



Chapter 5 Environmental

5.0 Environmental

Maintaining the proper environment for zebrafish is crucial to their well-being. Poor environmental conditions lead to stressed fish, and fish systems have the potential for most fish diseases. The fish are readily able to fight off most of these dangers as long as they are in the proper environment. If there is any problem with fish health one should first look for disruptions in their environment.

5.1 Photoperiod

Zebra fish are tropical photoperiodic breeders mating right after sunrise. To maximize this feature the laboratory lights are automated in all fish laboratories to turn on at 9:00 A.M. and off at 11:00 P.M. This photoperiod 14 hours light and 10 hours dark are intended to simulate optimal natural breeding conditions. Regulating the light and dark cycle also allows us to collect timed eggs if needed.

Do not override the light cycle of the laboratories because you decided to work late or wanted to get an early start on the day. **The only time the light cycle can be overridden is in the case of emergency.** The first indication that there is a problem with the light cycle is usually rather apparent the fish do not spawn.

5.2 Temperature

All laboratories maintain a 28°C air temperature again to maximize optimal breeding conditions. The ambient air temperature is used to warm the water because it would be impractical to warm the water directly only to warm the laboratories. This temperature is also crucial for developing nursery fish. There is evidence improper temperatures will lead to developmental problems in young zebrafish.

Water temperature controls the metabolic rate of every aquatic organism. As the temperature increases so does metabolism. An important factor to consider when looking at water temperature is the amount of dissolved oxygen. As the temperature increases, metabolism increases causing a higher demand for oxygen this leads to a precarious balance because the solubility of oxygen decreases at higher temperatures. This declining rate of solubility would be occurring in the face of higher biological oxygen demand (BOD). It takes increasing amounts of energy to maintain adequate levels of oxygen at higher temperatures.

Fish should never be subjected to rapid water temperature changes. This is very stressful to the fish leaving them more vulnerable to pathogens. If the water temperature needs to be changed higher or lower do it slowly. If you need to lower the water temperature by 5°C do it over a 24-hour period. Fish in too cold or too hot water tend to be lethargic and group together in a corner of the tank.



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5.3 RO and System Water

A clean source water supply is crucial to the fish facilities success. H221 and J083b laboratories are supplied with reverse osmosis (RO) water. In H221, the supply comes from the adjacent G-Wing roof. In J083b the supply is from the J-wing roof. Reverse osmosis water is produced by putting municipal water through a thin film composite (TFC) membrane at extremely high pressures. The end product is nearly 95-98% pure H₂O. This is important because it allows us to control what is in our water.

RO is used for filling the system water tower, rinsing dishes, and any other time clean water is needed (mixing reagents etc.). RO is also used to dilute fish system water conductivity, but this is only be done by trained staff. RO is not particularly good for fish though. It lacks nutrients, and has little to no buffers to resist pH changes. In fact, pure RO will readily pickup atmospheric CO₂ creating aqueous carbonic acid and a pH around 4.0.

To create system water (biological water) we add sea salts (instant ocean). Instant ocean contains sodium chloride, trace elements and buffers. Chloride is important component in fish osmoregulation and the trace elements are important for growth and health. Salts also buffers the water from CO₂.

System water is created in a 200 gallon water tower. The tower is filled with RO, next to the tower is a carboy of supersaturated salt solution 340 ppt. One hundred milliliters of this salt solution is added to every 10 gallons of RO water added to the tower. This creates water that has less than 1.0 ppm sea salt or as we normally measure it as conductivity of 1500 uS. The water in the tower is continually being mixed distribution/recirculating pump. This pump also delivers system water to sinks and hoses. When filling and salting the tower it is important to mix the super saturated salt solution with the stir pipe prior to decanting it from the carboy

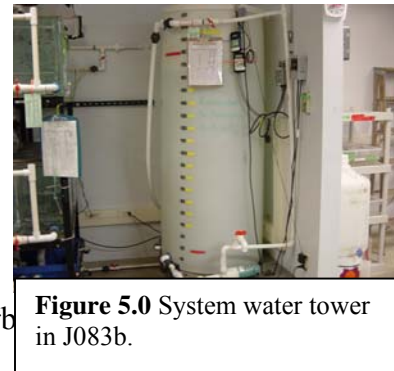


Figure 5.0 System water tower in J083b.

who might be using the system water that your filling the tower, and check the conductivity before after the tower is filled, for more details see chapter 6. System water is used for filling fish tanks, sumps, and spawning tanks, basically **any container that holds fish should have system water in it (not RO)**. There are numerous spigots, faucets and hoses in the fish facilities and all of them are labeled as RO or System.



