




	<b>Flinders University</b> School of Biological Sciences <b>Standard Operating Procedure</b> <b>For Working with Fish</b>					
				<b>Animal House</b>		
	SOP Number		RA Number		AWC Approval Date	
SOP-BIOL-3-08/2015-Fish		RA_		13 September 2016		
Contact Person		SOP prepared by			Review Date :	
Leslie Morrison		Leslie Morrison			July 2018	

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#### Legislation

- *Australian code for the care and use of animals for scientific purposes. 8<sup>th</sup> ed.*
- *Section 115 of the Fisheries Management Act 2007*
- *South Australian National Parks and Wildlife Act 1972*
- *Animal Welfare Act 1985.*
- *Animal Welfare Regulations 2012*
- *Work Health and Safety Act 2012*
- *Work Health and Safety Regulations 2012*

#### University policy

- Occupational Health, Safety and Welfare Policy
- NH&MRC Guidelines

#### Local policy

Use of the Biological Sciences Animal Facilities by all staff and students of the School of Biological Sciences, Flinders University of S.A. 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 submit a proposal to the Biological Sciences Animal Welfare Sub- Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee (AWC). Standardised application forms for Laboratory, Teaching and 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/researchersupport/ethics/committees/animal/animal\\_home.cfm](http://www.flinders.edu.au/research/researchersupport/ethics/committees/animal/animal_home.cfm)

- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Experienced Researcher Animal Welfare Seminars (ER-AWS).**

#### 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 Biological Sciences OH&S Manager.



The following are a list of the main SOP's governing working with animals in Biological Sciences- these must be complied with. An extensive database of specific technique SOP's are also available from the Animal House Manager and on the AWC home page.

- **Standard Operating Procedure and Safe Work Procedure for the Use of the Aquaculture/Marine Aquarium Facilities**
- **Standard Operating Procedure & Safe Work Procedure for the Use of the Animal House Facilities**
- **Standard Operating Procedure for Working With Fish / Aquatic Organisms**

#### General Information

- Prior to submitting an application to the AWC, you must discuss space requirements with the Animal House Manager. 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 House Manager.
- **Prior to animals arriving your space must 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](http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits)
- The Animal House Manager 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](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 School of Biological Sciences 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 House manager 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

#### **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, the dates on which the collections are to be made.
  - ❑ A signed **copy** of the Permit 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](http://pir.sa.gov.au/biosecurity/aquatics/aquatic_pests)

#### **Permitted equipment**

The Marine Exemption species 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](http://www.pir.sa.gov.au/fishing/fishing_gear/permitted_devices)



## Marine Specimen Collection Exemption

TAKE NOTICE that pursuant to section 115 of the *Fisheries Management Act 2007* (the Act), Ms Leslie Morrison, of the School of Biological Sciences at Flinders University, Sturt Road, Bedford Park (the 'exemption holder') or a person acting as her agent, are exempt from Sections 70 and 71(2) of the *Fisheries Management Act 2007*, Regulation 7 and clauses 38, 39, 40, 72, 118 and 123 of the 6<sup>th</sup> Schedule of the *Fisheries Management (General) Regulations 2007* but only insofar as they may engage in the activities specified in Schedule 1, using the gear specified in Schedule 2, (the 'exempted activity'), subject to the conditions specified in Schedule 3, from 24 August 2015 until 23 August 2016, unless varied or revoked earlier.

### SCHEDULE 1

For the purposes of teaching appropriate sampling techniques, the collection of aquatic organisms from all waters of South Australia, including the River Murray Protection Area, excluding aquatic reserves (unless otherwise authorised under the Act), marine parks (unless otherwise authorised under the *Marine Parks Act 2007*) and the Adelaide Dolphin Sanctuary.

For the purposes of undertaking the following research projects; monitoring populations of European Green Crab (*Carcinus maenas*), post-capture survivorship of Whaler Sharks (*Carcharinus brachyurus* and *Carcharinus obscurus*) and the monitoring of Southern Pygmy Perch (*Nannoperca australis*) and Yarra Pygmy Perch (*Nannoperca obscura*) populations.

