Fish Maintenance

Fish Maintenance is our principal system for ensuring the health and well-being of the stocks, and it is our first line of defense against catastrophe. To ensure the lab's continued productivity, all procedures must be followed exactly as written. Fish Maintenance has evolved over several years. Suggestions for further improvements will be considered carefully and should be brought to Dave or presented in lab meeting; but *no procedural changes may be implemented without prior approval from Dave*.

On each day, one senior lab member (typically a graduate student, postdoc or full time tech) is assigned to "Fish Maintenance". Fish Maintenance is assigned 1–2 months in advance. Days may be "traded" freely but any modifications should be recorded on the Fish Maintenance schedule. It is the ultimate responsibility of whoever has Fish Maintenance on any given day to see that all of the tasks described below are completed.

Overall tasks

Fish Maintenance comprises four major tasks:

1. thoroughly assessing health of the fish (once daily)
2. verifying environmental quality and mechanical components (twice daily)
3. feeding the fish (three times daily)
4. maintaining cleanliness and professional appearance of the room (once daily)

These are treated separately below, and a step-by-step protocol is provided at the end.

Fish health

We aim to use the smallest number of fish possible, both to minimize animal use per se, and to keep expenditures of time and funds as low as practicable. To achieve this goal means keeping very healthy stocks and intervening quickly to correct problems as they arise. Note that our "lab animal philosophy" (fewer, better fish) differs from a traditional "aquaculture philosophy" (more fish, with less attention to the health of each individual).

Health check: Fish health is evaluated by carefully observing the physical appearance and behavior of fish in every tank. Once per day the person conducting Fish Maintenance should start at one end of the room and progress row-by-row and rack-by-rack towards the other end of the room until every tank has been examined thoroughly. Typically, we check all of the tanks first, marking the ones that need attention, and then return to those tanks once the room has been surveyed. By convention, tanks are marked temporarily by moving the stock tag from the upper right corner to the middle of the tank.

During health checks, be alert for the following:

Healthy fish

- actively swimming with spread fins
- vigorous feeding

- displaying (flared fins)
- yellow-tinged ventrum for zebrafish males in breeding condition
- orange color of eggs near vent and pelvic fins for females in breeding condition
- red erythrophore coloration on fins or body for some species, particularly males in breeding condition (e.g., *D. albolineatus*, *D. kyathit*)
- courtship or breeding (rapid swimming in circular manner, males chasing females)
- aggressive interactions including biting
  Healthy fish often will attack sick fish, so examine fish being attacked for signs of disease. Aggressive interactions also occur more frequently in tanks with only 2–3 fish; under these circumstances fish may need to be split or additional fish added.

Runted fish

- abnormally small but otherwise healthy fish
  These should be removed and euthanized as they are particularly susceptible to disease. However: (i) young males are often small, so if it is a young stock (i.e., no sexually mature fish are apparent) be careful you are not removing males that may still be immature; (ii) mutants will often be runted compared to their wild-type siblings, so be sure you are not removing healthy but mutant fish. When in doubt, ask Dave, the person who bred the fish, or other senior personnel.

Sick fish

- body shape emaciated (“skinny”) or bent
- bloating with raised scales, resulting in a fuzzy appearance
- limp appearance, fins held close or folded rather than spread
- eye bulging
- open sores
- generalized internal hemorrhaging
- hemorrhaging at fin insertions or vertically along myosepta
- torn or abnormally truncated fins
- damaged or missing operculum (gill covering)
- flared, red gills and rapid breathing
- erratic swimming (head-up, twirling, etc.)
- “flashing” behavior, in which fish dart against the tank sides
- prolonged resting on tank bottom or floating at the surface
- gas bubbles adhering to body; gas bubbles on sides of tank or on surface of water (this latter “symptom” is an early stage in gas bubble disease which progresses *very* quickly; see below)
- being bitten or otherwise attacked
- any other symptoms of distress or poor physical condition
- tumor(s) covering more than 10% of body (note: melanoma-susceptible lines should have tumors; check with the researcher who bred the fish if you are unsure whether a tumor has exceeded the allowable size)

Dead fish

- isolated: tanks will occasionally contain dead fish because of disease, age, injuries suffered during tank changing, or breeding; a likely cause can be established by ascertaining if the tank is already marked for disease (see below), when the tank was last changed, or when the fish were last bred
- widespread: if numerous tanks (e.g., >5% of total) contain dead fish, or if individual tanks contain numerous dead fish, this can indicate a systemic mechanical failure or a fast-progressing epidemic

