Fish collection and dissection for the purpose of chemical analysis of tissues

1 Purpose and scope

The purpose of this document is to provide a standard method of fish dissection to collect tissues that can be used to assess whether toxic contaminants are the cause of a fish kill. Tissues will vary between and within species (e.g. size, colour). The primary tissues to be collected include the gills, liver, and muscle (flesh). If the fish are very small, whole fish should be collected.

It is important that you have a plan in place with a suitable analytical laboratory prior to a fish kill event. Contact the analytical laboratory prior to undertaking sampling and/or dissections to determine the amount of tissue required for a particular analysis.

This document does not outline methods for assessment of disease.

2 Associated documents

Sampling design and preparation:

- *Permits and approvals*
- *Record keeping, including taking field photographs and videos*
- *Choosing a laboratory and analytical method, holding times and preservation*

Biological assessment:

- *Sampling fish communities using fyke nets*
- *Sampling fish communities using bait traps*
- *Sampling fish communities using gill nets*
- *Sampling fish communities using electrofishing*
- *Sampling fish communities using seine nets*
- *Sampling fish communities using cast nets*
- *Fish holding, identification and measurement of length and weight*

3 Health and safety

Before following the methods contained in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the Queensland Work Health and Safety legislative obligations.

4 Permits and approvals

Permits and approvals may be required to conduct activities involving animals, plants and/or in protected areas (for example National Park/Regional Park, State Forest or State Marine Park). Specific to this procedure, a General Fisheries Permit, Scientific user registration and animal ethics approval are required to collect live fish samples. If fish are sick or dying (i.e. during a fish kill event), the collection of fish for initial diagnosis does not require animal ethics approval.

However, any further surveys outside of the initial fish kill event, such as sampling at control sites or for structured surveillance, will require animal ethics approval. See *Permits and approvals* document for more information on requirements.

5 Skills, training and experience

It is desirable that the person conducting this procedure, has some experience or training in the dissection of fish for target tissues and organs.

6 Equipment

See Appendix 1 for example equipment checklist.

7 Procedure

7.1 Collecting fish

Note: Taking fish or fish samples in excess of the minimum required is recommended because further investigations may not be possible if insufficient samples are taken.

- As a rule of thumb, a tissue sample of at least 20g is suggested for each analyte type (i.e. inorganics/organics). It is recommended to collect enough tissue for both organic and inorganic analyses. Providing smaller amounts of tissue sample may lead to higher limits of reporting.
- If dissection is not possible within 24 hours, whole fish may be frozen and tissue samples taken prior to chemical analysis, although freezing may lead to rupture of internal organs.
- If organic contaminants are to be analysed, the lipid concentration of the organ must be measured by the laboratory.

1. Determine the number of fish to be collected, and how much tissue is required from each fish for the analyses to be conducted.
   - For toxicant analysis, collect *at least* three fish of the same species of approximately uniform size *per site* if possible (to enable a statistically sound test against standards or between sites).
   - If the fish are small, you may need to collect more than three (enough tissue per sample for the lab analysis to be done). If there is insufficient quantities of tissue in each fish for analysis, then multiple fish and organ samples per site should be pooled to produce three composite samples per site.
2. Collecting appropriate samples:
   - In order to obtain fresh tissue after a fish kill, it is preferable to choose fish that are sick or dying rather than dead (e.g. some might be moving but showing signs of lethargy or distress). When sampling live fish, ensure that the fish are handled and euthanized humanely (such as with the use of AQUI-S™[1]). If only dead fish are present, choose the least decomposed fish available.
   - If collecting fish for chemical analysis other than in response to a fish kill, follow the relevant fish sampling procedures listed in the *Biological assessments* documents within this manual.
3. Place individual samples into individual resealable bags with a label stating relevant information such as date, time, sampler details, site, species and replicate number.
4. Place samples in an esky with crushed ice to transport to a laboratory, or clean area if dissections are to

[1] Other acceptable euthanasia methods are described in ANZCCART, 2001, Euthanasia of Animals Used for Scientific Purposes Reilly (2nd edition), Department of Environmental Biology, Adelaide University. Available from: https://www.adelaide.edu.au/ANZCCART/docs/euthanasia.pdf

be carried out in the field.