### SCHEDULE 2

#### **Teaching Activities**

- Two plankton nets where each net is a funnel shaped, fine-meshed net that is towed through the water to collect plankton with a diameter not exceeding 1 metre, depth not exceeding 1.6 metres and mesh size not exceeding 38 mm.
- A Seine Net with a mesh size not exceeding 5mm and total length not exceeding 20 metres.
- Plastic corer (10cm diameter, 20cm length)
- Sweep nets
- Double-rig demersal prawn trawl with standard mesh and a maximum cod end mesh of 50 mm

#### **Research Activities**

- A longline (no more 2.2km long, 1.7mm leaders with a maximum of 200, 16/0 size hooks, floats marked with exemption holder or Ministerial exemption number)
- Hooped bait net, floats marked with exemption holder or Ministerial exemption number
- A fyke net (maximum 6m long, with addition 4m wing)
- A Seine Net with a mesh size not exceeding 5mm and total length not exceeding 20 metres.



## Marine Specimen Collection Exemption

### General conditions for all activities

- 1 Research completed pursuant to this notice must be within the waters of the State and related to or for the purposes of the administration of the Act.
- 2 Any equipment used to collect and hold fish during the exempted activity must be decontaminated prior to and after undertaking the research activities.
- 3 All species caught pursuant to this notice that are not being collected for scientific, education and research purposes must be returned to the water as soon as practical except for species declared as noxious under the Act. Noxious species must not be returned to the water and be disposed of appropriately.
- 4 All protected species incidentally taken while undertaking the exempted activity may be measured and recorded and must be returned to the water as soon as reasonably practicable. Protected Species must not be retained.
- 5 The specimens collected by the exemption holder are for scientific, education and research purposes only and must not be sold.
- 6 The exemption holder must not collect specimens for aquaculture research purposes pursuant to this notice.
- 7 Organisms collected pursuant to this notice must not be released once they have been kept separate to their natural environment at the University.
- 8 At least 1 hour before conducting an exempted activity, the exemption holder must contact PIRSA Fishwatch on **1800 065 522** and answer a series of questions about the exempted activity. The exemption holder will need to have a copy of this notice in their possession at the time of making the call, and be able to provide information about the area and time of the exempted activity, the specific gear to be used, vehicles and/or boats involved, the number of permit holders undertaking the exempted activity and other related questions. **Exemption number ME9902796.**
- 9 The exemption holder must provide a report in writing detailing the activities carried out pursuant to this notice to the Director, Fisheries and Aquaculture (GPO Box 1625, ADELAIDE SA 5001) by 23 September 2016 with the following details:
  - 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.



## Marine Specimen Collection Exemption

- 10 Failure to submit a report as per condition 6 may result in further exemptions not being supported.
- 11 While engaging in the exempted activity, the exemption holder must be in possession of a signed copy of this notice and carry their identification card issued by Flinders University. Such notice and identification must be produced to a PIRSA Fisheries Officer if requested.
- 12 A person acting as an agent of the exemption holder must possess a copy of a signed letter from the exemption holder stating that they are acting as an agent during the exempted activity and carry their identification card issued by Flinders University.
- 13 The exemption holder, or agent must not contravene or fail to comply with the Act or any regulations made under the Act, except where specifically exempted by this notice.

