The following Standard Operating Procedure must be followed by each researcher holding salmonid fish within the Hagen Aqualab.

This SOP is to be used in conjunction with the General Animal Holding SOP and the room specific SOP found in the Hagen Aqualab Users Manual.
HOUSING:
Fish may be held in a variety of fibreglass tanks:

1) 6' diameter insulated tanks generally used only in the general holding room
2) 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' fibreglass living streams (insulated)
5) 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 rooms 150, 151, 152, 154, 156, 160, 180, 182, 184, 185, 186 air is typically between 15°C-20°C. In the controlled environment rooms (Room 156, 160a-g, 163, 165, 181, 181a and 183) air temperature may be set by the researcher. Please discuss temperature requirements with the Hagen Aqualab Manager. (See Room specific SOP).

- **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 rooms 150-154, 156 160, 180 and 182-186. Water is not necessarily recirculated in other rooms and chambers (See Room specific SOP’s for information on water recirculation). Room 155 is the only flow through system in the building.

- **Water Temperature:** Temperature is controlled and monitored by the Argus™ system to within ±1°C, alarms are set to function at ±3°C. All rooms have at least one plate heat exchanger. Temperature is controlled by the Facility Manager. 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, ammonia, nitrite, nitrate. The first two 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).

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.

**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, especially in the two foot diameter tanks where uneaten food passes 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. We only need 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.**

**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.
- **bi-weekly -** All 2’ dia. tanks must be drained and the sides, bottoms, standpipes and wells scrubbed. Tanks are then rinsed and refilled.
- **monthly -** All 4’ and 6’ dia. tanks must be partially drained and the sides, bottoms and standpipes scrubbed. Tanks are then rinsed and refilled.
**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.

**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.

**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.

**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% dilution 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.

Once a year, or when necessary, 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, replacement of sand, gravel and charcoal. 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.

**ANIMAL IDENTIFICATION:**
- Tank cards identifying AUP #, species, source, number, primary and associate researcher(s), and emergency contact person etc. are to be posted either on each tank or within the confines of the room.
- Salmonids may be tagged using a variety of approved tagging methods. Fish may be tagged using 10 lb monofilament threaded through the dorsal fin and tying a disk tag to the fish. This procedure is followed by a salt bath (3% NaCl) to improve healing. Other tagging methods include coded wire tags inserted in the head; opercular tags; Floy spaghetti tags, these tags are 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 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.
- A record must be kept of species, supplier, numbers, arrival date and disposition.
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 widespread 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.

ENVIRONMENTAL ENRICHMENT:
Salmonids are not held individually, they 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>N°</th>
<th>Arrival Date</th>
<th>AUP N°</th>
<th>Supplier</th>
<th>Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinook Salmon</td>
<td>100</td>
<td>Sept 3, 2000</td>
<td>99R000</td>
<td>Silvercreek Fish Farm</td>
<td>30 euthanized Sept 25, 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 euthanized Nov 2, 2000</td>
</tr>
</tbody>
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TECHNICAL PROCEDURES

Care must be taken to ensure contaminated water is not dumped back into the system water. Waste water may be disposed of in the trenches in each room, these trenches are connected to the sanitary sewers not the system water.

- **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.

- **Euthanasia:** Fish are euthanized 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 placed in the containers in the dead stock freezer.

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

- **Fertilization and incubation of eggs:** (See SOP for Iodophor Disinfection of Salmonid Eggs). Eggs may be checked after 24 hours to remove unfertilized eggs but should not be disturbed again until after they are eyed up. Eggs may be treated with an antifungicidal agent to prevent fungus growing on dead eggs. Possible treatments include hydrogen peroxide.

- **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.

- **Individual Identification of Fish:** Fish may be marked (fin clip) or tagged, under anaesthesia, at the beginning of an experiment. A variety of approved 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 extraordinary 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.