else and when could the relationship be validly applied (how general is the relationship)? The type of scientific evidence used to generate ecological response relationships varies widely, is usually based on a blend of inductive and deductive reasoning (Susser 1986), and may include correlative / observational data, manipulative experimental data (laboratory or field), expert opinion derived through elicitation and multiple lines and levels of evidence, combining some or all of the above (see Norris et al. 2011). Each approach is valid but each has strengths and weaknesses in terms of the inferential strength of an ecological response relationship.

Key considerations concerning the inferential strength of information to draw conclusions or make predictions in new contexts also relate to issues of scope and scale (Mac Nally 2002, Corwin et al. 2006). Scope and scale typically concern the spatial extent of environmental or ecological systems and the duration of processes associated with them (Figure 154). Large systems tend to have long histories and long-period dynamics, whereas small systems may respond over shorter time frames, within the constraints imposed at larger/longer scales. Cross-scale interactions also mean that small-scale processes may have non-linear effects on large scale processes and vice versa (Corwin et al. 2006).

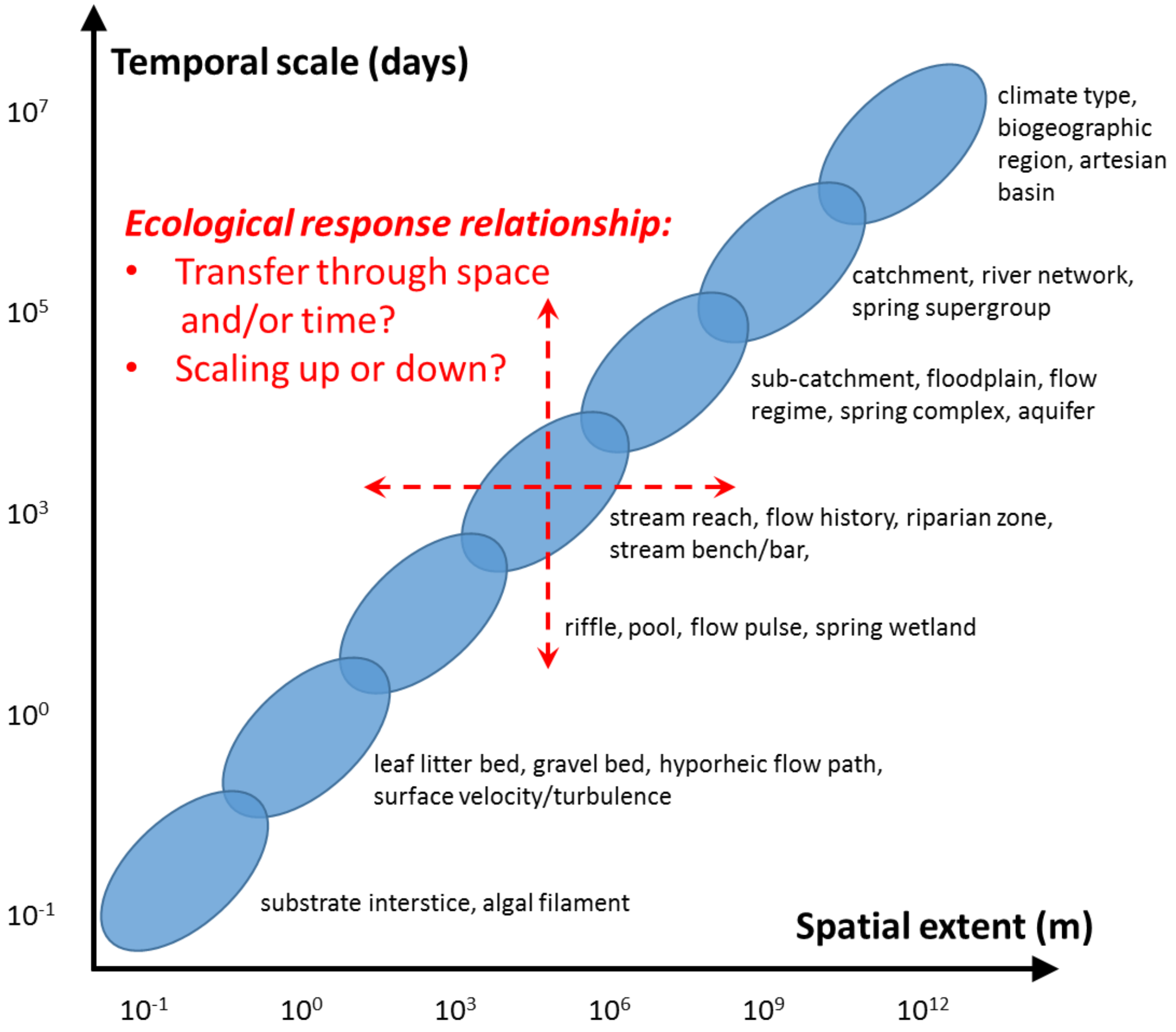

Figure 154. Conceptualisation of general spatial and temporal domains associated with environmental or ecological systems relating to groundwater influenced streams, rivers and spring wetlands. The inferential strength of ecological response relationships generated at a particular scale will decline with increasing departure from the spatio-temporal domain(s) from which data were collected and the ecological response generated (Source: modified from Boulton and Brock 1999 and Mac Nally 2002).

Issues of scope and scale highlights questions of whether ecological research is conducted at inappropriate scales for many ecological objects, especially in temporal terms (studies too short in duration), whether the choice of scale matches the needs of natural resource and conservation managers, or whether inferences drawn from restricted scales can be transferred to other situations and scales (upscaling for example). It is relatively straightforward to identify the overall spatial extent and duration of a particular study system (e.g. in this project, the upper Bremer River catchment was studied for one year), the size of sampling units in which data is collected (e.g. habitat patches within stream reaches), and the sampling frequency used (e.g. surveys were conducted bimonthly). While specification of sampling details provides precise information on the implied relevance of the study, it does not necessarily help to identify the extent to which this information can be transferred or scaled up (or down).

6.2.2 Transfer and scaling of responses through space and time

Ecologists have long sought to distinguish relationships that are general from those that are idiosyncratic to a narrow range of conditions (Peters 1991, Wenger and Olden 2012). Although relationships that are limited in scope and scale may be interesting and informative, they are not necessarily broadly applicable, or likely to constitute a general rule, or hence be useful for environmental management. Instead, models are sought that are variously (and interchangeably) said to have generality, generalisability and/or transferability to datasets or situations other than the one for which they were developed (Wenger and Olden 2012). Most ecologists view the generality of a model as being proportional to the number of biological systems or environmental conditions that a model can capture or to which its conclusions can be applied (Evans et al. 2013).

The issue of transferability has been the subject of ecological interest for a number of years, but has greatly increased with the rise of the field of species distribution modelling in the 2000s (Elith and Leathwick 2009). Researchers have investigated whether a species distribution model developed in one region can successfully predict distributions in a different region (e.g. Kennard et al. 2007) and whether models developed in one time period can predict distributions in a different time period with different weather or climatic conditions (e.g. Kennard et al. 2006). In some places, climates will shift to entirely novel ones that lack current analogues (Williams, et al. 2007), potentially further limiting transferability of a species distribution model calibrated under an entirely different set of environmental conditions. Such questions of generality are equally applicable to models of physical phenomena (e.g. models of temperature), of ecological processes (e.g. denitrification rates), or of population parameters (e.g. growth rates) (Wenger and Olden 2012). The key challenge is that there can be considerable spatial or temporal heterogeneity in environment-ecology response relationships, and this heterogeneity can limit model generality (Wenger and Olden 2012).

Key to the issue of transferability is that ecological response relationships can most reliably be transferred to situations with environmental conditions (through space and time) within the scale and scope (Figure 154) of the conditions in which the relationship was developed. Transfer beyond this range should be made with caution. Environmental or biological regionalisations or classifications (e.g. of hydrology, groundwater dependent ecosystem type, climate, bioregion; see Olden et al. 2012) can assist with deciding on appropriate situations for model/knowledge transfer, provided that the environmental attributes used to inform the assessment are functionally/mechanistically related to the response variables of interest (Poff et al. 2010).

Importantly, different ecological response variables likely vary in their transferability, and for different reasons. For example, measures of ecological processes (e.g. organic matter decomposition) may vary due to changes in resource supply and local physical properties of the environment, which are in turn linked to seasonal climate conditions and the nature of land–water connections, functional attributes of biotic communities (e.g. based on species' traits describing morphology, resource use, life history, etc.) may be constrained by phylogenetic history (Sternberg and Kennard 2013), and species distributions may be constrained by biogeographic factors such as regional species pools and spatial connectivity (Poff 1997, Sternberg et al. 2014).

These issues highlight the need to understand the factors responsible for shaping ecological responses generated in a particular setting and potential confounding influences, before the response relationship can be reliably applied elsewhere (e.g. to predict ecological responses to groundwater drawdown). Conceptual models that articulate ecological response relationships and include key drivers and potential confounding factors can aid in this process (Commonwealth of Australia 2015). The improved understanding of ecological responses to changes in surface water and groundwater hydrology generated through our project enabled the development of conceptual models that can facilitate the transferability of the findings from this research program to other situations for use by scientists, OWS, IESC, and development proponents. More detailed information is provided below regarding the potential transferability and scalability of findings for different ecological responses examined in this project.

6.2.3 Transferability and scalability of findings for different ecological responses

6.2.3.1 Hydrology and Biogeochemistry

The two locations studied in this project had differences in terms of input and chemistry of the regional groundwater. The regional groundwater discharge to the perennial gaining section of the Maules Creek catchment maintains continuous surface flow in this section of the system. In the Bremer River catchment the groundwater input and its chemical composition are temporally more variable and localised. Also, the regional groundwater in the Maules Creek Catchment is oxic, which has been observed in other groundwater basins (Winograd and Robertson, 1982). Despite these differences, both catchments showed similar trends in regard to biogeochemical processes related to OM mineralisation, nitrification and denitrification in the hyporheic zone. The studied sites in the Bremer River catchment were also generally oxic. The results obtained in this study support the conceptual models that have been developed for biogeochemical processes related to OM mineralisation, nitrification and denitrification in the hyporheic zone (for example Zarnetske et al., 2011), which highlights the generality and transferability of these processes.

6.2.3.2 Ecosystem processes

In this study, we used complementary experimental measures of organic matter processing that integrate the detrital processes of many organisms to maximise the transferability and upscaling of the findings. Organic matter processing, and more specifically decomposition of leaf litter, is a fundamental process occurring in aquatic ecosystems, has been widely studied globally, and has previously been shown to alter in response to altered hydrologic regimes. Leaf litter decay experiments are representative of natural detrital processes, and decomposition can be represented by a decay rate (*k*) that takes into account the length of the incubation, which often varies among studies. Decay rates are thus commonly used to compare rates of detrital processing among research from various biomes. However, because different studies often use leaves from local tree species to maximise biological relevance, and the chemical composition (i.e. quality or palatability for biota) of these leaves differs, caution is often required when making comparisons among studies. To overcome this concern, we combined leaf litter experiments with cotton strip assays. Cotton strip assays evaluate the decomposition of cellulose (constitutes 91% of cotton) which does not vary in its chemical composition. Together, these methods: a) assess biologically relevant detrital processes; and, b) employ spatially and temporally standardised approaches.

The key findings from the organic matter processing experiment lend support to its transferability to other regions and the upscaling from these patch and reach-scale experiments. Despite the climatic and ecological differences between our two study regions, we found the same ecological trends in response to hydrological changes. The breakdown of particulate organic matter was primarily driven by the degree of substrate saturation, with both habitat-scale and patch-scale measures of hydrological variability predicting variation in detrital processes. Consequently, cotton strip assays and leaf litter incubations should transfer well under diverse ecological settings. Furthermore, there is potential to upscale our patch- and reach-scale findings because hydrological variability was a key predictor of variation in detrital processes. Understanding the temporal and spatial variability in flow regime, and wetted habitat persistence, will enable the prediction of variation in rates of detrital processes throughout river networks. For example, this prediction could be achieved by combining local-scale estimates of organic matter processing rates during wet and dry periods with hydraulic and hydrologic models to estimate processing potential at larger spatial scales (e.g. stream reaches or river networks). This 'upscaling' will allow inferences about the outcomes of altered flow regimes for these particular measurements.

6.2.3.3 Groundwater-dependent vegetation

The relationship of plant functional types to environmental conditions has been widely researched and developed (Lavorel et al. 2011). Categorising species into plant functional types provides a convenient way of relating plants to their environment as it combines attributes of plant physiology, plant communities and ecosystem processes (Diaz et al. 2007). These plant functional groups based on attributes such as life form, life span, method of propagation, height, cover/function, leaf shape and texture can therefore be used as general indicators of responses to the environment systems (Dansereau 1959, Hutchison 1975). In general, classifications of plant functional groups are relatively easily transferable to any plant community given a reasonable botanical knowledge but does not require extensive or local knowledge of taxonomy. Our approach of developing a typology of plant hydrotypes can be used as part of a field assessment of plant community vulnerability to changes in hydrology such as groundwater drawdown. The development of a typology of plant hydrotypes will assist in assessing potential ecological impacts of changes to groundwater availability, as measuring the proportion of different hydrotypes within a habitat will provide an indication of the degree of reliance on groundwater. Using plant traits to understand ecosystem response is broadly applicable, although caution must be exercised to use trait linkages and trade-offs appropriate to the scale and environmental conditions. Therefore, other local or regional conditions other than groundwater and hydrology, such as soil type or disturbances including fire or herbivory are also likely to effect the distribution and classification of plant functional types.

Results presented here on use of groundwater by river red gum (*Eucalyptus camaldulensis*) are generally applicable for this wide-ranging species. Red gums are the dominant tree of river systems and floodplains throughout mainland Australia. Our results suggest that with some knowledge of depth and range in groundwater levels, stream flow regime and river and floodplain flood regime, together with knowledge of climate, geology, landscape position and soils we can make some assumptions on their likely dependence on groundwater. Similarly, from the analysis of plant communities provided here, with some knowledge of plant functional groups we can make an assessment of the likely proportion of a vegetation community that will be groundwater dependent.

The remote sensing analysis indicated that decadal variability of rainfall led to some reduction in vegetation greenness during the particularly dry periods (such as during the Millennium Drought of mid 2000s). During this time the groundwater levels also dropped, in some bores up to 4 m. However, both groundwater levels and vegetation greenness recovered in the wetter periods following the droughts. This recovery is likely to indicate that vegetation is well adapted to the decadal variability of the climatic conditions and variation in water availability associated with it. This remote sensing analysis can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation. Small-scale on-ground data can also be used to 'ground-truth' the remotely sensed data. The combination of on-ground studies and remote sensing provides a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales. Particular advantages of RS techniques include the availability of historical satellite datasets and the ability to monitor large areas at low cost. The remote sensing techniques we used here can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation. Small-scale on-ground data can also be used to ‘ground-truth’ the remotely sensed data.

6.2.3.4 Hyporheic fauna

While we expect that the subset of our key findings that is supported in the published literature (refer Component 3 key findings) will be transferable and scalable in a general sense, we advocate a precautionary approach when considering the transferability and scalability of the site-specific findings from our study, because we expect that local context is likely to be fundamentally important. We found that hyporheic invertebrate assemblages were locally highly variable across small vertical and lateral spatial scales. At sub-metre habitat patch scales, taxonomic richness may vary by one order of magnitude, and overall abundance by two orders, and the dispersion of individual taxa may be extremely “clumped” between neighbouring patches. This high localized variability in assemblages is associated with inherent habitat patchiness, the enormous ‘invisible’ heterogeneity that challenges groundwater ecologists worldwide (Griebler et al. 2014). This heterogeneity and variability across all spatio-temporal scales demands a careful approach be taken when considering the transferability and scalability of findings from this study. Additionally, the general paucity of baseline hyporheic ecology studies in Australia means there is little existing benchmark context against which to make comparisons and assessments. Notwithstanding these limitations, because biodiversity is typically positively correlated with rates of ecosystem function (e.g. Boulton et al. 2008), hyporheic invertebrate diversity indices such as richness, abundance, assemblage structure and functional group composition ought to be a useful tool for assessing and monitoring stream and groundwater ecosystem health and function.

While local and regional taxonomic differences limit comparisons of subsurface assemblages at generic or species level, classifications based on habitat affinities provide a ‘common currency’ that facilitates understanding of parallels and contrasts among different locations, as well as spatio-temporal changes within locations (Claret et al. 1999). Hyporheic invertebrate assemblages (hyporheos) and aquifer invertebrate assemblages (stygofauna) comprise a mixture of taxa with varying functional affinities to surface (epigean) and groundwater (hypogean) habitats respectively (Figure 63). Specialised stygofauna (stygophiles and stygobites) are taxa that, respectively, have an obligate or facultative, dependence on groundwater. They are universally characterized by the possession of conservative and convergent morphological characters that are adaptive for subterranean life (termed stygomorphic characters), such as reduced pigment and eyes and elongation of appendages. Stygoxenes are taxa that have specializations for life in surface waters, and they are typically pigmented and have functional eyes. Changes in conditions in subsurface habitats (e.g. through groundwater extraction and sedimentation) can alter the proportions of these categories, providing a potential indicator of subsurface community dynamics (Claret et al. 1999).

In the Maules Creek catchment it was observed that invertebrate abundance and richness decreased dramatically as conditions became more hypoxic, and invertebrates almost disappeared completely when streambed redox hydrochemistry suggested anoxic conditions. Anoxic conditions were reliably predicted by an increase in dissolved reduced iron (ferrous iron: Fe^{2+}). Because specialised groundwater invertebrates are tolerant of low DO concentrations (DO < 0.5 mg/L) (Malard and Hervant 1999, Hahn 2006) including concentrations below most field instrument detection limits (ca. < 0.2 mg/L), we suggest that ferrous iron may be a reliable indicator of completely anoxic conditions and therefore unsuitable habitat for invertebrates. As redox-sequences are well described globally, for a range of saturated subsurface aquatic environments (streams, estuaries, lakebeds – Appelo and Postma 2005), we expect that our observations in relation to ferrous iron and anoxia have general transferability. Nonetheless we recommend further field studies to test the wider applicability of our observed associations between ferrous iron and groundwater invertebrate richness and abundance.

