

Purdue University Department of Biological Sciences Zebrafish Facility Manual



Updated September 2016, D. Bieseimer

Biology Department Zebrafish Personnel

Facility Manager:

Deb Biesemeier
Office: Lilly 2-122
Office phone: (765) 496-3383
Cell phone: (765) 491-6888
Email: biesemei@purdue.edu

Professors:

Dr. Donna Fekete
Dr. Yuk Fai Leung
Dr. Joseph Ogas
Dr. Claudio Aguilar
Dr. Daniel Suter
Dr. Qing Deng
Dr. Estuardo Robles

Lab Techs/Contacts:

Fekete: Deb Biesemeier/biesemei@purdue.edu
Leung: Skye Brown/brown810@purdue.edu
Deng: Xiaoguang Zhu/zhu410@purdue.edu

This manual covers the Purdue University Department of Biological Sciences Zebrafish facility located in the Lilly Small Animal building, rooms 139 and 164.

Fish Facility Overview

Fish Maintenance is our principal system for ensuring the health and well-being of the fish stocks, and it is our first line of defense against catastrophic loss. To ensure continued productivity, all procedures must be followed exactly as written. Our Fish Maintenance procedures have evolved over time and improvements will always be considered. However, any changes must be brought to the Fish Facility Manager for careful consideration and no procedural changes may be implemented without prior approval.

Fish Maintenance includes four major tasks:

1. Assessing the health of the fish
2. Maintaining optimal environmental quality and mechanical components
3. Feeding the fish
4. Maintaining cleanliness and professional appearance of the room

These are addressed separately below. Detailed protocols are available for some tasks.

Fish Health

Many of our fish are specially bred and genetically unique. They are important both in terms of time spent to generate particular strains and potential research value. Our aim is to maintain very healthy stocks and to intervene quickly to correct problems as they arise.

Fish health is evaluated by carefully observing the physical appearance and behavior of the fish in every tank. Dead fish must be removed immediately, logged appropriately and disposed of properly. All live fish should be observed for common symptoms of illness:

- body shape – skinny or bent
- bloating with raised scales – a fuzzy appearance
- fins held close to body, rather than spread
- bulging eyes
- open sores
- torn fins or tail
- damaged, discolored or missing operculum (gill covering)
- rapid breathing
- erratic swimming
- prolonged resting on tank bottom or floating at the surface
- rubbing body on sides or bottom of tank (except when in breeding tanks)

If fish with any of these symptoms are found it should be immediately reported to the researcher in charge of the tank. Sick fish may be isolated in a separate tank, and possibly removed from system water flow. Any treatment with antibiotics or other chemicals must be done with tank removed from the system water flow. Severely affected fish may be euthanized.

Please note that some observations that appear to be symptoms of illness may be an expected genetic phenotypic trait. Always consult the owner of the tank before euthanizing or disposing of fish that appear sick.

Environmental quality

Our goal is to maintain the highest possible environmental quality for the fish at all times. Under normal conditions, fish grow rapidly and reproduce readily. By contrast, declines in environmental quality can kill fish directly, but can also retard growth, inhibit breeding, and impair the fishes' immune response. Deterioration in the environment can have effects that are acute and catastrophic, or chronic and initially hard to detect. Therefore, we take every precaution to prevent environmental problems before they occur.

Assessing environmental quality in the fish system is the responsibility of everyone who works in the fish room and begins the moment you walk into the fish room. Be aware of any unpleasant odors, air temperature, mechanical sounds, air handling noises, water on the floor, burned out lights, etc. If these or any other things seem abnormal, investigate immediately and thoroughly to find the source of the problem. If you can't identify it, consult with the facility manager or one of the professors. **Even seemingly innocuous changes can have serious consequences if not addressed as soon as possible.**

Clean vs. Quarantine Areas: The main system in LSA 139 is to be considered a 'clean system'. The free-standing system in LSA 164 is to be considered a 'quarantine system' and also serves as our "backup/redundant" system. Nothing (fish, feed, water, tanks, supplies or equipment) may be taken from the quarantine system to the clean system unless adequately cleaned or sterilized. There should be a complete set of supplies and equipment for each system. These must not be moved between clean and quarantine systems.

Only fish bred within the clean system or fish from bleached eggs from outside the clean system will be allowed into the clean system in LSA 139. Incoming adult fish must always be housed in the quarantine system and may not be moved to the clean system in LSA 139 under any circumstances! Fish may be moved from the clean system in LSA 139 to the quarantine system in LSA 164 to house breeding populations and/or redundant populations, but they may not be moved back to LSA 139 after being housed in LSA 164.

The floors are to be considered contaminated areas. No tanks, nets or other related fish supplies should be placed there. If a fish is dropped on the floor it must be moved to a tank on the quarantine system or euthanized. It cannot be returned to the clean system.

When moving between the clean and quarantine systems every effort should be made to avoid cross-contamination between the systems. Hands must be washed thoroughly with soap and water both before working in a room and when finished with your work, especially when moving from one room to another. When washing hands, make sure to thoroughly rinse them with water to remove ALL traces of soap, which is toxic to the fish.

Lab coats: It is optional to wear lab coats in the fish rooms. Do NOT wear lab coats between fish rooms (LSA 139 and LSA 164). Use a separate lab coat for each room.

Gloves: While wearing gloves is optional, it is highly recommended that you wear gloves when handling fish or fish system water. Our fish have been found to have mycobacteriosis, a common disease of laboratory zebrafish, as well as fish in the wild. Although a rare occurrence, mycobacteriosis can be transmitted to humans through cuts or abrasions on the skin. It is difficult to treat in humans due to resistance to antibiotics, so prevention is the best approach. You may use disposable latex or nitrile gloves, or re-usable rubber gloves. Please wash your gloves and/or hands, or remove gloves, after you have touched fish, system water, filter pads, etc. and before touching anything else, such as door knobs, water faucets, rack valves, squirt bottles, etc.

