Pre-Harvest Survey Program

Survey Guideline - Fish Crust (V2)

VICTORIA State Government | Environment, Land, Water and Planning

Acknowledgements

Lindy Lumsden, Jemma Cripps, Graeme Newell, Matt White, Arn Tolsma, Louise Durkin, Tarmo Raadik and Jenny Nelson of the Arthur Rylah Institute. Jamie Molloy. Project Manager Pre-Harvest Survey Program

Author

Tarmo Raadik Arthur Rylah Institute

Michael Nichol Arthur Rylah Institute

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1. Aquatic Survey A: fish and spiny crayfish

1.1 Context

The high priority species for aquatic fauna survey (excluding burrowing crayfish) are the 11 species of galaxiids in the Mountain Galaxias complex (see Section 11.10 e, below), and the various species of spiny crayfish (genus *Euastacus*), many of which in Gippsland are species complexes (see Section 11.10f, below). Bluenose (Trout) Cod and Macquarie Perch, both rated as high priority for the Pre-Harvest Program, are not included as no coupes in the TRP are located close to their distribution. For burrowing crayfish (*Engaeus*) see comments survey down, and for targeted surveys see survey guideline 11.9.

The River Blackfish in Gippsland and the Alpine Spiny Crayfish, Murray Spiny Crayfish, and South Gippsland Spiny Crayfish are considered Medium Priority species for targeting in the PHSP and they can be detected by this survey design. The South Gippsland Spiny Crayfish is the only species with a prescription and will be targeted in the program. None of the other taxa have prescriptions, and whilst not being specifically targeted, it is highly likely they will be sampled when targeting high priority species, as they may be present in the same waterways.

Many other species of aquatic fauna (fish, crayfish, freshwater mussels) may also be detected during survey, and while not the target of the PHSP, these observations are to be reported. Signs of burrowing crayfish presence within upslope or riparian areas of a coupe may be noted as distinctive, conical soil chimneys at the entrance to their underground burrows, or they may be found walking along the ground. Further, burrowing crayfish soil chimneys may also be present within dry stream beds, and individuals of four species may be found outside of their burrows, underwater along the edges of waterways. These detections should also be noted.

For species with a prescription the trigger is detection of the species. Therefore, the aim of the PHSP aquatic component is the detection of the species at a location, which may involve a single individual, or multiple individuals spread over a longer distance, depending on the specifics of the prescription with respect to protection to that species, and the length of waterways within or bounding a coupe.

Many of the species of galaxiids in the Mountain Galaxias complex and spiny crayfish will present identification issues. They are cryptic species and very similar in external morphology, poorly known as they are narrow range endemics and therefore infrequently found, have complex taxonomic keys, and are recently, or in the process of being, formally described and therefore not included in general field guides. However, it is a **fundamental requirement o**f the PHSP to at least identify the taxa to the species complex which would trigger a prescription, e.g. Mountain Galaxias, Orbost Spiny Crayfish, etc. It is therefore critical to retain a voucher of each for multiple benefits: 1) verify field based identification or provide an identification; 2) retain a specimen (curated in the Museum Victoria collection) to support the identification into the future when needed (e.g. court case, etc.); and, 3) contribute material for later taxonomic delineation of new species or comparative material for a species revision as the taxonomy is resolved.

1.2 Objectives

To detect high priority threatened fish and spiny crayfish using electrofishing and hand searching (where required) in waterways within, and immediately adjacent to, certain coupes in the PHSP.

1.3 Survey effort

Sampling of a coupe will be undertaken during a single visit, with sampling conducted during daylight hours.

The total number of, and distance between, sampling sites per coupe will vary according to the size of the coupe and length of waterways within or bounding, and species being targeted. For example, as the trigger for applying a prescription is detection, survey sites may be spread to maximize the extent of the buffer applied along the waterway within or alongside the coupe if the target species were to be detected and the prescription applied.