6.2.3.5 Surface macroinvertebrates and fish

The transferability and scaling of information collected for surface biotic assemblages is limited by the spatial and temporal extent of the sampling effort. For example, variation in fish and invertebrate assemblage composition among study sites in both the upper Bremer River and Maules Creek catchments was influenced by differences in the short and longer-term flow-regime characteristics: therefore, a comparison to other datasets would require a similar level of understanding of longer-term flow-regime characteristics as well as differences in shorter-term antecedent conditions. It can therefore be difficult to interpret compositional datasets, as recent flow history may in some cases have an over-riding influence, thus masking longer-term differences. Furthermore, the transferability of many indicator scores generated from macroinvertebrate assemblage datasets, such as the SIGNAL score, requires caution because they typically show values indicative of impairment (i.e. anthropogenic disturbance) when calculated for intermittent streams. From prior studies a more suitable approach to developing transferable relationships for fish and invertebrates may be to exploit large databases to develop statistical models of likelihood of occurrence based on average hydrologic conditions, and to use those models to predict the effects from modelled hydrologic changes.

6.3 Monitoring and assessment

6.3.1 Key principles for effective monitoring

Monitoring can be useful for detecting trends in ecological condition, identifying emerging threats, testing hypotheses, and evaluating the efficacy of management interventions (Orians and Soule 2001). It is important however, to distinguish between different types of monitoring programs for environmental management, namely targeted (or focused) monitoring and surveillance monitoring (Nichols and Williams 2006). Targeted monitoring is defined by its integration into management practice, with monitoring design and implementation based on a-priori hypotheses and associated models of system responses to management. Surveillance monitoring on the other hand, is not guided by a-priori hypotheses and corresponding models and risks an inefficient use of funds and effort (Nichols and Williams 2006).

Monitoring is a key component of adaptive management and can help management agencies make decisions. Unfortunately, however, monitoring practices have generally been poorly connected with decision making, and this disconnection has led to an inability to assess the effectiveness or efficiency of the management actions (Field et al. 2007). As part of the environmental assessment for a new CSG or large coal mining development proposal, proponents are required, as a condition of project approval, to develop a monitoring plan that details how they will assess performance of proposed measures to mitigate potential impacts of the development (IESC 2015). In this context, clear articulation of the monitoring programs' goals is critical to its success. Monitoring goals relating to groundwater drawdown, changes to subsurface and surface flows, and other threats associated with CSG extraction or large coal mining may include (but are not limited to):

- maintaining the ecological or conservation values in priority areas identified as being at risk from impacts (e.g. Component 4),
- identifying threatening processes that might pose risks to ecological or conservation values,
- assessing responses to management interventions intended to protect or restore ecological or conservation values or mitigate threats to those values.

The choice of candidate monitoring indicators will vary depending on the goal of the monitoring program, as will constraints to implementation imposed by available budget, human resources and technical capacity (see next section).

The design of an effective monitoring program should ideally follow a series of systematic steps (Possingham et al. 2001):

1. Specify goals
2. Identify stressors
3. Develop conceptual models
4. Select indicators
5. Establish sampling design
6. Define methods of analysis
7. Ensure links with decision making

An explicit statement of the goals of the monitoring program is essential and may include goals relating to detecting trends, identifying emerging problems, testing hypotheses, or evaluating the efficacy of particular management interventions. A conceptual model describing the role of natural and human-induced stressors and ecosystem responses provides an overview of scientific understanding of how the system works and is useful to determine what measures of system performance are likely to be useful monitoring indicators. For monitoring programs to be useful they must be informative, reliable and be linked with decision-making objectives in a cost-efficient manner (i.e. in an adaptive management framework).

Active adaptive management places an explicit value on learning about the effectiveness of management by monitoring its outcomes and differs from passive adaptive management in which learning occurs serendipitously and is then incorporated into management plans (McCarthy and Possingham 2007). Monitoring programs designed and embedded in an active adaptive management framework offer the best chances of effectively detecting negative impacts on water resources and water-dependent assets. These can then be targeted for effective management and mitigation and to reduce the uncertainty of predicted impacts (IESC 2015).

6.3.2 Monitoring indicators

The design of a monitoring program requires careful consideration of candidate species (or species assemblages), ecosystem processes and/or threats for measurement, as well as possible confounding variables. Selection of indicators should therefore be informed by the overall goals and underlying conceptual models of the ecosystem, as well as constraints on their implementation (Possingham et al. 2001). Some candidate indicators are likely to be more or less responsive to the key drivers of interest (e.g. responses to threats or management interventions), vary in their ease and or cost of data collection and interpretation, and differ in their sensitivity to other factors (e.g. natural or unrelated factors that influence spatio-temporal variation in the indicator) that may confound or obscure their interpretation.

One example of this issue in our study was the potential confounding effect of livestock access to the study streams and/or the catchment upstream. Livestock have a detrimental effect on streams (Armour et al. 2001) in many ways including: a) eroding channel banks, thus increasing sedimentation in streams; b) defecating and urinating in the wetted channel, leading to altered carbon inputs and elevated in-stream concentrations of dissolved and organic nutrients; and, c) spreading non-native plants and pathogens. All these factors can influence stream ecosystem structure and function, and are considered a confounding factor when investigating ecosystem responses to reduced baseflow. However, in many instances, these confounding factors can be assessed, or accounted for, during monitoring or in experiments. For example, nearby streams impacted and not impacted on by livestock can be incorporated into experimental designs in order to quantify livestock impact and account for these differences. In the present study, however, it is clear that changes in surface water persistence was the primary mechanism causing changes in many of the structural and functional response variables assessed. This notion is supported by the fact that most ecological response variables responded most to changes in substrate moisture rather than those parameters altered by livestock access (i.e. dissolved and organic nutrient concentrations).

Indicators can also differ in their ability to integrate processes and impacts that are occurring over different spatial and temporal scales. For example, water quality can degrade and recover almost instantaneously in response to local point-source pollution inputs, but such impacts may be reflected in invertebrate assemblages days or weeks after a pollution event. Monitoring and assessment of aquatic ecosystem health traditionally focuses on changes in structural indicators, such as physico-chemistry or species assemblage composition. However, streams and rivers also have functional components, whose rates, patterns, and relative importance can offer a greater level of understanding of ecosystem responses to perturbations. Increasingly, research advocates the use of both structural and functional measures for the assessment of monitoring of streams and rivers, because stressors may cause changes in ecosystem function but not structure, and vice versa (Gessner and Chauvet, 2002; Young et al., 2008).

Below we highlight ways in which some of our findings and research methods offer potential for monitoring impacts of water-related threats associated with CSG extraction and coal mining.

6.3.2.1 Hydrochemistry

In Maules Creek catchment existing infrastructure allowed for sampling from a range of monitoring bores and hence the regional groundwater chemistry could be characterised. In addition to being able to sample different depths in the hyporheic zone, our sampling regime enabled understanding of redox processes that were occurring as a function of hydrologic regime and in some cases hyporheic flow direction. For the Bremer River catchment however, the absence of nearby groundwater bores meant that the regional groundwater chemistry could not be characterised. Therefore, groundwater characterisation (through bore sampling or use of existing groundwater databases if available) is an important step in evaluating the potential linkages between regional aquifers and adjacent hyporheic zones.

For this project, paired fauna and hydrochemistry sampling was conducted, as opposed to separate collection (time and site) of fauna and hydrochemistry samples for each location. The advantage of paired fauna and hydrochemistry sampling is strengthened interpretation of the associations between biotic and abiotic variables. Also, physico-chemistry parameters (DO, EC, pH) were measured in the field with an in-line flow cell. This setup ensures the accuracy of the measurements by minimising any interaction with the atmosphere, which is of particular importance for obtaining accurate low DO measurements.

As mentioned previously, our results indicated that the presence of ferrous iron was a better predictor of the absence of fauna (or very low abundance) than DO depletion. This finding indicates that field testing for ferrous iron using a ferrozine-based assay could be used as a screening test for the likely absence of fauna in a collected sample.

6.3.2.2 Cotton strip assays and leaf litter incubations

Ecosystem process-based methods, such as cotton strip assays and leaf litter incubations, are ideal for detecting natural and anthropogenic disturbances, as well as for monitoring spatial and temporal trends in ecosystem functioning, because they integrate the activity of many taxa and are often primarily affected by the physicochemical changes that result from perturbations. In this research, both cotton strip assays and leaf litter incubations were ideal for assessing spatial and temporal patterns in ecosystem functioning in response to baseflow reductions and drying conditions. Therefore, they are recommended for assessing ecological impacts associated with anthropogenic perturbations in freshwater environments. Cotton strip assays (cellulose decomposition potential) have been advocated for freshwater monitoring purposes because they are economical, easy to deploy, quantify the decomposition of naturally occurring cellulose (a major component of leaves), and use a standard cloth substrate (less chemical variability than leaf litter) that results in less within-site variability and thus greater power to detect spatial and temporal patterns in ecosystem functioning (Boulton and Quinn 2000, Young et al. 2008, Tiegs et al. 2013). Despite variation in composition and hence breakdown rates in leaves within a single species, leaf litter incubations are a common technique for assessing ecosystem functioning in streams because leaves can be an important food resource that fuels many aquatic food webs. Young et al. (2008), however, suggest that indicators of ecosystem function should be complementary to traditional measures of ecosystem structure, because measurements of both structural and functional attributes provide a more holistic view of ecosystem health.

6.3.2.3 Stable isotope analyses

While stable isotope analyses are appropriate for investigating connections between organisms and their food sources in streams, sample collection is time consuming and laboratory preparation and analysis are relatively expensive. Therefore, we recommend stable isotope analyses be used only to characterise differences in food webs among distinct flow regime types (i.e. intermittent versus perennial) and/or following press disturbances (i.e. long-lived anthropogenic perturbations). We do not recommend stable isotopes analyses for regular monitoring purposes.

6.3.2.4 Groundwater-dependent vegetation

We suggest a simple and rapid method for the assessment of groundwater-dependent vegetation based on a number of relatively easily observable factors including landscape position, climate, soil, geology, groundwater depth and proportion of plant hydrotypes within a plant community. However, the degree of certainty will increase with the level of knowledge of plant traits and physiology, such as root morphology, xylem anatomy, water use efficiency and productivity. As a first step in assessment we provide a decision tree for broadly categorising plants within a community into hydrotype functional groups, and identifying potential groundwater-dependent plants. Caveats on the development of a plant hydrotypes typology are the requirement for good taxonomic and ecological knowledge of species, as well as information on ecophysiological traits and responses. However, the typology developed here can work as a general guide to categories of hydrological plant types in a continuum of groundwater dependence at the plant community level. Our approach of developing a typology of plant hydrotypes can be used as part of a field assessment of plant community vulnerability to changes in hydrology such as groundwater drawdown. The development of a typology of plant hydrotypes will assist in assessing potential ecological impacts of changes to groundwater availability, as measuring the proportion of different hydrotypes within a habitat will provide an indication of the degree of reliance on groundwater. Furthermore, detected changes over time in the proportion of different hydrotypes in a community will indicate possible changes in the hydrological regime (e.g. groundwater drawdown) operating within that area.

Some of the methods used here are more suitable for long-term measurement of plant dependency on groundwater such as water stable isotope analysis and tree dendrometers. Also, specialist equipment and knowledge are required to analyse and interpret results. These methods are therefore probably not appropriate as direct management tools for environmental impact assessment. To assess potential groundwater use by trees we suggest that ideally sources of tree water use must be assessed over several different seasons and years. Clearly this is rarely practical for environmental impact assessments and a possible compromise is to substitute space for time, so that assessment of groundwater use by trees can be done at different locations with a gradient of groundwater depths. Although measuring groundwater use of a plant species at different groundwater depths is possible and will provide a range of depths at which this species can access groundwater, it will not provide information on the effects of the rate of groundwater change. To assess plant species resilience to rapid and long-term groundwater drawdown would require the setting up of groundwater drawdown experiments.

The abundance of the naturally occurring stable isotopes ^{18}O and ^{2}H can also be used to represent the relative importance of groundwater as a plant water source in different hydrological conditions (Dawson and Ehleringer, 1991, Adams and Grierson, 2001). Assessing the importance of groundwater for plants can be done by measuring spatial variability of the isotope ratios $\delta^{18}O$ and $\delta^{2}H$ in plant xylem water, soil water and groundwater. As for food web analysis, it can be a relatively time consuming and costly method. Hydrogen and oxygen isotopes can also be used to measure the relative importance of groundwater in maintaining stream pools.

Remote sensing analysis of vegetation condition using such measures as Normalised Difference Vegetation Index (NDVI) and Normalised Difference Wetness Index (NDWI) can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation. Small-scale on-ground data can also be used to ‘ground-truth’ these remotely sensed data. The combination of on-ground studies and remote sensing will provide a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales.

The development and application of a web-based toolset to support the analyses provided in this report (see Section 7.1, Appendix 1 for full description) can enable non-specialists to remotely assess vegetation condition. This toolset adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. It is implemented in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications. The developed GEE-CSIRO-GDV tools include:

Tool 1 - creating image thumbnails for image quality control;
Tool 2 - identification of dates of cloud-free Landsat imagery from the GEE remote sensing database and generation of cloud free NDVI and NDWI data cubes covering the selected time periods;
Tool 3 - NDVI and NDWI time series extraction at selected point locations;
Tool 4 - Principal Component Analysis of multi-temporal NDVI and NDWI datasets;
Tool 5 - Multiple Linear Regression Analysis to model relationships between NDVI temporal profiles at each NDVI grid pixel location and local climate parameters (e.g. temperature, rainfall).

6.3.2.5 Hyporheic fauna

The paucity of hyporheic ecology studies in Australia means there is little existing baseline or benchmark information against which to plan monitoring studies and interpret the results. However, the methods are well established and published in numerous studies conducted in Europe and North America (e.g. Gibert et al. 1994). The inherent heterogeneity in hyporheic habitats and localized high variability in hyporheos assemblages means that surveys and monitoring need to ensure adequate spatio-temporal replication within and across sites, supported by pilot studies and progressive, iterative evaluation of results along the way. We advocate a flexible and adaptive approach that is responsive to site-specific conditions and context, rather than a prescribed survey effort. Good practice would require that each location is assessed on a case-by-case basis, and initial desktop studies are validated by field study. For data analysis, non-parametric permutational analysis of multivariate data is appropriate for typically highly variable and patchy invertebrate abundance and distribution patterns, and complements parametric treatment of univariate indices such as richness and abundance. Evaluation of sampling adequacy and completeness during surveys and monitoring is effectively undertaken using taxon accumulation curves, and the various commonly used estimators of total species richness (e.g. Colwell et al. 2004).

6.3.2.6 Surface macroinvertebrates and fish

Fish and macroinvertebrate sampling are traditional measures of aquatic ecosystem condition. The use of these indicators has been based on their demonstrated success in detecting a range of human impacts, especially those resulting from pollution, catchment land-use change, and flow reductions. However, in spite of the demonstrable sensitivity of invertebrate assemblages to land-use change in many regions, the use of invertebrates as indicators in intermittent and ephemeral streams has proved problematic (Sheldon 2005). Two particular issues are that: a) the often harsh environmental conditions during the dry phase in intermittent streams can mean that the fauna is typically dominated by those same types of taxa that can also cope with the physio-chemical changes often caused by human impacts; and, b) the high temporal variability of intermittent and ephemeral streams means that defining an appropriate reference condition can be extremely difficult. A number of studies have evaluated the ability of standard invertebrate biomonitoring approaches applied to intermittent streams, and found them to be inadequate to differentiate impaired sites (e.g. Chessman et al. 2006, 2010). It is thus perhaps not surprising that we could not detect clear patterns in macroinvertebrate and fish assemblages in relation to short-term hydrological changes in the current study.

One alternative approach to using fish and invertebrate datasets would be to use model-based predictions in the planning phase of assessing potential ecological impacts. For example, despite considerable short-term variation in abundance within individual sites, over the long-term the distribution and abundance of both fish and invertebrates are often strongly related to the degree of flow permanence (Bond et al. 2011, 2012), and such patterns can (on average) be quite predictable. Our findings support this notion, with fish and macroinvertebrate assemblage structure being influenced by longer-term discharge characteristics. Thus, rather than seeking to test for impacts from altered hydrology, an alternative approach would be to use models built using existing large-scale datasets to set limits to hydrologic alteration, and to focus investment on monitoring and modelling efforts to better understand the likely hydrologic impacts that may arise from CSG and coal mining developments. Such approaches have been advocated for setting environmental flow targets for regulated rivers (e.g. Poff et al. 2010) but in fact may be more appropriate for unregulated rivers subjected to broad-scale water withdrawals where the patterns of flow change may be far less complex than in regulated systems where flows can be manipulated via reservoir releases.

6.4 Key project findings

Here we consolidate the key findings from each project component.

A key step in developing suitable experimental approaches for this multi-disciplinary project was through the creation and revision of a series of conceptual models. In addition to helping formalize the experimental design, we found that reviewing and refining those conceptual models at key stages in the project to be an efficient way to consolidate our collective findings.

Component 1 – Ecohydrology of groundwater dependent terrestrial vegetation. (Short title: Groundwater dependent vegetation).

