



**Guidelines for
Vertebrate Experiments at
Oregon State University
John L. Fryer
Aquatic Animal Health Laboratory**

34347 NE Electric Road

Corvallis, OR 97333

541-737-0743

Updated Feb 2022

Table of Contents

1. EMERGENCY CONTACT NUMBERS	5
2. DIRECTIONS TO AAHL.....	7
3. OVERVIEW OF THE FACILITY	8
4. AAHL TANK RENTAL RATES	9
5. AAHL TANK CAPACITIES.....	11
6. THE LIFE OF A RESEARCH PROJECT AT AAHL	12
7. CHECK-LIST FOR IACUC COMPLIANCE:	14
APPENDIX.....	17
AAHL FORM: LABORATORY INDIVIDUAL TRAINING RECORD.....	18
AAHL SOP: USER GUIDELINES AT THE AAHL.....	20
AAHL SOP: FISH ACQUISITION	22
AAHL FORM: TANK REQUEST	24
AAHL SOP: FISH TRANSPORT	25
ODFW FISH TRANSPORT PERMIT	26
AAHL SOP: TANK CLEANING	27
AAHL SOP: FEEDING	31
AAHL SOP: FORMALIN TREATMENT	33
AAHL SOP: Maintaining zebrafish broodstock.....	28
AAHL SOP: RIVER EXPOSURES	36
AAHL SOP: SIGNS OF STRESS AND DISEASE IN FISH	38
AAHL SOP: RECOGNITION AND TREATMENT OF ICH (WHITE SPOT DISEASE)	40
AAHL SOP: EUTHANASIA AND SEDATION.....	42
AAHL SOP: EUTHANASIA AND SEDATION.....	42
AAHL SOP: WATER QUALITY	46
AAHL SOP: EFFLUENT TREATMENT SYSTEM MAINTENANCE	48
AAHL FORM: EFFLUENT TREATMENT SYSTEM MAINTENANCE RECORD	53
AAHL SOP: DAILY, WEEKLY, QUARTERLY, YEARLY TASKS.....	54

AAHL SOP: AUDIT FOR BIOSECURITY AND TO PREPARE FOR IACUC INSPECTIONS 55

AAHL SOP: VERMIN MONITORING AND CONTROL 56

AAHL SOP: MAINTAINING ZEBRAFISH.....52

AAHL FORM: ANIMAL CARE LOG 58

OREGON PROHIBITED SPECIES APPLICATION 60

AAHL FORM: TANK LABELS617

AAHL SOP: EMERGENCY AND ALARM RESPONSE 62

ODFW HATCHERY ASSIGNMENTS FOR FISH HEALTH SERVICES PERSONNEL 68

1. EMERGENCY CONTACT NUMBERS

In the case of an Emergency contact the [Manager](#) straight away anytime of the day or night.

Refer to [Emergency Response Procedures](#) SOP for action to be taken in the event of an emergency.

Name and Email	Designation	Office Phone	Cell Phone
Alarm	OSU Public Safety Dispatch	Emergency: 541-737-7000 Non Emergency: 541-737-3010 (ask for Alarm shop)	
Alarm (phone line)	Service Desk, Information Services Oregon State University	541-737-8787	SteveFowler (head IT services) 541 737 9630
Jerri Bartholomew bartholj@science.oregonstate.edu	Director of AAHL	541-737-1856	541-758-7508 541-224-2743
Ruth Milston-Clements aahl.manager@ oregonstate.edu milstonr@science.oregonstate.edu	AAHL Manager	541-737-0743	541-224-3019
Facilities Services after hours	Public Safety Dispatch (for Facilities Services after hours)	Emergency: 541-737-7000 Non-Emergency: 541-737-3010	
OSU Facilities Services in hours	Facilities Services in hours	541-737-2969	
Dr. Jennifer Sargent	Attending Veterinarian/ Director LARC	541-737-6213	

Tim Miller-Morgan, DVM tim.miller-morgan@oregonstate.edu	Clinical Veterinarian – Aquatics (HMSC)	541-867-0265	541-270-4218
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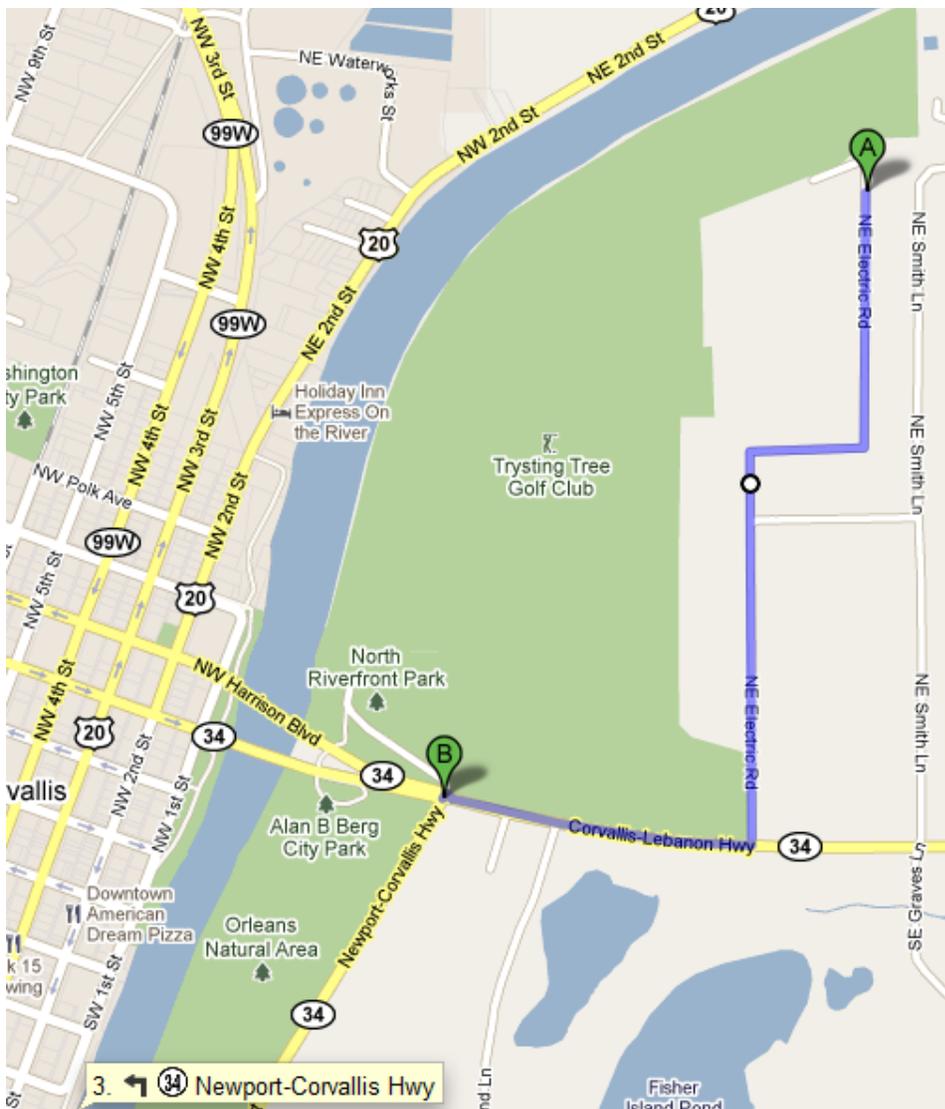
2. DIRECTIONS TO AAHL

From Corvallis, go east on Hwy. 34, cross the Willamette River. In .3 miles turn left at NE Electric Road (Trysting Tree Golf Course). Follow .8 mile, road turns right at Peach Place, left at T, and right at Y. You'll see a sign for our facility.

AAHL physical address:

34347 NE Electric Road
Corvallis, OR 97333

541-737-0743



3. OVERVIEW OF THE FACILITY

The John L. Fryer Aquatic Animal Health Laboratory (AAHL) is a 9000 sq. ft. [AAALAC](#) accredited regional fish disease research facility dedicated, but not limited to, the study of organisms infectious for salmonids and other species of freshwater fish. The AAHL has wet laboratory capacity for maintaining fish, as well as providing space for an analytical laboratory, a [Biosafety Level 2](#) (BSL2) laboratory, a microscopy room, office space and a conference room/library.

The laboratory is supplied with specific-pathogen free fresh water from two wells, each with a capacity of more than 300gpm. The water, which has an ambient temperature of 12.8°C, is processed through UV sterilization and a degassing tower to eliminate pathogens, gas supersaturation, and allow oxygen to saturate into the water. Effluent from the laboratory is treated with chlorine to prevent the escape of any pathogens from the facility. All systems are redundant and can be operated with a diesel-electric generator in the event of commercial power failures.

The wet laboratory is divided into two sections, one for stock fish and experiments not involving infectious agents; the other for fish to be used in disease experiments. The stock area has 49 tanks of various sizes (Table 1). The experimental area has 25 and 100-liter tanks (Table 2). The wet laboratory has some flexibility in tank configuration and numbers. Each section of the wet laboratory has storage space for equipment and fish food.

Table 1: Outside tanks available

Tank Size (Diameter)	Number Available
3 ft	36
4 ft	9
5 ft	3
12 ft	2

Table 2: Inside tanks available

Tank Size (Volume)	Number Available
25L	120
100L	128

In addition, an isolation/quarantine laboratory provides an area to work with non-endemic aquatic pathogens. The isolation facility has 24 twenty-five liter tanks and over 300 square feet of space for specialized studies, plus a separate necropsy room.

Heated water provides high-quality fresh water for warm water species and for research on the effects of temperature on disease processes. Tanks can be programmed to be heated with water from 5-30°C, making this laboratory ideally positioned for conducting studies on the effects of climate change on aquatic organisms. Temperature controls can also be programmed to replicate diurnal temperature fluctuations, allowing researchers to closely emulate natural conditions. The laboratory also has some capacity for chilling water below ambient temperature. UV treated Willamette River water is also available at the AAHL, which has proven useful in sustaining invertebrates and with experiments wanting to incorporate the natural microbiome.

4. AAHL TANK RENTAL RATES

OSU Research and State of Oregon Research

Tank Size	Location	Number of Tanks Available	\$Cost/Tank/Day Ambient 12.8C	\$Cost/Tank/Day Heated or Chilled
<25Liter/Aquaria	Indoor	50	0.40	0.58
25 Liter	Indoor	144	1.85	2.09
100 Liter	Indoor	112	1.91	2.19
3 Feet dia.(380L)	Indoor	15	2.47	2.97
3 Feet dia.(380L)	Outdoor	35	0.85	inquire
4 Feet dia.	Outdoor	6	1.17	inquire
5 Feet dia.	Outdoor	4	1.17	inquire
12 Feet dia.	Outdoor	2	4.25	inquire
Incubator	Indoor	2	n/a	2.04

Additional charges will be assessed if special water conditions are necessary. Labor (cleaning, feeding, monitoring) will be billed at cost as needed.

BSL2 Containment Dry Laboratory: \$10/day

Includes exclusive use of room, biosafety cabinet (certification, service, decontamination), waste stream autoclave/incineration, and disposal, etc.

BSL2 Isolation Room/Containment Wet Laboratory: \$10/day

Facilities lease for BSL2 isolation room or using non-native species/pathogens or BSL2 level pathogens. Includes exclusive use of isolation tank room, necropsy area, refrigerator, decontamination, wste stream autoclave/incineration and disposal etc. Doe not include tank fees.