### **Specific Teaching Activity - Double-rig demersal prawn trawl**

- 14 The exempted activity must be conducted using the SARDI research vessel Ngerin.
- 15 The exempted activity may undertake a maximum of six shots over three nights of no more than 45 minutes duration each.
- 16 The exempted activity is to be undertaken in the waters contained within Gulf St Vincent commencing at position latitude 34°45.00' S, longitude 138°17.00' E then to position latitude 34°47.00' S, longitude 138°17.00' E then to position latitude 34°47.00' S, longitude 138°15.00' E then to position latitude 34°45.00' S, longitude 138°15.00' E then to the point of commencement.
- 17 The exempted activity may not be undertaken in daylight hours between sunrise and sunset for Adelaide (as published in the South Australian Government Gazette pursuant to the requirements of the *Proof of Sunrise and Sunset Act 1923*).
- 18 The exemption holder must not conduct any other fishing activity including recreational fishing whilst undertaking the exempted activity.
- 19 All species caught during the exempt activity must be returned to the water as soon as practical.

### **Specific Research Activity – Monitoring of Southern Pygmy Perch and Yarra Pygmy Perch**

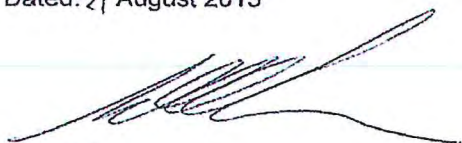
- 20 All tissue samples taken from any species must be taken in such a way to maximise the survival of the animal.



## Marine Specimen Collection Exemption

This notice does not purport to override the provisions or operation of any other Act including, but not limited to, the *Marine Parks Act 2007* and the *River Murray Act 2003*. The exemption holder and her agents must comply with any relevant regulations, permits, requirements and directions from the Department of Environment, Water and Natural Resources when undertaking activities within a marine park.

Dated: 21 August 2015



**Sean Sloan**  
**DIRECTOR, FISHERIES AND AQUACULTURE POLICY**  
 Delegate of the Minister for Agriculture, Food and Fisheries

## 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*, 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.



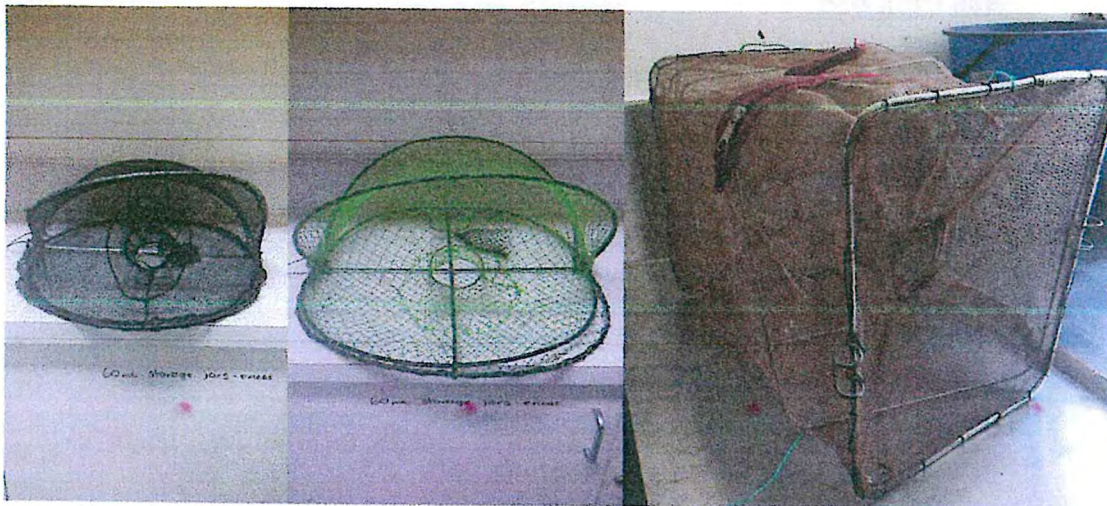
## Fish – Baited traps

### 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.

## Fish – Baited traps



Different sized Opera traps and an example of a box trap.

## Animal House 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 House 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 house 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 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 Uni) 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.



## Transport cont.

- 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.

### **During Transport**

- 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. This is particularly important with larger biomasses of fish. **If anaesthetics are being used then aeration must be provided.**
- 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.

### **Admission into the Flinders University School of Biology Animal Care Unit:**

- Animal House 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 house 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 Flinders University School of Biology Animal Care Unit, 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 monitoring sheet (see Quarantine).

