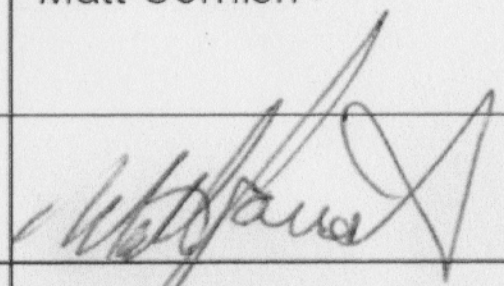
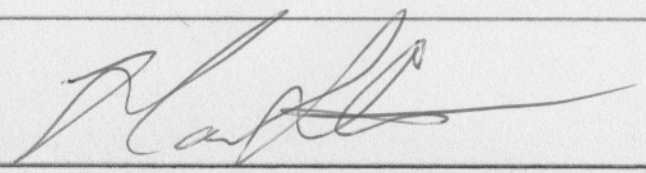
UOG Animal Care Guidelines
SOP: HAGEN AQUALAB
Submitted by: Matt Cornish (facility manager)
Last date revised: April 2021
Date approved by ACC:

Signatures:

	Facility Manager	Facility Veterinarian or Director, ACS
Name (please print)	Matt Cornish	Marcus Litman
Signature		
Date	13 May 2021	May 31/21

HAGEN AQUALAB FACILTY STANDARD OPERATING PROCEDURES

Revised by Matt Cornish April 2021

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First time users of Aqualab SOP-1

The purpose of this SOP is to introduce new users to the essential procedures and regulations for holding vertebrate animals and using Aqualab's facilities.

There are a number of procedures and regulations that first time users of Aqualab need to be aware of prior to, and during, their use of Aqualab's facilities, these include; Acquisition of a valid Animal Utilization

Protocol (AUP), use of proper operating procedures, acquisition of and use of keycards, record keeping and training to name a few.

A. Animal Utilization Protocol: Each Aqualab user utilizing a vertebrate as a model organism is required to obtain an Animal Utilization Protocol (AUP) from Animal Care Services (ACS). Vertebrate animals brought into Aqualab need to be listed on a valid AUP. Excellent information regarding many aspects of the Animal Utilization Protocol may be found at https://www.uoguelph.ca/research/for-researchers/ethics-and-regulatory-compliance/animals/animal-utilization-protocolswriting There will be 6-8 weeks between submission of the AUP and approval, so be sure to submit your AUP as far in advance of the project as possible if the project is time sensitive.

B. Aqualab Forms**:** There are a number of forms that Aqualab requires be filled out prior to and during a project. All of the following forms are included at the end of this document, are downloadable from the Aqualab website forms page, or can be obtained in hard copy format from the forms available outside the Aqualab offices.

i) **The Transfer and Acquisition Form:** This form must be completed and submitted to the Aqualab Manager when animals are either brought into Aqualab or are transferred from one protocol to another. This is for internal animal tracking purposes so that accurate numbers can be recorded for each protocol's animal usage. This form is included at the end of this document, is downloadable from the Aqualab website forms page, or can be obtained in hard copy format from the forms available outside the Aqualab offices.

ii) **The Daily Observation Sheet:** It is a legislative requirement (Ontario's Animals for Research Act) that all animals utilized in research and teaching be observed for well-being, **at least** once per day - this includes weekends, holidays and inclement weather days. This form provides space to record basic animal care (checking on animal and system, feeding, cleaning, etc.). A copy of these records, dating back 12 months (for long-term users) must be kept somewhere accessible in the room, or on the tank, with the animals.

iii) **Utilization & Mortality Sheet:** When animals are terminally sampled or die in captivity, they need to be recorded and disposed of properly (disposal of animals will be dealt with in another section). This form is a way for you to track these events and is another requirement of the Animals for Research Act. A copy of these records, dating back 12 months (for long-term users) must be kept somewhere accessible in the room, or on the tank, with the animals. This form is included at the end of this document, is downloadable from the Aqualab website forms page, or can be obtained in hard copy format from the forms available outside the Aqualab offices.

Users are not allowed to make any significant modifications to the rooms they are using. All significant room upgrades or modifications are performed by Aqualab Staff (Coordinator and Asst. Coordinator). This process can be communicated verbally with staff, but it is very helpful for convenience to fill out what is known as a Work Order Form.

iv) **Work Order Form:** If you have work that needs to be done for your project, please complete a work order form with detailed instructions so that we can most efficiently help you prepare. This form is also required for any consumable items that you would like to purchase from Aqualab, i.e. disinfectant, bleach, acid delimer, brine shrimp eggs, nets, salt etc.

v) **Trust Fund Authorization Form:** For first time faculty users, we will need a Trust Fund Authorization Form filled out. This details to which grant you would like room utilization charges and consumable charges applied, and once signed, also serves as an authorization for Aqualab to recover charges up to a predetermined amount.

C. Key Cards**:** Access to the Aqualab requires 2 cards to gain entry. A valid University of Guelph ID card, opens the front door, and an Aqualab issued keycard will open interior building doors. Aqualab keycards require a $20 deposit (refundable upon return of the card). Please see Mike Davies (Asst. Coordinator) to have a card issued in your name. This card will provide you with access to the area that you will be using as well as the Drylab. The cards issued by Aqualab are proximity cards and can be read from within your backpack or wallet without removal. The front door is on a timer and will be open between the hours of 8:30 and 12:00 and 1:00 and 4:30. The front door will be locked on weekends and holidays. If you should arrive at the building on a weekend or holiday to find the front door unlocked we would appreciate you contacting either **Matt (519-831-1321)** or **Mike (519-831-1671)** immediately, this is not the normal condition for this door lock. The main facility door is locked at all times only users with a valid access card will be allowed in. You are responsible for the card we issue to you so please do not loan the card to anyone else. You will be responsible for any problems that occur by someone else's unauthorized use of your card.

D. Animal Care**:** Users are responsible for the maintenance of their animals. Standard operating procedures for different species and tank cleaning can be found online at the Aqualab website.

i) **Training:** New users need to complete the ACS core modules prior to using animals. Users must be trained in all of the relevant procedures listed on the AUP for their project. Training records (mentor facilitated training) need to be kept and need to be accessible. Contact ACC@ugoguelph.ca if you have questions about training sessions or your status.

ii) **Daily Checking:** All users are required by Animal Care practices to check their

animals on a daily basis and maintain records to that effect (see record keeping). This daily checking includes weekends and holidays. If you are not able to meet this requirement you need to make arrangements for someone in your lab of group.

iii) **Feeding:** You can store your food in the walk-in freezer. This is common space. If you spill food on the floor, **CLEAN IT UP.** If you spill food in the corridor, **CLEAN IT UP.** If you spill food anywhere, **CLEAN IT UP.** If you are unsure on how to feed your animals please contact either Aqualab staff for advice or your supervisor for training. Each species that we have SOP's for has a section on feeding. If you can't find the SOP, please contact Matt or Mike.

iv) **Cleaning:** If you are using a tank that is on recirculation flow, there are special procedures for cleaning. Please read the SOP that is provided with the room you are using. If you can't find the Tank Cleaning SOP check the Aqualab website or contact Matt or Mike regarding proper procedures for cleaning your tank/s. If you are not sure what to do, **do not start without proper training**. Recirculation systems are affected by all of the tanks on the system, unlike flow-through or static systems were only the tank being cleaned is affected.

v) **Record keeping:** Mortality, animal utilization (procedures, sampling, disposition) and daily observation records **must be kept up to date,** and stored in the SOP binder in the room (or on the tank) you are using. Spare sheets can be found in wall bins outside the office or by download from the Aqualab website.

vi) **Disposal of dead animals:** After the mortality sheet has been filled out the animal needs to be disposed of. Fish die, if no disease is suspected the animal may be placed in the dead stock freezer located outside of the walk-in freezer near the main facility doors. If disease is suspected contact either Matt or Mike for advice on how to proceed.

Transfer of disease can become a problem if the general rules are not followed. If one or more of your organisms die, place only the animal(s) in the dead stock freezer - n**o paper towels or bags**. When transporting your animals to dead stock freezer**,** please **USE A BUCKET**. **DO NOT** carry dead animals with the net you used to take them out of the tank. Each room has its own nets. These nets are not to be transferred from room to room. Nets should be kept submerged in disinfectant between use.

vii) **Water Chemistry:** As the user of the system that your animals are in, it is your responsibility to make sure that the water quality is adequate for your animals. If you are going to conduct water quality testing, consultation with Aqualab staff is recommended. We have available a Hach DR2800, Hach chemicals, Oxygen meters, refractometers and pH meters to facilitate testing. This equipment is owned and operated by Aqualab. We require that you receive training in the use of this equipment prior to use. All chemicals used need to be recorded and submitted on work order form in the materials used section with a trust fund number

so that we can bill appropriately.

E. Standard Operating Procedures (SOP's)**:** There are a number of SOP's available online that are downloadable from the Aqualab website as PDF files. It is your responsibility to familiarize yourself with the operation of your research space. Relevant SOP's for facility usage are **Aqualab Facility SOP** or **ECARS Operating Instructions**. The **General Animal Holding Standard Operating Procedure** gives more detailed information pertaining to general practices in Aqualab. If there is no relevant documentation to be found on the Aqualab website you may have to write your own SOP, please contact the Aqualab Coordinator with help on this process. These documents are a good reference to help reinforce training received. They can be found at: https://www.uoguelph.ca/ib/infrastructure_and_facilities/aqualab/forms.

F. Walk-in Freezer**:** Immediately to the right after entering the facility door is a walk-in freezer. Please only store excess food in this freezer – all dead animals/tissues need to be stored away from food and should be put in the dead stock freezer. The walk-in freezer is a communal space so please clean up any food you may spill. There is a temperature alarm that is tied to the campus police which will activate if the door is left open too long and the temperature rises. Please be conscious of this when entering the room. It is not wise to close the door after yourself, as the emergency release has been known to stick on occasion. If you need to spend extended periods with the freezer door open, please contact either Aqualab staff or Campus Community Police at 52245 to notify them of the situation.

G. Facility Alarms**:** Much of Aqualab is computer controlled and on occasion alarm situations will arise that cause an audible alarm to sound within the animal holding portion of the building. During non-work hours either Matt or Mike are on-call and will respond to these alarms. There is no way for users to disable these alarms. There is another alarm that will also sound after hours. If a exterior door is left ajar, between 4:30 PM and 8:30 AM, an alarm in the front hall will sound until the door is closed. If alarms are ringing and you feel the need to call someone please contact Matt (519) 831 1321 or Mike (519)831-1671.

H. Maintenance and other problems arising from Aqualab usage**:** You are the person who will be in and out of the room you are using more frequently than any of the staff of AQUALAB. Please be aware of the normal operation of your room. If you notice or suspect any problems developing within your room, **do not hesitate to bring it to the attention of either Matt or Mike**. Small problems tend to escalate into large problems if left untended. Damage to equipment or mortalities can occur. If you have proposed upgrades to holding systems, new holding systems or problems that need to be fixed, please fill out the appropriate section (Work Requested) on a work order.

Daily Observation Sheet

Principal Investigator:

AUP # and species:

Year:

Month:	Time	Initials	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
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15			
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18			

Animal Acquisition / Transfer Form

Form to be filled out by Researcher receiving animals ONLY

Primary Investigator:	
Your Name:	
AUP #:	
Date of Arrival:	
Room number where animals will be housed:	
Species Name (Common & Scientific):	
Number of Animals:	
Supplier or Researchers Name	
Originating AUP #: Complete for transfers only	

Work and Materials Request Form

Thank you for taking the time to fill out the following information.

