Charles Darwin University, Animal Ethics Committee
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Charles Darwin University Animal Ethics Committee

Standard Operating Procedure:

GSOP 07.2024 TRaCK: Field Manual Including protocols for quantitative sampling of fish assemblages, habitat, water quality and sample preservation.

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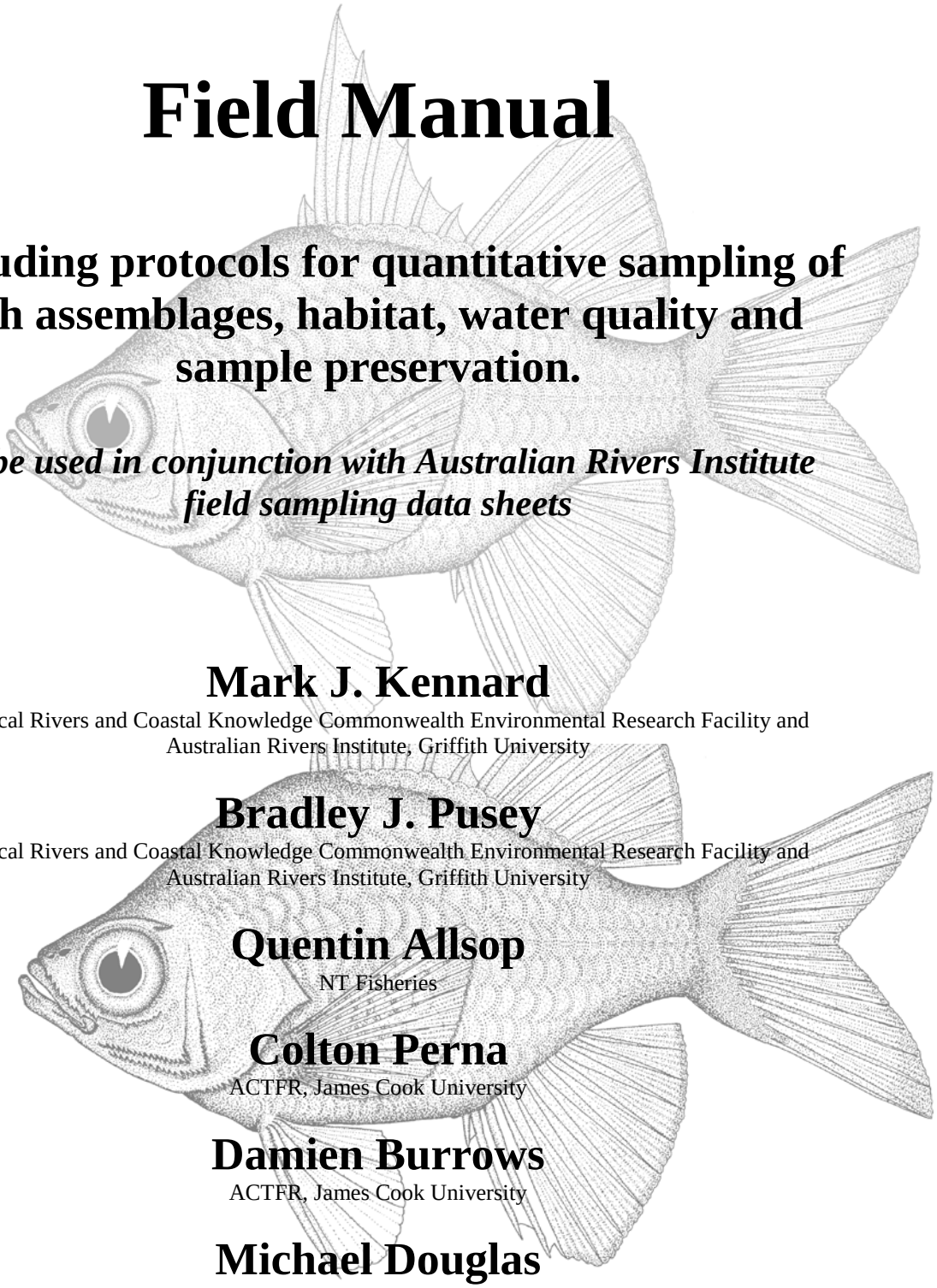
Field Manual

Including protocols for quantitative sampling of fish assemblages, habitat, water quality and sample preservation.

To be used in conjunction with Australian Rivers Institute field sampling data sheets



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1.0 Introduction

1.1 Overview and objectives of the manual

- This protocol manual explains the sampling methods used to sample fish and aquatic habitat in a number of TRaCK related projects across northern Australia. Whilst this sampling protocol has been developed for previous specific projects, it is a useful guide for designing future monitoring programs. The protocol is based on significant prior examination of the suitability of sampling methods across a variety of applications and is intended to provide a relatively rapid and rigorous framework for sampling programs using freshwater fish as monitoring targets.

1.2 Objectives of the research programs in which this protocol has been used

- This sampling protocol has been used in research programs in which information on the spatial and temporal variation in the composition of fish assemblages over a range of spatial scales has been collected. The key questions have been concerned with quantifying variation in the distribution and abundance of freshwater fish at various spatial scales how much do fish communities change over time. Such investigations must be mindful of various trade-offs between sampling intensity at individual locations necessary to accurately define the fish assemblage at that location and to allow a meaningful comparison of assemblage composition at any other time, and the extent of sampling undertaken at broader spatial scales (i.e. basin). Given that resources are often limited, decisions about how many sites are needed and how intensively individual sites are examined (which equates to how much time is spent at individual locations) are frequently necessary. What type of data is collected is another decision needing to be made.

- To satisfy the objectives of monitoring programs a range of data could be collected;
    - fish species composition
    - fish species abundances
    - fish species size (length) distributions
    - fish species distributions throughout the catchment
    - aquatic habitat characteristics of the study sites. Often this involves quantitative assessment of habitat at multiple points within each site (i.e. at each electrofishing shot location).

