UoM Commercial Ltd

Standard Operating Procedures for *Macroinvertebrate monitoring*

Version 2.0

Contact Details

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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 LTIM project and should be updated throughout the life of the Long Term Intervention Monitoring Program to reflect any agreed changes to method or procedure.

Objectives and hypotheses

The objective of macroinvertebrate monitoring in the LTIM Program is to determine how Commonwealth environmental water (CEW) has contributed to macroinvertebrate diversity and biomass 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 and increase biomass in the Goulburn River, which could have important implications for fish recruitment and other ecological processes dependent on macroinvertebrates. It is hypothesised that fresh and bankfull flows will increase macroinvertebrate diversity and biomass.

Indicators

The indicators to be monitored are:

Macroinvertebrate diversity – macroinvertebrate diversity can give an indication of the condition (health) of a waterway and the types of stressors that might be having an impact there. Until recently, this was a Category II monitoring indicator and the method had been specified by the M&E Advisor. It is now a Category III indicator.

Biomass and weight of key macroinvertebrate taxa – macroinvertebrate biomass – is monitored because it is a potentially sensitive measure of ecological impacts, and has important implications for other aspects of the ecosystem, including providing a food source for vertebrates such as fish. Until recently, this was a Category II monitoring indicator that used logs as artificial substrates. We will use a different type of artificial substrate (i.e. onion bags) and replicated edge sweep sampling, to measure the biomass of key macroinvertebrate taxa (e.g. shrimp, prawns, and other large individuals that be a significant component of native fish diets).

Biomass of large-bodied crustaceans – following the 2016 floods, monitoring effort was diverted to assessing whether there was a substantial increase in biomass of large-bodied crustaceans post-flood. These data are to be compared to equivalent data sets collected during (presumably) non-flood years in year 4 and 5 of the program.

These indicators will contribute to a better understanding of how environmental flow delivery in the lower Goulburn River can affect the diversity, biomass and lifecycle of macroinvertebrates, which has important implications for the river in terms of the services and functions provided by macroinvertebrates. One specific function is the role of both adult and juvenile macroinvertebrates as an important food source for other riverine species, especially fish. 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.

Locations for monitoring

The monitoring will occur at three sites (Table 1):

Zone 2 (McCoys Bridge, Goulburn River)

Zone 2 (Loch Garry, Goulburn River) – Crustacean biomass monitoring only

Outside of zones 1 and 2 (Central Avenue, Broken River) – control site for Goulburn River effects

The Broken River site was chosen as a reference site for investigating the effects of CEW flows on macroinvertebrates. There are many other factors present at a site that could affect macroinvertebrates (e.g. water and air temperature, seasons). The close proximity of the Broken River site to the Goulburn River site means many of these factors will be similar between the sites, while the factor of interest (CEW flows) will only affect the Goulburn River site. Thus a comparison between the two sites will give insight into how CEW flows, rather than variations in other factors, are contributing to changes in the macroinvertebrate fauna.

Table 1: Location of macroinvertebrate diversity and biomass assessment sites

Site Name	LTIM Zone	Map Zone	Easting	Northing
Goulburn River at McCoy's Bridge	2	55	330771	5994884
Broken River at Central Avenue	NA	55	362105	5966888
Goulburn River at Loch Garry	2	55	345933	5966033

Timing and frequency of sampling

The text below is based on the original SOPs for the Goulburn River LTIM Project macroinvertebrate monitoring, with the omission of references to the Yellow Sticky Trap (YST) method of data collection, which was removed from the program in Year 4. Methods for crustacean biomass monitoring, added to the program in Year 3, are detailed in an extended version of Attachment 4, which was added to the SOP in 2016 following the spring floods.

Two sampling methods will be used to assess macroinvertebrate diversity and biomass: Replicated Edge Sweep Samples (RESS), and Artificial Substrate Samplers. There will be two sampling events each year over the five year program for each sampling method, although the timing and frequency of sampling will depend on the method employed (Attachment 1). These sampling events will occur in late spring/early summer. The first sampling event will occur at least six weeks prior to the planned environmental flow release (pre-CEW sampling) to allow the removal of artificial substrates before the flow event. The second sampling event consists of sampling after the release once water levels have returned to base flow conditions (post-CEW sampling).