Mechanical Components

In the context of Fish Maintenance we pay close attention to the water mechanical system, biological filtration, water flow and conditioning throughout the system, and water flow and quality in individual tanks.

Mechanical system overview: We use recirculating water systems designed by Aquatic Habitats. The systems are termed 'recirculating' because most of the water is re-used, with only a comparatively small amount regularly replaced with fresh water. The main system in LSA 139 circulates water through all of the connected racks. The quarantine system in LSA 164 is a 'stand-alone' system that operates in the same way, just on a smaller scale.

These systems continuously circulate water in the following manner: from individual fish tanks the water drains out of each tank to horizontal drain troughs and vertical drain pipes, through filter pads and into sump tanks at the bottom of each rack. The sump at the bottom of each rack contains a biological filter, and some contain air diffusers. From the sump the water flows to a central filtration system where it is exposed to UV light and passes through a particle (mechanical) filter and a carbon (chemical) filter. After filtration the water flows through water carrying PVC pipes along the top of each rack, down a vertical manifold on each rack, to horizontal manifolds on each row, and through a control valve and blue hose into each individual tank.

Recirculating systems are very efficient and provide a high quality environment; however they can also be very vulnerable. Failures at any point can affect the entire system: foreign and/or lethal chemicals will be spread rapidly throughout the tanks, high loads of pathogens or parasites can overwhelm the UV lamps, and failures in the biological filtration can leave toxic levels of ammonia and nitrites in the water. For these reasons, we must be extremely vigilant in monitoring and maintaining all aspects of the system, and in preventing contamination from outside chemicals and pathogens.

The systems use four main types of filtration:

- 1) **Mechanical:** Particle filter pads are present in every sump and remove uneaten food, fish feces, and other solid matter. If these filters become saturated with debris they interfere with water flow and become ineffective. We change/rotate these filters three times per week, or more often as needed.
A second method of particle filtration occurs in a large filter vessel which contains a 50 micron filter bag. This traps solid matter that escapes the particle filter pads. This filter is changed as needed, depending on fish population.
- 2) **Chemical:** One of the large filter vessels contains a 100 micron filter bag containing activated carbon pellets. The carbon removes dissolved waste products from the water and the 100 micron filter bag further filters solid matter. This filter is changed as needed, depending on fish population.
- 3) **Biological:** Biological filters consist of denitrifying bacteria that colonize the bio-filter media (Siporax) located in every sump. As fish metabolize their food and as organic matter decomposes, ammonia is produced. Ammonia is toxic to the fish, but with a healthy biological filter, bacteria convert the ammonia to nitrite, (which is also toxic), and other bacteria convert the nitrite to nitrate, which is relatively harmless to the fish. For these denitrifying bacteria to survive, they must receive adequate oxygen. This is accomplished in the sumps through constant water flow and aeration. Lack of oxygen, as well as chemicals and antibiotics, will kill these bacteria, resulting in toxic levels of ammonia in the fish system. The biological filters are monitored by checking ammonia, nitrite and nitrate levels.
- 4) **Ultraviolet:** UV lamps are used to minimize the number of water-borne pathogens. They can also help reduce the risk of cross-contamination between tanks. LSA 139 has two UV sterilizers, each containing 3 UV lamps. LSA 164 has one UV sterilizer that contains 1 UV lamp. The lamps are changed annually.

Water flow and conditioning: Water flow for each system is achieved with water pumps that provide continual water flow to all the fish tanks. If water pumps are malfunctioning they will probably make unusual or alarming sounds, or they may make no sound at all if they have completely failed. Because

water flow is so important we have backup water pumps. Any problem with water pumps should be immediately reported to the facility manager or one of the professors.

Water levels of sumps will give an indication of the correct operation of the water flow system. Unusually low levels may indicate a malfunction or leak and should be investigated immediately. All of our systems are automatically replenished with RO water to compensate for evaporation. This water comes from the campus reverse osmosis (RO) system that deionizes and dechlorinates tap water. In LSA 139 the RO water is collected in a 165 gallon reservoir tank, and from there it empties into a nearby sump, as needed. In LSA 164 the RO water is manually added to a carboy, then empties into the sump, as needed. If there is a leak in either system, the addition of RO water to replace water volume will result in a gradual reduction of the conductivity levels if an appropriate amount of salt is not also added. During Fish Maintenance we monitor water volume and conditioning by checking system water conductivity and water levels in the sumps.

Water loss that occurs during tank washing, breeding, or any other cause, as well as additional water needed when new tanks are added to the system, is managed differently on the two systems:

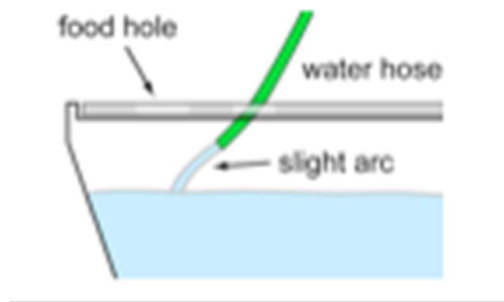
In LSA 139, both water and salt are added automatically to replenish the amount lost (including water lost from leaks) and/or additional amounts needed.

In LSA 164, RO water will be added automatically from the carboy to replace water from evaporation, leaks, or any other cause. However, **any water loss other than evaporation** requires the manual addition of both RO water and salt, in a concentration equal to the current system conductivity, to prevent a drop in conductivity. If you are breeding fish, adding tanks, or removing tanks in LSA 164, make sure you know the proper way to do this to maintain system conductivity **BEFORE** attempting to do any of these things.

Note on RO water: Although the campus RO water system has historically been quite reliable, there have been times when it has malfunctioned and failed to remove chlorine from the water. Even low levels of chlorine are toxic to fish and could result in a catastrophic loss of fish. During Fish Maintenance we monitor all sources of RO water for chlorine. If ANY chlorine is found in ANY RO water source, steps must be taken immediately to prevent RO water from entering ALL fish systems!

