

# **PROCEDURES MANUAL**

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**A. INTRODUCTION**

The John L. Fryer Salmon Disease Laboratory (SDL) is a regional facility providing space for the general use of many scientists. The efficient and smooth operation of any multi-use facility demands cooperation and mutual respect by all users. Trust is involved regarding the common use of equipment, reagents, etc. and failure to observe proper procedures could be disastrous.

This manual outlines procedures which must be followed at the SDL to ensure that all operations are carried out smoothly, to see that the facility is well-maintained, and, most importantly, to enhance experimental success of all users. All users of the SDL should be familiar with, and follow, the procedures outlined in this manual. However, the manual cannot nor is it intended to be all encompassing. If situations arise which are not addressed, it is important to request assistance from the laboratory manager.

The use of the SDL is a privilege which will be revoked if there is a failure to demonstrate sufficient competence, care, and responsibility towards the facility, its other users, and the research animals.

**B. OVERVIEW OF THE FACILITY**

The Salmon Disease Laboratory is a 9,000 ft<sup>2</sup> facility which is divided into three sections: 1) the wet laboratory, an inside area for work with infectious agents and an outside area for holding uninfected animals, 2) the preparatory (prep) laboratory and 3) the dry laboratory. The second floor consists of a conference room and offices for staff.

Two wells, each 48 feet deep, supply the SDL with fish pathogen-free water at an ambient temperature of 12.8°C. The water passes through a UV sterilizer before traveling through a packed column where excess gas is eliminated and supplemental oxygen can be added if necessary. This system provides consistent water quality. Effluent from the wet laboratory is treated with chlorine to ensure that no infectious agents used in the laboratory exit via the discharge water. The treated water is subsequently held in chlorine dissipation ponds prior to discharge into the Willamette River. This effluent water is monitored daily to meet D.E.Q. out fall standards.

The primary function of the SDL is to provide holding facilities for experimental fish used in disease research.

The outside circular stock tanks available are:

2	6 ft
6	4 ft
35	3 ft
4	5 ft
2	12 ft

The inside laboratory has:

120	25L tanks
128	100L tanks
18	30 gallon aquaria

Tank capacities are provided in Table 1. The wet laboratory has some flexibility in tank configuration and numbers. Each section of the wet laboratory is equipped with storage

space for equipment and fish food. The outside tanks are used for stock fish. The preparatory laboratory is for experimental manipulations of live fish and necropsies. The deep sink can hold two glass aquaria which can be used for anesthesia and recovery of experimental animals. The dry laboratory provides a basic microbiological laboratory. This area is provided with centrifuges, microscopes, spectrophotometers and other equipment for general use. **Fish or eggs intended for placement in rivers must be held outside in the stock area, and never inside**

## **C. FISH HANDLING**

The proper care of experimental animals is tantamount to the success of the research that uses them. This is especially true when fish are the experimental animal; therefore, appropriate fish husbandry is important to research at the SDL. Each scientist is responsible for her/his fish and is required to be familiar with their humane care. If experimental animals are neglected, not only will research results be jeopardized, but also the privilege to use the facility.

### **1. Tank allocation**

Prior to initiation of experiments at the SDL and the acquisition of fish, tank space must be requested and approved. The appropriate form is on page 13 and should be submitted to the laboratory manager as far in advance of tank need as possible.

Selection of appropriate size tanks for experimental use is dependent on numbers of fish, size and duration of the test, etc. Table 1 gives tank capacities and is based on conservative parameters so that fish will not be crowded, exposed to unnecessary stress and related potential complications. The guide is based on densities of 2 lbs (0.9 kg) of fish per cubic foot (28.3 l) of water. For every one-gallon (3.8 l) per minute inflow, 5 lbs (2.3 kg) of fish may be stocked. Fry should be stocked at about 3 lbs (1.4 kg) per every gallon (3.8 l) per minute inflow.