Actions to take when sick or dead fish are discovered:

- if there are signs of widespread death or disease (e.g., gas bubbles, hemorrhaging), immediately inform Dave (206-734-7331) and contact the on-call veterinarian (pager: 206-583-1853) to request an emergency visit; additional procedures are required on a case-by-case basis as determined by Dave or the vet.
- sick or dead fish must be removed from their tank and recorded in the Sick Fish log
- remaining healthy fish in a tank should be transferred to a new, clean tank and placed back on flowing water with a yellow tag, labeled “D” (disease) and with the date and your initials to allow further monitoring; if a tank already is labeled in this way, add your initials and the new date and place the same tag on the new tank (do not re-use tags on new tanks, without crossing out any old data)
- if you are not sure whether fish are sick, check with Dave or other senior personnel
- some mutant phenotypes can resemble sick fish phenotypes (e.g., *puma* and *ednrb1* mutants appear to be missing their operculums but only because they lack iridophores there); other mutants actually are more susceptible to disease than are wild-type fish and must be watched closely (e.g., *renoir*); check with Dave or other personnel if you are unsure about a particular stock
- sick fish must either be euthanized (see **Euthanasia SOP**) or held separately in properly labeled tanks or beakers (off of flowing water) for examination by Dave or the veterinarian
- sick fish may not remain overnight in the fish room; fish that must be kept for longer than one day (e.g., precious fish that need to be out-crossed), should be provided with a tank in the quarantine room
- any tank, nets, or other materials that have been in contact with sick or dead fish must be set aside for bleaching and should not come into contact with sponges etc. used for sanitizing tanks with healthy fish
- fish that have been found dead or euthanized must be disposed of properly (see **Dead Animal SOP**)

Environmental quality and mechanical components

We strive 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 the fish directly, and also can retard growth, inhibit breeding, or impair the fishes' immune response (thereby causing increased susceptibility to disease). As deterioration in the environment can have effects that are acute and catastrophic, or chronic and initially hard to detect, we take every precaution to prevent environmental problems before they arise.

Assessing environmental quality for the fish begins the moment you walk in the fish room: are there unpleasant odors? does the air temperature seem normal? are the normal mechanical sounds present? are there new sounds? is there water on the floor? If these or any other things seem abnormal, investigate immediately and thoroughly to find the source of the problem; if you cannot identify it, consult with Dave, or if necessary, other senior lab personnel. Even *seemingly* innocuous changes can have catastrophic consequences!

In the context of Fish Maintenance, we pay close attention to the water mechanical system and biological filtration, water flow and conditioning throughout the system, and water flow and quality on a tank-by-tank basis.

Fish water mechanical system overview. We use recirculating-type systems designed and installed by Aquaneering. These systems continuously circulate water: from the fish tanks, into drain troughs and pipes, through prefilter pads, into sumps, to FSI particle filters, past UV sterilization lamps, through a biological fluidized bed filter, and back to the tanks. We have a main system that circulates water through most of the racks as well as two "stand-alone" systems that operate using the same principles on a smaller scale. The systems are termed "recirculating" because most of the water is re-used, with only a comparatively small amount of water replaced with fresh water each day.

While recirculating systems are exceptionally efficient and provide a very high quality environment, they can be extremely vulnerable. Failures at any point can affect the entire system: foreign and potentially lethal chemicals will be spread rapidly throughout the tanks, high loads of pathogens or parasites can overwhelm the UV lamps, and failures in biological filtration can leave toxic levels of ammonia and nitrites in the water.[1] For these reasons, we remain extremely vigilant in monitoring and maintaining all aspects of the system, and in preventing the introduction of outside chemicals and pathogens.

The system uses two main types of filtration:

1. Particle filters (prefilter pads, and FSI particle "sock" filters) remove uneaten food, fish feces, and other solid matter. When prefilter pads become saturated, water flows over them instead of through them and they cease to remove solid material. When FSI filters become saturated, they impede water flow through the

[1] Ask Dave for some catastrophic examples from other facilities.

whole system, thereby resulting in the buildup of waste products within the tanks themselves. We check both of these filters during Fish Maintenance (see below).

2. Biological “filters” consist of denitrifying bacteria that colonize sand suspended in the fluidized bed filters. As fish metabolize their food and as organic matter decomposes, ammonia is produced. Ammonia is toxic to the fish but in 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 and this is accomplished by suspending the sand in a “fluidized bed”. If the sand is no longer suspended, the bacteria will quickly begin to die, and with this toxic levels of ammonia can rapidly accumulate, killing the fish. Any chemical or antibiotic that kills the bacteria will have a similar effect. During Fish Maintenance, we monitor the biological filter both chemically, by sampling ammonia and nitrite, and physically by observing the state of sand suspension and relevant water pressure. Departures from normal conditions must be reported immediately to Dave so that corrective action can be taken.