- A small number of individuals of certain fish species may also be retained (and preserved) from each site for the following potential reasons:
    - To enable accurate identification in the laboratory
    - To weigh and measure in the laboratory such that length-weight relationships can be generated if required for biomass calculations (for those species for which we don't already have LW equations)
    - Genetic analysis

  - Diet analysis
  - Reproductive status, fecundity, egg size, etc

- This Field Manual outlines the following
  - Sampling design and site selection (Section 2)
  - Fish sampling protocol including sample preservation (Section 3)
  - Habitat sampling protocol (Section 4)

2.0 Sampling Design

2.1 What constitutes a study site?

- A study site is defined here as being a section of river/stream (hereafter termed a study reach) that is broadly similar in terms of fluvial geomorphology, hydrology, etc. In practice, study reaches will usually be less than 1km in length.
- Within each selected study reach, fish and habitat sampling is conducted with the intention of characterising as much of the environmental and biological variation possible.
- Multiple samples of fish and habitat are collected within each study reach. These data allow within-site variability to be quantified, and the data from multiple samples can be summarised for the entire site.

3.0 Fish Sampling Methodology

3.1 General sampling issues

- Electrofishing is generally the most non-destructive, effective and cost-efficient means of sampling freshwater fish (Pusey et al 1998, Schramm et al. 2002).
- Electrofishing (boat and backpack) using a pulsed direct current (DC) waveform is recommended here as the principal fish sampling method used in monitoring programs.
- The following electrofishing apparati may be available for use in monitoring programs:
  - electrofishing boat with ETS or Smith Root electrofisher
  - Small punt (<3m) with a backpack electrofisher temporarily mounted at the prow
  - Backpack electrofisher
  - In sites consisting of deep pools and shallow wadeable riffles, a combination of boat electrofishing and backpack electrofishing may be used (see backpack protocol below), provided that wading with a backpack electrofisher is deemed not be hazardous (i.e. due to the presence of estuarine crocodiles or extreme high velocities).

  - The choice of sampling device is dependent on what is available but **must** be considered in relation to, and in accordance with, your organisation's health and safety requirements.

- The sampling effort required (i.e. number of electrofishing shots within a site and the number of sites required within a catchment at a given level of within-site sampling intensity) to estimate various fish assemblage parameters (such as species richness, species composition, species relative abundances) is likely to depend on a number of environmental and biological factors. These factors include within- and between- site diversity of physical and chemical characteristics and within- and between- site variation in fish species richness and diversity, etc. Most importantly, the sampling effort required to estimate fish assemblage parameters is dependent on the desired accuracy and precision of theses estimates required to satisfy the particular project objectives.
- We recommend that a single pass electofishing method be used and that the pass should be broken up into a number of 5 minute blocks (hereafter referred to as 'shots'). Breaking the entire sampling period up into smaller sampling blocks allows collected fish to be processed in a timely fashion to avoid stressing them (important from both an ethical perspective and from a methodological perspective – if you want to examine mortality over time, it is best if the sampling method isn't a contributing factor). It also allows a finer scale assessment of spatial variation within a sites and its relationship to habitat structure to be assessed.
- We recommend, based on analyses of sampling efficiency (accuracy and precision) that at least 10 and **preferably 15** electrofishing shots are required for each sites (see below). This has implications for the number of sites that can be sampled in a limited time. However, it is always desirable to do more if this is possible, especially if it is apparent that new species are being collected in the last 2 or 3 electrofishing shots.
- If only a few sites are included in a monitoring program (thus time and money are not limited), then a more intensive sampling regime may be implemented. This might entail multiple pass electrofishing. Pusey et al. (1998) describe such a sampling design for wadeable streams.
- The mix of electrofishing gear types (i.e. boat or backpack electrofishing) used at a given site is proportional to the estimated proportion of that site that is suitable for each sampling method (see Table 1, Section 3.5).
- Electrofishing dipnets are standardised across all teams and electrofishing boat types with the dimensions of the net-head being 500mm wide X 400mm long, net depth of 300mm and a knotless mesh diameter of 12mm stretched
- Dipnets used for backpack electrofishing will be of the same mesh size (12 mm), and the net heads will be the same as boatshock nets. The net mesh attached to the anode pole ring will also be 12mm stretched.
- A net (12mm mesh size) **IS** to be placed on the anode ring (when backpack electrofishing) to maximise fish catches and minimise potential harm to fish (by minimising the duration of time that electricity is required to immobilise the fish before it is dipnetted) but ensure that the anode ring is kept clean and polished (using a mild acid to remove calcareous deposits)
- Individual electrofishing shots at a site **do not** need to be located in exactly the same location on successive sampling occasions as it is assumed that the sampling program is sufficiently intensive (i.e. do enough electofishing shots) that fish assemblages in a study reach will be accurately characterised. In

practice, it often results that individual shots do occur in the same location if the starting point on each occasion remains constant. This is recommended.

- It remains to be resolved whether electrofishing shot data collected by boat or backpack are considered comparable for data analysis and reporting (this approach is used in the *Sustainable Rivers Audit* – hereafter *SRA*). It has been be argued that although there is potentially great variation in electrofishing efficiency between boat and backpack electrofishers (due to the higher power, voltage, and amperage output of the boat electrofisher and variation in fright bias between gear types), the lower output of backpack electrofisher may be compensated by the smaller volume of habitat being sampled by this method. Two alternative approaches could be used:
    - Treat boat and backpack electrofishing shot data separately in analyses and reporting
    - Treat shot data collected by each electrofishing method as comparable by arguing that we used the electrofishing method most suited to the particular habitat type while recognising the potential limitations of this approach
    - It is **essential** that the type of electrofishing (boat or backpack) used is noted down on the data sheets.
- Note that Dip Net catch data or any other supplementary sampling method is always to be treated separately.
- In the case where electrofishing cannot be undertaken for what ever reason, then as full a range of additional sampling techniques should be employed. These may include sampling by gill netting, angling, seine netting, fyke netting, traps, visual observation from stream bank, snorkelling, etc. Each technique can only be employed where it is safe to do so. For example, seine netting or snorkelling is not appropriate in habitats used by estuarine crocodiles.
- Any fish observed but not actually collected during sampling should be classified to species level where possible, an estimate made of their abundance, and **noted on the data sheets** as 'observed'
- There are inherent biases and uncertainties in which species get recorded as 'observed' and how accurate the estimates of abundances are (e.g. few individuals of large and easily recognised species will be more accurately assessed than abundant but small and difficult to identify species). Post-sampling exploratory analyses can be undertaken to establish whether the 'observed' catch data should be included in the final analyses and reporting for any montoring projects (note that the *SRA* does not use 'observed' catch data).
- **All** fish over **15mm** total length sampled and/or observed should be identified and counted (fish < 15mm are excluded from the catch unless they represent the only example of a species collected at the site). Small fish are not well sampled by elctrofishing unless the output settings (waveform, voltage etc.) are specifically adjusted to enable their capture – this will however compromise the capture of species over a larger size range. Sampling programs must be mindful of the specific objectives in mind and tailor their sampling regime to suit.
- Some species may be difficult to identify in the field (particularly some small gobies and gudgeons). In this event, several example specimens should be retained for later laboratory identification. See additional notes on sample preservation below.

