



Marine Fish Standard Operating Procedures



June, 2000

Revised: December 2005

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

- 1) 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.
- 3) 7' fibreglass trays (non-insulated)
- 4) 7' square fibreglass tanks (non-insulated)

Other housing may be available, however arrangements must be made with the Aqualab Manager prior to use.

ENVIRONMENTAL CONDITIONS:

- Light: Photoperiod may be determined by the individual researcher (See Room specific SOP).
- Air temperature: In room 154 & 182 air is between 15°C-20°C.
- Water: Water from campus wells is pumped into the building where it is filtered prior to being supplied to each room. This ground water is recirculated within each room. All of these rooms have gravel bed filters that rarely require backwashing.
- Water Temperature: Temperature is controlled and monitored by the Argus™ system. Room 154 is capable of supplying three water temperatures to within $\pm 1^\circ\text{C}$, room 182 has only one water temperature and is typically set to 10°C. The water temperature deviation alarm is set to function at $\pm 3^\circ\text{C}$. 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 tanks is provided by two regenerative air blowers. Air pressure is monitored and alarmed if the pressure drops below a preset point. A third blower is present in case of failure of the other two.

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 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 DR2000 portable spectrophotometer for the analysis of nitrogenous compounds. Procedures for the use of this instrument may be found in the dry lab. Alkalinity can be measured by a buret titration method. Instructions for this may also 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. Salinity should be 33 ‰. Other tests that could be performed are copper, hardness, total suspended solids and phosphate, however these tests are generally not necessary in this system.

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

FEEDING: Large fish are fed chopped herring, squid or krill (See specific SOP for Little Skate Care). Smaller fish may be fed small pieces of herring or live feed such as black or white worms (See SOP's for culturing white worms and black worms). 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 is stored in bags, or appropriate containers, in the freezer.

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, Westcodyne™ 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. 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.

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

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.

▪ **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.

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.

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.

TECHNICAL PROCEDURES:

■ **Anaesthesia:** Fish are anaesthetized prior to all handling procedures using a 0.05 - 0.1% (50-100 mg/L) solution of MS-222 (tricaine methane sulphonate) or a 0.025% (1 mL / 4 L water) solution of 2-phenoxyethanol (stored in research lab). After procedure fish are placed in a recovery tank (if available) prior to being returned to their experimental or holding tank. Waste water is disposed of into the trench. **Care must be taken to ensure contaminated water is not dumped back into the system water.**

■ **Euthanasia:** Fish are euthanised in a 0.1% solution of 2-phenoxyethanol or via extended exposure to an anaesthetic dose of 0.1% MS-222. After the animal is dead all waste tissue must be disposed of in the dead stock containers in the freezer. Waste water is disposed of into the trench. **Care must be taken to ensure contaminated water is not dumped back into the system water.**

■ **Blood Sampling:** (See: Fish Blood Sampling SOP).

■ **Body weight:** changes may be monitored on a monthly basis. Larger fish should be anaesthetized and weighed on an electronic balance, smaller fish may be transferred to a beaker of water on an electronic balance.

■ **Identification of fish:** Fish may be marked (fin clip) or tagged, under anaesthesia, at the beginning of an experiment. A variety of approved tagging methods may be employed.

■ Fish may be tagged using 10 lb monofilament threaded through the dorsal fin and tying a disk tag to the fish. Follow this procedure with a salt bath (3% NaCl) to improve healing.

■ Opercular tags;

■ Coded wire tags inserted into the head;

■ Floy spaghetti tags inserted into the dorsal musculature posterior of the dorsal fin;

■ PIT tags (passive integrated transponder tags) placed sub-cutaneously generally on the left side posterior of the dorsal fin. PIT tags may be safely implanted into fish as small as 2-3 inches in length.

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.