Water flow and conditioning. Water flow for the whole system is monitored at pressure gauges on the fluidized bed filter, at the main water line going to the racks, and at the FSI filters. Water levels also should be checked in the sumps: unusually low levels may indicate a malfunction or leak and should be investigated immediately. Because the systems are recirculating and only a limited volume of water is added daily, any spills or leaks result in the loss of water that may not be replenished. Even a seemingly small leak can mean large volumes lost (e.g., as much as 25% of the system volume, or 500 gallons, overnight).

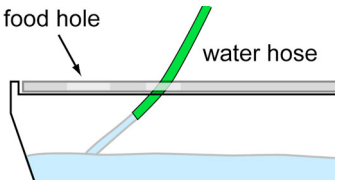
During normal operation, the system receives new water through a carbon filter that removes chloramine and other impurities from city tap water. In our system, hot and cold tap water are blended in a mixing valve to achieve the desired temperature upstream of the carbon filter. Water from the carbon filter is injected into a degassing column above the main sump, and this flow is controlled by a solenoid-dependent valve that is opened and closed automatically several times a day (via a standard timer). Typical daily on-cycles total 5–6 hours per day at 5–10 liters per minute. If incoming water is found to be too hot or too cold, the temperature should be adjusted at the mixing valve while the water is actually running; this may require overriding the timer. Do NOT add tap water to the system that has not passed through the carbon filter.

The system also has a reverse osmosis (RO) system that deionizes and dechlorinates building tap water. After adding back salts to appropriate concentrations, this water is used for embryo and larval rearing media, or as emergency make-up water for the main system. The RO water is collected in a reservoir that sits on the main sump, and from there empties into the main sump itself. We pay close attention to the RO unit’s operation; if filter’s upstream become clogged this can cause inadequate water flow to the RO system that will destroy the pump motor. This is often indicated by a grinding noise when the motor is on (if this is heard it must be immediately turned off and the filters replaced). The RO system should be turned on manually when the reservoir has reached ~50% of total volume and during normal operation it should take

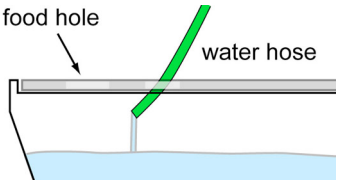
less than one hour to fill. If the RO reservoir overflows into the main sump unintentionally, or when RO is intentionally added to the main sump, dosing pumps automatically add salt and sodium bicarbonate to maintain in the main fish system a constant salinity and pH, respectively. We monitor these parameters, as well as the reservoirs of concentrated salt and sodium bicarbonate solution.

Water flow at the tanks. During Fish Maintenance we check water flow and environmental conditions in each tank.

1. Tanks containing fish receiving any adult food (see below) should have a continuous stream of water from the hose; hoses should be above the water line and should not be positioned immediately below the food hole. If adjusted correctly, water should flow in a relatively straight line out from the hose (see illustration). This amount ensures that left over food is flushed out of the tank.



2. Tanks containing larval fish receiving only juvenile food or rotifers should have a low flow of water from the hose; i.e., water runs straight down from the hose tip in a continuous stream (*not* a drip). This amount of water ensures that rotifers are not flushed out of the tank too quickly, and that fish receive an adequate input of fresh, aerated water.



3. Tanks containing larval fish receiving rotifers only should not have any flowing water until ~10 days post fertilization. See the **Embryo and Larva Rearing SOP** for details.

In addition to flow into the tanks we verify that every tank is seated correctly on the rack supports and is thereby emptying into the drain trough (i.e., not onto the tanks below, into the sump, or on the floor). If a tank supply valve is broken (e.g., leaking even when closed), then affected tank should be moved immediately to a new location and a yellow flag labeled “V” should be placed on the valve to mark it for change out by whoever is overseeing animal room organization at that time.

Finally note that non-zebrafish danios are often housed in brown-bottomed tanks (tank outside bottoms are spray painted outside of the facility). If you see brown-bottomed tanks in use for zebrafish please let the researcher now that the tanks should be swapped out for ones with clear bottoms at there are only a limited number of the painted tanks.