- Standard safety equipment such as rubber gloves and rubber waders should be used when backpack electrofishing and rubber gloves and gumboots when operating from a boat. Polaroid sunglasses should also be worn to reduce reflection and make fish easier to see and catch. Floatation devices may be required when operating from a boat – check your organisation's requirements.
- A sign saying "Collection in Progress Under ??…agency name…?? Permit #….." should be displayed on the vehicle and as close as possible to the site.
- On the data sheets provided make a note of any factors affecting the efficiency of the sampling (see data sheets)
- If a major flood event occurs in the weeks leading up to sampling a site this should be noted on the data sheets. In the event that a flood occurs immediately prior to or during a sampling period, it is suggested that sampling re-commence as soon as possible after the flow recedes, providing this is still within the sampling window and time permits.
- Electrofishing is potentially dangerous, take care and consider your safety and that of your colleagues – don't be a goose!

3.2. Sampling approach

- There are many possible sampling approaches using boat and/or backpack electrofishing that are potentially suitable for fish survey and monitoring programs. Each has a number of possible advantages and disadvantages, and the choice of methods ultimately depends on the objectives of the study and the potential existing and future uses of the data to be collected. The following sampling protocol has been used by the authors after critical examination of the various sampling approaches currently used in existing State and Regional monitoring and assessment programs and after considering our project requirements.

3.3 Boat electrofishing methods

- Boat electrofishing teams will consist of one operator and one or two netters (depending on boat size).
- Ideally (if sufficient personnel and space & on the boats is available), one additional person will be present on the boat to assist with data recording and fish measuring
- If sufficient field personnel are available, habitat data for each shot will be collected by a two-person team following in a small boat, otherwise the boat electrofishing team will collect this data in addition to fish sampling at the cessation of electrofishing (shot locations will be marked with buoys - see habitat data recording protocol in Section 5)
- The approach recommended for this study is as follows:
- At least 15 electrofishing shots are to be made at each site, where the mix of electrofishing gear types (i.e. boat or backpack) used at a given site is proportional to the estimated proportion of that site that is suitable for boat electrofishing (see Table 1).

- The location of electrofishing shots is to be selected based on the protocol used in the Sustainable Rivers Audit: each major habitat type at a site must be sampled at least once and then remaining sampling effort should occur in the most abundant habitat types. Such habitats within a site could include riffles, runs, pools, macrophyte beds, stretches of mid-channel open water, undercut banks, woody debris piles, etc. ·
- The length of each shot is not prescribed, but each shot should be restricted to as homogenous an area as possible (i.e. if possible avoid sampling multiple meso-microhabitat types within a single shot – this is because it is desirable to quantify fish habitat use using the electrofishing catch data & habitat survey data, using each shot as a sample or replicate).
- Each electrofishing shot (including both boat and backpack) is to be fixed at **five minutes** duration (elapsed time).
- On the data sheets provided, record the 'power-on' time at the start and end of each shot.
- Electrofisher Output settings are NOT to be standardised (i.e. using Power Transfer Theory) but rather are to be set to maximise efficiency at each site but with the minimum power required to elicit a response.
- The following Electrofisher output information is to be recorded for each shot
    - AC/DC (Note that AC power will rarely, if ever, be used, and only in conditions of extremely low water conductivity (i.e. < 50μS.cm^{-1}). Note also that the booms can be set as anode and cathode thus focusing effort in front of the boat.)
    - Volts (usually low in water conductivities $\geq$150 μS.cm^{-1} and higher in conductivities < 150 μS.cm^{-1})
    - Pulses per sec (should usually range from 60-250)
    - % duty cycle (range) (usually no higher than 25% unless in very high conductivity)
    - Amps (usually low in low conductivity and high in high conductivity)
- At the start and end points of each electrofishing shot respectively, deploy small marker buoys (to mark the position and extent of the shot for subsequent habitat assessment) or record start and finish using a GPS. This is not necessary if an additional team in a separate boat is able to follow the elctrofishing boat. Note however that this team should not follow too closely –safety first!
- During each shot the boat is slowly driven along the river with one operator at the back controlling the boat and the electrofisher settings, whilst the dip netters at the front of the boat control the passage of the electric current into the water (using a foot on/off switch) and remove any immobilised fish
- Use short intermittent pulses. Do not constantly depress the ON switch when electrofishing as reduces the element of surprise needed to catch many fish species and tends to herd fish without actually stunning them.
- Immobilised fish are immediately dip-netted from the water and placed in an aerated (if available) holding tank on board the boat to recover prior to identification, measurement and release
- Care must be taken to ensure independence of each electrofishing shot (i.e. that sufficient distance is maintained between shot locations such that fish in a given shot location have not been affected by electrofishing and/or releasing fish in the previous shot location)

- In wide streams (> **15m**), shots are conducted on alternate banks to maintain independence.
- Mid-channel shots are also undertaken where necessary, but are spaced at least **25m** away from the preceding shot.
- In narrower streams (**<15m**) where sampling on alternate banks does not ensure independence, adopt a zigzag coverage of sample area. Shots are also spaced by at least **25m** to maintain independence
- All fish collected are enumerated and recorded on the data sheets by the assistant. To avoid confusion with fish identification on the data sheets, use the first letter of the Genus and the full species name (e.g. *H. fuliginosus*)
- DO NOT electrofish in the same location as previous shots!!!!!!!