Macroinvertebrate diversity will be assessed twice a year using artificial substrates and Replicated Edge Sweep Sampling.

Macroinvertebrate biomass will be assessed using Artificial Substrate Samplers based on those used in macroinvertebrate monitoring of the Murray River by Cook *et al* (2011), as well as Replicated Edge Sweep Sampling. Artificial substrates are deployed at the beginning of each sampling period (pre-CEW sampling and post-CEW sampling) and are to be retrieved from the field six weeks after deployment.

Water quality monitoring would occur with each site visit, which would mean at the beginning and end of each sampling period when artificial substrates or yellow sticky traps are being deployed and retrieved.

Responsibilities – 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 Kallie Townsend in conjunction with a qualified research assistant and a CMA employee. Subsequent field surveys will be conducted by the qualified research assistant and CMA employee. 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 BioSciences, University of Melbourne. Macroinvertebrate identifications will be conducted by a qualified research assistant. The contact for the laboratory is Dr Vincent Pettigrove. 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 during 2014 sampling events and a Coxswains certificate in subsequent years due to legislative changes. Staff involved in Replicated Edge Sweep Sampling (RESS) 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 RESS and Artificial Substrate Samplers 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.

Monitoring methods

Field methods

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

1. Hydrological and water quality measurements

2. Deployment of Artificial Substrate Samplers

3. Replicated Edge Sweep Samples

4. Retrieval of Artificial Substrate Samplers and final Replicated Edge Sweep Sample.

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

Artificial Substrate Samplers are adapted from Cook *et al.* (2011) and have been used in ongoing monitoring of sites along the River Murray since 1980. Each sampler consists of a cylinder of black plastic

"Gutterguard" (mesh size 10 mm, 180 mm height, 240 mm diameter) containing one and a quarter new, commercially available onion bags (1000 mm X 420 mm) as a substratum. The sampler also contains a clean river rock as ballast. The bottom of a basket is secured to the cylinder using a nylon cord, which is also used to seal the top of the sampler when it is pinched closed. The sampler is placed on the river bed at a depth of less than 1.5 m (in the photic zone) and is left for colonisation by burrowing, drifting and actively swimming macroinvertebrates for a period of six weeks. At each site 15 samplers are to be deployed. At retrieval, five randomly selected substrates are processed for diversity and abundance. This involves placing the samplers in a macroinvertebrate sorting tray. The cylinder and the onion bag are gently scrubbed to remove any macroinvertebrates into the sorting tray, which are then emptied into a sampling jar and preserved in ethanol. The jar should be given an internal and external label specifying the site, sample type, replicate and date. Any remaining substrates are processed for biomass/weight samples. A similar method is followed as above, with the exception that once the sample is in the sorting tray, large macroinvertebrates (>5mm) are live-picked from the sample and stored in an appropriately labelled jar containing 100% ethanol.

Replicated edge sweep sample (RESS) involves the use of a hand net to sample edge habitats and was formulated by the Murray Irrigation Limited Aquatic Ecosystem Monitoring Program (Gigney et al. 2007a, 2007b). 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 estimate their relative cover within the reach; from this an estimation of how much of each habitat is sampled is determined. Sampling begins downstream and moves upstream. Three to five replicate samples should be collected for each site, contingent on the extent of habitat with the lowest available cover; if there is insufficient of one type of habitat to form three samples this should be recorded as composing <10% of habitat and the area should not be resweeped. Each sample consists of ten sweeps, with a sweep covering an area of habitat of 300 mm^2 (the width of the opening on a standard sweep net). A sample should consist of each habitat type that is present in the area, with the contribution of each habitat sampled in proportion to how much of that habitat is available (e.g. in a reach with 50 % bare edge, 30 % macrophytes and 20 % snags, five sweeps should be taken in bare edge habitat, three in macrophytes and two over the swags). Samples should only be collected from water that is waist deep or shallower. Each sweep should be conducted using vigorous sweeping motions in a 300 mm^2 section with two sweeps in an upstream direction, two sweeps towards the bank at a right angle to the upstream sweeps, and one final sweep in the upstream direction. In larger rivers 50 % of a sample should be taken from each bank. The netted sample should then be rinsed in the river to remove fine sediments from the sample. Invertebrates attached to the sides of the net should be brushed down and coarse leaves and twigs discarded. The contents of the net should then be emptied into a bucket half full of river water than has been sieved to remove invertebrates. The contents of the bucket should then be washed through a 10 mm sieve followed by a 250 µm sieve. In 2014-15, the sieve samples and sluice sediment should be rinsed three times to remove fine sediments, and then the sieved contents should be transferred to a sample jar, preserved in ethanol, and labelled internally and externally. To decrease the time taken to process these samples in the laboratory, from 2015-16 onwards these macroinvertebrate samples were live-picked in the field by a qualified and experienced research assistant. Macroinvertebrates were picked from the sample for a total of 30 minutes and stored in appropriately labelled jars containing 100% ethanol. Information about each sample should be recorded on a macroinvertebrate data sheet, including percentage composition of habitats within the reach, and the composition of sweeps in each sample.