SERVICES AND SUPPORT RATES

Hourly Student Support \$11.00/hour

Provide support for requested project assistance. Can include animal care and husbandry, health checks, feeding, tank set up, cleaning and disinfecting and collection of samples if needed.

Technician/Staff Support \$32.00/hour

Provide support for requested project assistance. Can include husbandry, health checks, feeding, tank set up, cleaning and disinfecting and collection of fish samples or filtering of water samples if needed. May also include assistance with ACUPs, literature searches. May include minor procedures such as tagging, necropsy, tissue collection, formalin or hydrogen peroxide treatments, bacterial streaks etc. Can include assistance with ACUPs, limited literature searches, experimental set up fabrication/minor plumbing changes.

5. AAHL TANK CAPACITIES

Commonly available tanks used at the Aquatic Animal Health Laboratory and their loading capacities for fish.

Size	Capacity	Flow Rate	Loading Capacity Based on Flow Max	Loading Capacity Based on Density & Flow to Yield Adequate DO
	Cu Ft (L)	GPM (LPM)	lb (kg)	lb (kg)
25 L	0.9 (24)	0.58 (2.2)	1.9 (862 g)	1.8 lbs (817 g)
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)
3 ft Reduced volume	(178)			
4 Foot	25 (769)	3.4 (12.9)	17 (7.8)	50 lbs (22.7)
4 ft Reduced volume:	(355)			
5 Foot	40 (1112)	3.4 (12.9)	17 (7.8)	100 lbs (45.4)
6 Foot	56 (1600)	3.4 (12.9)		
12 Foot	282 (7755)	(to be determined)		

6. THE LIFE OF A RESEARCH PROJECT AT AAHL

Please follow AAHL Standard Operating Procedures (SOPs) and use AAHL forms. An IACUC compliance checklist follows providing links and forms to help you meet requirements of vertebrate animal research.

1. Discuss your project with our director, Jerri Bartholomew, bartholj@science.oregonstate.edu: 541 737-1856, and contact OSU Aquatic Animal Health Lab Manager Ruth Milston-Clements AAHL.manager@science.oregonstate.edu; 541-737-0743 to confirm tank availability and see how we can meet your needs.
2. Prepare an [ACUP](#); see IACUC website for forms, guidelines and training requirements: <http://research.oregonstate.edu/iacuc>
Note: Outside users may consider adding a Co-PI from OSU to your ACUP to ease the process and allow continuation of your project in the event your PI is unavailable. See [Jerri Bartholomew](#) for details.
NO VERTEBRATES MAY BE HELD AT THE AAHL WITHOUT PRIOR ACUP APPROVAL.
3. Obtain Institutional Biosafety Committee ([IBC](#)) approval for your pathogen
4. Complete all [training](#) required for PI and people involved with husbandry or procedures
5. Employees, students and volunteers involved only in cleaning and feed need to complete an Occupation Health and Safety Survey and Animal Handler Safety Training. <http://oregonstate.edu/ehs/bio/animal-handler>
6. Provide a copy of the approved ACUP, training confirmation, and tank request to lab manager AAHL.manager@science.oregonstate.edu
7. Acquire fish
 - a. Order from commercial supplier (e.g. www.troutlodge.com) or contact Oregon state hatcheries for availability. Fill out ODFW Transport Permit. <https://nrimp.dfw.state.or.us/FishTransportPermit/>
 - b. Are your fish a non-native species to Oregon? Contact ODFW Invasive Species Coordinator, Rick Boatner Rick.J.Boatner@state.or.us. You may have to submit a [Prohibited Species Application](#).

If you your species are being transported into to Oregon, you may need to have a sub-sample tested for pathogens prior to transporting your experimental animals. Allow up to 1 month for testing. Contact ODFW Rick Stocking richard.w.stocking@state.or.us 541 325 - 5343 for more details or ODFW Senior Fish Health Specialist Craig Banner bannerc@onid.orst.edu

- a. Let [Attending Veterinarian](#), helen.diggs@oregonstate.edu know about your project and proposed delivery date of fish, she may want to inspect them.
 - b. Transport fish. Refer to the AAHL SOPs: [Fish Transport](#) and Obtaining an ODFW [Fish Transport Permit](#). Small fish from a commercial source will be shipped.
8. Get the access code to the Keypad so you can access the building after hours, see Ruth Milston-Clements
 9. Prepare [labels](#) for every tank and [daily husbandry and mortality logs](#) to be kept near tanks. Labels are in drawer in Prep Lab, in addition to your husbandry care and mort records, you must update the lab record sheet kept in Prep Lab.
 10. Care of fish: Researcher is responsible for husbandry: health checks, daily feeding, tank cleaning, and monitoring of their experiments

Need Help? Researchers may request support from AAHL staff in any or all of these areas. Contact the lab manager in advance to arrange assistance AAHL.manager@oregonstate.edu

Using the dry lab

- We have basic supplies, but researcher is responsible for purchasing project-specific supplies
- If you're using equipment or lab space for more than a few hours, let other lab users know
- Clean up and put away every day.
- Communicate with manager about your needs
- Inspections occur periodically. IACUC inspects spring and fall (announced), manager intermittently (unannounced)

7. CHECK-LIST FOR IACUC COMPLIANCE:

The use of fish or other vertebrates for research, teaching, or testing at AAHL is supervised by the Oregon State University Institutional Animal Care and Use Committee (IACUC). This body must approve and oversee any vertebrate research and your Animal Care and Use Proposal ([ACUP](#)) must be approved before any animals are acquired or manipulated. The approval process may take up to eight weeks to complete, so it is essential for you as a researcher to submit your Protocol to the committee far in advance of the initiation of your project. The ACUP must be renewed every three years and requires yearly reporting.

In addition, the [Institutional Biosafety Committee](#) needs to approve the pathogen, biohazard or recombinant DNA products you are working with. This approval needs to be attained before the IACUC can approve your ACUP, so you will need to submit these forms concurrently with your ACUP proposal.

There are certain [training](#) requirements in order to have your ACUP approved and work at the AAHL

Below is a simplified IACUC check-list. More complete instructions are available at: <http://research.oregonstate.edu/iacuc>

ACUP Forms

<http://research.oregonstate.edu/iacuc/iacuc-forms>

[Animal Care and Use Proposal \(ACUP\) Form 2013](#)

This form is for new or continuing projects. It must be completed and approved by the IACUC prior to initiating vertebrate animal projects. (may take up to 8 weeks)

Information on responding to questions on the new ACUP form. Recommended as a reference when completing or submitting the new ACUP form.

[Animal Care Use and Renewal Form/Final Report Form](#)

An ACUP renewal form is required every year for renewal of approved projects and when an ACUP is closed/expired.

[Animal Care and Use Amendment Form](#) (2-8 weeks)

For addition of a new protocol or pathogen, new personnel processing or manipulating fish to an existing ACUP. Staff members simply cleaning tanks don't need to be on ACUP.

1. **IBC Institutional Biosafety Committee** forms <http://oregonstate.edu/ehs/bio/ibc-registration-instructions-forms>

Approval needed for work involving: Biohazards, infectious agents, recombinant DNA products, or the creation or breeding of genetically modified animals. See Website: <http://oregonstate.edu/ehs/bio/institutional-committee>

2. **Training** for everyone processing or manipulating fish, training must be renewed every five years

Students participating in classes and volunteers do not need to enroll in this program, but should be informed of any risks.

It is the responsibility of each PI to insure that they and their staff have the required training before performing manipulations or husbandry tasks. Please provide the lab manager with documentation of this training.

You will need to complete the following training requirements (1 - 3 are required in order to obtain your ACUP, 4 and 5 will be carried out at the AAHL before you start your project).

- I. Health surveillance form for the Occupational Health and Safety Program.

The form may be accessed at: <http://oregonstate.edu/occupationalhealth/animal-handler>. Please submit this form to Martha Adams, Occupational Health, R.N., as directed on the form. Martha.Adams@oregonstate.edu, Annual renewal required.

- II. Animal Welfare Education (AWE) training. (Animal Care and Use Training).

Classes are offered monthly in the Kerr Administration building, during the 2nd Tuesday of each month beginning at 3:00 p.m. Please send a training request to the IACUC inbox at <mailto:IACUC@Oregonstate.edu> (in-person training is preferred.) However, if a schedule conflict occurs or personnel are located off-campus, AWE requirements may be met by completing the on-line CITI (Collaborative Institutional Training Initiative) module, "Working with the IACUC." Please submit your request to the IACUC Staff at IACUC@Oregonstate.edu to pursue this option (the website is www.citiprogram.org and incurs no fee).

- III. Environmental Health and Safety (EH & S) Animal Handler training.

Classes are offered monthly by Matt Philpott in the Oak Creek building during the first Thursday of each month. Contact Kay Miller Kay.Miller@Oregonstate.edu to register. Alternatively, this training may be completed on-line and accessed via the following link: <http://oregonstate.edu/ehs/training>.

IV. AAHL in-house training (as required by IACUC):

On your arrival at the Aquatic Animal Health Laboratory, prior to starting your project you will be given training by the lab manager or other personnel on AAHL laboratory safety, fish care and husbandry procedures and other SOPs pertinent to your project.

Included in this training is the following reading material:

- [Care and Use of Fish \(PDF\)](#)
- [Biosafety Spill Response Guide \(PDF\)](#)
- [Biosafety in Microbiological and Biomedical Laboratories 5th Ed \(PDF\)](#)
- [Fisheries Safety Handout \(PDF\)](#)

Each Individual must complete an [individual training record](#) and submit to the PI and the Lab Manager

3. **Contact OSU's Attending Veterinarian** /Director LARC: Dr. Helen Diggs, 541-737-6462, helen.diggs@oregonstate.edu, she must be aware of:
- i) Planned work with animals
 - ii) Planned acquisition of fish and proposed delivery date
 - iii) Housing, husbandry care
 - iv) Health issues as they arise. Any medical treatments not included in your ACUP proposal must be approved by the veterinarian.
 - v) Fish moved from one ACUP to another or one researcher to another—submit an animal transfer form: http://oregonstate.edu/dept/larc/transfer_internal_form.php
 - vi) If an unexpected negative event happens to the fish, submit an Adverse Event form: [Animal Care and Use Committee Adverse Event Form](#) 

The lab manager is the point-person for local oversight and communication with veterinarian.

- Accident report if any personnel are injured while working at the lab: [Accident Report Form](#)

APPENDIX

AAHL
STANDARD OPERATING
PROCEDURES & FORMS

Training for any personnel who works in the laboratory:

Laboratory Safety Training Requirements (EH&S)

1. Hazardous waste training and quiz (video and quiz) Date:
2. Laboratory Safety Training and quiz (video and quiz) Date:
3. Hazard Communication :SDS and GHS (global harmonizing system) video and quiz) Date:

Signature _____ Date _____

Provide PI and Laboratory Manager with copy

AAHL SOP: USER GUIDELINES AT THE AAHL

Preparatory and Dry Laboratory Use

The preparatory and dry lab 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 1/2 day). Use of any lab equipment at a location other than the AAHL must be approved by the lab manager.