- We developed a typology of plant functional groups from the international literature that characterised plant communities as a mix of hydrotypes indicative of hydrological requirements and associated with landscape position. We then applied this typology to plant communities from the study sites at Maules Creek and the Bremer River. The typology provides us with a convenient first step in identifying the water sources important to maintaining vegetation and the potential for groundwater dependence for EIA. Using this typology, it is possible to predict, for management purposes, the importance of groundwater to a given plant community. The typology can be based on a number of somewhat easily observable factors including landscape position, climate, soil, geology, groundwater depth and proportion of plant hydrotypes within a plant community.

- We provide a decision tree for broadly categorising plants within a community into hydrotype functional groups, and identifying potential phreatophytes. The decision tree uses life history traits (annual or perennials) and life form (woody or herbaceous) as well as habitat, depth to groundwater, duration of inundation and drought adaptation to identify the different hydrotypes. Consequently, this type of approach can be used to assess potential ecological impacts of groundwater drawdown as a result of coal seam gas (CSG) and coal mining developments in a variety of biophysical settings.

- Our results indicate the importance of antecedent weather (particularly rainfall) and hydrological conditions to the ecohydrological state of the trees and their water sources. Our dendrometer study of red gum trees in different landscape positions suggests that sites with shallow groundwater for most of the year had greater growth rates over the year and larger daily stem changes indicating greater short-term growth rates. Although these trees have responded to rainfall events (and corresponding flow), they show less response to drought periods, suggesting they are less dependent on rainfall and may also be accessing groundwater continuously. Riparian trees at the intermittent site, where groundwater is seasonally deeper, may also be accessing groundwater at some periods during the year. Stable isotope analysis provided further evidence that there were strong links to groundwater for trees in the riparian area of Maules Creek at the site of a perennial pool as well for trees on the nearby floodplain.

- Plants using groundwater (phreatophytes) are generally restricted to environments with permanent groundwater supplies within a depth accessible to plant roots. Therefore, phreatophytes can occur in most habitats where groundwater is shallow enough for roots to reach and plants opportunistically use groundwater to a greater or lesser extent when the energetic balance is favourable to extracting deeper soil water at the capillary fringe of the water table, rather than from the soil water. It is generally assumed therefore that if plants can access groundwater then some plants within the ecosystem will develop some degree of dependence. Therefore, phreatophytic behaviour seems to be more related to the prevailing environmental conditions than to the capabilities of a given plant species.

- Remote sensing analysis indicated that riparian and floodplain vegetation in the study area is likely to have access to groundwater, though at various depths: more than 8m below ground level (BGL) in the north-west and within a few meters below ground level in the south-east. Decadal variability of rainfall in the study area led to some reduction in vegetation greenness during the particularly dry periods (such as during the Millennium Drought of mid 2000s). During this time the groundwater levels also dropped, in some bores up to 4 m. However, both groundwater levels and vegetation greenness recovered in the wetter periods following the droughts. This recovery is likely to indicate that vegetation are somewhat resilient to the decadal variability of the climatic conditions and variation in water availability associated with it.

- This remote sensing analysis can assist in the development of a baseline for investigation of the potential impacts of groundwater drawdown from mining activities on vegetation. Small-scale on-ground data can also be used to 'ground-truth' remotely sensed data. The combination of on-ground studies and remote sensing will provide a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales.

- We also developed and applied a web-based toolset to support the analyses (see Section 7.1, Appendix 1 for full description). This toolset adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. It was implemented in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications.

Component 2 – Ecological values of baseflow and surface water-groundwater connectivity regimes in non-perennial streams. (Short title: Baseflows).

- Intermittent river systems are important locations for detrital processes and the hyporheic zone sustains critical ecosystem processes even when surface flow-paths cease.

- Short-term and long-term reductions in flow may affect the availability and nutritional quality of important sources of energy for aquatic food webs. Given that algae require wetted environments, extended periods of reduced flow (and cease-to-flow events) may reduce the availability of preferred energy sources for consumers, leading to less productive food webs.

- The intermittent streams studied contained diverse biotic assemblages, and continual subsurface baseflow appears to be instrumental in sustaining these assemblages. This finding challenges the commonly held assumption that intermittent rivers and ephemeral streams do not contribute to local and regional aquatic biodiversity.

- Longer-term patterns in flow variability are an important predictor of fish assemblage structure. Streams with a lower discharge will likely be more intermittent and have low wetted habitat persistence and longitudinal connectivity, which may act as a broad-scale filter in determining fish species composition and assemblage structure. However, groundwater contributions to baseflow may play an important role in sustaining fish populations by buffering against periods of flow cessation by maintaining residual wetted habitats.

Component 3 – Hydrogeochemistry, biogeochemical processes and the hyporheos / stygofauna (Short title: Subsurface ecology).

- Baseflow is important. Groundwater is essential for sustaining baseflow, hydroecological processes and ecosystem structure and function in surface and subsurface (hyporheic) habitats in both intermittent and perennial streams.

- Hyporheic exchange flows are important. The hyporheic zone plays an important role in hydroecological processes and stream ecosystem functioning in both intermittent and perennial streams.

- Groundwater recession (drawdown) is a stressor. Groundwater recession/drawdown reduces baseflow and changes hyporheic exchange flows, which may deplete ecological values and the hydroecological functioning of streams and associated groundwater-dependent ecosystems (GDEs). The very narrow vertical range (generally < 1 to 2 m depth) of the hyporheic zone means that only small amounts of groundwater drawdown may have a significant impact on the ecological functioning and baseflow connectivity of streams.

- Ecosystem assets and services that are potentially depleted by groundwater drawdown include biodiversity, detrital processes, and important biogeochemical processes mediated by microbes such as carbon and nutrient transformations.

- Conceptual models were useful for integrating and synthesizing the multidisciplinary findings to aid understanding of hydroecological responses in streams due to changes in groundwater regime.

The additional key findings below are context-specific to the case studies at Maules Creek and Bremer River, and are expanded and generalised, with precautionary inferences, where possible.

- Intermittent streams are ecologically important. Intermittent streams with a groundwater baseflow component harbor a rich biodiversity in both surface and subsurface (hyporheic) habitats.

- Even when surface flow has ceased, important ecosystem processes still occur in the subsurface (hyporheic) zone of intermittent streams.

- Obligate groundwater invertebrates (stygobites) are an ecological functional group that are ‘signature’ indicators of baseflow connectivity between streams and permanent groundwater (aquifers), and they respond to changes in groundwater condition and quality (DO and redox state), hyporheic exchange and stream flow regime.

- Streams with only a thin alluvial cover and hence shallow hyporheic / groundwater habitat may harbor a rich hyporheos. At Bremer River, we found a rich hyporheos inhabiting thin veneers of saturated alluvium (< 1m) overlying relatively impermeable and uninhabitable strata (clay or tight bedrock). In contrast at Maules Creek, the hyporheic habitat is comparatively deep (> 10m) alluvium. The shallow hyporheic habitats are likely to be more sensitive and vulnerable to changes in groundwater levels.

- Groundwater (baseflow) connectivity confers resistance and resilience in stream ecosystems, but resistance and resilience is dependent on the nature, context and regime of stream, baseflow and aquifer connectivity. At Maules Creek, compared with Bremer River, enhanced resistance and resilience is inferred from the deep alluvium and strong baseflow inputs of oxic regional groundwater.

Component 4 – Biodiversity and risk assessment of Great Artesian Basin spring wetlands (Short title: GAB springs).

Biodiversity assessment of GAB springs

- Describing and revealing undocumented diversity will help to preserve its evolutionary potential

Taxonomy is key to conservation of GAB spring flora and fauna. Numerous species are collected and await description. Whole phyla remain unexplored. Understanding the distribution and ecology of endemic taxa cannot progress until species are described. For conservation of GAB springs to progress, efforts need to be made to ensure taxonomists are supported. Effective conservation also needs to embrace the fact that diversity is not fixed – species have diverged and will continue to diverge in springs as geographical isolation and environmental change shape their evolution. Conserving biodiversity and understanding species will be complicated if cryptic species complexes are present but remain unidentified. Therefore, understanding species is not only about diversity across taxa, but also within them.

- Update conservation listing

This review has highlighted that GAB-dependent species that are listed individually as well as being listed as part of the 'threatened community' are the focus of more dedicated conservation attention and efforts than those species that are not presently listed. Our study highlights additional taxa belonging to the 'community of native species dependent on natural discharge of groundwater from the GAB'. There is a scientific basis for an assessment and re-evaluation of all taxa (in addition to those considered here but excluding plants reviewed previously by Silcock et al. 2011) using EPBC and IUCN guidelines for consideration by the Threatened Species Scientific Committee. This assessment was beyond the scope of our study but should now be readily achievable for some taxon groups (particularly the invertebrates).

- Broaden the focus to other organisms and increase the spatial resolution of the assessments

The data we have regarding endemic GAB species are focussed on particular groups of taxa and their complex-scale distributions. Finer-resolution distribution data (i.e. springs within complexes) for a broader range of taxon groups is required for effective conservation management of springs and their resident biodiversity. There are also uneven levels of knowledge available on the ecology and conservation of taxon groups in different parts of the GAB (see Component 4 for further details). This review has highlighted types of data we currently lack, and the locations and species for which this data is most urgently needed. It has also highlighted that such efforts need not be dispersed across the entire basin, but can be focussed upon biodiversity 'hot-spots'. Emphasis needs to be placed on prioritising research that fills these knowledge gaps.

- Improve collaboration across research effort

For organisms or complexes where research programs currently exist, the types of questions being asked are contingent on the interests of the research group. In the Lake Eyre complexes, molecular biologists are providing detailed information about species boundaries and population connectivity but there is little ecological data to complement these models. The opposite can be said for the northern complexes, where conservation, diversity and biogeography are a focus. This research divide extends beyond biology, with those that study the organisms that live in the surface manifestation of springs and those that study the hydrodynamics of the GAB. Likewise, those assessing potential impacts on springs are not necessarily engaging with taxonomists or ecologists with the expertise to identify potential new species, comment on sampling designs or provide comment on the ecological mechanisms behind biodiversity patterns. This lack of collaboration means surveys of spring complexes may be overlooking diversity. It also means we still cannot answer vital questions about the relationship between groundwater pressure within the GAB and the size of springs or the species that occupy them. Collaborations across on-ground managers, consultants and research groups with interest in springs need to be fostered and will facilitate identification of knowledge gaps and research priorities.

- Focus on the ecology of species and their responses to threats

Information regarding the ecology of GAB spring endemic species, their potential responses to threats, and even variation in the nature and intensity of threats themselves, are the most deficient. Even basic information regarding abundance, environmental requirements (beyond the need for GAB fed springs), the effects of introduced species, or changes in spring flow is deficient for most species, even though these threats have been emphasised as major threats to the system since its conservation listing. This lack of information limits our ability to prioritise species for conservation or manage species persistence. Endemic GAB spring taxa have restricted distributions, have very few populations within protected areas, and may have high exposure to threats meaning they may be at substantial risk from the cumulative impacts of a range of threats. The lack of data, the size of the GAB, and the diversity of species in question, has previously precluded efforts to systematically evaluate threat exposure to endemic species, understand species' vulnerability to threatening processes and prioritiese risks.

Cumulative risk assessment of GAB springs

- Some GAB springs and their endemic species at clearly at high risk – strategic, coordinated, inclusive and committed management is urgently required to address current and future threats

Evidence from past practices reveals that unrestricted and unmanaged access to groundwater (through bore extraction) and other threats led to a high proportion of springs to go extinct. Many of these historical impacts were concentrated in particular areas that still contain highly restricted endemic taxa (i.e. to a single spring), so it is extremely likely that undocumented biodiversity has already been driven to extinction. Our cumulative threat exposure analysis and risk assessment results further highlight the urgency of the problem, given the continued, widespread, and pervasive threats that springs are currently exposed and the likelihood of these threats being exacerbated by climate change and expansion of the coal seam gas and coal mining industry.

Threat management is required at different tiers with different stakeholders. For example, feral animals can be easily managed by on-ground practices like fencing, trapping and shooting. In contrast, groundwater drawdown is a basin-wide problem that needs national leadership and management to complement state-based and regional initiatives because losses in one place can lead to drawdown in another. Some localities stand as testament to the ability of multiple stake-holders to work together to preserve 'hot-spots' of biodiversity in this system (e.g. the Edgbaston portion of the Pelican Creek complex). However, this report emphasises that such activities need to be fostered elsewhere and provides a basin-wide perspective for facilitating this process.

On-ground action to mitigate threats cannot be effective without complementary efforts to protect from further losses of groundwater GAB pressure. A number of initiatives currently exist to facilitate more effective and strategic management, including the Great Artesian Basin Sustainability Initiative (GABSI), the Great Artesian Basin Strategic Management Planning process currently underway involving federal and state/territory governments and the Great Artesian Basin Coordinating Committee (GABCC).

- Science is needed to establish ecological responses to threats and benefits of actions

This review has highlighted that quantitative data is lacking concerning population trends, responses to threats and benefits of management actions to mitigate those threats for most GAB endemic species. Targeted monitoring of population trends is needed in order to understand natural temporal variation within spring systems and populations within springs act as metapopulations that shift across the landscape. Targeted monitoring can in turn inform managers on how species are or are likely to be responding to environmental changes and highlight conservation concerns that would require management actions. Examples of ongoing monitoring programs exist in some South Australian springs and at Edgbaston springs. However these programs concern only two of 33 unique 'assemblages' of species that exist in GAB springs. Fostering a basin-wide approach to monitoring that ensures that biodiversity 'hot-spots' and localities with the most pertinent exposure to threats will help to expand on these past successes and fill knowledge gaps. Our expert elicitation revealed that most species are highly vulnerable to groundwater drawdown. However, we lack precise evidence of actual species responses and how cumulative interacting threats may exacerbate vulnerability. Some of these knowledge gaps are relative straightforward to address, at least for some species and threats (e.g. using experimental mesocosms to simulate water drawdown and recovery and monitoring ecological responses such as physiology and behaviour). These questions come hand-in-hand with research regarding the ecology of endemic GAB species – another literature gap at a basin-wide scale.

Monitoring programs are not only important for management; they provide knowledge that is useful to both academic science and community conservation. Long-term monitoring programs in Lake Eyre have facilitated cutting-edge research into the evolutionary origins and patterns of population connectivity at landscape scales. They have allowed ecologists and taxonomists to officially describe over 50 species that prior to the 1980's were unknown to science, and to continue to identify at least 50 more. These species are ecologically and taxonomically diverse – some have transitioned from rainforest creek-dwelling species to arid-zone spring specialists others have colonised the surface after persisting within underground aquifers. By understanding them, researchers have been able to document the quaternary changes in Australia's climate, document speciation and document the adaptations needed to persist in the Australian arid-zone. This in turn provides information and understanding about species that increases the concern of the general public for their conservation (e.g. 'friends of the mound springs' are a voluntary organisation that conserve South Australian mound springs, ~20 individuals volunteer every year to aid in conserving the red-finned blue-eye, and numerous property owners manage populations of threatened species or have entered into voluntary conservation agreements). Greater understanding and awareness also facilitates community learning and cultural well-being (e.g. undergraduate science students from the University of Queensland facilitate annual invertebrate sampling at Edgbaston and Indigenous Custodians are strongly involved in the management of Witjera and Finniss Springs in South Australia).

- Many on-ground management actions to mitigate threats can be implemented now

The high concentration of endemic species in relatively small areas means that it should be feasible to identify appropriate threat management strategies and successfully implement them (notwithstanding the challenges identified above). Our research identified a small set of highest risk spring complexes that could be prioritised for targeted management in the short term. So many springs of conservation importance are on private property (freehold and leasehold) and many threats are localised and can be relatively easily managed at a local scale. Engaging and incentivising private property owners about values of their own springs and the need to protect and monitor them would be a major leap forward in this regard.

6.5 Project outcomes

The expected outcomes from the project are:

- improved identification and understanding of the potential water-related ecological responses to coal seam gas extraction and coal mining in Australia.
- information that will strengthen IESC advice on project proposals concerning the adequacy of methods of prediction, assessment and mitigation of impacts on:
    - subsurface biogeochemistry and water quality,
    - groundwater dependent vegetation,
    - ecological values of non-perennial streams (including ecosystem processes, and hyporehic and surface fauna),
    - GAB springs and the biodiversity they sustain.
- strengthened regulator and industry understanding of:
    - the water-related requirements of, and impacts on, groundwater dependent vegetation

  - the ecological importance of groundwater discharge and surface water-groundwater connectivity regimes in non-perennial streams,
  - the ecological relevance of subsurface fauna and biogeochemistry,
  - the biodiversity and conservation values of GAB spring wetlands and the vulnerability of resident taxa to hydrological changes and other threats.
- research that complements the bioregional assessments and other research activities coordinated by OWS that will collectively improve the knowledge base regarding the potential water-related impacts of CSG extraction and coal mining,
- a rigorous foundation for future research to fill critical research gaps,
- improved capacity to evaluate relative ecological and biodiversity values of an area subject to proposed mining development compared with elsewhere,
- information for future updates to conservation listing of GAB discharge spring ecological community (and individual species) by DoE Threatened Species Scientific Committee.

6.6 References: Synthesis

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7 Appendices

7.1 Appendix 1: Component 1: Groundwater dependent vegetation supplementary material

7.1.1 Remote sensing report commissioned as part of this project

The full report can be accessed via CSIRO or by directly contacting the authors:

Emelyanova I., Barron O., Vleeshouwer J. and Bridgart R. (2016) Application of remote sensing techniques to support delineation and characterisation of groundwater dependent vegetation: Technical report. CSIRO, Australia.

Executive Summary

The reported research is contributing to the project “Research to inform the assessment of eco-hydrological responses to coal seam gas (CSG) extraction and coal mining” funded by the Office of Water Science (the Department of Environment) and particularly to Component 1 – “Eco-hydrology of groundwater dependent vegetation”, led by the Edith Cowan University, WA. This component aims to understand the impacts of altered groundwater regimes as a result of CSG and coal mining developments on groundwater-dependent vegetation (GDV) in different biophysical settings.

