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**Commonwealth Environmental Water Office**

Monitoring, Evaluation and Research Program

SOP Macroinvertebrates

Standard Operating Procedures
Macroinvertebrates v1.0

23 June 2019

1. Introduction

The Standard Operating Procedure (SOP) for Macroinvertebrates describes the purpose of macroinvertebrate surveys in the lower Goulburn River, how the monitoring will be conducted, who is responsible for specific tasks and how the collected data will be analysed and reported. The document is intended to be taken in the field during any macroinvertebrate surveys for the MER Program and should be updated throughout the life of the MER Program to reflect any agreed changes to method or procedure.

1. Objective and Hypotheses

The objective of macroinvertebrate monitoring in the MER Program is to determine how Commonwealth environmental water (CEW) has contributed to macroinvertebrate composition and abundance at the Basin-scale and within the lower Goulburn River region. More specifically macroinvertebrate monitoring will be used to investigate what combination of freshes and low flows are required to maintain macroinvertebrate diversity, abundance and biomass in the Goulburn River, which could have important implications for fish recruitment and other ecological processes dependent on macroinvertebrates. It will also investigate how flow events contribute to crustacean growth, reproduction and biomass in the Goulburn Catchment. Large-bodied crustaceans are likely to be an important food source for native fish, including the Golden Perch. Hence the monitoring will help to understand the effects of the CEW and other natural flows of these large-bodied crustaceans and is directly relevant to the future flow management of the Goulburn and other lowland rivers.

1. Indicators

The indicators to be monitored are:

Composition and abundance of key macroinvertebrate taxa – is monitored because it is a potentially sensitive measure of ecological impacts and has important implications for providing a food source for vertebrates such as small and juvenile fish.

Large bodied crustacean life history and biomass (shrimp, prawns and yabbies) – size, abundance and biomass. It is believed that crustaceans are an important food source for fish, including the Golden Perch (*Macquaria ambigua*), with literature confirming they may eat macroinvertebrates and large bodied crustaceans and or gudgeons (Hebert, 2005). The information specifically targeting large-bodied crustaceans will provide information on how these potential key food sources for fish respond to environmental flows.

These indicators will contribute to a better understanding of how environmental flow delivery in the lower Goulburn River can affect the abundance and composition of macroinvertebrates and the lifecycle (reproduction and recruitment) of large bodied crustaceans, which has important implications for the river in terms of the services and functions provided by macroinvertebrates. The role of bank vegetation, macrophytes and biofilms play an important role in sustaining these populations, while it is likely large-bodied crustaceans that are likely to be an important food source for other riverine species, especially Golden Perch. Macroinvertebrate monitoring, particularly biomass assessments, could thus complement fish monitoring and provide a mechanistic explanation for how environmental flows are affecting fish larvae by affecting a critical food resource.

1. Locations for Monitoring

To measure macroinvertebrates and large-bodied crustaceans a total of eight sites will be sampled within the catchment. Specific sites are being confirmed at the moment and the SOP will be updated prior to field sampling to reflect the chosen sites.

1. Timing and frequency of sampling

One sampling method will be used to assess macroinvertebrate composition and abundance: Rapid Bioassessment Edge Sweep Samples (RBA). One sampling method will be used to assess large-bodied crustaceans – size, abundance and biomass: bait trapping. There will be five sampling events each year. Sampling events will occur pre and post CEW fresh usually in late/spring early summer and the other events will occur in each season.

1. Responsibilities and identifying key staff

***Field program***

Dr Vincent Pettigrove will be responsible for overseeing the planned monitoring. Field and laboratory activities will be coordinated by Dr Claudette Kellar/Dr Kallie Townsend in conjunction with qualified research assistants. Relevant training and safety plan preparation will be provided by a post-doctoral researcher. Data collation, checking and uploading will be performed by a qualified research assistant under the supervision of a post-doctoral researcher, who will also perform data analysis and interpretation in partnership with Dr Angus Webb.

***Laboratory requirements***

Macroinvertebrate samples will be processed in the freshwater laboratory at the School of Science, RMIT University. Macroinvertebrate identifications will be conducted by a qualified research assistant. The contact for the laboratory is Dr Claudette Kellar. The chain of custody procedures for transfer of samples from the field to the laboratory will involve written documentation of who collected the samples, where the samples were collected and when they were collected, where the samples are stored in the receiving laboratory, who has processed the samples and when they were processed.

