Chapter 8 Duties/Protocols of Zebrafish Staff

8.0 Organization of Staff

The *Fish Laboratory Manager*, reports directly to Dr. Raible. He manages the use the use of the facility by approximately 30 researchers (including faculty, technicians and students). He works with Comparative Medicine to establish and maintain guidelines for fish care and use. He designs, builds, operates, maintains the recirculating fish systems. He monitors fish health under the direction of Comparative Medicine. He is responsible for the purchase of supplies and equipment. He maintains fish room databases and logs and general fish records, and is the primary contact for 24-hour monitoring/alarm systems. Other duties include cleaning of tanks, feeding, interaction with comparative medicine, and training of researchers to ensure the safe and successful use of zebra fish research at the University of Washington.

The fish technician, reports to laboratory manager. His responsibilities include cleaning and maintenance of tanks, autoclaving of spawning traps and paramecium water, performs fish health inspections, inspects water flow volumes to all tanks. He performs minor repairs to equipment as needed, helps to train and oversee part-time staff.

There are also two part-time workers who perform morning and afternoon feedings, record water quality (pH, conductivity, and temperature), refill water tower as needed and culture Artemia (live fish food). A third part-time worker functions as a food-prep and quarantine lab helper. Primary duties include the culture of paramecium (live baby fish food), the making of chemical reagents. This person also cleans tanks, feeds fish as needed.

No personnel should perform any procedure with out proper training.

8.1 Zebrafish Lab Technician Check List

Daily H and J Laboratories
++ Easily done simultaneously . Follow the individual laboratory checklist every laboratory has one refer to Appendix C for list.

++Assess fish health on every system, remove and record mortalities. You must look into every tank to accomplish this it can be time consuming. Make a prioritized list of what systems need cleaning during this time.

++Check all tanks to make sure they are not discharging through overflow drains (change out/wiggle baffles), and that water is flowing into all tanks. J big system make sure water and air are flowing. Make sure tanks are not leaking or about to leak (clean bulk head strainer). Check and replace dirty Zmod screens. Water must be running to all tanks, on every system. Clean any dirty tanks.

Adjust water quality to given parameters of pH 7.0-7.5, conductivity 1500 ± 200 μs. Never make drastic changes in water quality parameters. Each fish system has a green card attached with details on how to manipulate parameters also refer to Chapters 5 & 6.

Clean systems, this varies from system to system, but generally it involves vacuuming out tanks, cleaning lids, shelves, and tank faces. Not all systems can be cleaned every day work on those that need it the most first see Chapter 6 for more details on tank cleaning and fish system details.

Euthanize fish labeled for disposal. Use MS-222 or ice no other method is acceptable, see Chapter 7. Fish are to be placed in a bag and stored in the freezer immediately after euthanizing. Carcasses need to be moved to dead animal storage D607.

Keep the lab organized, clean up after yourself and others. Generally make sure things have been done for the day ex. Net soak has been changed etc… Keep labs clean.

Dishes see directions below.

As needed (often daily)
Autoclave traps see directions below
Concentrated salt solution 6400 grams/20 L RO water (hose off water tray)
Fill water towers
Top off sumps

Weekly
Change out bleach baths in both labs (Q room is changed out monthly). Drain baths by attaching a hose to the bath's spigot run the other end of the hose to a floor drain. Open the bath valve and drain completely. Close the valve and disconnect the hose. Fill the bath with 20 gallons of RO water and add 1.5 L sodium hypochlorite (bleach). Record the date of change out on top of the bleach bath lid.

AHAB and J Big system need filtration maintenance weekly, see chapter 6.

Disinfect tank shelving, J lab big system, J larval system, any surface with a solid shelve.

Monthly
All other fish systems are on a monthly schedule to have micron filters, and media replaced check the computer database and see Chapter 6 for directions.

Calibrate water meters.

Quarterly
Hose down back panels on all Zmods and wash off or replace sponges in biological filters see Chapter 6.

8.2 Feeding (Morning, Afternoon and Weekend-Duty)

Adult fish are feed dry salmon feed, and artemia. Nursery fish are feed paramecium, "shaker" food (spirulina, OSI artificial plankton, rotifers), and when older than 10 days artemia. Fill out the feeding checklists on the H221 door when you're done feeding. Set aside a paper cup full of artemia and paramecium if need be for the quarantine laboratory, which needs to be fed after H and J laboratories and just before you leave the facility. Cover the cups with aluminum foil to transport them see quarantine room feeding below.