Requested by:	**Aqualab Room #:**
Department:	**Your Phone #:**
Date Submitted:	**Date Required:**

Work Requested:

Materials Requested: (eg Bleach, Acid Delimer, Disinfectant, Nets)

Materials Used:

Office use only

Cost:_____

Work completed by:	Date Completed:

Charge to:	Trust Fund Number:

COLLEGE OF BIOLOGICAL SCIENCE
DEPARTMENT OF INTEGRATIVE BIOLOGY
HAGEN AQUALAB

This document provides authorization to invoice your grant with costs associated with room utilization and cost recovery for consumables and work orders from the Hagen Aqualab.

Name: _____

Signature: _____

Date: _____

Grant to be used for room utilization charges: _____

Grant to be used for work orders and consumables: _____

The above signature authorizes the Hagen Aqualab Coordinator to recover quarterly room utilization and consumable charges up to, but not exceeding:

$_____

$_____

Any quarterly bill that exceeds the allocated limit for room utilization **or** consumable cost recovery, will require an additional signature from the Faculty member named on the grant as authorization.

General Animal Holding SOP-2

Aqualab Staff:

Matt Cornish	**Coordinator**	**Room 140 Aqualab**	**Ext 52714**
Mike Davies	**Assistant Coordinator**	**Room 138 Aqualab**	**Ext 52714**

PRIOR TO ARRIVAL

- Consultation with the Aqualab Coordinator is required prior to bringing any animals into Aqualab.
- An approved **Animal Utilization Protocol** is required prior to ordering or collecting any animals.
- All animals entering Aqualab must be covered under an approved **Animal Utilization Protocol**
- Aqualab and the Aqualab Coordinator must be listed on each AUP that is active in the building.
- An **Animal Acquisition/Transfer Form** (containing information on animal numbers, supplier etc.) should be submitted to the Aqualab Coordinator **24 hours prior** to animal arrival.

PURCHASING ANIMALS FOR AUP'S

Animals purchased from local distributors must be authorized by the local Animal Care Committee.

- A purchase requisition must be submitted to the departmental purchasing clerk who will forward a copy to the Animal Care Committee.
- Once approval has been secured, the Animal Care Committee will notify the purchasing clerk to move forward with the transaction.
- This process takes some time to accomplish so it must be started well in advance of the need for animals.
- This is an **Animal Care** requirement.

Each researcher is responsible for tracking their own animal usage. By use of the Animal Acquisition/Transfer forms, Aqualab tracks animals coming into and being used within Aqualab. This information is available to researchers as a cross reference to check their records.

UPON ARRIVAL

Animals arriving in Aqualab are to be moved directly into the receiving lab as soon as possible and placed in appropriate housing (tanks, cages, aquaria, etc).

Fish

Fish arriving in live wells/tanks are to be moved directly into the receiving lab, through the overhead door in room 174. There is no loading dock dedicated to animal usage in Aqualab. Fish may be moved to the room in Aqualab's clean transfer bin or in sterilized garbage cans on wheeled carts. **Any water spilled in the halls or room 174 must be cleaned up by the user**.

- Fish may also arrive in a variety of other containers.
- Fish arriving in shipping bags should be placed into a separate tank while still in the shipping bag to facilitate temperature equilibration; if necessary an air stone can be added to the bag to ensure adequate aeration. Fish may be released once the temperature in the shipping bag and the tank reach equilibrium.
- Fish may also be transported to the facility in coolers or splash tanks; the water in these containers should be supplied with supplemental aeration or O_2 during transport. Fish arriving this way may be placed directly into holding tanks.
- Fish arriving from the field need to receive prophylactic treatment for external parasites (see SOP prophylactic treatment of fishes).
- Eggs fertilized in the field or in the lab, or "eyed" eggs, are transported in plastic jars and must be disinfected prior to being placed into the vertical incubators (See Aqualab SOP for Iodophor Disinfection of Salmonid Eggs).

DAILY ANIMAL CARE

ALL ANIMALS HELD IN AQUALAB ARE UNDER THE DIRECT RESPONSIBILITY OF THE PRIMARY RESEARCHER (P.I.) AND MUST BE MONITORED BY THE USER, OR A DESIGNATED PERSON, 7 DAYS-A-WEEK.

Weekend and weekday animal care are similar. An individual in each lab must be identified to be on call to deal with extraordinary problems which might occur overnight or on weekends. Procedures for contacting the person(s) responsible must be posted on the door to the animal holding room.

BIOFILTRATION:

Aqualab is a recirculation facility and as such these systems are provided with biofilters to reduce nitrogenous wastes to less toxic forms. New biofilters need time to grow bacterial cultures. *Nitrosomonas* sp. grows first, converting ammonia to nitrite. There is a lag time before *Nitrobacter* sp. starts to grow. It is during the time that *Nitrobacter* sp. is becoming established that elevated levels of nitrite could become dangerous to fish. *Nitrobacter* sp. converts nitrite to nitrate a much less toxic form of organic nitrogen.

Biofiltration in Aqualab is achieved by growing the aforementioned denitrifying bacteria in one of two ways. First, in recirculation rooms, the bacteria are grown and cultured in the gravel bed filters, which also serve as mechanical filtration. In the ECARS systems, the denitrifying bacteria is grown in bubble bead filters.

WATER QUALITY:

Every room (fresh water or marine) that has its own recirculation system must have its water quality monitored regularly. It is the responsibility of the research team members of each recirculation system to ensure that these tests are being performed and the results recorded (a copy of these results should be stored and made available to those in the room).

When any room or system is first put into use, water quality testing should be carried out on a regular basis, until a stable state is reached. Further testing will become necessary as the rooms biomass increases (growth or greater numbers of animals) or density within tanks increases. Testing should be done at approximately the same time of the day, as diurnal fluctuations do occur in the production of nitrogenous wastes and the utilization of oxygen.

In a freshwater room with a functioning biofilter and adequate water replacement, **ammonia and nitrite** should be **near zero**, with **nitrate** levels **< 10 mg/L**. **Oxygen** should be **> 7 mg/L** with 9-11 mg/L as optimal. **pH** should be between **8 and 9**. In a marine room **salinity** should be about **33 ‰**. Other tests that could be preformed are copper, hardness, alkalinity, total suspended solids and phosphate.

Tests include dissolved oxygen, pH, salinity, ammonia, nitrite, nitrate. The first three tests can be carried out using hand held metres. These metres can be located either in the lab or the Aqualab Office. Aqualab has a Hach DR2800 portable spectrophotometer for the analysis of nitrogenous wastes (ammonia, nitrite and nitrate). Procedures for the use of this instrument (Aqualab Water Chemistry SOP 2009) may be found in the Dry Lab (room 166).

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MANAGEMENT PRACTICES

Sanitation:

IT IS THE RESPONSIBILITY OF THE RESEARCHER TO MAINTAIN CLEANLINESS IN THEIR ROOMS, TANKS, CAGES OR AQUARIA.

Disinfectants or detergents are not used in the routine cleaning of tanks. Tanks are scrubbed with brooms, brushes or abrasive pads and clean water to remove accumulations of algae, faeces, uneaten feed, bacteria and light build-ups of calcium.

ALL TANKS MUST BE INSPECTED DAILY TO ENSURE ADEQUATE WATER QUALITY AND PROPER WASTE REMOVAL BETWEEN THE STAND PIPES.

All large tanks should be cleaned, at least monthly, and more frequently if required. Partially drain the tank and scrub the sides, bottoms, stand pipes and wells. Tanks are then rinsed and refilled.

The two foot diameter tanks should be cleaned bi-weekly. The fish should be removed completely from the tank and the screen at the bottom should be removed and the lower cone cleaned. For more detailed explanation please see Aqualab's tank cleaning SOP.

Note: It is important to ensure that disinfectant and rinse water are not mixed with system water. Contamination of system water may result in fish death as well as biofilter death. Valves are placed in the drainage trench to allow water from the tanks to be diverted from the system directly to the sanitary sewers. Please ensure that the valves are positioned properly. **If you are unsure how they must be positioned or the valve is stuck please ask for assistance from the Aqualab staff**.

Walls and floors of each room should be rinsed weekly and washed with a disinfectant (A33™ at a concentration of 12 mL/L) monthly. Mildew on the bottoms of the walls or outsides of tanks may be removed with sodium hypochlorite (bleach) or A33™ at a concentration of 12 mL/L whenever build-ups become noticeable.

Footbaths are provided in aquatic rooms. They must be maintained by cleaning when necessary and replacing the germicidal solution that can be found under the sink in the anteroom (A33 Dry: dissolve two packets in the clean water of the footbath). Footbaths have a volume of approximately 5L. If no A33 Dry can be found, the liquid version is suitable as well.

Net care: Nets should be stored dry between uses. Nets can be dipped in a solution of Westcodyne™ (at manufacturers recommended concentration) or A33 for at least **10 minutes** to be effectively cleaned, rinsed and hung to dry after use.

Net disinfectant solutions need to be changed every 10 days – this is the responsibility of research team members.

Completion of an experiment:

At the completion of each experimental use of a room, tank, cage or aquaria, it must be fully disinfected. All tanks, air stones, air hoses, stand pipes, nets and water hoses must be scrubbed and disinfected with a quaternary ammonia disinfectant at the manufacturers recommended concentration; A33™ at a dilution of 12 mL/L. Tanks may also be disinfected by acid washing with a 50-100% solution of an acid (eg Servac™, Muriatic acid, Lime-A-Way™, CLR™, Airkem Brawn™. The system must be thoroughly rinsed afterward to ensure removal of residual disinfectant or acid.

Animal and Project Information:

Sheets identifying species, AUP №, source, number and age of animals, primary and associate researchers, and emergency contact person and number are posted on every door of research rooms and in the case of multi-user rooms (General Holding or the ECARS room) white boards and tank specific information sheets have been provided.

Records must be kept up to date for: species; supplier; animal numbers; arrival date; procedures performed; disposition; & tank mortalities.

i) Daily Observation Sheets: these sheets must be filled out every day and are used for keeping track of husbandry procedures (ex. Tank cleaning, draining water) and feeding. Records of animal utilization (sampling) should be tracked, as well as any animals that are sick, under observation/treatment, where presumptive problem, treatment and eventual outcome must be recorded.

ii) Mortality Records – should be used to track all mortalities. These sheets also provide extra space (in addition to the Daily observation sheets) to record animal utilization (sampling) and any additional health observations or outcomes.

A copy of the **Daily Observation Sheet** and the **Mortality Record** can be found at the end of this document or on the wall outside the Aqualab office.

Each room has a binder that should contain copies of the:

- **Animal Utilization Protocol(s)**
- **Standard Operating Procedure(s)**
- **Mortality Record**
- **Daily Observation Sheet**
- **MSD sheets** for chemicals utilized in the room.
- **Assessment score sheets** if necessary.

Feeding:

THROWING FOOD AT FISH SHOULD BE AVOIDED AT ALL COSTS.

UNEATEN FOOD STAYS IN THE SYSTEM AND CAUSES A DETERIORATION OF WATER QUALITY. POOR WATER QUALITY CAUSES DISEASE.

All animals should be hand fed whenever possible. Animals should be fed to satiation. This is best accomplished by introducing small amounts of feed in short burst. This will allow for all of the food to be eaten, before any more is introduced. It should be noted that depending on the diet (growth or maintenance) mature animals do not need to be fed every day, but that growing juvenile animals need to be fed more frequently. Care should be taken to provide only as much food as is necessary, large quantities of uneaten food will cause the quality of living spaces (cages, aquaria, tanks) to deteriorate.

Veterinary Care**:**

All mortalities in the Aqualab must be reported to Aqualab Staff. Any animal that dies of unknown causes or is suspected of dying of a disease related problem must be bagged, tagged and taken immediately for a post mortem examination, the **results** of which **must be reported to Aqualab Staff**. It is of vital importance that PM's be done on animals that die of unknown causes in this facility. There are several users and an unknown infection has the potential to cause wide spread disease problems not only for the individual researcher but also to other users. Reports including diagnosis, numbers of mortalities, treatment and success or failure of treatment are required for all outbreaks of infection and disease.