Tank environmental quality. Besides water input, several additional factors also impact environmental quality for the fish. Tanks are designed to flush debris from the bottom between a solid baffle and the back of the tank. For this to work well, every tank must have a solid baffle and this baffle must be in the slot closest to the back of the tank. Occasionally the space between the baffle and tank will become clogged so that water spills through overflow holes near the top of the baffle. In such instances the tank or

baffle should be changed. If debris is observed between the baffle and tank, it can sometimes be flushed out simply by raising the front of the tank so that extra water is forced through this space in the back.

Be especially on the look-out for tanks missing baffles as well. We have had many whole stocks of precious fish swept away into the sumps because their tanks were placed on racks without first installing baffles.

Besides baffles, the lab uses mesh screens of two different sizes to ensure that small fish are retained in their tank and not lost in the tank outflow: small 400 μm screens for very small larvae and large 1000 μm screens for larger larvae and juveniles. Tanks with new larvae should be 'set up' with a 400 μm screen, a 1000 μm screen and a baffle, respectively in front-to-back order. When flowing water is first provided, tanks will likely still have both screens but should already be large enough that they will be retained by the 1000 μm screen alone (see **Embryo and Larva Rearing SOP**). Therefore, *if a 400 μm screen is present and clogged, it should be removed* after verifying the presence of a 1000 μm screen behind it. (If larvae are too small to retained by the 1000 μm screen it is the responsibility of the person who bred the fish to see that the 400 μm screen is free of debris and replaced as needed). In contrast, *if a 1000 μm screen becomes clogged it must be replaced*; only the person who bred the fish may decide when to stop using the wider screen.

Finally, tanks may need to be changed during daily fish maintenance, although the lab performs a weekly changing of large numbers of tanks. Tanks must be changed during maintenance if in danger of overflowing because of accumulated debris, if there is excessive bacterial or fungal growth or uneaten food, or if the view of fish is obscured for any reason.

Young larvae. Special attention also should be paid to small larvae not yet on flowing water, or that have recently transitioned to flowing water. For fish on static water, the water should be relatively clear; if turbid this indicates bacterial growth, which can rapidly be lethal. For fish on flowing water, there should be food tags for rotifers but there must also be tags for juvenile food or brine shrimp. Be sure to alert whoever bred the larvae if something is amiss.

Tank marking conventions. During Fish Maintenance you may come across tanks that have been marked by moving tags from their normal location or orientation:

- stock tag rotated 90° counterclockwise: tank needs changing
- stock tag moved to middle of tank: sick fish or other tank condition that needs immediate attention
- stock tag flipped upside down and moved to bottom center: whole tank to be euthanized (disease or culling); note that only Dave or someone he designates should mark tanks in this way and that such tanks should be taken care of immediately (if sick) or during the next tank changing (if healthy)
- post-it notes or lab tape: specific instructions for culling or sorting fish, combining stocks, or moving tanks; note that Dave will periodically mark tanks in this way,

particularly if they are not being managed properly; depending on severity of the problem action should be taken immediately or during the next tank changing at the latest

Because marking of tanks potentially impacts the health of all fish, no tanks may be "unmarked" (i.e., action not taken) without first consulting with Dave; note that anyone unmarking tanks without approval will lose their fish room privileges.

Feeding the fish

Fish are fed two to three times per day if health and environmental conditions permit. In general, it is better to underfeed than overfeed. NEVER feed the fish if there are problems with water quality, mechanical components, or questions about system-wide health. If necessary, fish can last several days without food; by contrast, adding more biological material (food) to a system in trouble can tip the balance towards disaster. Always ask Dave or other senior personnel if there is any doubt as to whether or not the fish should be fed, and always take care not to overfeed as uneaten food fouls the tanks, clogs the filters, and costs a lot in time and labor (in addition to the cost of the food itself).

To ensure that fish receive the appropriate amount of food, Dave or other designated personnel evaluate food levels received by every tank several times per week. Designated amounts of food are indicated by post-it flags on the right side of each tank. There are several types of tags:

blue	—	adult food (large grain); tag may be large or small
yellow	—	juvenile food (small grain); tag may be large or small
pink	"R"	rotifers (fortified with Rotimac)
blue	"AR"	thyroid hormone-free rotifers (fortified with Artemac)
orange	"S"	supplemental feeding of adult food at midday
purple	"B"	brine shrimp
blue	"F"	tropical fish flake food (non-zebrafish only)
yellow	"W"	bloodworms

Adult and juvenile food is made up from dry stocks and is kept refrigerated or frozen when not in use.[2] These dry foods must be returned immediately after feeding as ambient humidity in the fish room can promote the growth of toxic molds.