Specific survey effort requirements are detailed below (Section 11.8.9) in the methods section for electrofishing and hand searching.

1.4 requirements

A field survey team of two people qualified to be part of an electrofishing team (i.e. electrofishing training certification, appropriate medical clearance, wader training, defibrillator training, first aid level II certification)

Both team members to have a reasonably high level of physical fitness to walk long distances over rough terrain to reach sampling sites and to carry a large amount of sampling equipment.

Sound, practical knowledge and experience in all aspects of small fish and spiny crayfish electrofishing sampling methodology in small streams in forested catchments, particularly the practical limitations of electrofishing, and the modifications required to the technique to improve detection probability based on species rarity and local catchment conditions (e.g. water permanency, stream size, stream morphology, elevation, degree of vegetation cover, in-channel disturbance, etc.).

Experience with, and expertise in accurate identification of freshwater fish and crayfish in small foothill to upland catchments (e.g. River Blackfish, Tupong, Pygmy Perch, Freshwater Eels, Flatheaded Gudgeon etc.), and specifically with species of galaxiids (Galaxiidae), and spiny freshwater crayfish (Parastacidae). Due to the similarity of species in the *Galaxias olidus* complex, and spiny crayfish in the Gippsland area, that there are known but undescribed species and a high probability that additional undescribed species are present, it is **essential** that the common and non-threatened taxa in both groups can be accurately identified and separated from unknown specimens, so that vouchers of the 'unknown' taxa can be taken for confirmation in the laboratory.

Attention to detail to ensure that the above requirement of identifying and vouchering unknown and potentially new taxa is followed

1.5 Equipment for the technique

- ☐ Portable, commercially available backpack electrofishing unit, battery powered, suitable for low conductivity water (maximum >800V output and >2000W power), with adjustable frequency and able to operate at 80–120 Hz, with quick release safety harness
- ☐ Anode ring covered with <6 mm stretch mesh
- ☐ Anode pole of 1.5 m or greater in length
- ☐ Spare complete anode pole and anode ring
- ☐ Electrofisher unit battery plus at least two spares (fully charged), including battery charger
- ☐ Siliconized rain cover for electrofishing unit when in damp vegetation
- ☐ Multimeter for testing batteries and electrofishing unit/poles
- ☐ Lineman's electrical gloves rated and tested at a minimum of 1,000 volts, for each team member
- ☐ 1 x waterproof chest high waders with cleated sole boots and 1 x spare, per team member
- ☐ 1 x polarized sunglasses per team member to reduce glare from the water surface
- ☐ Electrofishing safety equipment (portable defibrillator, EPIRB, portable first aid kit)
- ☐ Small (aquarium) fine mesh (1 mm) dipnets (2 +) and larger (2 mm mesh x 200 mm x 200 mm) dipnet on 1.2 m long handle to collect stunned fish or crayfish
- ☐ Large, solid plastic bucket with tight fitting lid for temporary storage of collected fauna
- ☐ Large, appropriate hiking backpack to carry non-electrofishing field equipment
- ☐ Dry bags to protect smaller electronic equipment
- ☐ Waterproof or water resistant digital camera for habitat and general images of fauna
- ☐ Calibrated water quality meter suitable to record water temperature, and water electrical conductivity (reported as $\mu S.cm^{-1}$ @ 25 ^{o}C) to guide adjustment of electrofishers settings. Other water quality parameters optional (dissolved oxygen, turbidity, pH, total hardness, etc.)
- ☐ Vouchering equipment for galaxiids and crayfish: follow details in attached protocol for fixation (fish) or preservation in ethanol (crayfish) of specimens for laboratory identification or verification
- ☐ 2 x GPS units
- ☐ 1 x hand-held compass
- ☐ Appropriate spare batteries for non-electrofishing equipment
- ☐ Appropriate aquatic fauna field guides, including more specialised texts for target fauna, i.e. Coughran et al. 2015, Horwitz 1990, Morgan 1986, Morgan 1997, Raadik 2014.
- ☐ Aquatic fauna data sheets on waterproof paper on clipboard x 1, including HB pencils, sharpener, eraser, etc., or rugged waterproof laptop, tablet, PDA or mobile phone for digital data collection
- ☐ Waterproof field pocket-notebook plus pencils
- ☐ Large plastic bags suitable to carry voucher specimens back to the vehicle for vouchering
- ☐ Suitably sized (height, width, depth) plastic jars in which to place voucher specimens so that they are not bent, twisted or crammed.
- ☐ 100% ethanol for preservation of crayfish (burrowing and spiny) voucher material