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 AAHL has been purchased with grant funds for specific projects being done at the lab. When you are coming to the AAHL 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 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.

Wet Lab requirements

Individual principal investigators and their research staff are responsible for monitoring the health of their animals, as well as performing cleaning, feeding, and record keeping. Users must use the [daily animal care log](#) and keep these records by your tanks at all times for inspection.

Your staff will be provided with [training](#) for feeding and care of fish. If you have questions contact the lab manager.

Every tank must be [labeled](#) with: principal investigator, researchers involved, ACUP number, infectious agent, initiation date, projected termination date, species and number of fish, contact phone numbers. Pre-printed waterproof labels are available in the top drawer of the prep room.

Fish should be fed appropriately: fry up to 7 times a day, when they're eating 4ml food they can be fed 6 times a week; fish more than 1 year old can be fed every-other-day, or as approved on your ACUP. Fish tanks must be cleaned weekly and flushed 3-4 times a week, or as approved on your ACUP. Refer to AAHL SOP: [Feeding](#)

Cleaning, feeding, and mortalities must be recorded on our [form](#). Records must be kept on or near tanks. Records must be retained for six years and we request that you leave them with the lab manager when your project concludes.

Prevent inadvertent spread of disease by using sanitized nets and brushes for each tank, wash hands/gloves between tanks, and prevent spatter of water to neighboring tanks. Wear gloves when feeding fish. Use footbaths when entering or leaving a fish holding area.

In addition to your own lab notebook, several aspects of a research project must be tracked:

- [Daily Health, Husbandry Mortality Record](#): Keep records updated daily, place at end of row for manager to check. Report mortalities that are unexpected or that occur after a procedure.
- ACUP and [revisions](#): Provide manager with copies of revisions and apprise her of changes.
- [Formalin treatments](#) and amount of use (record and give to lab manager, who makes quarterly reports to DEQ).

Waste Disposal:

NO FISH LEAVES THE FACILITY ALIVE (except for caged river exposures)
NO FISH OR OTHER BIOLOGICAL WASTE LEAVES WITHOUT BEING AUTOCLAVED
OR INCINERATED:

Fish from the outside area of the facility will be autoclaved.

Fish from inside the facility will be incinerated.

Fish, gloves, samples, messy necropsy paper all go into the chest freezer just outside the prep lab. Use autoclave bags for waste from the outside area of the facility and use the red bio-waste bags for waste from inside the facility.

The manager will autoclave your bio-waste or send it for incineration as necessary.

AAHL SOP: FISH ACQUISITION

ACUP approval, amendments, and personnel training must be completed before fish are brought to the facility. Procurement of fish must be approved by the Attending Veterinarian, who may also wish to inspect the fish upon arrival (see Page 12). Capture of fish must be approved by IACUC. If you have an urgent situation or an unusual opportunity to acquire unique animals, contact the AAHL manager aahl.manager@oregonstate.edu (Ruth Milston-Clements) who will assist you with contacting the IACUC to discuss your options. If your project is not described in your current ACUP, you may contact the Attending Veterinarian for approval and to request putting your fish on her holding ACUP or the AAHL holding ACUP until the ACUP amendment has been accepted. Contact Rob Gabel if an [amendment](#) needs to be expedited, he may be able to shorten the approval time.

Sources of fish:

Fish or eggs may be purchased from an aquaculture company such as <http://www.troutlodge.com>, (253) 863-0446 or acquired from the Oregon Department of Fish and Wildlife (ODFW). ODFW has been very helpful in providing fish or eggs without costs from their hatcheries if they have surpluses to their programs. The AAHL manager (Ruth Milston-Clements) can help you locate a hatchery that may have the type of fish that you need. The next step would be to contact the hatchery manager for availability and numbers.

The Aquatic Animal Health Laboratory occasionally has fish stocks available for researchers. Check with the manager for availability.

Acquiring fish:

1. Get approval from your PI before you get fish.
2. Contact Ruth Milston-Clements aahl.manager@oregonstate.edu for assistance with this process
 - a. Are your fish a non-native species to Oregon? Contact ODFW Invasive Species Coordinator, Rick Boatner Rick.J.Boatner@state.or.us for assistance. You may have to submit a [Prohibited Species Application](#).
 - b. If your species are non-native to Oregon, you may need to have a sub-sample tested for pathogens prior to transporting your experimental animals. Allow up to 1 month for testing. Contact ODFW Aimee Reed aimee.reed@oregonstate.edu for more details
3. The AAHL manager will Contact [Attending Veterinarian](#) about your plans if necessary
4. Complete [Transport Permit](#) and ODFW fish request form. You can ask the AAHL manager to do this for you as they already have an account.

Oregon Department of Fish and Wildlife
3406 Cherry Avenue NE
Salem, Oregon 97303
(503)-947-6249

If you have any questions Trevor Clerk trevor.r.clark@odfw.oregon.gov at ODFW is in charge of approval of transport permits.

5. Fill out a [tank request](#) at AAHL at least one month before fish arrive
6. If fish are from AAHL stock or another research project, keep track of the numbers of fish you transfer to a different ACUP. The ACUP that you transfer fish to must account for the number of fish you transfer. If it does not, you will need to submit an [amendment form](#) for “Change in number of animals” in the ACUP you transfer fish to before you commence experiments.
7. Don’t get or use more fish than you’ve asked for in your ACUP. Extra fish may be transferred to the AAHL holding ACUP so they can be used for other projects, but check to see if this will require an ACUP amendment.

AAHL FORM: TANK REQUEST

AQUATIC ANIMAL HEALTH LAB – TANK REQUEST
Submit to the laboratory manager
AAHL.manager@science.oregonstate.edu

DATE: SUBMITTED BY:

Name of researcher/ primary investigator:

Laboratory affiliation:

Funding source and index number:

ACUP number:

- Submit a copy of your ACUP to laboratory manager on paper or by email

Project description:

Length of study: Start date: End date:

Fish species:

Type of pathogen:

Number and size of tanks requested:

Special needs (temperature, flow, lighting, feed, etc.)

APPROVAL:

Date tank available;

Tanks allotted:

Tank costs:

Manager's signature:

AAHL SOP: FISH TRANSPORT

The weight of fish that 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 AAHL.

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 airlines, 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) such as Amquel or Stress Coat

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, causing as little stress as possible. Use hatchery equipment for fish handling and inquire as to proper disinfections procedures. Nets and buckets from AAHL 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 AAHL, drain previously cleaned and sanitized tank(s) that have been assigned by the lab manager.

Add fish in transport water and supplement with oxygen. Slowly introduce AAHL 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). Ideally, no more than 2C temperature change per hour, and allow an hour for tank volume to turn once.

All equipment that came in contact with fish or water during transportation should be thoroughly disinfected for 15 min with a solution of 75 ppm iodophor or 1 ppt Virkon AQ. The pressure remaining in the oxygen cylinder should be recorded on a cylinder tag before returning the cylinder to storage.

ODFW FISH TRANSPORT PERMIT

<https://nrimp.dfw.state.or.us/fishtransportpermit/>

You will need to create an account and get a user name and password to get to obtain the form.

AAHL SOP: TANK CLEANING

Before you perform any of these tank cleaning procedures at the AAHL, you will need to undergo training by the AAHL manager or other trained personnel. Included in the training course is watching the AAHL tank cleaning DVD (see manager for a copy).

There are 3 levels of tank cleaning:

Type of tank cleaning	Frequency
Flushing	2-4 times a week
Scrubbing	Weekly
Disinfecting	At the end and before an experiment

***IMPORTANT – to prevent cross contamination*:**

- wear gloves
- you must wash your hands/gloves (or replace gloves) in disinfectant and thoroughly rinse them between EACH tank
- Only open the lid of one tank at a time (splashes can go over from one tank to another and spread disease!)

ONLY USE INSIDE BRUSHES AND BUCKETS FOR INSIDE TANKS AND OUTSIDE BRUSHES FOR OUTSIDE TANKS

Flushing tanks (lifting standpipe to flush feces and uneaten food)

- To lift standpipe, pull inner standpipe up by the wire loop.
- Use the piece of standpipe to direct debris to drain.
- Create vortex to swirl debris toward center by directing spray of water across top of tank water on one side.
- When most debris has been flushed (drain tank to about six inches), replace standpipe.
- Check tank a few minutes later to make sure the water level is rising. Update the husbandry record sheet.

Scrubbing tanks (scrubbing lids and standpipes to remove biofilm)

- Gather clean brushes
- Always rinse brushes to make sure that no disinfectant remains from previous use.
- Rinse brushes for at least 1 minute and until there are no “foamy bubbles” evident.

- Lift inner standpipe to flush loose debris and lower the water level.
- Before removing the outer standpipe, allow water level in the tank to drop below the water level in the inner standpipe (if you do not do this, small fish will get suctioned down the inner standpipe when you remove the outer standpipe).
- ****Do not remove the inflow hose at anytime and do not turn off the inflow water****
- As the water level is dropping, follow the water down just above the water line and scrub the tank with large brush or cloth glove to remove biofilm.
- Pay attention to the corners, drain hole, and the 'bath tub' ring, especially in the back.
- Replace the inner standpipes when level is down to approx 6 inches (dependant on how many/size of fish in the tank, this could be higher).
- Remove outer standpipe and scrub inside and out with smaller brushes.
- Rub tank and standpipes with fingers. They should be squeaky clean.
- Do not stress the fish unnecessarily with loud noises, abrupt motions, or water level lower than six inches.
- Replace outer standpipes.
- When tank is clean, rinse brushes and gloves, immerse in disinfectant for at least 15 minutes before re-use.
- Check the tank a few minutes after you've completed cleaning to make sure the water level is rising.
- Hose off the floor thoroughly.
- Update the husbandry record sheet with the cleaning or standpipe lift that occurred that day.
- Wear gloves and/or wash your hands in disinfectant and thoroughly rinse them between EACH tank.
- Rinse and put away brushes, standpipes, or other equipment after disinfection.

Tank Disinfection:

BE EXTREMELY careful not to splash iodine into any adjacent tanks that have fish in them.

- Tanks must be disinfected within two days of termination.
- Mark tanks to be disinfected with a piece of red tape and remove tape when completed.
- Leave water running until tank is disinfected (otherwise biofilm sets hard on the surfaces and is very difficult to remove).
- Use iodine for tank and wooden bench disinfection, it stains biofilm and will help you get every little spot clean.
 - Make up a bucket of Iodine solution (stored in a lidded bucket, outside under the disinfectant bath): fill bucket with water from the hose, add about 50 to 100ml iodine (estimate by eye is OK) mix with a brush.
 - Make the Iodine the color of strong tea (add more if you think it is not strong enough). You can re-use the iodine solution the next day, but you may need to top it up as it breaks down in light.
- Remove: airline, airstone, water hose, inner and outer standpipes, lids
 - Take these items to the sink next to the virkon bath
 - Using pipe cleaning brushes scrub clean of any biofilm
 - Separate the airstones from the airlines
 - Separate the inner standpipe into 2 halves
 - Draw the pipe cleaning brushes of appropriate size through the standpipes to clean
 - Draw the very small pipe cleaning brush with the really long handle through the water line – you need to feed the handle in first and pull back towards you else it won't go through.
 - Do a quick rinse under the faucet to remove any remaining biofilm
 - Place the brushed clean items in the virkon bath (DO NOT PUT DIRTY ITEMS IN THE VIRKON BATH)
 - Disinfect items for at least 15 minutes in virkon bath
 - Rinse the disinfected items thoroughly in the sink (Virkon is VERY toxic to fish at very low concentrations so you need to make sure they are clean of Virkon.
 - Allow items to dry on drying rack overnight.
 - Once dry, put items away in the “clean storage area” (boot room).
- Remove the tank from the rack and place on the floor
- Thoroughly scrub inside and outside surfaces of the tank with an iodine soaked brush.
- Rinse any biofilm away using the hose
- Once it is ‘clean’ of any biofilm- drench the surfaces with Iodine and allow 15 minutes contact time
- Scrub the wooden support rack, get all evidence of dirt off the wooden support rack.
- Scrub the surrounding floor.