This component of the project uses remote sensing (RS) data to delineate GDVs and their dependency on various elements of the water regime in selected areas of New South Wales (NSW) and Queensland (QLD). The RS analysis includes identification of the determinants of vegetation resilience and recovery following altered groundwater regimes in different biophysical settings and at different scales.

Particular advantages of RS techniques include the availability of historical satellite datasets and the ability to monitor large areas at low cost. The combination of on-ground studies and remote sensing provides a critical linkage in understanding how plant communities respond to groundwater drawdown at the local and catchment scales.

The GDV’s relationship with water regime (and particularly with groundwater) results in distinct signatures in remotely sensed surface reflectance images, which differ spatially and/or temporally. Remotely sensed reflectance data allow analysis of ‘active greenness’ provided by the multispectral NDVI (Normalised Difference Vegetation Index) time series. Time series RS data are well suited for exploration of differences in the ‘phenology’, or cyclical growth rate, of terrestrial vegetation with and without access to groundwater. This analysis makes use of time series of NDVI and NDWI (Normalised Difference Wetness Index) as indicators of vegetation greenness and wetness patterns, respectively. In this study, we mainly used Landsat data, which has the longest record of data acquisition.

This report presents the outcomes of analysis undertaken within two selected study areas: the Maules Creek and Bremer River regions located in northern NSW and South East QLD, respectively. As more on-ground information was available for the Maules Creek study area, a wider range of analyses was carried out for this region. Some interpretations presented in this report were based on general knowledge of the eco-hydrological conditions in the region. More localised ecological analysis would add value to the reported outcomes.

Most of the identified remnant vegetation is associated with the riverine systems, including riparian and foodplain vegetation. In the Maules Creek study area, the depth to groundwater reduces from the north west to the south east. Following this pattern, the ephemeral Maules Creek becomes perennial, providing annual recharge to groundwater in the north west and being a gaining stream in the south east. As a result, all riparian and floodplain vegetation in the study area is likely to have access to groundwater, though at various depths: more than 8 mBGL in the north west and within a few metres BGL in the south east.

All analysis indicated that vegetation wetness (NDWI) is a more sensitive variable to spatiotemporal variation in groundwater levels than NDVI, likely due to a greater effect of depth to groundwater on evapotranspiration rates by plants than on their greenness.

Decadal variability of rainfall led to some reduction in vegetation greenness during particularly dry periods (such as during the Millennium Drought of mid 2000s). During this time groundwater levels also dropped, in some bores by up to 4 m. However, both groundwater levels and vegetation greenness recovered in the wetter periods following the droughts. This recovery suggests that the vegetation is well adapted to the decadal variability of climatic conditions and the variation in water availability associated with it.

Groundwater is locally used for irrigation, which has an effect on groundwater levels in the south west of the study area outside of the riverine systems. Seasonal changes in the groundwater level may be more than 7 m, and there is a long-term trend in the groundwater drawdown in this area. However, this trend has not had an impact on vegetation greenness within the study area.

In conclusion, decadal and seasonal variations in groundwater levels, even when induced by groundwater use for irrigation, did not have a detectable impact on remnant riverine vegetation. The lack of impact is likely to be due to position in the landscape of the vegetation (within groundwater discharge zone or localised groundwater discharge zone associated with the riverine systems). Unless groundwater abstraction, climate change or their combination lead to substantial changes surface and groundwater interaction processes (e.g. reversing streams from gaining to losing), seasonal river flow and regional groundwater discharge will provide a sufficient water source for vegetation within the riverine systems.

The main results for the two study areas can be summarized as follows.

1. Maules Creek study area:

- The spatial extent of remnant vegetation including riparian, floodplain and terrestrial was delineated. Seven types (classes) of remnant vegetation were defined by eco-hydrological conditions with a suite of spatiotemporal statistical techniques applied to multi-date Landsat imagery. These classes were perennial vegetation along perennial creeks, perennial vegetation along intermittent creeks, remnant floodplain vegetation within along perennial creeks, remnant floodplain vegetation along intermittent creeks, and terrestrial vegetation outside of the riverine systems.

- The NDWI range indicated that this index is more sensitive to variability in depth to groundwater than NDVI. However, both RS indices are substantially lower where groundwater is deep. Vegetation with access to groundwater (or permanent surface water) can be characterised by high intercept and low slope parameters in linear regression models describing relationships between vegetation greenness (NDVI) and wetness (NDWI).

- The mapped vegetation has greater productivity in the early winter (June-July) than in late spring–early summer (November-December). The greenness of remnant vegetation is more sensitive to annual than to concurrent meteorological conditions. Sensitivity to changes in vapour pressure deficit, maximum temperature, vapour pressure and pan evaporation is greater for vegetation associated with the riverine system.

- At a decadal scale, reduction in vegetation greenness and wetness was detected at the end of the prolonged dry periods (1992 to 1995, 2001 to 2006 and 2012 to the present time). After such dry periods, the NDVI and NDWI values recovered to their previous levels. Greenness of vegetation dependent on deeper groundwater was more sensitive to historical regional variability in groundwater levels. Changes in groundwater levels due to groundwater abstraction for irrigation did not affect remnant vegetation.

2. Bremer River study area:

- Five types (classes) of remnant vegetation were delineated and described in terms of their eco-hydrological conditions with a suite of spatiotemporal statistical techniques applied to multi-date Landsat imagery. These classes included riparian vegetation and terrestrial vegetation outside of the riverine systems.

- The mapped vegetation has greatest productivity in the late summer (February-March) and lowest in spring (September-October). This phenology differs from that in the Maules study area.

The research activities also included the development and application of a web-based toolset to support the analyses. This toolset adapted the methodology for GDV mapping (CSIRO-GDV), previously developed by CSIRO for the Pilbara Water Resources Assessment (PWRA) project. It was implemented in the Google Earth Engine (GEE) environment, an open source monitoring platform for development of web-based Earth observation applications.

Table 43: Location of the vegetation overstorey and understorey plots at the study sites on Maules Creek (NSW) and the Bremer River (Qld).

Maules Creek					**Bremer River**				
Site Code	**Stream Type**	**Landscape Position**	**Latitude (◦)**	**Longitude (◦)**	**Site Code**	**Stream Type**	**Landscape Position**	**Latitude (◦)**	**Longitude (◦)**
MCI_F1	Intermittent	Floodplain	30.4514	150.17119	BCCI_F2	Intermittent	Floodplain	28.0866	152.45766
MCI_F2	Intermittent	Floodplain	30.45132	150.17165	BWCI_F1	Intermittent	Floodplain	28.10538	152.51460
MCI_F3	Intermittent	Floodplain	30.45149	150.17215	BCCI_F3	Intermittent	Floodplain	28.08571	152.45796
MCI_F4	Intermittent	Floodplain	30.4942	150.11879	BWCI_F4	Intermittent	Floodplain	27.98433	152.44293
MCI_F5	Intermittent	Floodplain	30.49398	150.11816	BWCI_F5	Intermittent	Floodplain	27.98445	152.44469
MCI_R1	Intermittent	Riparian	30.45121	150.17560	BCCI_R4	Intermittent	Riparian	28.08681	152.45735
MCI_R2	Intermittent	Riparian	30.45183	150.17488	BUBI_R1	Intermittent	Riparian	27.94108	152.45604
MCI_R3	Intermittent	Riparian	30.45091	150.17569	BUBI_R2	Intermittent	Riparian	27.94185	152.45580
MCI_R4	Intermittent	Riparian	30.46519	150.16405	BWCI_R1	Intermittent	Riparian	28.10514	152.51430
MCI_R5	Intermittent	Riparian	30.49406	150.12006	BWCI_R2	Intermittent	Riparian	28.10593	152.51389
MCI_T1		Terrestrial	30.46638	150.16743	BWCI_R3	Intermittent	Riparian	28.10716	152.51290
MCI_T2		Terrestrial	30.46873	150.17416	BWRI_R1	Intermittent	Riparian	27.98488	152.44682
MCI_T3		Terrestrial	30.48102	150.12706	BCCI_R5	Intermittent	Riparian	28.08583	152.45781
MCI_T4		Terrestrial	30.47998	150.12794	BUBI_T1		Terrestrial	27.94578	152.45811
MCI_T5		Terrestrial	30.48037	150.12590	BUBI_T2		Terrestrial	27.94596	152.45715
MCP_F1	Perennial	Floodplain	30.48732	150.08434	BWCI_T1		Terrestrial	28.1035	152.51332
MCP_F2	Perennial	Floodplain	30.48712	150.08459	BR7_T1		Terrestrial	27.75654	152.44942
MCP_F3	Perennial	Floodplain	30.48684	150.08423	BR7_F1	Perennial	Floodplain	27.75778	152.45369
MCP_F4	Perennial	Floodplain	30.49223	150.08327	BRCP_F1	Perennial	Floodplain	28.10565	152.51677
MCP_F5	Perennial	Floodplain	30.49257	150.08369	BRCP_F3	Perennial	Floodplain	28.10265	152.51718
MCP_R1	Perennial	Riparian	30.49677	150.08112	BRCP_F4	Perennial	Floodplain	28.10041	152.51816
MCP_R2	Perennial	Riparian	30.49588	150.08295	BR7_R1	Perennial	Riparian	27.75778	152.45418
MCP_R3	Perennial	Riparian	30.49484	150.08441	BRCP_R1	Perennial	Riparian	28.10527	152.51790
MCP_R4	Perennial	Riparian	30.49752	150.07925	BRCP_R2	Perennial	Riparian	28.10635	152.51720
MCP_R5	Perennial	Riparian	30.4987	150.07637	BRCP_R3	Perennial	Riparian	28.10723	152.51613
					BRCP_R4	Perennial	Riparian	28.10265	152.51781
					BRCP_R5	Perennial	Riparian	28.10052	152.51782

Table 44: Species list of plants (including hydrotype, life form and landscape position categories) found in quadrats at the Maules Creek and Bremer River study sites (n = 27 and 25 sites, respectively).

Hydro-type[1]	Life Form[2]	Species	Sites[3]	Landscape position[4]	Hydro-type	Life Form	Species	Sites	Landscape position
-	ag*	*Bromus diandrus*	M	Rp	2	ph	*Adiantum hispidulum*	B, M	Ri Rp
-	ag*	*Bromus hordeaceus*	M	Rp	3	ph	*Ageratina adenophora*	B	Ri Rp
-	ag*	*Hordeum sp*	M	Ri	3	ph	*Arthropodium* sp	B, M	Fi Fp T
-	ag*	*Lolium sp*	M	Fi Fp Ri Rp	6	ph *	*Asclepias curassavica*	B	Fp Ri Rp T
-	ag*	*Anagallis avensis*	M	Ri	1	ph	*Azolla filiculoides*	B, M	Rp
-	ag*	*Argemone ochroleuca*	B	Ri	2	ph	*Bolboschoenus fluviatilis*	B, M	Fi Fp Ri Rp
-	ag*	*Aster subulatus*	B	Rp	2	ph	*Carex apressa*	B, M	Ri
-	ag*	*Bidens bipinnata*	M	Fi Ri	6	ph	*Harrisia* sp	M	Ri T
-	ag*	*Bidens pilosa*	B, M	Fi Fp Ri Rp	3	ph	*Commelina cyanea*	B, M	Fp Ri Rp
-	ag*	*Conyza bonariensis*	B, M	Fi Fp Ri Rp	5	ph *	*Ipomoea indica*	B	Fi
-	ag*	*Crinum* sp	B, M	Fi Fp Ri Rp T	2	ph	*Cyperus difformis*	B, M	Fi Fp Ri Rp
-	ah	*Asteraceae sp*	M	T	2	ph	*Cyperus exaltatus*	B, M	Fi Rp
2	ah	*Cyperus* sp1	B, M	Fi Fp Ri Rp	2	ph	*Cyperus vaginatus*	M	Rp
-	ah *	*Daucus glochidiatus*	B, M	Fp Ri	3	ph	*Dichondra repens*	M	Ri
-	ah *	*Echium plantagineum*	M	Ri Rp	2	ph	*Eleocharis acuta*	B, M	Ri Rp
-	ah *	*Galium aparine*	M	Fp Ri Rp	2	ph	*Fimbristylis nutans*	B	Rp
-	ah	*Gnaphalium luteo-album*	B, M	Fi Ri Rp	2	ph	*Fimbristylis* sp	B	Fi
-	ah *	*Hypochaeris glabra*	B, M	Fi Ri	6	ph*	*Gomphocarpus fruticosus*	B, M	Fi Fp Ri
2	ah	*Isolepsis sp*	B, M	Fp	2	ph	*Histiopteris incisa*	B	Ri Rp
-	ah *	*Leonotus nepetifolia*	M	Fi	2	ph	*Juncus* sp	B, M	Fp Ri Rp
-	ah	*Lepidium sp*	M	Fi Fp	2	ph	*Lindsaea incisa*	B	Ri
-	ah *	*Medicago polymorpha*	M	Fi Fp Rp	5	ph	*Lomandra longifolia*	B, M	Fi Fp Ri Rp
1	ah	*Najas tenuifolia*	B	Rp	6	ph	*Lomandra elongata*	M	Fp
-	ah	*Oxalis corniculatus*	B	Fi Fp Ri Rp	2	ph	*Ludwigia peploides*	M	Fp Rp
-	ah *	*Petrorhagia velutina*	M	Fi Ri	3	ph	*Marsdenia* sp	B	Ri
-	ah *	*Physalis sp*	B	Fi	1	ph	*Ottelia ovalifolia*	B	Rp
-	ah	*Spermacoce* sp	B	Ri	1	ph	*Persicaria decipiens*	B, M	Fp Ri Rp
2	ah	*Ranunculus* sp	B	Rp	1	ph	*Potomageton tricarinatus*	B	Rp
-	ah *	*Sonchus* sp	M	Fi Ri	1	ph	*Potomegeton perfoliatus*	B	Rp
-	ah *	*Stellaria media*	M	Ri	6	ph	*Secamone elliptica*	B	Ri
-	ah *	*Tetragonia tetragonoides*	B, M	Fp Ri Rp	4	ph	*Pteridium esculentum*	B	Ri
-	ah *	*Tridax procumbens*	B	Fi Fp	3	ph	*Rostellularia adscendens*	B, M	Fi Fp Ri Rp
-	ah *	*Trifolium arvense*	M	Fi Fp Rp	2	ph	*Schoenoplectus validus*	B	Rp
-	ah *	*Urtica incisa*	B, M	Fi Fp Ri Rp	6	ph	*Solanaceae* sp	B	Rp
1	ah	*Utricularia* sp	B	Rp	6	ph *	*Stylosanthes humilis*	B	Fi
-	ah	*Eclipta prostrata*	B	Ri	2	ph	*Typha domingensis*	B, M	Rp
-	ah *	*Xanthium occidentale*	B, M	Fp Ri	6	ph	*Vicia sativa*	M	Rp
-	ah	*Xerochrysum bracteatum*	M	Fi T	6	ph	*Walhenbergia stricta*	M	Fi Fp Ri
5	pg	*Aristida calycina*	M	Fi	2	ph *	*Cyperus polystachyos*	B, M	Fp Ri Rp
5	pg	*Aristida ramosa*	B, M	Fi Fp Ri T	4	ph *	*Macroptilium atropurpureum*	B	Fi Ri
6	pg	*Astrostipa scabra*	B, M	Fi Fp Ri Rp T	3	ph *	*Melilotus indicus*	M	Ri
3	pg	*Bothriochloa bladhii*	B	Fp Rp	2	ph *	*Phyla nodiflora*	M	Ri
6	pg	*Bothriochloa* sp	B	Fi Fp Rp T	1	ph *	*Polygonum aviculare*	B	Rp
3	pg	*Cynodon dactylis*	B, M	Fi Fp Ri Rp	4	ah *	*Stellaria media*	M	Ri
4	pg	*Austrodanthonia* sp	B	T	6	sh*	*Asparagus africanus*	B	Fi Fp Ri
4	pg	*Dichanthium sericeum*	B, M	Rp T	6	sh	*Atriplex* sp	M	Fi Rp T
6	pg	*Enteropogon acicularis*	B	Fp Rp	4	sh	*Beyeria viscosa*	M	Fp T
6	pg	*Bracharia* sp	M	Fp	5	sh	*Breynia oblongifolia*	M	Ri
6	pg	*Heteropogon contortus*	B	Fi T	3	sh	*Caesalpinia* sp	B	Ri
6	pg *	*Hyparrhenia hirta*	M	Fp	5	sh	*Capparis* sp	B	Fp Ri Rp
3	pg	*Imperata cylindica*	B	Fi Ri Rp	6	sh	*Dodonea viscosa*	M	Fp Ri
6	pg	*Iseilema* sp	M	Fp T	6	sh	*Eremophila debilis*	M	Fp
6	pg *	*Chloris gayana*	B	Fi T	6	sh	*Rhynchosia minima*	B	Fi T
6	pg *	*Melinis repens*	B	Fi	6	sh	*Croton* sp	B	Rp
4	pg	*Panicum decompositum*	M	Fi Fp Ri Rp	6	sh	*Grevillia* sp	B	Ri
6	pg	*Paspalidum jubiflorum*	B, M	Fi Rp	6	sh	*Grewia latifolia*	B	Ri
2	pg	*Paspalum dischium*	B, M	Rp	2	sh	*Hardenbergia violacea*	B	Ri
4	pg *	*Paspalum urvellei*	B, M	Fi Rp	5	sh	*Indigofera* sp	B, M	Fi Fp
5	pg *	*Eragrostis curvula*	B	Fi Ri T	6	sh	*Cryptandra* sp	M	Fp T
6	pg	*Phalaris aquatica*	B	Ri Rp	6	sh *	*Macfadyena unguis-cati*	B	Fp Ri Rp
5	pg	*Sporobolus creber*	B	Fp Ri Rp	6	sh *	*Opuntia stricta*	M	Fi

Hydro-type[1]	Life Form[2]	Species	Sites[3]	Landscape position[4]	Hydro-type	Life Form	Species	Sites	Landscape position
5	pg	*Themeda australis*	B	Fi Fp Ri T	6	sh	*Sclerolaena* sp	M	Fi
6	pg	*Triodia scariosa*	M	T	6	sh	*Sida acuta*	B, M	Fi Fp Ri Rp
4	pg	*Leptochloa digitata*	M, B	Fi Ri Rp	6	sh*	*Abutilon auritum*	B	Fp Ri
4	pg *	*Megathyrsus maximus*	B, M	Fi Fp Ri Rp	3	sh	*Abutilon oxycarpum*	B, M	Fi Fp Ri

* introduced species. [1]hydrotypes – 1 = hydrophyte; 2 = helophyte; 3 = mesophyte; 4 = semi-mesophyte; 5 = semi-xerophyte; 6 = xerophyte. [2] Life form – ag = annual grass; ah = annual herb; pg = perennial grass; ph = perennial herb; sh = shrub; t = tree. [3]Sites – M = Maules Creek; B = Bremer River. [4]Landscape position - Rp = riparian perennial; Ri = riparian intermittent; Fp = floodplain perennial; Fi = floodplain intermittent.