☐ Formaldehyde solution (buffered, 35–38% w/w AR grade) for fish fixation.

☐ Waterproof labels for use with voucher material with the following fields: Waterbody Name, Site Code, Date, Collector Names, and indicate if sample is 'DRY', 'Formalin' or 'Ethanol'

1.6 Site preparation

The general location of the survey sites should be pre-determined (e.g. via desktop assessment), based on aspects of the stream network which may maximise available aquatic habitat, consider distance from access tracks (e.g. avoid sites more than 1.5 km from access tracks or more than 1 hour to walk in, etc.), and the coverage of the stream network (permanent stream, intermittent stream, drainage line) afforded if a prescription is triggered by the detection of a particular species.

Sites may be located within or adjacent to a coupe, they may also be located further downstream of necessary, but within the distance over which a triggered prescription would apply to ensure the detection was relevant to the coupe. For example, a detection of a species which has a prescription to apply a buffer 1 km upstream and downstream of a detection point would be relevant to a coupe if the detection site was up to 1 km downstream of a coupe, though this would not afford the waterways alongside or within the coupe any protection, whereas a detection point less than 0.5 km downstream of the coupe would provide a buffer to waterways extending at least 500 m upstream into the coupe.

1.7 Navigation

Use a smart phone, PDA, tablet or laptop with geo-referenced 1: 25,000 scale (or similar detailed) topographic raster maps (e.g. Avenza or OruxMaps smartphone applications, ESRI arcpad, Windows software, etc.), or a GPS, tablet or laptop with suitably detailed vector topographic maps loaded to navigate to the pre-selected survey point, or to additional survey sites established based on survey results.

1.8 Conducting the survey

Survey from late October to late Autumn, depending on streamflow/rainfall, though sampling can be undertaken during dry times from September to early June. Avoid instream sampling during periods of higher stream flows and turbid water (usually from recent rainfall events), as this will reduce survey efficiency and decrease the probability of detection of target taxa.

Undertake surveying during daylight hours.

Ensure that the electrofishing equipment is operational before entering the coupe.

On arrival at a survey location, determine if water is present and whether there is a defined stream channel (as per Code specifications) or a drainage line without channel.

Record if site is dry (this is a valid survey outcome and data can be entered on the VBA as 'Misc Dry'), and visually search the channel and nearby stream banks (also lift rocks, timber debris, vegetation and dry leaves) for signs of spiny crayfish and/or burrowing crayfish burrow entrances or exoskeletons (approximately 20 minutes or over a distance > 15 m, focussing on appropriate habitat). Record whether there is a defined stream channel (as per Code specifications) or a drainage line without a channel.

If the site contains water, record if it is still or flowing, and note clarity (e.g. clear, tannin, turbid) and select a location to commence electrofishing and record its GPS location (decimal latitude/longitude).