***Procedure for transferring knowledge to new team members***

New team members will be formally inducted into the project where a post-doctoral researcher or suitably qualified and experienced staff member will demonstrate the techniques used for sampling macroinvertebrates. They will also be shown how to access relevant information pertaining to the project, such as the location of standard operating procedures and safety information (site risk assessments, laboratory risk assessments). New team members will also be given safety inductions in the laboratory and the field by the laboratory manager and the post-doctoral researcher, with appropriate training provided where necessary (e.g. first aid training, wader training).

All staff must undertake a safety induction for the laboratory and be familiar with any Material Safety Data Sheets (MSDS) for chemicals used in the sampling (e.g. ethanol) as well as the location of MSDS hard copies, site risk assessments, and other safety information. All staff involved in field work should have up-to-date first aid and CPR training. Staff driving vehicles must have a current Victorian Drivers Licence. Staff operating the boat must have a current Victorian Boat Licence. Staff involved in RBA Edge Sweep Sampling must have successfully completed the EPA Victorian AusRivAS and Rapid Biological Assessment Competency course or have a minimum of five years’ experience conducting sweep sampling or be supervised by someone with the above qualifications. Similarly, staff involved in sorting and identifying macroinvertebrates from RBA Edge Sweep Samples must have successfully completed the EPA Victorian AusRivAS and Rapid Biological Assessment Competency course or have a minimum of five years’ experience conducting sweep sampling or be supervised by someone with the above qualifications.

1. Monitoring Methods

***Field methods***

The advised order of activities at each field site is as follows:

1. Hydrological and water quality measurements
2. Rapid Bioassessment (RBA) Edge Sweep Samples
3. Large-bodied crustacean Bait Trapping Sampling

Hydrological and water quality measurements will be obtained from existing flow gauge stations and routine water monitoring as needed.

**Rapid Bioassessment edge sweep sample (RBA)** involves the use of a hand net to sample edge habitats as outlined by EPA Victoria (2003). At each site the operator needs to identify the major edge habitat types within the reach (bare ground, snags, macrophyte beds, leaf litter deposits) and sample each of these for a total of 10m. The contents of the sample should be placed in a sampling tray. Macroinvertebrate samples are live-picked in the field by a qualified and experienced research assistant. Macroinvertebrates greater than 5mm should be targeted and all animals should be picked from the sample within the given time allocation. Macroinvertebrates are picked from the sample for a total of 30 minutes and stored in appropriately labelled jars containing 100% ethanol. If a new taxon is found in the last 10 minutes, the sample should be picked for another 10 mins. Information about each sample are recorded on a macroinvertebrate data sheet, including percentage composition of habitats within the reach, and the composition of sweeps in each sample.

**Bait trap monitoring** involves quantitatively sampling large-bodied crustaceans by deploying 5 bait traps (see Figure) overnight in each selected sub-habitat at each monitoring site. No baits will be used in the traps as previous experience with fish surveys has demonstrated that they are very effective at catching shrimp without a specific attractant. Because the nets are a standard size, sample the environment passively and can be deployed for a finite period, the method will produce replicate samples at each site. These quantitative data will lend themselves to several forms of univariate statistical analyses and provide a good measure of abundance and biomass. The nets will be deployed in edge sub-habitats (stands of emergent macrophytes, submerged snags, deposition areas, bare edge) that are present at the site and in water that is 30-70 cm deep and weighed down with a rock or similar to prevent them being moved or turned over by the current. The relative proportion of each habitat may vary between sites and may vary at a site from year to year. Each net will be labelled with the appropriate fisheries permit number and the name of the research group to comply with Fisheries Victoria research permit requirements. The nets will be deployed during on one day, left overnight and collected the following day. The time of deployment and collection will be recorded and every effort should be made to deploy nets at each site, and in each habitat type, for approximately the same amount of time, even though the deployment and collection times will vary between sites. It is expected that each net will be deployed for approximately 17-20 hours. All of the crustaceans caught in each net will be transferred to a sample jar (that is clearly labelled with the site, habitat type and net number), preserved in ethanol and transferred to the laboratory for sorting and processing. Any yabbies’ caught in the bait traps should be weighed on the scales, have the carapace length measured and then be placed in the bucket of water for release. Yabbies and any native fish should be returned to the river.