- After at least 15 minutes of contact time of iodine on tanks and wooden bench- hose the iodine off the tanks and benches being EXTREMELY careful not to splash iodine into any adjacent tanks that have fish in them.
- Replace the tank to the wooden rack
- Place tank label in plastic pocket near the door in the necropsy room.

- If the lid or any area of the tank does not come clean of black mildew (especially around the hinge area), the “water line” inside the tank and the outside of the tank –
 - Use chlorine solution from the chlorine vat to remove any black mildew staining.
 - Wearing gloves and eye protection and protective clothing (like yellow raingear), get a small bucket of chlorine solution, scrub the area, allow to penetrate for at least an hour then rinse and dry.
- Wash your hands.

USE YOUR TIME WISELY: i.e. don't sit around waiting for 15 minutes for one tank to disinfect, go on to the next one and get several scrubbed then return to the first one to rinse.

AAHL SOP: FEEDING

Care and feeding is the responsibility of each researcher unless otherwise requested and prearranged with the manager. As they grow, fish may need a feed adjustment based on observations of body conformation and appetite, so frequent observation of feeding by researchers is important. Feed frequency and rates are dependent on age/size of animal (refer to the [Feeding Table](#)).

Fry should be fed 2 to 7 times a day and the AAHL has timed feeders available. Fish 3-18 inches should be fed at least six times a week.

Large fish may be fed every other day.

For growth, fish require 2.5% of their body weight per day in feed, 1-2% body weight per day is considered maintenance. Overfeeding can be as bad as underfeeding.

Various sizes of feed are provided at the AAHL and stored in a walk-in cooler. Speak with the manager to be sure that the size or type of food you require will be on hand. Food must always be kept in covered containers in the walk-in cooler. Leave no food outside of the walk-in cooler.

- Prevent cross-contamination by wearing gloves;
- Feed stock fish first, experimental controls next, and infected fish last.
- Feed fish slowly.
- Make sure water from the tank does not splash into your feed container
- Generally, any food left on the bottom of the tank will not be eaten.
- Fish species have varying feeding habits. For example, rainbow trout will eat eagerly to the point of death and Chinook salmon prefer small meals and may need to be fed small amounts several times a day.

Ask the lab manager if you have questions.

Food

- Feed should be used or discarded 24 months (depending upon storage conditions) after date of opening. Store food in the walk-in cooler. Some feeds have shorter or longer shelf-lives (For example Medicated food is usually 12 month shelf life and is best stored in the upright freezer). Consult manufacturers' recommendations for additional information if needed.
- Let the manager know in good time if food is running low and needs to be reordered.
- Make sure feed is stored in plastic feed bins off the floor and away from walls.
- **The mill date and expiration date must be written legibly on all containers used to hold animal feed.**

- Use the scoop provided in each bin to decant the feed into your own personal Tupperware container. Store the Tupperware on the top shelf in the walk-in cooler.
 - **Prevent moisture and contamination from entering the food bins: Do not use your Tupperware to scoop food from the bin, use the scoop provided. Make sure feed containers are properly closed. Moist food grows mold and is expensive to replace.**
- On your feed container lid write:
 - Mill date
 - Expiration date
 - Food Size
 - The tanks numbers to be fed with that food
 - Amount and frequency of feeding

Your initials **Recommended Feed Size related to Fish Size: % to feed: kg (lbs) per 100 kg (lbs) fish per day**

Fish Size Weight (g)	Fish/lb	Pellet Size mm	Water Temp F (C)						
			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

AAHL SOP: FORMALIN TREATMENT

Suggested treatment for ectoparasites:

Formalin is an aqueous solution of 37%-40% formaldehyde gas. Formalin is volatile and irritating. It causes cancer in laboratory rodents and can cause contact hypersensitivity and lung damage in humans; solution should be tightly sealed during storage and not allowed to contact human skin. Formalin should only be used in well-ventilated areas.

Formalin should be stored in a dark cool place to prevent formation of paraformaldehyde, a white precipitate highly toxic to fish. Formalin should never be used if paraformaldehyde is present. Methanol 12-15% is added to formalin to inhibit paraformaldehyde formation.

Formalin is an effective parasiticide for bath treatment of most ectoparasitic protozoa and monogeneans. It has moderate-to-weak antibacterial activity. It is used to control molds on eggs but is not antifungal at doses that are nontoxic to fish.

Formalin can be irritating to the gills and water should be well aerated during treatment. Some fish are more sensitive to formalin, and while a common treatment concentration is 125-250ppm (0.125-0.250ml/L), trout require lower levels and careful monitoring.

The practice at the Aquatic Animal Health Lab is to treat for three days. Treat for one hour with aeration, day one 1:12000 (83ppm), day two treat one hour 1:10000 (100ppm), day three one hour 1:8000 (125ppm). Check every 5-10 minutes and discontinue treatment if fish show signs of distress.

Record all treatments and total volumes of formalin used and submit to lab manager. DEQ requires effluent water testing after formalin treatments and the manager will need to keep records and conduct water tests within one day of treatment.

Formaldehyde = commercially available 37% solution

Formalin = 37% formaldehyde solution added to water

The 100 L tanks are closer to 95 L when filled so the volume of formaldehyde is based on that.

Procedure:

- 1) Flush tank of any debris before treatment.
- 2) Do NOT feed fish before treatment. Fish may be fed later in the day after the treatment.
- 3) Place a 1-L beaker on top of each tank with label of tank number (keep for duration of treatment).
- 4) Take tank off water flow.

- 5) Make sure airstone is aerating the water.
- 6) Fill all 1-L beakers with respective tank water (do not cross-contaminate tanks).
- 7) Add appropriate volume of 37% formaldehyde (Table X) to each beaker using a pipette.
- 8) Stir solution in beaker to mix and then add contents of beaker to the appropriate tank. Stir the water in the tank to avoid any hot-spots and to evenly distribute formalin. Repeat on next tank. It is very important to mix well, as fish are very sensitive to formaldehyde.
- 9) Check after 5-10 minutes to make sure tank is not leaking. Check fish every 15 minutes to ensure the fish are not in distress. If fish are in distress, flush tank immediately and put tank back on water flow. Try treatment next day with less formaldehyde.
- 10) Let each tank sit for 45 minutes.
- 11) After the 45 minutes elapse, turn water flow back on for 15 minutes to start diluting formalin.
- 12) After the 15 minutes elapse, drain tank to a sufficient level to drain formalin but not stress fish.
- 13) When the 5- or 6-day treatment is completed, send AAHL manager an email detailing how much formaldehyde was put into the system (example shown in Table Y).

Table X. Volume of formaldehyde to add to each tank based on tank size and concentration.

Tank Size	Final Concentration	Volume of 37% formalin to add to tank
25 L	1:12000	2ml
	1:10000	2.5ml
	1:8000	3.1ml
100L	1:12000	7.7ml
	1:10000	9.2ml
	1:8000	11.5ml
3ft	1:120000	36ml
	1:10000	45ml
	1:8000	56ml

Table Y. Example of information AAHL manager should receive after treatments are completed.

Klamath River (Keno Eddy) exposure groups, October 2012

Date	Tank(s)	No. tks/size	Treatment conc.	Formaldehyde (mls)/tank	Total volume of formaldehyde (ml)
10/09/12	A1,2,4,5,6	5/ 100L	1:12,000	7.9	39.5
10/10/12	A1,2,4,5,6	5/ 100L	1:10,000	9.5	47.5
10/11/12	A1,2,4,5,6	5/ 100L	1:8,000	11.9	59.5
10/12/12	A1,2,4,5,6	5/ 100L	1:8,000	11.9	59.5
10/13/12	A1,2,4,5,6	5/ 100L	1:8,000	11.9	59.5
					265.5

AAHL SOP: RIVER EXPOSURES

Check [fish transport SOP](#)

- Reserve a truck from Motor Pool 7-4141
- Get a partner - never go alone unless you're meeting a co-worker at the river site
- If prophylactic treatments are planned for fish after river exposure make sure you've written them into your ACUP proposal. If medical treatment not described in your ACUP is required, you must get the Attending Veterinarian to prescribe treatment.
- Obtain permission to access public and non-public lands
 - Coordinate with land-owners for river access and to expose fish on their land
- Submit fish transport permit to ODFW
- Order fish or check that appropriate stock fish are available at AAHL
- Set up tanks in wet lab prior to returning fish – have water running slowly

Equipment Checklist:

- | | |
|---|--|
| <input type="checkbox"/> Large coolers (~50 qt) for transport of fish to field | <input type="checkbox"/> Latex/Nitrile gloves |
| <input type="checkbox"/> Oxygen tank, regulators, air hoses, splitters and air stones | <input type="checkbox"/> Furanase |
| <input type="checkbox"/> Small buckets | <input type="checkbox"/> Plastic beaker |
| <input type="checkbox"/> Nets | <input type="checkbox"/> Forceps |
| <input type="checkbox"/> Cages – check for holes years and cable and cable clamps | <input type="checkbox"/> Carry fish transport permit with you during the trip. If you're going to California, carry permits for both states. |
| <input type="checkbox"/> Wrenches for O ₂ , live cage and cable U-bolt | <input type="checkbox"/> Field kit: spare parts, First Aid |
| <input type="checkbox"/> DO meter | <input type="checkbox"/> O ₂ regulator |
| <input type="checkbox"/> GPS unit | <input type="checkbox"/> Cable clamps |
| <input type="checkbox"/> Rite in the Rain notebook | <input type="checkbox"/> Hand wipes, paper towels |
| <input type="checkbox"/> Walking (snake) stick | <input type="checkbox"/> Thermometer |
| <input type="checkbox"/> Spare O ₂ cylinder | <input type="checkbox"/> Maps labels |
| <input type="checkbox"/> Ice | <input type="checkbox"/> pens and pencil |
- While transporting fish, stop every 1.5 – 2 hours and check coolers and O₂ tanks to ensure adequate oxygen delivery and check to see that fish are alive and well. Adequate oxygen = small bubbles steadily stream out of the air stone. If it's a very warm day check the water temperatures of the coolers and add ice to keep below 16°C or close to expected natural conditions.
 - At your site find a suitable tree, rock, or other sturdy inanimate object to loop the live cage cable around. Make sure the water is deep enough to completely submerge the cage and flow is not too fast as to pin fish against the cage. Secure the cable around object and place live cage near the water.

- Net out desired number and species of fish, make a label with site name, date, and species of fish for each cage and place in the live cage along with fish.
- Tighten live cage lid/bolt and gently push the cage out into the river.