5.4 Water Quality

Better understanding of biological process and technological advances has lead to the development of fish systems that can safely house thousands of fish in a relatively small amount of recirculated water. There are many fine balances that must be maintained in fish systems. A good understanding of what is going on in the water in necessary.

Alkalinity

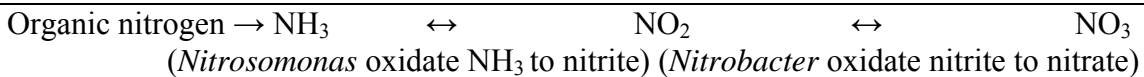
Alkalinity is a measurement of the water ability to resist a pH change due to acid addition. It is composed of a variety of bases mainly bicarbonates, carbonates, and hydroxides that can react with free hydrogen. Calcium carbonate in the form of crushed coral is most commonly used by the facility to control alkalinity levels. It is used not only to prevent detrimental shifts in pH, but it is also important component in nitrification process (used by nitrifying bacteria). Measured in CaCO₃ ppm (mg/l).

Ammonia

Un-ionized ammonia NH₃ is highly toxic to aquatic vertebrates (Malone and Rusch, 1997). Concentrations as low as 0.02 ppm can be problematic. This form predominates at high pH levels. Around pH 7 and below ammonia in the presence of any excess H⁺ mainly exist as non-toxic ammonium NH₄⁺. Ammonia is produced from bacterial breakdown of nitrogen bearing organic molecules. It is also produced as a waste from protein metabolism. The major sources of ammonia in fish systems are directly from the fish in the form waste products, and uneaten food. This is why it is important not to overfeed fish tanks and disrupt a rather delicate balance.

Ammonia poisoning becomes apparent in fish by hemorrhages first in the gills then on the epidermis and internal organs (Untergasser, 1989). The fish stop eating and become lethargic. Reducing the levels of ammonia can be done chemically by ammo-lock or by simply making water exchanges. Ammonia can be stripped by resins and removed by degassing towers but these methods are either difficult to maintain or have limited capacities. Biological filters containing nitrifying bacteria are by far the most productive and easily maintained system for controlling ammonia. The ammonia is not stripped out of the water rather it is converted to a less harmful forms in an important process called nitrification. Measured in NH₃ ppm (mg/l).

Table 5.0 Nitrification of organic nitrogen. Un-ionized ammonia is only one part of the nitrogen cycle, organic nitrogen is broken down in to nitrite and eventually into relatively harmless nitrate. The process relies on nitrifying bacteria.



Nitrite

Nitrite is also toxic to fish. In water nitrite exists either as the nitrite ion (NO) or as nitrous acid (HNO₂). The prevalent form of nitrite depends on the pH of the water. At a high pH the nitrite ion prevails, at a lower pH nitrous acid. Fish do not directly produce nitrite it is typically only found at high concentrations as the intermediate product of



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nitrification, although excessive algae growth in fish tanks may also produce nitrite. Nitrite is readily converted to nitrate in a properly sized and functioning biofilter. It is toxic to fish because it oxidizes hemoglobin to methemoglobin making the blood and gills turn brown and causing labored respiration (Malone and Rusch, 1997). To temporarily reduce nitrite levels reduce feedings and increase water exchanges. Measured in NO_2 ppm (mg/l).

Nitrate

Nitrate is the stable end product of the nitrification process. It is generally considered to be non-toxic to fish even at levels greater than 500 ppm, but it depends on the fish species. For zebrafish concentrations below 50 ppm should be maintained. Nitrate can be removed by bacteria under anaerobic conditions, but regular water exchanges will readily dilute nitrate concentrations. Measured in NO_3 ppm (mg/l).