3.4 Backpack electrofishing methods

- Backpack electrofishing protocols are generally similar to those prescribed for boat electrofishing
- Backpack electrofishing teams will consist of one operator and two netters (ideally)
- At least 15 electrofishing shots are to be made at each site, where the mix of electrofishing gear types (i.e. boat or backpack) used at a given site is proportional to the estimated proportion of that site that is suitable for boat electrofishing (see Table 1).
- The location of electrofishing shots is to be selected using the same approach as for boat electrofishing (each major habitat type at a site must be sampled at least once and then remaining sampling effort should occur in the most abundant habitat types. Such habitats within a site could include riffles, runs, pools, macrophyte beds, stretches of mid-channel open water, undercut banks, woody debris piles, etc.)
- The length of each shot is not prescribed, but each shot should be restricted to as homogenous an area as possible (i.e. need to avoid sampling multiple meso-microhabitat types within a single shot)
- Each electrofishing shot is to be fixed at the same duration (i.e. **five mins elapsed time**) as that prescribed for boat electrofishing.
- On the data sheets provided, record the 'power-on' time.
- Electrofisher Output settings are NOT to be standardised (i.e. using Power Transfer Theory) but rather are to be set to maximise efficiency at each site but with the minimum power required to elicit a response.
- Use the minimum VOLTAGE required to do the job as the more power you use, the faster the battery will discharge. Under normal use one battery will usually last between 2-3 hours.
- The Electrofisher will overload and automatically switch off if too much power is being drawn from the Electrofisher, (ie. VOLTAGE setting is too high for the Conductivity of the water). To rectify this, reduce the VOLTAGE and/or PULSE WIDTH settings, switch the Electrofisher back On and resume shocking.
- Never change Electrofisher settings whilst the current is switched on. This will terminate your session and result in damage to the unit (very costly).

- The Electrofisher must be operated within 45 degrees of vertical otherwise it will automatically cease outputting a charge and emit a distinctive noise. Try to avoid this.
- DO NOT touch the CATHODE rat tail with the ANODE ring as you will short circuit and may damage the Electrofisher.
- We recommend that a portable multimeter be carried in the field to assist with detection and repair of any small problems.
- Use short intermittent pulses. Do not constantly depress the ON switch when electrofishing as reduces the element of surprise needed to catch many fish species and tends to herd fish without actually stunning them.
- Quietly approach a likely fish habitat (such as a snag, undercut bank or aquatic macrophyte bed) and carefully thrust the Anode probe towards it whilst depressing the ON switch for sufficient time to stun any fish in the vicinity (10 seconds is usually more than adequate). Occasionally stunned fish deep within undercuts may be drawn out by withdrawing the anode with current on.
- Avoid using the anode ring as a net to dig in and upward under weeds or submerged grasses – the anode ring is made of soft aluminium and welds will quickly break if it is used in this manner. It is best to modify the ring by having a ring brace welded to the ring and which is inserted 3 or four inches up the pole.
- All stunned fish are netted (usually by the electrofishing operator and less frequently by the assistants wielding dip nets) and placed immediately in a live fish-well (a 70 L plastic container part-filled with fresh stream water and floated within an inflatable rubber truck-tyre inner tube) towed behind the assistant to recover prior to identification, measurement and release.
- It is often difficult for the assistants to tow the fish-well in high gradient, fast-flowing riffles. In these situations, use buckets with lids instead.
- At the start and end points of each electrofishing shot respectively, deploy small marker buoys (to mark the position and extent of the shot for subsequent habitat assessment)
- If sufficient field personnel are available, habitat data for each shot will be collected by a two-person team following behind, otherwise the backpack electrofishing team will collect this data in addition to fish sampling at the cessation of each electrofishing shot (see Section 4)
- Care must be taken to ensure independence of each electrofishing shot (i.e. that sufficient distance is maintained between shot locations such that fish in a given shot location have not been affected by electrofishing and/or releasing fish in the previous shot location)
- In wide streams (> **15m**), shots are conducted on alternate banks to maintain independence.
- Mid-channel shots are also undertaken where necessary, but are spaced at least **25m** away from the preceding shot.
- In narrower streams (**<15m**) where sampling on alternate banks does not ensure independence, adopt a zigzag coverage of the sampled area. Shots are also spaced by at least **25m** to maintain independence.
- In narrow streams, commence electrofishing at the downstream end of the shot area and progress upstream in a zig-zag pattern
- All fish collected are enumerated and recorded on the data sheets by the assistant.

- When probing undercut banks or root masses, swirl the probe, ring and net repeatedly through the structure to capture fish that may be stunned but not visible (whilst maintaining the bag in the net so as to avoid loss of fish)
- The field assistant should remain behind but slightly to the side of the electrofishing operator at all times so as to avoid scaring fish or stirring up bottom sediments. However, the assistant should avoid walking directly behind the operator to avoid stepping on the cathode wire, an action which can damage the cathode connection or trip the operator (or at the very least annoy him or her enormously)
- Remember to recharge the backpack electrofisher batteries each night or during travel to the next site (where if vehicles have been fitted with appropriate charging facilities).
- Wear appropriate safety gear.

3.5 Combination of backpack & boat electrofishing methods

- The mix of electrofishing gear types used at a given site is proportional to the estimated proportion of that site that is suitable for boat electrofishing (i.e. based on habitat characteristics such as depth and velocity, and the likely presence of crocodiles) (see Table 1).

Table 1. Recommended number of electrofishing shots required with each electrofishing gear type based on a total of 15 and 10 electrofishing shots per site, respectively.

	% of site suitable for boat electrofishing					
	100	**80**	**60**	**40**	**20**	**0**
15 shots/site						
Number of Boat electrofishing shots	15	12	9	6	3	0
Number of Backpack electrofishing shots	0	3	6	9	12	15
10 shots/site						
Number of Boat electrofishing shots	10	8	6	4	2	0
Number of Backpack electrofishing shots	0	2	4	6	8	10

3.6 Comparative assessment of sampling effort and efficiency to characterise fish assemblages

- Figure 1 compares the relative gain in information (accuracy and precision) gained with increasing number of electrofishing shots. Data is derived from a study undertaken in the Daly River.
- Parameters examined include the number of species, the composition of the assemblage defined by presence or absence of individual species and the composition of the assemblage as defined by the relative abundance of individual species.
- Data were collected at 10 sites in which 20 electrofishing shots were conducted. Estimates of the "true" value of these parameters were based on data collected from all 20 shots. A randomisation procedure was then used to compare how well a randomised selection of shots compared to the true value (see Kennard et al. (2006) for further details on this approach). That is, a

single shot was randomly selected from the 20 shots for each site and compared to the true value. This was repeated 100 times. Next, two shots were randomly selected and repeated 100 times and so on.

- Accuracy and precision both improved as more shots were added (although less quickly for estimates of species richness).
- On the basis of these data, we recommend that 10 and preferably 15 shots are needed to characterise a fish assemblage with acceptable levels of precision and accuracy.