Water flow and quality at the tanks: During Fish Maintenance we check water flow and environmental conditions in each tank. All tanks containing fish must have water flow, unless they are being contained in static tanks for breeding or genotyping. Some empty tanks may be kept 'running' on the stand-alone system is LSA 164 to maintain a large total volume of water, if the system is operating at less than half capacity. However, as a rule, only tanks with fish should contain water.

- 1) Tanks containing juvenile and adult fish (not including breeding or nursery tanks) should have a continuous flow of water from the blue hose. The end of the hose should be above the water line and the water flow adjusted to form a moderate arc as it leaves the hose. It should not jet straight out from the hose or fall straight down (see illustration below). Ten-liter tanks may require a stronger water flow and that water flow may generate a few bubbles. As a rule, 3-liter and 1-liter tanks should not have water flow that is forceful enough to generate bubbles.



- 2) The water flow should be directed at the front edge of the tank by inserting the hose in the second, center hole of the tank lid (shown in dark blue below). Some tanks will have a small, angled hole behind the second hole (shown in green below). If this hole is present, the hose should be inserted into this hole, which will correctly angle the hose and hold it firmly in place. The amount and direction of water flow should provide enough water flow to flush away waste and uneaten food, yet not produce excessive turbulence for the fish. The front, left hole (shown in yellow) is for feeding the fish.

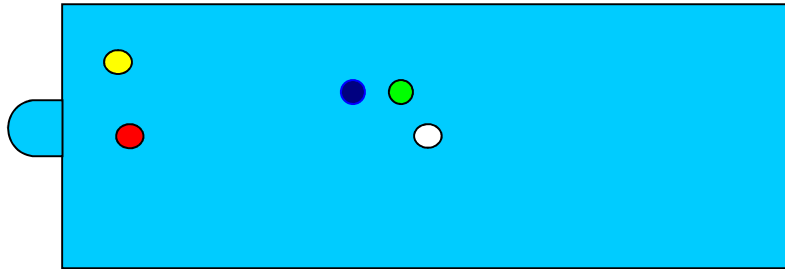


Diagram of 3L tank lid – other sizes are slightly different

- 3) Nursery tanks containing larval fish may or may not have water flow, depending on the age of the fish. See the instructions posted on the wall near the nursery tanks.

In addition to water flow, tanks should be checked to verify that every tank is stable and seated correctly on the rack to allow proper emptying into the drain trough at the back.

Maintaining clean tanks: Tank owners are responsible for keeping their fish tanks clean and in good operating condition. Tanks should be checked a minimum of once weekly!

Our tanks are designed to flush debris from the bottom of the tanks, front to back. Water and debris leave the tank through a gap at the bottom of the solid baffle at the back of the tank. Occasionally the space between the baffle and the tank will become clogged with debris that restricts the water flow and interferes with the flushing of the tank. This debris can be observed and/or the water level of the tank may rise when water flow is restricted. Tanks with restricted water flow should be cleaned to avoid the tank overflowing.

Fish tank cleaning protocol: Fish should be transferred to a clean tank and the dirty tank washed to remove debris and algae. Dirty tanks are washed with RO water only (no soap) and a scrub pad. Hand washed tanks may be re-used or placed in the storage room to eventually be run through the cage washer for hot water sterilization. **All solid debris and algae must be scrubbed from the tanks BEFORE being placed in the storage room.** The cage washer will not remove dried debris and algae, and the heat will cause this debris and algae to permanently adhere to the surface of the tank!

Important: Never use tap water, soap or detergent on any fish system parts or anything that comes into contact with fish or fish system water! Chlorine, soap and detergent are toxic to fish.

Breeding tank inserts are sometimes used to separate multiple fish in a single 3-liter tank. The use of the breeding tank insert interferes with the self-cleaning design of the tank, causing debris to accumulate on the bottom of the tank at a faster rate than usual. Anyone setting up a tank in this way must check it on a weekly basis and clean it when solid debris accumulates.

Tanks containing juvenile fish should have a baffle with a screen insert that sits tightly against the bottom of the tank. This allows water flow through the screen, but prevents small fish from being washed out of the tank. The use of screen baffles interferes with the self-cleaning design of the tank causing debris to accumulate on the bottom of the tank at a faster rate than usual. Anyone setting up a tank in this way must check it on a weekly basis and clean it when solid debris accumulates on the surfaces of the tank and/or interferes with water flow through the screen. Please replace the screen baffle with a regular baffle when the fish are large enough to not be washed under the baffle.

Note: *The screen baffle must be pressed firmly against the bottom of the tank or small fish may be washed out of the tank! When the fish have reached adult size, remove the screen baffle and replace it with a regular solid baffle.*

Dirty tank baffles (solid and screen) will restrict the water flow leaving the tank and will result in a high water level in the tank. If the water level is high enough fish will be washed over the top of the baffle and out of the tank. Protect your fish by keeping your tank baffles clean!

If excess food and other debris has accumulated on the bottom of a tank, there are 4 possible causes:

- 1) Water flow in that tank may be insufficient to flush debris out of the tank.
- 2) Fish may be overfed.
- 3) The baffle at the back of the tank may be plugged with debris.
- 4) There may be a screen baffle in the tank, so some food accumulation at the bottom of the tank is unavoidable.

The cause should be determined, if possible, and steps taken to correct the problem.