Veterinary care is on a consultative basis only. Advice for the treatment of diseased fish may be sought from either

- **Dr. John Lumsden** (X54519) in the OVC Fish Pathology Lab
- **Dr. Marcus Litman (**X58856) the UofG Veterinary Director.
- Prescriptions for the treatment of disease can be received from Dr. Lumsden or Dr. Litman.

Unusually high mortalities must be reported to the Animal Care Committee (within 24 Hrs of each occurrence). This may be done by completion and submission of an **Animal Care Incident Form**. (Included)

Water samples should be taken from tanks in which animals die of unknown causes. In several instances testing for copper has revealed measurable amounts in water when copper normally should not be present.

Surface scrapes, from dead animals, may be conducted if external parasites are suspected and the mucous viewed under a microscope.

BIOSECURITY

Rooms 155 and 156 are restricted access rooms. Greater care is given to disease prevention in these rooms. Guidelines for the use of “isolation” rooms must be strictly followed (Included).

Rubber boots should be worn in all animal holding rooms.

Footbaths are available for all aquatic research rooms. To reduce the risk associated with introduction of disease and or transmission to and from other rooms please use rubber boots and the footbath while inside the holding room. Care should also be taken around the sump pit as this has direct connection to your tank water.

Prior to **entering and leaving** animal rooms, hands should be washed in a germicidal soap or alcohol based sanitizing solution. Extra care should be taken to wash hands before leaving the room after you have finished handling your animals.

UV sterilizers must be on and functional.

Transfer of fish between rooms should be kept to a minimum. Transfer only healthy, disease free fish. Consult with the Aqualab Coordinator prior to transferring fish between rooms.

In the event of disease outbreak, nets used for diseased fish must be isolated and disinfected separately. Tanks and all associated equipment must also be thoroughly cleaned and disinfected with a quartinary ammonia disinfectant at the manufacturers recommended concentration. **Care must be taken not to allow disinfectant into the system water as this will have a deleterious effect on the biofilters.**

Soak nets for a minimum of 10 min in disinfectant solution after each use, rinse with clean water and hang to dry. (See Net Care; page 3).

EXIT FROM AQUALAB

Live animals:

Fish are rarely transported from the laboratory; if so, only healthy, uncontaminated specimens are selected. These animals are transported in approved containers and maintained in clean, oxygenated water of appropriate pH (≈8.2) and temperature (≈10°C) for the duration of the trip.

Uncontaminated animals: Uncontaminated animals which have died naturally or have been euthanized (for euthanasia procedures see animal specific SOP) are to be placed in the dead stock containers in the freezer for disposal at OVC Post Mortem. **Only animal tissue may be placed in the dead stock containers.**

Contaminated animals: Specific arrangements must be made to dispose of contaminated carcasses (e.g. radioactive, viral infections, biohazardous). Radioactive carcasses are specially handled by the local Health and Safety Officer for dedicated disposal at an approved site (e.g. burial at Chalk River). All such procedures must be pre-approved by the Coordinator and any other regulatory agencies, prior to the start of the project.

Others: Animals may be preserved in formalin, Bouin's, etc. and stored in alcohol for later examination. These animals are stored at the researcher's discretion outside of the Aqualab.

EMERGENCIES

Aqualab has a suite of alarms that are active for each animal holding room. These alarms are monitored by the Argus Control System. After hour alarms are directed to Aqualab staff either at home or to a Cellular phone. Aqualab staff are on call 24 hours a day, 7 days a week, 365 days a year, except leap years when they are on call 366 days a year. If a problem arises contact either:

Matt Cornish	**X52714**	**Office**
	519 831-1321	**Cell Phone**
Mike Davies	**X 52714**	**Office**
	519 831-1671	**Cell Phone**

Animal Incident Report

<u>Important</u>: Form must be completed within 24 hours of knowing of the incident -

Email to ACS, acc@uoguelph.ca

Facility _____ AUP #

Reported by _____ Position

Time of Incident _____ D/M/Y of incident _____ D/M/Y reported

DESCRIPTION OF INCIDENT State exactly what was leading up to the incident, where the incident occurred etc.:

ANIMALS AFFECTED:

Total #	**Gender**	**Species**

MORBIDITY / MORTALITY #'s - Describe how the animals were affected

CAUSE OF SICKNESS OR DEATH (IF KNOWN)

ACTION PLAN:

Tests to be Performed:

By Whom:

Contributing Factors What conditions contributed to the incident:

Control Measures:

Recommendations for Corrective Measures:

Signature of Person Reporting Incident: _____ Date:

Mortality Record Sheet

Researcher: **Room #:**

Date	Species	Tank №	№ Dead	Disease Suspected Yes	Disease Suspected No	Disposition PM/Dead Stock

Principal Investigator:

AUP # and species:

Year:

Room/Tank #:

Month:	Time	Initials	Comments
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Hagen Aqualab Marine Collection Feeding SOP-3

Step 1: Collecting materials

Gather:

- Office fridge - bottle of Shellfish Diet 1800
- Walk-in freezer – Frozen fish and/or squid along with container, cichlid food container and trout pellet container. LPB frozen shellfish in the walk-in freezer in 50mL falcon tubes.
- Cupboard outside marine room (room 154) - cutting board, graduated cylinder, long forceps, knife, small yellow plate empty container and plastic pellet pestle.
- Room 139: using the Rubbermaid container from the cupboard outside of Rm 154, grab some dried kelp.
- Frozen fish can be found in the large cardboard box on the shelf in the back right hand side of the walk-in freezer on the second shelf. If contents are very hard and frozen drop box on floor to break up the frozen fish. The squid are located in a bucket on the floor in front of the shelf. The fish pellets are in labelled bags on the wire shelf.
- Bring all items into the marine room

Step 2: Feeding with Shellfish diet

- There are two types of Shellfish diet used to feed the marine collection. Shellfish diet 1800 and LPB Frozen Shellfish diet. Shellfish diet 1800 is not frozen and is kept in the office fridge in 1L bottles. It has smaller cells and is therefore preferable for feeding smaller life stages. LPB frozen shellfish diet has larger particles and must be kept frozen. It is kept frozen in the walk-in freezer in 50mL falcon tubes. It is the more commonly used form of algae due to its appropriate use with larger life stages. LPB must be thawed prior to use.
- Turn off the inflow to the tanks that need the shellfish diet. Measure the described volume into a graduated cylinder and then dilute (i.e. fill up the rest of the cylinder) with salt water. Invert several times to thoroughly mix and then evenly pour onto the surface water of each tank.
- Leave filter feeder inflows off for the rest of the time it takes you to complete feeding (~2 hours)
- When done feeding remember to **TURN INFLOW BACK ON!!!!**

N.B. Calculations of volumes to feed each tank vary depending on the type of animal and their density per tank. Consult with Matt or Mike to calculate 'per tank volumes' to be delivered at the beginning of every semester.

Animals that need shell fish diet

- Burrowing sea cucumbers
- Frilled Anemones
- Scallops
- Sea cucumbers
- Horse mussels & blue mussels
- Quahogs

Step 3: Feeding with herring chunks

- For animals listed in Table 1.2, it is necessary to present herring chunks with tweezers. Obtain an appropriately sized herring piece with tweezers and approach mouths of animals gently and slowly.
- **Preparation**:
  - Run herring under saltwater for ~5 minutes to thaw
  - When thawed enough to cut, start slicing. Remove from each piece all bone and internal organs so that all that is left to dice is fish meat (see figure 1).
- Slice thin (~.3cm), medium (~.5cm) and thick slices (~1cm) of herring
- Once sliced, dice each slice into chunks (see Figure 1 and table below)
- Feeding directions as follows:

Table 1.2 Size and Amount of Herring Chunks to be Fed to Each Individual of a Carnivorous Species.

Tank	Size of Fish Chunk	Amount to each	Thickness
Rock Crabs	Medium (~1cm)	To satiation	Medium
Toad crabs	Small (~.7cm)	To satiation	Medium
Hermit crabs	Small (~.7cm)	1 piece each	Medium
Green crabs	Small (~.7cm)	To satiation	Medium
Lobster	Large (~1cm)	To satiation	Thick
Burrowing anemones (on wet bench)	Very small (~.5cm)	1 piece each	Thin
Northern-Red Anemones (bottom and middle tray)	Very small-small (~.5cm)	1 piece each	Thin

N.B. These sizes are standard guidelines. Exact measurements are just to give you an idea of appropriate sizing. In most circumstances, feed according to the size of animal.

Step 4: Fish Feed Distribution

- Mummichogs, Sand dollars, Sand shrimp, Brittlestars and Rock gunnels are fed trout chow until satiation (this usually amounts to a small handful of feed).
- Cichlids are fed pellets of cichlid chow. Some pellets should be crushed with a mortar and pestle to provide appropriate sized pieces for the small fish. Dump contents in the side of the tank by the filter

Step 5: Feeding with Kelp

- Using the chef knife, cut seaweed into medium sized pieces. Place seaweed under rocks in any tank that has urchins. Remove any seaweed that was not previously eaten.
- Use 1 large leaf for approximately 10 urchins.
- For the two littorinid snail tanks, place a couple of pieces of seaweed in each tank and remove any uneaten seaweed from previous feeding. CLOSE THE LID!

Double check-did I forget to…?

- …turn the inflows back on for all tanks?
- …put the shellfish diet and trout chow away?
- …check to make sure all airstones are back in each tank and turned on?
- …place any deadstock in bins located in freezer beside walk-in freezer? Record in room binder the deaths of any animals (vert. or invert.)
- … take all fish feed and fish meat back to the freezer with tightly sealed lids?
- … put the shellfish diet back in the fridge?
- … clean all equipment used for feeding with fresh water?
- … place any nets used back into the disinfectant bin?

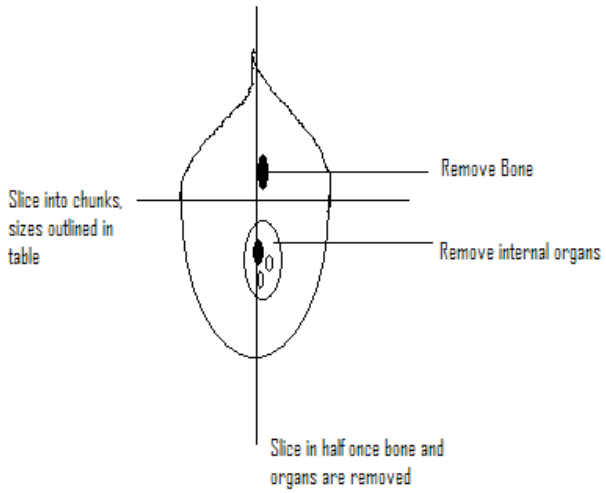


Figure 1. Figure illustrating appropriate sizes of herring slices and chunks

Stocking Density SOP 4

Recommended stocking densities depend on many factors including: tank size, flow rate, species, biomass per tank, etc. The following recommendations have been adapted from Section 4 of the Alma Aquaculture Research Station SOP, 2013.