Nursery Fish (H and J)

1) Fish in H and J-lab nursery need to have their water shutoff to their tanks in the morning, and have it turned back on in the afternoon. To do this simply open and close the red water manifold valves on each shelf. In the afternoon both laboratory nurseries need to have their water turned back on and the water line valve to each tank needs to be dripping water ~1 drip/2 seconds. Make sure that the tank screens (drainage) are clean. Replace if dirty. See **Chapter 6** if need be for more details.
2) Feed all nursery fish paramecium the amount of feed depends on the density of the paramecium culture. In H221 on the shelf adjacent to and above the sink take down the tub of paramecium furthest to the left. If the density has already been estimated skip to step 3. Lightly spray a spatula with 70% isopropyl and shake dry. Lift and slide back the lid of the tub and vigorously stir the culture with the spatula. Obtain a clean 5 cc pipette and pump from H225 and collect a 1 ml sample from the tub. Go to the dissecting scope in H225 and dispense the sample in to 1 of the cells in the 6-cell dish located next to the scope. Set the dish on the scope platform and turn on the light. Each dish cell is divided in to 4 quadrants on low-magnification count all of the paramecium in two of the quadrants. The paramecium generally are found moving along the perimeter of the dish. If need be there is a hand-counter and proto-slow (viscous fluid) to slow the paramecium to aid in counting. After you have counted all of the paramecium in 2 quadrants multiply this amount by two. This is the density of the culture per ml (cc). Zoom in on the sample (highest magnification), scan around the entire sample looking for any other living organism. If anything but paramecium are discovered in the sample do not use this tub it's contaminated. See **Chapter 4** for more details. If the sample is clean-not contaminated get some labeling tape write the days date, the paramecium density, "clean" to indicate the sample was inspected for other organisms and your initials. Place this label on the lid of the paramecium tub.
3) All nursery tanks are 1.0 L and are fed 4000 paramecium/1.0 L fish water. To calculate the volume paramecium feed per tank take the density of the paramecium to be fed and divide it into 4000. An example, the paramecium tub has a density 150 para/ml then divide 4000/150=26.6 ml. For practical reasons always round to the nearest 5 ml when calculating feed 30 ml. If the densities are low requiring you to feed more than 50 ml to a nursery tank try counting a new tub to find a higher density, but do not dispose of the tub with low densities simply place it back on to the shelf (the tub maybe too old or too young) and inform the lab manager. The volume of paramecium needed for each laboratory

can be calculated by multiplying feed per tank by the total number of tanks in each laboratory. This information (tank counts) can be found on the H221 door.

4) To collect the paramecium for feed stir the tub again with a spatula as described above. Place a clean beaker in the sink, place the paramecium net (labeled and hanging above the sink) over the beaker, slide back the lid on the tub and decant the paramecium culture from the tub through the net in to the beaker. The paramecium net allows paramecium to pass but catches larger organic debris. From the large beaker pour the desired volume in to a clean 100 ml beaker and carefully pour the calculated amount of feed through the hole in the nursery tank lids. To transport paramecium between labs pour the paramecium in to a clean pitcher and place the lid on top or seal the opening of the beaker with aluminum foil.
5) Feed all larval fish dry (spirulina & artificial plankton) food located in the falcon tube modified as a "shaker" in the refrigerator. Give each tank one shake.
6) Feed all larval fish 10 days and older instar I Artemia. Using a 3 ml dropper add 6 drops to each tank.
7) Count the number of tanks in H, J, and Q labs and update the white board in H-lab on the total number of nursery tanks in use in each lab.

Adult Dry Feeding

1) Locate the small and large adult salmon crumble fish food in the refrigerator; H and J labs each have their own refrigerator with this food.
2) Feed all adult fish this food first (before feeding artemia). Start out feeding lightly across the top row of a system. Then start feeding the shelf just below it, while doing so look up at the row you just fed and if their food is already eaten go ahead and give them another pinch of food. It will take some time to get the hang of this. Reminder (always) fish food belongs in the tanks, not on the lids or on the floor.
3) For small fish use the small food, for larger fish use large fish food if its questionable what size to use give them a pinch of each size. Feed the fish not the tank i.e. look at the fish how many and how big are they before you feed them.