Tanks should be inspected by the user on a **weekly** basis for accumulation of debris on the bottom, algae and other deposits on tank surfaces, and solid waste that blocks water flow through the baffle. A layer of debris on the bottom of the tank can harbor bacteria and/or parasites, creating an unhealthy environment for everyone's fish. Deposits of algae are generally not harmful to the fish, but can make it difficult to effectively observe the health of the fish. Blocked baffles will restrict water flow and raise the water level in the tank, causing fish to be washed out of the tank and/or the tank to overflow. When necessary, fish should be transferred to a clean tank and the dirty tank properly washed. All tanks in the facility will be inspected frequently by the facility manager. If it is determined a tank is in need of cleaning, a dated, red Post-It flag will be placed on the tank. Tank owners must check their tanks frequently and clean any flagged tank within one week. An email will be sent to the owner of any tank requiring immediate attention. To avoid being inconvenienced, please check your tanks on a regular basis and keep them in good condition.

Feeding the fish

Food is prepared in LSA 139 for all fish rooms. An adequate amount can be carried in a paper cup to LSA 164.

Fish are fed live food and dry food twice each day, Monday through Friday, between 8:30 a.m. and 10:30 p.m. (automatic light cycle schedule). As much as possible, effort should be made to allow a minimum of 6 hours between the beginning of the morning feeding and the beginning of the afternoon feeding. On weekends and holidays fish are fed live food and dry food once each day. This may be done at any time during the light cycle, but the brine shrimp hatching cone should be set up before 12:00 noon to allow adequate time for hatching before the following day's feeding.

Note: Tanks in photoperiod boxes may be on a different light schedule than the room light cycle. Please check the sign posted on the front of the box before opening the door. The door should not be opened during the dark cycle, except in an emergency. Fish in photoperiod boxes may be fed through the blue tube protruding from the top of the door.

NEVER feed the fish if there are problems with water quality, mechanical components, or issues with system-wide health. If necessary, fish can last many days without food, but adding more biological material (food) to a system in trouble can tip the balance toward disaster. Always ask the facility manager or a professor if there is any doubt as to whether or not the fish should be fed.

Fish should be fed enough to stay healthy and breed well. In general, feed all fish conservatively. Try to avoid overfeeding as uneaten food will foul the tanks, clog the filters and waste money. In addition, overfed fish will not breed well. Take the time to observe individual tanks after feeding to see if the fish are consuming all the food. Make adjustments accordingly.

Live food is in the form of brine shrimp, which are decapsulated prior to hatching. Brine shrimp are grown in a hatching cone in a concentrated salt solution with continuous aeration. Brine shrimp are harvested 24-36 hours after a culture is set up. Unhatched, decapsulated brine shrimp are safe to feed to the fish. Brine shrimp have the most nutritional value before and shortly after hatching, and that nutritional value decreases with time after hatching. Hatched brine shrimp should not be fed to the fish 48 hours or more past set-up as they have minimal nutritional value.

The dry food is a mixture of fish feeds, specially formulated for use in aquatic research facilities. The bulk stock of this feed mixture is kept frozen at -20°C in LSA 143. Only small amounts are kept in the fish rooms to avoid contamination caused by high humidity. The small supply of fish food that is kept in the fish room should be dated when the container is refilled. Fish food stock components are to be dated on arrival and stored in a cool, dry place. The fish food mixture is dated when prepared and stored at -20°C in LSA 143. The facility manager should be notified when this food mixture is in low supply.

Live food (brine shrimp) and dry food are mixed in a squirt bottle (labeled for this use ONLY) and the squirt bottle used to feed the fish. The food mixture should be delivered through the hole at the front, left corner of the tank lid. Fish feeders are responsible for keeping tank lids clean and should use a paper towel to clean the lid of spilled feed. If you find a tank lid that is excessively dirty or has mold growing on it, use RO water and a paper towel to clean it, then replace it on the tank.

Room cleanliness and organization

The fish room is a professional research facility and should be treated accordingly. While cleaning up after oneself is everyone's responsibility, the person assigned to Fish Maintenance on any day verifies that things are clean and orderly. This includes the following responsibilities:

- 1) All tank lids must be clean and dry after feeding. Even when care is taken, small amounts of food inevitably end up on tank lids. A damp paper towel can be used to wipe food and water from the top of the lid. If lids are wet this may indicate a leak. Inspect tubing and tanks above the wet tank to pinpoint and correct the problem.
- 2) Everyone using the fish room should clean their own glassware and plastic ware after use. If necessary, people will be asked to return to the fish room to clean up after themselves.
- 3) Monitor the sinks for clutter and cleanliness. Hoses should be looped around the faucet to keep them from lying in the bottom of the sink where they will develop mold. Check paper towel dispensers and fill when running low.
- 4) Monitor the stock of regularly used supplies: transfer pipets, water test dipsticks, sea salt, filter pads, paper cups, trash can liners, etc. As needed, replace or place on re-order list. Stock of some of these items are kept in LSA 143.
- 5) The floor should be swept and mopped on a regular basis.
- 6) The trash should be emptied as needed. Waste baskets should be lined with TWO trash bags to minimize leaking. When full, trash liner should be tied shut, then placed with the tied end up in the trash dumpster near the elevator. Make sure there are no holes in the outer trash bag that will allow liquid to leak onto the floor or into the trash dumpster! Place two new trash liners in the waste basket. Extra trash liners are on the shelf above the sink in LSA 139 and on the shelf in LSA 164.

Disposal of dead fish

Dead fish should be disposed of in the dead animal disposal box inside the freezer in LSA 110. Dead fish can be transported in a paper cup, paper towel or plastic beaker. **Only dead fish** are allowed in the dead animal disposal box. Make sure the fish go inside the box, and are not stuck on the cardboard lid! Make sure to close the lid of the freezer before walking away. Paper cups and paper towels go in the trash barrel next to the freezer. Plastic beakers should be returned to the fish room and washed with RO water.

Security of the fish rooms

The fish rooms are located in the Lilly Small Animal (LSA) building, which is a locked animal facility. You need a key to access LSA and you should not give access to anyone you are not certain has official business in LSA.

Doors to both fish rooms should be locked after the last feeding of the day on week days, and on weekends and holidays. The doors may be kept unlocked on regular work days when the rooms are frequently occupied.