In addition to the samples collected above, from 2015-16 onwards an additional five RESS samples were collected for biomass/weight of key taxa. The methods followed are the same as those outlined above, although the area covered for each replicate sweep sample was recorded, and only large macroinvertebrates (>5mm) are live-picked from the samples.

Laboratory methods

Artificial Substrate samples and RESS samples are processed in the laboratory by sorting and identifying macroinvertebrates within the samples. The process involves tipping the entire contents of a sample jar 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 in tap water, with larger debris removed by hand after these have been checked for macroinvertebrates. After no more fine particulate matter can be rinsed from the sieve and all large debris have been checked and removed, the contents of the sieve are then transferred to the 100-cell sub-sampler (Marchant sub-sampler) or a quarter tray for smaller samples. The sub-sampler is filled with water to just below the cell walls and then the sub-sampler is shaken vigorously to evenly distribute macroinvertebrates across the cells. A random numbers table is used to select which 5 cells will have their contents identified. This gives a sub-sample of 5 %. The 5 % sub-sample is transferred

into a 150mL screw top jar and preserved in 69 % ethanol. The sample details are recorded on a Species Level Identification Sheet. Some of the subsample is transferred to 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 and removed from the channel and placed into 5 mL vials. The macroinvertebrates should be placed into vials according to major taxonomic groups (e.g. Order), and a tally should be made of how many macroinvertebrates are placed in each vial on the Species Level Identification Sheet. Every tray should be scanned for macroinvertebrates more than once; if a macroinvertebrate is found during the second scan then a third scan should be conducted. Once all macroinvertebrates have been removed the residue should be stored in a 500 mL sample jar and labelled with sample details, % sub-sample sorted and "RESIDUE" for quality control purposes. The process is then repeated for the rest of the subsample until the entire subsample has been searched for macroinvertebrates. If more than 300 macroinvertebrates have been found then subsampling is complete; if less, then another random numbers table is generated to pick five more cells that will sorted. This means the total sub-sample becomes 10 %. The subsample is then sorted for macroinvertebrates as described above. If at the end of this process there are still fewer than 300 macroinvertebrates repeat the process again. At the end of the sorting process record what percentage of the sample was subsampled, and return any remaining part of the sample that has not been sorted to the 500mL sample jar, ensuring it is labelled with sample details, remaining % unsampled, and the word "UNSORTED". The macroinvertebrate vials should then be filled with 69 % ethanol for preservation until identification; only 50 % of the vial should be filled with macroinvertebrates to ensure preservation, so some groups may need to be divided across several vials. The contents of the vials can then be identified to the lowest taxonomic levels possible using current macroinvertebrate identification keys. The taxon name and number of individuals belonging to that taxon should be recorded on the Species Level Identification Sheet. From this the number of individuals sorted for each major taxonomic group/Order should be calculated and compared to the original number previously sorted, and a note should be made to account for any discrepancies between these. Once identifications are complete the operator should write their initials in the column next to the applicable major taxonomic group/Order, and a dot marked on each vial that has had its contents identified. Coleoptera adults and larvae of the same taxon should be sorted and recorded separately, with identifications specifying "adults" or "larvae". Chironomidae are often numerous and can be subsampled before identification and counting so that only 60 randomly chosen individuals are identified. Chironomidae may need to be mounted on slides for identification. Pupae of all taxa except Chironomidae 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.