- Assess and record water electrical conductivity/temperature and adjust electrofishers settings for voltage and percent of range to maximise electrofishing efficiency for small fish and spiny crayfish (note: frequency should be set to >80- Hz). Record all these details. Reset electrofishing power-on or seconds counter to zero.
- Commence electrofishing, moving in an upstream direction, sampling areas of within-channel habitat appropriate for the target fauna to maximise detection. Sampling may be non-continuous (spot sampling) and should target the stream edges/banks and substrate, including areas of dense structural habitat. The dip netter should follow the electrofishing operator searching for missed animals, and for signs of crayfish which have been disturbed and may emerge from cover a short time following the application of electricity.
- If target taxa are located (e.g. fish and or spiny crayfish), cease sampling, unless additional sites/detections nearby are required to increase the extent of waterway protection based on the prescription for the taxa. Otherwise undertake sampling up to 30–45 minutes, depending on stream size and habitat attributes related to the target taxa.

- If no spiny crayfish are detected during sampling, target shallow instream areas composed of rock (boulder, cobble, pebble) for additional sampling by hand: remove rocks searching for the presence of spiny crayfish (duration 15–30 minutes per person x 2).
- Use bright (e.g. >700 lumens) head-torches (both team members) in low light conditions, such as early morning/late evening, heavy shading by vegetation, cloudy day, etc.
- For broader biodiversity benefit, also record the species and number of individuals of non-target fauna detected during sampling. This includes the presence of spiny crayfish or burrowing crayfish burrows along the water's edge, burrowing crayfish burrows up the banks, and the presence of live or dead mussel shells instream, or dead shells along the banks (these can be placed into plastic ziplock bags for later identification).
- Record the 'time start' and 'time end' for the sampling event (= elapsed time in minutes), the electrofishing power-on time (seconds), the sample end GPS location, estimate the distance travelled (metres) and average wetted channel width, and estimate average and maximum water depth (m). Also record whether electrofishing was undertaken as 'spot' or 'continuous' sampling along the survey reach.
- Retain voucher specimens of target fauna (i.e. galaxiids in the Mountain Galaxias species complex (3 representative larger specimens), species of spiny crayfish other than Gippsland Spiny Crayfish (2–3 representative larger/mature specimens, preferably with both claws and including **at least one male**). Take sharp and clear digital images of the specimens before vouchering. A hand lens may be required to help in determining the sex of spiny crayfish.

Depending on survey results, coupe size (e.g. for prescription coverage to maximize protection for target taxa), etc. determine if additional survey sites are required before completing the sampling program for the coupe.

Ensure that all required information is entered onto the data sheet (hard copy or electronic).

1.9 Preparation of Voucher Material

Fish

The accurate identification of the cryptic galaxiid species requires examination of voucher material which retains the body morphology as close to life-like as possible. This is achieved by a process of fixing, where water is replaced in the cells with a compound (Formaldehyde) which solidifies the cells and does not cause bending, twisting or shrinkage. A 10% formalin (Formaldehyde + water) solution is used.

1. Select a suitably sized container to avoid bending or twisting the specimens and allow > 40% of the container volume for the liquid.
2. Half fill the container with freshwater and the specimens required for vouchering, including a waterproof label (details above).
3. Add more water until you estimate the container is 85-90% full.
4. Carefully fill the rest of the container with the Formaldehyde solution, seal the lid, and place into an airtight storage vessel.

In this way the specimens are in a ~90% formalin solution (1 part Formaldehyde solution : 9 parts water). This method also minimises exposure to the Formaldehyde solution.

When the voucher specimens are returned to a taxon expert (within a week of collection), they will need to be soaked in freshwater over two days to remove any traces of unfixed formalin, and then placed into 70% ethanol for longer term storage.

Protocols for the safe decanting, fixation process and washing process for formalin will be supplied.

Spiny Crayfish

Spiny Crayfish have a hard exoskeleton and are therefore not prone to shrinkage or deformation when vouchered. Consequently, laboratory grade ethanol can be used, and this will penetrate the specimen and preserve tissue. A 100% solution of ethanol is used.

1. Select a suitably sized container to avoid bending or twisting the specimens and allow > 40% of the container volume for the liquid.
2. Place the specimens required for vouchering inside the container, including a waterproof label (details above).
3. Add enough 100% ethanol to cover the specimens by approximately 40 mm.
4. Place the container into an airtight storage vessel, though separate from the formalin container.

