JAMES COOK UNIVERSITY
SINGAPORE

Research Institute

Aquaculture Research and Teaching (ART) Facility

Standard Operating Procedures (SOPs)

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1. Introduction

1.1. Objective

1.1.1. The objective of this Standard Operating Procedure (SOP) is to provide the operational framework necessary towards promoting good health conditions for aquatic animals housed in James Cook University Singapore's Aquaculture Research and Teaching Facility (ART Facility) located at Block E. All facility users must develop and maintain an up-to-date SOP specific to their respective areas. The SOP is enforced as a condition of a NACLAR (National Advisory Committee for Laboratory Animal Research) license.

1.1.2. This document does not replace any regulatory requirements for the husbandry of aquatic animals, including the transportation, purchasing, and disposal thereof, but is intended to help facility operators write their own SOPs. Applicable local legislation and regulations are included in Appendix 3.

1.2. Definitions

1.2.1. Terms used in this document are derived as defined in the latest NACLAR guidelines and from the latest Animal and Veterinary Services (AVS) documents on the subject. In addition, this document includes the following definition:

1.2.2. **Best Management Practices (BMP)**:

(a) must include a description of specific management practices and SOPs,
(b) must be reviewed and endorsed by the Institutional Animal Care and Use Committee established at James Cook University Singapore (JCUS-IACUC), and
(c) must have the individuals responsible for implementation of the plan understand and be trained in the plan.

1.3. Target Audience

1.3.1. This document is intended as an SOP for each operator's site staff in their training and day-to-day contact with aquatic animals, for health staff who are responsible for keeping good health status of the animals, and for management officers who have to make decisions about animal health and husbandry.

1.4. Document Structure

1.4.1. This document is generic and includes sections for outdoor and indoor facilities. Sections requiring an operator-specific SOP are noted.

1.4.2. In developing an SOP, operators should reference Appendix 1 and Appendix 2 of this document. In some instances, an SOP may apply to more than one section; in these cases, the same SOP can be used to address multiple requirements (e.g., the same feed storage SOP may be used for saltwater marine and freshwater hatchery sites). Operators may also combine related SOPs, e.g., isolation/quarantine procedures and infectious disease emergency procedures.

1.5. Annual Review

1.5.1. This document will be subject to annual review by the (Institutional Animal Care and Use Committee) IACUC to make sure they are up to date.

1.5.2. Where these SOPs are a condition of license, operators can anticipate periodic review by regulators.

1.5.3. The animal housing facilities are subjected to IACUC members' inspection annually. The inspection forms for IACUC members for the facility can be found under appendices 5.11.

1.6. Living Document

1.6.1. Changes will be made to this document as required.

1.7. Personnel Duties and Responsibilities

1.7.1. Attending Veterinarian

(a) The Attending Veterinarian, in conjunction with fish health staff, is responsible for overall fish health management for the operator. The Veterinarian must be licensed in Singapore and will retain a veterinarian-client-patient relationship with the operator.

(b) The Attending Veterinarian is responsible for disease diagnoses and writing prescriptions. He/she is expected to exercise good professional judgment in fish health matters. In the event of significant disease outbreaks, the Attending Veterinarian will report these to the proper authorities.

(c) The Attending Veterinarian shall advise on the appropriate care and use of aquatic animals and provide adequate specialist veterinary care when and where necessary.

(d) The Attending Veterinarian will be appointed by the Chair of the JCUS-IACUC as a committee member. The Attending Veterinarian will be engaged on a part-time basis.

(e) The formal arrangements must include a written programme of veterinary care to be provided in this guideline. In the case of a part-time Attending Veterinarian, the formal arrangements will be set out regularly during scheduled visits to the Aquaculture Facility.

i. Regularly scheduled visits are at least one visit per semester of one academic year (3 semesters per academic year)

ii. The semester schedule is as follows: March—June, July—October, and November—February.

(f) If the Attending Veterinarian is on leave or will otherwise be unavailable to provide any general or emergency veterinary care, the stand-in Attending Veterinarian will be activated.

i. JCUS Attending Veterinarian: Dr Fred Chua
(Contact No.: 9863 4258)

ii. JCUS stand-in Attending Veterinarian: _____
(Contact No.:_____)

(g) The Attending Veterinarian or other veterinarians engaged on a part-time or *ad hoc* basis must be persons with qualifications in veterinary science and licensed by the AVS.

1.7.2. Components of Veterinary Care

(a) There are 3 types of veterinary care applicable to the James Cook University Singapore Aquaculture Facility:

- Semestral Scheduled Veterinary Care
- Emergency *ad hoc* Veterinary Care
- Telecommunication Veterinary Care

(b) **Semestral Scheduled Veterinary Care** can only be done by the James Cook University Singapore duly-appointed Attending Veterinarian. The job

scope for this veterinary care includes:

i. Verification of whether IACUC-approved quarantined aquatic animals are ready for experimental use. The Attending Veterinarian will make sure that the aquatic animals are in good health prior to commencement of experiment. A quarantine form must be signed to indicate the verification.

ii. In situations of significant mortality and other signs of poor health of the aquatic animals during the quarantine period, the Attending Veterinarian must make the respective diagnosis, prescribe medication and treatment, administer vaccines, and euthanize as necessary.

iii. In monitoring the situation following diagnosis and/or the prescription of medication and treatment, a schedule for the Attending Veterinarian to visit the facility must subsequently be made.

(c) **Emergency *ad hoc* Veterinary Care** can be done by the Attending Veterinarian or the stand-in Attending Veterinarian. In view of mortality between 25-35% of aquatic animals in each approved IACUC project, Emergency *ad hoc* Veterinary Care will be activated. The arrival of the Attending Veterinarian is recommended to come to the affected facility within 24 hours from the time of activation. The job scope for this Veterinary Care includes:

i. In situations of significant mortality and other signs of poor health of the aquatic animals during the quarantine period, the Attending Veterinarian must make the respective diagnosis, prescribe medication and treatment, administer vaccines, and euthanize as necessary.

ii. In monitoring the situation following diagnosis and/or the prescription of medication and treatment, a schedule for the Attending Veterinarian to visit the facility must subsequently be made.

(d) **Telecommunication Veterinary Care** can be done by the Attending Veterinarian or the stand-in Attending Veterinarian. In case the Attending Veterinarian is not available, consultation through telecommunication can be done via phone, multimedia instant messaging, or email. The job scope for this Veterinary Care includes:

i. Give advice and make sensible diagnoses, and prescribe medication and/or treatment where warranted, based on communicated observations and multimedia, i.e., digital photographs or movies, of sick, dying, or deceased aquatic animals.

ii. In monitoring the situation following diagnosis and/or the prescription of medication and treatment, a schedule for the Attending Veterinarian to visit the facility must subsequently be made.

(e) It is reasonable to expect that the Attending Veterinarian will have an unpredictable availability schedule, thus it is recommended for sake of practicality that a **Telecommunication Veterinary Care** be done as a first response prior to deciding whether any **Emergency *ad hoc* Veterinary Care** is warranted.

(f) Execution of Unforeseen Circumstances

i. In a scenario where both the Attending Veterinarian and the stand-in Attending Veterinarian are not available (e.g., both are not in Singapore) during a health problem alert at the facility, the first line of activation will be commencing a partial water change, removal of mortalities, which will be placed in the -20°C freezer by the responsible caretakers at the facility.

ii. At such time when the Attending Veterinarian or the stand-in Attending Veterinarian is back, **Emergency *ad hoc* Veterinary Care** will be activated immediately.

iii. It is recommended to activate **Emergency ad *hoc* Veterinary Care** within 7 working days from the point of discovery of the health problem.

1.7.3. Facility Staff

Job descriptions for Facility Manager, Technical Support Officers, and other positions are detailed as below:

(a) be responsible for managing the day-to-day care of the animals, supervise the work of other staff, and act as liaison between Principal Investigators, Researchers, and other staff;

(b) contribute to the development and maintenance of the University's animal care policies and procedures;

(c) ensure that there is reliable monitoring of the well-being of all animals by staff, and be knowledgeable regarding signs of pain, distress and illness specific to each species housed (Note: after animals are allocated to a project, that project's Principal Investigator has primary responsibility for ensuring adequate monitoring of the animals' well-being);

(d) ensure that ill or injured animals are treated promptly, and any cause of death is properly investigated if the animal dies unexpectedly;

(e) ensure that staff are provided with appropriate protective clothing or PPE, that staff follow high standards of personal hygiene, and that they do not eat, drink, or smoke in the animal areas;

(f) document procedures used in the management and care of animals. These procedures should take into account the requirements of the species and the experiments being conducted. The procedures include transport, quarantine, and disposal of animals; routine husbandry; prevention, diagnosis, and treatment of disease; monitoring of health status and genetic constitution; and maintenance and recording of physical environmental factors. These procedures should be made known to all staff involved in the care and use of the animals and should be reviewed regularly.

(g) maintain a regular schedule of tanks, equipment, and facility sanitization to ensure that potential pathogens are kept at minimum levels in the environment.

(h) ensure that adequate records are maintained of:

i. the source, care, allocation, movement between locations, use and disposal of all animals, and development of any diseases;

ii. the fertility, fecundity, morbidity, and mortality in animal breeding groups, in order to monitor the management of the groups, and assist in the detection of the origin and spread of disease; and

iii. the health status, genetic constitution, and the physical environment of the animals, when definition of these is required.

(i) ensure that records maintained must be made available to Investigators and IACUCs.

(j) ensure that Investigators are informed of any changes to the conditions under which animals are held as these may affect their experiments.

1.7.4. Aquatic Animal Health Management

Aquatic Animal Health Management refers to those personnel, including the Veterinarian, who have responsibility in making decisions impacting aquatic animal health, specifically fish. Aquatic Animal Health Management is responsible for identifying and managing risk factors so as to minimize their effect on aquatic animal health.

1.7.5. Technical Support Officer Job Specifications

Frequency	Job descriptions	Record keeping
Daily from Monday to Friday (except PH)	Feed all live stocks for their AM and PM schedules as instructed by PI or Lab Manager	Form M - Husbandry Care Form
	Move animal feeds from the fridge and storage area	Form C - Animal Feeds Inventory Record
	Monitor all live stocks by doing an inventory count of live and dead animals	Form H - Daily Livestock Inventory Form
	Clean tanks and racks as required if found to be dirty	Form G - Tank Maintenance Record
	Ensure security of the facility and make sure that access doors are locked after office hours	
	Ensure all timers for lights and feeds are well set before weekends or PH	
	Top up all tanks (indoor and outdoor facility)	
Every Monday (if it falls on a PH, then next working day)	Inspect facility premises for pests such as rodents, cockroaches, etc.	Form B - Pest Inspection Record
	Monitor water quality parameters of all systems	Form E - Water Monitoring Record
	Clean tanks and racks and conduct partial water change as scheduled	Form G - Tank Maintenance Record
Weekends and PH	Standby for activation between 9 am to 12 pm	
Monthly	Schedule sea water re-supply and ensure it is safe to use for	Form F - Reservoir Top Up Form

	all aquatic animals	
Scheduled	Set appointments for regular maintenance with external vendors for equipment servicing	Vendor Service Form
	Set appointments with PI to perform quarantine process for newcoming aquatic animals	Form A - Quarantine Process Record
	Prepare or order re-supply of feeds for aquatic animals	Form D - Feeds Supply Record
	Coordinate with testing service providers to test wastewater	
***Ad hoc* and Special Requests**	Transfer of aquatic animals from one tank to another tank within the same facility	Form K - Livestock Transfer Form
	Ensure proper reporting when mortality is higher than 35%	Form I - Livestock Anomaly Report
	Assist in preparation of logistics and live feed for practicals and events	
	Assist in procuring for practicals and normal operations such as soliciting quotations and raising PRs.	
	Ensure that the facility and its record keeping is in accordance to NACLAR and AVS standards	
	Ensure that equipment is serviceable and calibrated	

1.7.6. ART Facility Manager Job Specifications

Frequency	Job descriptions	Record keeping
Weekends and PH	Standby between 9 am to 12 pm to activate Technical Support Officer	
Monthly	Perform a random check on the records	Forms A to M
Scheduled	Set appointments for regular maintenance with contractors for equipment	Vendor Service Form

	servicing	
	Set appointments with PIs to conduct the quarantine process and allocate animals into new tanks	E-mail
	Set appointments with the veterinarian to conduct the quarantine process for newly acquired aquatic animals	
	Ensure PIs have booked ART Facility calendar for usage	
	Lead and conduct IACUC audit for the facility	Internal Audit Checklist for Animal Holding Facility Semi-Annual Review Form
***Ad hoc* and Special Requests**	Ensure proper reporting when mortality is higher than 35%	Form I - Livestock Anomaly Report
	Plan the budget for ART Facility operation and upgrading costs	
	Solicit and sonsolidate feedback from users of the facility	
	Arrange for AVS audit for license renewal	

1.7.7. ART Facility Users (PIs, Lecturers, and Students) Job Specification

Frequency	Job descriptions	Record keeping
Daily Monday to Friday except PH	Feed all live stocks for AM and PM as instructed	Form M - Husbandry Care Form
	Transfer aquatic animals from one tank to another tank within the same facility if required	Form K - Livestock Transfer Form
	Clean tanks and racks and perform partial water change as required	Form M - Husbandry Care Form
	Prepare feeds for aquatic animals if required	Form D - Feeds Supply Record
	Use the facility	Lab Usage Form

1.7.8. On Site Staff

Facility staff may be assigned animal health duties from time to time. Facility staff are expected to follow good hygiene and aquatic animal health procedures.