Only personnel fully trained and approved/documentated by the fish room manager are allowed to work independently (without supervision) in any fish rooms. Personnel that are untrained or in-training must be supervised by someone trained and approved/documentated. To obtain training and approval/documentation, contact the facility manager.

As always, if you have a question, please ask!

Regular fish room chores are assigned by room, feeding (morning or afternoon), day of the week, and/or weekend or holiday, as shown in the charts below. Details and descriptions of the chores are listed following the charts, and ordered by the number in the first column.

Note: These charts may be printed and used as guidelines while working in the fish rooms.

LSA 139/Main fish room:

Chore #	Chore	Weekday AM	Weekday PM	Weekend/Holiday
1	Harvest, rinse & prepare brine shrimp & dry food mix for adult and nursery fish.	X	X	X
2	Feed the adult and nursery fish.	X	X	X
3	Check & clean tank lids of adult and nursery tanks.	X	X	X
4	Set up brine shrimp hatching cone for the following day.	X		X
5	Wash the empty hatching cone.		X	X
6	Check level of brine solution carboy. Refill if remaining amount is 10 liter.	X		X
7	Check water parameters on YSI & TGP monitors.	X		X
8	Use dipstick to check nitrate & nitrite levels in system water – Monday, Wednesday and Friday only.	X		
9	Use dipstick to check for chlorine at the water source for each room.	X		X
10	Use dipstick to check ammonia level in system water – Tuesday only.	X		
11	Check pressure levels in filtration cylinders while water flow is ON to all fish tanks.	X		X
12	Check UV lamp LED indicator lights.	X		X
13	Check levels of conductivity & pH dosing tanks.	X		X
14	Change/rotate filter pads in each sump – Monday, Wednesday and Friday afternoon only.		X	
15	Check/empty trash.	X	X	X
16	Check all tanks/blue hoses after restoring water flow.	X	X	X
17	Check sink & room for general neatness.	X	X	X
18	Record the room temperature and humidity.	X		X
19	Remove and dispose of dead fish.		X	X
20	Lock the door.		X	X

LSA 164/Main fish room:

Chore #	Chore	Weekday AM	Weekday PM	Weekend/Holiday
2	Feed the adult and nursery fish.	X	X	X
3	Check & clean tank lids of adult and nursery tanks.	X	X	X
8	Use dipstick to check nitrate & nitrite levels in system water – Monday, Wednesday and Friday only.	X		
21	Manually take and record conductivity of system water.	X		X
22	Manually take and record pH of system water.	X		X
23	Check and record the system water temperature.	X		X
9	Use dipstick to check for chlorine at the water source for each room.	X		X
24	Check the level of the sump.	X		X
25	Manual water change – Monday, Wednesday and Friday afternoon only.		X	
10	Use dipstick to check ammonia level in system water – Tuesday morning only.	X		
11	Check pressure levels in filtration cylinders while water flow is ON to all fish tanks.	X		X
14	Change/rotate filter pads in each sump – Monday, Wednesday and Friday afternoon only.		X	
15	Check/empty trash.	X	X	X
16	Check all tanks/blue hoses after restoring water flow.	X	X	X
17	Check sink & room for general neatness.	X	X	X
18	Record the room temperature and humidity.	X		X
19	Remove and dispose of dead fish.	X	X	X
20	Lock the door.		X	X

Explanation of Chores by Chore #:

1. Harvest Brine Shrimp/mix with dry food

Use the chart posted on the wall to determine amount of harvest appropriate for the day:

- a) Get a 2L beaker from the dish drainer on the wall above the sink or you may use one of the white buckets on the bottom shelf below the hatching cones.
- b) Pull both air tubes out of the brine shrimp culture, but do not turn off the air to these lines. The lines can rest at an angle in the cone while harvesting the brine shrimp. Drain appropriate amount of hatched brine shrimp from the cone. Replace the air tubes at the bottom of the cone and use the brine solution squirt bottle to rinse down any brine shrimp stuck to the sides of the cone.
- c) Pour the hatched brine shrimp through the green brine shrimp strainer. Rinse the collected brine shrimp with RO water from the RO faucet to remove the brine solution.
- d) Distribute the rinsed brine shrimp as follows:
 - a. Rinse the brine shrimp into the three squirt bottles labeled for fish feeding, distributing as evenly as possible. You may use a funnel and RO water to facilitate this. Don't fill the squirt bottle to the top: leave room to add dry food!
 - b. Pour 50 ml of brine shrimp into the 100 ml plastic beaker (drain rack above the sink) for the nursery fish greater than 15 dpf. Dry food will be added following the instructions below.
- e) In the morning only, take a sample from one squirt bottle, using a transfer pipet, and use the microscope near the hatching cones to estimate the hatching percentage. Record that number on the "Brine Shrimp Hatch Record" sheet.
- f) Add dry food to squirt bottles for adult fish AND beaker for nursery fish:

Adult fish:

 - a. Weigh out the appropriate amount of adult dry food (see the chart posted on the wall) and distribute evenly between the three squirt bottles. Fill the squirt bottles to the top with RO water.
 - b. Pour an appropriate amount of brine shrimp/dry food mixture into a paper cup for the quarantine room.

Nursery fish:

 - a. Use the small, white scoop (stored in large container of fry food in the refrigerator in LSA 143) to measure a level scoop of Otohime A fry food. Add it to the beaker of brine shrimp.
 - b. Use a transfer pipet to mix the brine shrimp and fry food thoroughly, then use the transfer pipet to feed 4-6 drops of this food mixture to the tanks with fish 15 dpf and older. Stir the mixture periodically to keep both the brine shrimp and fry food suspended in the beaker.
 - c. If there are nursery fish in the quarantine room, use the remainder to feed these fish.