A. Stocking Rates and Carrying Capacity

i) Carrying capacity:

a. **Definition**: the fish load a system or tank can support and is expressed as the weight of fish per volume (i.e. kilograms per cubic metre or pounds per cubic foot). Note that reference is also made to the weight of fish per unit inflow (i.e. kilograms per litre or gallon on influent water).

b. **Factors affecting Carrying Capacity**: water flow, tank volume, exchange rate, dissolved oxygen content, pH, size and species of fish, and accumulation of metabolic products and feed wastage.

c. **Exceeding Carrying Capacity:** slightly exceeding the carrying capacity can place *stress* on the fish which may result in reduced growth rates and chronic disease episodes. Greatly surpassing the carrying capacity can result in catastrophic mortalities due to hypoxia and/or bacterial disease outbreaks.

d. **Main components of carrying capacity:**

i. ***Flow Index*** The flow index refers to the relationship of fish weight and size (biomass) to *water inflow* and deals specifically with the amount of oxygen available for life support and growth.
ii. ***Density Index***. The density index refers to the relationship of fish weight and size (biomass) to *water volume* and indicates the special relationship of one fish to another.

There are clear differences in the effects of these two expressions. Even though **water flows** may be adequate to provide sufficient oxygen and to flush excessive wastes form the tank – overcrowding can cause behavioral and physiological problems.

This is clearly illustrated by comparing the carrying capacities of Rainbow trout and Arctic charr.

- Rainbow trout appear to be ***density*** *dependent* in that critically high densities result in clinical disease outbreaks (swim bladder stress syndrome, various gill diseases, Columnaris or peduncle diseases, etc.) even when water flows are sufficient to maintain dissolved oxygen levels and waste removal.
- Arctic charr, however, appear to be ***flow*** *dependent* in that they are able to tolerate higher densities than rainbow trout and do not present with clinical diseases unless the water quality becomes compromised.

B. Loading Density

a) **Definition**: loading density refers to the *density* of fish in a tank/rearing unit.

b) Optimal Loading Density is the density that allows for maximum growth rates. It is suggested in the literature that the optimal loading density for Arctic charr is 40-60 kg/m^3 - due to the gregarious social nature of this species. Growth rates can be negatively impacted if the fish are held at less than this density. Comparatively, the optimal loading density for Rainbow trout is significantly lower as social interactions between individual fishes introduce stressors (dominance hierarchies that can lead to intraspecific aggression) that result in the reduction of growth rates.

c) Maximum Loading Density is the density that should not be exceeded if normal growth rates are to be maintained. Once the maximum loading density is achieved, the density should be reduced by culling or increasing the holding volume. *As densities increase in excess of the suggested maximum loading densities, growth rates will decline progressively and, ultimately, clinical diseases will occur*. The maximum loading density will vary as it is dependent on the carrying capacity of the system.

C. Suggested Maximum Loading Densities for Rainbow Trout

Table 5. Maximum loading densities for rainbow trout (kg/m^3) at 8^N - 10^NC

Water Flow (turnovers/hour)	Fish Size (g)			
	1.5 - 5	10 - 60	90 -100	> 350
< 1	8	14	20	27
1 - 2	13	23	32	40
2 - 3	19	32	48	64
> 3	24	42	62	85

In most cases, carrying capacity and maximum loading densities are a function of oxygen availability. As such, it is imperative that the dissolved oxygen content of the water within the rearing system be known. For most salmonids, the rule of thumb is that the DO should never be less than ***5 mg/l.*** *Oxygen should be monitored continuously. Aqualab is in the process of installing optical dissolved oxygen sensors facility-wide, which will be connected to the ARGUS monitoring system, to provide continuous monitoring. If spot monitoring is done, the water should be tested at least once a day when the researcher anticipates the greatest oxygen demand*.

Marine Vertebrates SOP-5

A. HOUSING**:** Marine fish may be held in a variety of fibreglass tanks:

- 4' and 2' circular fibreglass tanks generally only used in the research rooms. The 2' tanks have cone shaped bottoms and require a grid. Perforated PVC or egg-crate may be used depending upon fish size. The 4' tanks are not insulated.
- 7' fibreglass trays (non-insulated)
- 7' square fibreglass tanks (non-insulated)
- Always consult with the Aqualab staff prior to use so that the correct size tank and stocking density can be achieved.

B. ENVIRONMENTAL CONDITIONS**:**

- Light: Photoperiod may be determined by the individual researcher (See Room specific SOP).
- Air temperature: In rooms 154 (marine collection) & 182 (skate room) the air temperature is approximately 17.5C, but can be adjusted several degrees if necessary.
- Water: Fresh water from campus wells is pumped into the Aqualab where it is filtered prior to being supplied to each room, or utilized for the mixing of salt water. Freshwater and salt water is recirculated within each room. All animal rooms have gravel bed filters that, depending on bio-load, could require backwashing once per year.
- Water Temperature: Temperature is controlled and monitored by the Argus™ system. Room 154 is capable of supplying three water temperatures to within ±1℃; room 182 has only one water temperature and is typically set to 10℃. The water temperature deviation alarm is set to function at ±2C. Temperature is set by the Facility Manager at the beginning of the project. Data

is logged into the computer and a print-out is available upon request (See Room specific SOP).

- Aeration: Air for diffusers in all animal tanks is supplied regenerative air blowers. Air pressure is monitored and alarmed if the pressure drops below a pre-set threshold. A backup blower is onsite in case of any catastrophic failure.

C WATER QUALITY**:**

When the room is first put into use, water quality testing should be carried out on a regular basis, until a stable state is reached. Further testing will become necessary as the room's biomass increases (growth or greater numbers of fish) or density within tanks increases. Testing should be done at approximately the same time of the day, as diurnal fluctuations do occur in the production of nitrogenous wastes and the utilization of oxygen.

Tests should include: dissolved oxygen; pH; salinity; ammonia; nitrite; nitrate and alkalinity. The first three tests can be carried out using hand held metres. These metres can be located either in the Dry Lab (room 166) or the Aqualab Office. Aqualab has a Hach DR2800 portable spectrophotometer for the analysis of nitrogenous compounds. An SOP (Water Chemistry SOP) for the use of this instrument may be found in the dry lab.

In marine rooms, with a functioning biofilter, salinity should be 33 ‰, ammonia and nitrite should be near zero, but commonly nitrate values will need to be managed over time. Different species have different thresholds for maximum nitrate tolerance, but values above 50mg/L are not unusual. Nitrate increases can be controlled by the addition of new water (dilution) or the introduction of appropriate plant matter into the recirculation system (algae, mangroves etc.). However, once critical levels of nitrate are reached, the best intervention is to dump and replace up to 90% of all water in the system. Dissolved oxygen will vary with temperature but generally should be above 7 mg/L. pH for most marine systems should be maintained between 8.2 and 8.3. Other tests that could be preformed are copper, hardness, total suspended solids and phosphate, however these tests are generally not necessary in these systems.

***As marine rooms do not have automatic water replacement, salt water must be made up and added periodically.**

D. FEEDING**:**

All animals should be fed by hand to satiation. Remember this is a recirculation system and everything that is added must eventually be removed again to prevent contamination. Over-feeding will result in clogged filters, cloudy water and increased levels of nitrogenous waste. Fish food should be stored in bags, or appropriate containers, in the freezer.

Whatever food you are feeding to your marine animals, you are trying to deliver all the ingredient necessary for growth (optimal growth or maintenance growth) and optimal health. In order to

accomplish this, you will want to deliver a mixture of protein, carbohydrates, fats, vitamins, and minerals.

If you are not feeding pellets to your fish, then the choice of what to feed your marine vertebrates will vary depending on the species and life stage you are holding. This SOP cannot cover all the different requirements for so a variable group, however there are some general guidelines that will help increase the health of your model organism.

i) Commercially produced feeds (i.e. pellets)
ii) Frozen marine fish and/or frozen seafood is a great basis to begin planning how to feed you marine verts. Check what is available at local sources (grocery stores, pet stores, aquarium supply) and determine what best fits your budget as well as the dietary needs of your study species.

Ex. Frozen fish/animals

Round herring (cheap and available at local grocery stores)
Mackerel (more expensive than herring, and has a higher fat content, available at local grocery stores)
Commercially available mixes of frozen animals (Ex. Seafood Medley: octopus, squid, clam and crab meat, available at local grocery stores)

NB. Additives for frozen fish:

Vitafish - It is recommended, regardless of the type of meat being fed to marine fish, that prior to feeding, the meat be soaked in Vitafish™ ("Vita Fish is a highly concentrated vitamin complex containing 10 essential vitamins. It is highly soluble, easily absorbed and can be used as a food additive to enhance the nutritional quality of dry or live food. Dosage rate is 2 drops per gallon"). Please contact Aqualab staff for supplier in the area.

iii) Marine Zooplankton: High survival and growth, normal pigmentation, and low frequencies of skeletal deformities are characteristics of marine fish reared on natural assemblages of marine zooplankton. Different life stages may benefit more from being fed one type of zooplankton (larval fish have higher survival when reared on mostly copepods (Støttrup, 2003; Lee et al., 2005)

Ex. Live/frozen zooplankton
Daphnia
Copepods
Brine shrimp
Rotifers
Mysis shrimp

Each of these inverts can be purchased commercially as frozen products (Angelfins, Guelph, ON). The variety listed here reflects a size difference. Smaller animals or life stages have smaller mouths and therefore require smaller feed.

Whichever combination of feeds you chose, it is highly encouraged to keep supplies of all feeding items well stocked and to replace any depleted items well in advance of running out in order to protect against shipping or supply-chain delays.

Aqualab has undertaken a live feed production project. We are currently successfully producing copepods, brine shrimp and rotifers, and plan to begin producing Daphnia. We will scale up production as space permits, so please contact Aqualab staff to inquire as to whether there is sufficient production to be able to provide/augment your current feeding regime.

Refs:
Støttrup, J.G., 2003. Production and nutrition value of copepods. In: Støttrup, J. G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. BlackwellPublishing, Oxford, pp. 145–205.

Lee, C.-S., O'Bryen, P.J., Marcus, N.H., 2005. Copepods in Aquaculture. Blackwell Publishing, Oxford. 269 pp

Virginia Cooperative Extension, Publication 420-256. Understanding Fish Nutrition, Feeds, and Feeding. https://fisheries.tamu.edu/files/2019/01/FST-269.pdf

E. SANITATION**:** Disinfectants or detergents are not used in the routine cleaning of tanks. Tanks are scrubbed with brooms, brushes or abrasive pads and clean water to remove accumulations of algae, faeces, uneaten feed, bacteria and light build-ups of calcium.

Daily - All tanks must be inspected daily to ensure proper waste removal and water quality.

Monthly - All tanks must be partially drained and the sides, bottoms, standpipes and wells scrubbed. Tanks are then rinsed and refilled.

Footbaths - footbaths are provided in aquatic rooms. They must be maintained by cleaning weekly and replacing the germicidal solution.

Net care - Nets are stored dry between uses and dipped for at least 10 minutes and rinsed between tanks in a solution of A33™ (at a concentration of 12.5 mL/L). "Tamed" iodine formulation may also be used eg. Germ Kill™ at a concentration of 1.5 mL/L, Wescodyne™ at a concentration of 25 mL/L or Argentyne™ at a concentration of 1 mL/L.

Disinfection - At the completion of each experimental use of a room or tank the user is required to disinfect the room or tank. Failure to do this will result in Aqualab's staff being responsible for the disinfection of the room or tank. This will result in a bill to the primary researcher for time and materials. All tanks, air stones, air hoses, stand pipes and water hoses must be scrubbed and disinfected with a quaternary ammonia disinfectant at the manufacturers recommended concentration; A33™ at a dilution of 12.5 mL/L, or Quatto 44 at a concentration of 16mL/L. Calcium may also be removed by acid washing with a 50-100% solution of Brawn™ acid de-limer or 50% solution of Muriatic acid. The tanks must be properly rinsed afterward to ensure removal of excess disinfectant or acid.

Note: It is important to ensure that disinfected and rinse water is not mixed with system water. Contamination of system water may result in fish death as well as biofilter death. Valves are placed in the drainage trench to allow for water from the tanks to be diverted from the system directly to the sanitary sewers. Please ensure that the valves are positioned properly. If you are unsure how they must be positioned or the valve is stuck please ask for assistance from the Aqualab staff.

