Flinders University

College of Science and Engineering

Standard Operating Procedure

Working with Aquatic Organisms

Science & Engineering Animal Facilities

SOP Number	RA Number	AWC Approval Date
SOP-BIOL-3- Aquatic Organisms	RA_	24/03/20

Contact Person	SOP prepared by	Review Date
Leslie Morrison	Leslie Morrison	March 2023

The SOP **Working with Fish** contains the following sections:

Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed*.
- *Animal Welfare Act 1985*
- *Animal Welfare Regulations 2012*
- *Gene Technology Act 2000*
- *Gene Technology Regulations 2001*
- *Work Health and Safety Regulations 2012*
- *Fisheries Management Act 2007* (Section 115)
- *National Parks and Wildlife Act 1972*
- *National Parks and Wildlife Regulations 2019*
- *National Parks and Wildlife (Protected Animals- Marine Mammals) Regulations 2010*

University Policy

- Work Health and Safety Policies and Information
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the Flinders University College of Science and Engineering Animal Facilities, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must initially submit a proposal to the College of Science and Engineering Animal Welfare Sub- Committee (AWS-C) for review. Following this review, the application may then be submitted to the University Animal Welfare Committee (AWC). No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Laboratory or Wildlife work with animals can be found on the Flinders University Animal Welfare Committee website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc.

  http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal- ethics_home.cfm

- From 2019, Applications will be transitioning to ResearchNow Ethics & Biosafety, located on OKTA. As long as the forms are still available at the link above, they will be accepted too.

- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Standard Operating Procedures

Refer to Risk assessments, Standard Operating Procedures and Safe Operating Procedures for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Chief Investigator and submitted to the College of Science and Engineering OH&S Manager.

The following are a list of the main SOP's governing working with animals in the College of Science and Engineering. An extensive database of specific technique SOP's is also available from the Animal Facilities Coordinator and on the AWC home page.

- **Standard Operating Procedure and Safe Work Procedure for the Use of the Animal Facility, Aquaculture and Marine Aquarium Facilities**
- **Standard Operating Procedure for Working With Fish**
- **Standard Operating Procedure for Working With Reptiles**
- **Standard Operating Procedure for Working With Birds**

General Information

- Prior to submitting an application to the AWC, you must discuss space requirements with the Animal Facilities Coordinator. Available facilities can then be matched to your project (with consideration of compatibility with other users, temperature, light cycle, housing type, length of project, etc).

- No animals can be housed in the facilities until your project has approval from the Animal Welfare Committee (if required) and *you* have a confirmed booking with appropriate housing for the animals, signed and submitted to the Animal Facilities Coordinator.

- **Prior to animals arriving, your space must be set up, housing ready, water quality stable, and food and equipment (eg water quality measuring) organised.**

Permits and Exemptions

- Any research to be undertaken in the field may require a permit from Department for Environment, Water and Natural resources (DEWNR) http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits

- The Animal Facilities Coordinator holds a Marine Specimen Collection exemption, that may be used by nominated delegates, or you may obtain your own at http://www.pir.sa.gov.au/fishing/permits_and_exemptions

- Collection and live transport/holding of noxious species/declared pests will require a specific permit from The Department of Water, Land and Biodiversity Conservation (DWLBC) and The Department of Primary Industries and Resources of South Australia (PIRSA).

- The College of Science and Engineering has previously held an Aquaculture licence that covers all species endemic to South Australia. If you wish to hold endemic or non-endemic species; with an intention to sell them, please speak with the Animal Facilities Coordinator to apply for a new licence.

While your research may not involve animals as defined by the Australian Code, and therefore not require an application for the use of animals, it is necessary to provide details of organisms you propose to use to the AWC, so as to register their use and identify potential situations where an application will still be required. For example: marine or terrestrial invertebrate collecting, which includes the 'by catch' of non-target animal species, will require an application must be submitted to the AWC.

Marine Specimen Collection Exemption

The Animal Facilities Coordinator holds a Ministerial Exemption and associated permits, for the collection of aquatic organisms in South Australian waters.

Please contact the Animal Facilities Coordinator for advice on using this exemption or applying for a new exemption, if your Teaching or Research falls outside the scope of the existing conditions.

Prior to collection of any organisms:

- The PIRSA fisheries compliance unit on 1800 065 522 must be advised of the following:
    - Details of the proposed locations, number of people collecting, car registration, and the dates on which the collections are to be made.
- A signed **copy** of the Exemption and any associated Permits must be obtained from the exemption holder (Animal Facility Coordinator x 12196).
- Details of the collection must be completed as follows:
    - the date and location of sampling;
    - the gear used;
    - the number and description of all species caught and their fate;
    - the number and description of any samples/biopsies collected;
    - any interactions with protected species and their fate; and
    - any other information regarding size, breeding or anything deemed relevant or of interest that is able to be volunteered.

Declared Pest Species

Please be aware that any declared pest species of fish captured must be euthanased.
http://pir.sa.gov.au/biosecurity/aquatics/aquatic_pests

Permitted Equipment

The Marine Exemption specifies a list of equipment that can be used that is outside the scope of recreational fishing equipment. As well as the equipment on the exemption, you may also use the equipment listed at the link below.

http://www.pir.sa.gov.au/fishing/fishing_gear/permitted_devices

If your activity uses permitted equipment and is not within an aquatic reserve, marine park, or other protected area, and does not involve the collection of protected or noxious species, you may not require an exemption - Please see the Animal Facilities

Coordinator for advice.

Expected Mortality Rates

These are examples of parameters that can affect mortality rates in fish. Some of the issues can be mitigated with stable biological filters and temperature control, quality feeds, and routine water quality monitoring and water changes.

- Transport
- Handling
- Freshwater
- Saltwater
- Temperature
- Recirculating water
- Static water
- Feed type and quantity
- Age
- Microorganisms in environment

However, even with careful management, activities that induce a stress response (transport, etc), microorganisms in the environment and sub-par immune systems (juveniles and aged) will impact mortality rates.

- Capture/ Handling - **5-10%**
- Transport - up to **40%**
- Quarantine - **10-40%** (this upper range is likely due to the impact of transport and will more commonly be seen in fresh water fish up to 2 weeks after arriving).
- Ongoing holding - **5-10%** (reflects age range (often unknown), behavioural issues, naturally occurring physiological issues, and length of time population has been held).
- Species specific holding- Clownfish- 30% until stable pairs established
    - When juvenile/sub-adult clownfish are paired, ongoing aggression will occur until the dominant female has established hierarchy. The smaller male fish can receive minor bite injuries, misadventure, minor injuries, or other unknown during this period and attempt to jump out of the tank (based on historical data)
- Minor intervention- sedation, swabbing, sampling- **10%**

When writing your application, please use these rates as a guide. In particular, during the ongoing holding period, the rates are cumulative, therefore add ongoing holding (10%) to minor intervention (10%) to capture/handling (10%) = 20-30%

Facility Communication and Compliance Workflow

Glossary of terms describing the daily monitoring program, associated documentation and work flow of Facility staff and Facility Users.

Door monitoring chart

Each room has a chart on the door describing what the animals are monitored for daily or weekly.

Door calendar

The calendars are **located on the door to each animal room**. Outside pens share a calendar, this is pinned up above the Terrestrial communications book. Next to the calendars are a list of animal and physical environment parameter lists, a calendar signature indicates these have been checked each day. Accompanying the signature will be a (C) checked and (F) to indicate animals and environ have been checked and fed.

If monitoring indicates there is an issue, the calendar date signature will be highlighted to indicate staff should go to the communications book for further information.