### **2. Preparatory and Dry Laboratory Use**

The preparatory and dry labs' counter space and equipment is available for general use. Please check with the lab manager for availability especially for periods of extended use (i.e. over ½ day). Use of any lab equipment at a location other than the SDL must be approved by the lab manager and listed on the check-out clipboard in the prep lab.

The Laboratory does not have a budget for tools (i.e., auto-pipettes, knives, syringes, needles, gloves and etc.) and materials (i.e., chemicals). Everything at the SDL has been purchased with grant funds for specific projects being done at the lab. When you are coming to the SDL to perform procedures on your fish, PLEASE bring your own supplies. If you need something you have forgotten, please ask and then replace what you have used. If you need some storage space for items you will be using often at the lab, please ask and a place will be provided. It is best to clearly mark your tools and materials with your name.

### **3. Fish Acquisition**

Experimental fish may be obtained from a variety of sources but the most common are hatcheries operated by the Oregon Department of Fish and Wildlife (ODFW). The ODFW requires that requests be made and approved in advance (see sample form on page

15). Fish from hatcheries not operated by the ODFW should be requested through appropriate channels.

The ODFW invests significant amounts of time and money rearing the fish donated to research. Proper appreciation is to be extended to them.

#### **4. Fish Transport**

The weight of fish which can be safely transported is a function of parameters that include aeration system efficiency, transport time, fish size, water and air temperature, transport container size and fish species. Consultation with experienced personnel is suggested. A conservative rule of thumb is to transport no more than 0.25 lb (1.1 kg) of fish per gal (3.8 l) of oxygenated water. Use the maximum volume of water possible.

Several different containers are used to transport fish depending on number and size. There is a transport unit available for an 8' pickup bed. Disinfect transport unit/containers before leaving the SDL.

##### **Equipment necessary for fish transport:**

1. sanitized transport container
2. oxygen cylinder(s) with known volume of gas  
- with backup cylinder for long trips
3. cylinder cradle or holder
4. two gas regulators for oxygen cylinder – one as a backup
5. wrench
6. disinfected air lines, air stones and manifold
7. rope and/or bungee cords
8. thermometer
9. ice which is chlorine free (optional)
10. nets
11. buckets
12. transport buffer (optional)

At the facility where fish will be obtained, assemble the oxygen system, rinse and fill transport containers with hatchery water and check the water temperature. Start oxygen to supply a gentle stream of bubbles. Too much oxygen can be as detrimental as not enough. Add fish with as little stress as possible. Use hatchery equipment for fish handling and inquire as to proper disinfections procedures. **Nets and buckets from SDL should not be used to load hatchery fish.**

During transport, monitor the water temperature (adding ice if required), oxygen flow, and general condition of the fish. Intervals of 30-45 min are not excessive.

At the SDL, drain previously cleaned and sanitized tank(s) which have been assigned. Add fish in transport water and supplement with oxygen. Slowly introduce SDL water until approximately one tank volume has been added, increase water flow and remove oxygen. This tempering process will decrease the amount of stress experienced by fish from changes in water quality (temperature, pH, gas).

All equipment which came in contact with fish or water during transportation should be thoroughly disinfected for 15 min with a solution of 75 ppm iodophor. The pressure remaining in the oxygen cylinder should be recorded on a tag before the cylinder is returned to storage.

Label tanks with researcher's name, laboratory affiliation, size and number of fish species, pathogen (if any) used, ACUP number, emergency contact information, and initiation date. Upon termination of tank, write termination date and place in envelope on tank map board. Labels are in the prep laboratory. A tank map located in the preparatory laboratory is to be labeled with the researchers initials, initiation date and termination date when the tank is empty and clean. Fish in inappropriately labeled tanks or in unallocated tanks will be eliminated.

## 5. Feeding

Care and feeding is the responsibility of each researcher. Table 2 gives recommendations on type of feed for different sizes of fish. Ideally, fish should be fed 2-3 times per day, more often for smaller fry. For growth, fish require 2.5% of their body weight per day in feed; 1-2% body weight per day is considered maintenance.