### 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 water's 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.

### Quarantine and health monitoring

- All animals should be assessed by experienced staff and confirmed as healthy prior to transport to the Animal House.
- Animals should be placed in their specific quarantine tanks which will be allocated by the Animal House 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 and numbers.
- Water quality and animal health should be monitored daily for the quarantine period. The parameters that need to be monitored are included in the monitoring record sheet.
  - The monitoring 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 house 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).

Quarantine and health monitoring cont.-  
Clinical Record Coversheet EXAMPLE

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) CLINICAL RECORD SHEET

- YES a Clinical Record Sheet is attached.  
 NO a Clinical Record Sheet isn't attached.

3) SPECIES / PHENOTYPE / MODEL ISSUES

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4) MONITORING CRITERIA

List monitoring criteria.			
Monitoring Criteria	No obvious deviation from normal	Slight or intermittent or possible deviation from normal	Moderate or consistent or definite deviation from normal but not marked
Score	0	1	2
<b>Environmental</b>			
Temperature °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
<b>Clinical - animal</b>			
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



Quarantine and health monitoring cont -  
Clinical Record Coversheet EXAMPLE

<b>Other Condition</b> (not documented above, but impacting on welfare)			
<b>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.</b>			

**5) MONITORING FREQUENCY**

Describe monitoring regime.
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**6) RESEARCHER NOMINATED ACTIONS AND INTERVENTIONS**

Score	Assessment	Actions/Interventions
<b>Environmental</b>		
<b>0 - 1</b>	Environment within acceptable limits	No interventions required.
<b>2</b>	Ammonia/nitrite/nitrate/salinity →	Approximately 50% of tank water to be replaced
	Oxygen →	Aeration to be increased to tank
	pH →	Bicarbonate or acid to be added
<b>Clinical - animal</b>		
<b>1</b>	Animal demonstrates minor level of deviation from normal	Notify animal house manager and/or PI and/or AWO – commence treatment if recommended by AWO
<b>2</b>	Animal demonstrates moderate or major deviation from normal	Immediate euthanasia. Notify the AWO and complete an Unexpected Adverse Event Report if incident is unexpected

**7) 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 euthanasia or consultation with the AWO 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.



**Quarantine and health monitoring cont.  
Clinical Record Sheet EXAMPLE**

**1) ANIMAL DETAILS**

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

**2) MONITORING**

<b>Day/date</b>									
<b>Time</b>									
<b>Procedure</b>									
<b>Criteria – environmental/water</b>									
Temperature °C									
DO saturation %									
Salinity (ppt)									
Ammonia mg/L									
Nitrite mg/L									
Nitrate mg/L									
pH									
<b>Criteria - animal</b>									
Appearance									
Behaviour									
Other									
<b>Total</b>									
<b>Prescribed AEC Intervention Criteria</b>									
<b>Signature</b>									
<b>OFFICE USE ONLY AWO CHECK</b>									

**3) COMMENTS:**

Biology Animal House Standard Operating Procedure for Working With Fish



## Quarantine and health monitoring cont.

### Standard Ongoing Maintenance Monitoring parameters

• Appropriate room temperature and air circulation/ water parameters
• Any signs of abnormal body shape
• swelling/ fight injuries
• abnormal movement(loss of equilibrium)
• significant change in appetite
• abnormal level of activity (erratic swimming)
• Abnormal respiration (gaspings at surface)
• Water parameters - pH, salinity, NH <sub>4</sub> , NO <sub>2</sub> /NO <sub>3</sub>

- All rooms are labelled with a chart describing the species held in the room, project approval numbers, number of animals held and Chief Investigator contact details. This must be kept updated at all times.
- A folder for each room is located on the aquaculture hallway bench. All animal movements, births, deaths, health issues and treatments must be recorded in this folder and kept up to date for monthly submissions to the AWC.