Communications Book

The Terrestrial and Aquatic communications books are for recording all animal related activity (e.g. births, deaths, ill-health, room movement, arrivals, transfers, initiation of medical treatment, etc). Please use the appropriately dated page and initial your notes.

These books are **located on the benches in the Terrestrial and Aquatic hallways**.

Approved application

Approved applications are **located on pin up boards** either near the rooms housing the animals or near the communication books. Copies are also available on the **share drive**. Bear in mind there may have been modifications or animal welfare officer directives since the original approval but it does give you a baseline to inform further enquiries.

Clinical Record Sheet

These are initiated for animals undergoing experimental procedures or presenting with signs of ill health. Copies are available on the **share drive and adjacent to the communication books**.

Running Mortality Sheet

These apply to a limited range of projects with a fixed number of identifiable animals (e.g. individually housed, non-breeding, etc). Templates are located on the **share drive** and spare hard copies are in a folder **next to the communication book.**

Facility Communication and Compliance Workflow

Daily Duties

This excel spreadsheet is **located within Microsoft Teams** (Animal House Team). The document can be edited in real time by multiple team members and is where you can find information on tasks being undertaken each day- including monitoring and maintenance of all animals in the facility.

Medication Book

Located next to the Communication book. Information recorded here- Animals details, dosage regime of medication approved by the AWO, record of medications held.

Health Records

Excel spreadsheet located on the share drive. This notes all animal's location, numbers held, health status, arrival, transfer, departure. The information is obtained from the Communications books and is used to compile the Health Report to the Animal Welfare Committee, the annual Fauna Permit returns/ permit renewal and, occasionally, the Marine Collection Exemption.

These records are also a visual indicator of potential patterns in health anomalies.

Health Report

Compiled monthly from the Health records and submitted to the Animal Welfare Committee. **This document is stored on the share drive.**

Share drive

Permission restricted folder **on the CSE share drive** for Animal Facility staff to access records and maintain monitoring and compliance information. Routinely backed up to the Facility Managers computer. AWO has access to view health records and daily duties archive.

Unexpected Adverse Event

An incident impacting on the health and welfare of animals held in the facility that is not part of the approved ethics application. Eg equipment failure, mortalities exceeding approved numbers, ill health. A template of this form is **located on the Animal Ethics Committee webpage.**

Calendar and Daily Duties signatures/ symbols

(C) Checked. (F) fed are used on the door calendars, as well as staff initials. Staff initials are used to sign off on the daily duties.

Facility Communication and Compliance Workflow

Communication Workflow

- Upon arrival, communicate with other team members about any animal related matters and determine who is working where (terrestrial or aquatic).
- Check Daily Duties for any notes from previous day.
- Check emails/messenger/office phone for Facility related messages.
- Check communications book in terrestrial and aquatic hallways for notes regarding animals/facilities. (if you are only rostered to one area just check the relevant book initially, check the other area later if assisting in that area).
- Commence animal monitoring and husbandry tasks as per directions in the Daily Duties spreadsheet.
- As tasks are completed in the animal rooms- as per the parameters listed on the doors to the rooms- sign off with your initials on the calendar and whether you have (C) checked and (F) fed.

    - If there are any issues (births, deaths, ill health, equipment failure, potential unexpected adverse event, etc), highlight the day on the door calendar, sign off and proceed to the Communication book to note the issue (if it is an urgent animal welfare matter, please attend/ resolve the issue first and complete noting afterwards).
    - If the approved application has a running mortality sheet, record deaths on the sheet as well as in the Communication book. If the running mortality hits a milestone, notify the Animal Facilities manager and in their absence- contact the Chief Investigator and the Animal Welfare officer.
    - If the approved application has a clinical record sheet, please fill in details on the sheet and follow the monitoring protocols outlined on the sheet.
    - Running mortality and clinical record sheets are stored next to the communication books.

    - The AWO must authorise courses of medication. Once authorised, please note this in the Communication book and then record the animals details and dosage regime in the Medication book. There is a column to sign off in the medication book after each dosage is given.

Facility Communication and Compliance Workflow

Communication Workflow

- If your monitoring detects an issue that is not in compliance with the approved application, please advise the Facility Manager. If this is not possible, Please contact the Chief Investigator to discuss the appropriate course of action to resolve the issue; you can also advise the CI to notify the Animal Welfare Office. If the CI is uncontactable, advise the Animal Welfare Office in their absence.
- When work has been completed, please sign off on the Daily Duties spreadsheet, located in **Microsoft Teams**. Please include notes for staff rostered on the next day.
- Information from the Communication books is then transferred to the excel spreadsheets file "Health records" located in the **share drive**. This spreadsheet allows us to keep track of animal numbers.
- The monthly report to the Animal Welfare Committee is created with the data from the Health records and stored in the **share drive**.

Copies of Legislation

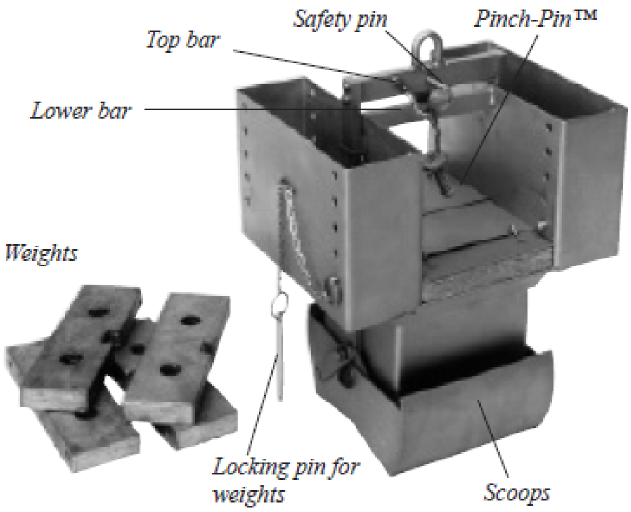
- Hard copies of the *Australian Code for the Care and Use of Animals for scientific purposes, 8th ed 2013* are available in the Animal Facilities Office and can also be found online https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes **and on the share drive.**
- A Hard Copy of the Animal Welfare Act is available in the Animal Facilities Office and can also be found **on the share drive.**
- A Hard copy of the Animal Welfare Regulations is available in the Animal Facilities Office and can also be found **on the share drive.**
- A Hard copy of the Marine Specimen Collection Exemption is available in the Animal Facilities Office and can also be found **on the share drive.**

Collecting Techniques

General - Box or Opera House Baited Trapping

- Opera House or Box traps are used to capture and determine which scavenging species are present within protected waters and include streams, rivers, lakes, and estuaries.

- Scavenging species include native crustaceans, the invasive crab *Carcinus maenas*, and small bodied fish.
- Opera house or box traps are commonly baited with frozen pilchard or cat food, and deployed for a soak time of 24 hours before being retrieved.
- Detrimental effects of Box or Opera House trapping can be reduced by:
    1. Ensuring traps are submerged for entire soak time, particularly with tidal movement along the intertidal zone;
    2. Minimising capture by restricted entrance hole diameters (<7.5cm) and soak times of 24 hours; and
    3. Minimising handling time of animals captured which are then released immediately to the waterway where they were captured. The exception being the invasive European shore crab *Carcinus maenas*, which are not allowed to be returned to the environment according to Australian biosecurity policies.