7.2 Preparing for dissection

1. Ensure there is a clean working area and that equipment can be rinsed between each sample.
2. Clean sampling equipment to be used for the dissection:
    - Tools, work surface, and sample containers[2] must be clean and not likely to contaminate the samples with an analyte of interest (for example, if nickel or chromium are of interest, then stainless steel tools may be inappropriate).
    - After each fish is dissected, all equipment should be cleaned and rinsed, and the cutting board covering and gloves need to be changed.
3. Clear an area to conduct the dissection.
4. Place clean aluminium foil (for organics/pesticides analysis) or plastic (for metal analysis or other) on the cutting board or tray prior to placing the fish on the work area.
5. Set up the work area to ensure all equipment is easily accessible once dissections begin.
6. Place a waste bin in an area easily accessible to the person conducting the dissections.
7. Identify a procedure for naming each sample/replicate/organ, and relating these back to the individual sample. See the *Preparation for sampling* document for information regarding naming sites and samples.

7.3 Fish dissection

1. Measure and weigh fish in accordance with the *Fish holding, identification and measurement of length and weight* document. Record details.
2. Put on powder-free gloves. Gloves must be stored in a clean environment (e.g. in a resealable plastic bag).
3. Lay fish flat on one side with the dorsal fin facing away from you.

7.3.1 Gill samples

If the gills are to be collected:

1. Lift the operculum (gill cover) (Figure 1) and cut this off at its base to expose the gills (Figure 2).Take care not to damage the gills when doing this.
2. Carefully cut out the gills at their base (Figure 3), taking care not to damage these when doing so.
3. Rinse gills with de-ionised water.
4. Place gills in labelled storage container/bag (see Section 7.4).

Note: Gills on larger fish may not require the operculum to be removed.

[2] If high concentrations of contaminants are suspected, laboratory grade plastic bags should be adequate to store tissues in. However, if low concentrations of an analyte are suspected it may be more appropriate to store the fish organs in glass jars that have been supplied and cleaned specifically for the analyte of interest by the analytical laboratory.