1.7.9. Contact Names and Numbers

Contact names and numbers for all key personnel, including emergency numbers, will be posted at easily identifiable locations near the doors.

1.8. Emergency Response Plan (ERP)

1.8.1. The facility will adhere to JCUS university-wide Emergency Response Plan (ERP) and Fire Emergency Plan (FEP). JCUS Facilities Deprtment (during office hours, 8:30am to 6pm) or JCUS Security (outside of office hours) will serve as the contact point for incidents, accidents, crises, emergencies, and disasters. Upon receiving alert of an incident, the responsible department will mobilize personnel as required depending on the nature and severity of the situation. The ART Facility Manager will activate JCUS Facilities staff in the event of a power supply disruption. The support staff will implement the appropriate power recovery processes.

Facilities Hotline: 6709 3890
Security Guardhouse: 6709 3892

1.8.2. The saving and preservation of human lives shall take precedence over all other considerations. In the event of an emergency, all animals within the research facility will be left behind during evacuation.

2.1. Biosecurity

2.1.1. Maintaining a clean and safe work environment will reduce the possibility for exposure and spread of the aquatic animals to pathogens. Pathogens may be spread by sick animals and wild-caught animals through the water, through shared equipment, or by inadvertent contact by personnel, visitors, or their gear. Entrance of potential pathogens will be prevented or minimized by an effective biosecurity "barrier" at each facility. Biosecurity applies to all personnel (staff and management), to all visitors, and all equipment.

2.1.2. Biosecurity includes three components:

(a) Keeping the animals healthy
(b) Keeping pathogens out
(c) Keeping disease from spreading within the site

2.1.3. The personnel granted access to the facility includes the following:

(a) Designated Technical Support Officers
(b) ART Facility Manager
(c) JCUS-IACUC Committee Members (JCU Staff)
(d) Principle Investigators using the facility
(e) Safety Committee Members

2.2. Keeping Aquatic Animals Healthy

Keeping aquatic animals as healthy as possible is critical to keeping disease from coming on site and/or spreading within a site.

2.2.1. Suitable Facility Environment

(a) The operator's management is responsible for ensuring a suitable rearing environment for the aquatic animals, so they can stay healthy. Facility requirements include:

- Freshwater system with life support system with 15 fiberglass tank of 500 liters each
- Seawater system with life support system with 15 fiberglass tank of 500 liters each

(b) The life support system for freshwater system includes the following equipment:

- Filtration tanks with filtration sponges, carbon, and buffering system
- Air pump for aeration
- Ultraviolet sterilizers

(c) The life support system for seawater system includes the following equipment:

- Filtration tanks with filtration sponges, carbon, and buffering system
- Protein skimmer
- Air pump for aeration
- Ultraviolet sterilizers

(d) Nettings will be used to cover the tanks when the water level is high. This is to prevent fish from jumping out from the tanks.

(e) The room temperature of the facility should be set between 23°C to 28°C.

2.2.2. Quarantine Environment

(a) Quarantining is the process of isolating newly acquired aquatic animals from the existing population of animals in the facility for a period of time to ensure that they are in good health. For the introduction of new animals into the facility, the operators are required to perform the quarantine process as follows:

i. Tanks labelled Q1 and Q2 are assigned for acclimatization and quarantine process for both seawater and freshwater.

ii. To use the quarantine tanks, shut off the valve connecting the tanks to the main filtration system. Optionally turn on the valve connecting the tanks to the auxiliary filtration system dedicated to quarantining.

iii. Perform the acclimation process.

iv. Add the new aquatic animals (e.g., fish) to the quarantine tank. Let

the animals rest or get used to their new environment for 24 hours. Very light feeding several hours after introduction may be attempted.

2.2.2.1. *Acclimatization Process*

(a) Floating acclimatization method

i. Turn off any light which is applicable to the quarantine tank.

ii. Dim the lights in the room where the shipping box will be opened. Never open the box in bright light. Severe stress or trauma may result from sudden exposure to bright light.

iii. Float the sealed bag in the aquarium for 15 minutes. Never open the shipping bag at this time. This step allows the water in the shipping bag to adjust slowly to the temperature in the quarantine tank, while maintaining a high level of dissolved oxygen.

iv. After floating the sealed shipping bag for 15 minutes, cut open bag just under the metal or plastic clip and roll the top edge of the bag down one inch to create an air pocket within the lip of the bag. This will enable the bag to float on the surface of the water. For heavy pieces that will submerge the shipping bag (e.g., corals), place the bag in a plastic bowl or specimen container.

v. Add 1/2 cup of tank water to the shipping bag.

vi. Repeat step (v) every 4 minutes until the shipping bag is full.

vii. Lift the shipping bag from the aquarium and discard half the water from the bag.

viii. Float the shipping bag in the aquarium again and proceed to add 1/2 cup of aquarium water to the shipping bag every four minutes until the bag is full.

ix. Net aquatic life from the shipping bag and release into the aquarium.

x. Remove the filled shipping bag from the tank and discard the water. Never release shipping water directly into the tank

(c) Drip acclimatization method (for sensitive aquatic animals)

i. Start with Steps (i-iii) of the floating method to acclimatize water temperature.

ii. Carefully empty the contents of the bags (including the water) into a bucket or multiple buckets as required, making sure to keep the sensitive animals in the water at all times. Depending on the amount of water in each bag, this may require tilting the bucket at a 45 degree angle to make sure the animals are fully submerged. You may need a prop or wedge to help hold the bucket in this position until there is enough liquid in the bucket to put it back to a level position.

iii. Using airline tubing, set up and run a siphon drip line from the main aquarium to each bucket. You'll need separate airline tubing for each bucket used. Tie several loose knots in the airline tubing, or use a plastic or other non-metal airline control valve, to regulate flow from the tank.

iv. Begin a siphon by sucking on the end of the airline tubing you'll be placing into each of the buckets. When water begins flowing through the tubing, adjust the drip (by tightening one of the knots or adjusting the control valve) to a rate of about 2-4 drops per second.

v. When the water volume in the bucket doubles, discard half and begin the drip again until the volume doubles once more – about 1 hour.

vi. At this point, the specimens can be transferred to the tank. Sponges, clams, and gorgonias should never be directly exposed to air. Gently scoop them out of the drip bucket with the specimen bag, making sure they're fully covered in water. Submerge the bag underwater in the aquarium and gently remove the specimen from the bag. Next, seal off the bag underwater by twisting the opening, and remove it from the aquarium. Discard both the bag and the enclosed water.

(d) Quarantine with methylene blue (recommended for small ornamental fishes)

i. On day 2 of quarantine, **medicate with methylene blue**. Follow the manufacturer's directions. Methylene Blue (MB) helps boost fish immune system as well as prevents

bacterial, fungal, or parasitic outbreaks. MB is a chemical with many benefits for stressed and/or sick fish.

- It is gentle to fish and does not cause them any stress.
- It increases oxygen absorption in fish. Fish that have been in high ammonia environments such shipping bags often have damaged gills. Methylene Blue helps fish recover their ability to absorb oxygen from the water.
- Both a bactericide and fungicide, it is often used by fish breeders to prevent fungus infection on eggs. It is also an anti-protozoan that kills a common parasite, ich *(Ichthyophthirius multifiliis)*.
- It is easily absorbed through the fish's gills and skin to help fight internal infections. It also assists the absorption of antibiotics when used together in a bath.
- Since Methylene Blue does not work as a typical antibiotic drug, pathogens do not build up a resistance to it.

ii. For every day from day 2-7, perform a 50% water change with new saltwater / freshwater only. Make sure the new water is the same salinity and temperature as the quarantine tank. Re-dose 50% of the chemicals and/or drugs every time.

iii. Observe the fish for 1 week. If you see any signs of bacterial infection such as fin rot or cloudy eyes, inform the veterinarian to administer an antibiotic. All administration of antibiotics must have approval from the veterinarian who is a member of NACLAR.

iv. The operator is required to fill in **FORM A** for the tracking of every quarantine process. The form will be sent to the PI, veterinarian, and the lab manager.

(e) Quarantine with formalin (recommended for bigger fin fishes)

- Fish being treated should not have been recently fed. They should be in good health. Finally, the treatment should be done under dim lighting and the tank kept with dim lighting in

between treatments.

- When Formalin is added to seawater, it removes oxygen by reacting with the dissolved oxygen in the water. Thus, to get oxygen back into the water, this treatment requires the use of an air stone.

- In the presence of Formaldehyde (Formalin), oxygen is restricted in part by the temperature of the water. This is one case where temperature is very critical to the availability of oxygen in the water. The higher the temperature, the less dissolved oxygen is available even with proper mixing. Controlling the temperature is essential to a proper treatment. The temperature must not exceed 26.6°C at any time.

- Formaldehyde chemical activity is affected by pH. Maintain pH between 8.0 and 8.4 during treatment and in the quarantine tank.

- The final concentration in the treatment bath is: 1 ml of Formalin (37 percent) to every 3.78 L of treatment water; or 250 ppm Formalin in the treatment water.

  i. Make sure the pH and temperature of the treatment water is in the preferred zone (see above).

  ii. Prepare the formalin treatment bath (which can be the tank beside the quarantine tank).

  iii. Mix-aerate with air stone for 2 hours before using the bath and continuously during the treatment.

  iv. Check pH and be sure it is between 8.0 and 8.4.

  v. Dim or turn off the light. Capture fish in bag, drain excess quarantine tank water from the bag, and let fish slip into the bath with air stone running. It is ideal to put only 1 fish through this at a time because of the limited oxygen. Don't treat multiple fishes at the same time. However, it is possible to treat one fish immediately after another in the same bath, provided bath is controlled as noted.

  vi. Monitor fish closely. If fish show signs of stress, this may be acceptable to a degree. If there is concern that fish are dying, then abandon the treatment.

  vii. Leave fish in bath for 45 minutes.

viii. Capture fish under water in bag. Return them to the quarantine tank (make sure there is another air stone in the quarantine tank running or a corner sponge biofilter running by air and circulating the water in the tank. Monitor the fish.

ix. On the second day after treatment, repeat the process starting with (i) using a fresh preparation of the treatment bath. This will be the second treatment.

x. On the second day after this second treatment, treat fish again, starting at (i). Repeat pattern (treating every other day) until fish has had 5 such treatments (but no less than 3 total).

xi. Hold fish in quarantine for no less than 4 more weeks for observation to verify treatment has been successful.

xii. Dispose of all bath water (and any unused Formalin) properly. That is, take it to a proper disposal site and follow disposal requirements of your national and local government.

xiii. The operator is required to fill in **FORM A** for the tracking of every quarantine process. The form will be send to the PI, veterinarian and the lab manager.