2. Feed the adult and nursery fish

Adult Fish:

- a) Water flow to the fish tanks is turned off during feeding:

In LSA 139 this is done by turning off the grey knob at the left or right end of each tank row. **Do not use the red emergency valve at the top of each rack to control water flow while feeding!** The grey knob is imprinted with the word "Quest". "Quest" will read right-side-up when the water flow is on and upside-down when the water flow is off. Do not turn off more than half of the racks at one time.

In LSA 164 this is done by turning the red toggle valve at the right end of each row. The valve will be horizontal when the water is on and vertical when the water is off. Do not turn off more than **half** of the tanks or rows with water flow at a time. When possible, some tanks without fish are

kept on this system to enable turning off all tanks with fish for feeding at one time. If the system is full, only turn off and feed half the tanks or rows at a time.

- b) When feeding tanks in a photoperiod system in LSA 139, pay attention to the instructions on photoperiod system door. This information will tell you how many fish are in each tank and when you can open the door. Water flow is not turned off in the photoperiod system during the 'dark' cycle. If the door must remain closed, an appropriate amount of feed mixture is squirted through each blue hose sticking out of the top of the door to the photoperiod system. Use the RO squirt bottle to rinse the hose following feeding. If the door can be opened, feed like the other system tanks, turning off the water flow and feeding through the hole in the tank lid.
- c) Use the squirt bottle to feed brine shrimp/dry feed to each fish tank. Gently shake the bottle periodically to keep the food suspended. Carry a paper towel and clean up any mess you make on tank lids! **Each feeder is responsible for keeping tank lids clean!**
- d) Amount to feed: a single, adult fish should be fed 3-4 drops of the feed mixture. This amount is adjusted to accommodate the number and size of the fish in each tank. Overfeeding will be obvious if a large amount of food collects on the filter pads or at the bottom of a tank, or if the baffle plugs frequently. **Make every attempt to NOT overfeed the fish!**
- e) Each set of racks should be allowed 15 minutes (timer on the back of each room door) to eat before turning the water back on. Then the water flow to the next set of racks may be turned off and the fish fed, following the same protocol.
- f) When finished feeding, use the ladder to check the top of every tank (both adult and nursery) and clean any food spills using a damp paper towel.
- g) Make sure water flow to all fish tanks is restored after the fish have been fed. Make a check of the proper position of the blue water hoses in ALL fish tanks before leaving the room! Sometimes water pressure will dislodge them when water flow is restored.

Nursery fish:

Nursery fish have different water flow requirements and are fed differently dependent on their age:

0-14 dpf: Water flow is off except for immediately before each feeding, when it is turned on at a slow drip for 10 minutes. Turn on dripping water for 10 minutes before each feeding. Turn water off after 10 minutes and feed one micro scoop of fry food twice per day during the week and once on weekends and holidays (stored in Petri dish in refrigerator in LSA 143).

15-60 dpf: Water stays on at a gentle, but steady, flow at all times and are fed a mixture of brine shrimp and Otohime A fry food following the instructions below.

Use a transfer pipet to feed 4-6 drops of the feed mixture to each nursery tank 15 dpf and older. Periodically swirl the mixture to keep the brine shrimp and dry food suspended.

When finished feeding the nursery fish, check all nursery tank lids for spilled food and clean with a paper towel.

In LSA 164 the nursery tanks are basket inserts. Remove the lid from these baskets before cleaning. Replace gently so as to not dislodge the basket, which may cause it to drop into the tank. There may also be nursery tanks temporarily moved from LSA 139 and these should be fed the same as those in LSA 139.

After feeding all the fish, write your initials on the "Checklist for Fish System" in LSA 139 and the "Twice Daily Checklist" in LSA 164 under "Fish Feeder Initials/PM".

3. Check & clean tank lids of adult and nursery tanks.

Fish feeders are responsible for making sure ALL tank lids are clean of food debris when they are finished feeding. Use the ladder to check any lids not visible while standing on the floor.

Fish feeders who discover lids left dirty from the previous feeder may contact that person and request they return to the fish room to complete this job. Ultimately, each person who feeds the fish is responsible for leaving the lids clean when they are finished, regardless of whether they found them in that condition or not.

4. Set up brine shrimp hatching cone for the following day

Use the chart posted on the wall to determine amount of brine solution and decapsulated brine shrimp appropriate for the day:

- a) Use the clean, empty hatching cone to set up the next harvest. Make sure the hatching cone, drain tube, air hoses and air stone are clean. Make sure the drain at the base of the hatching cone is closed before adding brine solution.
- b) The air stone should be placed into the drain hole at the bottom of the cone and the straight air tube placed at the base of the cone, off to one side.
- c) Use a beaker or bucket to measure premixed brine solution (carboy next to the hatching cones) and pour into the hatching cone. The amount needed is listed on the chart posted on the wall. The air flow shouldn't need to be adjusted, but if needed adjust air to both airlines to moderate, but steady turbulence.
Note: When the brine hatching solution carboy reaches 10L or less, refill it following the directions in #6 below.
- d) Get the storage bottle of decapsulated brine shrimp from the refrigerator in LSA 143. Shake the bottle well to re-suspend the brine shrimp cysts. Pour the appropriate amount of decapsulated brine shrimp into a small beaker. Wait for cysts to settle to get an accurate measurement. The amount needed is listed on the chart posted on the wall. **Do not use more than the posted amount!**
- e) Rinse the sides of the decapsulated brine shrimp storage bottle with brine solution and place it back in the refrigerator in LSA 143.
- f) Pour the measured cysts into the hatching cone. Rinse the beaker and sides of the cone with brine solution, using the brine solution squirt bottle.
- g) Put the hatching cone lid in place, making sure the airlines remain properly placed.
- h) Place a label indicating the day the culture will be harvested on the rim of the cone stand. Pre-made labels are on the wall behind the cones and have Velcro on the back to attach to the Velcro on the rim of the cone stand. The harvest day is the day AFTER the hatch is set up. (ie. Set up on Monday; harvest on Tuesday.)
- i) If you see cysts sticking to the sides of the cone at any time during the day, rinse them down with brine solution.