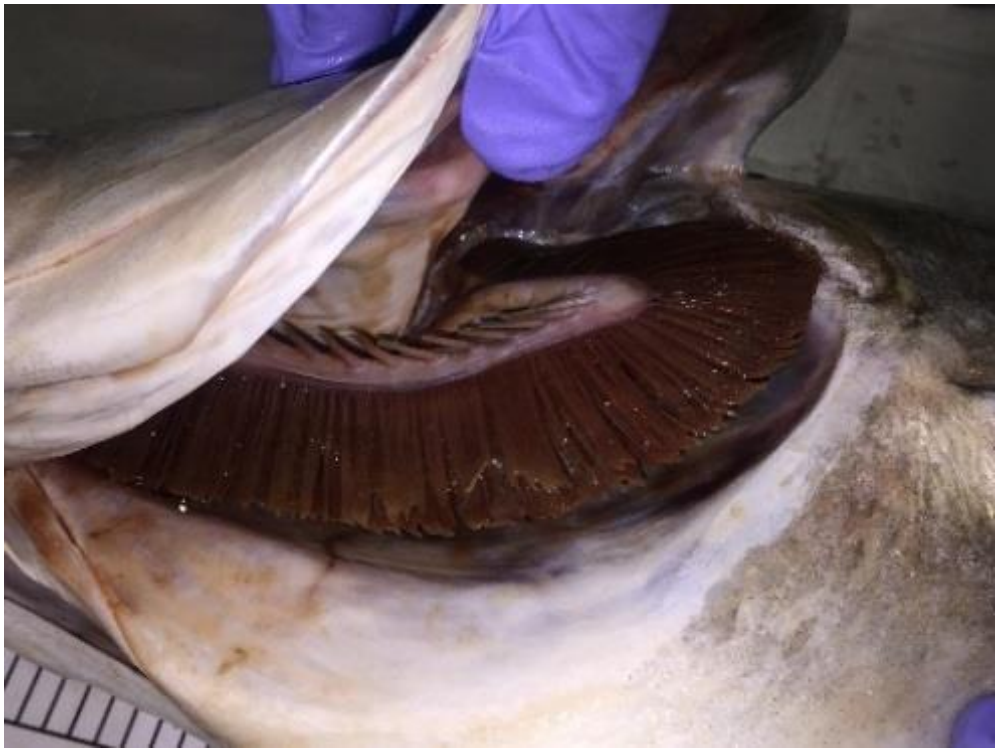

Figure 1: Lifting the operculum (gill cover)

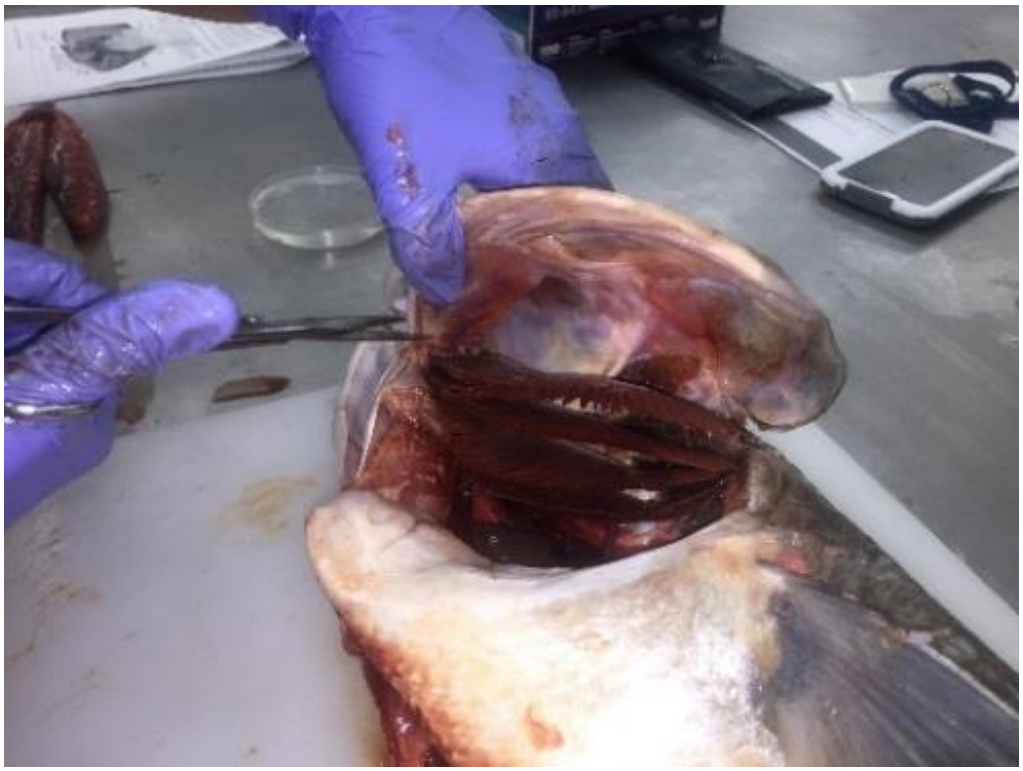

Figure 2: Cut at the base

Figure 3: Cut gills at the base

7.3.2 Muscle samples

Muscle (flesh) samples should be collected above the lateral line, between the dorsal fin and the caudal fin. This will maximise the amount of muscle tissue collected and reduce the risk of accidentally piercing internal organs. Avoid cutting below the fish's lateral line to ensure the lower intestine or other internal organs are not pierced. If the intestine is cut open, this will lead to contamination of the organs and the sample will not be usable.