After the exposure

- Check the river temperature and compare to the temperature in the transport tank.
- To minimize stress in the fish due to the temperature change between the river temperature and the temperature at which they will be held at the AAHL, adjust the transport water temperature to an average of the 2 temps by mixing river water and/or cube ice to the transport water.
- When river water temps are high, cooling the water slightly with ice will lower the fishes' metabolism and reduce the risk of escapees.
- Fill bucket with clean water from cooler and tip fish from live cage into the bucket.
- Pour fish and water into cooler and turn on air supply.
- Make sure tag from live cage is in appropriate cooler and label top of cooler using tape and Sharpie.
- Make frequent stops on the return to check fish, air supply and water temperature.
- If treating for external bacteria (w/ Furanase) – stop about 1 – 2 hours from the AAHL:
 - (While wearing gloves), mash 1 tablet (2 for large white coolers) in cooler water
 - Pour into cooler and mix thoroughly.
 - At the AAHL transfer fish to designated cages and dump cooler water onto grass.

AAHL SOP: SIGNS OF STRESS AND DISEASE IN FISH

- ❖ Off feed
- ❖ Lethargy
- ❖ Increased respiratory rate
- ❖ Isolation from group
- ❖ Opercular flaring
- ❖ Excess mucous production
- ❖ Gasping at the surface
- ❖ Clamped fins
- ❖ Reddened areas on fins or body
- ❖ Changes in color
- ❖ Scales raised or lost
- ❖ Buoyancy problems

Mortalities and Sick Animal Reporting:

Mortalities must be recorded.

If an unexpected negative event or mortality occurs contact the veterinarian and submit an adverse event form.

[Animal Care and Use Committee Adverse Event Form](#) 

Unexpectedly sick animals must be reported immediately to the clinical veterinarian. Disease or treatment included in the ACUP need not be reported.

Federal law requires that a veterinarian, specifically an Attending Veterinarian (AV) oversee animal care and provide guidance on animal related procedures. These include animal husbandry SOPs; appropriate surgical methods and materials; the use of anesthesia/analgesia; mechanism to be alerted to health issues; and preventative medicine, surveillance, diagnosis, treatment, and control of disease. Oregon State University's Attending Veterinarian is Dr. Helen Diggs. Also available for consult is Dr Tim Miller-Morgan.

Dr. Helen Diggs
Attending Veterinarian/ Director of
LARC
541-737-6462
Helen.Diggs@oregonstate.edu

Tim Miller-Morgan, DVM
Clinical Veterinarian - Aquatics
Oregon State University
Hatfield Marine Science Center
2030 Marine Science Drive
Newport, OR 97365
(541) 867-0265 (office)
(541) 270-4218 (cell)
tim.miller-morgan@oregonstate.edu

Also available for consult:

- For emergencies or after hours, Department of Public Safety & Oregon State Police at 541-737-3010 or 7-7000 afterhours. Veterinary care is available 24-7.
- Emergency/Weekend Contact Information
 - All tank labels must have current emergency contact phone numbers in order to reach PI or researcher.

- A staff member is available after hours and on weekends to assist in care or treatment of research animals in times of emergency.
- To reach emergency AAHL on-call staff person call Security Alarm Service 541-967-6200, Acct 10771, or check the whiteboard in the prep lab.

Animal Welfare Concerns and Non-compliance

- The responsibility for appropriate animal care and use involves all participants. The IACUC has a mechanism to report animal care and use concerns. If the IACUC determines that a report describes a threat to the well-being or appropriate use of animals, actions are taken to resolve and prevent the situation from recurring. These actions can include retraining, development of SOPs, additional monitoring, and suspension of individuals or entire projects. Signs detailing the reporting process are posted in all animal areas and available at: <http://oregonstate.edu/research/ori/animal/welfare.html>
- Non-compliance issues are the result of not following approved procedures in the ACUP. These can include unapproved changes to procedures, staff not authorized to work with animals, collection of animals without IACUC approval, mistreatment of animals, etc.

AAHL SOP: RECOGNITION AND TREATMENT OF ICH (WHITE SPOT DISEASE)

Ich is a common name for the parasite *Ichthyophthirius multifiliis* and the disease that it causes. The parasite is capable of killing large numbers of fish in a short period of time. Early diagnosis and treatment are essential for controlling Ich and reducing fish losses.

All species of freshwater fish can carry the Ich parasite.

Identification of Ich

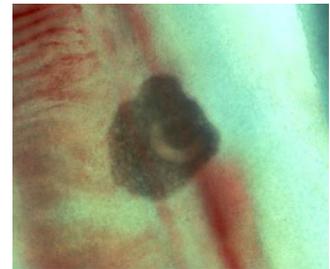
Often the only indication of Ich's presence may be dead and dying fish.

But be on the look out for the following signs of Ich: Fish

- appear lethargic
- often gather around inflowing water,
- gasping at the surface
- refuse to eat.
- exhibit "Flashing" (making quick rubbing or scratching movements showing their underbelly).

You may be able to see:

- white specks on skin
- skin may look bumpy.
- large amounts of mucus sloughing off skin,
- mature forms of the parasite are large (up to 1 mm or 1/32 inch across) and can be seen without magnification.
- In some Ich cases the parasite may be present only on the gills and not on the skin.



Ich trophont on gill filament

What to do if you suspect Ich:

- Inform the manager
- Preserve any freshly dead fish on ice in the refrigerator, do not freeze
- Examine a scraping of skin and gills under low power of microscope for parasite stages.
- Contact a fish pathologist if necessary to confirm diagnosis and recommend treatment. Contact either Rich Holt, holtr@onid.orst.edu, or [ODFW pathologists](#).



Ich trophont with characteristic horsehoe-shaped nucleus.

Treatment

Because not all stages in the life cycle of Ich are affected by treatments, multiple treatments must be administered to catch individual Ich organisms in the vulnerable stages of their life cycle.

Table 1.

Water temperature °F

24°C and higher

18°C to 24°C

13°C to 18°C

7°C to 13°C

Treatment interval

Treat every day.

Treat every other day.

Skip 2 days between treatments.

Skip 3 or 4 days between treatments

AAHL SOP: EUTHANASIA AND SEDATION

All euthanasia and sedation techniques that are utilized must be approved on the researcher's ACUP.

This SOP refers to euthanasia nad anesthesia by the Intentional overdose via immersion in anesthetic solutions of buffered Tricaine Methanesulfonate (MS-222).

- This SOP is in accordance with current AVMA guidelines (2020) S6.2 FINFISH P82

<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

- This procedure details how to prepare and effectively use Tricaine Methanesulfonate (MS-222 Formula $\text{NH}_2\text{C}_6\text{H}_4\text{COOC}_2\text{H}_5 \cdot \text{CH}_3\text{SO}_3\text{H}$ Mol. Weight 261.29) for use as a fish anesthetic. MS-222 is an FDA approved fish anesthetic (FDA ANADA 200-226) used for the temporary immobilization of fish, amphibians, and other aquatic cold-blooded animals (poikilotherms).
- MS-222 is a fine crystalline powder fish anesthetic that is mixed with water prior to use. Wear eye protection, nitrile gloves and lab coat. Preparation of MS 222 stock solution should take place under an extraction hood in Nash 528. Caution: Irritant. Irritating to eyes, respiratory system and skin
- Solution must be buffered to a neutral pH. The AAHL has standard stock solutions of MS222 and Sodium Bicarbonate Buffer. Both kept in the Prep Lab refrigerator. Label date made and date to discard. Keep stock solutions for up to one month in refrigerator.
-

Reagents and Equipment needed

- Finquel® / MS-222® Tricaine Methanesulfonate.
- Buffer: Sodium Bicarbonate NaHCO_3
- Distilled Water
- Weigh balance to accurate to 1 decimal place.

- Dedicated plastic weigh boats
- Dedicated spatulas
- Measuring cylinders (1 L and 20 ml).
- Dedicated 1 Liter plastic bottles.
- Refrigerator for storage of stock solutions.
- Container for carrying out anesthesia e.g. bucket or beaker (depending on size and number of fish).

Preparation of Stock solutions:

1) MS 222: 20g/L

Using a 1 liter measuring cylinder, measure 1 liter of distilled water and transfer to the dedicated plastic 1 L bottle. Under a chemical fumehood, weigh 20g of Tricaine Methanesulfonate powder into a dedicated weigh boat and transfer to the 1 Liter of water. Cap bottle and shake to dissolve. Label with date made, discard date (if kept in the refrigerator, stock solutions are good for 1 month), your initials and the concentration (20g/L).

2) NaHCO₃ :50 g/L

Using a 1 liter measuring cylinder, measure 1 liter of distilled water and transfer to the dedicated plastic 1 L bottle. Weigh out 50g of sodium bicarbonate into a dedicated weigh boat and transfer to the 1 Liter of water. Cap and shake to dissolve. Label with date made, discard date (if kept in the refrigerator, stock solutions are good for 1 month), your initials and the concentration (50g/L).

The following are the concentrations used for either anesthesia or euthanasia of salmonids (note: double the concentrations for Tilapia):

	MS222	NaHCO ₃
Stock Solution	20g ms222/ 1 liter H ₂ O	50g NaHCO ₃ / 1 liter H ₂ O
Anesthesia (sleep)	2.5 ml stock/ Liter H ₂ O	2.5 ml stock/ Liter H ₂ O
Euthanasia (kill)	10ml stock/ Liter H ₂ O	10ml stock/ Liter H ₂ O
Quick kill	20ml stock/ Liter H ₂ O	20ml stock/ Liter H ₂ O

To anesthetize fish (sleep):

- Add a known number of liters of water to an appropriately sized container for the size and number of fish to be anesthetized. Fish should have enough room to be able to swim freely prior to anesthesia without becoming stressed.
- Add the stock solutions of buffer then the MS 222 in equal proportion (2.5 ml stock Buffer per Liter H₂O plus 2.5 ml stock MS 222 per Liter H₂O).
- Using a net carefully transfer fish from tank to the container of buffered MS 222.
- For anesthesia, allow enough time for the fish to lose equilibrium before sampling.

To euthanize (kill) fish showing signs of morbidity or for lethal sampling:

- Add a known number of liters of water to an appropriately sized container for the size and number of fish to be euthanized. Fish should have enough room to be able to swim freely prior to anesthesia without becoming stressed.
- Add the stock solutions of buffer then the MS 222 in equal proportion (see above).
- Using a net carefully transfer fish from tank to the container of buffered MS 222.
- Allow a minimum of 30 minutes after cessation of opercular movement before necropsy or transferring fish to biohazard freezer for disposal or use a secondary adjunctive method to complete euthanasia (such as decapitation, pithing, or freezing) if sampling must take place within less than 30 minutes

To euthanize a tank of fish when terminating an experiment:

- Drop the water level somewhat in the tank by removing the inner standpipe. Fish should have enough room to be able to swim freely prior to anesthesia without becoming stressed. Estimate the number of liters of water left in the tank.
- Add an appropriate amount of the buffer then the MS 222 in equal proportion (see above).
- Allow 30 minutes after the fish has stopped operculating before necropsy or transferring fish to biohazard freezer for disposal.