Note for Formalin Usage:

Formaldehyde is a very active chemical. It is a poison to healthy fish in excessive quantities, however more notably it is very liable to kill fish with open wounds at treatment-level concentrations. Thus, fish with any kind of exposed injury—no matter how small—must not be given any kind of formaldehyde treatment regardless of how much it may be needed. In other words, fish with observable lesions and showing signs of skin loss must not be treated. This is the reason for recommending that the fish not be net-caught, rather they ought to be captured by using a clear plastic bag while underwater. The fish is not harmed and not exposed to the air in this manner.

2.2.2.2. *Disinfection of Quarantine Tank*

(a) After each quarantine process, clean the tank by scrubbing walls and base to remove remnant waste and algae.

(b) Disinfect the tank and equipment with **2-5% bleach solution** and allow to soak for 15 minutes.

(c) Rinse the tank and equipment thoroughly with freshwater until there is no trace of bleach.

2.2.3. Normal aquatic animal behavior

(a) Aquatic animals will be routinely monitored for signs of health and disease. All staff should be familiar with normal animal behavior. Key behaviors that indicate animal health include, but are not limited to:

- Physical – changes from normal, i.e. scale loss, parasites, external injury
- Behavioral – swimming and schooling behavior, increased respiration
- Feeding – normally aggressive feed response when feed is presented

(b) Aquatic animals will be kept at reasonable densities. Changes in behaviors and physical condition will be reported to the facility staff-in-charge immediately.

Table 1. Stocking density and survival rate of seabass larvae and fry at different ages.

Age (days)	Total length (mm)	Stocking density (per m^3)	Survival rate (%)
1 – 14	1.5 – 5	60,000 – 80,000	70 – 80
15 – 20	5 – 8	20,000 – 40,000	60 – 80
21 – 28	8 – 10	10,000 – 20,000	70 – 80
29 – 35	10 – 13	5,000 – 10,000	80 – 90
36 – 42	13 – 30	1,000 – 5,000	80 – 90

Source: http://www.fao.org/docrep/field/003/ab889e/AB889E02.htm

2.2.4. Predators

Predators will be excluded from the site. Predators include birds, other fish and marine mammals. The Technical Support Officer should do a weekly routine check of the facility for signs of birds and rodents droppings and nests. They should be removed immediately and reported to facility staff in charge using **FORM B**.

2.2.5. Feed and Nutrition

(a) The objective of good nutrition is to keep animals healthy. The Technical Support Officer and other facility operators have procedures in place for healthy feeding of the animals, including type of feed, and different feed delivery methods. Proper storage of these diets is essential to maintaining their nutritional value. Feed will be stored in secure buildings where wildlife can be excluded and spillage prevented.

(b) All feeds preparation is strictly to be performed in the Preparation Room. The storage of the following feeds should be as followed:

Types of feeds	Storage of feeds
Live feeds (Artemia, macroalgae)	Enclosed container with aeration with or without an incubator at room temperature 25°C
Frozen feeds	Freezer -20°C
Freshly prepared feeds	Fridge 4°C or Freezer -20°C
Dried feeds	Dry cabinets or enclosed dry cupboards

(c) Technical Support Officer should perform a monthly stock taking of the feeds in storage and the documents will be posted in an easily identifiable location at the storage doors using **FORM C**.

(d) Nutrients essential to aquatic animals, such as fish, are the same as those required by most other animals. These include water, proteins (amino acids), lipids (fats, oils, and fatty acids), carbohydrates (sugars, starch), vitamins, and minerals. In addition, pigments (carotenoids) are commonly added to the diet of salmonid and ornamental "aquarium" fishes to enhance their flesh and skin coloration, respectively. The general proportions of various nutrients included in a standard fish diet are given in Table 2.

Table 2. General amounts of nutrients incorporated into diets for growing fish.

Nutrients	Requirement (percent by dry diet)
Proteins (10 essential amino acids): lysine, phenylalanine, arginine, valine, leucine, isoleucine, methionine, threonine, tryptophan, and histidine.	32–45%
Fat: Used as a source of energy and polyunsaturated fatty acids. In general, freshwater fish require fatty acids of the linolenic (ω-3) and linoleic (ω-6) series.	4–28% (should contain at least 1–2% of the ω-6 or ω-3 essential fatty acid series)

Saltwater and coldwater fish require EPA and DHA (ω-3).	
Carbohydrates: These are an inexpensive source of energy and is a binding agent. No essential requirements have been identified. These are poorly digested when fed raw. Highest digestibility is achieved when cooked. Major carbohydrates are starch, cellulose, and pectin.	10–30%
Minerals: Some 20 inorganic mineral elements, including calcium, phosphorus, magnesium, iron, copper, manganese, zinc, iodine, and selenium.	1.0–2.5% fed as a multi-mineral premix
Vitamins: These are inorganic substances required in trace amounts that can be divided into fat-soluble (vitamins A, D, E, and K) and water-soluble (vitamins C and the B-complex [thiamin, riboflavin, pyridoxine, panthothenic acid, cyanocobalamin, niacin, biotin, folic acid, choline, and myoinositol]).	1.0–2.5% fed primarily as a multi-vitamin premix. Vitamin C and choline are added separately from the premix because of their chemical instability.

(e) Procedure for the preparation of gelatin based fish feed for fresh water and salt water fishes:

i. Ingredients (includes substitute ingredients)—For every 450 ml of water,

- 200 mL of canned shrimp (may replace with sardines, clams, flakes or pellet feeds, and beef heart)
- 60 mL of frozen spinach (may replace with peas or chopped kale)
- 30 mL of grated carrots (may replace with spirulina)
- 1 tablespoon of oats cereals
- 1 tablespoon of brewer yeasts
- 1 teaspoon of liquid vitamins (may use ½ tablet multivitamin supplement)
- 1 mL of cod liver oil

- 60 mL of unflavored powdered gelatin

ii. Blend 150 mL of water with shrimp, spinach, carrots, cereal, brewer yeast and vitamins with a blender machine.

iii. Boil remaining 300 mL of water with a hot plate.

iv. Add boiling water to gelatin powder in a bowl.

v. Cool gelatin mixture until warm (not cold).

vi. Add contents of the blender to partially cooled gelatin mixture and mix thoroughly.

vii. If needed, add medication to cooled mixture if instructed by veterinary personnel for disease treatment.

viii. Pour into an ice try and refrigerate at 4°C for 1–2 hours.

ix. Cut gelatinized mixture into smaller cubes for feeding and store in a freezer at -20°C. The maximum storage of the feeds in freezer is 30 days.

(f) Operators preparing the fish feeds should wear disposable masks and apron. Gloves are also required to prevent contaminating the feed. All procedures must be performed in the preparation room.

(g) Every preparation of fish feeds must be recorded in **FORM D** for inventory and tracking purposes.

(h) Frequency of feeding:

With the request of feeding services, Technical Support Officers and operators should employ the following feeding frequency. Technical Support Officers and PIs doing the husbandry care, such as feeding or performing partial water change, should indicate this clearly on **FORM M**.

Day of week	Feeding time	Remarks
Monday–Friday	0900–1000 1600–1700	

Saturday–Sunday Public Holidays	0900–1000	Automatic feeders can be used Dry pellets only

(i) Procedure for preparation of live feeds culture:

i. Fill the 1 L culture vessels with sea water of 35-40 ppt (specific gravity 1.024-1.028)

ii. Add the approximate amount of *Artemia salina* (brine shrimp) eggs into the 1 L culture vessels according to the manufacturer guidelines (approx. 1 g of eggs into 1 L of seawater).

iii. The initial pH should be between 7.5 and 8. The pH is likely to fall during the culture period. Adjust as required by adding sodium bicarbonate powder.

iv. Three days after hatching, feed the *Artemia salina* (brine shrimp) with 1 ml of macroalgae into the 1L of culture vessels. Feed twice per week.

v. Harvest the *Artemia salina* (brine shrimp) as required according to the needs for the live animals.

vi. Replace the amount of sea water with every harvest volume taken from the culture vessels.

vii. To start a new culture, discard the remaining *Artemia salina* (brine shrimp) and disinfect with 1% of hypochlorite and rinse with sea water.

viii. Repeat (i).

(j) Feeding menu:

Technical Support Officers and operators have the option of providing different types of feeds for aquatic husbandry.

Day of week	Feeding time	Feeding menu A	Feeding menu B
Monday	0900–1000	Dry feed	Fresh prep feed
	1600–1700	Fresh prep feed	Frozen feed
Tuesday	0900–1000	Dry feed	Fresh prep feed
	1600–1700	Fresh prep feed	Frozen feed
Wednesday	0900–1000	Dry feed	Fresh prep feed
	1600–1700	Fresh prep feed	Frozen feed
Thursday	0900–1000	Dry feed	Fresh prep feed
	1600–1700	Fresh prep feed	Frozen feed
Friday	0900–1000	Dry feed	Fresh prep feed
	1600–1700	Live feed	Live feed

Saturday–Sunday Public Holidays	0900–1000	Dry feed	Dry feed

2.3. Aquatic Animals Handling Technique

2.3.1. Common Fish Handling Techniques

(a) The operators will maintain good standards in accordance with the guidelines and training from Responsible Care and Use of Fish for Scientific Purposes (RCUF) (e.g. grading or seining, including minimizing the risk of escape while the fish are being handled). Handling—including equipment maintenance—will be done to minimize causing injury to the fish and/or predisposing them to disease.

(b) Fish will be monitored while being handled as well as for a period after handling to ensure any negative effects are identified and steps are taken to minimize impact. Staff will minimize the time fish are exposed to stress such as crowding and time out-of-water (i.e. handling, counting, grading, tagging, and injecting).

(c) <u>Handling fish:</u>

i. Use a landing soft net to scoop out small fish from the water to minimize stress.

ii. Quickly place the fish into another container that contains the same water parameters of its husbandry tank to minimize exposure to the air.

iii. Maximizing aquatic animals' survival during handling process for large fin fishes as in Figure 1[1]:

Figure 1. Correct handling of fin fish.

[1] Source: http://www.fpc.org/bon_jda/handleid01.html#GENERAL_FISH_HANDLING

iv. Minimize the length of time the fish is out of the water.

v. Handle the fish firmly and carefully. Avoid dropping fish onto the bottom of boats and other hard surfaces.

vi. Use wet hands or wet soft gloves when handling fish to minimize damage to its skin.

vii. Do not hold fish by the gills or the eyes.

viii. Take care to revive fish upon release if they appear exhausted (struggling to hold themselves upright or unable to swim away).

ix. Gently hold or push the fish through the water so that it obtains a good flow of water over its gills. If there is any water current, hold the fish upright facing towards the current until it starts to show signs of recovery.

(d) Maximizing aquatic animals' survival during handling process for large crustaceans (lobsters) as in Figure 2[2]:

Figure 2. Correct handling of lobsters.

i. If possible, do not handle undersize lobsters (due to their tendency to lose appendages).

ii. Avoid contact with the antennae of lobsters during hand collection and removal from traps. Handle lobsters by the body to limit breakage of antennae.

[2] Source: http://chilipaper.com/FWharf/Lobster/Lobster_Handling/lobster_handling.htm

(f) Maximizing aquatic animals 'survival during handling process for corals:

i. A pair of gloves or a disposable gripper sleeve is highly recommended when handling all corals, and it will reduce the possibility of irritating the coral when handling it.

ii. Minimize the length of time the coral out of the water.

2.3.2. Fin Fish Measurement

2.3.2.1. Total Length Measurement

(a) The image below[3] depicts the most commonly used measurements for fish. For fish, the measurements that you need to use are total length and girth.