F. ANIMAL IDENTIFICATION**:**

- Tank cards identifying species, source, number, primary and associate researcher, and emergency contact person etc. are to be posted on each tank **OR** within the confines of the room.

- A record must be kept of species, supplier, numbers, arrival date and disposition.

Hagen Aqualab Animal Utilization Record

Researcher:

Species	№	Arrival Date	AUP №	Supplier	Disposition
Little Skates	100	Sept 3, 2000	03R000	Huntsman Marine Science Centre, NB	30 euthanized Sept 25, 2000
					30 euthanized Nov 2, 2000

G. VETERINARY CARE:

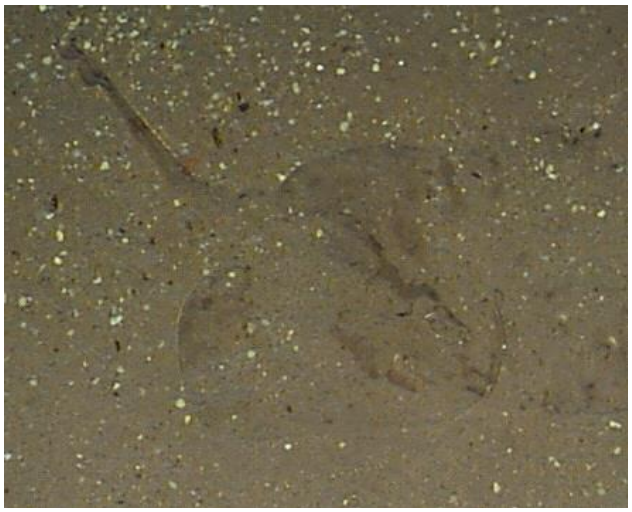
Veterinary care is on a consultative basis only. Advice for the treatment of diseased fish may be from either:

Dr. John Lumsden (X54519) in the OVC Fish Pathology Lab
Dr. Marcus Litman (X58856) the UofG Veterinary Director.
Prescriptions for the treatment of disease can be received from Dr. Lumsden or Dr. Litman.

<u>All mortalities</u> in the Aqualab must be reported to Aqualab Staff. Any animal that dies of unknown causes or is suspected of dying of a disease related problem must be bagged, tagged and submitted for a post mortem (PM) examination, the **results** of which **must be reported to Aqualab Staff**. It is of vital importance that PM's be done on animals that die of unknown causes in this facility. There are several users and an unknown infection has the potential to cause wide spread disease problems not only for the individual researcher but also to other users. Reports including diagnosis, numbers of mortalities, treatment and success or failure of treatment are required for all outbreaks of infection and disease.

- **Unusually high mortalities** must be reported to the Animal Care Committee (within 24 Hrs of each occurrence). This may be done by completion and submission of an **Animal Care Incident Form**. (See Animal Incident SOP)
- Water samples should be taken from tanks in which animals die of unknown causes. This allows for investigation/analysis of water chemistry to determine what if any parameters (e.g. nitrogenous wastes and/or chlorine) contributed to the incident.
- If external parasites are suspected, surface scrapes from dead animals may be conducted and the mucous viewed under a microscope.

H. ENVIRONMENTAL ENRICHMENT**:** Marine fish are generally held in large tanks with others of the same species. Water flow may be directed in a manner to promote swimming behaviours. Tanks are covered to decrease ambient light levels thus providing more natural lighting conditions.



Rock gunnels, sticklebacks and mummichugs are held communally in a tank with a large numbers of rocks. The rocks provide hiding places for the gunnels and an underwater terrain for the sticklebacks and mummichugs.

Skates are provided with sandy substrate on the bottom of the tank in which to hide.

I. WEEKEND RESPONSIBILITIES**:** Fish held in Aqualab must be monitored 7 days-a-week. Weekend and weekday tank care are similar. An individual in each lab must be identified to be on call to deal with extra-ordinary problems which might occur overnight

or on weekends. Procedures for contacting the person(s) responsible is to be posted on the tank card or on a poster near the door of the anteroom.

Salmonids SOP 6

Rainbow trout	*Oncorhynchus mykiss*
Chinook salmon	*Oncorhynchus tshawytscha*
Atlantic Salmon	*Salmo salar*
Arctic charr	*Salvelinus alpinus*

A. HOUSING:

Fish may be held in a variety of fibreglass tanks:

- 6' diameter insulated tanks
- 4' and 2' circular fibreglass tanks, generally used in the research rooms. The 2' tanks have cone shaped bottoms and require a grid. Perforated PVC or egg-crate may be used depending upon fish size. Not all of the 4' tanks are insulated.
- 7' fibreglass trays (non-insulated)
- 7' fibreglass living streams (insulated)
- 7' square fibreglass tanks (non-insulated)

- Always consult with the Aqualab staff prior to use so that the correct size tank and stocking density can be achieved.

B. ENVIRONMENTAL CONDITIONS:

- Light: Photoperiod may be determined by the individual researcher (See Room specific SOP).

- Air temperature: In rooms 160, 180,182,184,185,186 air is set between 15C-23C. In the controlled environment rooms (Rooms: 160a-g, 156 163,165 and183) air temperature may be held constant between 5C-25C or set to fluctuate on a diurnal cycle (See Room specific SOP).

- Water: Animal water (raw water) is supplied from campus wells. It is pumped into Aqualab where it is filtered prior to being supplied to each room. Most research rooms have gravel bed filters that act as both mechanical and biological filters. (See Room specific SOP).

- Water Temperature: Water temperature control, in all recirculation rooms, is achieved via plate heat exchangers. Temperature is set by the Facility Manager at the beginning of the project. In recirculating systems, water temperature is controlled and monitored by the Argus™ system. This system can maintain a +/- 0.1C band of control in most systems. All recirculation systems are monitored and alarmed – meaning that if any environmental variable (water temperature or flow rate) deviates from pre-set values, the system will alert facility staff within minutes of the situation.

- Aeration: Air for tanks within Aqualab is provided by regenerative air blowers. Operation of these low pressure, high volume air blowers is continually monitored by the Argus™ system.

- Stocking Density: See Stocking Density SOP for specific details and recommendations

C. WATER QUALITY:

When any recirculation system/room is first put into use, water quality testing should be carried out on a near daily basis until a stable state is reached. Once the biofilter is established, weekly testing should generally be

sufficient. Further testing might become necessary as the rooms biomass increases (growth or greater numbers of fish) or density within tanks increases. Testing should be done at approximately the same time of the day, as diurnal fluctuations do occur in the production of nitrogenous wastes and the utilization of oxygen.

The most common measurements are: dissolved oxygen, pH, ammonia, nitrite & nitrate. The first two tests can be carried out using hand held metres. These metres can be located either in the dry lab (Rm 166) or the Aqualab Office. Aqualab has a Hach DR2800 portable spectrophotometer for the analysis of nitrogenous compounds. Procedures for the use of this instrument may be found in the dry lab.

In a room with a functioning biofilter and adequate water replacement, ammonia and nitrite should be near zero, with nitrate levels below 10 mg/L. Oxygen should be above 7 mg/L with 9-11 mg/L as optimal. pH should be between 8 and 9. Other tests that could be preformed are copper, hardness, total suspended solids and phosphate, however these tests are generally not necessary in this system.

D. FEEDING:

All fish should be hand fed whenever possible. Animals should be fed to satiation, a little bit at a time, to allow all of the food to be eaten. This is especially true in the two-foot fiberglass tanks, where uneaten food can pass through the screen at the bottom of the tank.

Mature fish do not need to be fed every day. Growing juvenile fish need to be fed more frequently. Fish will, however, grow at a rate related to how much they are fed. This is not a production facility where we want to maximize growth and minimize holding time. Our goal is to maintain healthy! fish.

Use of automatic feeders should be restricted to fry - where the need to feed more frequently is necessary. Care should be taken to provide only as much food as is necessary, as large quantities of uneaten food will cause water quality to deteriorate**.**

THROWING FOOD AT FISH SHOULD BE AVOIDED AT ALL COSTS.

UNEATEN FOOD STAYS IN THE SYSTEM AND CAUSES A DETERIORATION OF WATER QUALITY. POOR WATER QUALITY CAUSES DISEASE.

E. SANITATION:

Disinfectants or detergents are not used in the routine cleaning of tanks. Tanks are scrubbed with brooms, brushes or abrasive pads and clean water to remove accumulations of algae, faeces, uneaten feed, bacteria and light build-ups of calcium.

- ***Daily*: All tanks must be inspected daily to ensure proper waste removal and general water quality.**
- ***Weekly:* All tanks must be partially drained and the sides, bottoms, standpipes and wells**

scrubbed. Tanks are then rinsed and refilled.

I. ***Walls and floors*** of each room must be rinsed weekly and washed with a disinfectant (A33™ at a concentration of 12 mL/L) monthly. Mildew on the bottoms of the walls or outsides of tanks may be removed with sodium hypochlorite (bleach) or A33™ at a concentration of 12 mL/L whenever build-ups become noticeable.

II. ***Footbaths*** are provided in aquatic rooms. They must be maintained by cleaning when necessary and replacing the germicidal solution, such as A33 or Iodine. These chemicals should be stored under the sink in the anteroom. The footbaths have a volume of approximately 5L. Please contact the Aqualab staff to purchase disinfectant.

III. ***Net care:*** Nets should be stored dry between uses. Nets must be dipped in a solution of Iodine or A33 at the manufacturers recommended dosage for at least 10 minutes, rinsed and hung to dry after use.

IV. ***Completion of an experiment:***
At the completion of each experimental use of a room, tank, cage or aquaria, it must be fully cleaned. All tanks, air stones, air hoses, stand pipes, nets and water hoses must be scrubbed and disinfected with a quaternary ammonia disinfectant at the manufacturers recommended concentration; A33™ at a dilution of 12 mL/L. Tanks may also be disinfected by acid washing with a 50% solution of an acid (eg Servac™, Muriatic acid, Lime-A-Way™, CLR™, Airkem Brawn™. The system must be thoroughly rinsed afterward to ensure removal of residual disinfectant or acid.

V. General housekeeping:
Ideally once a year, or when possible, a room may be shut down to be completely cleaned and disinfected. At this time all animals must be relocated or euthanized before the room is cleaned. Cleaning may involve the removal of tanks from waste lines, fishing waste lines, circulation of bleach throughout the system. At this time heavy deposits of lime build up are also removed. After the room is brought back on line, care must be taken to monitor water quality for build-ups of ammonia and nitrite.

F. ANIMAL IDENTIFICATION:

Sheets identifying species, AUP# and expiry date, source and number of animals, primary and associate researchers, and emergency contact personnel etc. are to be posted on the door to each room or in the containers provided with each tank.

Salmonids may be tagged using a variety of approved tagging methods. Fish may be tagged using Visible Implant Elastomer (VIE) tags, injected subcutaneously. Floy spaghetti tags which are inserted into the dorsal musculature posterior of the dorsal fin. Or PIT tags (passive integrated transponder tags) placed subcutaneously or in the intraperitoneal space. PIT tags may be safely implanted in fish as small as 2-3 inches in length.

For Salmonids held in schools numbering in the thousands records of certain animals, cohorts, year classes, *etc* must be maintained.

An animal utilization record must be kept detailing species, supplier, number of individuals, arrival date, any procedures conducted, and disposition. Additionally, a daily observation sheet detailing year, month, day, time, initials of observer and any comments must be kept on the tank or in each room. Templates for each of these sheets are available on the Aqualab webpage.