Adult food is fed to the fish with a grey, plastic, v-shaped spatula with notches to indicate units of food. Note that notches are not all equally spaced so that. Food on the spatula should always be level with the top of the v-shaped trough (heaping food results in twice the correct amount).

- Large blue tags indicate one level spatula of adult food.
- Small blue tags indicate one notch-worth of adult food.

[2] See **Recipes SOP**.

- Half-length small blue tags indicate half of the first notch of adult food or less (e.g., just a few grains for a single small fish).

To feed, deliver the appropriate amount of food for each tank through the lid feeding hole. For example, 3 small blue tags would indicate the tank should receive three notches-worth of adult food. One large blue tag and two small blue tags would indicate one full spatula plus two notches-worth of adult food. Be very careful to keep the spatula and the food itself from getting wet; dry the spatula as needed and discard any food that has been splashed. Note that it is always better to err on the side of underfeeding rather than overfeeding, which pollutes the water, requires changing of tanks, and risks the health of the fish.

Juvenile food is fed to the fish as a slurry made up in water, as the smaller grain size otherwise keeps the food on the surface and unavailable to small fish that will only feed in the water column. The juvenile food suspension is prepared immediately before feeding to the fish by adding 100 ml fish system water per 7.6 g of dry food and stirring well with a pipette. Juvenile food in suspension is delivered using a graduated 3 ml disposable pipette. You should place the food into a graduated glass beaker and add water immediately before administering it to the fish. When feeding the fish, be sure to keep the slurry of food well-mixed by stirring frequently.

- Small yellow tags indicate 0.5 ml of juvenile food suspension
- Half-length small yellow tags indicate 0.25 ml of juvenile food suspension

Brine shrimp are given to fish that are used frequently for breeding, that cannot handle standard juvenile or adult food, or that must be grown especially rapidly. Typically 0.5 to 1 3 ml pipette-full is appropriate unless a tank contains many adults in which case 2 pipette-fulls may be acceptable. When feeding brine shrimp be sure to avoid the left-over cysts, which are not digestible by the fish.

Bloodworms are given to fish on a thyroid-hormone free diet and to some other *Danio* species. Bloodworms come in a dry powder and are resuspended in system water to the amount specified on the whiteboard in the food prep room.

Rotifers are fed to the fish after being supplemented with either Rotimac or Artemac (≥45 min) then filtered on a mesh screen and resuspended in rotifer water containing ChlorAm-X (see **Rotifer High Density Culture SOP**). Dispense rotifers to the fish using a 3 ml plastic pipette and attempt to disperse the rotifers across each tank; make sure rotifers remain suspended by swirling occasionally them with the pipette. For tanks not yet on flowing water, several drops of concentrated rotifers will suffice (see **Embryo and Larva Rearing SOP**). For larvae on flowing water 0.5 to 2 pipettes-full are appropriate. Tanks having two rotifer tags contain larvae at high density and should receive more rotifers. If you are unsure how many rotifers to feed, check with Dave or other senior personnel.

Feeding sequence. We first feed the larger, adult food to all the fish so marked, and then feed the smaller, juvenile food as indicated. Since some tanks will receive both, this helps to ensure that larger fish in those tanks will be satiated by the adult food,

allowing weaker fish more access to the juvenile food. If any food type is limiting (e.g., rotifers), give priority to fish designated for receiving that food and no other.

Feeding times. All fish are fed at least twice a day; once in the morning and once in the evening. Larvae, fish that need to be grown rapidly, and fish that are being bred frequently are given "supplemental" food and are at midday as well. Feeding times are:

- morning: start by 8:30 to finish by 10 am
- midday: start no earlier than noon and finish by 2 pm
- evening: start after 4 pm

Adhering to these times ensures that feedings are spaced far enough apart that fish will eat all of the food they receive.

Midday "supplemental" feeding. Fish with any of the following tags are fed at midday, using the amounts indicated:

yellow	—	half the amount of juvenile food indicated on the tank
orange	"S"	half the amount of adult food indicated on the tank
pink	"R"	same amount of rotifers as morning and evening
blue	"AR"	same amount of TH-free rotifers as morning and evening
purple	"B"	same amount of brine shrimp as morning and evening
blue	"F"	same amount of tropical fish flake as morning and evening
yellow	"W"	same amount of boodworms as morning and evening

Room cleanliness and organization

The fish room is a professional research facility and is treated accordingly. While cleaning up after oneself is each individual's responsibility, the person conducting Fish Maintenance verifies that things are clean and in order, and has several specific responsibilities in this regard.