Artemia

Harvesting

1) First find the hatchery with the oldest date of setup. Dates including AM or PM and are found on the lids of the individual hatcheries.
2) Shut off air supply and remove the airline. Let the hatchery set stagnant for 10 minutes. The cysts should settle down into the bottom most layer, the mesa layer should be Artemia, and the upper most layer will be Artemia along with hatched cyst shells.
3) Place a waste vessel under the drain valve at the bottom of the hatchery. Open the valve and drain out the unhatched cysts usually the first liter of culture in to the waste vessel. Discard these cysts down the sink. Drain the rest of the hatchery into a clean beaker(s).
4) Take the beaker to the sink and get the two tuber ware screens (Artemia screens) labeled top and bottom. Place the top screen onto of the bottom screen and decant

1.0 liter of Artemia mixture from the beaker. Grab the white system hose and gently spray the Artemia through the top screen in to the bottom screen. Remove the top screen and decant the contents of the bottom screen in to a clean 2.0 L beaker using the system hose to wash the screen in to the beaker. Continue this process until all of the Artemia have been put through the screen and decanted into the beaker. Occasionally, flip over the screen and spray them with the hose from the other side to remove debris that is wedged into the screens, a small brush can also be used for this. The concentrated Artemia in the 2.0 L beaker are then diluted with system water to a volume of ~2.5 liters. Feed 1 liter to H and J laboratory fish and save a cupful for the quarantine laboratory. Transport Artemia in pitchers between H and J labs use a paper cup covered with aluminum foil for transportation to the quarantine lab.

Setting up a new Hatchery

1) Get Artemia cysts out of the refrigerator in H225. For the 5 L hatcheries #1,2, and 3 weigh out 12 grams of cysts in to a 250 ml beaker using the scale in the H-lab office. Then add enough system water to cover the cysts in the beaker. Hydrate the cysts for 20 minutes.
2) Go to the empty hatchery that is going to be set up. With the drain valve at the bottom of the hatchery open hose out the empty hatchery with RO water (green hose) in to a waste bucket and then empty the bucket down the sink. Connect the green airline to the drain valve. Then fill the hatchery to the 5-liter mark with RO water. The water should be vigorously bubbling with air.
3) Go to the carboy containing the super salt solution in H lab. Stir the contents of the jug with the PVC pipe at the top. Decant 750 ml of salt solution and pour it into the hatchery.
4) Add with a dropper 5 ml ammo-lock to the hatchery.
5) Decant the hydrated cysts into an Artemia net (white mesh nets located above the sink and labeled). Fill the glass bowl near the faucet with sodium hypochlorite (bleach) located below the sink. Swirl the net containing the cysts in the bleach for 90 seconds. There is a stopwatch on the shelf above the sink. Then rinse the cysts off under the faucet for an additional 90 seconds.
6) Take the net to the hatchery and invert it in to the hatchery releasing the cysts. Date the lid with the date of setup including AM (morning feeder) or PM (afternoon feeder). Place the lid back on top of the hatchery.

Feeding Adults Artemia

Artemia is then decanted into a squeeze bottle and feed to adult fish. Tanks that have a majority of “fat fish” should be fed lightly. Tanks with emaciated (skinny) fish should be fed extra along with any juvenile/small fish, which are primarily located on the bottom two rows of the Zmods in H and J lab.

Monthly Artemia

The artemia hatcheries need to be bleach disinfected monthly. Do this after the hatchery has been harvested and before it has been set back up again. Fill the hatchery with RO

water, add 100 ml bleach and put the lid back on top. Write on a paper towel 'bleaching' with the date and time and hang on it on the hatchery. Let the hatchery run (air on) for at least one hour. Then shutoffs the airsupply and drain the hatchery in to a waste bucket. With the drain valve still open rinse out the hatchery several times with RO water. Then close the drain valve, restart the air supply, fill the hatchery with 5.0 L of RO water and add 30 grams of sodium thiosulfate (dechlorinator). Let the system run for 10 minutes, drain, and rinse with RO water again. The hatchery is now ready to be used again, remove the sign and record the date of disinfection on the tape located on the hatchery housing.

Water Quality

Water quality parameters of temperature, pH, and conductivity (afternoon only) need to be recorded for each individual fish system and water towers. To do this locate the conductivity and pH meter in each laboratory see **Figure 8.0**, lightly spray the sensors (cell) with 70% isopropyl alcohol and shake off any excess alcohol. Place the sensors in the sump of the fish system to be tested, turn on the meters. Let the readings settle, Oakton meters take about a minute, Pinpoint meters take 5 minutes, and record the information on the water quality sheet located on each system. Spray isopropyl on the sensor every time you move it to a new system. Adjust the pH if need be by following the directions on the green card attached to the system. When you're done with the pH meter store the sensor in a conical vial labeled "cell storage". The conductivity probe can be left in the sump of the last system tested. There are separate digital thermometers on each system for water temperature. Water towers have there own conductivity and pH meters. Never place the meters themselves anywhere they can get wet.