Biomass will be measured by removing collected macroinvertebrates from the sample jars and ethanol, and allowing these to air dry for 24 hours (ensuring samples do not become mixed up). Each sample is then placed on a small aluminium foil dish and dried in the oven at 60°C for 48 hours. The sample is then removed, allowed to cool, and then the sample (on the foil dish) is weighed. The sample is then removed from the dish and the dish is re-weighed. The weight of the animal can be determined by subtracting the weight of the dish from the weight of the dish with the animal.

Data analysis

Macroinvertebrate diversity is to be calculated and assessed by using Australian Rivers Assessment System (AusRivAS) for RESS samples only, where a prediction of the expected number of taxa for a site is compared to the number of taxa observed at that site, with comparisons then made between pre-CEW and post-CEW periods for each site to determine what effect CEW has on macroinvertebrate diversity. This involves the construction of a list of taxa that are expected to occur at that site based on the literature and expert opinion. The flow habitat requirements of each species should be determined based on literature and expert opinion, and should be classified into one of the following six categories: critically dependent (specialist taxa requiring flow throughout their lifecycle), significantly dependent (taxa requiring flow for at least part of their lifecycle), dependent (taxa with life stages not directly affected by flow but where individual

fitness and species abundance can increase), tolerant (taxa with life stages not directly affected by flow but with abundance decreased), minimally disturbed (taxa with at least one life stage impacted by flow), disturbed (taxa cannot tolerate flow).

Macroinvertebrate abundance is to be calculated by determining the number of taxa and abundance of individual taxa observed in the Artificial Substrates Samples and RESS (which is semi-quantitative), and comparing this between pre-CEW and post-CEW sampling events. Multivariate and descriptive statistics should be used to compare the influence of CEW on macroinvertebrate abundance and community composition during single sample events, and the cumulative influence of flow on macroinvertebrate abundance of the entire sampling period.

Macroinvertebrate weight and biomass are determined for the artificial substrate samplers by comparing the weights of different key taxa groups collected in pre- and post-CEW sampling to determine if the environmental watering caused an increase in the growth of taxa or encouraged the colonisation of more small individuals. Biomass can also be determined for the RESS samples by reporting the weight of the key invertebrate taxa as a function of the area of edge habitat sampled, allowing comparisons between sites and before and after a CEW event.

Reporting

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

Macroinvertebrate diversity reporting using the RESS protocol should include data for the site, date of assessment, sample type, number of taxa, abundance of taxa, AusRivAS scores, and composition of communities (e.g. number of taxa and abundance of taxa belonging to each of the flow habitat types).

Macroinvertebrate biomass (abundance) reporting should involve specifying the results of analyses for investigating the effects of CEW flows on macroinvertebrate abundance and community composition, in addition to specifying the sample types (Artificial Substrate Samplers or RESS) being analysed, site details, sample dates, number of taxa and number of individuals per 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.

Quality assurance/quality control

- All persons conducting the RESS sampling must have successfully completed to EPA Victorian AusRivAS and Rapid Biological Assessment Competency course or have a minimum of five eyars experience conducting sweep sampling or be supervised by someone with the above the qualifications.

- All persons conducting Artificial Substrate Sampler deployment and retrieval must have experience with this method or be trained by someone with experience.

- 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 (Attachment 2).

- 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 defined in Attachment 3. 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.

References

Cook, R., Paul, W., Hawking, J., Davey, C. and Suter, P. (2011). River Murray Biological (Macroinvertebrate) Monitoring Program – review of monitoring 1980-2009. Final Report prepared for the Murray-Darling basin Authority by the Murray-Darling Freshwater Research Centre, MDFRC Publication 34/2011, May, 150pp.

Gigney, H., Hawking, J., Smith, L., and Gawne, B. (2007a). Murray Irrigation Region Aquatic Ecosystem Monitoring Program Development: 2005 Pilot Study Report. A report for Murray Irrigation Limited.