- If sampling is required before 30 minutes (for example for blood) a secondary method of euthanasia must be carried out such as

Fish need a higher dose of MS-222 at lower temperatures, but it is safer to use at lower temps. Crowding increases the required dosage. MS-222 causes a decrease in pH and should be buffered in order to prevent acidosis in fish. Stock solutions should not be buffered because this causes chemical dissociation of the sulfonate group. Tricaine solutions are unstable in light and should be discarded when they become yellow or brown. Physical euthanasia must be approved on your ACUP and handlers must be trained in its use. It must be followed by an adjunct method, such as pithing or decapitation, to ensure death.

It is essential that euthanasia be performed by personnel who are skilled in methods for the species in question and that it is performed in a professional and compassionate manner. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanatized. Euthanatizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they are involved in performing euthanasia repetitively or if they have become emotionally attached to the animals being euthanatized. When delegating euthanasia responsibilities, supervisors should be aware of this as a potential problem for some employees or students.

AAHL SOP: WATER QUALITY

The laboratory is supplied with specific-pathogen free fresh water from two wells, each with a capacity of more than 300gpm. The water, which has an ambient temperature of 12.8°C, is processed through UV sterilization and a degassing tower to eliminate pathogens, gas supersaturation, and allow oxygen to saturate into the water.

Water is cultured bi-annually for bacteria and fungi, using the SOP in Standards Methods for the Examination of Water and Wastewater. 21st Ed. Eaton, et al., 2005 pp 9.38-9.40. For testing, samples are taken from four locations in the facility (the well (just inside the building, near sink), from the UV unit in upstairs mechanical room, from the post packed column valve near tank K24, and from an end-of-the-line tank, A8).

The incoming well water is tested annually for microbiologicals, physical factors, inorganic analytes, organic analytes and pesticides by Edge Analytical Laboratory, 540 SW 3rd Street Corvallis, OR 97333 www.edgeanalytical.com.

In addition, the water, tanks and fixtures are tested quarterly for ATP by OSU.

Effluent water is tested for temperature, pH, conductivity, alkalinity quarterly, suspended solids. Yearly tests of the well are provided by DEQ and check for pesticides and herbicides in our water source.

Tanks should be checked for dissolved oxygen (DO) using a YSI DO meter whenever a group of fish shows unexpected signs of distress (color change, disease, rapid gilling). DO lower than 5.0 is dangerous. Trout and salmon are healthiest when DO is above 7. Split the fish into additional tanks or reduce numbers through euthanasia to meet DO needs.

WATER QUALITY	tropical	cold	tropical	coldwater
PARAMETERS for FISH	freshwater	freshwater	marine	marine
TEMPERATURE C/F	25/77	15/60	25/77	<15/<59
SPECIFIC GRAVITY	1.000	1.000	1.023-1.025	1.020-1.024
DISSOLVED OXYGEN (ppm)	>8.0	>5.0	>7.0	>7.0
CARBON DIOXIDE (ppm)	<10	<5.0	<5.0	<5.0
pH	6.0-8.0	7.0-8.5	8.0-8.3	8.0-8.3
ALKALINITY (ppm)	100-120	>25.0	125-200	125-200
HARDNESS (ppm)	>20	>20	125-200	125-200
UNIONIZED AMMONIA (ppm)	<0.03	<0.01	<0.02	<0.02
NITRITE (ppm)	<0.01	<0.01	<0.01	<0.01
NITRATE (ppm)	<50.0	<50.0	<20.0	<20.0
CHLORINE/CHLORAMINE (PPM)	0	0	0	0

AAHL SOP: EFFLUENT TREATMENT SYSTEM MAINTENANCE

1.0 The effluent treatment system at the AAHL treats waste water from the research facility. Regular maintenance and monitoring assures accurate dosing and readings, and minimized equipment problems. If equipment isn't working properly, untreated pathogens, excess chlorine or other lab chemicals may be released into the Willamette River.

2.0 SCOPE:

1. Maintain effective levels of chlorine by filling reservoir, monitoring Cl levels, maintenance of pumps and other mechanical systems in chlorine room (AAHL Rm 123).
2. Disinfection trough cleaning and care. (Adjacent to chlorine room in Outside Holding)
3. Settling pond water testing for DEQ, dike maintenance. (north of AAHL, near river)

3.0 RESPONSIBILITIES:

Ruth Milston-Clements will have primary responsibility, student workers will assist as requested.

4.0 DEFINITIONS:

- Hach CL17: a wall mounted spectrophotometer that monitors our effluent. It takes a reading every three minutes; the intake for the CL17 is near the outflow of the treatment weir. The CL17 will send an alarm if levels are below 1.0 or above 5.0ppt.
- Treatment weir: A serpentine concrete trough that allows sufficient contact time between chlorine and pathogens.
- The ponds: Two ponds receive our effluent, they are located due north near the river. They serve as settling ponds to remove suspended debris and chlorine is broken down by sun, air, organic materials. Our water then flows into the Willamette.
- Pulsafeeder peristaltic pumps: Chlorine dosing pump which works by using rollers to squeeze flexible tubing

5.0 MATERIALS and/or EQUIPMENT:

Chlorine 12.5% Supplier: Cascade Columbia Customer #81818 503-625-5293

Hach CI17 reagents, hoses

Supplier: Hach

Pulsafeeder hoses, lubricant, annual maintenance kit

Supplier: EngineeredControl Products, 503-656-4880

Instruction manual for Hach CI17 and chlorine system are in cabinet drawer in room.

6.0 PROCEDURES:

Weekly:

Fill chlorine reservoir:

- Put on all safety gear: boots, glove, face shield, plastic apron or rain gear (chlorine we use is 12.5%, twice the strength of household bleach).
- Place the rigid end of the drum pump into a drum of chlorine; place the hose in the reservoir.
- When the pressure comes on, the hose may jump out of the reservoir and spray chlorine everywhere. Make sure the hose isn't going anywhere (hold it, have someone else turn the pump on and off) don't plug the pump in until the pump and the hose are in place, just in case the pump's in the "on" position.
- Fill reservoir with about 2/3 of the volume you need.
- Remove hose from reservoir; allow bleach in hose drain back into drum-careful, it wants to start to siphon out, creating a chemical spill.
- Remove rigid end of drum pump from drum, rinse off the drum pump and hose.
- Fill a bucket with water, stick the hose out to the driveway, run a bucket of water through pump to rinse it.
- Hose off the deck and floor onto the parking lot.
- Fill the remaining 1/3 reservoir with water.
- Stir with length of PVC you found in the bucket.
- Reset peristaltic pumps to about 1/3 of the level they were at (during the week, it takes more and more chlorine to keep levels up. Fresh chlorine is stronger).

Potential problems:

A spill that's hosed into the drains can raise the chlorine level and trigger the alarm and take a long time to equilibrate. Hose spills onto the parking lot.

Rinse yourself and your gear—protect the next person by leaving clean and safe protective equipment.

Maintain monitoring equipment: The Hach CI17

Clean spectrophotometer cell:

Clean intake screen: At far end of trough

- remove screen, clean.
- Unscrew inner screen, clean.

Lube Pulsafeeder pumps:

- turn off the pump
- remove cover plate,
- wipe off and lube shaft and gasket,
- use pipe cleaner to place small amount of lube on hose in three places (turn on and off to move rotor).
- Replace cover plate.
- Tighten fully, then back off a couple turns to reduce friction, but don't be loose enough to create wobble.

Trough maintenance:

Vacuum trough about once every **three weeks**. During vacuuming, switch pump to well water until water clears up to prevent debris from entering chlorine sensing system. Shunt alarm to chlorine system (Zone 3).

Clean Y-strainer near pump when flow meter shows reduced flow or after winter weather has dumped pine needles that may have gotten into strainer. Check at least quarterly.

Clean intake screen daily, it will clog with pine needles or other debris.

Use a net to remove leaves and branches after winter storms.

Keep baskets clean **daily**, autoclave material collected from basket at beginning of trough.

To switch to well water:

Simultaneously

Close valve 1—supply line from disinfection trough, then

Open valve 2—supply line from lab carrying well water

Turn off pump-- switch right above pump, labeled 'Chlorine Pump'

To switch back to disinfection trough water:

Close valve 2—supply line from lab carrying well water

Open valve 1—supply line from disinfection trough

Turn on pump

If the above sequence is done in the wrong order or too slowly the pump may lose its prime.

To clean Y-strainer:

Close valve, turn off pump, remove Y-strainer and clean out pine needles and other debris, replace, turn on pump, open valve 1. Check pump pressure (should be 25-40psi) and prime if necessary.

To restore prime:

Turn pump off

Remove intake screen (intake is near outflow at end of trough),

Hold charged garden hose on intake,

Turn on pump, hold hose in place until pressure gauge on pump indicates 25-40psi.

Check intake with finger, will be able to feel suction.

Replace intake screen.

Monthly:

Change Hach CI17 reagents.

- New materials are delivered every six months on a repeating order; supplies are in the cabinet in the chlorine room.
- In the Hach CI17, the Buffer is on the left hand side and the Indicator is on the right hand side
- Unscrew the cap (with tubes attached) from the old buffer bottle and remove the bottle from the Hach CI17.
- Replace with new buffer, insert tubes into the bottle (attached to the cap), screw the cap on and write the date on the bottle with sharpie
- Unscrew the cap off the indicator bottle in the Hach 17 and remove from the Hach CI17
- Add the indicator powder to the new Indicator solution bottle using a plastic funnel. Cap the bottle and shake to mix.

- Remove the cap and put the new indicator in the Hach CI17, insert the tubes (attached to the cap), screw the cap on and write the date on the bottle with a sharpie.

Quarterly:

Replace Pulsafeeder hoses. Instructions are in book on counter in chlorine room.

Replace Hach CI17 hoses, materials are in cabinet in chlorine room.

7.0 REPORTING AND DOCUMENTATION:

Document your work by initialing and dating the record sheet posted in the chlorine room.