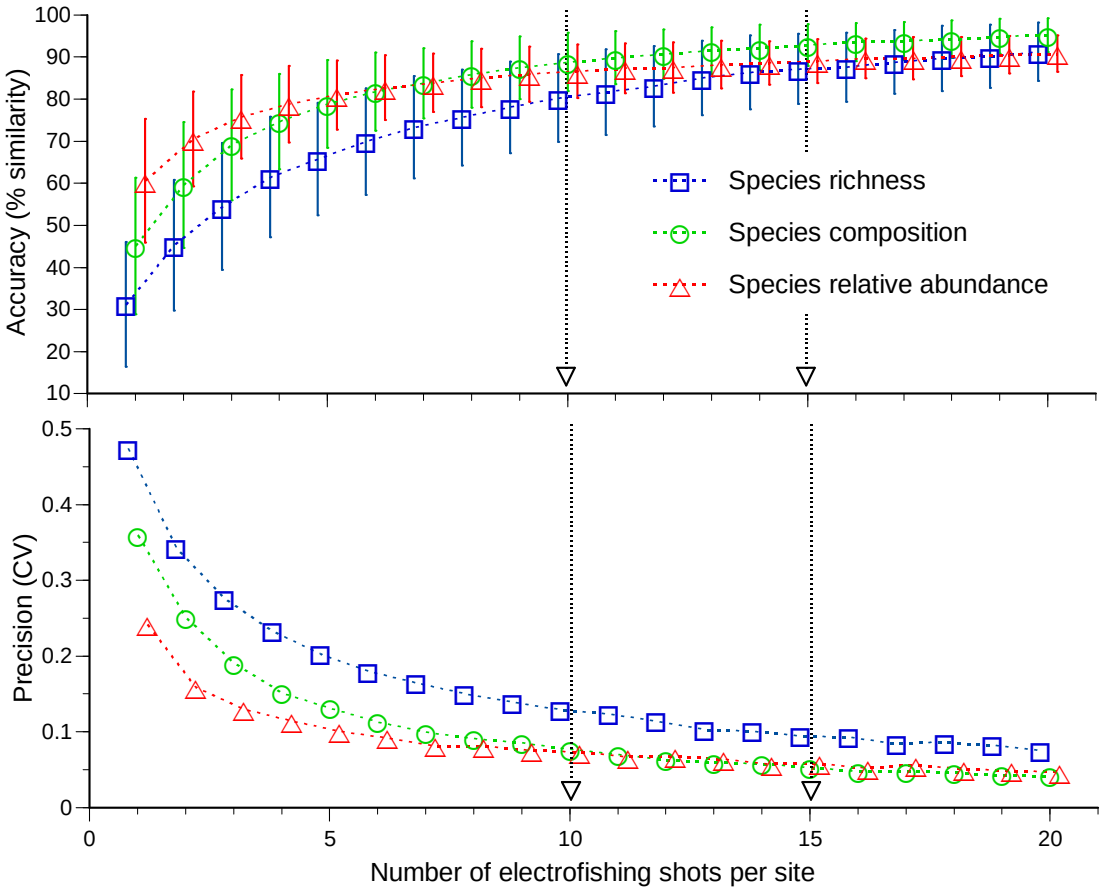

Figure 1. Changes in average sampling accuracy (± s.d.) and average precision v. number of electrofishing shots to estimate total fish species richness (blue squares), species composition (green circles) and species relative abundance (red triangles). Accuracy for each cumulative number of electrofishing shots sampled is represented by the mean (± s.d.) percentage of total fish species richness (closed circles) or mean (± s.d.) Bray–Curtis similarity with total species composition (closed squares) and species relative abundance (closed triangles) respectively. Precision for each cumulative number of electrofishing shots sampled is represented by the coefficient of variation (mean/s.d.). Results are averaged across 10 sampled stream reaches in the Daly River. Dashed lines facilitate comparison between fish assemblage attributes for two levels of sampling effort (10 and 15 electrofishing shots).

3.7 Measurement of fish after collection

- A sub-sample of 30 individuals per species captured by each method used at each site (boat electrofishing, backpack electrofishing and Dip Netting) should be measured for **Standard Length** (to the nearest mm) (see Figure 2).

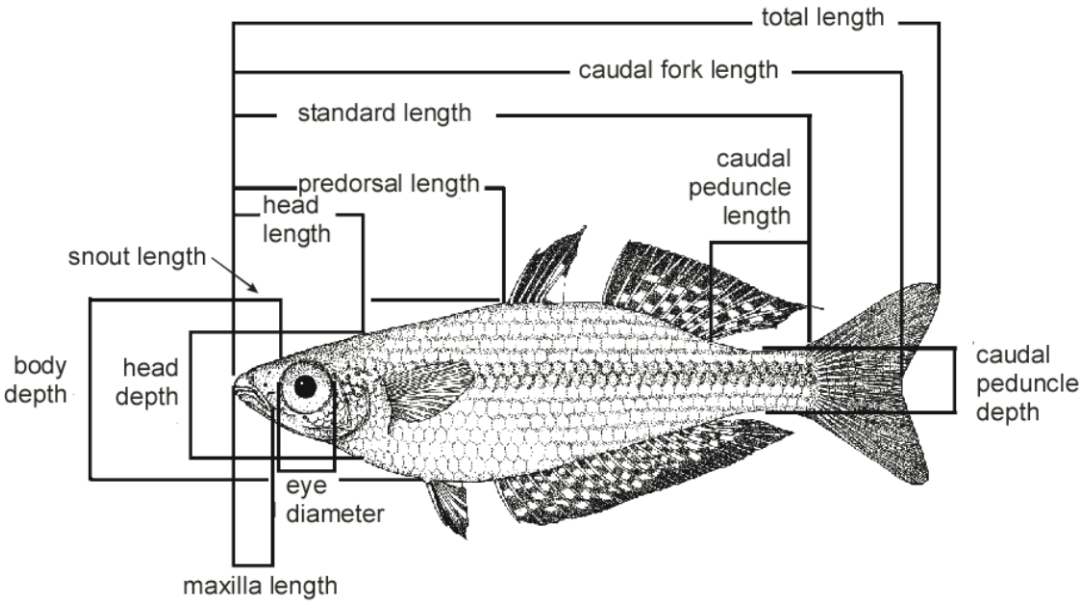

Figure 2. Commonly used morphometric characters (from Pusey *et al*. 2004)