## 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 House Rooms 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 House 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).



## Handling

- 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 and 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- 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 House Manager 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-

- Scales.
- Data recording supplies (eg notebook, pen, laptop).
- Dip net.
- Clear ruler.
- Bucket containing water from the system holding the fish or seawater that has been acclimatise to the temperature of the room.
- Aqwi-s.
- Small dish (eg a large petri dish) containing sponge or paper towel and small amount of tank water to keep it wet.
- Battery or mains powered aerator.
- Ideally, have a second person present to record data and assist in capture and monitoring fish post sedation.
- 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-

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

- Tare the container of water.
- Add fish and record weight.
- 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 Animal Handling section.
- All medications and how they are administered must be approved by 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 House staff and the Animal Welfare Officer.

### Anaesthesia

- Sedating fish prior to handling, administering medication or undertaking surgery will minimise potential physical trauma (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 respire 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.



### Anaesthesia cont.

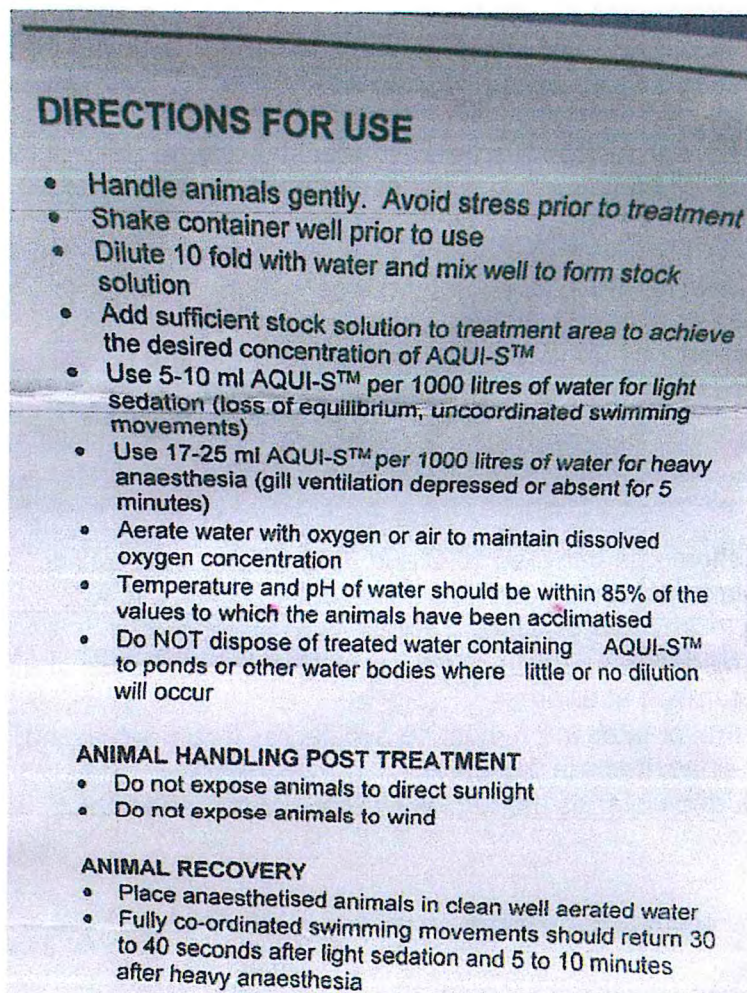
- The recommended initial concentration of Aqui-S is 10mg/L to sedate most fish for handling, but can be increased to up to 20mg/L. This concentration allows safe handling of the fish and will reduce the fish's stress response to most procedures and methods of administering medication.
- To evaluate 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) must be monitored at least 1 hour after the procedure and 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) 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 anaesthetised eg. for surgery, must be monitored closely for 1 day (1hour 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-(quiet, low traffic area).
- Sick fish that have been anaesthetised for treatment must be monitored closely for 2 days (1hour 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 (quiet, low people traffic) environment appropriate for recovery from disease.
- The anaesthetic most commonly used in the animal house is Aqui-S. The concentrations for different stages of anaesthesia are included on the following page.



