

GENERAL ANIMAL HOLDING STANDARD OPERATING PROCEDURE



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Aqualab Staff:

Bob FrankManagerRoom 140 AqualabExt 52714Matt CornishAssistant ManagerRoom 138 AqualabExt 52714

PRIOR TO ARRIVAL

■Consultation with the Aqualab Manager 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, a copy of which must be submitted to the Aqualab Manager.

■An **Animal Acquisition/Transfer Form** (containing information on animal numbers, supplier etc.) should be submitted to the Aqualab Manager **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 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 clean garbage buckets 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 treated 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 SOP for lodophor Disinfection of Salmonid Eggs).

DAILY ANIMAL CARE

ALL ANIMALS HELD IN AQUALAB ARE UNDER THE DIRECT RESPONSIBILITY OF THE PRIMARY RESEARCHER 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.



Water quality monitoring must be performed on a regular basis: pH, dissolved oxygen, ammonia, nitrite, and nitrate. Ammonia and nitrite must be monitored closely in newly set-up systems as the risk of nitrite poisoning can be high.

Biofilters should not be allowed to dry out. This is particularly important in marine systems, as dry-out will result in death of the biofilter.

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.

There is an SOP specifically for tank cleaning located on the Aqualab website.

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 (A33TM 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 A33TM 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: <u>Nets should be stored dry between uses</u>. 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.

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 quartinary ammonia disinfectant at the manufacturers recommended concentration; $A33^{TM}$ at a dilution of 12 mL/L. Tanks may also be disinfected by acid washing with a 50-100% solution of an acid (eg ServacTM, Muriatic acid,

Lime-A-Way[™], CLR[™], Airkem Brawn[™]. The system must be thoroughly rinsed afterward to ensure removal of residual disinfectant or acid.

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 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 DR2000 portable spectrophotometer for the analysis of nitrogenous compounds. Procedures for the use of this instrument may be found in the Dry Lab (room 166).

Animal and Project Information:

Cards identifying species, AUP №, source, number, primary and associate researchers, and emergency contact person and number are to be posted either on each tank or within the confines of the room. White boards have been provided in some of the multiuser rooms for this purpose.

Records must be kept up to date of species, supplier, numbers, arrival date, disposition and mortality. 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 a little bit at a time to allow all of the food to be eaten.

Mature animals do not need to be fed every day.

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:

• <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 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. Roz Stevenson** in the Fish Health Lab in Microbiology (X52517)
- **Dr. Marcus Litman (**X58856) the staff veterinarian.
- Prescriptions for the treatment of disease can be received from Dr. Lumsden or Dr. Litman.
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 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

•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 Manager 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 (\approx 8.2) and temperature (\approx 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 Manager 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:

Bob Frank	X 52714 519 831-1671	Office Cell Phone
Matt Cornish	X52714 519 831-1321	Office Cell Phone

"ISOLATION ROOM" PROCEDURES April, 1999 (revised 2006)

EVERYONE USING THIS ROOM MUST FOLLOW THESE PROCEDURES.

■One person from each lab must be designated to have responsibility for the room.

■Only authorized personnel are allowed into "isolation" rooms. Authorization must be obtained from the Aqualab Manager and the Primary Researcher.

■Footbaths must be used.

■Footbaths must be cleaned and the activated iodine replaced either as necessary or on a weekly basis.

Prior to <u>entering and leaving</u> the room hands must be washed with a germicidal soap.

■Rubber boots, clothing, equipment, etc used in conjunction with fish handling, must be sterilized prior to entering this room.

■Lab coats must not leave the room until they are removed for cleaning. At such time they must be taken directly out of the building.

■Rubber boots must be worn while inside the holding room. These boots **must not** leave the confines of the animal holding room or anteroom.

■All nets must be disinfected after each use.

Tank cleaning and water quality are paramount. Remove all uneaten food, this results in fungal mats if left unattended.

■UV sterilizers must be operating.

In the event of disease outbreak the Aqualab Manger must be notified.

■In the event of serious mortality or morbidity an Animal Incident Form must be filled out and sent to the Animal Care Committee with a copy to the Aqualab Manager. Forms are available in the Aqualab Office.

■In the event of disease outbreak, nets used for the 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.

■All mortalities must be recorded and sent for post mortem.

Animal Incident Report

Important: Form must be completed within 24 hours of knowing of the incident -Fax to ACS, Dr. D. Benn (519) 837-2341

Facility		AUP #		
Reported by		Position		_
Time of Incident		D/M/Y of incident	D/M/Y reported	
DESCRIPTION OF IN	CIDENT State exactly	y what was leading up to the inciden	t, where the incident occurred etc.:	
ANIMALS AFFECTED):			
Total #	Gender	Species		
MORBIDITY / MORTA	LITY #'s - Describe I	now the animals were affected		
CAUSE OF SICKNES	S OR DEATH (IF K	(NOWN)		
ACTION PLAN:				
Signature of Person R	eporting Incident:		Date:	



Mortality Record Sheet

Researcher:

Room #:

Date	Species	Tank №	Nº Dead	Disease Su Yes No	uspected	Disposition PM/Dead Stock

Aqualab	
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Daily Observation Sheet:

Year:

Room #:

Month:	Time	Initials	Comments
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