- The sub-sample begins with the first individual of each species collected from each method and continues until the 30th individual. During electrofishing operations, in the shot where the 30th fish is found all individuals present should be measured in order to avoid any bias in the size of fish selected for the sub-sample.
- More fish may be measured if this is a particular requiremtn of your sampling program (i.e. you want to asses changes in fish size structure over time for a aprticualr species)
- If a sample of fish from an electrofishing shot or Dip Net shot contains individuals of a species of noticeably different sizes to those already collected (by electrofishing), but 30 individuals of this species have already been measured in previous electrofishing shots, at least **10** of these fish should also be measured also.
- We recommend that when species present at a site cover a wide range of sizes, and particularly when different size classes preferentially use different habitats, that captures be recorded on the data sheets as juveniles or adults (e.g. *H. fuliginosus* JUV and *H. fuliginosus* ADULT). Future analyses can use this data when species within individual size classes are considered separate taxa or the data can be pooled and considered as a single species. This approach provides some flexibility. We have found that 150mm SL is often a good size limt for many species (e.g. grunters and plotosid catfishes) and that 300 mm sL is adequate for larger species such as barramundi and long tom.
- Small fish (< ~ 200mm SL) are to be measured using callipers, larger fish can be measured using a fish measuring board.

- All native fish are then returned alive to the water at the point of capture, unless specimens for alter examination in the laboratory are required. Alien fish species are anaesthetised (benzocaine - MS222 or clove oil), euthenased and not returned to the water.
- Extreme caution should be taken when handling certain species as they have sharp and sometimes poisonous spines, large teeth and/or pugnacious dispositions. Be particularly careful with the freshwater catfishes, bullrouts, stingrays, sawfishes and sharks.
- Don't overshock fish. Electrofishing can injure fish if injudiciously applied.

3.8 Supplementary fish sampling

- Seine netting, angling, snorkelling and other methods may be used to collect fish wherever conditions and time allow. Fish species caught using these methods are to be enumerated and noted in the appropriate part of the data sheets. A sample is to be measured only if a sample collected by electrofishing has not also been collected.
- If however, electrofishing is not used and all fish are collected by supplementary means then a sample of 30 individuals per species is to be measured.
- If seine netting, ensure that the area is free of estuarine crocodiles and is relatively free of woody debris, large and dense aquatic macrophyte beds and other structures which may impede effective seining. **Note - if there is even the slightest concern about the presence of estuarine crocodiles, do not enter the water to pull a seine.** Whilst data is precious, it aint that precious!
- A seine net (11mm stretched multifilament mesh) is pulled through the site starting at the upstream end and hauling downstream with the current (Fig. 3).
- Extreme care should be taken to ensure that the lead line of the seine net is kept in contact with the substrate at all times (otherwise fish will escape).
- The number of seines pulled at each site must be noted and records kept of the catch for each seine pull. **Data must be kept that will allow later computation of catch per unit effort.** This applies to all supplementary methods. For example, if gill nets are used then the number of nets, length, drop, mesh size, number of hours soaked and time of deployment must all be noted. Similarly, if traps are used, then trap type, number, hours of deployment, whether baited or not, depth and type of habitat, should all be recorded.

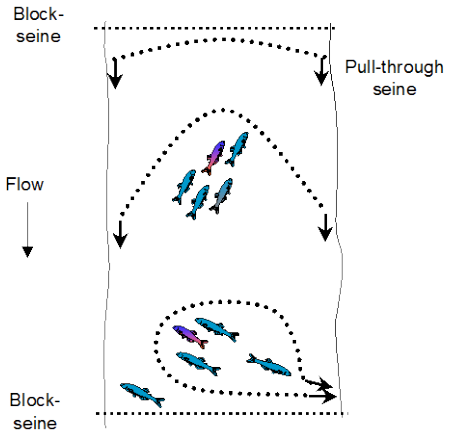

Figure 3. Example of seine netting methodology.

3.9 Safety

- All electrofishing procedures are to carried out following the Australian Code of Electrofishing Practice (Anon. 1997)
- All electrofishing gear to comply with AS/NZS 60335.2.86:2002
- An operation manual must be present on the boat at all times.
- A log book must be present on the boat and filled in after each session.
- A safety log must be present on the boat at all times and filled out prior to operation for each session.
- It is essential that the electrofishing operator and assistants establish a system to communicate whether the electrofisher needs to be turned on or off quickly.
- For safety, stop electrofishing immediately if members of the public (or their pets) approach the sampling area. Take the time to explain what you are doing and ensure they stay well away from the water while you are electrofishing.
- A field first aid kit must be carried on all field trips.
- Always follow your Institutions safety guidelines.
- Don't rely on any one else to ensure your working in a safe manner. Do it yourself.
- Keep an eye out for the safety of your workmates.
- Don't be a goose!

3.10 Ethics

- All sampling procedures must be conducted in accordance with the animal ethics approval for the project.
- For animal-ethics reasons, an aerator may be used in the bucket if available.
- Attempt to use electrofisher settings that minimise harm to fish while at the same time effectively stun fish.
- Care should be taken not to unnecessarily stress all fish collected, irrespective of sampling method. Wherever possible use the following procedures:
    - Stress to fish caught by seine netting can be minimised by very gently extracting fish that are gilled in the seine mesh and by placing all fish collected in buckets as soon as possible after capture,
    - keep holding tanks/buckets in the shade where possible to reduced high temperatures, and
    - measure the fish and release them back to the stream as soon as possible.
- As the sampling will be done in the public arena, every effort should be made to prevent excessive stress, damage or death to organisms sampled.