If muscle is to be collected:

1. Make a cut with the scalpel blade from just below the start of the dorsal fin down to the fish's lateral line (Cut 1, Figure 4).
2. Cut from just above the lateral line of the fish toward the tail (Cut 2, Figure 4).
3. Cut from where the first incision was made just below the dorsal fin across the top of the fish and down toward the tail (Cut 3, Figure 4), to meet the cut from step 2.
4. Remove the skin of this section of cut flesh using forceps and a scalpel blade (Figure 5). Take care not to touch this exposed muscle.
5. To remove the muscle sample, make incisions around the dissected area, cutting underneath the flesh to detach it from the small bones and allow it to be removed (Figure 6).
6. Once the muscle has been removed from the fish, rinse it in deionised water.
7. Place muscle sample in labelled storage container/bag (see Section 7.4).

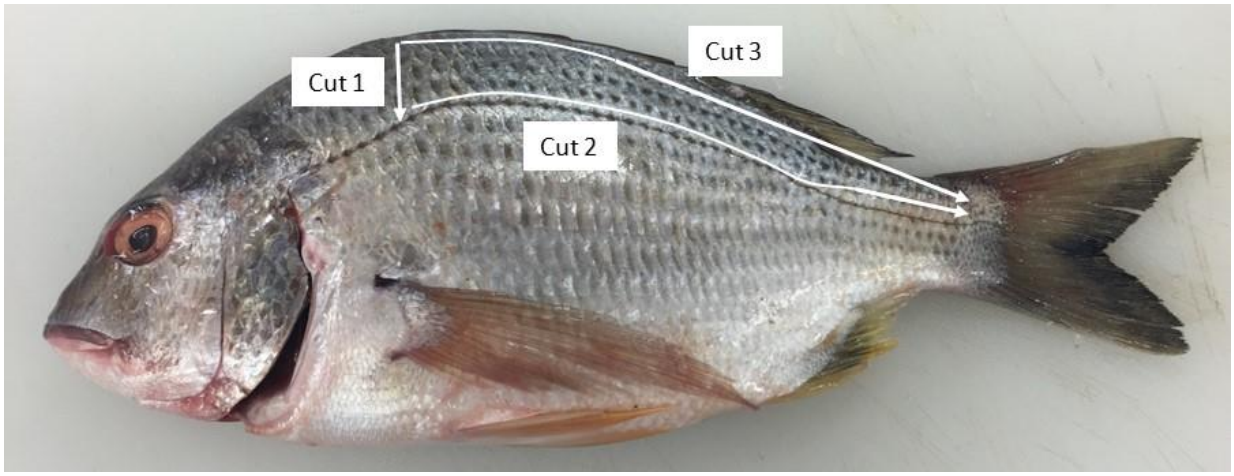

Figure 4: Outline of area to be removed from the fish for muscle sample

Figure 5: Removing skin

Figure 6: Removing muscle

7.3.3 Other internal organ samples

If other internal organs (i.e. primarily liver) are to be collected:

1. Make a small cut just in front of the anus (vent) to open the abdominal cavity.
2. With blunt-ended scissors, cut along the belly (ventral midline) of the fish, forward to the middle of the lower the jaw (Figure 7).
3. Remove the flap of skin covering the abdominal cavity by cutting from the small cut in front of the anus upwards, across the body of the fish and toward the head of the fish (Figure 8). This should expose the heart and abdominal organs for examination and removal.
4. Carefully cut out the organ for examination, taking care not to damage these when doing so.

Note: Organs can be located in differing/varying places depending upon the body shape of the species (e.g. Figures 9, 10). The kidney is a relatively difficult organ to locate and dissect successfully. It is usually located up close to the spine and may be hidden by the swim bladder.

5. Rinse the removed organ with de-ionised water.
6. Place the removed organ in labelled storage container/bag (see Section 7.4).