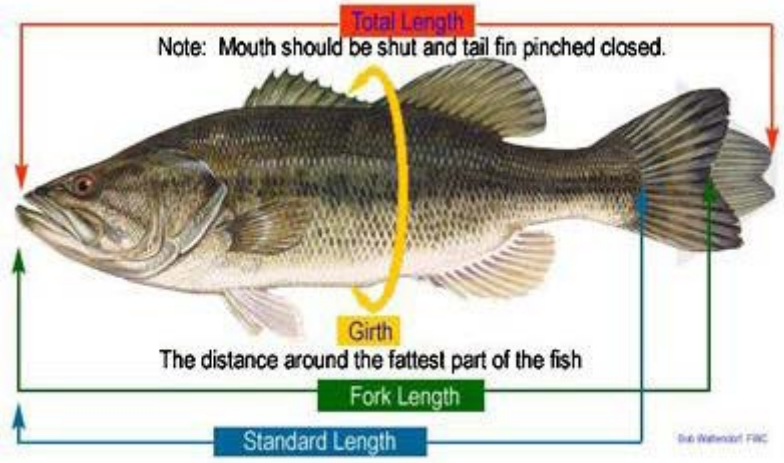

Figure 3. Fish common measurements.

(b) The total length is the maximum length of the fish, with the mouth closed and the tail fin pinched together. The best way to obtain this length is to push the fish's snout up against a vertical surface with the mouth closed and the fish laying along a tape measure, then pinch the tail fin closed and determine the total length. Do not pull a flexible tape measure along the curve of the fish. The image below[4] shows a bass on a measuring board with the mouth held shut. Prior to getting a final measurement the caudal (tail) fin will be pinched shut.

[3] Source: http://myfwc.com/fishing/freshwater/fishing-tips/measure/
[4] Source: same as above

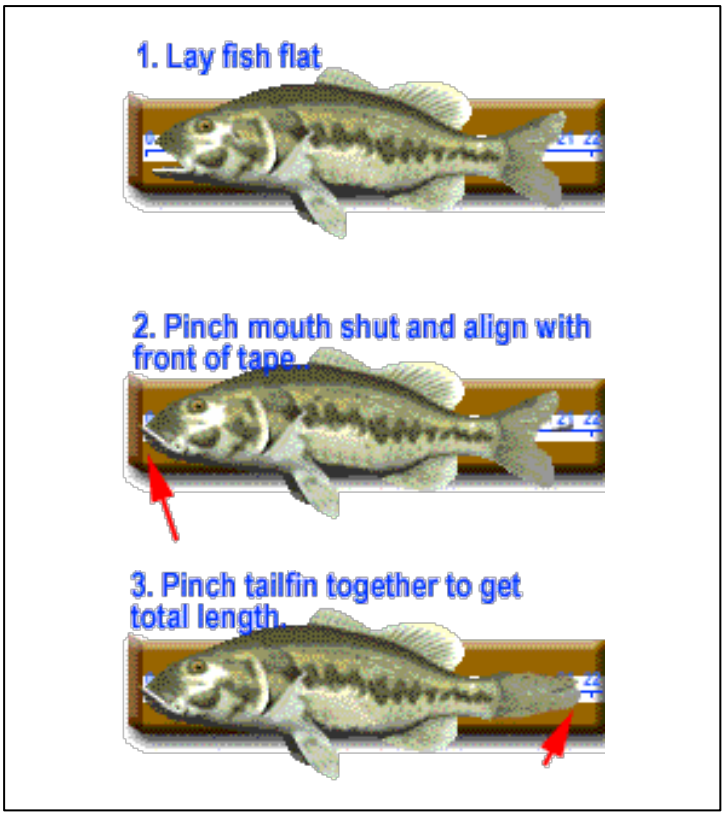

Figure 4. Procedure to measure fish total length.

(c) Many sources alternatively refer to the "fork length", and scientists often use "standard length", which is to the end of the fleshy part of the body. Standard length has the advantage of not being affected by minor damage to the tail fin, nor does it give too much credit to a fish for the relatively light weight tail when calculating a fish's condition.

2.3.2.2. *Girth Measurement*

(a) "Girth" is best measured with a fabric ruler, such as those that tailors use. It can also be determined by drawing a string around the fish at its widest point marking where the string overlaps and then measuring the distance between the overlapping points on a straight edge ruler (See Figure 5[5]). The measurement should be taken perpendicular to the length of the fish. This measurement is analogous to measuring the circumference of someone's waist. Knowing the girth is important when trying to certify a fish for a record, and provides useful information to biologists about the relative condition of a fish.

[5] Source: http://myfwc.com/fishing/freshwater/fishing-tips/measure/

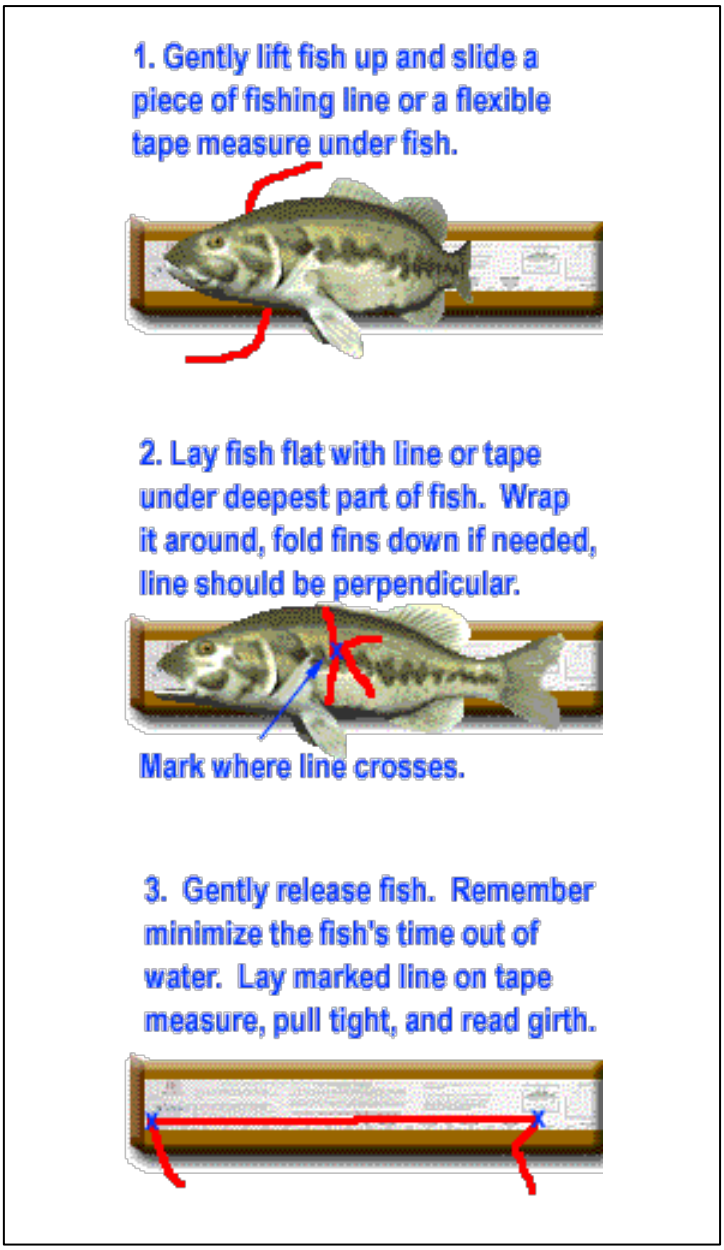

Figure 5. Procedure to measure fish girth.

2.3.3. Blood Sampling for Fin Fish[6]

(a) All fish are anaesthetized prior to blood sampling.

(b) Place anaesthetized fish on a "V" board fish holder or have an assistant hold fish ventral side up. The board should be clean and moist, do not place

[6] This procedure was taken from the Aquatic Animal Diseases Lab Manual by Dr. Hugh Ferguson. Image was taken from http://www.uoguelph.ca/~aqualab/forms/fishbloodsamplingSOP.pdf

absorbent material in contact with the fish as skin damage may occur.

(c) Use of 22 Ga. hypodermic needles are recommended for larger fish. Smaller gauge needles (upwards of 22 Ga.) are recommended for smaller fish.

(d) Blood may be sampled following four separate procedures: dorsal aorta, cardiac puncture, caudal vein, and caudal severance.

Blood sample provenance	Procedure
Caudal Vein	The sample is taken midline just posterior of the anal fin. Insert the needle into the musculature perpendicular to the ventral surface of the fish until the spine is reached or blood enters the syringe. If contact with the spine is made withdraw the needle slightly. The vein is ventral to the overlying spine. This blood vessel can also be sampled laterally.
Dorsal Aorta	Insert needle at a 30–40° angle into the dorsal midline in the roof of the mouth at about the 3rd to 4th gill arch. Depending on the size and species of the fish, insertion between the 1st and 2nd arch may be more suitable. Recovering fish tend to bleed at the mouth. This site may be used for indwelling catheterization.
Cardiac Puncture	Blood is collected from the heart ventricle. Insert the needle perpendicular to the ventral surface of the fish in the center of an imaginary line between the anterior part of the base of the pectoral fins.
Caudal Severance	Dry the caudal peduncle. Completely sever the tail posterior to the anal fin. The first few drops are discarded, the rest is collected in micro-hematocrit tubes. After the sample is collected, return the fish to a separate container of anesthetic for euthanasia.

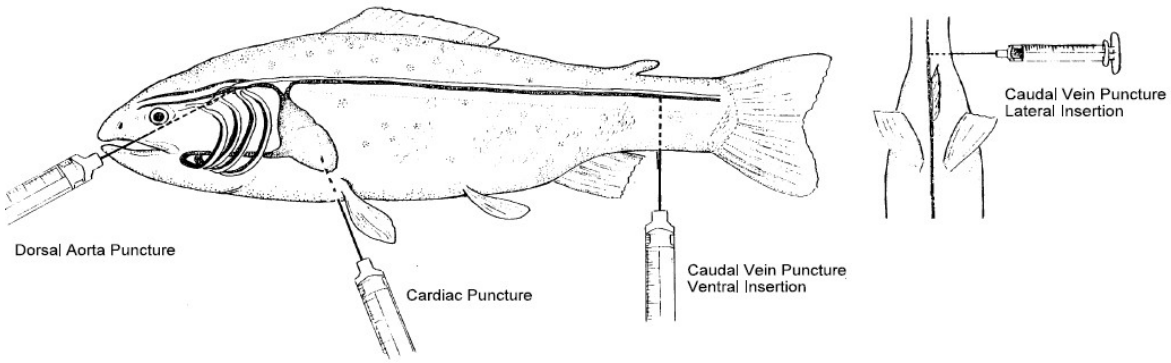

Figure 6. Blood sampling in salmonid fish

2.3.4. Anesthetizing fish

A variety of fish health procedures require that fish be anesthetized. Anesthetics are obtained through the operator's veterinarian. Netting or seining of fish prior to anesthesia will be done in as stress-free a manner as possible. Exposure to anesthetic will be minimized while ensuring the anesthetic level is adequate for the procedure. Anesthetized fish will be monitored carefully at all times. Water quality of the anesthetic bath—in particular, oxygen levels—will be monitored.

2.3.4.1. Materials

- Anesthetic agent [e.g., Isoeugenol (Aqui-S), pharmaceutical grade tricaine methane sulfonate (MS-222)], sodium bicarbonate (if using MS-222)
- Gloves (when handling animals, wear non-powered gloves pre-moistened with distilled or de-chlorinated water)
- Transport, anesthetic, and recovery tanks
- Oxygenation equipment (e.g. air pump, tubing, and air stone)

2.3.4.2. Procedures: General Considerations

(a) If using a new anesthetic protocol or species, anesthetize a small cohort of animals and follow them through full recovery to ensure drug dosages and techniques are safe, and provide sufficient anesthetic depth for the intended procedures.

(b) Do not disturb the mucus layer of fish. Wear non-powdered, pre-moistened gloves when handling animals. Do not apply detergents or solvents to the skin, and limit contact with abrasive materials (e.g., dry paper towels).

2.3.4.3. Procedures: Fish Anesthesia

(a) Fast fish for 12–24 hours prior to anesthesia, which reduces fecal contamination and risk of regurgitation.