Gigney, H., Hawking, J., Smith, L., and Gawne, B. (2007b). Murray Irrigation Region Aquatic Ecosystem Monitoring Program: Protocols handbook. Report prepared for Murray Irrigation Limited.

Attachments

Attachment 1. Frequency and timing of macroinvertebrate monitoring activities. Pre-CEW sampling period is 6 weeks prior to planned Commonwealth environmental water (CEW) release. Post-CEW sampling period is a 6 week period after CEW release once water levels have returned to base flow levels. The additional collection of adult emergence samples during 2015 onwards would be collected as part of a PhD, and is not a directly funded component of the LTIM project.

Sampling period

Activity	Method	2014			2015			2016			2017			2018		
		Pre-CEW	During CEW	Post-CEW	Pre-CEW	During CEW	Post-CEW	Pre-CEW	During CEW	Post-CEW	Pre-CEW	During CEW	Post-CEW	Pre-CEW	During CEW	Post-CEW
Water quality		Twice		Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice	Twice
Diversity	Replicated Edge Sweep Sample	Twice	-	Twice	Twice	-	Twice	Twice	-	Twice	Twice	-	Twice	Twice	-	Twice
Biomass	Artificial Substrate Sampler	6 weeks	-	6 weeks	6 weeks	-	6 weeks	6 weeks	-	6 weeks	6 weeks	-	6 weeks	6 weeks	-	6 weeks

Attachment 2. Field data sheets to be completed for each sampling method.

Attachment 2.1 Artificial Substrate Sampler

Project:	
Site name:	**Site number:**
Sheet completed by:	**Sheet checked by:**
Deployment date:	**Retrieval date:**
River/wetland height deployment:	**River/wetland height retrieval:**

Artificial Substrate Sampler replicate number	Image numbers	Locator notes	Retrieval notes
1			
2			
3			
4			
5			
6			
7			
8			
9			
100			

Attachment 2.2 Replicated edge sweep sample data sheet

Project:		
River:	**Site:**	**Number:**
Date:	**Samplers:**	**Sheet checked by:**

Edge habitat composition in reach

Habitat	% present	Metres per sweep sample
Macrophyte		
Litter		
Bare		
Snag		

% habitat within reach	0%	25%	50%	75%	100%
No. 1 metre sweeps required	0	1	2	3	4

Edge habitat composition in samples

Sweep composition	Bank	Description
1 metre	Near [ ] Far [ ]	
2 metre	Near [ ] Far [ ]	
3 metre	Near [ ] Far [ ]	
4 metre	Near [ ] Far [ ]	

Sweep no.	No. of jars
1	
2	
3	

Comments

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Note: near bank = bank where boat is launched

Far bank = bank opposite where boat is launched

Attachment 2.4 Mud map. This is a sketch of the site showing major landscape features (including roads, access points, vegetation types etc), the location of Artificial Substrate Samplers and Yellow Sticky Traps, and the location where Replicated Edge Sweep Samples were taken.

Attachment 3. Standard data format for macroinvertebrate data to be submitted to the CEWO.

Each row of data will contain the following information

Variable	Description	Type	Required	Range
AssessmentUnitID	A site that covers two 100m stretches of the channel, within which the diversity of habitats are represented and sampled	string	Yes	
dateStart	Start data (inclusive) that these measures were observed	dateTime	Yes	
dateEnd	End date (exclusive) that these measures were observed	dateTime	Yes	
HigherTaxaName	Latin name of Order, Class, Phylum for taxa that cannot be identified to family or below	string	Yes	
FamilyName	Latin name of Family	string	No	
GenusName	Latin name of Genus	string	No	
SpeciesName	Latin name of species	string	No	
NumberIndividuals	Total number of individuals (after multiplying up subsamples)	Number (8 decimals)	No	
SampleType	The types of sampling used to determine the total number of individuals	Category	Yes	RESS Artificial Substrate sampler

Attachment 4. Variation to monitoring from 2016/17 onward following spring 2016 flooding

Introduction and purpose

Conceptually, the 2016 floods in the Goulburn River were likely to have delivered a large pulse of carbon from the floodplain to the river channel. Such inputs are potentially important for riverine foodwebs and could lead to a large increase in the abundance and biomass of macroinvertebrates.