## Anaesthesia and Euthanasia of Fish Using Aqui-s

### Equipment

- Container to house animal during procedure.
- Aeration supply for container.
- 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).
- Measuring pipette/syringe.
- Clean container to mix stock solution in.
- Oxygen/ temperature meter.
- Stirring rod/paddle.



- Where the directions reference the quantity of Aqui-s to use eg "5-10 ml for light sedation", they are referring to using the full strength Aqui-s **NOT** the stock solution.



### Anaesthesia and Euthanasia of Fish Using AQUI-S cont

Euthanasia must only be undertaken by trained personnel and must be in consultation with the Animal Welfare Officer if being undertaken outside of approved application (e.g. emergency situations).

- AQUI-S is strongly recommended for euthanasing fish.
    - As a backup method if AQUI-S is unavailable or impractical.
  - MS-222 and Benzocaine are also recommended by the NH & MRC.
  - Methods such as immersion in icy brine, stunning and brain destruction, cervical dislocation and decapitation are "Acceptable with reservations" (NH&MRC guidelines) but must be justified in lieu of recommended methods and whenever possible, fish should first be anaesthetised.
  - For Euthanasia - Keep the fish in anaesthetic even after showing no signs of life- no response to touch, no gill and mouth movement or swimming for at least 30 minutes.
- 
- Alternatively, after euthanasia with AQUI-S, perform a secondary euthanasia technique of decapitation or destruction of the central nervous system (brain spike.) Brain Spike must only be undertaken by individuals assessed as competent and approved by the Animal Welfare Committee.

### Anaesthesia- General Instructions

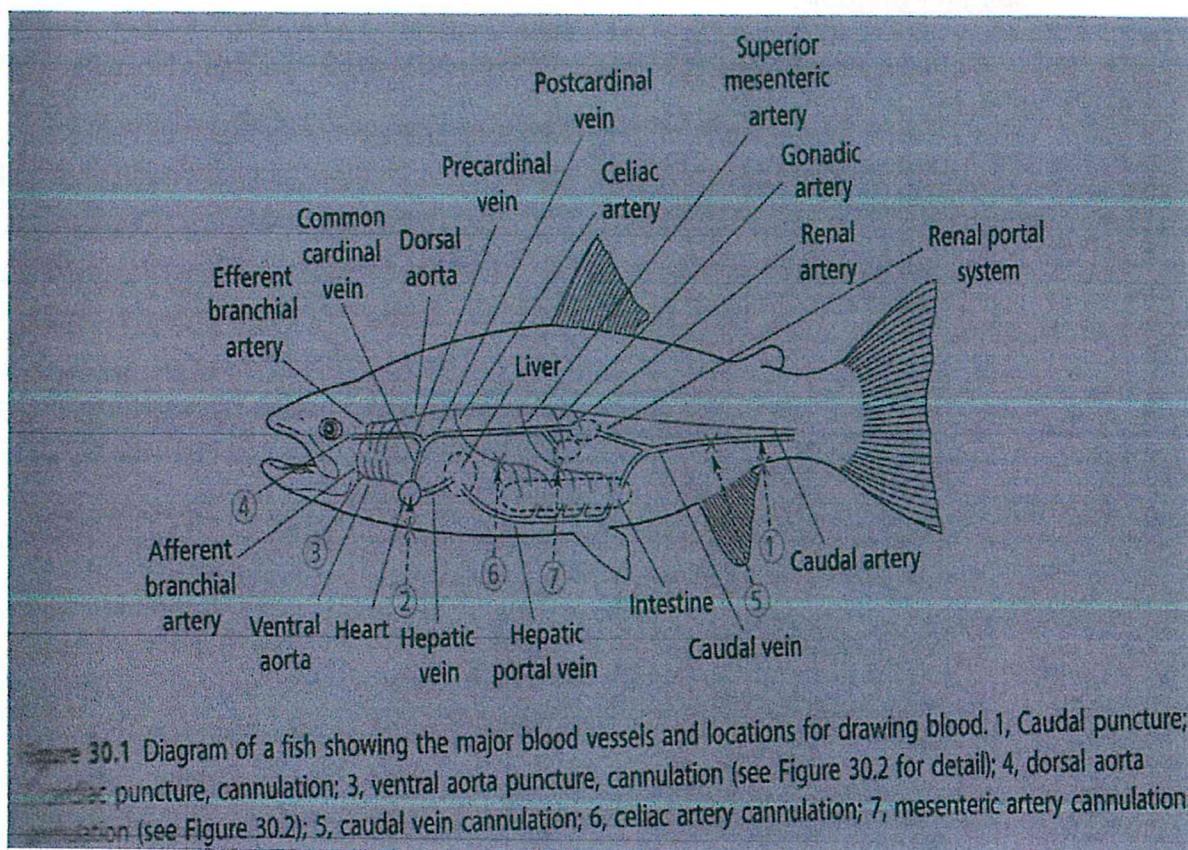
- Water temperature should be between 10°C and 30°C when using AQUI-S.
- Stock solution should be added below the surface of the water- this aids mixing and reduces losses due to foaming and surface evaporation.
- Aeration must be provided to maintain dissolved oxygen concentration at a minimum of 80% 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.