3.11 Sample preservation

- Fish collected during sampling may be needed for associated studies. Any fish collected that is of uncertain identity is to be fixed in 10% formalin (preferably buffered formal saline – see recipe below) and preserved in 70% ethyl alcohol. If the fish is longer than 10 cm, either formalin is injected into the gut cavity or the gut wall is neatly slit open (minimising damage to fins and pelvic girdle).
- ***The recipe for Buffered (ph 7.0) 10% formal saline per litre is:-*** Formalin *(40% formaldehyde soln)* 100 mls. Distilled water 900 mls. Na H2 P04 2H20 4.5 g Na 2H P04 6.5g.
- The fish must be dead prior to these actions, euthenasia is preferably achieved by pithing, MS 222, benzocaine or clove oil (must be in accordance with your institution's ethics requirements.
- In the case where species of uncertain identity are collected, then a sample of up to ten individuals is to be preserved.
- Keeping a sample for later genetic examination is becoming increasingly popular. Prior to preservation a fin clip (left hand pelvic fin) is to be taken and stored in 90-100% ethyl alcohol. **Do not** use **methylated spirits** as this denatures DNA. Fin clips of 5-10 individuals of each species from each site can be collected and stored in 90-100% ethyl alcohol. The upper limit of the number of individuals per species from which fin clips are collected will depend on the conditions of your permit.
- Do not overfill sample jars with specimens, always ensure that there is a 1:1 ratio of formalin to specimen.
- Samples are to be clearly labelled with site number, name, lat/long, collector's name, date and number and type of species in each jar. Ensure that a water proof pen is used and that labels are made of good quality paper. Genetics samples are to be given a unique number. **Information on samples is to be recorded on the data sheets also, taking great care to relate whole specimens to the appropriate fin clip.**
- When handling formalin, take great care. Use gloves, goggles and a facemask if necessary. Always follow your institution's safety guidelines.
- Formalin is a dangerous chemical. In the case of contact with the skin, wash affected area very thoroughly. If formalin comes in contact with the eyes, it is important that the surface be thoroughly washed with clean water and preferably with a saline solution from your first aid kit. It may be appropriate to seek medical advice. Similarly, if accidentally swallowed, it would be prudent to seek medical advice. Always act in accordance with your institution's safety guidelines.
- Always ensure that stores of formalin are kept secured and away from the public, especially children. Similarly, always ensure that supplies of ethyl alcohol are kept secured, preferably in a locked container.
- During and at the end of the field trip, all specimen jars are to be checked to ensure that no cracking of vials or leaking of formalin has occurred in transit. This is not uncommon when long distances over rough terrain are travelled.

4.0 Environmental Data Collection

4.1 Why collect this information?

- A range of catchment and local scale environmental variables is estimated for each site
- Estimation of these variables is necessary to satisfy the following objectives:
    - To document spatial and temporal variation in aquatic habitat structure and water chemistry and relate these to variation in fish assemblage structure
    - To quantify habitat availability at each site such that fish species habitat use can be quantified
    - To record factors potentially influencing fish sampling efficiency
- Local-scale habitat and water chemistry variables will be quantified during the field sampling at each site

4.2 **Habitat sampling methodology** - at each electrofishing shot location

- It is absolutely essential that GPS location data are collected at each sampling location.
- At each electrofishing shot location, data describing various aquatic habitat attributes are collected (see Table 2).
- If sufficient personnel are available at each site, a habitat assessment team (consisting of two people) will be dedicated to this task (naturally, personnel should be rotated between electrofishing and habitat assessment teams)
- If sufficient personnel are not available at each site, habitat assessment will be undertaken once the electrofishing has finished
- Everyone should know the location of each electrofishing shot because the electrofishing teams mark the start and end points of each shot with marker buoys or tape. If tape is used, then ensure it is all collected and disposed of properly after sampling.
- Wetted channel width is measured once at the central point of the electrofishing shot. We recommend that cheap and effective laser measuring devices (i.e. rangefinders) are used.
- Water column depth and water velocity are measured at **five** randomly (haphazardly) selected points within the electrofishing shot area (up to 5m either side of the shot)
- Mesohabitat type, substrate composition and microhabitat type are visually estimated and allocated to categories as a proportional contribution to the entire surface area of the electrofishing shot
- It is assumed that this intensive sampling scheme (i.e. 15 shot locations with multiple habitat measurements at each shot) will be sufficient to accurately and precisely estimated habitat characteristics for each site.

Table 2. Habitat parameters estimated or measured at each electrofishing shot location.

Parameter	Number of replicates/shot	Method of estimation
Wetted channel width (m)	1	measuring tape or laser range-finder
Mesohabitat type (% shot area) • Riffle • Run • Glide • Pool • Backwater	1	visual estimation
Water column depth (m)	5 random points	graduated depth probe or depth sounder
Water velocity ($m.sec^{-1}$)	5 random points	water velocity meter
Substrate composition (% shot area) • Mud (<0.06mm) • Sand (0.06-2mm) • Fine gravel (2-16mm) • Coarse gravel (16-64mm) • Cobble (64-128mm) • Rock (>128mm) • Bedrock	1	visual estimation
Microhabitat type (% shot area) • Aq. macrophytes • Leaf litter • Submerged marginal veg. (e.g. terrestrial grasses, weeds) • Submerged overhanging veg. (e.g. tree branches / leaves) • Emergent vegetation (e.g. sedges, rushes) • Root masses (% bank) • Undercut banks (%bank) • Large woody debris(>15cm stem diameter) • Small woody debris (<15cm stem diameter) • Filamentous algae	1	visual estimation

4.3 Environmental factors potentially influencing fish sampling efficiency

- Factors potentially influencing fish sampling efficiency at the study site (see Table 3) are to be subjectively assessed and rated on a five-point scale according to the following criteria: 0=no influence, 1=minor, 2=moderate, 3=major, 4=very major

Table 3. Factors potentially influencing fish sampling efficiency

Factor	Rating
Excessive depth	
Slipperiness/bogginess	
Woody debris	
Macrophytes	
Conductivity (High/Low?)	
Turbidity/colour	
High discharge/velocity	
Other	

4.4 Water chemistry

- Measures of a range of water chemistry parameters (listed in Table 4) should be measured at a least three random locations throughout the study site. This will depend on the requirements of individual studies.
- Time of day at which measurements were made **must** be noted on the data sheets.
- Turbidity may be measured in nephelometric turbidity units or cms secchi disk depth. Specify what method is being used.
- Note that some parameters change greatly over short time scales, however conductivity is less labile over short time periods. It and turbidity are most likely to influence electrofishing efficiency. These parameters **must** be measured to allow you to set your electrofishing parameters appropriately and to later assess your sampling effectiveness.