8.0 REVIEWS AND REVISIONS:

This procedure shall be reviewed for compliance and effectiveness and revised as necessary

ATTACHMENT A. Effluent treatment system maintenance record

AAHL FORM: EFFLUENT TREATMENT SYSTEM MAINTENANCE RECORD

Task	Freq	Date								
Chlorine ordered	As req.									
Chlorine delivered	As req.									
Clean CL17	/wk									
Clean intake screen	/d									
Fill reservoir	/wk									
Lube peristaltic tubes	/wk									
Vacuum trough	/3 wk									
Replace CL17 tubes	/Qt									
Replace peristaltic tubes	/Qt									
Replace reagents	/Mnth									
Task	Freq	Date								
Chlorine ordered	As req.									
Chlorine delivered	As req.									
Clean CL17	/wk									
Clean intake screen	/d									
Fill reservoir	/wk									
Lube peristaltic tubes	/wk									
Vacuum trough	/3 wk									
Replace CL17 tubes	/Qt									
Replace peristaltic tubes	/Qt									
Replace reagents	/Mnth									
Task	Freq	Date								
Chlorine ordered	As req.									
Chlorine delivered	As req.									
Clean CL17	/wk									
Clean intake screen	/d									
Fill reservoir	/wk									
Lube peristaltic tubes	/wk									
Vacuum trough	/3 wk									
Replace CL17 tubes	/Qt									
Replace peristaltic tubes	/Qt									
Replace reagents	/Mnth									

AAHL SOP: DAILY, WEEKLY, QUARTERLY, YEARLY TASKS

Daily

- Health checks
- Input health report on computer
- Feed fish <10" big
- MWF feed all fish, large and small
- Check chlorine level in pond and trough
- Clean strainer basket
- Flush tanks 2-4 times a week as needed
- Clean tanks, all must be cleaned 1x week
- Check room and water temps, record
- Record morts, feeding, cleaning

Monday:

- Autoclave biohazard waste,
- Check pond pH, TSS, well output,
- Service chlorine system: fill chlorine reservoir, clean chlorine intake, lube peristaltic tubes,
- Check eyewash,
- Check disinfectant levels and replace or refresh as needed
- Blow off air compressors

Thursday:

- Blow off air compressors
- Check disinfectant levels and replace or refresh as needed

Weekend:

- Health checks
- Submit health report on computer
- Check water and chlorine levels
- Feed fish

Monthly

- Vacuum disinfection trough (may need to be done 2x month)
- DEQ report
- Tank charges
- DEQ water tests: alkalinity, TSS, formalin, NH3
- Purge sand filter

Quarterly

- Disinfect wet lab floor with chlorine

Yearly

- Change UV tubes in both units
- Make sure compressors, generator, and chlorine pumps have been serviced
- By end of June, submit updated chemical inventory

AAHL SOP: AUDIT FOR BIOSECURITY AND TO PREPARE FOR IACUC INSPECTIONS

- Limited entry signs
- Public entry limited to low risk areas
- Foot and hand baths functional
- Doors locked to outside entry when not in use
- Separate or disinfected equipment for each tank, trough, room.
- Disinfection troughs functional
- Feeding procedure:
 - On time
 - Gloves
 - Separate buckets
- Fish treatment chemical storage
- Treatment regimes and chemical/ drug concentration recorded
- Vector exclusion fencing, lids operational
- Mortality and diagnosis recorded
- Densities monitored and adjusted, dates noted
- Fixtures scrubbed and disinfected
- Disinfection protocols posted
- Water tested for bacterial count
- Logs for husbandry and mortality up-to-date
- SOPs current and available
- SOP for routine husbandry and care, including weekend, holidays, emergency
- Husbandry logs current and available; husbandry, feeding, cleaning,
- Identification on all occupied tanks with ACUP number
- All active ACUPs available
- Medications labeled and stored properly, no outdated materials
- Information available regarding animal handler safety, zoonotic disease, reporting abuse
- Prep Lab clean
- Wet Lab clean
- Outside Holding clean
- Animal waste and mortality disposal available
- All tanks clean
- Food dated with mill date and pull date. Size marked. All food covered and stored in vermin proof containers
- Security and emergency contact information posted
- Emergency generator functional

AAHL SOP: VERMIN MONITORING AND CONTROL

A monitoring and control program for vermin must be an ongoing part of every animal housing facility. Monitoring for insects and wild rodents is vital to research animal and human health.

- Rodent poison bait is set out in likely thorough-fares quarterly even if no vermin are evident.
- If insects are found in a food area, all food is disposed of and Facilities is called in for vermin control.
- If burrowing animals are present in pond, contact Facilities for pest control.

AAHL SOP: MAINTAINING ZEBRAFISH BROODSTOCK

Groups of adult zebrafish are maintained at densities of 30 per 10 gallon tank or 100 per 30 gallon tank. Flowing well water passed through a degassing tower and disinfected with ultraviolet light is added to fish tanks to achieve at least 2-3 water replacements per day. Each tank is aerated with an airstone or other comparable device. Zebrafish are held at the optimal breeding temperature of 28 °C using either the AAHL heated water system supply or by 2 immersion heaters placed into each zebrafish broodstock tank to ensure temperature control. Temperature of each tank is recorded daily. Water quality parameters including total ammonia, nitrite, pH, and alkalinity are recorded weekly.

Broodstock groups are housed with both sexes in one tank, approximately half females, half males. Breeding is done in polypropylene breeding tanks that contain 4 females and 2 males in the upper partition. A mesh beneath the fish allows collection of eggs in the bottom of the breeding tank. Broodstock are left in breeding tanks overnight and tanks are checked for eggs the following morning. Broodstock can be left together for another 24 hours if they are not fighting. Individual broodstock can be bred weekly if necessary, but biweekly breeding is less stressful. Broodstock are fed Aquatox flake diet (Ziegler) twice daily and *Artemia* (brine shrimp) nauplii once daily.

Once fish enter production, they should be bred for 1 year and then retired to maintain optimal health. Fish are euthanized by immersion in buffered MS222 >200 ppm. Then they are placed in a freezer.

F= Fed, H= Health Check, C= Cleaned, S = Standpipe raised/flushed

OREGON PROHIBITED SPECIES APPLICATION



Oregon Department of Fish and Wildlife

406 Cherry Ave., NE

Salem, OR 97303

(503) 947-6300

FOR OFFICIAL USE ONLY

Date _____

Approved By _____

Oregon Prohibited Species Application For Universities and Colleges

Name of University or College

Name of Department:

Name of Person Responsible For Permit:

Address:

City: State: Zip Code:

Telephone:

Email:

Prohibited Species Common Name:

Prohibited Species Scientific Name: Please use taxonomic nomenclature from source identified in OAR 635-056-0000

Time Period during which prohibited species will be held:

Beginning:

Ending:

Important: Please attach a written description of your project which includes:

- a) How the facility is constructed to minimize escape of prohibited species,
- b) Short description of security programs, equipment and procedures which minimize the possibility of escape,
- c) Short discussion of record keeping to aid in tracking of confined animals or recovery of escaped animals,
- d) Short description of veterinary care used to identify and minimize the spread of diseases, and
- e) What the final disposition of the specimen(s) will be once the study is over.

Signature: _____ Date: _____

AAHL FORM: TANK LABELS

<p>Investigator: _____ Tank # _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>	<p>Investigator: _____ Tank # _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>
<p>Investigator: # _____ Tank _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>	<p>Investigator: _____ Tank # _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>
<p>Investigator: _____ Tank # _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>	<p>Investigator: # _____ Tank _____</p> <p>Disease Agent:</p> <p>Initiation Date:</p> <p>Termination Date:</p> <p>Fish Species and Number:</p> <p>ACUP#</p> <p>After-hours contact#</p>

*Print labels on Rite in the Rain Paper (available at AAHL). At the start of the experiment, fill in the label for each tank and place above the tank. When tank is no longer in use, note "out" date on label and turn it in to the manager.

AAHL SOP: EMERGENCY AND ALARM RESPONSE

Go to alarm panel at rear of wet lab.

Red constant light	Armed and ready (it should always be in this mode)
Green constant light	Disarmed (will not send)
Red flashing	Alarm at zone shown – sending
Green flashing	Alarm at zone shown – not sending
Fire-trouble	smoke and heat detectors

If alarm is sounding, enter the disarm code 3654 slowly.

Repeat after five seconds if necessary.

Security Alarm will call when an alarm occurs. Identify yourself and indicate if you can handle the alarm or want them to call others.

After the problem that triggered the alarm has been addressed clear the alarm by holding down 9 for two seconds, then re-arm the alarm system by pressing 3654.

- Make a note of the alarm on the record sheet near the alarm panel in the wet lab.

Alarm zones:

1. Power failure, very low water, control air pressure
2. Air blower – no longer in use
3. Chlorine levels
4. Isolation building
5. Temperature control equipment (heated water set-up in mechanical room)

To call 911, simultaneously push * and # on the control panel. Then the response list is called.

When an alarm occurs, Security Alarm Company (541-967-6200, Acct 10771) is dialed and they call the following response list:

1. BENTON COUNTY SHERIFF Global
2. LINN COUNTY SHERIFF Global
3. OSU Aquatic Animal Health Lab 541-737-0743
4. Lab Manager

*If you can't reach the manager during the week, contact someone on the weekend call list

- Call the lab manager for guidance.
- Do not call out with the phone at back of wet lab during an alarm event; the line is used for alarm system calling.
- Phone numbers for professional services are posted near each telephone at the lab.
- There are lanterns and flashlights in the prep lab and a flashlight near the mechanical room door.
- The disaster plan and equipment (lanterns, airstones & hose) are in the prep lab.

Zone 1: Power failure, very low water, control panel air pressure

Don't delay in getting to the lab or getting help solving the problem. A loss of water flow will cause fish death in 20 minutes

Power failure:

If only part of the system is dead, first check breakers, especially the E panel.

Call the electric company; they may not know that we've lost power: 541-888-221-7070.

1. If the lights come on, then the generator is running. It has a 3.5 second delay after power fails. If generator fails to start, check instrument panel inside south door of generator. To respond to trouble light or start it manually, see manual in file drawer under microscopes. Call security 7-7000 to get facilities to come get it running if you can't or don't want to try.
 - a. If the generator is running but water is no flowing, proceed to well pump panel on east wall of mechanical room and note pumps in on position. The well pumps may need to be reset or a fuse replaced. The fuses are inside the cabinet. To reset:
 - i. Turn off the hand-off-auto switch
 - ii. Pull the disconnect lever down to the off position
 - iii. Press the reset button
 - iv. Go to panel C and check the breaker #15 and #21, reset if necessary
 - v. Turn on the disconnect lever
 - vi. Switch the hand-off-auto switch to "Hand. Be sure breaker #16 in panel C and #1 in panel E are on.
 - vii. If this fails to supply water, call Mainline Pump 541-929-3870 and tell them we need emergency pump service.
 - viii. This same procedure may be needed to reset pressure pumps behind the head tank. They can be heard if they are running. The disconnects are inside the pressure pump panel, breakers are in panel C, #27 and #33.
 - b. If generator is running, check heated water pump, which will not be supplied with emergency power. If in use, check tanks using heated water to be sure they are getting adequate air. If necessary, turn on lab water or put in airstones.
 - c. Turn off or unplug anything that may be pulling power unnecessarily: incubator and fridge in dry lab, fridge in prep lab, freezer near rain gear, freezer in chlorine building, upstairs fridge. Don't open freezers while the power is out; plug them back in when power is restored.
 - d. Turn off the intake pump from the Willamette (the switch is on a pole near the pump, down by the river, just north of the far pond). Turn off the other Willamette pump inside the isolation room using the switch right above it. Unplug the UV lights next to the pump. UV will overheat if no water is going through them. When power comes back on the pump will start back up, but will have lost its prime and be dry. If the pump runs with no water going through it, the pump will burn out. The pump will need to be re-primed, which you don't need to worry about. Tell AAHL staff that the pump is off so that they can get it running again the next morning.
 - e. Stay at the lab until power is restored. When it comes back on, check all systems.