Table 44 continued Species list & hydrotypes

Hydro-type[1]	**Life Form[2]**	**Species**	**Sites[3]**	**Landscape position[4]**
6	sh	*Solanum cinereum*	M	Fi Rp
4	sh	*Stephania japonica*	M	Ri
5	sh	*Swainsona greyana*	M	Fi Fp
5	sh	*Xanthosia pilosa*	M	Fp
4	sh *	*Lantana camara*	B	Fi Fp Ri Rp
6	t	*Acacia* sp	B, M	Fi Rp
6	t	*Acacia deanei*	M	T
4	t	*Alectryon oleifolius*	M	Fp Fi Rp
5	t	*Angophora floribunda*	B, M	Fi T
6	t	*Archontophoenix cunninghamiana*	B	Ri
6	t	*Brachychiton populneus*	M	Fp Rp
6	t	*Callitris glaucophylla*	M	Fi Ri T
3	t	*Casuarina cunninghamiana*	B, M	Fi Ri Rp
4	t	*Celtis sinensis*	B	Ri
6	t	*Corymbia tessellaris*	B	Fi Rp T
5	t	*Corymbia trachyphloia*	B	Fi T
5	t	*Cupaniopsis* sp	B	Fp
5	t	*Eucalyptus albens*		T
3	t	*Eucalyptus camaldulensis*	M	Fi Fp Ri Rp
6	t	*Eucalyptus dealbata*	M	T
5	t	*Eucalyptus melanophloia*	B, M	T
4	t	*Eucalyptus populnea*	M	Fi T
4	t	*Eucalyptus resinifera*	B	Fi
4	t	*Eucalyptus tereticornis*	B	Fi Fp Ri T
5	t	*Flindersia collina*	B	Fp
4	t	*Geijera parviflora*	M	Fi Fp T
5	t	*Hymenosporum flavum*	B	Fp Ri
6	t	*Jacaranda mimosifolia*	B	Rp
4	t	*Lophostemon suaveolens*	B	Ri
5	t	*Mallotus* sp	B	Fp
3	t	*Melaleuca bracteata*	B, M	Fi Fp Ri Rp
3	t	*Melaleuca viminalis*	B	Ri Rp
4	t	*Melia azedarach*	B, M	Fi Fp Ri
5	t	*Notelaea microcarpa*	M	Ri
5	t	*Schinus terebinthifolius*	B	Ri Rp
5	t	*Streblus brunonianus*	B	Ri
4	t	*Syzygium* sp	B	Fi Ri
5	t	*Trema tomentosa*	M	Ri

* introduced species. [1]hydrotypes – 1 = hydrophyte; 2 = helophyte; 3 = mesophyte; 4 = semi-mesophyte; 5 = semi-xerophyte; 6 = xerophyte. [2] Life form – ag = annual grass; ah = annual herb; pg = perennial grass; ph = perennial herb; sh = shrub; t = tree. [3]Sites – M = Maules Creek; B = Bremer River. [4]Landscape position - Rp = riparian perennial; Ri = riparian intermittent; Fp = floodplain perennial; Fi = floodplain intermittent.

7.2 Appendix 2: Component 2 Ecological values of baseflow and surface water-groundwater connectivity regimes in non-perennial streams.

7.2.1 Supplementary material

Table 45: Output of two-way analysis of variance (ANOVA) models (for dry gravel bar and pool habitat) and a linear mixed-effect model (LMM; for riffle habitat) investigating the mean differences among depth treatments for cotton strips in each habitat. Tukey multiple comparisons of means are conducted when significant terms are present. The 'Intercept' for the LMM represents the term for Surface depth in Experiment One.

Cotton strip assays: Pool and gravel bar habitat (Two-way ANOVA)

	Gravel bar			Pool		
Treatment	df	F	P	df	F	P
Experiment	3	0.54	0.655	3	5.66	0.003
Depth	1	75.34	0.000	1	25.09	0.000
Experiment X Depth	3	1.03	0.395	3	1.03	0.394
Residuals	32			32		

Cotton strip assays: Riffle habitat (LMM)

```
Linear mixed model fit by REML
t-tests use  Satterthwaite approximations to degrees of freedom ['lmerMod']
REML criterion at convergence: 71.5

Random effects:
 Groups   Name        Variance Std.Dev.
 Site     (Intercept) 0.004123 0.06421
 Residual             0.072593 0.26943
Number of obs: 199, groups:  Site, 5

Fixed effects:
                              Estimate Std. Error        df t value Pr(>|t|)
(Intercept)                    0.49754    0.06106  35.73000   8.148 1.15e-09 ***
ExperimentTwo                  0.20951    0.07621 186.99000   2.749  0.00656 **
ExperimentThree                0.11955    0.07621 186.99000   1.569  0.11840
ExperimentFour                 0.35903    0.07621 186.99000   4.711 4.79e-06 ***
DepthHyporheic                -0.18655    0.07621 186.99000  -2.448  0.01529 *
ExperimentTwo:DepthHyporheic  -0.04497    0.10834 187.02000  -0.415  0.67854
ExperimentThree:DepthHyporheic 0.02942    0.10777 186.99000   0.273  0.78517
ExperimentFour:DepthHyporheic -0.32816    0.10777 186.99000  -3.045  0.00266 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Leaf litter incubations: Pool and gravel bar habitat (Two-way ANOVA)

	Gravel bar			**Pool**		
Treatment	**df**	**F**	**P**	**df**	**F**	**P**
Experiment	1	53.5	0.000	1	21.6	0.000
Depth	1	193	0.000	1	3.13	0.085
Experiment X Depth	1	10.3	0.003	1	7.58	0.009
Residuals	36			36		

Leaf litter incubations: Riffle habitat (LMM)

```
Linear mixed model fit by REML
t-tests use  Satterthwaite approximations to degrees of freedom ['lmerMod']
```

```
REML criterion at convergence: 1406.8

Random effects:
 Groups   Name        Variance Std.Dev.
 Site     (Intercept) 19.23    4.385
 Residual             84.49    9.192
Number of obs: 194, groups:  Site, 5

Fixed effects:
                             Estimate Std. Error      df t value Pr(>|t|)
(Intercept)                    79.952      2.353   6.660  33.981 1.02e-08 ***
ExperimentTwo                 -21.887      1.868 186.030 -11.715  < 2e-16 ***
DepthHyporheic                -17.026      1.838 185.990  -9.261  < 2e-16 ***
ExperimentTwo:DepthHyporheic   12.607      2.643 186.050   4.770 3.71e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 46: List of macroinvertebrate taxa recorded at sites in the Maules Creek catchment and upper Bremer River catchment (NI refers to taxa Not Idenetifiable).

Phylum	Class	Order	SubOrder	Common	Family	SubFamily	Genus
Athropoda	Acarina			Mite			
Athropoda	Crustacea	Cyclopoida		Cladoceran	Chydoridae		
Athropoda	Crustacea	Cyclopoida		Cladoceran	Daphniidae		
Athropoda	Crustacea	Cyclopoida		Copepod			
Athropoda	Crustacea	Decapoda		Yabbie/Shrimp	Atyidae		Paratya
Athropoda	Crustacea	Decapoda		Yabbie/Shrimp	Parastacidae		Cherax
Athropoda	Crustacea	Podocopida		Seed Shrimp			
Athropoda	Insecta	Coleoptera		Beetle Adult	Dytiscidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Dytiscidae		
Athropoda	Insecta	Coleoptera		Beetle Adult	Elmidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Elmidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Gyrinidae		
Athropoda	Insecta	Coleoptera		Beetle Adult	Hydraenidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Hydrochidae		
Athropoda	Insecta	Coleoptera		Beetle Adult	Hydrophilidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Hydrophilidae		
Athropoda	Insecta	Coleoptera		Beetle Larvae	Psephenidae		Sclerocyphon
Athropoda	Insecta	Coleoptera		Beetle Larvae	Scirtidae		
Athropoda	Insecta	Diptera		True fly larvae	Ceratopogonidae	Ceratopogoninae	
Athropoda	Insecta	Diptera		True fly larvae	Ceratopogonidae	Forcipomyiinae	
Athropoda	Insecta	Diptera		True fly larvae	Chironominae		
Athropoda	Insecta	Diptera		True fly larvae	Culicidae	Anophelinae	
Athropoda	Insecta	Diptera		True fly larvae	Culicidae	Culicinae	
Athropoda	Insecta	Diptera		True fly larvae	Dolichopodidae		
Athropoda	Insecta	Diptera		True fly larvae	Empididae		
Athropoda	Insecta	Diptera		True fly larvae	Muscidae		
Athropoda	Insecta	Diptera		True fly larvae	Simuliidae	Simulinae	
Athropoda	Insecta	Diptera		True fly larvae	Stratiomyidae	Stratiomyinae	Odontomyia
Athropoda	Insecta	Diptera		True fly larvae	Tanyderidae		
Athropoda	Insecta	Diptera		True fly larvae	Tipulidae		
Athropoda	Insecta	Ephemeroptera		Mayfly	Baetidae		NI
Athropoda	Insecta	Ephemeroptera		Mayfly	Baetidae		Bungona
Athropoda	Insecta	Ephemeroptera		Mayfly	Baetidae		Cloeon
Athropoda	Insecta	Ephemeroptera		Mayfly	Baetidae		Jappa
Athropoda	Insecta	Ephemeroptera		Mayfly	Caenidae		NI
Athropoda	Insecta	Ephemeroptera		Mayfly	Caenidae		Irpacaenis
Athropoda	Insecta	Ephemeroptera		Mayfly	Caenidae		Tasmanocoenis
Athropoda	Insecta	Ephemeroptera		Mayfly	Leptophlebiidae		NI
Athropoda	Insecta	Ephemeroptera		Mayfly	Leptophlebiidae		Atalophlebia
Athropoda	Insecta	Ephemeroptera		Mayfly	Leptophlebiidae		Austrophlebioides
Athropoda	Insecta	Ephemeroptera		Mayfly	Leptophlebiidae		Nousia
Athropoda	Insecta	Ephemeroptera		Mayfly	Leptophlebiidae		Tillyardophlebia / Riekophlebia
Athropoda	Insecta	Hemiptera		Water Boatman	Corixidae		Agraptocorixa
Athropoda	Insecta	Hemiptera		Toe Biter	Corydalidae		
Athropoda	Insecta	Hemiptera		Toad Bug	Gelastocoridae		Nerthra
Athropoda	Insecta	Hemiptera		Water Strider	Gerridae		Calyptobates
Athropoda	Insecta	Hemiptera		Water Strider	Gerridae		Rhagadotarsus
Athropoda	Insecta	Hemiptera		Water Strider	Gerridae		Rheumatometra
Athropoda	Insecta	Hemiptera		Water Scorpion	Nepidae		Laccotrephes
Athropoda	Insecta	Hemiptera		Back Swimmer	Notonectidae		Anisops
Athropoda	Insecta	Hemiptera		Back Swimmer	Notonectidae		Enithares
Athropoda	Insecta	Hemiptera		Back Swimmer	Notonectidae		Paranisops
Athropoda	Insecta	Hemiptera		Small Water Strider	Veliidae		NI
Athropoda	Insecta	Hemiptera		Small Water Strider	Veliidae		Drepanovelia
Athropoda	Insecta	Hemiptera		Small Water Strider	Veliidae		Microvelia
Athropoda	Insecta	Lepidoptera		Caterpillar	Pyralidae		
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Corduliidae		Hemicordulia
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Gomphidae		Hemigomphus
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Gomphidae		Austrogomphus
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Libellulidae		Diplacodes
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Libellulidae		Nannophya
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly	Synthemistidae		Eusynthemis
Athropoda	Insecta	Odonata	Anisoptera	Dragonfly			NI
Athropoda	Insecta	Odonata	Zygoptera	Damselfly			
Athropoda	Insecta	Trichoptera		Caddisfly	Calamoceratidae		Anisocentropus
Athropoda	Insecta	Trichoptera		Caddisfly	Ecnomidae		Ecnomus
Athropoda	Insecta	Trichoptera		Caddisfly	Hydropsychidae		
Athropoda	Insecta	Trichoptera		Caddisfly	Hydroptilidae		Hellyethira
Athropoda	Insecta	Trichoptera		Caddisfly	Leptoceridae		NI
Athropoda	Insecta	Trichoptera		Caddisfly	Leptoceridae		Triplectides
Athropoda	Insecta	Trichoptera		Caddisfly	Leptoceridae		Oecetis
Athropoda	Insecta	Trichoptera		Caddisfly	Philopotamidae		Chimarra
Athropoda	Insecta	Trichoptera		Caddisfly			NI
Athropoda	Ostracoda			Clam Shrimp			
Mollusca	Bivalvia	Veneroida		Clam			
Mollusca	Gastropoda	Hygrophila		Snail	Lymnaeidae		
Mollusca	Gastropoda	Hygrophila		Snail	Planorbidae		
Mollusca	Gastropoda	Hygrophila		Snail	Planorbidae		Ferrissia
Annelida	Hirudinea	Rhynchobdellida		Leech	Glossiphoniidae		
Annelida	Oligochaeta			Segemented worms			

Phylum	Class	Order	SubOrder	Common	Family	SubFamily	Genus
Nematoda	Nematoda			Round worm			

7.3 Appendix 3: Component 3 – Subsurface ecology supplementary material

Figure 155: East Lynne study site in upper Middle Creek with intermittent losing flow regime at time of sampling. The bores and pool were sampled for hyporheic invertebrates and hydrochemistry.

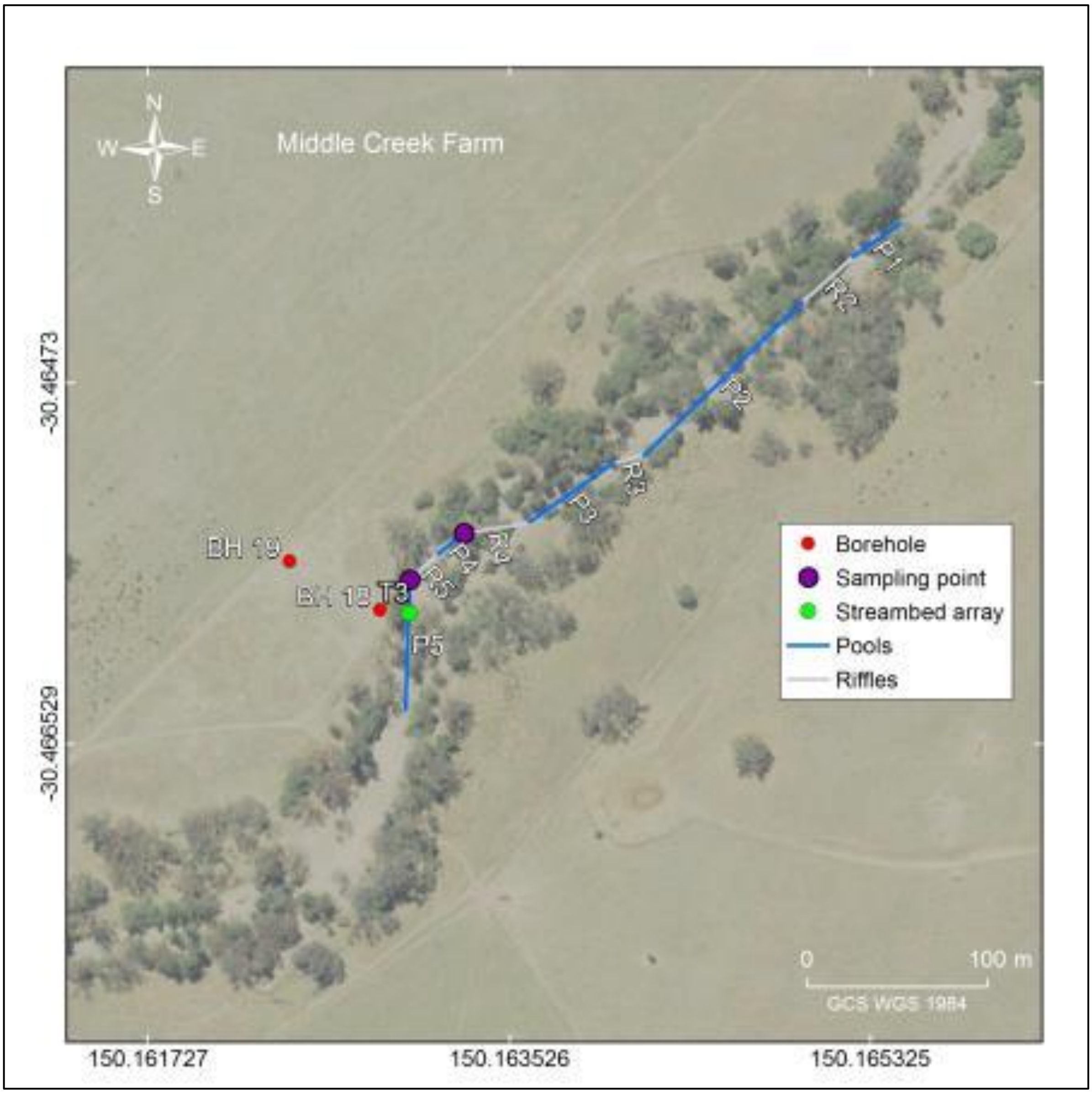

Figure 156: Middle Creek Farm study site in upper Middle Creek. Pools (P) and riffles (R) are numbered sequentially from upstream. Pools P4 and P5 were sampled for hyporheic invertebrates and hydrochemistry.