(b) Use water taken from original fish holding tank for transport, anesthetic and recovery chambers. If using another water source, closely duplicate the water quality parameters (i.e., chlorine, temperature, pH and ammonia) of the original holding tank.

(c) Maintain adequate oxygenation of holding tanks throughout induction, anesthesia, and recovery. Supply oxygen via air pump and air stone, or

similar device.

(d) Maintain water temperature at the species' normal optimum during both anesthesia and recovery.

(e) Anesthesia is achieved by immersion in an anesthetic solution. See table below for anesthesia properties and preparation.

Anesthetic agent	**Dose** (mg/L of buffered aqueous solution)	**Comments**
Isoeugenol (Aqui-S)	1-5 ppm (light sedation) and 10-15 ppm for normal sedation	Typically, no need to buffer to neutral pH.
MS-222 (tricaine methane sulfonate)	75–125 mg/L (induction) and 50–75 mg/L (maintenance)	Buffer with equal weight of sodium bicarbonate. This is the only FDA approved anesthetic for fish (21 day withdrawal)
Benzocaine hydrochloride	25–100 mg/L	Small margin of safety between effective and lethal doses. Buffer solution with sodium bicarbonate to maintain neutral pH.

Further notes on MS-222:

- Always buffer solution with an equal weight of sodium bicarbonate to maintain neutral pH.
- In solution, MS-222 will lose efficacy if kept longer than 7 days.
- MS-222 is light-sensitive, it must be kept in a dark container.
- It has a wide margin of safety.

Further notes on Benzocaine:

- Dissolve powder in appropriate solvent (e.g., water or ethanol) to create a stock solution.
- As with MS-222, buffer solution with sodium bicarbonate to maintain neutral pH.

(g) Stages of anesthesia in fish:

Stage 1	Stage 2	Stage 3
Deep sedation	Deep narcosis	Surgical anesthesia
• Cessation of voluntary swimming • Decreased response to stimuli	• Decreased muscle tone • Equilibrium loss • Appropriate level for fin and gill biopsies	• Slow respiration and heart rate • Total loss of response to stimuli • Firmly squeeze at the base of the tail to determine response to stimuli

(h) Allow the animal to reach the appropriate level of anesthesia for planned procedures.

(i) While performing procedures, keep the fish's skin moist and the gills submerged or regularly flushed with well oxygenated water.

(j) Evaluate respiratory rate and gill color throughout anesthesia:

- Observe movement of the operculum (rigid flap that covers the gills) as it opens and closes to assess rate.
- Observe gill color; should be dark pink to light red.
- If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume.

2.3.4.4. Post Anesthetic Care

(a) Closely monitor fish recovering from anesthesia until they are swimming/moving normally and have completely regained their righting response.

(b) Place the fish in well oxygenated and unmedicated water in a holding tank.

(c) To speed recovery, create a flow of oxygenated water over the gills by:

- Moving the fish back and forth in the water, or
- Opening and closing the mouth several times

(d) Maintain water temperatures at the species' normal optimum throughout recovery.

2.3.4.5. MS-222 (tricaine methane sulfonate) Safe Practices

(a) Wear protective clothing, gloves, and goggles when handling MS-222 powder. Wear gloves to handle animals exposed to MS-222.

(b) If possible, work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water. Wear gloves and use a utensil to stir until all powder is dissolved. Dilute the stock solution further as required.

(c) Dispose of MS-222 waste by flushing down the drain to a sanitary sewer with an excess of water. Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.

(d) If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land, in a location away from water.

2.3.5. Vaccinating fish

Vaccines are used to boost immunity against certain infectious diseases (e.g., Furunculosis) and are part of an integrated fish health management program. Vaccines are biological substances that will be stored (refrigerated) and handled as per manufacturer's instructions so as to maintain their effectiveness. Staff will be appropriately trained prior to undertaking the vaccination procedure.

Dip vaccination will be done in accordance with manufacturer's guidelines. Fish will be handled in as stress-free a manner as possible.

Intraperitoneal vaccines will be administered in accordance with manufacturer's guidelines.

2.3.5.1. Vaccine Handling and Storage Guidelines

(a) General considerations:

- Designate primary and backup personnel to be in charge of vaccine inventory, storage, and handling.
- Maintain a vaccine inventory log that notes the vaccine's quantity, date of arrival, and arrival condition.
- When a shipment arrives, check the temperature inside the shipping box and immediately refrigerate the vaccines.
- Use only the diluent supplied with the vaccine.

- Do not reconstitute or draw up the vaccine in the same syringe unless licensed for that use.
- Avoid mixing different vaccines in the same syringe unless licensed for that use.
- Properly dispose of syringes and needles in a sharps container.

(b) Transportation considerations:

- Use an insulated cooler to transport vaccines.
- Keep a thermometer in the cooler.
- Maintain the temperature between 2–7°C.
- Use refrigerated or frozen packs as needed to maintain the appropriate temperature in the cooler.
- Place insulation (e.g., bubble wrap) between the vaccine vials and the frozen pack to prevent direct contact.
- Keep the vaccines in their original packaging.
- To minimize exposure to extreme temperatures, keep the cooler in the interior of the vehicle instead of the trunk or truck bed.

(c) Storage considerations:

- Keep vaccines in a standard-size refrigerator with a separate freezer compartment.
- Keep a good-quality thermometer in the vaccine storage refrigerator.
- Maintain the refrigerator between 2–7°C.
- Do not store vaccines in a mini 'dormitory-style' refrigerator.
- Do not keep vaccines in the door, in vegetable bins, against the walls or against the cold air inlet of the refrigerator.
- Do not overpack the refrigerator.

2.3.5.2. General Guidelines for Dip Vaccination for Fish

(a) Shake vaccination bottle well before use.

(b) Dilute the vaccine with clean hatchery water according to manufacturer guidelines.

(c) Drain and weigh a net full of fish and dip the fish in the diluted vaccine for

30 seconds ensuring that they are totally immersed in the vaccine (timing may be subject to manufacturer guidelines).

(d) After 30 seconds of exposure, lift net and allow to drain and then return the fish to the holding tank.

(e) Spent vaccine is discarded after use.

2.3.6. Euthanasia

In the uncommon situation where fish should be euthanized (e.g., certain fish health sampling), euthanasia should be done in a humane manner. The method used should result in rapid and irreversible loss of consciousness.

2.3.6.1. Materials

- Dependent upon method used
- Topical euthanasia agent [e.g., Aqui-S]
- Mechanical euthanasia tool (e.g., ice and bucket, guillotine, pithing tool)

2.3.6.2. Procedures: Chemical Method

Note: Prior to disposing of euthanized fish, perform a physical means of euthanasia to observe that opercular movement has ceased for at least 10 minutes.

Note: Chemical methods of euthanasia may require up to 3 hours before death results. A physical method, as per section 2.3.6.3 below, can be applied once unconsciousness is achieved.

(a) For juveniles and fries, 25 ppm is used for an indefinite period or the fish's spinal cord is cut instead.

(b) For adults, use 100 ppm for 40 min or 175 ppm for 20 min.

2.3.6.3. Procedures: Physical Method

Note: Anesthesia or heavy sedation must be applied prior to the use of physical techniques unless otherwise approved by the IACUC.

(a) Decapitation: Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is separated from the body rapidly and completely. Follow decapitation with pithing via the open spinal canal.

(b) Thermal shock (only for specimens that are <5 cm in total body length):

- Rapid freezing: Anesthetize animal as per Section 2.3.4 and then immerse in liquid nitrogen.
- Rapid Chilling (hypothermic shock) in tropical fresh water fish:

  i. Set up an ice bucket or cooler with ice slush.

  ii. Form a depression in the ice to expose water and prevent direct animal contact with the ice.

  iii. Pour the anesthetized fish and/or larvae into the depression.

  iv. Use a minimal amount of water for transfer of animals into ice water to prevent local warming of ice water by adding room temperature water to the ice.

  v. Use a net for adults and a minimal volume of embryo medium for embryos/larvae.

2.4. Monitoring Water Quality

Maintaining good water quality is vital to good aquatic animal's health. The operator maintains a regular program for monitoring and recording water quality at the sites.

2.4.1. Contingency Plans

The operator maintains a contingency plan in the event of acute deterioration of water quality. Depending on cause, the aquatic animals will usually be taken off feed immediately. Water quality monitoring is immediately increased to determine the cause and to estimate how long the problem may persist. The animals will be monitored more closely for the duration of the event and will not be further handled until water quality is acceptable. Records will be kept in **FORM E**.

System	Status	Volume of partial water change	Frequency
All tanks	Normal	100% change of 4 tanks per shared system	Monthly
	Emergency[7]	100% change of 8 tanks per shared system	Emergency

2.4.2. Schedule of Water Quality Testing

The testing of water quality for the facility has to be followed as per the schedule below:

Type of water quality test	Type of system	Normal Range	Frequency of test
Ammonia Test	Freshwater & Seawater	0 ppm	Weekly or Emergency care test[7]
Nitrate Test	Freshwater & Seawater	< 10 ppm	Weekly
Phosphate Test	Freshwater & Seawater	< 0.05 ppm	Weekly
pH Test	Freshwater	6.8 – 8.0	Weekly or Emergency care test[7]
	Seawater	7.8 – 8.5	
Dissolved Oxygen Test	Freshwater & Seawater	7.0 – 9.0 ppm	Weekly
Temperature measurement	Freshwater & Seawater	22 – 26°C	Weekly
Salinity Test	Seawater	35 ppt	Weekly or Emergency care test[7]
Alkalinity Test (Hardness Test)	Seawater	7 –11 dKH (2.5 – 4.0 meq/L)	Weekly
Calcium Test	Seawater	> 380 ppm	Weekly

[7] In the event of massive fish death and high ammonia above range.

2.4.3. Water Quality Test Methods

2.4.3.1. There are two methods to perform water test in Aquaria facility: water testing using electrode and colorimetric test kit.

2.4.3.2. Water testing using electrode is only available for the following water parameters testing:

- Ammonia
- Nitrate
- pH
- Calcium

2.4.3.3. Colorimetric test is only available for the following water parameters testing:

- Phosphate
- Alkalinity

2.4.4. Water Quality Test using Electrode

2.4.4.1. Electrode Preparation

(a) Remove the sensing module from the vial and save the vial for storage. Make sure that both O-rings are in place on the module. Remove the electrode handle from the box.

(b) Unscrew the electrode cap. Slide the cap and spring down the electrode cable.

(c) Hold the outer body sleeve and gently push the inner stem through the outer body. Slide the outer body sleeve down the electrode cable until it is beyond the inner stem.

(d) Grasp the middle of the inner stem without touching the reference pellet. If a red storage tip is connected to the inner stem, unscrew it, and save it for storage.

(e) Screw the sensing module into the stem until it stops and the module is flush against the stem. Tighten the module an additional one quarter turn. The module should be firmly attached to the stem. Do not over tighten the module.

(f) Hold the electrode cable and slide the outer body, spring, and cap over the inner stem.

(g) Grasp the outer body sleeve, without touching the sensing membrane, and gently screw the cap onto the inner stem while pulling on the cable. Stop when an opposite force is felt. Do not overtighten or continue to turn the cap. The cap will not completely stop. If the inner body turns at all, the cap is too tight. Remove the cap and reassemble.

(h) Press on the top of the cap with your thumb to make sure that the electrode has a smooth flushing motion and the outer body sleeve returns to its original position.

(i) Install the flip spout cap onto the Ion Electrolyte A reference filling solution bottle and lift the flip spout to a vertical position. Insert the spout into the electrode fill hole and add a small amount of filling solution to the reference chamber.

(j) Hold the electrode body and use your thumb to push down on the electrode cap to allow a few drops of filling solution to drain out of the electrode. Release the electrode cap.

(k) If the sleeve does not return to its original position, add filling solution and repeat step (j) until the sleeve returns to its original position.

(l) Add filling solution to the electrode up to the fill hole.