5. Wash the empty hatching cone.

- a) Take the empty hatching cone and the airlines to the sink. Use the RO water and a white scrub pad to thoroughly clean the inside of the hatching cone, the airlines and air stone. Do NOT use soap! Clean the air stone gently as they break easily. If the stone breaks there are replacements on the shelf behind the hatching cones. The air stone is attached to the rigid air tube using a flexible piece of tubing. Additional flexible tubing is in one of the plastic drawers under the sink.
- b) If necessary, remove the drain tubing from the bottom of the cone and clean with a brush.
- c) Clean the hatching cone lid.
- d) Put the clean hatching cone back in the hatching stand. Place the clean airlines in the cone, feeding them through the holes in the lid. Place the lid off center on top of the cone so the cone can air dry overnight. Reconnect the air tubing to the air lines. The air flow may be left on at all times.

6. Check level of brine solution carboy. Refill if remaining amount is 10 liters or less.

- a) Refill the brine solution carboy at 10L or less. To refill, use the hose from the 165 gallon RO water reservoir.
- b) Follow the instructions regarding the amount of Instant Ocean salt and sodium bicarbonate (stored in LSA 143) posted on the front of the carboy.
- c) Place air tube back in the carboy and make sure the air is on full.
Note: The brine shrimp solution carboy should be cleaned, as needed, by dislodging solidified salt and rinsing thoroughly with RO water. Do not allow the solidified salt to go down the sink drain!

7. Check water parameters on YSI & TGP monitors.

- a) Record water parameters from the YSI and TGP monitors on the "YSI Monitoring System Check List", located on top of the pH dosing tank.
- b) All water parameter readings should be checked against the chart showing acceptable and unacceptable readings posted next to the YSI monitor.
If an unacceptable reading is found it must be reported to the facility manager or one of the PI's.

8. Use dipstick to check nitrate & nitrite levels in system water.

Nitrate/nitrite levels are checked on Monday, Wednesday and Friday morning only.

- a) Use the 5-in-one water test strips located in the plastic drawer under the sink. The stock of these test strips are in the gray bin on top of the refrigerator in LSA 143. Notify the facility manager when you take the last bottle of test strips from the gray bin.
- b) Test for nitrate/nitrite by dipping a test strip in a fish tank located on the system. Follow the instructions on the bottle regarding the time to wait before taking the recording. Record the values on the water value checklist located on top of the water filter cylinders.
- c) Notify the facility manager if the nitrite value is above "20" and/or the nitrate value is above "80".

9. Use dipstick to check for chlorine at the water source for each room (separate instructions for each room are below).

Chlorine must be prevented from entering the fish systems, so the water source of each room is checked for chlorine daily. Chlorine levels should be undetectable. If there is ANY chlorine detected, immediately follow the directions to completely isolate the system water as outlined in the "Emergency Procedure" protocol, located in each fish room. After doing this, report IMMEDIATELY to the facility manager or a PI. **Note: This is an emergency situation. Report in person or via phone call. Do not email!**

- a) LSA 139: Use a chlorine detection dipstick to check for chlorine in the 165 gallon reservoir tank and record on the checklist located on top of the water filter cylinders. While doing this, note the level of water in the reservoir tank and make a check on the checklist to note that the water level is normal (normal level is noted on the reservoir).
If the water level in the reservoir tank falls below normal levels it may indicate a disruption in the RO water supply or recent use of water from the reservoir. Wait at least 15 minutes and check again to see if it is refilling. If it is not refilling, report PROMTLY to the facility manager or a PI. If the situation is not resolved within a few hours it may be necessary to follow the directions to turn off the automatic water exchange system as outlined in the "Emergency Procedure" protocol.
- b) LSA 164: Use a chlorine detection dipstick to check chlorine from the **RO water hose** and record on the appropriate check list. After verifying that there is no chlorine in the RO water, check the level of the RO carboy/head tank located on the table. If it is below the indicated level, use the RO water hose to refill to the top. Place a check on the "Once Daily Checklist" under "Tank levels/Head".

If chlorine is found in ANY water source, follow the emergency instructions for isolating the fish systems from incoming RO water.

10. Use dipstick to check ammonia level in system water.

Ammonia levels are checked on Tuesday morning only.

- a) Use an appropriate dipstick to check ammonia (follow instructions on the bottle) in the system water and record on the appropriate check list. If ammonia levels are above 0.25 ppm (mg/L), report to the facility manager.

11. Check pressure levels in filtration cylinders while water flow is ON to all fish tanks.

Each fish room has two filtration cylinders. In LSA 139 these cylinders are located inside the door where the nursery is located. In LSA 164 these cylinders are mounted on the wall to the right inside the door.

Read the pressure valve on top of each cylinder and record the values (left & right) on the checklist.

12. Check UV lamp LED indicator lights.

While both fish rooms have UV lamps, only LSA 139 has LED indicator lights to show if all 6 lamps are operating. There are 3 red lights on each of the two ballast boxes located below the UV lamp housings. Look at the lights to make sure all 6 are on. If any are not lit, report to the facility manager. This observation does not have to be written on the checklist.

13. Check levels of conductivity & pH dosing tanks.

The dosing tanks are located in LSA 139 on the cart below the YSI monitor. The pH (sodium bicarbonate) tank on the upper shelf of the cart and the conductivity (salt) tank is located on the lower shelf of the cart.

Check the levels of these tanks using the measurements shown on the outside of each tank. If necessary, remove the lid on the top of each tank and verify the level by looking inside. Record the level of each tank on the checklist on top of the filter canisters. If the level of either tank is below 20L, report to the facility manager via email.

14. Change/rotate filter pads in each sump.

The sumps are located at the bottom of each rack. Water from the fish tanks flows through a square or rectangular filter pad before entering the sump. The filter pads are changed or rotated on Monday, Wednesday and Friday afternoon, or more often if needed.