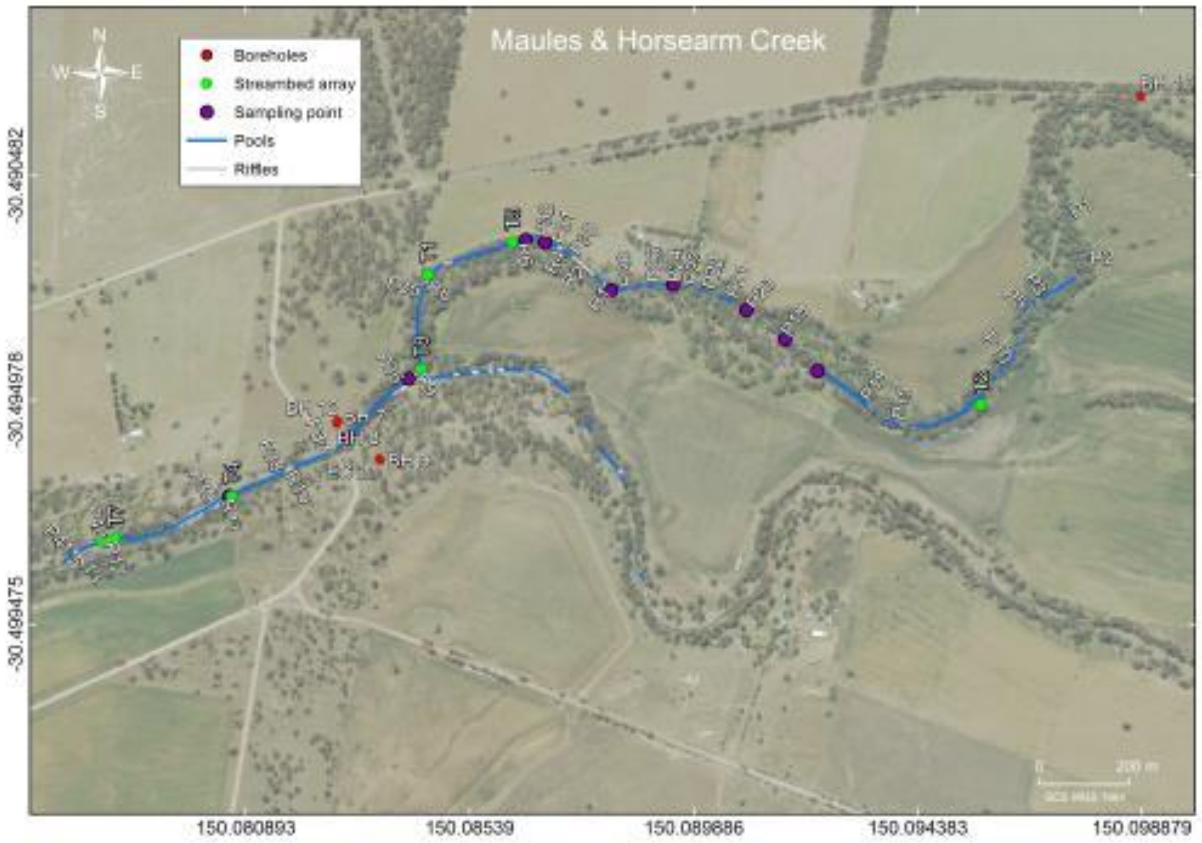

Figure 157: Maules and Horsearm Creek. Horsearm Creek perennial gaining and Perennial losing from Horsearm - Maules confluence. Pools P18, P19, P20, P24, P27, P32 (The Spring), Bar 6, the Confluence (P35-R9), P36 (Elfin Crossing), P38 and riffle R11 were sampled for hyporheic invertebrates and hydrochemistry.

Table 47: Summary of survey effort in the Bremer River catchment, including the predominant direction of hyporheic exchange flow inferred from vertical hydraulic head difference (VHH) measured at the time of sampling: upwelling (UW), downwelling (DW), Neutral, or Dry (hyporheic water not intercepted).

Location	**Field Campaign**	**Date**	**Site Date Code**	**Regime**	**UW/DW/ Neutral/Dry**	**n Subsamples / site**	**Notes**
Coulson Creek	Trip 6 (R1)	11/10/2015	CC_Oct15	Intermittent	Neutral	6	
	Trip 8 (R2)	1/03/2016	CC_Mar16		Neutral	6	
Reynolds Creek	Trip 6 (R1)	8/10/2015	RC_Oct15	Intermittent	near neutral	6	
	Trip 8 (R2)	2/03/2016	RC_Mar16		near neutral	6	
Upper Bremer	Trip 6 (R1)	9/10/2015	UB_Oct15	Intermittent	DW?	6	
	Trip 8 (R2)	29/02/2016	UB_Mar16		DW	6	
Warrill Creek	Trip 6 (R1)	9/10/2015	WC_Oct15	Intermittent	Neutral-DW?	3	Near dry
	Trip 8 (R2)	1/03/2016	WC_Mar16		near neutral	6	
Wild Cattle Creek	Trip 6 (R1)	10/10/2015	WCC_Oct15	Intermittent	Neutral-UW	6	
	Trip 8 (R2)	2/03/2016	WCC_Mar16		near neutral	6	
	Trip 6 (R1)				Total subsamples	57	

Table 48:Summary of survey effort Maules Creek catchment, including the predominant direction of hyporheic exchange flow inferred from vertical hydraulic head difference (VHH) measured at the time of sampling: upwelling (UW), downwelling (DW), Neutral, or Dry (hyporheic water not intercepted).

Location	Field Campaign	Date	Site Date Code	Regime	UW/DW/Neutral/ Dry	n Sub-samples / site	Notes
Middle Creek	Trip 5	17/09/2015	ELP1_Sept15	Intermittent Losing	UW	6	
	Trip 8 (R2)	25/02/2016	ELP1_Sept15		Dry	0	Dry
	Trip 3	24/07/2015	MCP4_July 15		Neutral	3	
	Trip 6 (R1)	14/10/2015	MCP4_Oct15		Neutral-DW	3	see also P5
	Trip 8 (R2)	25/02/2016	MCP4_Feb16		Dry	0	Dry
	Trip 5	16/09/2015	MCP5_Sept 15		UW	4	
	Trip 8 (R2)	25/02/2016	MCP5_Feb16		Dry	4	Dry
	Trip 6 (R1)	15/10/2015	MCP5_Oct15		Neutral	2	see also P4
	Trip 8 (R2)	25/02/2016	MCP5_Feb16		DRY	0	Dry
Horsearm Creek	Trip 2	30/04/2015	P18_Apr15	Perennial Gaining	DW	6	
	Trip 2	29/04/2015	P19_Apr15		UW?	6	
	Trip 3	21/07/2015	P19_July15		UW?	4	
Horsearm Creek	Trip 2	30/04/2015	P20_Apr15		UW?	2	
	Trip 3	21/07/2015	P20_Jul15		UW?	6	
	Trip 2	29/04/2015	P24_Apr15		UW?	6	
	Trip 6 (R1)	15/10/2015	HCB6_Oct15		Neutral	6	
	Trip 8 (R2)	25/02/2016	HCB6_Feb16		Neutral-DW?	6	
	Trip 1 pilot	25/02/2015	P27_Feb15 AB		DW	7	AB samples 10L
	Trip 1 pilot	25/02/2015	P32_Feb15 AB		UW	32	AB samples 10L
	Trip 1 pilot	25/02/2015	P35R9_Feb 15AB	Perennial Losing	DW	32	AB samples 10L
Maules Creek	Trip 5	15/09/2015	ECP36_Sept 15	Perennial Losing	DW	2	
	Trip 6 (R1)	25/02/2016	ECP36_Feb 16		Neutral-DW?	4	
	Trip 8 (R2)	26/02/2016	P38_Feb16		Neutral-DW?	6	
	Trip 3	22/07/2015	R11_July15		DW	6	
	Trip 6 (R1)	13/10/2015	R11_Oct15		DW?	6	
	Trip 8 (R2)	25/02/2016	R11_Feb16		DW?	0	Dry, see P38
					Total subsamples	159	

Table 49: Deployment sites and logger details for the organic matter degradation study.

	Location	**Number** of Plots	**Logger**	
			Round 1	Round 2
Intermittent	MCBar 3	2		
	MCBar 4	2		
	MCBar 5	1		
	MCPool 3	2	1 X Surf - Temp & Light 1 X Hyp - Temp	1 X Surf - Temp & Light 1 X Hyp - Temp 1 X Hyp - Temp & Light
	MCPool 5	3	1X Surf - Temp & Light 1 X Hyp - Temp	1X Surf - Temp & Light 1 X Hyp - Temp
	MCRiffle 3	2	1 X Surf - Temp & Light	1 X Hyp - Temp
				1X Surf - Temp & Light 1 X Hyp - Temp
	MCRiffle 4	2		
	MCRiffle 5	1		
Perennial Gaining	Bar 6	2		
	Bar 7	1		
	Bar 8	2		
	Pool 32	1		
	Pool 33	2	1 X Hyp- Temp	1 X Hyp - Temp & Light
	Pool 34	2	1 X Surf - Temp & Light	1 X Surf - Temp & Light
	Riffle 6	2		
	Riffle 7	1		
	Riffle 8	2		
Perennial Losing	Bar 11	3		
	Bar 13	2		
	Pool 38	1	1 X Surf - Temp & Light	
	Pool 39	4	2 X Surf - Temp & Light 2 X Hyp - Temp	2 X Surf - Temp & Light 2 X Hyp - Temp
	Riffle 11	3	1 X Surf - Temp & Light 1 X Hyp - Temp	
	Riffle 12	2		

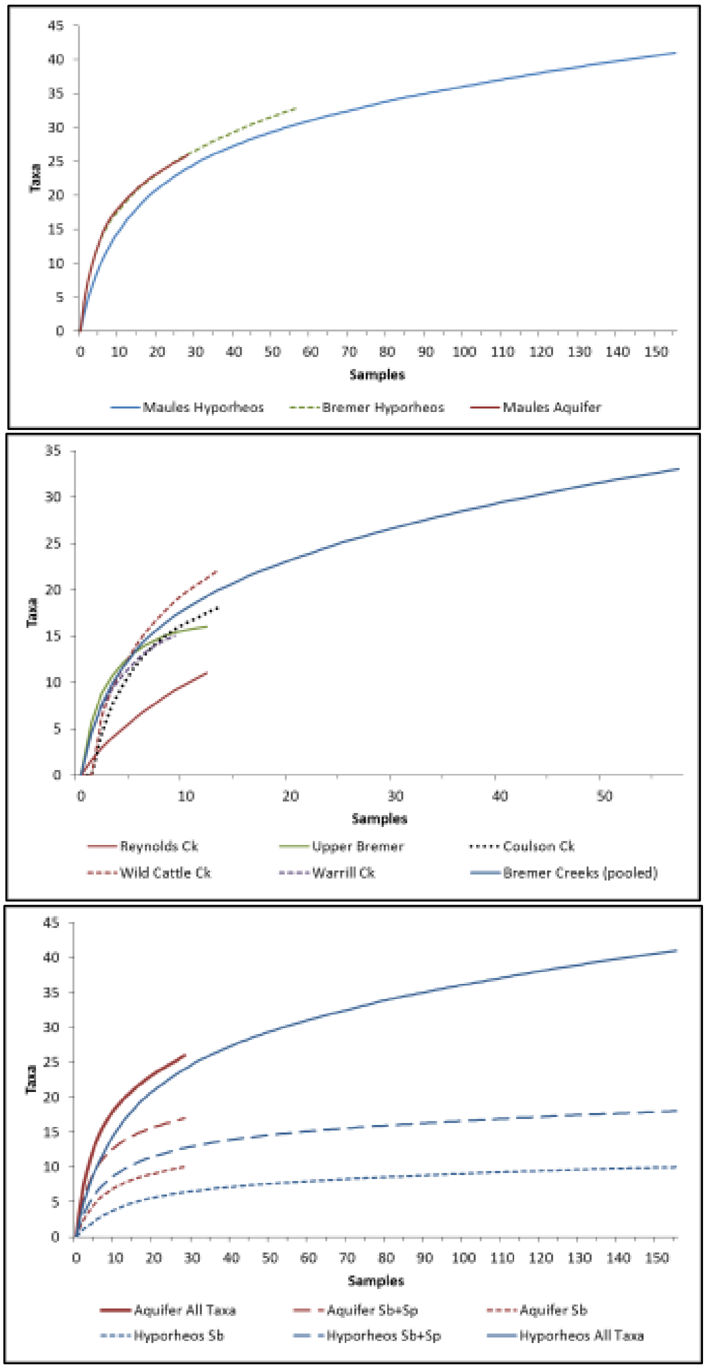

Figure 158: (top) Taxon accumulation curves (Sobs) for all hyporheos and aquifer samples: Bremer River (upper dashed line), Maules Creek aquifer (upper solid line), Maules Creek hyporheos (lower solid line); (mid) Taxon accumulation curves (Sobs) for Bremer River catchment hyporheos samples, plotted by individual creeks, and creeks pooled; (lower) Maules Creek taxon accumulation curves (Sobs) for hyporheos (blue) and aquifer (brown) samples: all taxa (upper solid lines), stygobites (Sb) + stygophiles (Sp) (mid dashed lines), stygobites only (lower dotted lines). Bremer River hyporheos functional group trajectories were very similar (plot not shown).

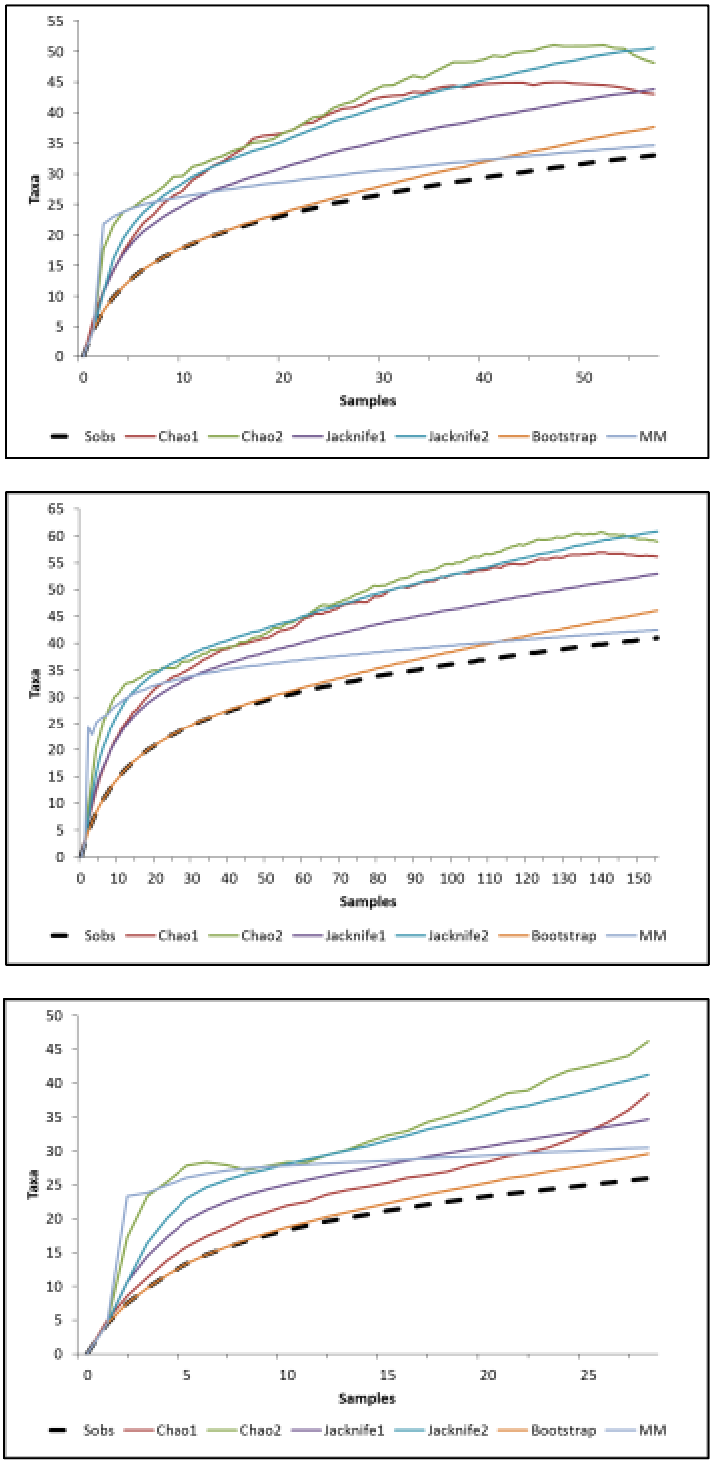

Figure 159: (top) Taxon accumulation curve (Sobs) for Bremer River catchment hyporheos samples (creeks pooled) and estimators; (mid) Taxon accumulation curve (Sobs) for Maules catchment hyporheos samples (all sites pooled) and estimators; (bottom) Taxon accumulation curve (Sobs) for Maules catchment aquifer samples (bores pooled) and estimators.