(m) Rinse the electrode with distilled water and soak it in a 100 mg/L standard solution (used for the specific probe) for 1 to 2 hours prior to use.

2.4.4.2. Checking Electrode Operation (Slope)

This procedure measures the electrode slope. Slope is defined as the change in millivolts observed with every tenfold change in concentration. The slope value provides the best means for checking the electrode operation.

(a) If the electrode has been stored dry, prepare the electrode as described in the Electrode Preparation in section 2.4.4.1.

(b) Connect the electrode to a meter with an mV mode. Set the meter to the mV mode.

(c) Add 100 mL of distilled water and 2 mL of ISA into a 150 mL beaker. Stir the solution thoroughly.

(d) Rinse the electrode with distilled water and place the electrode into the solution prepared in step (c).

(e) Select the desired probe measurement standard (either 0.1 mol/L or 1000 mg/L). Pipette 1 mL of the standard into the beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

(f) Pipette 10 mL of the same standard into the same beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

(g) There should be a 25 to 30 mV difference between the two millivolt readings when the solution temperature is between 20 to 25 °C. If the millivolt potential is not within this range, refer to troubleshooting section.

2.4.4.3. Small Volume Direct Calibration Setup

The electrode is able to measure sample volumes as small as 5 mL using a modified direct measurement procedure. Because less solution volume is required, the chemical usage of calcium standards and ISA is reduced. All samples should have a concentration greater than 1 mg/L or 1.0×10^{-4} mol/L. A two point calibration is sufficient although more points can be used. The following procedure recommends using 25 mL of sample. Smaller sample volumes can be used, as long as the final volume of solution is sufficient to cover the bottom of the electrode.

(a) Prepare the electrode as described in section 2.4.4.1.

(b) Connect the electrode to the meter.

(c) Prepare at least two standards that bracket the expected sample range and differ in concentration by a factor of ten. Standards can be prepared in any concentration unit to suit the particular analysis requirement.

(d) To prepare a 100 mg/L standard: Pipette 10 mL of the 1000 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

(e) To prepare a 10 mg/L standard: Pipette 10 mL of the 100 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

(f) To prepare a 1 mg/L standard: Pipette 10 mL of the 10 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

2.4.4.4. Small Volume Direct Calibration using Meter with ISE

(a) Add 25 mL of the less concentrated standard and 0.5 mL of ISA to a 50 mL beaker and swirl the solution to mix.

(b) Rinse the electrode with distilled water, blot it dry, and place it into the beaker with the less concentrated standard. Wait for a stable reading and adjust the meter to display the value of the standard as described in the

meter user guide.

(c) Add 25 mL of the more concentrated standard and 0.5 mL of ISA to a second 50 mL beaker and swirl the solution to mix.

(d) Rinse the electrode with distilled water, blot it dry, and place it into the beaker with the more concentrated standard. Wait for a stable reading and adjust the meter to display the value of the second standard as described in the meter user guide.

(e) Record the resulting slope value. The slope should be between 25 and 30 mV when the standards are between 20 and 25°C.

(f) Add 25 mL of sample and 0.5 mL of ISA to a clean 50 mL beaker and swirl the solution to mix.

(g) Rinse the electrode with distilled water, blot it dry, and place it into the sample. The concentration of the sample will be displayed on the meter.

2.4.4.5. Electrode Storage

For storage between measurements and up to three days, store the electrode in a $1.0x10^{-2}$ mol/L or 100 mg/L probe standard. The filling solution inside the electrode should not be allowed to evaporate, as crystallization will result. For storage longer than one week, drain the electrode, flush the reference chamber with distilled water, disassemble the electrode, and store the sensing module in the glass vial.

(a) Grasp the outer body sleeve and unscrew the electrode cap. Slide the cap and spring assembly down the electrode cable.

(b) Push the inner stem of the electrode handle out through the outer electrode sleeve, exposing the sensing module.

(c) Rinse the inner stem and module well with distilled water. Gently blot dry to prevent damaging the sensing module.

(d) Carefully unscrew the sensing module from the inner stem, taking care not to touch the sensing membrane.

(e) Place the calcium sensing module in the glass vial until it is needed again. Gently blot dry the inside of the inner stem and O-ring area, reassemble the electrode handle without the module, and store it dry.

2.4.4.6. Replacing the Probe Sensing Module

The sensing membrane of plastic membrane electrodes will wear over time,

indicated by low slope values, drift, poor reproducibility, and loss of response in low level samples. The electrode response can be restored by replacing the sensing module. Each sensing module will last about six months with normal use, but the actual lifespan of the sensing module will depend on the type of samples that are measured.

(a) Drain the electrode and flush the reference chamber with distilled water.

(b) Hold the outer body sleeve and unscrew the electrode cap. Slide the cap and spring assembly down the electrode cable.

(c) Push the inner stem of the electrode handle out through the outer electrode sleeve, exposing the sensing module. Rinse the inner stem and module well with distilled water.

(d) Gently blot dry to prevent damaging the sensing module. Carefully unscrew the sensing module from the inner stem and dispose of the old sensing module.

(e) Obtain a new probe membrane module from supplier and refer section 2.4.4.1 for detailed instructions on assembling the electrode.

2.4.4.7. Flushing the Combination Electrode

If the area between the outer body and inner cone becomes clogged with sample or precipitate, flush the area with filling solution or distilled water.

(a) Hold the electrode body and use your thumb to push down on the electrode cap until all the filling solution is drained.

(b) Fill and drain the reference chamber with distilled water. Repeat this procedure until all of the sample or precipitate is removed from the electrode.

(c) Fill the electrode with fresh filling solution up to the fill hole.

2.4.5. Water Quality Test using colorimetric test kits

2.4.5.1. Perform colorimetric test kits based on the instructions provided from the kit. No calibration is required for the test kits.

(a) Clean the test vials provided by the kit with distill water.

(b) Rinse the test vials with the water sample.

(c) Fill the desired amount of water sample and test according to the instructions provided in the test kit.

2.5. Tanks and Life Support System Maintenance

All necessary precautions will be taken to ensure disease is kept out of a facility with proper tanks maintenance and water monitoring.

2.5.1. Reservoir and Equipment Maintenance

Technical Support Officers and operators are to ensure the water level in the reservoir is maintained at the minimal level through daily checks. Equipment will be kept clean at all times. This is to prevent possible spread of pathogens by livestock, personnel, or via water borne routes. Equipment will be properly disinfected after each use and put away in its proper place. Refer to Annex A for facility map.

2.5.1.1. Make sure both freshwater and seawater reservoirs are maintained at least at one metric ton level at all times. It can be observed through the markings made beside the reservoir (plastic tube).

2.5.1.2. Topping up freshwater reservoir: top up the reservoir only when it reaches one metric ton level. This is to ensure that all the matured water in the reservoir is used up before adding the new water.

(a) Turn the reservoir top up valve to top up freshwater reservoir.

(b) Ensure that the water level reaches the 4 metric ton level before leaving the facility. Turn the valve off.

(c) It is required to wait for at least 24 hours before circulating the freshwater reservoir into the tank system. This is to allow time for dechlorination and maturation of the water. Make sure the UV light in the reservoir is turned on.

(d) Fill up **FORM F** for tracking of topping up of reservoir water.

2.5.1.3. Topping up seawater reservoir: top up the reservoir only when it reaches one metric ton level. This is to ensure that all the matured water in the reservoir is used up before adding the new water.

(a) Engage with a seawater supplier to top up water into the reservoir. Please check with the purchasing department on the regulations and budget of seawater supplies before engaging them.

(b) Use up all the remaining seawater in the reservoir before the scheduled trip from supplier. The water level is preferably right above the water reservoir pump. You may use the seawater for either:

- Partial water change
- Storage in carboy
- Cleaning or rinsing purposes

(c) Lead the supplier to anchor the hose to the seawater reservoir input pipe. The input tube is located outside the facility.

(d) Ensure that the water level reaches the 4 metric ton level before leaving the facility. Turn the valve off.

(e) It is required to wait for at least 48 hours before circulating the seawater reservoir into the tank system. This is to allow time for disinfection and maturation of the water. Make sure the UV light in the reservoir is turned on for the first 2 hours and switched off after that for each week.

(f) Fill up **FORM F** for tracking of topping up reservoir water.

2.5.1.4. Cleaning of reservoir: there is minimal cleaning requirement in the reservoir, however the schedule of basic maintenance is as below. Every maintenance task completion must be recorded in **FORM G**.

Task Code	Maintenance description	Frequency
R1	Cleaning of exterior of reservoir including cover and side walls	Weekly
R2	Cleaning and disinfection of internal linings of reservoirs which includes drainage of existing water Cleaning of mechanical filters	Every 6 months (by vendor)
R3	Cleaning and disinfection of internal linings of reservoirs which includes drainage of existing water Cleaning and replacement of at least 50% of mechanical filters or similar materials Replacement of UV light disinfection system	Every 1 year (by vendor)

2.5.2. Outdoor Life Support Equipment Maintenance

Technical Support Officers and the operators are to ensure the cleanliness of the life support equipment. Equipment will be kept clean at all times. This is to prevent possible spread of pathogens by livestock, personnel or water borne routes. Equipment will be properly disinfected after each use and put away in its proper place.

2.5.2.1. Cleaning schedule: the schedule of basic maintenance is as below. Every maintenance task completion is require to be recorded in **FORM G**.

Task Code	Maintenance description	Frequency
C1	Cleaning and disposal of protein wastes from skimmer (seawater system)	As required
C4	Cleaning of mechanical filters, cleaning of biological filters, and 100% replacement of all carbon media Replacement of all buffering agents only as required Servicing of air pumps, servicing and replacement (if required) of circular air rings Servicing and deep cleaning of protein skimmer; this includes the skimmer return pump and needle pumps	Every 6 months (by vendor)
C5	Cleaning and replacement of at least 50% of mechanical filters, cleaning and replacement of at least 50% of biological filters, and 100% replacement of all carbon media Replacement of all buffering agents only as required Servicing of air pumps, servicing and replacement (if required) of circular air rings Servicing and cleaning of external pumps Servicing and deep cleaning of protein skimmer (for seawater LSS); this includes the skimmer return pump and needle pumps	Every 1 year (by vendor)

2.5.2.2. *Disinfection of Quarantine Tank for Freshwater and Seawater*

(a) After each quarantine process, disinfect the tank and equipment with 2-5% bleach solution.

(b) Wash the tank and equipment thoroughly with freshwater until there is no trace of bleach.

2.5.2.3. *Daily Cleaning of Protein Skimmer:*

i. Switch off the main power supply of the seawater system protein skimmer.

ii. Drain the wastes using the attached valve connected to the head of the skimmer into the pail.

iii. Detach the protein skimmer cap by a slight turn and wash with tap water. Assemble back the cap and switch the pump back on.

2.5.2.4. *Cleaning of Protein Skimmer Body*

iv. Switch off the main power supply of the seawater system protein skimmer.

v. Drain the wastes using the attached valve connected to the head of the skimmer into the pail.

vi. Detach the protein skimmer cap by a slight turn and wash with tap water.

vii. Using a long tubed brush, clean the interior protein skimmer body with freshwater. Remove the residual proteins and algae. (You may need to carry the whole skimmer to ground level for washing and cleaning).

viii. Use vinegar (weak acetic acid) to remove stubborn stains in the skimmer body. Rinse the internal body again after cleaning with vinegar.

ix. Reassemble back and switch the pump back on.

2.5.3. Indoor Tanks and Equipment Maintenance

Technical Support Officers and the operators are to ensure the cleanliness of the life support equipment. Equipment will be kept clean at all times. This is to prevent possible spread of pathogens by livestock, personnel, or via water borne route. Equipment will be properly disinfected after each use and put away in its proper place.

2.5.3.1. Cleaning of the freshwater and seawater tanks: the schedule of basic maintenance is as below. Every maintenance task completion must be recorded in **FORM G**.