Introduction:

- The patented Wildco® box corer is designed to take larger samples in harder bottoms more easily and safely than spring-powered grabs. It is especially effective in finely divided muck, clays, mud, ooze, submerged marl, or fine peaty materials.
- The sole driving force is the box corer's weight, which can total 44 kg. The body itself weights about 14 kg, which is augmented by up to twelve extra weights, each weighing 4 kg, securely fastened in two (2) side bins. The heavy duty linkage

and scoops dig as deep as the weight will allow. The smooth interior allows an acrylic liner to easily slip in and out. *Ship weight: 32 kg.*

- We recommend one or two trial samples as a means of determining whether added weights are advisable to make certain that the dredge will bite deep enough into the bottom being sampled.

Operation Requires:

- 100' stainless steel cable (61-B14 rec.)
- Winch and depth meter (85-E10 recommended)

Accessories or Parts:

- 191-A91 extra weights pack of 4, 8# each
- 188-E50 Wash frame to sort sample
- 191-L12 Replacement screen, 500 micron
- 1728-L12 Replacement release pin
- 910-A26 Plywood carry case
- 191-B10 Acrylic liner

How to Operate:

1. **Inspect the dredge** before using to make sure it is in working order. Make sure it is securely attached to the cable on the winch/crane.
2. Carefully keeping clear of the jaws and other working edges of the dredge, **move the scoops to the open position.** To open the scoops, hold the corer by the lower bar and push down on the top bar until the holes in the two bars are aligned.
3. **Stack the included weights** in the side pockets on either side of the box corer, an equal number of weights on each side. To hold the weights in place, insert the locking pins through the hole on the side of the weight box and the actual weight. The large holes in the weights are used as fingergrips for handling the weights. [Additional weights are available from *Wildlife Supply*®].
4. **Insert the safety pin at this time**. To do so, push the pin into the hole in the lower bar until it is through both bars, then hold into place.
5. When ready to sample, remove the safety pin and **insert the Pinch-Pin™**.
6. Pull up on the top bar to allow the weight of the corer to pinch the pin and hold it into place.
7. Use the winch/crane to lift the dredge clear of the boat deck and then outboard.

8. **Lower the dredge slowly** into the water. Top surfaces are covered with 500 micron mesh screen to reduce shock wave and drift, yet prevents sediments and organisms from escaping.
9. When the dredge reaches the bottom, allow a moment for it to sink into the sediments. **Keep tension on the cable** for penetration to occur.
10. **Slack off on the cable** to release the tension on the upper bar. This permits the Pinch-Pin™ to slide out, thus allowing the sampler to close.
11. Now **winch the cable** to exert a closing motion, transmitted mechanically through the bars and to the jaws of the dredge.
12. This mechanical action, plus the force exerted downward by the weights bolted to the jaws (plus any additional weights) tends to force the jaws **deeper into the bottom** as they are moving to close. The machine tapered cutting edges on the jaws add to the ease of movement through bottom materials.
13. **Maintain tension on the cable** by operating the winch. This completes the closing of the sampler and raises it back to the surface. This should be in a steady, slow lift.
14. When the dredge reaches the surface, **lift it clear and swing it inboard** to position over a tub placed to receive the sample, such as the 188-E50 washframe.
15. Taking care to stay clear of the edges of the jaws, **open the sampler and discharge the sample** into the tub. The liner allows easy removal of the sample. You can pull the liner out with the sample contained within. Samples should be screened, sieved, separated, bottled, labelled and otherwise processed for analysis and classification studies by the standard procedures outlined for the work in progress.
16. At the conclusion of sampling operations, **replace the "Safety Pin"** to prevent accidental closing of the jaws in handling or shipping. Then wash and inspect the grab and make necessary repairs or adjustments in preparation for the next use. The unit should be decontaminated between each unique sampling location.

Maintenance:

Barring loss through accident or abuse, this dredge will give long years of trouble-free service. The 316 stainless steel construction resists corrosion.

1. **Wash the dredge** after each sample drop; at the close of the day's work, give the entire apparatus a thorough washing with fresh water. This is particularly essential after sampling in **salt water**. Do the same with all equipment - cable, crane, winch, boats, etc.
2. **Inspect the cutting edges** after each sample drop. Severe nicks or dents may require re-working of these edges to assure a good cutting action and tight closure.
3. **Lubricate** pivot points occasionally. When the bottom dredge is to be out of service for a long time, we recommend applying a coating of oil or other rust barrier to protect the unit's metal surfaces. Coat all surfaces, joints, bolts and stud-bolt holes if these are to be left open.

Fish – Baited Traps

Equipment Familiarisation

- Aquatic baited traps include:
    1. Opera House traps (64cm length, 47 cm wide, mesh sizes 2 - 3mm and 15mm mesh sizes).
    2. Box traps (47cm length, 25 cm wide, mesh size 2 - 3 mm).
- You will need to be familiar with the baiting, deployment, and retrieval of traps.
- Upon retrieval of traps, you need to be shown by an experienced person on the correct handling procedure for all fauna, and in particular the release of fish and cephalopods back to the waterway.
- You will also require datasheets to record your observations.

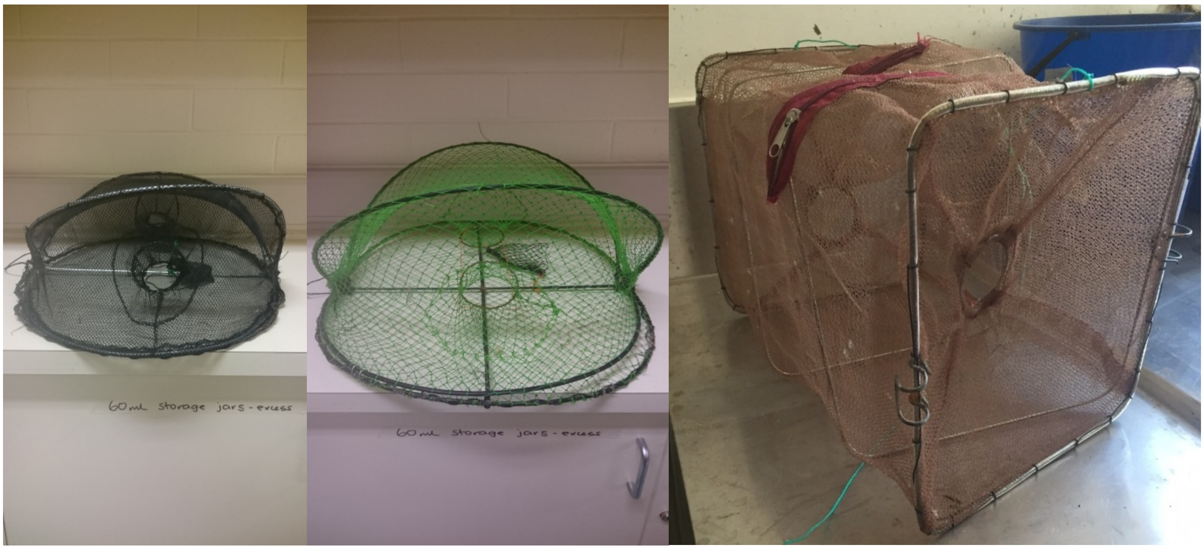


PHOTO: Different sized Opera Traps (left and middle), and an example of a Box Trap (right).