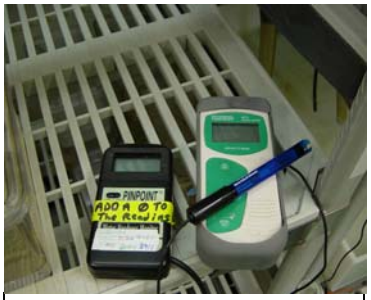

Figure 8.0 Left conductivity meter (black). Right pH meter (green and white).

Check and top off sumps if need be with system water on every fish system.

Refill water towers in H and J laboratories. Fill the tower by opening the RO valve and adding 100 ml of concentrated salt solution for every 10 gallons of RO added to the tower see section 6.8 for more details. The conductivity needs to be at 1500 µs when your done filling the tower.

Q-Room Feeding

The quarantine room needs to be kept completely separate from the main colonies. Follow procedures below carefully and always feed this laboratory last.

1) Take ~50 ml of harvested Artemia, paramecia (if any nursery fish) in paper cups covered with aluminum foil, a prox card that will gain you access to the comparative medicine wing G617, and the door key. Keep the prox card and key in your pocket when your not using it do not place it on any surface in the quarantine room.

2) Wash your hands upon entering the lab, put on a pair of latex-free gloves and a lab coat.
3) Feed all adult fish dry salmon food first, then feed Artemia. Feed any larval fish.
4) Take water quality readings on the flow-through fish system, nursery if there is any fish present and on the water tower. Record the temperature, pH, and conductivity information on the door. Note, clean all probes off with isopropyl before moving water meters to different fish systems. On the door there is a feeding checklist fill this out. The water tower has separate water meters they are located on the wall next to the tower.
5) Dispose of any paper cups in the trash can Remove the lab coat, gloves and wash your hands before leaving the lab. Do not take any items (dishes, tanks..etc) from the quarantine back to the main colonies in H and J laboratories.

8.3 Washing Dishes

Laboratory dishware and fish tanks are disinfected in bleach bathes, which are found in all 3 fish laboratories. Disinfection of equipment is crucial from a preventive medicine standpoint, but also carries some dangers. These hazards are due to the fact that sodium hypochlorite (bleach) is highly toxic to fish in very small quantities (and most organisms this is why we use it), but the risks are easily managed with adequate care.

Dirty dishes are thoroughly rinsed out with RO water to remove any organic debris, a sponge made be used to assist in removal, particularly if algae is involved, place dirty sponges in the net soak. Rinsed dishes are submerged in the bleach bath or if the bath is full they can be placed on the dirty dish cart (in q-lab there is a dirty dish shelf). Once in the bleach bath items need to soak for one hour before being removed. Always keep the lid sealed on the bleach bath when you're not using it.

To clean disinfected tanks put on a pair on rubber gloves, and a lab coat. Remove the items from the bleach bath and place them in the sink. Using the RO water hose thoroughly rinse inside and out all of the dishes. Generally, water should pass across every surface of every item at least 3 times. Minimize the backsplash from the hose to the immediate sink area. Then place the wet dishes on the drying rack above the sink (in q-lab there is a shelf). Let the items dry before using again.

8.4 Autoclaving

The autoclave is located next to J083b fish lab.
TO USE THE AUTOCLAVE YOU MUST BE SIGNED UP FOR IT
PUT GLOVES ON

1) Open the door, and slide the cart onto the lock mechanism (two small hooks on either side of the cart).
2) Slide the carriage out onto the cart.
3) For spawning traps load them directly onto the carriage. For any container that contains liquids find an autoclavable tray, usually located below the sign up notebook, and fill it with ~1.5" of water from the nearby sink. Place the containers in the water bath and LOOSEN ANY LIDS to relieve pressure.
4) Once the carriage is loaded, slide it back into the autoclave. Unhook the cart from the autoclave and slide it back.
5) Close the door, and seal it by turning the handle clockwise. Hand tighten, if during the start up phase the autoclave does not start try tightening this handle more.
6) Select the time for autoclave, it's just to the left of the door. Make sure the dry time clock is set to 0 minutes.
   Times:
   Spawning Traps: 25 minutes
   Water vessels: 45 minutes
7) Press the white ISO reset button
8) Choose the cycle, just above the timer, for traps press gravity, for water containers select liquids.
9) You should hear the autoclave start up immediately, if not repeat steps 6-8.
10) Both water and traps will be done in roughly 1 hour.
11) Before unloading check the digital pressure gauge top left, it should read less than 1 atmosphere. There will also be red illuminated letters that say DONE.
12) Open the door by turning the handle counter clockwise, BE CAREFUL SOME STEAM WILL EXIT THE MACHINE NEAR THE TOP OFF THE DOOR.
13) PUT GLOVES ON, and CAREFULLY UNLOAD THE HOT ITEMS. Hot water can be dumped down the near by sink.