Task Code	Maintenance description	Frequency
C1	Cleaning and disposal of protein wastes from skimmer (seawater system)	Daily
C2	C2.1 Cleaning of exterior of tank including cover and side walls. C2.2 Cleaning of interior walls using a sponge to remove residual algae. C2.3 Topping up evaporated water.	Weekly
C3	Partial water change	Monthly
C4	Cleaning of mechanical filters, cleaning of biological filters, and 100% replacement of all carbon media Replacement of all buffering agents only as required Servicing of air pumps, servicing and replacement (if required) of circular air rings Servicing and deep cleaning of protein skimmer; this includes the skimmer return pump and needle pumps	Every 6 months (by vendor)
C5	Cleaning and replacement of at least 50% of mechanical filters, cleaning and replacement of at least 50% of biological filters, and 100% replacement of all carbon media Replacement of all buffering agents only as required Servicing of air pumps, servicing and replacement (if required) of circular air rings Servicing and cleaning of external pumps Servicing and deep cleaning of protein skimmer (for seawater LSS); this includes the skimmer return pump and needle pumps	Every 6 months (by vendor)

2.5.3.2. *Disinfection of Quarantine Tank for Freshwater and Seawater*

(a) After each quarantine process, disinfect the tank and equipment with 2-5% bleach solution.

(b) Wash the tank and equipment thoroughly with freshwater until there is no trace of bleach.

2.5.3.3. *Daily Cleaning of Protein Skimmer:*

i. Switch off the main power supply of the seawater system protein skimmer.

ii. Drain the wastes using the attached valve connected to the head of the skimmer into the pail.

iii. Detach the protein skimmer cap by a slight turn and wash with tap water. Assemble back the cap and switch the pump back on.

2.5.3.4. *Cleaning of Protein Skimmer Body*

iv. Switch off the main power supply of the seawater system protein skimmer.

v. Drain the wastes using the attached valve connected to the head of the skimmer into the pail.

vi. Detach the protein skimmer cap by a slight turn and wash with tap water.

vii. Using a long tubed brush, clean the interior protein skimmer body with freshwater. Remove the residual proteins and algae. (You may need to carry the whole skimmer to ground level for washing and cleaning).

viii. Use vinegar (weak acetic acid) to remove stubborn stains in the skimmer body. Rinse the internal body again after cleaning with vinegar.

ix. Reassemble back and switch the pump back on.

2.5.3.5. *Partial Water Change*

(a) Switch off the main pump of the system. There will be a back flow of the water return to the sump tank. Please monitor the flow.

(b) Turn the drain valve located at the side of the rack.

(c) Turn the PVC drain pipe in the tank that is selected for partial water change.

Depending on the partial change, total removal of the PVC drain pipe results in a > 80% water change of the selected tank.

(d) Refer to the below tank for the estimation of partial water change for the whole system:

Partial water change	No. of tanks drained 100%
20%	4 tanks
50%	6 tanks
80%	10 tanks

(e) After draining the desired water volume, secure back the PVC pipe tightly. Turn the drainage pipe valve to lock.

(f) Select the correct water source to top up individual tanks until it reaches the PVC return pipe level.

2.5.4. Equipment hygiene

Equipment will be kept clean at all times. This is to prevent possible spread of pathogens by livestock, personnel, or via water borne route. Equipment will be properly disinfected after each use and put away in its proper place. Refer to ANNEX A for full equipment maintenance for the facility.

2.5.5. Equipment movement

Where possible, equipment will not be shared between sites. This includes animal handling equipment, vessels, feeding, monitoring, and other equipment. Vessels and equipment, which must be used at multiple sites, will be subject to strict biosecurity and disinfection measures between uses.

2.5.5.1. Facility personnel shall determine whether or not the equipment or furniture requires decontamination. For example, if the equipment was used in a facility office or had no potential for exposure to biological, chemical, or radioactive materials, no additional precautions are required. All equipment originating from a laboratory or laboratory support area must be documented as free of contamination.

2.5.5.2. Facility personnel shall clean and decontaminate the equipment appropriate for the potential hazard.

2.5.5.4. Biological Decontamination

(a) The minimum personal protective equipment (PPE) to wear when decontaminating lab equipment shall be a lab coat, gloves, and chemical splash goggles. Additional PPE may be required depending upon contaminants and disinfectants used.

(b) All equipment used to handle or store biological agents (such as aquatic animals) or located in a laboratory (ex. freezers, incubators, centrifuges, etc.) must be decontaminated with an appropriate disinfectant for the contaminant and/or equipment.

(c) Allow disinfectant to remain on the equipment for the appropriate contact time. In most cases, 15-30 minutes is sufficient.

2.5.5.5. Chemical Decontamination

(a) The minimum personal protective equipment (PPE) to wear when decontaminating lab equipment shall be a lab coat, gloves, and chemical splash goggles. Additional PPE may be required depending upon chemical hazard and decontamination method used.

(b) Generally, mild detergent and water will suffice for removing chemical residues from work surfaces and equipment. Consult manufacturer recommendations for instructions on cleaning equipment surfaces. Use caution and consult Material Safety Data Sheets for information on the specific chemical hazards with which the equipment or work surface may be contaminated (i.e., for water reactive chemicals).

(c) Equipment that contains oil or refrigerants (e.g., air conditioners or refrigerators) or that has a water jacket, must be properly drained of its contents prior to disposal. It is the owner's responsibility to make arrangements to have the equipment drained and to ensure chemicals are properly disposed.

2.5.6. Livestock movement

During cases where aquatic animals must be relocated between tanks within the same facility, the users (including the Technical Support Officer and PIs) required to fill in the **FORM K**.

2.7. Minimizing Disease within the Site

All efforts will be made to minimize disease on a site. Adequate hygiene, disinfection, and mortality collection help to keep aquatic animals healthy and exposed to as few pathogens as possible.

2.7.1. Hygiene and Disinfection – Personnel

All personnel will adhere to the facility hygiene and disinfection procedures as below:

2.7.1.1. Hand Hygiene

(a) Wash hands when they become contaminated with aquatic animals' secretions or are visibly dirty, at the beginning of the working day, after using the toilet, and before meal. Wash hands with liquid soap. Dry them carefully with clean paper tissues.

(b) Wash hands before and after submersion into the tank water.

(c) Use gloves when exposed to aquatic animal's secretions or blood and when handling wounds or mucous membranes. The gloves are for single use only. Always apply a hand disinfectant after taking off the gloves.

(d) Take care of the skin of your hands. Use a moisturizing lotion after a working day. Keep your nails short and clean.

(e) When performing animal husbandry work, do not wear rings or bracelets, because they hamper effective hand disinfection. They can cause injuries to fish skin as well.

2.7.1.2. Hand Disinfection

Hand disinfection with a disinfectant is an easier and quicker way of cleaning hands than washing with soap, if hands are not visibly dirty. However, if you have diarrhea or are in contact with someone with diarrhea, hand washing prevents the spread of microbes more effectively than mere disinfection.

(a) Squeeze 3-4 ml of hand disinfectant (push the pump of the bottle 2 3 times) on you palm.

(b) Dip you fingertips on disinfectant.

(c) Rub fingertips and nails carefully with disinfectant. Do not forget thumbs and little fingers. Also scrub the gaps between fingers.

(d) Keep on scrubbing until your hands are completely dry. Do not wipe the

disinfectant away with a towel or tissue.

2.7.2. Hygiene and Disinfection – Equipment

Equipment must be kept clean, in good working order and disinfected as per section 2.5.4.

2.8. Monitoring Aquatic Animal Health

Aquatic animals will be monitored at least once daily for any unusual behavior, visible lesions, or other signs of disease. Changes in behavior and physical condition will be reported to facility management. Water quality will also be routinely monitored as per section 2.4.

2.8.1. Mortality Collection and Livestock Inventory

Mortalities will be collected on a routine and frequent basis to minimize the potential spread of disease and to minimize attractiveness to predators. The operator needs to follow the timing for mortality collection. Disinfection procedures will be adhered to after each mort procedure for personnel.

(a) Collection of mort should be done daily every morning before feeding time. Use a proper net to collect the deceased animals into a plastic bag.

(b) Label the plastic bag with the date and store in a freezer for 7 days.

(c) Fill in **FORM H** for livestock inventory.

(d) Management of unusually high mortalities will be as below:

i. When more than 25% of the aquatic animals in a tank die, it will be classified as a case of high mortality. Fill in **FORM I** and alert the facility manager and the PI.

ii. Perform the usual mort collection procedure as described above (section 2.8.1, subsections (a) to (c)).

iii. Perform water quality test and partial water changes based on the contingency plan described in section 2.4.1.

2.8.2. Mortality Inspections

Mortalities collected from daily routine (as per section 2.8.1) will be examined for signs of disease. Suspected causes of mortality must be recorded and Animal Health Management will be notified of any unusual numbers or types

of mortalities.

Routine sampling may be done as per the operator's procedures and/or on the instructions of the operator Veterinarian and/or Animal Health Management.

2.8.2.1. Guidelines for Preparation of a Aquatic Animal Health Inspection

(a) Refer to USFWS/AFS-FHS Standard Operations for Aquatic Health Inspections. (Website: https://www.fws.gov/policy/aquatichandbook/Volume_1/Chapter_2.pdf)

(b) Fill in **FORM J** for aquatic animal health inspection report or send for external agency to perform a post-mortem test, if required.

2.9. Aquatic Animal Disease Outbreaks

An aquatic animal health emergency is any situation where the health of the animal population is suddenly at risk. This may be due to significant pathogens such as IHN virus or sudden, severe decreases in dissolved oxygen levels. Vigilant monitoring and early detection are key to good management of emergencies.

2.9.1. First Steps

If there is a system failure, all efforts will be directed to restoring sufficient water quality for the aquatic animals. Sufficient oxygen levels must be restored. The site will immediately activate the Contingency Plan (section 2.4.1).

If a serious infectious disease problem is suspected, the Attending Veterinarian and/or Animal Health Management will be immediately notified. If the problem is not easily discerned, diagnosis and management needs to be done hand in hand.

2.9.2. Infectious Disease Emergencies

An outbreak is defined as an unexpected occurrence of mortality or disease. Not all outbreaks are health emergencies. Diseases may differ in how infectious they are and therefore how easy or difficult they are to control. Rapid response is essential, but will be determined on a case-by-case basis in conjunction with the Attending Veterinarian and/or Animal Health Management. Once an emergency has been recognized, certain steps will be followed. The objective is to keep the pathogen load as low as possible and to prevent spread of the problem within or off the site.

2.9.2.1. *Isolation/Quarantine*

At the Veterinarian's recommendation, the site may be officially isolated/quarantined. Isolation/Quarantine remains in effect until such time when the problem has been diagnosed and/or managed.

2.9.2.2. *Cease Livestock Movement and/or Handling*

The movement of all livestock on/off and within the site should cease. Aquatic animals will not be further handled. No visitors or non-essential staff will be allowed on site unless previously authorized by Management.

2.9.2.3. *Disinfection and Hygiene*

Hygiene and disinfection on site, including procedure for personnel and equipment will be strictly enforced.

2.9.2.4. *Suppliers*

Suppliers and vendors (e.g., feed or oxygen delivery) will be instructed to visit the site last or to make special arrangements.

2.9.2.5. *Mortality Collection*

The frequency of mortality collection will be increased. Affected tanks will be accessed last and staff will adhere to disinfection procedures between tanks and rearing units. Where possible, separate gear will be designated for the affected unit. All equipment, surfaces, and clothing that come in contact with infected animals or infected material will be thoroughly disinfected after use. Mortality collection and disposal procedures will be strictly adhered to, and provisions made for increased mortality pick-ups and disposal.

2.9.2.6. *Outbreak Investigations*

The Attending Veterinarian may require records and appropriate sampling to determine the cause of an outbreak and the best course of action in response. The Attending Veterinarian and/or Animal Health Management will give instructions regarding material sampling. Water and feed samples may be requested. Avoiding contamination and tampering, samples will be properly handled, properly stored, and promptly shipped as per the Attending Veterinarian's or Animal Health Management's instructions.