Table 50: Bremer Catchment Pearson correlations of each invertebrate taxon, univariate biotic indices and environmental variable with non-metric multidimensional scaling (nMDS) axes MDS1 and MDS2. Variables with Pearson r > 0.4 are in bold. Abbreviations: 'Sqr' = square root transformation. Refer to Table 51 and Table 52 for all other abbreviations.

Variable	MDS1	MDS2
Taxa		
Neonphargidae_QLD_Sb	-0.156	-0.191
Bathynellidae_sp 2_Sb	-0.130	-0.202
Bathynellidae_sp 3_Sb	-0.031	-0.364
Parabathynellidae_sp 3_Sb	0.014	0.221
Harpacticoida_indet_sp 2_Sb	**-0.077**	**-0.520**
Cyclopoida_indet._Sp/Sb?	**-0.814**	**0.053**
Candonidae?_indet._cf sp1B QLD_Sp/Sb?	**-0.398**	**-0.218**
Ostracoda_Candonidae?_indet._Sp/Sb?	-0.097	-0.227
Turbellaria_indet._Sp/Sb?	-0.371	0.121
Nematoda indet._Sp	**-0.215**	**-0.771**
Oligochaeta_indet._Sp	**-0.671**	**-0.153**
Aturidae_indet._Sp?	-0.194	0.139
Oribatida_indet._Sp?	**-0.367**	**-0.291**
Acarina_indet_Sp?/Ac?	-0.086	-0.173
Ceratopogonidae_indet_Sx	**-0.340**	**-0.271**
Chironomidae_indet._Sx	**-0.776**	**0.147**
Psychodidae_indet_Sx	-0.317	-0.176
Tipulidae_indet_Sx	-0.229	-0.031
Ephemeroptera_indet._Sx	**-0.588**	**0.017**
Diptera_indet._Sx?	-0.108	0.099
Insecta_indet._Sx?	-0.287	-0.130
Elmidae_indet._Sx?	-0.337	0.076
Hydraenidae_indet_Sx?	-0.314	0.018
Hydrophilidae_indet_Sx?	-0.206	-0.034
Coleoptera_indet._Sx?	-0.196	-0.149
Ecnomidae_indet_Ep	-0.340	-0.108
Trichoptera_indet._Ep	-0.097	-0.227
Tardigrada_Ep	-0.134	0.003
Rotifer_indet._Ep	-0.264	0.224
Hydridae_indet_Ep	-0.340	0.135
Chydoridae_indet_Ep	-0.372	0.000
Branchiura_indet._Ep/P	0.054	0.025
Univariate biotic indices		
Total richness	**-0.885**	**-0.248**
Sb/Sp richness	**-0.732**	**-0.423**
Sx/Ep richness	**-0.843**	**-0.009**
Total abundance	**-0.679**	**-0.089**
Sb/Sp abundance	**-0.614**	**-0.148**
Sx/Ep abundance	**-0.700**	**0.153**
Sb/Sp richness ratio	0.025	-0.344
Sx/Ep richness ratio	**-0.600**	**0.325**
Sb/Sp abundance ratio	**-0.190**	**-0.410**
Sx/Ep abundance ratio	**-0.319**	**0.437**

Variable	MDS1	MDS2
Hydraulic gradient & sediments		
VHH norm (cm)	-0.009	0.121
Sqr(Fine Sed. (ml))	0.090	-0.321
Hydrochemistry		
Temp	-0.268	0.177
EC (µS/cm)	**0.482**	**-0.153**
pH	-0.226	-0.075
Sqr(Sqr(DO (mg/L)))	**-0.656**	**0.160**
Sqr(Sqr(NOx (mg/l)))	-0.121	0.138
Sqr(Nitrate (mg/l))	0.140	0.131
Sqr(Sqr(P (ug/l)))	0.200	-0.149
Sqr(Phosphate (mg/l))	-0.142	-0.188
Sqr(DOC (mg/l))	0.137	0.267
Sqr(Factor 1)	-0.076	-0.048
Sqr(Factor 2)	0.277	-0.178
Sqr(Factor 3)	0.275	-0.192
Sqr(Abs @ 253nm)	0.172	-0.077
Sqr(S(274-295nm))	-0.020	-0.130
Sqr(S(350-400nm))	0.046	-0.169
Na (mg/l)	**0.375**	**-0.192**
Ca (mg/l)	**-0.436**	**0.029**
Mg (mg/l)	**-0.447**	**0.213**
K (mg/l)	-0.269	0.223
Alkalinity	0.248	-0.253
Chloride (mg/l)	**0.414**	**-0.074**
Sulfate (mg/l	-0.042	0.231
Sqr(Ammonia (mg/l))	0.279	-0.063
Si (mg/l)	-0.141	0.054
Sqr(Fe (mg/l))	0.262	0.020
Sqr(Mn (mg/l))	0.296	0.099
Sr (mg/l)	0.266	-0.022
Sqr(Al (ug/l))	-0.084	0.226
Sqr(Cu (ug/l))	-0.239	-0.065
Sqr(Zn (ug/l))	0.120	0.172
Ba (ug/l)	0.151	0.017
Sqr(Cr (ug/l))	0.226	-0.092
Sqr(Co (ug/l))	**0.415**	**0.051**
Sqr(As (ug/l))	**0.449**	**-0.240**
Fluoride (mg/l)	0.030	-0.144
Bromide (mg/l)	**0.582**	**-0.221**

Table 53: Maules Catchment hyporheic zone Pearson correlations of each invertebrate taxon, univariate biotic indices and environmental variable with non-metric multidimensional scaling (nMDS) axes MDS1 and MDS2. Variables with Pearson r > 0.4 are in bold. Abbreviations: 'Sqr' = square root transformation. Refer to Table 54 and Table 55 for all other abbreviations.

Variable	MDS1	MDS2
Taxa		
Neoniphargidae_MC_Sb	**0.428**	**-0.186**
Psammaspididae_indet._Sb	**0.382**	**-0.209**
Bathynellidae_indet._Sb	0.124	0.003
Bathynellidae_sp 1_Sb	**0.121**	**-0.433**
Parabathynellidae_sp 1_Sb	0.105	-0.060
Janiridae_indet. NSW Sb_Sb	0.328	-0.076
Phreatoicidae_indet. Sb	0.073	-0.118
Pezidae_indet_Sb	0.131	-0.137
Harpacticoida_sp_LongThin_Sb	0.325	0.025
Harpacticoida_indet_Sb	**0.575**	**0.267**
Cyclopoida_indet._Sp/Sb?	**0.777**	**-0.223**
Candonidae?_indet._sp1/1B NSW_Sp/Sb?	0.336	0.043
Candonidae?_indet._sp2 NSW_Sp/Sb?	0.172	-0.041
Candonidae?_indet._sp4 NSW_Sp/Sb?	0.120	-0.060
Ostracoda_Candonidae?_indet._Sp/Sb?	0.141	0.008
Turbellaria_indet._Sp/Sb?	**0.388**	**0.444**
Nematoda indet._Sp	**0.528**	**0.565**
Oligochaeta_indet._Sp	**0.620**	**0.097**
Acarina_sp1_shield_Sp?	-0.089	-0.185
Oribatida_indet._Sp?	**0.316**	**0.337**
Oxidae?_indet._Sp?	0.197	0.111
Unionicolidae?_indet._Sp?	0.272	-0.210
Gomphodella_sp._Sx/Sp?	0.099	-0.179
Acarina_indet_Sp?/Ac?	0.275	0.250
Ceratopogonidae_indet_Sx	0.084	0.136
Chironomidae_indet._Sx	0.299	0.121
Psychodidae_indet_Sx	0.231	0.128

Variable	MDS1	MDS2
Hydraulic gradient & sediments		
VHH norm.	0.328	0.060
Fine Sed.	0.124	-0.119
Hydrochemistry		
Temp	-0.364	0.096
EC (µS/cm)	**-0.598**	**-0.421**
pH	-0.342	0.003
Sqr(Sqr(DO (mg/L)))	**0.387**	**-0.314**
Sqr(Sqr(NOx (mg/l)))	**0.202**	**-0.454**
Sqr(Nitrate (mg/l))	**0.236**	**-0.643**
Sqr(Sqr(P (ug/l)))	**-0.540**	**-0.064**
Sqr(Phosphate (mg/l))	-0.047	-0.390
Sqr(DOC (mg/l))	**-0.275**	**0.449**
Sqr(Factor 1)	**-0.245**	**0.640**
Sqr(Factor 2)	**-0.597**	**0.499**
Sqr(Factor 3)	**-0.632**	**0.506**
Sqr(Abs @ 253nm)	**-0.501**	**0.308**
Sqr(S(274-295nm))	0.065	0.350
Sqr(S(350-400nm))	-0.119	0.304
Na (mg/l)	**-0.605**	**-0.403**
Ca (mg/l)	**-0.616**	**-0.432**
Mg (mg/l)	**-0.616**	**-0.417**
K (mg/l)	**-0.466**	**-0.230**
Alkalinity	**-0.562**	**-0.257**
Chloride (mg/l)	**-0.539**	**-0.476**
Sulfate (mg/l	**-0.369**	**-0.546**
Sqr(Ammonia (mg/l))	**-0.458**	**0.131**

Variable	**MDS1**	**MDS2**
Ephemeroptera_indet._Sx	0.048	-0.045
Diptera_indet._Sx?	-0.124	0.057
Insecta_indet._Sx?	0.159	0.049
Elmidae_indet._Sx?	-0.067	0.001
Simulidae_indet._Ep	0.194	0.085
Odonata_indet._Ep	-0.039	-0.087
Trichoptera_indet._Ep	-0.128	-0.016
Tardigrada_Ep	0.238	0.094
Rotifer_indet._Ep	0.269	0.248
Gastropoda_indet_Ep	0.021	0.079
Amphipoda_indet._MC_Ep	0.081	-0.049
Cladocera_indet._Ep	0.126	0.179
Nematomorpha_indet_P	0.100	0.079
Univariate biotic indices	**MDS1**	**MDS2**
Richness Tot.	**0.901**	**0.131**
Richness Sb+Sp	**0.893**	**0.053**
Richness Sx/Ep	**0.373**	**0.277**
Abund. Tot.	**0.623**	**-0.028**
Abund. Sb+Sp	**0.611**	**-0.084**
Abund. Sx/Ep	0.272	0.152
Sb+Sp richness ratio	**0.506**	**-0.085**
Sx/Ep richness ratio	-0.105	0.156
Sb+Sp abund. ratio	**0.516**	**-0.088**
Sx/Ep abund. ratio	-0.133	0.177

Variable	**MDS1**	**MDS2**
Si (mg/l)	**-0.392**	**-0.325**
Sqr(Fe (mg/l))	**-0.511**	**0.107**
Sqr(Mn (mg/l))	**-0.547**	**0.122**
Sr (mg/l)	**-0.564**	**-0.492**
Sqr(Al (ug/l))	-0.058	0.037
Sqr(Cu (ug/l))	**0.388**	**-0.098**
Sqr(Zn (ug/l))	-0.034	-0.150
Ba (ug/l)	-0.613	-0.305
Sqr(Cr (ug/l))	-0.146	-0.098
Sqr(Co (ug/l))	-0.295	-0.083
Sqr(As (ug/l))	-0.584	0.031
Fluoride (mg/l)	**-0.389**	**-0.358**
Bromide (mg/l)	-0.342	-0.118
Delta 2H	-0.135	0.235
Delta 18O	**-0.464**	**0.130**

Table 56: Maules Catchment aquifer Pearson correlations of each invertebrate taxon, univariate biotic indices and environmental variable with non-metric multidimensional scaling (nMDS) axes MDS1 and MDS2. Variables with Pearson r > 0.4 are in bold. Abbreviations: 'Sqr' = square root transformation. Refer to Table 57 and Table 58 for all other abbreviations.

Variable	MDS1	MDS2
Taxa		
Neoniphargidae_MC_Sb	-0.361	-0.150
Psammaspididae_indet._Sb	-0.114	-0.276
Bathynellidae_indet._Sb	**-0.393**	**0.405**
Bathynellidae_sp 1_Sb	**0.129**	**-0.445**
Parabathynellidae_indet._Sb	**-0.346**	**0.600**
Parabathynellidae_sp 1_Sb	-0.180	-0.196
Parabathynellidae_sp 4_Sb	**0.338**	**-0.553**
Janiridae_indet. NSW Sb_Sb	-0.294	-0.189
Harpacticoida_indet_sp 2_Sb	-0.114	-0.276
Harpacticoida_indet_Sb	**-0.550**	**0.100**
Cyclopoida_indet._Sp/Sb?	**-0.796**	**-0.386**
Candonidae?_indet._sp1/1B NSW_Sp/Sb?	**-0.138**	**-0.400**
Candonidae?_indet._sp2 NSW_Sp/Sb?	-0.160	-0.092
Ostracoda_Candonidae?_indet._Sp/Sb?	**-0.492**	**0.300**
Turbellaria_indet._Sp/Sb?	-0.224	0.167
Nematoda indet._Sp	**-0.415**	**-0.047**
Oligochaeta_indet._Sp	**-0.439**	**-0.062**
Acarina_sp1_shield_Sp?	-0.180	-0.196
Hygrobatidae_indet._Sp?	-0.114	-0.276
Oribatida_indet._Sp?	**0.059**	**-0.511**
Unionicolidae?_indet._Sp?	-0.160	-0.092
Gomphodella_sp._Sx/Sp?	-0.054	-0.007
Acarina_indet_Sp?/Ac?	**-0.494**	**0.563**
Diptera_indet._Sx?	**-0.415**	**-0.114**
Rotifer_indet._Ep	**-0.446**	**0.104**
Turbellaria_indet. (eyed)_Ep	-0.022	-0.190
Univariate biotic indices		
Total richness	**-0.871**	**-0.072**
Sb/Sp richness	**-0.829**	**-0.083**
Sx/Ep richness	**-0.502**	**0.025**
Total abundance	**-0.706**	**-0.164**
Sb/Sp abundance	**-0.718**	**-0.168**
Sx/Ep abundance	**-0.408**	**-0.083**
Sb/Sp richness ratio	**-0.433**	**-0.281**
Sx/Ep richness ratio	-0.381	0.100
Sb/Sp abundance ratio	**-0.526**	**-0.273**
Sx/Ep abundance ratio	-0.129	0.111

Variable	MDS1	MDS2
Hydrochemistry		
Temp	0.210	-0.079
EC (µS/cm)	0.141	0.056
pH	**0.109**	**0.467**
Sqr(Sqr(DO (mg/L)))	-0.186	-0.098
Sqr(Sqr(NOx (mg/l)))	-0.037	-0.087
Sqr(Nitrate (mg/l))	-0.041	-0.134
Sqr(Sqr(P (ug/l)))	-0.107	-0.053
Sqr(Phosphate (mg/l))	-0.050	-0.068
Sqr(DOC (mg/l))	0.023	-0.326
Sqr(Factor 1)	0.048	0.128
Sqr(Factor 2)	-0.099	-0.058
Sqr(Factor 3)	-0.122	-0.083
Sqr(Abs @ 253nm)	**0.099**	**-0.519**
Sqr(S(274-295nm))	-0.014	-0.394
Sqr(S(350-400nm))	0.087	-0.285
Na (mg/l)	0.201	0.234
Ca (mg/l)	0.083	0.011
Mg (mg/l)	0.075	-0.041
K (mg/l)	0.119	0.114
Alkalinity	0.245	0.137
Chloride (mg/l)	-0.045	-0.072
Sulfate (mg/l	0.006	0.048
Sqr(Ammonia (mg/l))	0.152	0.071
Si (mg/l)	0.129	0.040
Sqr(Fe (mg/l))	0.365	-0.010
Sqr(Mn (mg/l))	0.177	0.062
Sr (mg/l)	0.163	0.143
Sqr(Al (ug/l))	-0.060	0.225
Sqr(Cu (ug/l))	0.045	0.056
Sqr(Zn (ug/l))	0.250	0.097
Ba (ug/l)	**0.415**	**0.321**
Sqr(Cr (ug/l))	-0.255	0.139
Sqr(Co (ug/l))	0.261	-0.064
Sqr(As (ug/l))	0.166	0.158
Fluoride (mg/l)	0.202	0.320
Bromide (mg/l)	-0.011	-0.041
Delta 2H	-0.238	-0.026
Delta 18O	**-0.208**	**-0.578**

Figure 160: Middle Creek Farm intermittent losing section study sites in July 2015 showing continuous riffle–pool surface flow, filamentous algal growth and borehole BH18 located next to pools 4 and 5. Hyporheic sampling was undertaken in the upwelling zone at the head of pool 4 (top right).

Figure 161: Middle Creek Farm pool 5 study site showing changes in water levels and algal growth from September 2015 to March 2016. Hyporheic sampling was undertaken in the bank on the right.

Figure 162: Horsearm Creek perennial gaining section study sites Pool 32 (The Spring) - Riffle / Bar 6 – Pool 33.

Figure 163: Maules Creek at Elfin Crossing showing pool 36 hyporheic sampling and sampled bores BH12, BH7, BH8 on north bank.

Figure 164: Maules Creek perennial losing section study sites Pool 38 - Riffle 11 – Pool 39 from July 2015 to February 2016, showing the cessation of riffle flow and gradual drying and contraction of pool 38. Top right image shows head of pool 39 and tail of pool 38 with hyporheic exchange flow path of length < 10m under riffle 11.

Figure 165: Maules Creek perennial losing section study site Pool 39 from Sept 2015 to February 2016, showing the contraction of pool 39 with the concomitant lengthening (to > 200m) of the hyporheic flow path between pools 38 and 39. Upwelling water at the head of Pool 39 in February 2016 was hypoxic / anoxic and contained Fe^{2+}*, which on contact with air becomes rapidly oxidised (by iron bacteria?) producing the yellow deposit. These deposits are typical at the upwelling end of hyporheic flow paths in Maules Creek.*

7.4 Appendix 4: Component 4: GAB Springs supplementary material

7.4.1 Supplementary material

Table 59: Taxa considered but not included in this review and biodiversity assessment.