BRUVS

Please refer to the approved manual on the Flinders University Animal Ethics Webpage

MARINE SAMPLING FIELD MANUAL FOR BENTHIC STEREO BRUVS (BAITED REMOTE UNDERWATER VIDEO)
Tim Langlois*, Joel Williams‡, Jacquomo Monk§, Phil Bouchet, Leanne Currey,
Jordan Goetze, David Harasti, Charlie Huveneers, Daniel Ierodiaconou, Hamish Malcolm, Sasha Whitmore

Sharks- Handling and Tagging

Please refer to the approved Code of Practice on the Flinders University Animal Ethics Webpage

CMAR Code of Practice for Tagging Marine Animals
Bradford, R. W.; Hobday, A. J.; Evans, K., Lansdell, M. 01 November 2009

Animal Facility Equipment

- Holding tanks/aquaria
- Transport tanks
- Water quality measuring equipment
  - Oxygen meter
  - pH/salinity/temperature/conductivity meter
  - Chemical test kits for ammonia, nitrite, and nitrate
- Food
- Species specific habitat/enrichment
- Lab equipment

- The Animal Facility will supply you with basic cleaning equipment (siphon hoses, sponges, buckets, bleach, ethanol), basic medication, tank labels, gloves, etc.

Transport

Before Transport:

- Only a person that has previous experience with transporting fish (and other aquatic animals covered under the Code) may collect animals. This may include Animal Facility staff, researchers with past experience in fish transportation, and other staff or students under the guidance of an experienced supervisor.

- No food should be given on the day of transportation. Feed restriction will decrease water fouling during transport. Fish should also not be crowded or have undergone significant stress prior to transporting (e.g. grading, handling, etc).

- Whenever possible, contact the supplier the day before pickup to confirm last feeding time, and that fish will be packed with supplemental oxygen. **Battery powered aerators must still be taken when picking up fish in case of air leak from bag.**

- Only healthy fish should be transported. Any fish with deformities and/or showing signs of disease should not be transported. The likelihood of problems during transportation is increased when fish have had recent disease problems or have physical deformities.

- Fish transportation from interstate or outside metropolitan Adelaide (more than 1 hour from the University) must only be undertaken when forecast air temperatures during transport will not exceed 35°C, and all stages of transport and housing during transport can maintain temperatures **within an acceptable range for the specific species.**

- Insulated foam boxes and 20-40L plastic buckets or drums (both with lids) are the commonly used transport containers.

- You MUST transport fish in a vehicle with temperature control, so that fish can be maintained within their ideal temperature range. This does not mean placing them in the tray of a ute where they are exposed to varying ambient temperature and radiant heat from the vehicle exhaust, it means transporting them in the back seat of a car or cargo area of a station wagon. Car boots, trays of utes etc, are not appropriate unless you have evidence they will stay within the fishes ideal temperature range, and the equipment to monitor it.

- Fish picked up from a local supplier (within 1 hour of the University), or arriving by bus or plane, that have been bagged with supplemental oxygen and placed in an insulated box at a standard stocking density (research the species/size you are working with) may be transported in a climate controlled vehicle directly to the University without providing battery powered aeration or sedation. However, you must still take battery powered aerators with you and monitor fish and supply supplemental oxygen if they appear stressed.

- If the Biology Fish Transporter is being used, it must be cleaned and sterilised with ethanol or bleach (bleach must then be neutralised with sodium thiosulphate) prior to use. This will eliminate potential source of infection if fish receive any physical damage. The person supervising the transport must take and provide some form of aeration (oxygen cylinders for fish transporter), and on longer trips (over 2 hours) water quality monitoring equipment should be taken to ensure water quality can be monitored hourly during the trip. Fish waste must be siphoned out (if possible) and water exchanged if ammonia levels exceed safe limits. “Complete Water Treatment” is a product that removes ammonia from water and is also recommended for long trips as a contingency plan if replacement water is unavailable.

<u>During Transport:</u>

- Fish transported in the fish transporter should be inspected every 2-3 hours. The fish should be inspected for physical signs of stress like increased respiration, equilibrium, swimming behaviour, and colour, and the oxygen level and temperature of the water checked.

- For large numbers of fish being transported in the Fish Transporter, it is recommended a dose of 10mg/L to 20 mg/L of Aqui-S be put in the water during trips. <u>This is particularly important</u> with larger biomasses of fish. **<u>If anaesthetics are being used, then aeration must be provided.</u>**

- If fish are showing significant signs of stress (gasping, erratic swimming, loss of equilibrium) during transportation, then an increase in the anaesthetics dose should help sedate them to reduce stress. Increasing the dose (Aqui-S in this example) slowly by 1-2mg/L should allow the level of sedation to be closely controlled. The overall level of Aqui-S anaesthetic administered should not exceed 20mg/L, as they will slowly lose equilibrium.

<u>Admission into the Flinders University College of Science and Engineering Animal Facility:</u>

- Animal Facility staff must be notified in advance of expected arrival day and time. Space booking and housing with appropriate environmental parameters must be confirmed prior to transport commencing.

- The Animal Facility staff will have allocated space to quarantine the fish for 2 weeks, so they must not come into contact with other fish housed in the facility. This will help reduce the transfer of disease. Quarantined fish must have their separate nets, hoses, and cleaning equipment allocated.

- Once animals are transported to the Flinders University College of Science and Engineering Animal Facility, they must be placed into quarantine tanks that are within their optimal water parameters, including temperature, and allowed to equilibrate. The pH should be checked and adjusted to the same pH as the transport water. Animals must not be offered food until they have acclimatized for at least 24 hours, and must be monitored daily as per the Clinical Record Sheet (see *"Clinical Record Sheet Example"*).

Housing

- Housing design must allow for routine monitoring of animal health. Glass and clear plastic tanks must be used for shelving style housing (no black tubs).

- Housing may include invertebrates that fish have a symbiotic relationship with (e.g. Clownfish and anemones).

- Numbers to be held must take into consideration that waters oxygen carrying capacity decreases as temperature increases.

- A system generally will be able to accommodate a greater total weight of large fish than small (in regards to available oxygen).

- Oxygen consumption is directly related to the amount of food fed. Uneaten food reduces oxygen carrying capacity through BOD (biochemical oxygen demand). BOD measures the amount of organic compounds in water.

- All applications in which new aquaculture systems are to be established must mention the pre-conditioning of water and biofilters. In addition, applications must mention that all tanks, water recirculation equipment, and facilities should be tested <u>prior</u> to arrival of aquatic animals.

Quarantine and Health Monitoring

- Please refer to the Clinical Record Sheet for Monitoring Parameters, Scoring, Intervention Points and Actions

- All animals should be assessed by experienced staff and confirmed as healthy prior to transport to the Animal Facility.
- Animals should be placed in their specific quarantine tanks, which will be allocated by the Animal Facility staff. The tanks should be biologically separated from other holding animals, and all equipment used for quarantine should not be used for anything else or shared with other rooms.
- Aquatic animals should be quarantined for 2 weeks and then assessed to be healthy before releasing into a project. Most potential disease issues should start to show signs of infection within that period. If disease is found during the quarantine period, it may be necessary to extend the quarantine period for treatment.
- All tanks must be labelled with the following:
    1. Project approval number.
    2. Species.
    3. Animal numbers.

- Water quality and animal health should be monitored daily for the quarantine period. The parameters that need to be monitored are included on the Clinical Record Sheet.
- The Clinical Record Sheet is to be filled in for the quarantine period and placed with the approval notice on the front of the facility doors.
- After the quarantine period, please see Animal Facility staff to assess the health of the animals for release to the project.
- You may use the Clinical Record Sheet for your daily monitoring during quarantine, and then adapt it as required for your specific project (eg. water quality may only need weekly checking).
- Clinical Record Sheets must be kept with the animals in the Animal Facility **at all times.**

- Once quarantine is complete, monitoring will continue as described in the Facility Communication and compliance workflow section of this Standard Operating Procedure.