Hagen Aqualab Animal Utilization Record

Researcher: **Location:**

Species	#	Arrival Date	AUP #	Supplier	Procedure/Disposition
Chinook Salmon	100	Sept 3, 2000	99R000	Silvercreek Fish Farm	30 blood sampled, Sept 25, 2000
					30 euthanized Nov 2, 2000

G. VETERINARY CARE:

- **All unusual mortalities in the Aqualab must be reported to the Coordinator**. Any fish that dies of unknown causes, or is suspected of dying of a disease related problem, should immediately taken for a post mortem (PM) examination, the results of which must be reported to the Coordinator. In order to maintain high levels of biosecurity, it is of vital importance that PM's be done on animals that die of unknown causes in this facility. Any pathogen has the potential to cause wide spread disease problems throughout the facility. Reports including diagnosis, numbers of mortalities, treatment and success or failure of treatment are required for all outbreaks of infection and disease.

- Unusually high mortalities must be reported to the Animal Care Committee (within 24 Hrs of each occurrence). This may be done by completion and submission of an animal care incident form (See Animal Incident SOP).

- Water samples should be taken from tanks in which animals die of unknown causes. In several instances testing for copper has revealed measurable amounts in water when copper normally should not be present.

- Veterinary care is on a consultative basis only. Advice for the treatment of diseased fish may be sought from Dr. John Lumden - the OVC Fish Pathology Lab (x 54640 / 52566) or through the UofG Veterinary Director (Dr. Marcus Litman X58856). Prescriptions for the treatment of disease can be received from the Fish Pathology Lab in OVC or Dr. Litman.

H. ENVIRONMENTAL ENRICHMENT:

Salmonids are not held individually, but are generally held in circular tanks with others of the same species. Water flow is directed in a manner to promote swimming behaviours. Tanks are covered to decrease ambient light levels providing more natural lighting conditions.

I. WEEKEND RESPONSIBILITIES

Fish held in Aqualab must be monitored 7 days-a-week. Weekend and weekday tank care are identical. An individual in each lab must be identified to be on call to deal with extra-ordinary problems which might occur overnight or on weekends. Procedures for contacting the person(s) responsible is to be posted on the tank card or on a poster near the door of the anteroom.

J. SOURCES OF SALMONIDS IN ONTARIO

i) **Ontario Ministry of Agriculture and Food and Rural Affairs:**
Alma Research Station, Alma, Ontario-Rainbow Trout, Arctic Charr, Atlantic Salmon

ii) **Ontario Ministry of Natural Resources:**
Blue Jay Creek, Tekummah-Lake Charr

Chatsworth Hatchery, Chatsworth -Lake Charr

Dorian Hatchery, Dorion-Lake Charr,Brook Charr

Harwood Hatchery, Harwood-Lake Charr, Brown fingerlings

Hills Lake Hatchery, Englehart-Lake Charr, Brook Charr, Aurora Trout

Normandale Hatchery, Vittoria-Atlantic Salmon, Rainbow Trout, Brown Trout

North Bay Hatchery, Redbridge-Lake Charr, Brook Charr

Ringwood Hatchery, Stouffville-Chinook Salmon (Credit River Stock), Rainbow fingerlings

Tarentorus Hatchery, Sault Ste Marie-Lake Charr, Brook Charr

White Lake Hatchery, Sharbot Lake-Lake Charr, Brook Charr, Splake, Brown Trout

Whitefish, Walleye, Atlantic Salmon

OMNR's animals are not certified disease free however we can get a listing of past disease problems.

iii) **Commercial suppliers** of rainbow and speckled trout in Ontario. For a more complete listing refer to Fish Farm Supplies website "Fish Stocking and Fish Out Contacts" https://www.fishfarmsupply.ca/pages/fish-stocking-and-fish-out-links.

Rainbow Spring	J Stevenson	Thamesford	(519)283-6222
Lydndon Fish Hatchery	Clarke Rieck	New Dundee	(519)696-3076
Humber Springs		Orangeville	(519)941-2453
Three T's Fishing Hole		Elmvale	(705)322-3174
Georgian Bay Aquaculture		Victoria Harbour	(705)534-3971
Kinmount Fish Farm		Kinmount	(705)488-2660
Cedar Springs Trout Farm	Lloyd Brauman	Waterloo	(519) 576-5737
Mimosa Springs	Don Astill	Hillsburgh	(519) 855-6819
			or (519) 821-1257

K. LOCAL SUPPLIERS OF AQUACULTURE EQUIPMENT

Fish Farm Supply Julia Weber (877) 669-1096

Nets, air stones, tanks etc. catalogue is available in Aqualab office.

Angelfins — Jarmila Johnston (519) 546-6911

Aquaria and filters, immersion heaters, brine shrimp cysts and lots of stuff related to aquarium fish keeping.

SS Filtration — Austin Carroll (905) 827-3171

Pumps, tanks, filters, UV sterilizers etc.

Marks Supply — (519) 824-0320

Plumbing supplies catalogue is available in Aqualab office.

L. TROUT FEEDING SUPPLEMENT: GENERAL RECOMMENDATIONS

Pre-Starter Fish Food: Feed as soon as sac fry have utilized all nutrients in the yolk sac and have begun to swim to the surface. Feed frequently but in small rations to avoid water quality problems and excess waste. This diet has been formulated to provide an optimum balance of nutrition for newly hatched fish fry.

Recommended Pellet Size to Fish Size

Feed Size	Fish Size
Pre-Starter Starter	up to 5 days
0.5 GR	2.5 cm (0.2 gm)
1.0 GR	2.5 - 5 cm (0.2 - 1.5 gm)

Starter Fish Food: Feed to satiation. Sample fish weights regularly and monitor intakes to optimize feeding rates. Adjust rate according to water condition, population and growth rate. Feed larger pellets as they will accept them.

Recommended Pellet Size to Fish Size

Feed Size	Fish Size
1.5 GR	5 - 7.5 cm (1.5 - 5 gm)
2.0 GR	7.5 - 10 cm (5 - 12 gm)
3.0 GR	10 - 12.5 cm (12 - 23 gm)

Grower Fish Food: Feed to satiation. Sample fish weights regularly and monitor intakes to optimize feeding rates. Adjust rate according to water condition, population and growth rate. Feed larger pellets as they will accept them.

Recommended Pellet Size to Fish Size

Feed Size	Fish Size
3 PT	12.5 - 15 cm (23 - 40 gm)
4 PT	15 - 18 cm (40 - 60 gm)
4.5 PT	18 - 20 cm (60 - 90 gm)
5 PT	20 - 28 cm (90 - 250 gm)
6 PT	28□ cm (250□ gm)

Floating Fish Food: Fish may be fed small floating sizes after they have advanced from larger starter sizes. feed to set appetite and energy level.

Recommended Pellet Size to Fish Size

Feed Size	Fish Size
3 PT	12.5 - 15 cm (23 - 40 gm)
4 PT	15 - 20 cm (40 - 90 gm)
5 PT	20 - 28 cm (90 - 250 gm)
6 PT	28□ cm (250□ gm)

Brood stock Fish Food: Feed as a maintenance diet to a set appetite. This is a large floating fish food ideal for mature fish of larger size. It also contains Asthaxanthin, a naturally occurring carotenoid of physiological benefit to egg production and overall reproductive performance.

Recommended Pellet Size to Fish Size

Feed Size	Fish Size
8 PT	Feed to all Brood stock

Martin Feed Mills Nutrient Analysis:

Trout Food	Pre- Starter	Starter	Grower	Floating	Brood stock
Crude Protein (min)	52.0%	52.0%	42.0%	42.0 %	44.0%
Crude Fat (min)	17.0%	17.0%	16.0%	16.0%	9.0%
Ash (max)	7.0%	7.0%	4.8%	5.4%	5.8%
Fibre (max)	1.5%	2.5%	3.0 %	3.0%	3.5%
Moisture (max)	10.0%	10.0%	10.0%	10.0%	10.0%
Carbohydrate (calc)	16.0%	17.0%	29.0%	28.0%	33.0%
Calcium (actual)	1.5%	1.4%	1.2%	1.0%	1.3%
Phosphorous (actual)	1.0%	1.0%	0.9%	0.8%	0.8%
Sodium (actual)	0.8%	0.4%	0.4%	0.2%	0.4%
Vitamin A (min)	10,000 IU/kg	10,000 IU/kg	7,500 IU/kg	5,000 IU/kg	10,000 IU/kg
Vitamin D_3 (min)	3,000 IU/kg	3.000 IU/kg	3.000 IU/kg	2,500 IU/kg	3,000 IU/kg
Vitamin C (min)	300 IU/kg	300 IU/kg	200 IU/kg	200 IU/kg	200 IU/kg
Vitamin E (min)	110 IU/kg	100 IU/kg	100 IU/kg	100 IU/kg	100 IU/kg
Metabolic Energy	3900 Kcal/kg	3825 Kcal/kg	3400 Kcal/kg	3250 Kcal/kg	3050 Kcal/kg

Animal Incident Reporting SOP 7

A. What is an animal incident?

In determining whether or not an observed mortality pattern comprises an animal incident, the following criteria should be considered:

1) The number of animals involved. Is the number affected greater than what would normally be expected in the group of animals being observed? Ex. 2 fish found dead in a tank of 80 rainbow trout/zebrafish is not unexpected, whereas 15 dead fish in a tank of 30 fish is.

2) The emergence of a pattern or trend. Similar to the first criterion but subtly different. Ex. 2 fish found dead in a tank of 80 fish is not unexpected, but if it were to happen for several days in a row, it would constitute an incident.

3) Is the observed mortality / morbidity expected in terms of what has been approved for a given study? If in an infectious disease study where the AUP clearly states that a certain percentage of the animals will develop external lesions, an incident report is not required. However, if substantially more animals are affected than what has been predicted, and/or groups that are not supposed to be affected i.e. control groups, are showing signs, then an incident report is required.

B. What to do in the event of unexpected animal mortality?

In the event an animal health situation arises that might constitute an animal incident, please follow these steps:

1) Remain calm! You did not deliberately hurt your animals and will not be blamed for what has happened. This procedure is in place so that Aqualab researchers have another layer of animal care expertise to consult in order to solve the existing problem and if appropriate, to take steps so that the likelihood of reoccurrence is reduced or eliminated.

2) Collect as much information as you can or is relevant. Ex. water temperature records, previous mortalities (dates and numbers), water chemistry values (or water samples from the affected tank(s)), any observations about the holding system, and/or the behaviour and morphology of the fish. This information will be included on the animal incident form, and proves very informative in revealing patterns that might have contributed to the present situation.

3) The animal care committee (ACC) wants to be alerted to any animal incident within 24 hours of you becoming aware of the occurrence. Once an incident is deemed to have occurred (see section 1), you should immediately let the Aqualab Coordinator know and begin to fill out the incident report form.

Animal Incident Report (AIR) forms are available for download from the address below:
https://www.uoguelph.ca/research/document/animal-incident-report

C. How to initiate a pathology assessment with the Aqualab Facility Veterinarian

1) In order to provide as much information as possible to the pathologists, should a pathology report be in order, a number of preserved specimens (preserved in 10% buffered formalin) should be collected for histological examination, i.e. if you have dead fish and they are size appropriate, retrieve an appropriate container, fill it with the preservative (located in the chemical storage cabinet located outside the dry lab) and label it with all relevant information (your name, species of fish, date, etc.). If possible, open the abdomen of the fish to allow the formalin to preserve the organs and tissues (good for histology).

2) Contact the principle investigator (your professor), and the Aqualab Coordinator as soon as possible. By collectively assessing all available information, this group will provide advice for any immediate steps to be taken, assist you in the preparation of the animal incident report, and will decide if the facility veterinarian needs to be contacted to conduct a pathology assessment.