1. All tank lids must be clean and dry. Even when care is taken, small amounts of food inevitably end up on lids, in addition to dust, molds, etc. from the air itself. As these materials can attract vermin and also create an unhealthy environment and unprofessional appearance to the room, we keep them from building-up in the first place. If lids are wet, this may indicate a leak and this should be ruled out immediately. Even if just a small spill, the water should be dried as it can result in transfer of pathogens between tanks. At days' end, use the hand vacuum to remove debris from all tanks (including tanks without fish if they are below racks that have been fed). For each rack and starting from the top, pass the vacuum first in one direction across all the lids, then back in the other direction (which helps to dislodge food stuck between sides of lids and the tank). Repeat for the next row down, then the next rack, etc. The entire fish room can be cleaned in this way in just a few minutes. Lids in the quarantine room must also be cleaned and this can be done with a damp paper towel.

2. The fish room work stations are checked. The counter top should be cleaned and items returned to their correct location after every use. The person conducting Fish Maintenance makes sure this is so, and also wipes down the counter with 70% EtOH at the end of the Fish Maintenance in the evening. Additionally, microscopes normally covered when not in use must be covered at the end of the day. Finally, empty the trash can.
3. Any spills on the floor (water, food, etc.) must be cleaned immediately to keep materials from being tracked around the room, to verify that the problem is not on-going (e.g., a leaking valve or a hose out of place), and to maintain a safe working environment for lab personnel.

Fish Maintenance Protocol

Use the on-line Google document "Parichy Lab Spreadsheets" to record all data for the main fish system ("Fish main" tab) and the standalone racks in the main fish room as well as the fish incubator and quarantine room ("Fish-other" tab). For each, record the correct date when beginning (left), as well as your initials and time when fish maintenance is completed (right).

Specific data to be recorded are indicated below, with values in parentheses being normal conditions; situations resulting in deviations from these values should be corrected immediately or reported immediately to Dave or other senior lab personnel. A protocol with times is given at the end.

FISH LOOP

- Dechlorinated tap water flow rate (>4 lpm).
  This can only be read when the tap water solenoid is on and water is flowing so check the timer for the best time to observe.
- pH and salinity (pH 7.0–7.8 pH and 100–1800 ppm, respectively).
  Displayed on the +GF+ Signet meters. As long as the reservoirs are filled and the meters and pumps are working, relatively constant pH and salinity should be maintained. A red light on the meter indicates the pump is currently on (or should be, if working).
- UV lamps (both banks, all lights on).
  Verify that all three lamps in each of the two lamp housings are operational by examining the port on the lamp housing.
- Main system pressure (3–10 psi).
  The gauge is located on a pipe flowing out to the racks above the UV lamp housing. Less than 3 psi means insufficient water flow to the tanks; this can happen if many tank valves are open excessively or if the FSI particle filters are clogged. High pressures can result if many tank valves are closed or if there is some other block in the system.
- FSI particle filter pressure high (≤25 psi). Check the range and record the highest value.
  Gauges are on top of each filter housing. Higher than normal pressures imply the filters

*are clogged and need to be changed; consult senior lab personnel and the **Filter Change** SOP.*

- Prefilters clean (yes). Verify that prefilters above sumps are clean and that water is flowing through them not over them.
- Leak-free (yes). Certify the absence of leaks or puddles of unknown origin.
  Puddles on the floor are a safety hazard and can also indicate a leak in the system; even a slow leak can completely empty the system overnight!
- Water level, standpipe drainage ok (yes). Verify that water level in the sumps is normal so it is flowing into the top of the drainage standpipe, and that no water is flowing directly into the standpipe from filters or tanks overhead.
  When water flows directly from tanks into the standpipe it 'short circuits' the recirculation and this can quickly drain all the water from the system!

- pH (salt) and salinity (sodium bicarbonate) reservoir volume (both at least half full). Re-fill if needed.
  Use system water from the hose and stir in the appropriate amount of powder.

FLUIDIZED BED LOOP

- Fluidized bed pressure (normal: 3–6 psi). Check and record value.
  The gauge is located above the fluidized bed vessel. Less than 3 psi will not provide adequate suspension of the sand and the biological filter will begin to die.
- Sand suspension (normal: yes).
  Verify that sand in the fluidized bed filter vessel is in fact suspended by looking through the port hole.