1.10 Data reporting requirements

Data requirements are outlined in Aquatic Fauna datasheet.

PHSP data is to be reported back to the PHSP team in accordance with the procedures outlined in the SOP.

The additional aquatic survey data collected (see above), not required by the PHSP but valuable more broadly from a biodiversity perspective, must be included in the VBA data upload.

surveys that failed to detect the target species shall also be reported (e.g. record species, and number of each, of all other taxa sampled.

Also record if no aquatic taxa are sampled at a site with water (No Taxa)

Also record if an aquatic site was dry (Dry), as this is still an aquatic survey outcome.

2. Aquatic Survey B: Burrowing crayfish

2.1 Context

The high priority species of burrowing crayfish in forested catchments are the Narracan Burrowing Crayfish, Strzelecki Burrowing Crayfish, and the Curve-tail Burrowing Crayfish, with medium priority species being the Mallacoota Burrowing Crayfish and the Warragul Burrowing Crayfish. All except the Curve-tail Burrowing Crayfish have prescriptions. However, of the four with prescriptions, only the Narracan Burrowing Crayfish and Warragul Burrowing Crayfish potential have coupes on the TRP within their known or presumed rang, or nearby (1 and 8 respectively) and so will be targeted in the PHSP. No planned coupes are within or near the distribution of the Mallacoota Burrowing Crayfish or the Strzelecki Burrowing Crayfish.

The Curve-tail Burrowing Crayfish is considered high priority for the PHSP and will be targeted for sampling as its range extends the Yarra River at Warburton, past Noojee to Mount Baw Baw in the upper La Trobe River system.

Many other species of aquatic fauna burrowing crayfish may also be detected during survey, and while not the target of the PHSP, these observations are to be reported.

For species with a prescription the trigger is detection of the species. Therefore, the aim of PHSP aquatic component is the detection of the species at a location, which may involve a single individual, or multiple individuals spread over a longer distance, depending on the specifics of the prescription with respect to protection to that species.

Species of burrowing crayfish can be difficult to accurately identify as they are small and morphologically similar; accurate identification in the field is very difficult. They are cryptic species, spending most of their time underground, poorly known by most aquatic biologists, rarely encountered in stream surveys, and have a complex taxonomic key (Horwitz 1990), with many important taxonomic characters too small or difficult to see in the field, or hidden, such as pores between the legs. Consequently, a high level of taxonomic expertise and familiarity with burrowing crayfish morphological characters and taxonomy is required, as a **fundamental requirement** of the PHSP is accurate species identification. As the crayfish are not widely included in general field guides, it is essential to collect voucher material, which will have multiple benefits: 1) verification of field-based identification or provision of an identification; and 2) retention of a specimen (curated in the Museum Victoria collection) to support the identification into the future when needed (e.g. court case, etc.).

2.2 Objectives

To detect high and medium priority threatened burrowing crayfish using burrow tube traps and visual searching (where required) in riparian zones within coupes, and along waterways within and bordering certain coupes, in the PHSP.

2.3 Survey effort

Sampling of a coupe will be undertaken during a single visit, with searching conducted during daylight hours and tube trapping conducted over night.

The total number of, and distance between, sampling sites per coupe will vary according to the size of the coupe and length of waterways within or bounding, and species being targeted.

Specific survey effort requirements are detailed below (Section 11.9.9).

2.4 Staff requirements

A field survey team of two people, with at least one with previous expertise in visual surveys for signs of borrowing crayfish (crayfish tunnel opening soil mounds)

Previous expertise in the use of burrow tube traps to detect burrowing crayfish

Both team members to have a reasonably high level of physical fitness to walk long distances over rough terrain to reach sampling sites and to carry sampling equipment

Experience with, and expertise in burrowing crayfish taxonomic characteristics and accurate identification. Due to the similarity of species, their small overall size and the small size of diagnostic morphological characters, field identification will be extremely difficult. It is therefore **essential** that field identifications are validated more detailed examination in the laboratory.