Different sizes of feed are provided, but it is the responsibility of each researcher to ensure the appropriate feed for her/his fish is requested. Notify the lab manager if you open the last box of a particular size food. Feed containers are provided and should be labeled with the researcher's name, size and type of food, and tank numbers. Food in unlabeled containers will be discarded. Feed for daily use is stored in freezers either in the outside stock area or inside the wet lab. The walk-in cooler is for bulk feed storage only.

At the termination of each experiment, remaining food should be discarded; containers cleaned and disinfected. **DO NOT PLACE ANY FISH CARCASSES OR FISH TISSUES IN SDL FOOD FREEZERS.**

Feed stock fish first, then feed experimental controls and infected fish last. If gloves are worn, they should be changed or disinfected between each group. Feed small quantities of food spread over the surface of the water; do not pour food into the tank and do not feed all food at once. In general, feed on the tank bottom will not be eaten.

## 6. Routine Tank Cleaning

Tanks should be cleaned at a minimum of once or twice weekly. During sunny periods, outside tanks and water lines will require frequent cleaning because of algae growth. The sides and bottom of the tanks should be cleaned with a soft brush which has been disinfected. **No abrasive cleaners or scrub pads are to be used.** Care should be taken not to injure any fish. After brushing, remove the inner stand pipe to flush the tank. Clean the stand pipes if required. Hose down the floor around and under the cleaned tanks. The laboratory manager will show how this should be done.

If fish escape down the drain during cleaning, or during any other manipulations, they are to be recovered at the chlorination pond. Any fish which escapes is not to be returned to the tank and should be killed and placed in the freezer designated for mortality. If the floor becomes contaminated, disinfect with a working dilution of iodophor, wash with water and squeegee to remove excess water. All cleaning equipment is to be disinfected by submersion

in an iodophor bath for at least 15 min, then rinsed thoroughly before replacing it in the storage area. Disinfectant tanks are located inside and outside. **Equipment should not be left in the disinfectant.**

#### **7. Veterinary Care of Fish**

Fish are not traditional laboratory animals requiring standard veterinary care. Individual PI's and their employees are responsible for monitoring the health of their animals. The Oregon Department of Fish and Wildlife State Fish Pathologists, who are housed at OSU, and in conjunction with the lab manager will make determinations on the health of questionable fish. The laboratory is visited bi-annually by animal care personnel, and unannounced visits may occur. Be sure to follow Institutional Animal Care and Use guidelines. For more information see the website:  
<http://oregonstate.edu/research/osprc/rc/index.htm>

#### **8. Termination of Fish**

Fish are euthanized by an overdose of anesthetic. Lower the water level in the tank and add 10 ml/L of MS222 stock solution to remaining water. Place expired fish in autoclave bags and put them in the mortality freezer.

Fish or eggs brought to the SDL cannot be removed from the inside wet laboratory alive. The only exception is fish taken from the outside area, live-boxed at an approved site, and returned to the SDL. Once an experiment has been completed, surviving fish must be destroyed.

Because tank space is at a premium **NO PET FISH ALLOWED.**

### **D. CONTAINMENT AND SANITATION**

#### **1. Equipment**

Nets, buckets, lab carts, brushes, etc. are to be used only in their designated area. Do not interchange equipment between areas of the laboratory. Equipment from the outside can be brought inside only when transferring stock fish to experimental tanks. After the transfer equipment is disinfected it should be returned to the outside storage area.

Any equipment used in the wet laboratory must be brushed and disinfected with iodophor solution. Containers with 75 ppm are provided in both areas of the wet laboratory. Disinfect equipment a minimum of 15 min and thoroughly rinse before returning it to its storage area. **DO NOT LEAVE EQUIPMENT IN DISINFECTANT BATH.**

If fish are injected, bled, etc., in the wet laboratory, avoid contaminating your colleague's tanks. Work should be done on carts. Tank lids are not operating theaters. Disinfect carts and floor after use.