Table 4. Water chemistry parameters that may be recorded at each site

Parameter
Dissolved oxygen (mg/l)
Dissolved oxygen (% sat)
pH
Conductivity (μs.cm^{-1})
Temperature (^{o}C)
Turbidity (NTU's)
Secchi depth (cm)

4.5 Other variables

- Three replicate estimates of riparian cover should be made at random locations throughout the study site using a hand-held densitometer (at best).
- The total length of the study site is to be estimated (measured) using a range-finder
- The latitude and longitude of the upstream and downstream end of each study site is to be recorded using a hand-held GPS

4.6 Map of study site

- A rough sketch of the study site should include the following details:
  - access point
  - direction of flow
  - location where photos taken
  - water chemistry collection sites
  - electrofishing shot locations (numbered corresponding to the electrofishing data sheets)

- basic mesohabitat features (i.e. riffle, run, pool, etc)
- basic microhabitat features (i.e. LWD, macrophyte beds, etc)

5.0 Permits

- It is essential that the appropriate State/Territory Fisheries Permit is in possession of the sampling team at all times. Please abide by all requirements of the permit. Also ensure that the "Collection in Progress" sign is displayed in a prominent place at all times (e.g. the windscreen of the vehicle).
- If sampling on Aboriginal land, it is essential that appropriate permissions and permits have been obtained. It is also essential that the Traditional Owners are informed well in advance of upcoming field sampling on their land.
- If sampling in a state forest you may need a DPI Forestry Permit to Traverse State Forests
- **Always** seek permission of private landholders if you intend to traverse private property or sample on their land.
- Please conform to the ethics requirements of your institution – use some common sense and compassion.

6.0 Additional Notes

6.1 Notes on ETS Boat electrofisher settings

- At each electrofishing shot location, it is prefereable that to collect all species and size classes present with generally equivalent sampling efficiency (if possible)
- Therefore select electrofisher settings that provide a balance in the ability to catch small and large fish (if possible)
- If possible, do not select electrofisher settings that maximise catches of large fish at some shot locations and change the electrofisher settings to maximise catches of small fish at other shot locations (as this will compromise the fish species habitat use information derived from the capture data and the habitat availability data at each shot)
- We therefore suggest that prior to the commencement of actual quantitative sampling at a site, some time is spent trialling the electrofisher using a range of settings until optimal settings are found that maximises electrofishing capture efficiency for a range of species and size classes
- The table below can be used as a starting point to guide the selection of optimal settings for different water conductivity ranges

Troubleshooting notes:

- If having trouble shocking <u>smaller</u> fish – try <u>increasing Hertz</u>
- If having trouble shocking <u>larger</u> fish – try <u>decreasing Hertz</u>
- At low conductivities up to 150 *u*s.cm^{-1} use the high voltage setting and put as much anode into the water as possible.

- As conductivity increases decrease anode surface area.
- Conductivities more than 150 *u*s.cm^{-1} use the low voltage setting as it is much more versatile for setting outputs.
- 25% duty is optimal in most situations but as conductivity decreases increase the duty range.

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Table 5. Ranges of boat electrofisher settings for given levels of water conductivity that can be used to guide the selection of optimal settings to maximise electrofishing capture efficiency for a range of species and size classes.

	Water conductivity (μs.cm^{-1})				
	<50	**50-100**	**100-500**	**500-700**	**>1000**
Droppers	Yes (Large)	Yes (Large)	Yes (but decrease surface area as conductivity increases by pulling booms up)	No	No
Spheres	No	No	Yes (can be used at higher end of conductivity range).	Yes (Preferred)	Yes (Preferred)
Pulsed DC	Yes (High Voltage)	Yes (High Voltage)	Yes (Low Voltage)	Yes (Low Voltage)	Yes (Low Voltage)
AC	Yes (Preferred)	No	No	No	No
Volts (start at zero)	Increase voltage until taxis is achieved in smaller bodied fish (usually 300-600 Volts)	Increase voltage until taxis is achieved in smaller bodied fish (usually 300-600 Volts)	Increase voltage until taxis is achieved in smaller bodied fish (Usually 100-300 Volts)	Increase voltage until taxis is achieved in smaller bodied fish (Usually 100-300 Volts)	Increase voltage until taxis is achieved in smaller bodied fish (Usually 100-300 Volts)
Hertz	Increase HZ if only catching large specimens.	Increase HZ if only catching large specimens	Increase HZ if only catching large specimens	Increase HZ if only catching large specimens	Increase HZ if only catching large specimens
Duty Range (%)	25% or higher for low conductivity	25% is optimal	25% is optimal	25% is optimal	25% is optimal
Amps	Not important in low conductivity water. Voltage is much more important.	Not important in low conductivity water. Voltage is much more important	15 – 35 As conductivity increases, current (amps) is key to fishing	25 – 40 As conductivity increases, current (amps) is key to fishing	25 – 40 As conductivity increases, current (amps) is key to fishing

7.0 References

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8.0 Appendices

Appendix 8.1: Inventory of FIELD Equipment (list of gear required for field work)

Purpose	Item	Quantity
Habitat survey	50m tape measure 100m tape measure Range finder Graduated depth probe Water velocity meter Densiometer Transect Marker buoys & sinkers Small Tinny (boat) & outboard motor	
Boat electrofisher	Safety check list Safety gear (boots, gloves etc) Operation log for electrofisher Dip nets (maximum 12mm mesh) Anode arrays (1x droppers 1x spheres) Rubber gloves (linesman) Rubber boots	
Backpack electrofisher	Backpack Electofisher & accessories (anode pole, 30cm ring with 12mm mesh net, cathode rat's tail) Electrofisher batteries Battery charger Spare anode poles, rings, nets, cathode rats tail Rubber Waders Rubber gloves (linesman) Dip net (maximum 12mm mesh)	
Seine nets	Seine net (multifilament, 30m long x 2m drop x 11mm stretched mesh, with purse) Seine net (multifilament, 15m long x 2m drop x 11mm stretched mesh, no purse) Seine net (shadecloth, 3m long x 2m drop x 2 mm stretched mesh)	
Fish holding tanks	Aerated live-well on electrofishing boat 70Litre plastic Nally bin + lid Rubber inner tube (for towing Nally bin while backpack shocking 10 Litre buckets + lids	
Fish measurement	Vernier callipers Measuring board (with mm graduations) Hand scoop nets (for catching fish from holding tanks) Field balances	
Fish preservation	Clove oil (or MS222) anaesthetic Plastic sample vials (e.g. urine sample jars), range of sizes Plastic snap-lock bags, range of sizes Plastic drum (for large specimens) Formalin (40%, dilute to 10% in the field) Ethanol (100%, dilute to 90% in the field) Waterproof paper labels Dissection kit Pencils	

Other items	Hand-held GPS Fish identification keys & books Topographic Maps Permits to collect “Collection in Progress” permit sign Field data sheets Clipboards Pencils Camera First-aid kits Electrofisher manuals
Lunch gear	Fold-out table Fold-out chairs Esky (icebox) Plates, cups, cutlery Chopping boards Tin opener Insulated drink containers
Camping gear	
Food	