Standard Ongoing Maintenance Monitoring Parameters

1. Appropriate room temperature and air circulation/ water parameters
2. Any signs of abnormal body shape
3. Swelling/fight injuries

4. Abnormal movement (loss of equilibrium)
5. Significant change in appetite
6. Abnormal level of activity (erratic swimming)
7. Abnormal respiration (gasping at surface)
8. Water parameters (pH, salinity, NH4, NO2/NO3)

- Cleaning regimes vary considerably depending on the nature of the project. A general rule is 20% water change per week, supported by weekly water quality monitoring to ensure water quality remains stable.

- Algal growth will naturally occur in tanks, some more than others. Its growth is affected by nutrient load in the system/ water chemistry, lighting, water flow, and water type (salt/fresh). Manually removing algae from tanks walls with cleaning and siphoning- while the fish are in the tank- can be detrimental to their health. The small algae particles stirred up in the water irritate their gills, causing respiratory issues and can impact experimental data too (algae attaching to mucus coating on fish for example).

Clinical Record Coversheet and Record sheet (Pages 16-18) adapted with the permission of The University of Western Australia, Office of Research Enterprise. ["Monitoring Cover Sheet" at http://www.research.uwa.edu.au/staff/forms/animals]

Project Number	
Project Title	
Chief Investigator	
Monitoring Start Date / Animal Issue Date	

1) CONTACT DETAILS

Contact Type	Name	Contact Number
Emergency Contact		
Researcher (1)		
Researcher (2)		
Animal Facility Staff		
Animal Welfare Officer	Dr Lewis Vaughan	0450 424 143
Other (please specify)		

2) SPECIES / PHENOTYPE / MODEL ISSUES

3) MONITORING CRITERIA

List monitoring criteria.			
Monitoring Criteria	No obvious deviation from	Slight or moderate deviation from normal	Significant or sustained deviation from normal

	normal		
Score	**0**	**1**	**2**
Environmental			
Temperature ^{o}C	+/- 5 from optimum	+/- 10 from optimum	> 10 from optimum
DO saturation %	>80	70-80	<70
Salinity (ppt)	+/- 5 from optimum	+/- 10 from optimum	> 10 from optimum
Ammonia mg/L	<1	1-5	>5
Nitrite mg/L	<1	1-5	>5
Nitrate mg/L	<50	50 - 100	>100
pH	< 1 unit from optimum	1 – 1.5 units from optimum	> 1.5 units from optimum
Clinical - animal			
Appearance		Discolouration of scales (dulling or change in mucus) +/- slight scale lesions	Extensive loss of scales, generalised lesions
Behaviour		Flashing, spiral swimming, resting on bottom of tank, reduced eating	Loss of equilibrium, gasping, not eating
Other Condition (not documented above, but impacting on welfare)			
Note: Training by the AWO/PI or pictures or video footage of the monitoring criteria at the various scoring points is recommended to ensure all personnel are consistent in terms of scoring.			

4) MONITORING FREQUENCY

Describe monitoring regime.

5) RESEARCHER NOMINATED ACTIONS AND INTERVENTIONS

Score	**Assessment**	Actions/Interventions
Environmental		
0 - 1	Environment within acceptable limits	No interventions required.
1	Ammonia/nitrite/nitrate/salinity/pH →	Review historical records. If new readings are inconsistent, schedule a 20% water change and incorporate waste removal if required. Cross check on alternate equipment if available/reading unusual
	Oxygen	Increase aeration and schedule a 20% water change (incorporate waste removal if required.)
2	Ammonia/nitrite/nitrate/salinity	Approximately 50% of tank water to be replaced
	Oxygen →	Aeration to be increased to tank and a 20-50% water change undertaken (considering ambient temp/available water. Incorporate waste removal (excess feed, algae,etc)
	pH →	Bicarbonate or acid to be added
Clinical - animal		
1	Animal demonstrates slight or moderate deviation from normal	Notify Animal Facilities Coordinator and/or PI and/or AWO – commence treatment if recommended by AWO
2	Animal demonstrates significant or sustained deviation from normal	▪ Immediate consultation with the AWO or immediate euthanasia. ▪ Notify the AWO. ▪ Complete a Running Mortality Sheet if euthanised. ▪ If animal welfare compromise or mortality rates fall outside of approved conditions (section 10 of the application), an Unexpected Adverse Event (UAE) Report must be submitted.
Accumulative score of 3 or greater	Animal demonstrates significant or sustained deviation from normal	▪ Immediate consultation with the AWO or immediate euthanasia. ▪ Notify the AWO. ▪ Complete a Running Mortality Sheet. ▪ If animal welfare compromise or mortality rates fall outside of approved conditions (section 10 of the application), an Unexpected Adverse Event (UAE) Report must be submitted.

6) INSTRUCTIONS

a. Each parameter/animal/tank/enclosure is examined at each nominated monitoring time point.
b. Each criterion is scored and the score marked on the monitoring sheet. Training by the AWO and/or PI is required to ensure all personnel are consistent in terms of scoring.
c. Scores are then added together and a total score is recorded on the Monitoring Sheet.
d. Appropriate to the score, specific actions/interventions are undertaken.

e. Comments concerning abnormalities are recorded in the "Comments" section.
f. Any other abnormalities are recorded in the "Other" section.
g. Any abnormality that is observed to be of greater severity than the descriptors above, or a major deviation from impact or incidence Approved, requires immediate consultation with the AWO or immediate euthanasia and recorded as an unexpected adverse event.
h. All unexpected adverse events must be reported immediately to the AWO and an Unexpected Adverse Event Report completed.

Clinical Record Coversheet and Record sheet (Pages 16-18) adapted with the permission of The University of Western Australia, Office of Research Enterprise. ["Monitoring Cover Sheet" at http://www.research.uwa.edu.au/staff/forms/animals]

1) ANIMAL DETAILS

AEC Project #		Monitoring frequency	
Enclosure/tank #		Species/Strain	
Animal # (If applicable)		Age/DOB	
Animal Identification System		Sex	

2) MONITORING

Day/date										
Time										
Procedure										
Criteria – environmental/water										
Temperature °C										
DO saturation %										
Salinity (ppt)										
Ammonia mg/L										
Nitrite mg/L										
Nitrate mg/L										
pH										
Criteria - animal										
Appearance										
Behaviour										

Other										
Total										
Signature										
OFFICE USE ONLY AWO CHECK										

3) COMMENTS:

Handling

- Wash hands and arms thoroughly before, and after handling any animals, to reduce risk of infection to animals or transfer of zoonoses to users. Refer to the Animal Facility Safe Operating Procedures and Risk Assessment for any hazards or risks associated with animal handling.
- Hands and equipment must also be washed between handling different groups of animals with an unknown disease status.
- Detergents are not recommended for hand washing, as they may be toxic to aquatic animals and amphibians. Ethanol hand wash gel and a through rinse in water are recommended.
- The species being handled must be investigated by the researcher, and any additional risks like stings, bites, and potential zoonoses (not covered by the Animal Facility Risk Assessment) must be risk assessed in the project risk assessment prior to working with the particular species (e.g. anemone stings, poisonous spines, etc).
- Wear gloves and protective clothing when required - this should be identified during your risk assessment prior to commencing your project.
- Generally, fish must be lightly sedated until they reach handling stage, as per guidelines accompanying your chosen sedative (Aqui-S is recommended). You must research/seek advice on the species of fish you are working with, as some species (eg: small bodied fish such as pygmy perch) can be transported, briefly handled, and fin clipped without sedation. Using Aqui-S, which is delivered via immersion bath, on such a small species may present a greater risk of death than undertaking minor procedures without sedation.
- When handling fish, use equipment that will minimise external damage to the animal, and catch and hold the animal effectively. For animals with large scales or sharp spines, use a net with a fine mesh to minimise spines or scales catching in the net. Also, use a net appropriate for the size of a fish. Try to only catch 1 animal at a time, as more animals in the net will scrape against each other and cause external damage.