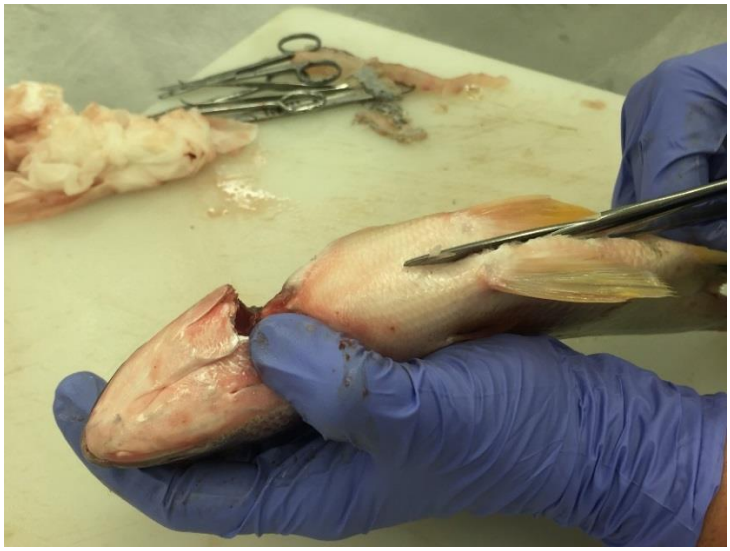

Figure 7: Cut along the belly

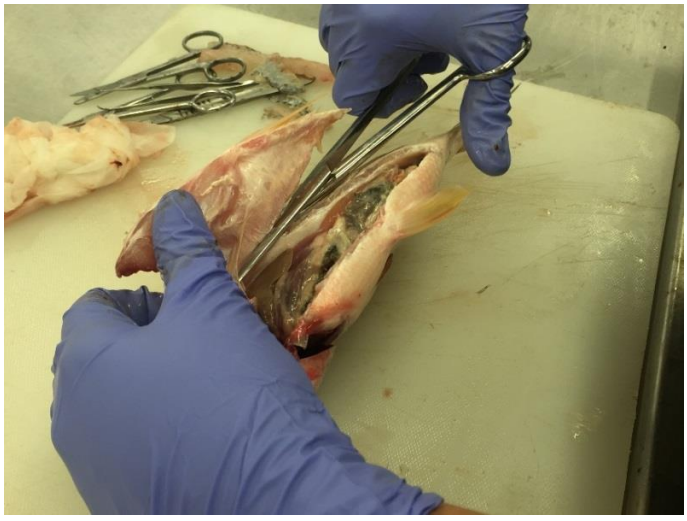

Figure 8: Expose the abdominal cavity