#### **2. Mortalities (sic)**

Remove mortalities from experimental tanks daily, more often during active epizootics. Wear disposable gloves (not those used in feeding) when removing dead fish; wash with iodophor between tanks. Use a separate disinfected net for each tank. Pick control or non-infected fish first, then infected mortalities. Place mortalities in separate bags labeled with researcher's name, tank number and date. Store in refrigerator/freezer in prep lab. Avoid

contaminated nets dripping on the floor before being disinfected. The chest freezer in the wet lab is for discard fish and is emptied on Tuesday of each week. Dead fish should **never** be placed in food freezers.

### **3. Tank Disinfection**

Following use, all tanks will be immediately cleaned and disinfected with iodophor (75 ppm). Do not turn off and drain tank until cleaning. If the tank must be left, reduce the flow and leave the tank filled until ready to clean. The tanks, stand pipes, hoses, and lids must be scrubbed inside and out. **Do not use abrasive cleansers or scrubbers.** After cleaning, disinfect tanks for a minimum of 15 min by keeping all surfaces wet by brushing. **Do not** fill tanks with iodophor solution and let run. Rinse tanks, stand pipes, hoses and lids. Replace stand pipes and hoses. Put tank labels with check out date in envelope in the prep lab. Update the tank status on map.

**TABLE 1: Tank Sizes and Loading Capacities**

Commonly available tanks used at the Salmon Disease Laboratory and their capacities.

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Tank Size	Capacity in Cubic Feet (L)	Flow Rate GPM (lpm)	Loading Capacity Based on Flow Max 1b (kg)	Loading Capacity Based on Density 1b (kg) & Flow to Yield Adequate DO
25 L	0.9 (24)	0.58 (2.2)	1.9 (862 gr)	1.8 lbs (817 gr)
100 L	3.5 (99)	0.73 (2.8)	3.6 (1.7)	7.0 lbs (3.2)
3 Foot	13.4 (379)	2.2 (8.4)	11 (5)	26.8 lbs (12.2)
Reduced volume:	(178)			
4 Foot	25 (769)	3.4 (12.9)	17 (7.8)	50 lbs (22.7)
Reduced volume:	(355)			
5 Foot	40 (1112)	3.4 (12.9)	17 (7.8)	100 lbs (45.4)
6 Foot	56 (1600)	(to be determined)	(to be determined)	(to be determined)
12 Foot	282 (7755)	(to be determined)	(to be determined)	(to be determined)
Transport Tanks				
Small white	11 (311)/side	--	--	TBD on air supply
Large green	30 (849) At 12" deep	--	--	TBD on air supply

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Conversion factors:

28.32 l/cuft  
454 gm/lb  
2.2 lbs/kg

**TABLE 2: Fish Size and Recommended Feed Size**

% to feed: kg (lbs) per 100 kg (lbs) fish per day

Fish Size		Pellet Size mm	Water Temperature F (C)						
Weight (g)	Fish/lb		39.2 (4.0)	42.8 (6.0)	46.4 (8.0)	50.0 (10.0)	53.6 (12.0)	57.2 (14.0)	60.8 (16.0)
1.0-1.4	454-324	1.0	0.9	1.5	2.1	2.7	3.2	3.8	4.0
1.4-2.4	324-189	1.3	0.9	1.4	2.0	2.6	3.0	3.6	3.8
2.4-5.0	189-91	1.5	0.8	1.4	1.9	2.4	2.8	3.4	3.6
5.0-8.5	91-53	2.0	0.8	1.3	1.8	2.2	2.6	3.2	3.4
8.5-12.5	53-36	2.5	0.8	1.3	1.6	2.0	2.4	3.0	3.2
12.5-20.0	36-23	3.0	0.7	1.2	1.5	1.9	2.3	2.9	3.1
20.0-30.0	23-15	3.0	0.7	1.2	1.4	1.8	2.1	2.7	2.9
30.0-45.0	15-10	4.0	0.7	1.1	1.4	1.6	2.0	2.5	2.6
45.0-75.0	10-6	4.0	0.7	1.1	1.3	1.5	1.9	2.3	2.4
< 450	< 1.0	5.0	0.5	0.6	0.7	0.8	0.9	1.0	1.1
450-900	1.0-2.0	5.0, 6.0, 8.0	0.3	0.4	0.5	0.6	0.7	0.8	0.9
900-1800	2.0-4.0	8.0, 10.0	0.2	0.3	0.4	0.5	0.6	0.7	0.8
1800-3600	4.0-8.0	10.0, 12.0	0.2	0.3	0.4	0.4	0.5	0.6	0.6
>3600	>8.0	12.0	0.2	0.3	0.3	0.3	0.4	0.5	0.5