- Ensure nets, hands, and any equipment that are going to come into contact with the animals/cephalopod, are wet at all times and in good repair. This will minimise damage to the mucous layer.

- When netting large or fast fish, 2 nets are recommended to reduce the time taken to effect capture, and thereby reduce stress. The help of another person can also be helpful to ensure quick, gentle capture. When being netted, they should be raised and lowered slowly, and not dropped into the water.

- Fish must not be out of the water for more than 3 minutes without sedation and supplemental aeration. For recovery, the animal must be placed in a clean, protected container with clean water and aeration.

- Fish must be monitored for the entire recovery time for any anaesthetics, and post anaesthetic monitoring every 1-2 hours until all physiological monitoring parameters have returned to the normal expected signs for the species (eg: respiration, swimming orientation, activity level, etc) is also essential. Monitoring the next day will also show any signs of external damage, and should be undertaken twice per day. If there is any skin damage or external damage, then the fish should be quarantined and treated as per standard husbandry practices in consultation with the Animal Facilities Coordinator and/or AWO.

- You must plan and research your work to ensure that fish have had sufficient time to recover from anaesthetic before night time, so that you are able to visually monitor them.

Weighing and Measuring Fish - Clownfish Example

Equipment:

1. Scales.
2. Data recording supplies (eg: notebook, pen, laptop).
3. Dip net.
4. Clear ruler.
5. Bucket containing water from the system holding the fish or seawater that has been acclimatise to the temperature of the room.
6. Aqui-S.
7. Small dish (eg: a large petri dish) containing sponge or paper towel and small amount of tank water to keep it wet.
8. Battery or mains powered aerator.
9. Ideally, have a second person present to record data and assist in capture and monitoring fish post sedation.
10. Plan your work so that all fish are returned to their tanks and can be checked 2 hours later to confirm recovery before you leave for the day.

Procedure:

1. Set up the weighing and measuring area as close as practicable to the fish holding room. You need to be able to complete all measurements and return the fish to its tank for recovery within 3 minutes.
2. Add Aqui-S to bucket as per SOP instructions.
3. Ensure dish with moist paper towel or cloth is tared/zeroed on the scales.
4. Capture fish as per SOP and place in bucket containing Aqui-S, and move to the weighing and measuring area.
5. Place air stone in the bucket and provide aeration (not so much that it creates a foam).
6. When fish appears sedated (loss of equilibrium, uncoordinated swimming movements, etc) quickly transfer it to the dish on the scales.
7. Record weight.
8. Align clear, plastic ruler with fish, measure, and record length.
9. Return fish to bucket, and then return to fish holding room and place fish in its original tank (which can also be used as a recovery tank).

Please note, if you only need to weigh fish it is recommended this be done in a small container of water.

1. Tare the container of water.
2. Add the fish and record weight.
3. Return fish to recovery tank and retare container of water for next fish.

Administering Medication

- Administering medication to animals may require handling. Users must specifically refer to the “Handling” section of this SOP.
- All medications and how they are administered must be approved by the AWC in the animal welfare application (when using animals that come under the animal ethics guidelines).
- Medication must only be administered by trained personnel.
- Prior to administration of medication, ensure you have a quiet location and all equipment required (e.g. appropriate gauge needles, alcohol swabs, collection vials (if you will be taking samples), sharps bin, protective clothing).
    - Animals requiring medication that is not part of the project protocol (e.g. due to ill health) must be in consultation with Animal Facility staff and the Animal Welfare Officer.

Anaesthesia (General)

- Sedating fish prior to handling, administering medication, or undertaking surgery, will minimise potential physical trauma (e.g. swimming into tank walls to avoid capture). Only anaesthetics that have been approved in your animal welfare application may be used.

- Anaesthetics are routinely used to reduce the impact of environmental change (eg. transport) on the fish. Stress within fish can have a significant impact on the animal's physiology and behaviour. The result can lead to a reduced immune function, so the aim of using anaesthetics is to try to minimise the adverse effects of stress.

- The three most commonly used anaesthetics for fish are Aqui-S, benzocaine, and MS-222-methane sulphonate (when available). The concentration of anaesthetics will depend on the size and species of fish. Researchers must investigate how susceptible a particular species is to being anaesthetised. Different gill structures effect anaesthetic uptake, as does respiration rate (e.g. a Murray Cod respires quite slowly compared to a Blue-fin tuna).

- When anaesthetising fish, there must be a constant supply of air to provide enough oxygen for the period of anaesthesia and recovery. The initial response of fish that have been placed in an anaesthetic bath will be to increase respiration and increase swimming speed (stress response behaviour in fish). Increased aeration can also assist in a quicker recovery time (this can be measured with an oxygen meter to indicate when 100% saturation has been reached). Aeration referred to is from electric/battery aerators, or air supply in Animal Facilities. If tanks of pure oxygen are used, then it must be in accordance with the SOP for the oxygen tanks.

- To evaluate when the fish has reached the suitably sedated stage, the tail hold method can be used (this is where the tail is gently held and if the fish does not try to escape, then it is sufficiently anaesthetised). The period of time that the fish remains anaesthetised must be as short as possible, and periods of time out of the water cannot be longer than a few minutes. A fish can remain lightly sedated for up to 48 hours without additional support. Deep anaesthesia without supplemental oxygen should last no longer than 5 minutes, before recovery is initiated.

- Once the fish has undergone any procedures, they must be recovered in a separate environment (container/floating cage) with aeration, and in the same water as they are housed in. Groups of fish normally held in the same tank may be recovered from anaesthetic in a group tank.

- Healthy fish that have been anaesthetised for non-invasive procedures (e.g. weighing, visual health check, light salt bath, etc) must be monitored at least 1 hour after the procedure, regularly throughout the day (every 2-3 hours), and checked the next day.

- Healthy fish that have been anaesthetised for invasive procedures (e.g. tagging, clipping, injection, surgery, etc) must be monitored closely (1 hour intervals) until normal clinical signs are consistently observed (respiration rate, swimming behaviour), and then monitored daily for at least 1 week.

- Healthy fish that have undergone deep anaesthetisation (eg: for surgery) must be monitored closely for 1 day (1 hour intervals during daylight hours), and monitored twice daily for at least 1 week. These fish must have a sheltered environment appropriate for recovery from surgery (i.e. quiet, low traffic area).

- Sick fish that have been anaesthetised for treatment must be monitored closely for 2 days (1 hour intervals during daylight hours on Day 1, and 2 hourly on Day 2 if condition has not deteriorated), and monitored twice daily for at least 1 week. These fish must have a sheltered (i.e. quiet, low people traffic) environment appropriate for recovery from disease.

General Instructions:

- Water temperature should be between 10°C and 30°C when using Aqui-S.
- Stock solution should be added below the surface of the water (this aids mixing and reduces losses due to foaming and surface evaporation).
- Aeration must be provided to maintain dissolved oxygen concentration at a minimum of 80% saturation at all times.
- Excessive aeration that creates foam must be avoided as it can reduce the amount of available anaesthetic in the water.
- Do not treat fish in turbid water as the turbidity will also affect anaesthetic dispersal and uptake.

Euthanasia

- Euthanasia should only be undertaken by trained personnel, and should be in consultation with the Animal Welfare Officer if being undertaken outside of approved application conditions (e.g. emergency situations).
- Aqui-S is recommended for euthanising fish.