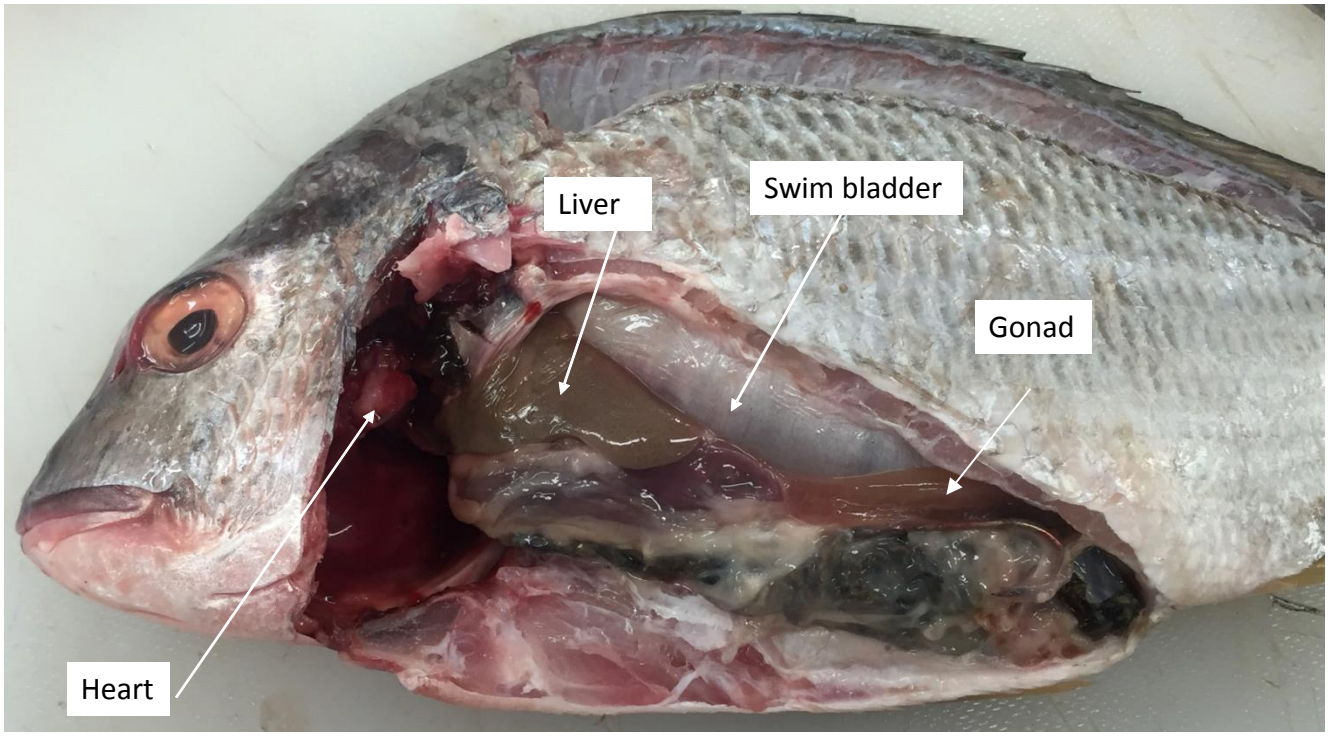

Figure 9 Internal anatomy of a yellowfin bream (*Acanthopagrus australis*)