Previous macroinvertebrate monitoring for the LTIM has shown that crustaceans (mainly *Paratya* and *Macrobrachium* shrimp) account for a large proportion of the macroinvertebrate community biomass in the lower Goulburn River. These shrimp species feed on biofilms that grow on macrophytes, snags and other hard surfaces in the water column. A large injection of bioavailable carbon is likely to increase biofilm production and therefore we hypothesise that the floods would have led to an increase in the abundance and/or biomass of shrimp in the river. Qualitative observations from our scheduled macroinvertebrate sampling event in early December 2016 indicated that shrimp were more abundant compared to 2015 and 2014.

Based on these observations and conceptual understanding, the Goulburn River LTIM M&E Providers will conduct a supplementary monitoring program to quantify the abundance and biomass of shrimp in the lower Goulburn River from 2016/17 onward. The purpose of the monitoring program will be to determine whether the abundance and biomass of shrimps following large floods was greater than subsequent years (which presumably will not have overbank floods). Because shrimp represent a large proportion of the macroinvertebrate biomass and feed on biofilms, we think they will be a good indicator of broader macroinvertebrate community responses to overbank floods. Moreover, they are an important food source for native fish and so understanding the effect of particular flows on this group is directly relevant to the future flow management of the Goulburn and other lowland rivers.

The remainder of this attachment describes the monitoring method.

Proposed method

Site and habitat selection

The monitoring will focus on two sites in the lower Goulburn River. The sites will be the primary macroinvertebrate monitoring site at McCoys Bridge and the vegetation/geomorphology/fish assessment site at Loch Garry.

At each site we will collect separate samples from four edge sub-habitats:
- Stands of emergent macrophytes
- Submerged snags
- Deposition areas or areas with large amounts of Coarse Particulate Organic Matter (CPOM)
- Bare edge substrates

These habitat types are most likely to support shrimp and are found at most sites in the lower Goulburn River, although the relative proportion of each habitat type may vary between sites and may vary at a site from year to year.

Sampling method

We will quantitatively sample macroinvertebrates by deploying 5-7 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 5-7 replicate samples from each habitat type 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 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. 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 (see details below).

In addition, replicated edge sweep samples (RESS) will also be conducted during each sampling following the methods outlined in the Standard Operating Procedure for Macroinvertebrate Monitoring for the LTIM program. These samples will be immediately preserved in 100% ethanol and processed in the laboratory. Only shrimp will be analysed in these samples for the same parameters outlined below (see Laboratory Processing). Comparisons between RESS and bait trap shrimp will be made in terms of abundance, biomass, reproductive status and size classes of shrimp to determine if there are habitat preferences and behavioural differences in shrimp at different life stages.

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

Sampling frequency

It is uncertain how long it will take for crustaceans to respond to the input of carbon from the recent floods, nor whether the abundance and biomass of crustaceans will steadily increase, plateau and decline or follow several peaks and troughs as different cohorts grow. For these reasons we propose to sample the crustacean community in December 2016 and then approximately monthly until March 2017 (4 trips), which is when we expect macroinvertebrate productivity to begin to decline. Sampling times in the following years will be largely matched to these times, with the addition of extra 'early' trips in 2018-19 (see budget).

Laboratory processing

Each preserved sample is to be processed separately so that data can be assigned to a single bait trap. The following measurements will be taken for each sample:

- All crustaceans 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).
- Total dry weight of each species from each sample will be recorded to determine biomass.

- The reproductive status of females will be recorded.

All data will be entered into prepared data spreadsheets and saved on the LTIM Central Data Repository at the University of Melbourne.

Budget

Budget for the crustacean monitoring program is sourced from the 'pre-flow' trips that were cancelled in 2016-17, and the removal of Yellow Sticky Trap monitoring for the final two years of the project. Collectively, these reductions provide sufficient funds for 14 samples as described above.

Four trips were undertaken in each of 2016-17 and 2017-18, from December to March. For the final year (2018-19), an extra two trips will be undertaken before December to better characterise the increase in crustacean biomass as waters warm during spring.