Method 1: (Anaesthesia and Euthanasia of Fish Using Aqui-S)

Equipment:

1. Container to house animal during procedure.
2. Aeration supply for container.

3. Fresh or salt water supply at temperature appropriate for species (e.g. Have a supply of preheated water prepared if working with a tropical species as stored salt water and fresh tap water are approximately 10-15°C).
4. Measuring pipette/syringe.
5. Clean container to mix stock solution in.
6. Oxygen/temperature meter.
7. Stirring rod/paddle.

Anaesthesia:

1. For:
   a) Light sedation = Use 5 to 10 mL of Aqui-S™ from the bottle per 1000 litres of water.
   b) Anaesthesia = Use 15 to 25 mL of Aqui-S™ from the bottle per 1000 litres of water.

2. Example calculation and steps for light sedation
   a) A 10 Litre container is a common sized container to use in our facilities, for small fish.
      i. 5 -10ml Aqui-S/ 1000L water
      ii. 0.5 -1.0ml Aqui-S/ 100L water
      iii. 0.05 – 0.1ml Aqui-S/ 10L water
   b) Fill a container with 10 Litres of water with parameters appropriate to the species
      i. Add a gently bubbling airstone
   c) Draw up 0.1ml of Aqui-S in a syringe and dispense into a 100ml container
   d) Add 10 ml of water (from 10L water container)
   e) Secure lid on 100 ml container and invert several times to mix Aqui-S with the water (do not shake)
   f) Add 50ml of this solution to the 10 L container and gently move airstone around tank to help solution disperse
   g) Add fish to the container for light sedation
   h) Observe fish for 5 minutes, if not suitably sedated, add half the remaining solution (25ml). If fish are still insufficiently after 5 minutes, add the remaining solution (25 ml)

Euthanasia:

1. Following the steps described in the anaesthesia section, expose the fish to an anaesthetic concentration of Aqui-S™ (15-25ml/ 1000L), and once signs of sedation are observed, add sufficient Aqui-S™ to reach a final ratio of 40 mL per 1000 litres. This will usually require adding 15 to 25 mL of additional Aqui-S™ per 1000 litres.
2. If no supplementary forms of euthanasia are used, keep the fish in Aqui-S™ treated water even after showing no signs of life (no response to touch, no gill and mouth movement, and no swimming for at least 30 minutes).
3. If fish are to be removed from treated water within 30 minutes of euthanasia with Aqui-S™, perform a secondary euthanasia technique (such as decapitation or brain spike). Brain spike should only be undertaken by individuals assessed as competent and approved by the Animal Welfare Committee (AWC).

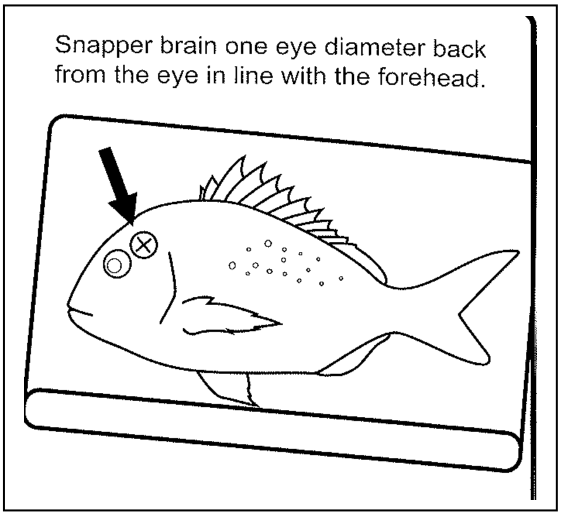
Method 2: (MS-222 and Benzocaine)

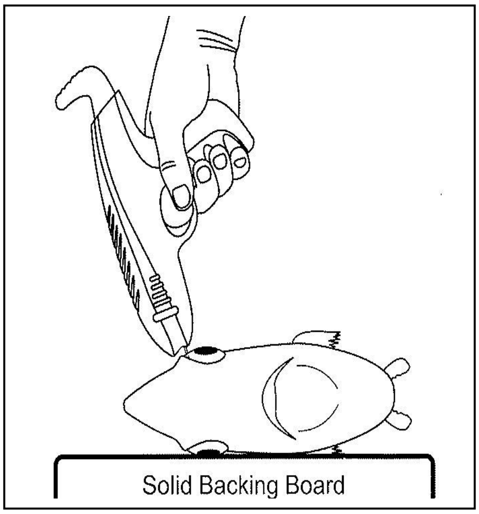
- As a backup method if Aqui-S is unavailable or impractical:
    - MS-222 and Benzocaine are also recommended by the NHMRC.

Method 3: (Brain Spike)

- The Ikigun® with Ikiboard restraint *(see image below)* are recommended when brain spike is used for euthanasia of fish from 200 to 350 mm in length.
- The Ikigun® is a dangerous instrument and should be used only once fish are restrained on a flat surface.
- See http://www.ikijime.com/ for photos of where to administer the brain spike for different fish species.

1. Place the fish in lateral recumbency on the Ikiboard, and secure by placing light pressure using the clasp.
2. Cock the bolt by pulling back on the ram (up to three clicks).
3. Press the gun muzzle onto the side of the head, one eye diameter caudodorsal from the eye, in line with the forehead.
4. Pull the trigger.
5. Withdraw the gun and clean with cold water.





(Picture from: Ikigun, Adept Ltd, Auckland, NZ)

Method 4: (Other)

- Methods such as immersion in icy brine, stunning, brain destruction, cervical dislocation, and decapitation are "*Acceptable with Reservations*" (NHMRC guidelines), but must be justified in lieu of recommended methods and, whenever possible, fish should first be anaesthetised.
- ***Method 5: Octopus Euthanasia with Magnesium Chloride***
  - 3.5% Magnesium Chloride solution (3.5mg magnesium chloride in 10 Litre of water) in chilled water for a minimum of 30 minutes.
  - Freeze at – 18°C for several hours
  - Refer to Guidelines for the Care and Welfare of Cephalopods in Research -A consensus based on an initiative by CephRes, FELASA and the Boyd Group.
    - Fiorito G[1], Affuso A[2], Basil J[3], Cole A[4], de Girolamo P[5], D'Angelo L[5], Dickel L[6], Gestal C[7], Grasso F[8], Kuba M[9], Mark F[10], Melillo D[11], Osorio D[12], Perkins K[12], Ponte G[4], Shashar N[13], Smith D[14], Smith J[15], Andrews PL[16].
  - These guidelines were used to develop the euthanasia protocol for Octopus, in conjunction with the AVMA guidelines for euthanasia
    - https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf.

Analgesia

- There are currently insufficient guidelines available on analgesic use in fish to provide general recommendations. Current practice is to sedate fish with Aqui-S anaesthetic to minimise potential trauma and stress of invasive procedures.