- a) Use the correct filter pad for each filter basket: square pads for square baskets; rectangular pads for rectangular baskets! Be sure to position the filter pad and filter basket under the spillway so the waste water flows through the filter pad, and not around it!
- b) Replacement filter pads are on the shelf above the sink in LSA 139 and on the bottom shelf of the cart in LSA 164. (Stock of filter pads is in LSA 143).
- c) Wring as much water as possible out of the dirty filter pads (wring over the sump) and deposit the dirty pads in the trash.

15. Check/empty trash.

Empty the trash, as needed. Trash must be double-bagged to avoid leaking in the hallway and trash dumpster. The trash dumpster is located in the back hallway near the elevator. Place the trash bag into the dumpster, keeping the open end up so it doesn't leak. Replacement trash bags are above the sink. Place two trash bags inside the waste basket.

16. Check all tanks/blue hoses after restoring water flow.

When water flow is restored to fish tanks after being turned off for feeding (or any other reason), pressure in the system may cause the hose to become dislodged from the hole in the lid. Always walk through the room and check for dislodged hoses before leaving.

17. Check sink & room for general neatness.

Before leaving the room, make sure all supplies have been washed and returned to their proper storage location. Pick paper and trash off the floor. Identify the source of any water on the floor. Refill the paper towel dispenser, if necessary.

18. Record the room temperature and humidity.

A digital temperature and humidity monitor is in each room. In LSA 139 record those values on the appropriate spaces on the "Brine Shrimp Hatch Record" sheet. In LSA 164 record those values on the appropriate spaces on the "Once Daily Checklist".

19. Remove and dispose of dead fish.

If you find dead fish while feeding and/or checking tanks, please remove them from the tank and dispose of them.

- a) Record the dead fish by batch number on the mortality sheet located on a clipboard on top of the filter canister.
- b) Transfer the fish in a paper cup, paper towel or plastic beaker to LSA 110. Dead fish should be placed in the dead animal box located INSIDE the freezer. Make sure the dead fish go inside the box and are not stuck to the lid of the box. Only dead animals may be put in the dead animal box. All trash (paper cups, paper towels, etc.) may be placed in the trash barrel next to the freezer. Close the lid to the freezer when finished.

20. Lock the door:

Doors may be left unlocked on week days. The afternoon and weekend fish feeders should lock the doors when they are finished.

21. Manually take and record conductivity of system water. (LSA 164 only)

Use the portable conductivity meter to measure the conductivity of the system water.

- a) Use a 500 ml beaker and remove 300 ml of system water from one of the fish tanks. Place the probe into the water. Alternatively, you may place the probe directly into one of the fish tanks that does not contain fish.
- b) Plug the temperature probe into the top of the meter and turn the meter on.
- c) Allow the reading to stabilize for at least 1 minute. Record on the appropriate check list.
- d) Rinse the probe with RO water. Turn the meter off and unplug the temperature probe when finished. Save the sample of water to measure pH.
- e) The conductivity reading should be checked against the chart showing acceptable and unacceptable readings posted on the wall.

If an unacceptable reading is found it must be reported to the facility manager or one of the PI's.

22. Manually take and record pH of system water. (LSA 164 only)

Use the portable pH meter to measure the pH of the system water.

- a) Use the same sample of water used to measure conductivity. Place the stir bar in the beaker and place on the stir plate. Set the stir speed to "4" or lower.
- b) Turn the pH meter on and rinse the probe with RO water. Position the pH probe in the water sample in the beaker, but do not allow the stir bar to contact the probe. Follow the directions posted on the wall to properly operate the pH meter and record an accurate pH reading.
- c) **Note: You must allow at least 15-20 minutes**, until the pH meter automatically shuts off, for the pH meter to "Log" the reading. You must then retrieve the logged number following the posted directions. Please follow these instructions to assure that everyone is taking the pH reading the same way.
- d) Check the pH reading against the chart on the wall showing acceptable and unacceptable readings.
If an unacceptable reading is found it must be reported to the facility manager or one of the PI's.
- e) The pH meter should be periodically calibrated using the 7.0 and 10.0 pH standards. Instructions are posted on the wall.

23. Check and record the system water temperature. (LSA 164 only)

The digital thermometer for the system water is located on the right side of the rack. Record the current water temperature, as well as the high and low temperatures over the past 24 hours. Record these values on the check list. Press the "reset" button on the thermometer to reset all values.

24. Check the level of the sump. (LSA 164 only)

The sump is located at the bottom of the rack.

- a) Check the level of the sump. The water level should be between the "high" and "low" tape levels. If the water level is acceptable, place a check on the "Once Daily Checklist" under "Tank levels/Sump".
- b) If the water level is above the "high" level or below the "low" level, report to the facility manager or one of the PI's.

25. Manual water change. (LSA 164 only)

This system does not have the ability to do automatic water changes, so they must be done manually. Manual water changes are done on Monday, Wednesday and Friday afternoon only.

- a) Remove 3 3-liter tanks that do not contain fish (for a total of 9 liters). If possible, remove tanks that need to be washed and wash them while doing the water change.
- b) Pour the water down the drain, clean the tank, and place it back on the rack - do not turn the water on yet.
- c) Fill the white bucket with 9 liters of RO water from the RO water faucet and add about 110 ml of salt solution from the small carboy on the floor under the sink. Measure the conductivity in the bucket, using the conductivity meter, and make adjustments until you have 9 liters of water with a conductivity between 625 and 675.
- d) Pour this bucket of water/salt into the sump (below the racks); you may need to use the 2 liter plastic beaker to get the last of the water into the sump.
- e) Turn the water flow on to the 3 tanks you just emptied and cleaned.
- f) Place a check on the "Once Daily Checklist" under "Water Change".

If you have any questions about any procedure in either fish room, please ask the facility manager or a PI.