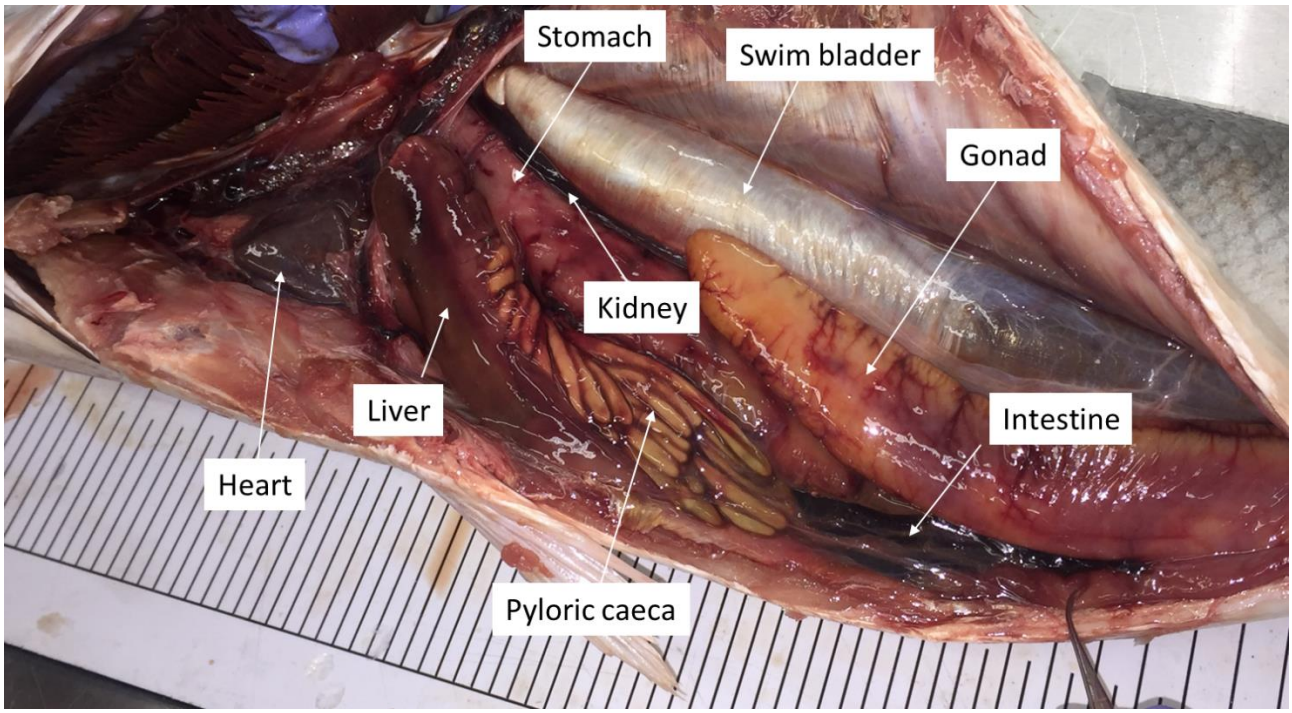

Figure 10 Internal anatomy of a tailor (*Pomatomus saltatrix*)

7.4 Preserving and packing samples

Packing samples will depend upon the analysis required and should be discussed with the analytical laboratory prior to dissections. Individual organs should be separately packaged, labelled and preserved prior to sending to the laboratory.

For toxicant analysis:

- where poisoning by pesticides or organic compounds is suspected:
    - wrap fish or samples in aluminium foil with the dull side of the foil inwards
    - place in laboratory grade container or bag
    - freeze as soon as possible (-20ºC freezer for longer term storage i.e. >24–48h)
- for other analysis, including metals
    - place fish or samples in laboratory container or bag
    - freeze as soon as possible (-20ºC freezer for longer term storage i.e. >24–48h)

8 References and additional reading

There is a vast array of online resources with information on fish anatomy for various species of fish, and step-by-step guides to fish dissections. These guides and videos will assist with fish dissections. Some examples include:

- European Association of Fish Pathologist (a highly comprehensive manual for fish necropsy) http://necropsymanual.net/en/
- http://www.popstoolkit.com/sops/methods/fish.aspx
- http://www.jove.com/video/1717/dissection-of-organs-from-the-adult-zebrafish

USEPA (United States Environment Protection Agency), 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1. Fish Sampling and Analysis. Third Edition. Available from: https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf

USGS (United States Geological Survey), Biomonitoring of Environmental Status and Trends (BEST), Filed Procedures for Assessing the Exposure of Fish to Environmental Contaminants. Available from: https://pubs.er.usgs.gov/publication/itr19990007

ANZCCART(Australian & New Zealand Council for the Care of Animals in Research and Teaching) 2001, Euthanasia of Animals Used for Scientific Purposes Reilly (2nd edition), Department of Environmental Biology, Adelaide University. Available from: https://www.adelaide.edu.au/ANZCCART/docs/euthanasia.pdf

Appendix 1

Table 1 Equipment checklist

Equipment	✓
Various sizes of laboratory grade resealable plastic bags (if analysing for inorganics/metals), and/or laboratory supplied and cleaned glass jars for the analyte of interest	
Aluminium foil (if analysing for organics/pesticides), or clean plastic sheet (metals or other)	
Scalpel (with disposal blades and sharps disposal unit)	
Scissors	
Forceps	
Disposable gloves (powder-free)	
Marker pen for labelling samples	
Measuring board and scales (to measure/weigh fish prior to dissection)	
Tray or cutting board to dissect upon (if possible)	
Table	
Waste bucket	
De-ionised water	
Squeeze bottles	
Portable fridge/freezer (-20ºC freezer for longer term storage i.e. >24-48h)	
Fish catching equipment and associated euthanasia equipment	