Continued monitoring will be required after the initial workup to determine the course of the outbreak and to assess whether treatment and/or

management measure are being effective. Frequent observations of the fish are essential. Feeding response and water quality should be monitored. All treatments and management changes should be noted as they occur. The Attending Veterinarian, Animal Health Management and site management will work together to review health records and make further management decisions. Any repeat sampling—including the results—should be duly noted.

2.10. Aquatic Animal Escape

In the unlikely event that aquatic animals escape into nearby streams or watersheds, the operator needs to inform the ART Facility Manager and AVS. As part of the Response Plan, health records—including relevant diagnoses and treatments—will be made available to the appropriate regulatory authorities as required. Please contact:

Department	Contact Number
JCUS ART Facility Manager	6709 3751
AVS Animal Response Centre (24-hours)	1800 476 1600

2.11. Handling Drugs and Chemicals

The goal of good aquatic animal health management is to have healthy and productive animals. However, if the animals do become sick, they may require treatment with drugs. As per section 1.7.1, the Attending Veterinarian retains a veterinarian-client-patient relationship with the operator and that is the basis for disease diagnoses and prescribing treatments.

2.11.1. Medicated Feed Storage and Inventory

Medicated feed will be stored in clearly marked bags separately from non-medicated feed. The storage area should be clean, dry, and free of predators. The label on the medicated feedbag states details about the feed, medication included, feed rate, name of the veterinarian, prescription number, and date it was milled.

Medicated feed will be inventoried separately from regular feed. Daily inventory records will be kept as the feed is fed to the animals according to prescription.

In the event that there is excess medicated feed after completion of the treatment, the Veterinarian will be contacted to determine proper handling

and disposal.

2.11.2. Handling and Administering Medicated Feed

Medication mixed into feed has a Material Safety Data Sheet (MSDS), which specifies handling and safety precautions. An MSDS for all medications used on site must be in a readily accessible binder. As per policy, all chemicals must be handled safely by trained staff e.g., by wearing appropriate protective gear and taking suitable precautions.

Medicated feed will be fed out in accordance with the Veterinarian's instructions. The appropriate tank(s) must receive the prescribed amount medicated feed for the duration of treatment.

2.11.3. Treatment Records

Provincial regulations require that treatment records for drugs include:

- Aquaculture license number and name of holder
- Location of facility
- Species of aquatic animal
- Name of the prescribing Veterinarian
- A log naming the drugs, including:
    - Name of the drug
    - Method of administration
    - Treatment schedule, including the commencement date
    - Date of last treatment
    - Name and signature of the person responsible for administering each treatment

Detailed records of medicated feed administration will be kept for the entire duration of treatment. Medicated feed records will be kept for the entire time the animals are on site.

A copy of the treatment records will accompany those animals to another site, if they are subsequently moved.

2.11.4. Chemicals and Biologicals

2.11.4.1. Disinfectants

Disinfectants will be stored in clearly marked containers. An MSDS for each disinfectant that is on site will be kept in a safe, readily accessible place, e.g., binder in the site office. As per policy, all chemicals must be handled safely by

trained staff e.g., by wearing appropriate protective gear and taking suitable precautions.

2.11.4.2. Chemicals

Chemicals include, but are not limited to, fixatives such as formalin or Davidson's solution used for preserving tissues. These chemicals will be stored in clearly marked containers. An MSDS for each chemical that is on site will be kept in a safe, readily accessible place, e.g. binder in the site office. As per policy, all chemicals must be handled safely by trained staff e.g., by wearing appropriate protective gear and taking suitable precautions.

2.11.4.3. Biologicals

Biologicals include vaccines. Where applicable, these products will be kept refrigerated and handled as per manufacturer's instructions. A product insert for each vaccine that is on site will be kept in a safe, readily accessible place. Trained staff must handle all biologicals safely e.g., by wearing appropriate protective gear and taking suitable precautions.

3.1. Disposal of Animal Carcasses and Related Wastes

3.1.1. Appropriate provision must be made for prompt and sanitary disposal of animal carcasses and waste material in accordance with current laws and any other guidelines or requirements of the National Biosafety Committee, the Ministry of Health and the National Environmental Agency.

3.1.2. All aquatic animal carcasses from approved IACUC projects should be removed immediately from the experiment and quarantine tanks within 24 hours and placed into biohazard bins. Waste containers should be readily accessible with tight fitting lids.

3.1.3. If waste must be stored while awaiting disposal, the storage area should be outside the animal holding and clean equipment areas. Animal carcasses and tissues should be disposed of within 24 hours.

3.1.4. Hazardous waste, including carcasses of animals exposed to radioactive or bio-hazardous agents, must be adequately sterilized and/or contained prior to removal and disposal. After adequate sterilization, pathogen-contaminated animal carcasses should be removed and disposed in the same way as the nonhazardous animal carcasses. In particular, animal carcasses contaminated with toxic chemicals and radioactive materials should be disposed following the guidelines of disposal of toxic chemicals and radioactive materials of the Ministry of the Environment and any relevant laws.

3.1.5. The biohazard disposal bags would then be send to Keppel Lab Central Services to be dispose with the authorized vendor for collection.

3.1.6. Monitoring of dead-stock (DS) is to be fill in the **FORM H**.

3.2. Waste water management

3.2.1. All trade effluent to be discharged into the public sewerage system must be done so with the written consent of the Public Utilities Board. The requirements for trade effluent discharge are spelt out in the Sewerage and Drainage Act, Chapter 294 and the Sewerage and Drainage (Trade Effluent) Regulations.

3.2.2. Water Quality Standards For Trade Effluent Discharge Into Public Sewer: The physical and chemical characteristics of the trade effluent to be discharged into the public sewer shall not exceed the following limits:

(a) The temperature of the trade effluent shall not exceed 45°C at the point of its entry into any public sewer.

(b) The pH value of the trade effluent shall not be less than 6 nor more than 9 at the point of its entry into any public sewer.

(c) The caustic alkalinity of the trade effluent shall not be more than 2,000 milligrams of calcium carbonate per liter at the point of its entry into any public sewer. One degree KH is equal to 17.848 mg/l (ppm), therefore not exceeding 112 degree KH.

(d) Maximum concentrations of certain substances in trade effluent:

List of Substances	**Max. concentration** in mg/L of trade effluent
5 Day Biochemical Oxygen Demand (BOD) at 20°C	400
Chemical Oxygen Demand	600
Total Suspended Solids	400
Total Dissolved Solids	3000

3.2.3. Waste water released from the experimental tanks and circular tanks require consistent monitoring and treatment before it can be released to the drainage system. Guidelines table for different waste water treatment is illustrated below:

Type of wastewater	**Treatment recommendation**
Facility tank with or without livestock (freshwater)	Send wastewater to treatment tank for 24 hours and perform dilution 1:1 before release to the drainage system.
Facility tank with or without livestock inclusive of introduced chemicals and biological agents (freshwater)	Isolate the waste water from the tank into a carboy and send to Keppel Lab Central Services to be dispose with the authorized vendor for collection.
Facility tank with or without livestock (seawater)	Send wastewater to treatment tank for 24 hours and perform dilution 1:1 before release to the drainage system.
Facility tank with or without livestock inclusive of introduced chemicals and biological agents (seawater)	Isolate the waste water from the tank into a carboy and send to Keppel Lab Central Services to be dispose with the authorized vendor for collection.

3.2.4. Periodic water tests must be done to wastewater before discharge to the main drainage area. This includes:

(a) The temperature

(b) The pH value

(c) The hardness of the water

(d) TDS / TSS (Total Dissolved / Suspended Solids)

(e) BOD (Biological Oxygen Demand)

(f) COD (Chemical Oxygen Demand)

3.3. Wastewater Treatment and Collection

3.3.1. Wastewater released from the tanks need to be held in the treatment tank at least 24 hours for carbon absorption. Afterwards, the wastewater must then be diluted with freshwater in a ratio of 1:1.

3.3.2. Wastewater will be collected periodically by an engaged vendor to perform the water tests as enumerated in section 3.2.4.

4. Procurement and Transport of Aquatic Animals

4.1. Aquatic Animals from a Local Source

4.1.1. All animals obtained locally must be from a licensed or otherwise legally permitted source.

4.1.2. Under the Wild Animals & Birds Act, all wild animals are protected by law except those listed in the Schedule.

4.2. Aquatic Animals from an Overseas Source

4.2.1. No animals are to be imported without a permit from the AVS as required under the Animals & Birds Act.

4.2.2. The source of the animal must be recognized by the exporting country as a legitimate supplier of the particular species of animal.

4.2.3. The transport, import, and use of genetically modified (GM) animals shall be in accordance with the Singapore Biosafety Guidelines for Research on GMOs as set out by the Genetic Modification Advisory Committee.

4.3. Particular Considerations in Procurement of Endangered Animals

4.3.1. No endangered animals are to be imported without proper CITES certificates and export permits from the exporting country and import permits from the AVA, as required under the Endangered Species (Import and Export) Act.

4.3.2. An endangered animal which is included in CITES Appendix II[8] must not be used in projects unless the project concerned will be of direct benefit to the conservation of that species or a closely related species and will not further endanger the species.

4.4. Particular Considerations in Procurement of Wildlife

4.4.1. Aquatic animals are to be taken from natural habitats only if alternatives bred in captivity are not available or are unsuitable for the specific scientific activity.

[8] http://www.cites.org/eng/app/appendices.php

4.4.2. Capture and restraint is stressful to most animals. Strategies must be employed to minimize distress during capture and disruption of the colonies from which they are taken. There must be careful choice of suitable capture techniques, skilled persons must be used, and appropriate and safe enclosures or caging must be utilized. Animals must be monitored for signs of distress following capture and appropriate measures taken to minimize the stress. Any animals suffering from capture-induced trauma should receive treatment without delay.

4.5. Transport of Aquatic Animals

4.5.1. In all cases of aquatic animals transport in open systems, it should be borne in mind that even a short-time transport of 10–30 m in open plastic or metal tanks should be done under the conditions of constant air or oxygen supply. This is very important to the welfare of aquatic animals even if the dissolved oxygen content in the water seems to be satisfactorily high in the tank to begin with. Transport longer than half an hour should be in completely filled and closed tanks to prevent splashing and injuries to individuals bumping into each other.

4.5.2. The weight of aquatic animals that can be safely transported in a tank depends on the efficiency of the aeration system, duration of the transport, water temperature, size, and species.

4.5.3. Please take note not to feed the aquatic animals at least 1 day before transportation to minimize waste production.

4.5.4. Examples: Weight (in kg) of channel catfish that can be transported per liter of 18°C water (Piper *et al.*, 1982)[9]

Number of fish (per kg)	**8h transit**	**12h transit**	**16h transit**
2	0.75	0.66	0.57
4	0.71	0.57	0.41
9	0.60	0.49	0.35
110	0.41	0.30	0.24
276	0.35	0.26	0.21
552	0.26	0.21	0.18
1100	0.21	0.20	0.15
2200	0.15	0.12	0.08
22000	0.02	0.02	0.02

[9] Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraven, L. G. Fowler, and J. R. Leonard. 1982. Fish Hatchery Management. U.S. Fish and Wildlife Service, Washington, D.C.

4.5.5. Transport Tank

(a) For transportation of live fish out of the facility, it is recommended that the covered transport tank as shown below be used. The transport tank is aerated with a portable pump, and could be fitted into a car if required.

Figure 7. Example of vessel for fish transport.

(b) **FORM K** should be accomplished to record the handing over of live fishes to another party.

Appendix 1.

Appendix 2.

Appendix 3. Applicable local legislation and regulations

ANIMALS AND BIRDS ACT (CHAPTER 7, SECTION 80), ANIMALS AND BIRDS (CARE AND USE OF ANIMALS FOR SCIENTIFIC PURPOSES) RULES

ANIMALS AND BIRDS ACT (CHAPTER 7, DEFINITION OF "DISEASE"IN SECTION 2), ANIMALS AND BIRDS (DISEASE) NOTIFICATION

Annex A. Facility Map

Annex A.2. Equipment in the Facility (Maintenance Schedules)