**TABLE 3: Treatment - Formalin**

Suggested treatment for ectoparasites

Formalin (37%)		
(3 ft tanks)	1:4000	94 ml (flush)
(1 hr w/air)	1:6000	63 ml
(100 l tanks)	1:4000	25 ml (flush)
(1 hr w/air)	1:6000	16 ml
(1 hr w/air)	1:8000	12.5 ml (fry)
eggs	1:600	
(15 min flow through)		

**Table 4: Tank Rental Rates Effective July 1, 2006**

1. OSU Research and State of Oregon Research

<u>Tank Size</u>	<u>Number of Tanks Available</u>	<u>Cost/Tank/Day</u>
<25 Liter/Aquaria	24	\$ .32
25 Liter	144	\$ .93
100 Liter	112	\$ 1.18
3 Feet dia.	35	\$ 1.66
4 Feet dia.	6	\$ 2.61
5 Feet dia.	4	\$ 2.61
6 Feet dia.	2	\$ 2.61
12 Feet dia.	4	\$ 3.34
Incubator	3	\$ 1.66

2. Private companies and other agencies.

<u>Tank Size</u>	<u>Number of Tanks Available</u>	<u>Cost/Tanks/Day</u>
<25 Liter/Aquaria	24	\$ .48
25 Liter	144	\$ 1.40
100 Liter	112	\$ 1.78
3 Feet dia.	35	\$ 2.49
4 Feet dia.	6	\$ 3.92
5 Feet dia.	4	\$ 3.92
6 Feet dia.	2	\$ 3.92
12 Feet dia.	4	\$ 4.77
Incubator	3	\$ 2.49

**Additional charges will be assessed if special water conditions are necessary.**

**Heated or chilled water charges = \$.0465/L/°F/day**

**Labor will be billed at cost as needed.**

**SALMON DISEASE LAB - USE REQUEST**

Submit two copies to the laboratory manager or email to [shawn.tucker@oregonstate.edu](mailto:shawn.tucker@oregonstate.edu)  
AND [stevensdo@oregonstate.edu](mailto:stevensdo@oregonstate.edu).

DATE: \_\_\_\_\_ SUBMITTED BY: \_\_\_\_\_

Name of Researcher:

Laboratory Affiliation:

Funding Source & Acct. #:

Lab Animal Use Form – Please bring a copy to the SDL - #:

ACUP #:

Project Description:

Length of Study: \_\_\_\_\_ Start Date: \_\_\_\_\_ End Date: \_\_\_\_\_

Fish Species:

Number & Size of Tanks Required:

Type of Pathogen:

Special Needs (temperature, flow, lighting, water treatment, etc.):

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APPROVAL

1. Date tanks available:
2. Tanks allocated:
3. Tank costs:

Fish Request Form

TO: Oregon Department of Fish and Wildlife  
Fish Culture Section  
P.O. Box 59  
Portland, OR 97207

FROM:

Subject:

Number, size, and species of fish:

These fish are to be used for the following experiments:

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Date fish are needed:

Signed: \_\_\_\_\_ Date: \_\_\_\_\_

ODFW review and comment:

Signed: \_\_\_\_\_ Date: \_\_\_\_\_