2. Does it look like everything is running properly? We may have had a power surge, which will trigger all the alarms.
 - a. Check to see that everything is running properly. Check breaker boxes to and reset tripped breakers. If a breaker won't reset and you can't figure out why, decide if it's crucial to have that equipment running. If it's a vital piece of equipment, call security 7-7000 and have facilities called in to get it running. Once the place is running functionally for the fish and the chlorine has stabilized, reset the alarm. Shunt an area if you know it's working properly but the alarm panel won't reset.
 - b. Check the outside 12' round to see if water coming in through the spray bar. If it's not, go to the river and turn off the intake pump from the Willamette- the switch is on a post nearby, close the valve closest to the river. If the pump is in the on position when power resumes it may have lost its prime. If the pump runs with no water going through it, the pump will burn out. The pump will need to be re-primed, which you don't need to worry about. Tell AAHL staff that the pump is off so that they can get it running again the next morning. The 12' round of Willamette water will be depleted in 6-8 hours, so turn off the Willamette pump in the isolation room if the river intake pump will be off for an extended period you'll need to shunt this area on the alarm panel). If fish are on the Willamette water, supplement with airstones or lab water.

3. Is it dead quiet and/or dark? A breaker may be tripped in the E panel (check E1)- check the panel and reset, or the E panel itself may be tripped,- call facilities. You probably need help, so call in someone else now. You may need to supply air to all tanks with fish in them, and you have 20 minutes before they start to suffocate.
 - a. Call the power company and tell them it's urgent that they restore power to us: 877-548-3768.
 - b. If generator fails to start, check instrument panel inside south door of generator. To respond to trouble light or start it manually, see manual in file drawer under microscopes.
 - c. Call OSU security and have them contact facilities services to tell them it's urgent that they come get the generator running: 7-7000.

Low and very low water warning lights:

1. Lights are mounted on the air control panel door (west wall of mechanical room), indicating water level in the head tank. When the water drops in the head tank, the alarm is triggered. This alarm indicates an excessive use of water or the loss of water to the head tank.
 - i. Check breaker boxes to and reset tripped breakers (probably E1 in the very corner of the mechanical room). If it's a vital piece of equipment and you can't get it running, call security 7-7000 and have facilities called in to get it running if the breaker won't reset.
 - ii. If it's mid to late summer, the water table may have fallen and we need an additional well pump to get adequate water. Go to gray box on the east wall of the mechanical room; turn on pump 1 with the big hand lever.

Check gauge on the control panel and if water levels are back to normal in a short time, you've solved the problem.

- iii. If the valve position gauge on the air control panel door is wide open, overuse or pump failure may be the problem (valves open wide in an attempt to increase flow). Reduce demand or put another pump online.
2. The well pump may have failed. You can tell if the pump is working by putting your hand on top and feeling for vibration; well pumps are outside, #2 is near generator and #1 is across the driveway towards Fish Performance Lab. Put another pump online by turning on well pump #1, monitor gauges, and put airstones in tanks if you still can't get enough water (low or very low water light will be on) Call Mainline Pump 541-929-3870 and have them come fix or replace the broken pump right away.
 - i. Turn on the aeration blower, the big blue machine in the mechanical room. It will supply air pressure throughout the lab.
 - ii. Get airstones and airline from under the counter in the prep lab, additional line and airstones are behind the walk-in cooler in the wet lab. Connect to air supply lines above the fish tanks both inside and outdoors. Use oxygen cylinders if you need air to fish tanks that don't have air supply from the blower.

Control panel malfunction:

1. If you've got power and the pumps are running, the control panel or the compressor that runs it may be malfunctioning and be the cause of low water alarms. The control panel is the box on the west wall. Gauges should be steady, mid range. If they fluctuate erratically, water levels will be low or unstable, valves on the ceiling over the exterior door may be shrieking. Call security 7-7000 and have them contact facilities to come fix it right away. The facilities guy that knows this odd system is Scott Williamson, so ask that he be contacted.
2. Check the pressure gauges on the control compressor, it should read 20psi. Check circuit breaker panel C #28. Check for a disconnected hose.
3. The big valves over the exterior door can be adjusted manually if the air control panel is totally out of commission or the compressor that supplies it is on the fritz.

Zone 2: Aeration blower

If this fails and you have running water then you are ok. Get Facilities out to service or repair it as soon as you can.

If there is no running water and no aeration blower, you will need to hook up the oxygen cylinders and smaller aeration units to critical tanks.

Zone 3: Chlorine level

This is the most common alarm and is not a threat to fish in the facility. Go to chlorine room by back gate; look at the digital readout on the Hach CL17 on the wall. CL17 should read between 1.5 and 2.0 ideally and the alarm sounds when levels are below 1.0 or above 5.0.

- a. Usually alarms are for chlorine too low, which is often a false alarm and settles in a few minutes back to normal levels with no action required other than resetting the alarm. If it's above 1.0, things are ok and you can return to the alarm panel at the back of the lab, clear the alarm by pressing 9, and reset by inputting 3675. A hot sunny day can degrade the chlorine in the trough, and you may need to increase input of chlorine.
- b. If levels are hovering around 1.0 or dropping below, increase the flow of chlorine by adjusting the dials on the blue pumps on the wall to your right. Only turn them up one tick mark each, wait a couple hours, and then turn up one more tick mark if needed.
- c. If it's above 5.0, there's too much chlorine going into the system. If the cause is not found and addressed, we may end up putting chlorine into the river with serious repercussions from DEQ and EPA. Find out how the excess chlorine got into the effluent system, (usually an accidental spill during reservoir filling); stop the input of excess chlorine. If the cause is a spill, hose it onto the parking lot, not into the drains. Be careful, chlorine can be deadly: use protective boots, gloves, face shield, apron or rain gear. Sodium thiosulfate neutralizes chlorine instantly but shouldn't go into the effluent system.
- d. If you don't believe the CL17, take a water sample and check the chlorine level using the LaMotte 1200 colorimeter located in the chlorine room. A sample taken near the outflow will tell you exactly what the CL17 is seeing, a sample taken near the chlorine building will tell you what the current level is and will reflect recent adjustments about 20 minutes after you've changed chlorine input.
- e. Other problem sources: chlorine reservoir nearly empty, chlorine concentration too low in reservoir, Hach CL17 reagents empty, clogged intake screen at far side of chlorine weir, the pump in the chlorine room on the floor breaker E2—pressure gauge should be 25-35.

Zone 4: Isolation building

1. Is there water on the floor? Find out why, stop the source, and use the shop vac to get the water off the floor. If there's quite a bit of water, use the trash pump located under the sink in the isolation room to pump out most of the water.
2. Is the floor dry?
 - a. Probably the E3 breaker has been tripped by overloading the circuit. Go to the mechanical room, check the small breaker box in the back corner, and reset the breaker. Sometimes this breaker is tripped by plugging the shop vac into the E3 outlets, so if you're vacuuming water out a flooded room, don't use the outlet marked E3.
 - b. The pump in the isolation room may be off. Check the breaker and see if the pump restarts (it won't need to be primed). If it won't restart it may need repair. This pump supplies Willamette water from the 12' tank to worms and may be supplying water to fish. See if any fish tanks are getting this water, supply them with air until water is restored, or supply them with well water. Check with the researcher to see what they want you to do. Worms can go a day or two without water, so don't worry about them.

- c. Important: Unplug the UV lights next to pump in isolation room. Without water running through them, they'll overheat and burn out. Plug them back in when water is restored.

Zone 5: Temperature control equipment (heated water set-up in mechanical room)

The alarm will sound if water temperature goes outside 21-25C. There are three reasons that this can happen:

- a. Too much demand has been placed on the heating system and it can't keep up. Some fish tanks will have to be taken off the heated water or temps in tanks reduced. The researchers with fish on heated water will need to be contacted (their number is on the card above each tank) and decisions made about temperature requirements. Reduce the demand on the system; when the digital readout near the heated water tank is back to normal range, you can reset the alarm.
- b. Too little demand has been placed on the heating system and it doesn't have enough flow to equilibrate. Open up a valve that will move water through the system, the 3' round near the door to the mechanical room is a good choice if there are no fish in it.
- c. The fuse may be blown in the disconnect boxes on wall. Circuit breakers for the heaters and control unit are in panel "A". If circuit breakers are not tripped, call facilities or security 7-7000 to send out an electrician. We will not have heated water until this unit is running. Shunt the alarm, notify researchers.

If you've got everything settled but you still can't get the alarm to reset, shunt the area causing the problem. When would you do this? If chlorine level is good (between 1 and 5ppm) but Hach CL17 is giving incorrect readings; if the Willamette pump is off and it will be awhile before river water can be restored; during repairs if a system has been shut down intentionally.

To shunt an area, see instruction book near alarm panel.

ODFW Hatchery assignments for Fish Health Services personnel



Oregon Department of Fish and Wildlife

Hatchery assignments for Fish Health Services personnel. Please note that there have been some reassignments. As you have done in the past, please call or email any of us if you cannot contact the person assigned to a specific facility or area.

Tony Amandi — 541-737-1855 amandia@onid.orst.edu

Marion Forks Hatchery South Santiam Hatchery McKenzie Hatchery

Dexter Ponds Willamette Hatchery

Craig Banner — 541-737-1857 bannerc@onid.orst.edu

Alsea Hatchery Roaring River Hatchery Wizard Falls Hatchery

Round Butte Hatchery Fall River Hatchery Rock Creek Hatchery

Klamath Hatchery Oak Springs Hatchery Oregon Hatchery Research
Center

Lead for the Naturally Reared Fish Health Investigations project

Sarah Bjork — 541-737-1863 bjorksa@onid.orst.edu

Parkdale Hatchery Clackamas Hatchery Sandy Hatchery

Bonneville Hatchery captive brood ChS

Jerry Jones — 541-737-6041 jonesge@onid.orst.edu

Bandon Hatchery	Big Creek Hatchery	Gnat Creek Hatchery
Nehalem Hatchery	Elk River Hatchery	Klaskanine Hatchery
Leaburg Hatchery	Cole Rivers Hatchery	Trask Hatchery
Trask Pond	CFC Complex	Tuffy Creek Pond
Indian Creek	Morgan Creek	Noble Creek
Coquille High School	Letz Creek	Gardiner Reservoir
Munsel Creek	Daniels Creek	Astoria High School
Warrenton High School	Whiskey Creek Hatchery	Millicoma Interpretive Center

John Kaufman — 541-737-1853 kaufmanj@onid.orst.edu

Bonneville Hatchery	Cascade Hatchery	Oxbow Hatchery
Cedar Creek Hatchery	Salmon River Hatchery	Rhoades Pond
Depoe Bay	Siletz School	Mt. Hood Community College

Lhuuke Ilahee (Siletz Nation)
Fish Virologist and erythromycin INAD coordinator.

Sam Onjukka — 541-962-3823 sam.t.onjukka@state.or.us

Irrigon Hatchery	Umatilla Hatchery	Lookingglass Hatchery
Wallowa Hatchery	South Fork Walla Walla Hatchery	
Little Sheep Creek	Imnaha facility	Minthorn Ponds
Threemile Dam	Big Canyon facility	Lostine River
Catherine Creek ponds	Thornhollow ponds	Upper Grande Ronde River
River Mile 56 facility	Imeqes C-mem-ini-kem facility	

Rick Stocking — 541-325-5343 richard.w.stocking@state.or.us

Round Butte Hatchery passage portion of production

Wizard Falls passage portion of production Opal Springs facility

Pelton/Round Butte Passage program

Even though the rest of the Fish Health Services staff does not have specific hatcheries assigned to them they are very capable of examining fish in situations when some of us are not available.

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Leslie Lindsay — 541-737-1863 smitlesl@onid.orst.edu

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FAX numbers:

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La Grande – 541-962-3873

Madras – 541-325-5335