2.5 Equipment for the technique

- ☐ 30 burrowing crayfish tunnel tube traps per site (maximum three sites) made to specification in Bryant et al. 2015.
- ☐ Small (aquarium) fine mesh (1 mm) dipnets (2 +) and larger (2 mm mesh x 200 mm x 200 mm) dipnet on 1.2 m long handle to collect stunned fish or crayfish
- ☐ Large, solid plastic bucket with tight fitting lid for temporary storage of collected fauna
- ☐ Large, appropriate hiking backpack x 2 to carry traps and equipment
- ☐ Dry bags to protect smaller electronic equipment
- ☐ Waterproof or water resistant digital camera for habitat and general images of fauna
- ☐ Vouchering equipment for crayfish: follow details in attached protocol for preservation in ethanol of specimens for laboratory identification or verification
- ☐ 2 x GPS units
- ☐ 1 x hand-held compass
- ☐
- ☐ Appropriate aquatic fauna field guides, including more specialised text, i.e. Horwitz 1990,
- ☐ Aquatic fauna data sheets on waterproof paper on clipboard x 1, including HB pencils, sharpener, eraser, etc., or rugged waterproof laptop, tablet, PDA or mobile phone for digital data collection
- ☐ Waterproof field pocket-notebook plus pencils
- ☐ Suitably sized (height, width, depth) plastic jars in which to place voucher specimens so that they are not bent, twisted or crammed.
- ☐ 100% ethanol for preservation of crayfish voucher material
- ☐ Waterproof labels for use with voucher material with the following fields: Waterbody Name, Site Code, Date, Collector Names, and indicate if sample is ‘DRY’, ‘Formalin’ or ‘Ethanol’

2.6 Site preparation

Specific to the three target species of burrowing crayfish is that their borrows are mainly found on the banks and floodplain of waterways, with the Curve-tail Burrowing Crayfish also found in burrows extending a short way upslope. Therefore, surveys for these taxa should be restricted to these zones. See also information Section 11.8.6.

2.7 Conducting the survey

Survey from Spring to late Autumn, with suspected higher catch rates during periods of greater crayfish activity (seasonal or local conditions such as rainfall or warm moist weather), though this is largely unknown for the target taxa.

Undertake visual searches during daylight hours.

On arrival at a survey location, search the waterway banks and floodplain for evidence of crayfish burrows by the presence of canonical soil chimneys at burrow entrances (applicable for Narracan Burrowing Crayfish and Curve-tail Borrowing Crayfish), or potential burrow openings with reduced to no soil chimneys (Warragul Burrowing Crayfish). This should also include a visual search for live crayfish or exoskeletons by searching at the base of vegetation (i.e. tree ferns), under timber debris, etc. (Approximately 30 mins over an area > 30 m in length x 5–10 m wide per person).

Where crayfish burrows are detected, deploy 30 burrow tube traps in the afternoon, placed > 1 m apart (potentially reducing number of traps placed into a single burrow with multiple entrances). Focus on burrow entrances with evidence of recent activity (e.g. fresh soil pellets). Deploy traps overnight for >12 hours.

Depending on coupe size, etc. determine if additional survey sites are required and repeat the process detailed above.

The following morning collect tube traps from each sampling location and check for captured crayfish.

Record the ‘time and date start’ and ‘time and date end’ for the sampling event (= elapsed time in minutes), and the sample reach start and end GPS location.

Record the GPS location of each trap with a burrowing crayfish and take a digital image.

Retain voucher specimens to confirm identification in the laboratory (see Section above for method of preservation). A hand lens will be required for preliminary identification.

Ensure that all required information is entered onto the data sheet (hard copy or electronic).

2.8 Data reporting requirements

Data requirements are outlined in the Aquatic Fauna datasheet.