TEMPERATURES

- Current, low and high water temperatures for main system and for room air temperature (27–29 °C). Record current values then RESET each thermometer.
  Low/high digital thermometers are used. Record the current temperature as well as the minimum and maximum temperatures recorded.
- High temperatures for heat shock tanks (37–39°C). Record high temperatures achieved then RESET thermometers.
  Heat shock promoters require at least 37–38°C to be functional and experiments fail when this temperature is not achieved consistently.
- Current temperatures for cold tank (23–25 °C). Record current values and RESET thermometer.
  Used for temperature-sensitive alleles.

RO SYSTEM

- RO system product flow rate (>4 lpm). If you turned on the RO water system, record the amount of RO water being produced. Otherwise enter N/A in the log.
  Read the vertical, right-most gauge on the RO unit where the floater is at its widest point.

- RO filter pressure low (>15 psi). If you turned on the RO system, record the lower pressure indicated on the two gauges above the blue filter
  The RO unit must receive at least 15 psi to operate properly; lower than this can destroy the motor. If the pressure has dropped too low, shut down the RO unit using the red switch; the most likely reason is clogging of the sediment filter attached to the incoming tap water line on the back wall of the fish room. See Filter Change SOP for how to change this. If this does not solve the problem, call for service (which is under contract; contact information should be on the RO unit itself).
- RO filter pressure difference (≤10 psi). If you turned on the RO system, record the absolute difference between the two pressure gauges above the blue filter.
  The pressure difference between gauges indicates flow across the blue filter; a large pressure difference means it is becoming clogged. Change if needed.

FISH and FEEDINGS. For these parts of Fish Maintenance it is a good idea to start on alternate ends of the room each time you perform these duties; this helps to ensure that no one group of fish is ever systematically neglected. Begin at one end and examine the tanks from one side to the other on every shelf, moving from top to bottom. Use a step stool or the rolling ladder and be sure to view tanks at an appropriate angle so that fish, as well as water flow and quality can be observed effectively.

- Healthy (yes). Verify that fish are alive, that they look normal and are behaving normally, and there are no overt signs of disease (see **Fish health**, above). Check water quality in every tank and look for excess debris. Take any corrective action necessary. Note that a cursory look during feeding does not substitute for a thorough health check, usually performed in the middle of the day.
- Hoses flowing and in position (yes). Make sure that water is flowing at the correct rate and that hoses are in their correct holes in the lids.
- Screens pulled or changed (yes). Verify that water is flowing through screens and not through the overflow holes above the screens. Small screens that are clogged should be removed or replaced. Large screens that are clogged must be replaced.
- Fish fed (three times daily; indicate times). Assuming health and environmental conditions are appropriate, feed the fish as described above (**Feeding the fish**) and note the time at which feeding is completed.

SUPPLIES AND SANITATION. These are to be performed as needed through the day and must be completed in the evening before leaving.

- Food for tomorrow (yes). Verify there is enough food to last the next day; make more if needed (see **SOP Recipes**). Be sure to date and initial.
- Items returned from second floor (yes). Collect and store materials left in the 'return to fish room' bin in the main lab.
- Hanks, larval water carboys filled (yes). Verify that solutions in stock and working strength carboys are above refill line. Empty and rinse carboys and make more

solutions as needed (see **SOP Recipes**). Note that solutions must be labeled with dates and initials when they are prepared!

- Breeding supplies, squirt bottles, tricaine ok (yes). Verify that all are ready to use for the following day. Nearly empty squirt bottles should be emptied, sanitized and left to dry.
- Lids cleaned (yes). After the last feeding, all lids must be vacuumed to remove food and other debris that may have accumulated during the day. Note that even unoccupied tanks must have their lids vacuumed.
- Fish dishes ok (yes). Make sure dishes are not overflowing and are being attended to by workstudy students, yourself, or other lab members. See **Fish Dishes and Sanitation SOP**.
- Counters clean, scopes covered (yes). Clean countertop, put away (or have put away) remaining items, cover microscopes,
- Trash removed (yes). Take out the trash and empty it in the dumpster behind the building.

CHEMISTRY. These checks are done on specific days. Use the test kit solutions or strips. Be sure to order new reagents when the numbers of tests remaining are 10 or fewer!

- Ammonia levels (daily, normal: 0); nitrite levels (weekly, normal: 0).
  Sample water from a tank with fish and examine indicator solutions in a well-lit part of the room. If the biological filter is healthy, ammonia and nitrites should be 0 mg/L. Ammonia levels above 0.5 mg/L are very toxic and require immediate intervention.
- Nitrate (weekly, normal: <30 mg/L).
- Total hardness (weekly, GH)
- Alkalinity (weekly, KH)

CERTIFICATIONS.