**Figure 1. Photo of type of bait trap that will be used to quantitatively sample crustaceans.**

***Laboratory methods***

**RBA samples** are processed in the laboratory by sorting and identifying macroinvertebrates within the samples. The contents of a jar is rinsed through into a 250 μm sieve held over a plastic beaker for collecting ethanol. The ethanol is disposed of into a waste ethanol container. The contents of the sieve are then rinsed and placed into a channel sorting tray, with the sample spread evenly in the channels and covered in 69 % ethanol (if required). Using a dissector microscope the microscope is focussed so that the field of view covers the internal width of the channel. Starting at one end of the continuous channel, all of the macroinvertebrates are scanned, picked, identified (major group/family) and removed from the channel and placed into 5 mL vials by major groups. The contents of the vials can then be identified according the EPA Victoria protocol (EPA Victoria 2003), mostly to family level where possible using current macroinvertebrate identification keys. The taxon name and number of individuals belonging to that taxon should be recorded on the Family Level Identification Sheet. Coleoptera adults and larvae of the same taxon should be sorted and recorded separately, with identifications specifying “adults” or “larvae”. Pupae of all taxa except should only be identified to Order. Exuviae and empty shells should be ignored. Immature or damaged specimens should not be assumed to be the same as more mature, undamaged specimens. If immaturity or damage prevents identifications being taken to a lower level this should be recorded as “indeterminate”. Only heads should be counted of broken specimens unless the body portion can be unequivocally identified as a particular taxon. Pupae and indeterminates should not be counted as distinct taxa. Representatives of Oligochaeta, Nematoda, Bryozoa, Cnidaria (Clavidae and Olindiidae) should be checked by staff but not individually identified or counted; numbers of Oligochaeta and Nematoda, and the presence of Bryozoa, Clavidae and Olindiidae should be recorded. The completed data sheet must then be signed and dated. Terrestrial specimens should not be counted or kept in the major group/Order vial of aquatic specimens.

**Bait traps** for large-bodied biomass will be identified (to species level where possible) and counted. Carapace length will be recorded for all individuals to support cohort analyses. Depending on the number of individuals caught and distribution of size classes, carapace length will be recorded to the nearest mm or else recorded as a size class category (e.g. 10-15 mm). The reproductive status of females will be recorded. Total dry weight of each species from each sample will be recorded to determine biomass, following the same procedure described above.

1. Quality assurance/ quality control
* All persons conducting the RBA Edge Sweep sampling must have successfully completed to EPA Victorian AusRivAS and Rapid Biological Assessment Competency course or have a minimum of five years’ experience conducting sweep sampling or be supervised by someone with the above the qualifications.
* A chain of custody is to be filled out *in situ* for sample collection, stating the sample identification, type of sample, replicate, location, date, time and the person who collected the sample. As a further precaution field sheets are to be filled out for each sampling method detailing the location of samplers.
* Macroinvertebrate identifications in the laboratory are to be performed by a person who has completed the EPA Victorian AusRivAS and Rapid Biological Assessment Competency course. The exception is for adult Chironomidae identifications, which can be conducted by a person with extensive experience identifying adult Chironomidae.
* 10 % of macroinvertebrate samples are to be identified by another suitably qualified person for quality control/quality assurance purposes.
* All laboratory data is to be entered into a Microsoft Excel spreadsheet that follows the data structure. Data will be cross checked against data sheets for accuracy by a post-doctoral researcher (not the person who entered the data). Data will be uploaded to a central database accessible to all consortium members as specified before.
1. Data Analysis

**Macroinvertebrate abundance** is to be calculated by determining the number of taxa and abundance of family level or higher taxa observed in the RBA sample.

**Large-bodied crustacean abundance, weight and biomass** are determined by comparing changes in crustacean presence, abundance and dry weights among different habitat types over different flow periods.

***Reporting***

**Water quality and hydrological data** should be reported for each sampling period as these could inform the interpretation of results.

**Macroinvertebrate composition and abundance** reporting using the RBA protocol should include data for the site, date of assessment, sample type, number of taxa, abundance of taxa, and composition of communities.

**Large-bodied crustacean abundance, weight and biomass** reporting should involve specifying the results of analyses for investigating the effects of CEW flows on large-bodied crustacean abundance, weight and biomass, in addition to specifying the sample type being analysed, site details, sample dates, number of crustacean taxa and number of individuals per crustacean taxon.

All data will be uploaded to the lower Goulburn River LTIM folder hosted on the University of Melbourne Server and on the Dropbox site so that other consortium members can access them. Data will be uploaded once per week during the sample processing phase each year and all data will be uploaded within three months of the final field sampling event each year. Processed data will be uploaded to the CEWO Monitoring data Management System (MDMS) as specified in the project contract.

1. References

Herbert, B. (2005) Feeding and growth of Golden Perch (*Maquaria ambigua*), and assessment of its potential for aquaculture. PhD thesis, James Cook University.

EPA Victoria (2003) Guideline for Environmental Management: Rapid Bioassessment Methodology for Rivers and Streams, Environment Protection Authority, Southbank, Victoria.



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