Anaesthetizing Aquatic Organisms SOP 8

Adapted from: section 9 of the Alma Aquaculture Research Station operating protocol the University of Michigan's guidelines on fish anaesthesia, analgesia and surgery

:GWACC-105 Fish Anesthesia Protocol, DFO/Environment and Climate Change Canada

:CCAC guidelines on the use of anesthetics on aquatic animals

I. Sedation/Anaesthesia

a) Specific Definition:

- Anaesthesia can be defined as the loss of sensation over all, or part of the body, resulting from pharmacological depression of nerve function. Anaesthetics are chemicals or physical agents that produce anaesthesia by preventing the initiation and conduction of nerve impulses.

b) General definitions:

- Anaesthesia can generally be defined by a temporarily loss of sensation with or without loss of consciousness.
- Analgesia can generally be defined as providing pain relief without an associated loss of consciousness.
- Sedation can be generally defined as a mild depression of the central nervous system in which the animal is awake but calm.

c) Stages of Anaesthesia

Stage I-light sedation-slight loss of reactivity to external stimuli; movement and opercular rate slightly decreased; equilibrium normal

Stage II-deep sedation-total loss of reactivity to external stimuli except strong pressure; slight decrease in opercular rate; equilibrium normal

Stage III-light anaesthesia-partial loss of equilibrium and muscle tone; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli

Stage IV-deep anaesthesia-total loss of equilibrium and muscle tone; loss of spinal reflexes; no swimming movements and may be upside down; slow but regular opercular rate.

Stage V-surgical anaesthesia-total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes

Stage VI-medullary collapse, opercular and cardiac movements stop. **Death**.

d) Anaesthetic and Sedative Drugs used with Fish

For a full listing of CCAC approved fish anaesthetics, please see: https://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Fish_Anesthetics.pdf

The most commonly used anaesthetic at the Hagen Aqualab is Tricaine.

A. Tricaine (MS-222™)

Common and proprietary names include tricaine methansulfonate, MS-222™ (proprietary name for a formulation produced by Sandoz Ltd.), Finquel™ (proprietary name for a formulation produced by Fort Dodge Laboratories and sold by Argent Chemical Laboratories), and Metacaine™. Tricaine should be used as the generic name.

Tricaine is a lipid-soluble drug that moves across the gill by diffusion or by coupling to specific transport systems. Tricaine is readily soluble in water, and acts as a general anaesthetic that depresses the central nervous system.

If any anaesthetic other than Tricaine is to be used (ex. 2-phenoxyethanol or clove oil), please refer to the CCAC document**:** https://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Fish_Anesthetics.pdf for instructions on proper use.

I. Cautionary Notes on the Use of Tricaine

- Cover anaesthetic baths - tricaine solutions are unstable and can form toxic compounds when exposed to light. Therefore, avoid mixing stock solutions and when possible, anaesthetic baths should be prepared immediately (from crystals) prior to use.
- Tricaine crystals should be kept dry and stored in sealed, light proof containers at 4˚C.
- When used in fresh water with weak acid-neutralizing capacity (i.e. total alkalinity less than 50 mg/L as $CaCO_3$), Tricaine will reduce the pH of the water. Water with a pH artificially lowered by the addition of Tricaine, can be a significant irritant to fish, and an extensive list of physiological effects of its use are documented. The pH of the bath should be checked prior to introducing fish, and if necessary the anaesthetic bath can be buffered with sodium bicarbonate (stored in the Dry Lab)

- Always aerate the bath. If processing a large number of fish, bath water should be changed often to reduce gill clogging materials (scales and slime/mucous). Aeration also removes dissolved CO_2.
- Never use any tricaine product in the presence of metals.
- Residues of tricaine decline to less than the detection limit of 0.1 mg/L in fish flesh within 24 hours of exposure. However, the FDA requires a 21-day withdrawal period for fish that will be used for food.

II. Dosage for Salmonids

The concentration needed to obtain sedation or full anaesthesia (loss of equilibrium and reflexes) by a given anaesthetic can be affected by many factors, but principally by the species of fish to be anesthetized. Fish differ greatly among species in the concentration of chemical required to bring them to a given level of anaesthesia, their tolerance of a given chemical, and their recovery time. Intraspecific variables, including age, sex, diet, and individual health factors, are also important. In addition, the optimum anaesthetic concentration varies with fish density, water temperature and hardness, induction time, and duration of exposure. *In general, higher temperatures reduce both induction and recovery times.*

Induction should take 1-3 minutes and recovery should take 3-10 minutes. Tricaine is rapidly excreted from the fish by diffusion across the gills. Recovery time varies according to tricaine concentration and exposure time, both of which affect the amount of anaesthetic absorbed by the fish. Generally, a recovery time longer than 10 minutes suggests that too much anaesthetic was used or that the exposure times were too long.

Loss of equilibrium and full anaesthesia of adult salmonids generally requires **50-100 mg/l**, the concentration depending on fish size, temperature, and desired induction time

III. Sedation Protocol

- Where possible, fish should be fasted 24-48 hours to prevent vomiting.
- Prepare a size appropriate water bath in proximity to the tank with target fish. It is important for calculating amount of anaesthetic to be used that you know the volume tof water in the bath. Prior to introducing fish, the bath should be aerated, and water temperature and pH should be checked.
- Tricaine should be dissolved in clean, influent water from tank with target fish.
- If unsure about the proper concentration of tricaine to be used, the anaesthetic bath should be initially prepared using 50 mg/l. A test fish from the population should be placed into the bath and observed. If loss of equilibrium does not occur within three minutes, the fish should be removed from the bath. Add additional tricaine (ex. an extra 25mg/L to start) to the bath and test another

fish. Should the fish lose equilibrium too quickly, i.e. under two minutes, the fish should be quickly removed and more water added to the bath to dilute the anaesthetic. This procedure is to be repeated until the proper dosage is achieved. It is critical that testing the bath with fish be conducted each time fish are to be sedated.

- If large biomasses of fish are to be sedated, the anaesthetic bath should be changed frequently. There are no guidelines suggesting what this frequency should be, but observations concerning the rates of induction and recovery and condition of the water (slime, suspended solids, colour, etc.) should be used as indicators.

Table 1.1 List of selected anesthetics and estimates for optimum doses, as well as induction and recovery times, for various fishes. (Table from CCAC document: Anesthetics, P. A. Ackerman, J. D. Morgan & G. K. Iwama)

Anaesthetic	Dose	Induction Time	Recovery Time	Test Fish
Tricaine	25-100 mg/L	<3 mins.	<10 mins	Salmonids, Carp, Minnows
	80-100 mg/L	2.6-6.8 min	2.5-1.2min	Tilapia
2-Phenoxyethanol	200-500 mL/L	3 mins.	2-10 mins	Salmonids
	100-500 mL/L	3 mins	<4 mins	Various species
Hypothermia	Instant drop of 6ºC			Tilapia
	Immersion in ice water			Various species
Clove oil & AQUI-S	40 mg/L	2.5-4 mins	3 mins	Rainbow trout (FW, 11ºC)
	40-60mg/L	3-4 mins	12-14 mins	Rainbow trout (FW, 9ºC)

Standard Operating Procedures for Euthanizing Fish SOP 9

Adapted from: section 9 of the Alma Aquaculture Research Station operating protocol

:GWACC-107 Fish Euthanasia Protocol, DFO/Environment and Climate Change Canada

:CCAC guidelines on the use of anesthetics on aquatic animals

I. General info and definition:

Euthanasia refers to humane death. According to guidelines put forth by the Canadian Council on Animal Care (CCAC), the procedure for humane death must meet the following criteria:

- death without signs of panic, pain or distress;
- minimum time to loss of consciousness, i.e., shortest lag time;
- reliability and reproducibility;
- safety for personnel involved;
- minimal undesirable physiological and psychological effects on the animal;
- compatibility with the requirement and the purpose of the scientific study;
- minimal or no emotional effects on the observer and the operator;
- minimal environmental or ecological impact; and
- simple, inexpensive mechanical equipment which is relatively maintenance free.

Fish are to be euthanized either by concussion (small numbers of large fish) or by chemical overdose (eyed eggs, fry and small fish, large numbers of fish).

II. Preparation for Euthanization

A. Materials to be assembled prior to commencing procedure

- Tricaine (aka MS222 or TMS) or Clove oil
- Gloves
- Safety glasses
- Lab coat
- Holding container for fish filled with tank water
- Aerator fish holding tank
- Balance to weigh fish post procedure

- Sodium bicarbonate (to buffer TMS)
- Method to measure pH of euthanizing bath (ex. Hand-held meter, pH strips etc.)
- Blunt object for stunning (bonker)
- Sharp knife

A. Tricaine procedure

Background:

TMS comes as a fine crystalline white powder that dissolves easily in water. A concentration of 200 to 250 mg/L is usually recommended to effectively euthanize fish. However, the optimum should be determined experimentally as it may vary with the size and the species of fish.

The use of distilled or deionized water is to be avoided, as well allowing the TMS mixture to come into contact with any metal (i.e. use plastic bins or totes for mixing and exposing fish to euthanizing compounds).

As TMS is light-sensitive and may form toxic by-products upon exposure to light, TMS solutions should be prepared freshly and used within 24 hours. Concentrated stock solutions (10g/L TMS) stored in amber bottles (or other means of protection from light) remain potent for 3 days at room temperature and a few weeks if refrigerated or frozen. These solutions must be replaced monthly and/or any time a brown discoloration is observed.

Step 1 – Preparation of the euthanasia bath

TMS is a benzoic acid derivative and, in water of low alkalinity (< 50 mg/L $CaCO_3$), the solution has to be buffered with sodium bicarbonate. Prior to exposing the fish to the euthanizing bath, the pH of the solution should be verified and adjusted to neutrality or to the pH level at which fish are housed (in the laboratory) or to which they are naturally exposed (in the field).

The volume of water in the euthanasia bath must sufficient to easily accommodate fish movement. Size and number of individuals that will be housed together in the euthanasia bath should be taken into consideration when determining the volume needed.

To minimize stress, it is recommended that euthanizing baths be prepared using water from the tank in which the fish are being held (i.e. same temperature and pH) if in the laboratory setting or with water at the site of capture (in the field).

Step 2 – Fish immersion

Fish should be handled with care and as smoothly and quickly as possible when transferred to the euthanasia bath.

Once in the solution, fish movement will gradually slow; the fish will lose its ability to stay upright in the water; or lie on the bottom of the tank.

The fish is left in the TMS solution until death is achieved. Veterinarians consider a fish to be dead 10 minutes after the last sign of gill movement.

Due to species differences in response to TMS, a secondary method of euthanasia (decapitation/spinal severance) is recommended in some fish species and amphibians to ensure death. When, for scientific reasons, fish must be withdrawn from the TMS solution less than 10 minutes after the last sign of gill movement, such secondary methods of euthanasia are also required.

B. Clove Oil procedure:

Background:
Clove oil is a product obtained from the distillation of clove tree; it is composed of a number of essential oils including eugenol (85-95%), isoeugenol and methyleugenol. For centuries, it has been used as a mild topical anesthetic and to help with toothache, headaches and joint pains. It is also known to exhibit antifungal, antibacterial and antioxidant activity and is incorporated in several aromatherapy preparations.

In recent years, clove oil gained popularity as a fish anesthetic because it is less expensive and easier to acquire than controlled chemicals. The Canadian Council on Animal Care and the American Veterinary Medical Association now consider clove oil as an acceptable method for fish euthanasia. However, clove oil or its components are not acceptable means of euthanasia for animals intended for consumption. Currently only TMS and metomidate are registered for veterinary use in Canada for fish that may be consumed by humans (with at least 5 days withdrawal); therefore investigators are individually responsible for the use of other anesthetic agents that have not been approved for such use.