At the end of Fish Maintenance certify with your initials and time completed. Also certify that any deviations from normal parameters have been reported to Dave. Daily supervisors certify that all checks have been done.

STANDALONE RACKS, INCUBATOR and QUARANTINE ROOM.

The two stand-alone recirculating water systems receive their feed water from the main system, but do not return water to the main system. These racks are used for temperature-sensitive studies and it is especially critical that they hold their set temperatures.

- The warm rack is set for 33 °C and should range between 32° C and 34°C.

- When operational, the cool rack is set for 24 °C and should range between 23 °C and 25 °C; if intentionally not operational, it should have the same temperature range as the main system.

Any deviation should be reported immediately.

The stand-alone systems use the same basic principles as the main system, and have their own UV lamps, fluidized bed filters, prefilters, and particle filters. They do not have FSI filters nor do they have pressure gauges. It is thus imperative that the water flow and level be examined qualitatively. A loss of water flow will result in rapid temperature change and the loss of experiments. Inform Dave or other senior lab personnel immediately if there appears to be a problem with water flow. The stand-alone units are set to automatically drain new water from the main system, and this is necessary for normal water change-over. Check the water level in the sump: if it is more than ~1–2 cm below the drain pipe, the unit probably is not receiving fresh water. Any real or suspected problems with the stand-alone units should be immediately reported to Dave or other senior lab personnel if necessary.

An additional incubator in Kincaid 262 can be used for fish and its temperatures (26–29°C) must be recorded.

Finally, the quarantine room is on the roof level of Kincaid. Record water temperatures (22–29°C); fish health (ok); certify that all stand alone filters are running (yes); that lids are clean and the floor is clean and free of spills (yes) and that the fish have been feed (3 times).

Feeding and maintenance times

The protocol below gives appropriate start times and durations for the various tasks in Fish Maintenance and Rotifer Harvesting, which need to be done in a coordinated manner. While various contingencies may cause delays at different steps, it is important that feeding times remain sufficiently spaced apart from one another, and that young fish, especially those receiving rotifers are fed as soon after the lights come on (7 am) as possible. Details of system checks for the room, main system and stand-alone racks are at the end.

• 7:30–8:15 am	harvest rotifers and perform rotifer system maintenance (see **Embryo and Larva Rearing SOP** and **Rotifer High Density Culture, Harvesting, and Fish Feeding SOP**); weigh out juvenile food and bring this and adult food to fish room
• 8:15–9:00 am	perform system checks; feed adult food to fish, then juvenile food
• 9:00–9:15 am	feed rotifers to the fish
• 9:15–10:00 am	tank-by-tank fish health and environment checks*
• 12:00–12:30 pm	perform supplemental adult, juvenile, and rotifer feeding

- 4:00–4:45 pm harvest rotifers and perform rotifer system maintenance
- 4:45–5:30 pm perform system checks; feed adult food to fish then add water to juvenile food and feed the resulting slurry to the fish
- 5:30–5:45 am feed rotifers to the fish
- 5:45–6:00 pm final room and sanitation checks

* miscellaneous tasks such as filter changes, dishes, etc. can be performed during late morning or early afternoon

Fish maintenance vs. Fish feeding

Finally, in addition to the person overseeing Fish Maintenance on any given day, junior lab members (typically undergraduates) sign up as “Fish Feeders” to assist with Fish Maintenance. Typically, each undergraduate is expected to sign-up on the Fish Maintenance schedule for three feeding shifts per week. These shifts can be chosen well in advance and can be changed up to one week in advance. Within one week of the chosen shift, the schedule is considered “frozen”; if a shift cannot be completed it is the responsibility of the person who has signed up to find a replacement. This policy allows other lab members to plan their research schedules accordingly. Fish Feeders are responsible for:

1. feeding the fish at the correct time and marking time started on the daily care log that the feeding has been done
2. during feeding, watching for any signs of ill health amongst the fish, inappropriate water flow to the tanks, or other problems, and remedying these problems or consulting with the Fish Maintenance person or other senior personnel to see that problems are remedied
3. for midday feedings, conducting water chemistry tests and changing system “pre-filters”, and recording these on the daily logs
4. for evening feedings, cleaning the tank lids and cleaning the rotifer dispenser in the rotifer room, and recording these on the daily logs

(If a Fish Feeder is not assisting with midday or afternoon feedings, the Fish Maintenance person will need to make sure these tasks are completed.)