### 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



- Consider the purpose of the sampling- 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.
- Vacutainer 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.



## Blood Sampling cont.

### 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 handle able-grading, harvesting, weighing) 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, benches must be cleaned with either 70% or 99% ethanol, or bleach or sodium hypochlorite, floors with bleach or hospital grade disinfectant. (Bleach and sodium hypochlorite used to clean aquatic tanks must be neutralised with sodium thiosulphate at a ratio of 1:3 (bleach:thiosulphate).

### Adverse Event Reporting

- An Unexpected Adverse Event is an event that is not expected and was not foreshadowed in the application approved by the AWC.
- You must advise the Animal House manager and the AWO as soon as possible when such an even occurs and no more than 24 hours after 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 House Manager 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:
  - An unexpected adverse event and the project may continue unmodified,
  - An unexpected adverse event and the project may continue with modifications, or
  - An expected adverse event and whether or not the project can continue and if modifications are required.

### SOP Review

This SOP currently applies to the animals housed in the Biological Sciences Animal House facilities 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 House Manager.

Position	Name	Contact Details
Animal House Manager	Leslie Morrison	X 12196 Office in Animal House <a href="mailto:Leslie.morrison@flinders.edu.au">Leslie.morrison@flinders.edu.au</a>
Animal Welfare Officer	Lewis Vaughan	0450 424 143 <a href="mailto:awo@flinders.edu.au">awo@flinders.edu.au</a>



Useful References:

- [http://www.pir.sa.gov.au/fishing/permits\\_and\\_exemptions](http://www.pir.sa.gov.au/fishing/permits_and_exemptions)
- <http://www.nhmrc.gov.au>
- <http://www.adelaide.edu.au/ANZCCART/>
- <http://www.australiananimalwelfare.com.au/content/aquatic-animals/aquatic-animal-welfare-guidelines>
- <http://www.environment.sa.gov.au>
- [http://www.environment.sa.gov.au/licences-and-permits/Animals\\_in\\_captivity\\_permits](http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits)
- [http://www.flinders.edu.au/research/researcher-support/ethics/committees/animal/animal\\_home.cfm](http://www.flinders.edu.au/research/researcher-support/ethics/committees/animal/animal_home.cfm)

Ross, L. G. and Ross, B., 2008. Anaesthetic and Sedative Techniques for Aquatic Animals, *Blackwell Publishing* (3<sup>rd</sup> Edition)

Ostrander, G., Bullock, G. and Bunton, T., 2000. *The Laboratory Fish*, Academic Press