Blood Sampling

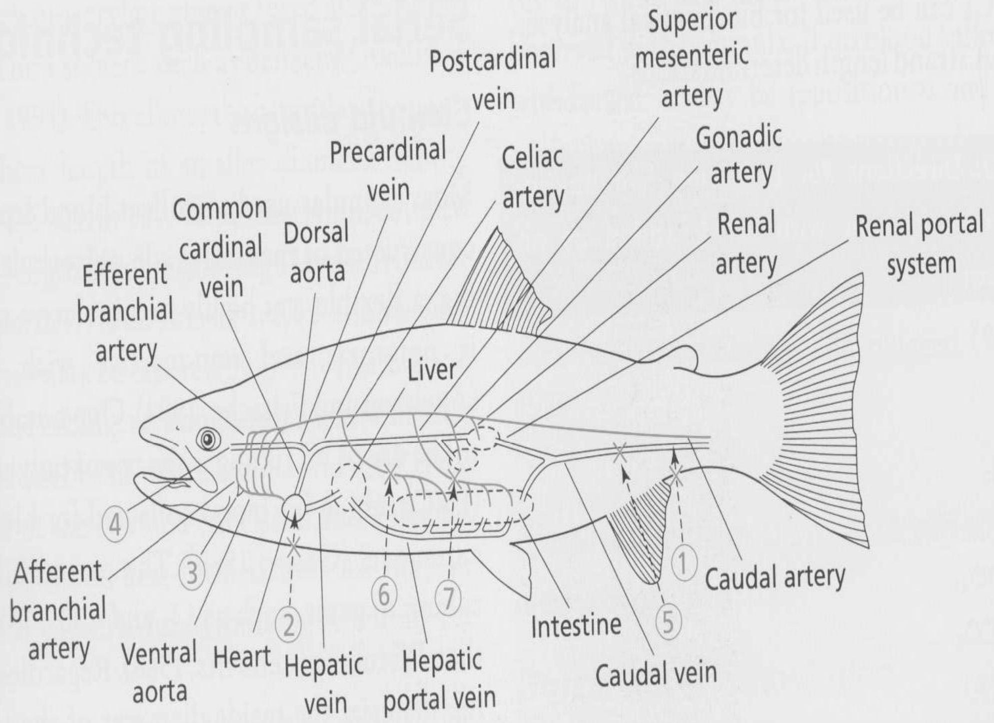


Figure 30.1 Diagram of a fish showing the major blood vessels and locations for drawing blood. 1, Caudal puncture; 2, cardiac puncture, cannulation; 3, ventral aorta puncture, cannulation (see Figure 30.2 for detail); 4, dorsal aorta cannulation (see Figure 30.2); 5, caudal vein cannulation; 6, celiac artery cannulation; 7, mesenteric artery cannulation.

- Consider the purpose of the sampling, as anticoagulants may be required to prevent the blood sample clotting during or after collection. Heparin dissolved in saline is the most commonly used substance to prevent clotting.

- Vaccutainer syringes are not recommended for blood collection in small fish, as amount of suction cannot be controlled and the vein may collapse.

- Weigh fish prior to sampling. No more than **1% of the fishes total body weight** can be taken in a blood sample, with a gap of 14 days between samples (e.g. **fish weight 50g** = blood sample volume not exceeding **0.5ml).**

- Ensure you have all necessary equipment prepared prior to sampling, and are familiar with the Safe Operating Procedure for administering medication/blood sampling.

Caudal Puncture:

- Will yield between 0.2ml and 10ml from most fish weighing more than 25g.
- After first anaesthetising as described in the previous section, place fish ventral side up on a stable surface (e.g. a plastic/metal dissecting tray lined with a wet, soft matting).
- Using an appropriate gauge needle, the insertion point is approximately 5mm posterior to the anal fin, along the midline of the body. Refer to diagram.
- Gently insert the needle, with the bevel facing the fish's posterior end, straight down into the caudal region of the fish. Needle gauge will vary depending on size of fish. As an example, a 22 gauge needle would be appropriate for a fish in excess of 1kg.
- Slowly guide the needle down until it just touches the spine, then withdraw approximately 1mm to position the needle in the caudal vein.
- Blood will pool in the needle hub when the needle is in the vein, but if it is not then draw back the needle point from the area and try increasing or decreasing the angle of the needle as it may be sitting just above or below the vein.
- Holding the syringe in place by the needle, gently apply light suction with the syringe until the desired amount of blood has been withdrawn.
- After the blood is drawn, gently invert the syringe several times to mix the blood (and anti-coagulant, if added). The blood may now be discharged from the syringe into another container (do not discharge via the needle as this may cause haemolysis of the blood sample).

Fish Tagging

- Handling of fish for anaesthesia, and subsequent tagging, will be performed according to this SOP.
- Fish will be anaesthetised according to this SOP, using a concentration of 10 mg/L – 20mg/L of Aqui-S (i.e. suitable for handling, harvesting, weighing, etc) to achieve total loss of equilibrium.
- The anaesthetised fish will then be tagged with an applicable tag type and size, depending on the purpose of the tagging, the species, and the size of the fish, which will be based on the tag manufacturer's guidelines (see http://www.hallprint.com/#home-section). The appropriate tag applicator will be used, also as per the tag manufacturer's guidelines (see http://www.hallprint.com/#home-section).
- Tagging of the fish typically takes only a few seconds.

- Immediately following tagging, the fish will then be recovered and monitored according to this SOP.

Disposal and Clean-Up

- Euthanased animals must be placed in a plastic bag and put in the carcass bin in the level 1 freezer (biology room 130). Disposal will be treated as medical waste and will be incinerated at Flinders Medical Centre, or by an appropriate service provider in Port Lincoln.

- At the end of the project, all tanks and benches must be cleaned with either 70% or 99% ethanol, bleach, or sodium hypochlorite (bleach and sodium hypochlorite used to clean aquatic tanks must be neutralised with sodium thiosulphate at a ratio of 1:3 (bleach:thiosulphate)). Floors must be cleaned with bleach or hospital grade disinfectant.

Adverse Event Reporting

- An Unexpected Adverse Event is an incident impacting on the health and welfare of animals held in the facility that is not part of the approved ethics application (e.g. equipment failure, mortalities exceeding approved numbers, ill health, etc).
- You must advise the Animal Facilities Coordinator and the AWO as soon as possible when such an even occurs, but within 24 hours of the event.
- You must submit a report to the Animal Welfare Committee within 3 working days.
- The reporting form can be found on the AWC website.
- All unexpected deaths must be necropsied.
- The Animal Facilities Coordinator and AWO will work with you in the short term to stabilise the situation and maintain the animals, until the AWC has reviewed the incident and decided whether the incident is:
    1. An unexpected adverse event and the project may continue unmodified,
    2. An unexpected adverse event and the project may continue with modifications, or
    3. An expected adverse event and whether or not the project can continue, and if modifications are required.

Emergency Evacuation

❖ **In the event of an emergency evacuation, staff must move to local assembly points to await further instruction. If there is sufficient prior notice, fish may be moved-in consultation with the project C.I. and as per *"SOP - Working with Fish"* to a temporary new location that can maintain their husbandry requirements; until such time as they can be returned to the College of Science and Engineering Animal Facilities.**

SOP Review

This SOP currently applies to the animals housed in the College of Science and Engineering Animal Facility and field sites. This SOP will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facilities Coordinator.

Position	Name	Contact Details
Animal Facilities Coordinator	Leslie Morrison	X 12196 Office in Animal Facility Leslie.morrison@flinders.edu.au
Animal Welfare Officer	Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References:

- http://www.ikijime.com/
- http://www.pir.sa.gov.au/fishing/permits_and_exemptions
- http://www.nhmrc.gov.au
- http://www.adelaide.edu.au/ANZCCART/
- http://www.environment.sa.gov.au
- http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits
- http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm
 (Link for Animal Incident Report forms, Teaching and Research Application Forms and all animal welfare related matters)

- Ross, L. G. and Ross, B., 2008. Anaesthetic and Sedative Techniques for Aquatic Animals, *Blackwell Publishing* (3rd Edition)
- Ostrander, G., Bullock, G. and Bunton, T., 2000. The Laboratory Fish, *Academic Press*