Clove oil is administered in water. This viscous liquid is highly lipophilic and therefore adheres to, and penetrates rapidly, into the gill epithelium and is absorbed by body tissues (such as the brain) once in the blood circulation. When exposed to clove oil at euthanizing concentrations - fish quickly lose consciousness, stop breathing and die from hypoxia. When compared with TMS, clove oil (eugenol) has a shorter induction time and remains effective at a wider range of water temperatures.

Step 1 – Preparation of the euthanasia bath

Because clove oil does not easily solubilize in water nor dissolve in water below 15ºC, it is usually mixed into a stock solution with 95 % ethanol as solvent (1:9 clove oil: ethanol).

This solution of clove oil in ethanol is then mixed with the desired volume of acceptable water (i.e. water the fish was held in). The required dose for fish euthanasia is 400 mg/L or 4ml/L. Whenever possible, products with standardized, known concentrations of each essential oil should be used so that more accurate dosing can occur.

The volume of water in the euthanasia bath must be sufficient to easily accommodate fish movement. Size and number of individuals that will be housed together in the euthanasia bath should be taken into consideration when determining the volume needed.

To minimize stress, it is recommended that euthanizing baths be prepared using water from the tank in which the fish are being held (i.e. same temperature and pH) if in the laboratory setting or with water at the site of capture (in the field).

Step 2 – Fish immersion

Fish should be handled with care and as smoothly and quickly as possible when transferred to the euthanasia bath.

Once in the solution, fish movement will gradually slow; the fish will lose its ability to stay upright in the water; or lie on the bottom of the tank.

The fish is left in the clove oil solution until death is achieved. Veterinarians consider a fish to be dead 10 minutes after the last sign of gill movement.

When, for scientific reasons, fish must be withdrawn from the clove oil solution less than 10 minutes after the last sign of gill movement, a secondary method of euthanasia such as spinal severance or decapitation is also required.

C. Physical/Concussive Procedure:

The procedure to euthanize a fish by concussive force is achieved by delivering a blow to the back of the head with an “fish bonker” (a general use fish bonker is located at the sink in General Holding) with sufficient force to produce massive cerebral hemorrhage and thus immediate depression to the CNS.

This technique should not be undertaken in the presence of casual observers or the uninformed as it is aesthetically unpleasant. However, when properly applied, it is both effective and sanctioned by the CCAC.

Remove the fish from the holding tank and restrain it in a net or by the tail. With the fish bonker, deliver a forceful blow to the head - the blow should be delivered on the top of the head between the eyes.

This procedure should be followed by spinal severance with a scalpel or knife at the location of the junction of skull and the first vertebrae.

III. Hazards

Tricaine is **not** considered a mutagenic or carcinogenic substance. It is however retinotoxic (significant eye irritant) and an irritant to skin and mucous membranes, including the upper respiratory tract.

When preparing solutions of **TMS**, individuals should wear personal protective equipment (PPE): nitrile gloves, lab coat and safety glasses.

When fish are immersed in a TMS bath, there is a potential for splash water to contact humans, therefore the above-mentioned PPE should be worn during procedure.

Clove oil is a natural substance used for centuries in human dentistry - was long thought to be a safe substance. However, recent studies suggest some of its components have the potential to cause health problems in mammals. In fact, the US National Toxicology Program (NTP) now considers eugenol as an equivocal carcinogen and methyleugenol as carcinogenic to rodents. This substance should therefore be handled with care:

When preparing solutions of **clove oil**, the personnel should always wear protective equipment including nitrile gloves, lab coat and safety glasses.

When fish are immersed in a clove oil bath, there is a potential for splash water to contact humans, therefore the above-mentioned PPE should be worn during procedure.

IV. Post Procedure

Once fish have been euthanized, they are to be placed into deadstock freezer in Aqualab. If you are planning in euthanizing a large number of fish, please alert Aqualab management (Matt or Mike) and they will be able to furnish you with sufficient deadstock bins for your carcasses.

V. Reagent Disposal

After being used, TMS and clove oil solutions must be collected in clearly labeled containers and are to be disposed of as hazardous waste through EHS.

Fish Blood Sampling SOP-10

This procedure was adapted from the Aquatic Animal Diseases Lab Manual by Dr. Hugh Ferguson and from the University of Saskatchewan's SOP 301 (Blood Sampling and Plasma Handling in Trout)

All fish are to be anaesthetized prior to blood sampling.

A. Responsibility: Individuals performing the procedure outlined in this SOP must be adequately trained. The principal investigator is responsible for ensuring all research personnel performing these procedures are properly trained, that training is documented via the mentor facilitated training form, and that each individual is comfortable completing the procedure.

B. Equipment to assemble prior to sampling:

I. General Procedure (without syringe)

- Buffered MS-222 or similar appropriate anaesthetic agent
- Appropriate sized container for anaesthetic bath
- Source of aeration for aneasthetic bath
- Dedicated dip net for anaesthetic
- Gloves and eye protection appropriate for anaesthetic used protection
- Paper towels
- Enamel tray or Vee-board
- Disinfectant for clean up after blood sampling
- Microcentrifuge tubes or heparinized blood tubes
- Centrifuge or microcentrifuge with a rotor appropriate for the size of collection tube used
- Heparin solution in normal saline (0.9% NaCl)
- P200 pipette and pipette tips (yellow)
- Ice and suitable container
- Biohazard sharps container for used needles or microcapillary tubes
- Suitable container for biohazardous waste disposal of blood or tissue contaminated materials, such as paper towels and syringes and carcasses

II. For blood sampling with a needle and syringe

a) If fish are to be recovered:

- 1 mL syringe or vacutainer needle (only fish > 300 g)
- Sterile needles (Recommend 22 gauge, 1-1.5" needle; however, size will depend on the weight of the fish)

- Recovery tank or container
- Proper aeration for recovery tank
- Dip net to be used for recovery container

ii) If blood sampling will be followed by euthanasia:
- Scalpel
- 1 to 3 mL syringe or vacutainer needle
- Sterile needles (Recommend 22 gauge, 1-1.5” needle; however, size will depend on the weight of the fish)

c. For blood sampling of small fish with very small blood volume (caudal severance)
- Scalpel or razor blade
- Cutting board or surface suitable for cutting
- Heparanized microhaematocrit tubules with caps or sealant
- Microhaematocrit centrifuge or rotor

C. Sampling Procedure

I. Introduction

Blood may be sampled following four separate procedures: dorsal aorta, cardiac puncture, caudal vein, and caudal severance (See Figure 1.1). Of the three sampling methods the caudal vein is the preferred method for salmonids and tilapia.

a) 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.

b) Dorsal Aorta:
Insert needle on a 30-40˚ angle into the dorsal midline in the roof of the mouth at about the 3rd to 4th gill arch. Depending upon size and species of 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.

c) Cardiac Puncture:
Blood is collected from the heart ventricle. Insert needle perpendicular to the ventral surface of the fish in the centre of an imaginary line between the anterior most part of the base of the pectoral fins.

d) 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 microhematocrit tubules. After the sample is collected return the fish to a separate container of anaesthetic for euthanasia.

All fish should be fasted for 24 hours prior to sampling to prevent regurgitation or defecation during the procedure

Care must be taken to avoid contamination of the sample with tissue fluids.

No more than 0.5-1.0 % of the fish's body weight should be removed from a fish that will be recovered from blood collection (See Table 1)

Recovery after blood sampling may be performed on fish greater than 200g

For fish under 200g, individuals may have to be sacrificed to ensure an adequate blood volume is collected.

General recommendations:

- fish weighing less than 300g are blood sampled using a *1 mL syringe*.
- fish weighing more than 300g are typically blood sampled using a vacutainer needle.

Fish must be allowed adequate time to recover and regenerate blood volume if serial blood samples are to be collected.

D. Heparin solution:

- Heparin is an anticoagulant (prevents clotting)
- Using heparin allows for the collection of *blood plasma*
- By centrifugation, cells are separated from the plasma, thereby allowing collection
- When heparin is not used, whole blood is allowed to clot
- Blood serum may be collected by allowing the blood to clot and then removing the clot by centrifugation.
- Final concentration of heparin in 0.9% NaCl is 5 mg/mL.

I. Procedure (if keeping fish for further testing):

- Reconstitute chemicals (in labeled glass bottles) into 100 mL of sterile saline from IV bags (solution will be stable with proper storage for approximately one month).

• Using a pipette transfer 100 µL of heparin solution into each microcentrifuge tube BEFORE you start to collect blood from fish. Keep the 'heparinzed' tubes on ice.

• Sedate fish in container with buffered 50 mg/L MS-222 (or similar anesthetic agent)

• Remove from bucket once righting reflex is lost and place fish on tray or on Vee-board.

• Firmly hold the fish in dorsal recumbancy with left hand, then use right hand to manipulate heparinized syringe.

• Collect appropriate amount of blood from caudal peduncle (See Table 1) using heparinized 1-2 ml syringes fitted with a 22 gauge needle. Carefully remove the needle and immediately empty all blood into the appropriate microcentrifuge tube. If the needle is kept in place, the red bloods cells may be lysed.

• Close tube and gently mix tube by flicking side of tube or invert several times. The heparin in the solution needs to be mixed immediately with the blood to prevent clots. Store tubes on ice at all times except during centrifuge.

- Immediately following the transfer of blood to the micorocentrifuge tube, place the fish in an aerated recovery tank - filled with system water.
- Gently holding the fish under it's belly with one hand and over tail with the other, begin gently moving the fish back and forth in the water to move water across gills. Once normal swimming behavior has been established, return the fish to its appropriate tank.

II. Procedure (if NOT keeping the fish for further testing)

- Once sampling is complete, transfer fish to a bin containing an overdose of anaesthetic, ex. 125 mg/L MS-222. Carcasses must be disposed of according to Aqualab deadstock procedures.
- Centrifuge tubes at 2500 x g for 10 min.
- Remove the supernatant (plasma) and transfer in a quantitative manner to a microcentrifuge tube for freezing at -80ºC until assay. Take care not to disturb the thin middle layer containing white blood cells. It is generally better to lose a little plasma than to accidently decant this middle layer.

E. Safety

- To reduce the risk of contamination, gloves should be worn at all times when handling blood collection equipment and blood.
- ***Never*** attempt to re-sheath needles. Always discard them into a SHARPS container immediately after use
- Do not overfill the SHARPS container.
- To reduce the risk of contamination, ensure you are working in a clean environment. Allow sufficient workspace to safety obtain blood sample without contamination
- Should a blood spill occur; wearing gloves, use paper towel to soak up larger

quantities of blood, wipe up any remaining blood and spray area with a 70 % alcohol solution and allow to sit for 10 minutes, repeat if necessary

F. Potential Complications and Troubleshooting

If initial attempts to obtain blood from the caudal vein are unsuccessful in a fish destined to be euthanized, reinserting the needle at a different angle or in a slightly more anterior location along the midline but still behind the caudal fin may alleviate the problem. If the fish was not fully anaesthetized and begins to revive as the blood sampling is commencing, return the fish to the anaesthetic container immediately and wait until the fish is fully anaesthetized.

Table 1. Maximum amount of blood that can be taken from recoverable fish[1], based on body mass

Body Mass (g)	Maximum blood sample (mL)
50	0.175
100	0.350
150	0.525
200	0.700
250	0.875
300	1.050
350	1.225
400	1.400
450	1.575
500	1.750
550	1.925
600	2.100
650	2.275
700	2.450
750	2.625
800	2.800
850	2.975
900	3.150
950	3.325
1000	3.500

[1]If fish will not to be recovered after collection, a rough guideline for collection is 1 mL of blood.

Table 2. Needle gauge to be used for maximum blood collection in fish

Body Mass (g)	Needle gauge	Syringe size
50-300	20-22	1 mL
300+	20-22	1-10 mL or vacutainer needle

Figure 1.1 Locations for blood sampling salmonids