Taxonomic group	Location (spring supergroup) and species	Source	Summary and justification for exclusion from the review
Diatoms	Dalhousie (~21 species occur there but no information on endemics to springs)	Ling 1989	Where they have been studied in detail, diatoms have been shown to be a key component of the endemic assemblages within springs (Cantonati et al. 2012). Knowledge concerning endemism in freshwater algae in general is poor in Australia (see Tyler 1996) and assessments of algae diversity in springs have only occurred in some areas. A basin-wide review of potentially endemic taxa is therefore not possible at present.
	Lake Eyre	Kinhill 1997	
Micro & macro-algae	Lake Eyre (red, green and blue-green occur there)	Kinhill 1997	Less is known about microalgae endemic to springs than is known concerning diatoms. Knowledge regarding endemism in freshwater algae in general is poor in Australia (Tyler 1996) and assessments of algae diversity in springs have only occurred in some areas. A basin-wide review of potentially endemic taxa is therefore not possible at present.
	Dalhousie (3 species green, 31 species blue-green occur there but no information on endemics to springs)	Symon 1984, Ling 1989	
Amphibia	Lake Eyre (rare)	Kinhill 1997	Few dedicated studies of the amphibian fauna of springs have been made. Though work conducted by Kinhill noted in 1997 that few frogs were present in Lake Eyre springs, frogs abound in other areas. Of those species that have been identified most are common in other waterways. A lack of studies across the basin and a general lack of endemism means this group were excluded.
	Edgbaston (abundant and diverse)	Rossini pers. obs.	
	Dalhousie	Ponder 1986	
Fishes	Lake Eyre (*Craterocephalus eyresii*)	Pers. Coms: Dr. Peter Unmack, Dr. Adam Kereszy	The Spangled Perch (*L. unicolor)* is excluded, as it is not endemic to springs. All fishes previously included in the list of endemic species are included here except for two species of Hardyhead (*C. eyresii* and the undescribed species from Myross). These two species were excluded following the advice of the consulted experts who argued that the extent of regional endemism previously suggested within *Craterocephalus* was not well supported by recent studies.
	Barcaldine *(*Craterocephalus sp. from Myross)		
	Numerous locations (*Leiopotherapon unicolor*)		
Decapod crustaceans	Lake Eyre (*Caridina sp.*)	Page et al. 2005	The taxonomy of *Caridina* within Australia is generally poorly resolved though there is strong evidence that *C. thermophila* is endemic to springs and is not the same as a currently undescribed species from Lake Eyre (Page et al. 2005). These taxa have been excluded as (Ponder et al. 2010) argue there is not currently enough evidence to suggest it is endemic.
	Barcaldine (Caridina thermophila)	Ponder et al. 2010	
	Lake Eyre (*Cherax sp.*)	Sokol 1987	Yabbies from Dalhousie have a different morphology from *Cherax destructor* (a broadly distributed arid-zone species) and resemble a more ancestral form. However, Sokol (1987) was unsure whether this morphological difference was a response to the extreme environment (i.e. morphological plasticity within *C. destructor*). Rossini has observed *Cherax* at Edgbaston but these have not been analysed in any way. *Cherax* yabbies from springs were excluded due to the uncertainty in Sokol's (1987) analysis and a lack of any other data regarding the group.
	Barcaldine (*Cherax* sp.)	Rossini pers. obs.	

Taxonomic group	**Location (spring supergroup) and species**	**Source**	**Summary and justification for exclusion from the review**
Micro-crustaceans			
Ostracods	Lake Eyre *(Ngarawa dirga)* and others	De Deckker 1979	Only one species of endemic ostracod has been described (De Deckker 1979), but experts have flagged putative endemic species from better-known complexes, some of which may be from new genera or families (Ponder et al. 2010). Unfortunately, due to the high proportion of undescribed species and biases in sampling toward a few complexes the ostracods have been excluded from this review.
	Barcaldine (~ 2 species)	Ponder et al. 2010	
	Dalhousie	Ponder 1986	
Cladocerans & Copepods	Lake Eyre (one species of cladoceran)	Mitchell 1985	Both of these extensive groups of fauna have been found in springs that have been sampled intensively. Ponder et al. (2010) argue most species are probably not spring endemics but at least two taxa that have been flagged as putative endemics. However, as this group are no doubt diverse and much more broadly distributed in springs other than Lake Eyre and Barcaldine they have been excluded pending further research.
	Barcaldine (*Paracyclops* sp.)	Ponder 1986	
	Barcaldine (numerous cladocerans and copepods)	Ponder et al. 2010	
Arachnids			
Spiders	Lake Eyre (9 species)	Framenau et al. 2006	The diversity of wolf spiders in springs can be quite high (Framenau et al. 2006) but no thorough surveys have been published outside of Lake Eyre. Species described from Lake Eyre are stated to occur in wetland types other than springs so the spiders have been excluded from this review. However, more extensive work is needed on the group.
	Barcaldine (~1 species)	Fensham et al. 2010	
Mites	Lake Eyre (Mamersella ponderi)	Harvey 1990	Mites form an extensive part of the endemic spring fauna in other regions (Sabatino et al. 2003) but only one species (and a potential congener) have been identified as endemic to GAB springs thus far (Harvey 1990). As this is a likely underestimate of the full diversity, mites have been excluded from the review.
	Barcaldine *(Mamersella* sp.)	Fensham et al. 2010	
	Eulo (*Mamersella* sp.)	Fensham et al. 2010	
Flatworms	Lake Eyre (*Promacrostomum palum*)	Sluys 1986	Sluys and colleagues have conducted significant investigations into the Planarian fauna in springs. Three species known to be endemic to springs and to be taxonomically distinctive within the Australian fauna have been described. They have not been included in this review because broad-scale sampling outside of their type localities appears to be rare, meaning that estimates of their range may not be accurate.
	Barcaldine (*Dugesia artesiana*)	Sluys et al. 2007	
	Eulo (*Weissius capaciductus*)	Sluys et al. 2007	
Oligochaetes	Barcaldine (erpobdellid and glossiphoniid leeches)	Ponder et al. 2010	This is the only reference to leeches in GAB springs though mention of oligochaetes and their diversity in general are made in Ponder (1986) and Ponder et al. (2010) and there are endemics in other regions (Blinn 2008). This group is excluded due to a severe lack of data.
Insects	Lake Eyre (up to 48 species)	Greenslade 1985	Insect diversity is high in springs but due to their broad dispersal capabilities few are or are expected to be endemic (Ponder et al. 2010). Some exceptions are a putative endemic dragonfly from Edgbaston (Ponder et al. 2010) and a diverse range of caddis flies which are a large component of springs faunas in other regions (Pauls et al. 2009).
	Barcaldine (wide diversity of species, putative endemic *Nannophya* sp, Hellyethira and Hemiptera)	Ponder et al. 2010	

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Table 60: The information types used to score the relative information base available from the literature for each endemic taxon included in this review.

Information type	**Information availability** High (Score = 4)	Moderate (Score = 3)	Low (Score = 2)	None (Score = 1)
Taxonomy	Full morphological description supported by genetic (DNA) assessment of relationship to other species and potential cryptic species complexes if it occupies >1 complex or supergroup	In depth morphological description with brief genetic analysis at species level; if range >1 complex no in-depth enquiry regarding potential cryptic species or evolutionarily significant units	Morphological descriptions but no genetic data	Remains undescribed
Distribution	Full survey of range, regular (>1) and/or ongoing surveys of patch occupancy in at least one part of the range	Rudimentary knowledge regarding patch occupancy within range from 1 or few disparate surveys and no ongoing surveying	No data regarding full range as yet, no ongoing monitoring	Single specimen from one or few visits
Population status	Temporally replicated (>1 time) systematically collected abundance assessments across >5 springs within the range	Anecdotal observations regarding relative abundance within most springs in range	One off or limited anecdotal observations within some parts of the range	No information.
Connectivity	Patch level data regarding population connectivity across at least 50% of the range	Spatially limited but detailed patch level data (i.e. one group within one complex)	Anecdotal observations regarding potential connectivity in the system or could be inferred from other species in the system.	No information.
Ecology	Extensive spatially and temporally replicated information regarding the state of and potential environmental correlates of occupation and abundance, responses to seasonal variance, trophic ecology, reproductive ecology, physiology or behaviour	Robust but not systematic observations regarding microhabitat preferences, environmental associations, responses to environmental variance within some part of the range	Anecdotal observations regarding potential associations with environmental characteristics or responses to environmental variance	No information
Threats	Experimental and/or long-term in situ observations regarding response to range of threats faced by taxa.	Robust knowledge regarding some threats but not the full range	Anecdotal and/or expert opinion (e.g. IUCN) regarding potential threats but no testing of these to date	No information.

Table 61: Details of the 76 Great Artesian Basin spring complexes identified as containing one or more endemic taxa (fishes, molluscs, amphipods, isopods and plants). Table includes supergroup membership, location (longitude and latitude), name and abbreviation (used in figures) of each complex. Also shown for each complex are the number of endemic species (ES), cumulative risk score (CRS), and number of springs within each land tenure type. Land tenure codes are: NC (nature conservation, qualifying as an IUCN Protected Area), PF (private freehold), PL (private leasehold) and RCL (reserved crown land – not elsewhere classified). Source for land tenure data: ABARES (2016) and CAPAD (2014).

Supergroup	Complex	Code	Longitude	Latitude	ES	CRS	Land tenure (# springs)			
							NC	PF	PL	RCL
Barcaldine	Pelican Creek	BA_EDG	145.44	-22.77	26	1.240	121	38	0	0
Barcaldine	Moses	BA_MOS	146.24	-22.08	10	0.196	80	0	70	0
Barcaldine	Wobbly	BA_WOB	145.37	-22.25	5	0.110	0	7	0	0
Barcaldine	Archer's	BA_ARC	145.36	-22.30	4	0.091	0	11	0	0
Barcaldine	Caring	BA_CAG	145.39	-22.15	3	0.073	0	1	22	0
Barcaldine	Caress	BA_CAR	145.41	-23.28	3	0.053	0	26	0	0
Bourke	Peery	BU_PEE	143.59	-30.73	2	0.014	189	0	0	0
Dalhousie	Dalhousie	DA_DAL	135.50	-26.46	9	0.176	141	0	0	0
Eulo	Yowah Mud	EU_YOW	144.78	-27.97	12	0.182	0	0	13	0
Eulo	Eulo Town	EU_EUT	145.03	-28.17	4	0.092	0	0	0	9
Eulo	Dead Sea	EU_DSEA	144.88	-27.91	3	0.089	16	3	5	0
Eulo	Granite	EU_GRAN	144.55	-28.33	4	0.068	0	0	6	0
Eulo	Tunga	EU_TUN	144.63	-28.22	4	0.061	0	3	3	0
Eulo	Wooregym	EU_WOOR	144.74	-28.25	2	0.039	0	6	0	0
Eulo	Carpet	EU_CARP	144.86	-28.14	1	0.032	0	10	0	0
Eulo	Jubilee	EU_JUB	144.53	-28.40	1	0.026	0	10	6	0
Eulo	Merimo	EU_MER	144.84	-28.19	1	0.024	0	7	0	0
Eulo	Tungalla	EU_TUNG	144.81	-28.18	1	0.023	0	0	1	0
Lake Eyre	Freeling North	LE_EFN	135.90	-28.06	7	0.303	0	0	23	0
Lake Eyre	Outside	LE_NOS	136.21	-28.26	8	0.293	0	0	9	0
Lake Eyre	Freeling	LE_EFS	135.90	-28.07	8	0.250	0	0	100	0
Lake Eyre	Billa Kalina	LE_KBK	136.45	-29.49	8	0.218	0	0	268	0
Lake Eyre	Big Perry	LE_NBP	136.35	-28.34	7	0.197	0	0	9	0
Lake Eyre	Kewson Hill	LE_CKH	136.83	-29.41	6	0.192	51	0	0	0
Lake Eyre	Welcome	LE_WWS	137.83	-29.67	6	0.171	0	0	34	0
Lake Eyre	The Fountain	LE_NTF	136.28	-28.34	8	0.167	0	0	2	0
Lake Eyre	Warburton	LE_BWS	136.67	-29.27	7	0.161	0	0	13	0
Lake Eyre	Hawker	LE_NHS	136.19	-28.42	7	0.150	0	0	104	0
Lake Eyre	Francis Swamp	LE_FFS	136.30	-29.14	7	0.148	0	0	884	0
Lake Eyre	West Finniss	LE_HWF	137.41	-29.60	7	0.141	0	0	122	0
Lake Eyre	Fanny	LE_NFS	136.24	-28.32	7	0.139	0	0	8	0
Lake Eyre	Twelve Mile	LE_NTM	136.26	-28.31	7	0.129	0	0	5	0
Lake Eyre	Strangways	LE_CSS	136.55	-29.16	6	0.123	0	0	433	0
Lake Eyre	Hermit Hill	LE_HHS	137.43	-29.57	6	0.122	0	0	429	0
Lake Eyre	Sulphuric	LE_HSS	137.40	-29.61	6	0.119	0	0	56	0
Lake Eyre	Davenport	LE_WDS	137.59	-29.66	6	0.114	0	0	75	0
Lake Eyre	Coward	LE_CCS	136.79	-29.40	7	0.109	14	0	0	0
Lake Eyre	Bopeechee	LE_HBO	137.39	-29.61	5	0.103	0	0	62	0
Lake Eyre	Beresford Hill	LE_BBH	136.66	-29.26	6	0.098	0	0	1	0
Lake Eyre	Old Finniss	LE_HOF	137.45	-29.59	5	0.093	0	0	266	0
Lake Eyre	North West	LE_HNW	137.40	-29.56	5	0.092	0	0	56	0
Lake Eyre	Old Woman	LE_HOW	137.45	-29.60	5	0.091	0	0	45	0
Lake Eyre	Brinkley	LE_NBS	136.31	-28.50	4	0.080	0	0	11	0
Lake Eyre	Spring Hill	LE_NSH	136.15	-28.42	2	0.078	0	0	5	0
Lake Eyre	Emerald	LE_LES	137.06	-29.38	4	0.071	0	0	2	0
Lake Eyre	Big Cadna-owie	LE_UBC	135.67	-27.86	2	0.065	0	0	9	0
Lake Eyre	Dead Boy	LE_HDB	137.41	-29.60	4	0.063	0	0	11	0
Lake Eyre	Birribiana	LE_PBI	135.71	-28.21	2	0.062	0	0	2	0
Lake Eyre	Jersey	LE_CJE	136.76	-29.34	8	0.050	9	0	0	0
Lake Eyre	Elizabeth North	LE_CEN	136.77	-29.35	8	0.050	179	0	0	0

Supergroup	**Complex**	**Code**	**Longitude**	**Latitude**	**ES**	**CRS**	**Land tenure (# springs)**			
							NC	PF	PL	RCL
Lake Eyre	Mt Hamilton Ruin	LE_CMH	136.90	-29.49	2	0.048	1	0	0	0
Lake Eyre	Blanche Cup	LE_CBC	136.87	-29.45	8	0.046	20	0	0	0
Lake Eyre	Finniss Well	LE_HFL	137.47	-29.58	3	0.041	0	0	1	0
Lake Eyre	Wangianna	LE_WWA	137.71	-29.67	3	0.040	0	0	8	0
Lake Eyre	Elizabeth South	LE_CEL	136.78	-29.36	7	0.039	76	0	0	0
Lake Eyre	Horse East	LE_CHE	136.92	-29.49	7	0.037	6	0	0	0
Lake Eyre	Emily	LE_FES	136.40	-29.04	2	0.036	0	0	3	0
Lake Eyre	Horse West	LE_CHW	136.91	-29.49	6	0.030	2	0	0	0
Lake Eyre	Levi	LE_NLS	136.15	-28.38	1	0.028	0	0	13	0
Lake Eyre	Gosse	LE_LGS	137.34	-29.46	1	0.027	0	0	6	0
Lake Eyre	Buttercup	LE_CBU	136.89	-29.48	5	0.024	1	0	0	0
Lake Eyre	Primrose	LE_NPS	136.38	-28.15	2	0.020	0	0	3	0
Lake Eyre	Milne	LE_NMI	136.08	-28.26	1	0.010	0	0	9	0
Lake Frome	Public House	LF_OPH	139.49	-29.76	1	0.036	0	0	152	0
Lake Frome	Petermorra	LF_OPC	139.54	-29.76	1	0.032	0	0	44	0
Lake Frome	Twelve	LF_OTS	139.66	-29.84	1	0.015	0	0	72	0
Lake Frome	Mulligan	LF_ZMS	139.97	-29.72	1	0.007	0	0	1	0
Mitchell/ Staaten	Gammyleg	MS_GAM	143.57	-16.36	2	0.034	0	0	1	0
Springsure	Cockatoo	SS_COC	150.25	-25.73	2	0.063	0	19	0	0
Springsure	LuckyLast	SS_LUC	148.77	-25.80	1	0.047	0	12	0	0
Springsure	Scotts Creek	SS_SCO	149.28	-25.89	1	0.024	5	0	0	0
Springsure	Prices	SS_PRI	150.13	-25.48	1	0.017	0	4	0	0
Springvale	Reedy	SS_REE	140.45	-22.92	4	0.092	0	0	4	0
Springvale	Spring Creek	SS_SPR	140.70	-23.57	1	0.042	0	0	1	0
Springvale	Elizabeth	SS_ELI	140.58	-23.34	6	0.007	87	0	0	0
Springvale	Mt Datson	SS_MDAT	140.43	-22.85	1	0.007	0	0	15	0

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