Carbon Dioxide

Carbon dioxide makes up only a small percentage of the air, but it is highly soluble in water, ~30 times more so than oxygen. CO_2 can be added to water through physical interaction between water and the surrounding air. It is also produced during respiration of fish, bacteria, and algae. Most fish system have supersaturated levels of CO_2 i.e. levels in the air are the same as those found in the water. Saturation concentrations depend on temperature and partial pressure of gaseous CO_2 .

CO_2 in water forms carbonic acid, hence the affects of it will depend on the alkalinity and the overall pH of the water. It can be removed by degassing towers. Since it at least some of it is produced by respiration, lower biological load on a fish system will reduce the levels of CO_2 . Buffering the waters with salts will also greatly reduce the affects of carbonic acid. This is one of the major reasons why we add salts to reverse osmosis water to make system water. Measured in $\text{CO}_{2(\text{aq})}$ ppm (mg/l).

Chlorine

Chlorine is highly toxic to fish even at levels as low as 0.1 ppm. Chlorine toxicity causes erratic movement in fish, their body color dulls and they stop breathing. Chlorine is found in municipal water supplies where it used as a disinfectant. It can be removed by activated carbon filtration, aeration, and chemically most commonly by sodium thiosulfate. Special note do not use sodium thiosulfate to remove chlorine from fish system with fish in it because it will drastically drop the pH. The fish facility uses large amounts of sodium hypochlorite (bleach) a source of chlorine for disinfecting tanks, equipment, floors and even embryos, also in decapsulating artemia cysts. Great care must be used when working with bleach. **Do not perform any procedures with bleach in the facility with out proper training.**

Chloramine

Chloramine is toxic to fish because it moves directly into their bloodstream through their gills. It is a water disinfectant intended to eliminate pathogenic organisms in municipal drinking water supplies. It is becoming the preferred disinfectant because it is less reactive than free chlorine. There are two methods that can be used to remove or



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neutralize chloramines. GAC filtration system specifically designed to remove chloramine, or conditioners or additives that contains dechlorinating chemical for both ammonia and chlorine.

Conductivity

Conductivity is a measurement of water's ability to transmit electrical current. It can be used to estimate the amount of total dissolved solids (TDS) in water. We use conductivity readings to measure the level of salts/nutrients. Measured in microsiemens (uS).

Dissolved Oxygen

Adequate levels of oxygen in the water are crucial to fish survival. They need oxygen just like us. Oxygen is added to water in the same fashion as CO₂ except that O₂ is much less soluble particularly at higher temperatures. Oxygen can be added to water by aeration. You can also increase oxygen levels by reducing the biological oxygen demand (BOD) of the water. It can also be added chemically by hydrogen peroxide etc but this method is not used or recommended. Measured in DO₂ ppm (mg/l).

Hardness

Hardness is a measure of the concentration of dissolved polyvalent cations in water. Since calcium carbonate is a commonly found divalent cation in water this measurement is often related to alkalinity both are measured in ppm CaCO₃. This may or may be true to check measure the alkalinity and compare it to the hardness. In the fish facility this is not at all true. The majority of our hardness comes from the addition of sea salts (Mg⁺² etc). Measured in CaCO₃ ppm (mg/l).

pH

pH is a measure of the acidity or alkalinity of a solution. The pH scale ranges from 0-14 with 7 being a neutral solution- acid and bases are in balance. At pH < 7.0 acid predominate at pH > 7.0 bases prevail. Water is inherently unstable constantly disassociating in to hydrogen ions (H⁺) and hydroxide ions (OH⁻). $H_2O \leftrightarrow H^+ + OH^-$ Great care must be taken not to alter the pH of water by more than 0.2 in an hour or by 1.0 in a day. Drastic sudden changes in pH can irreparable damage the fish. There are many chemicals that can alter the pH of water. **The only that should be added to fish water is a little salt, fish and the occasional net.**

Signs of acidosis (pH too low) in fish are heavy breathing, erratic swimming (darting through the water), and gasping for air at the surface. With alkalosis (pH too high) fish develop a whitish skin, and frayed fins followed later by skin and gill corrosion. With alkalosis ammonia poisoning is also possible.

Phosphorous

Phosphorous levels appear to have no affect on fish health. It is however the limiting nutrient in water for algae. High levels of phosphorous will lead to increased algae growth that in turn will increase the BOD of the system lowering the DO (dissolved oxygen) and altering the pH. The main source of phosphorous in fish system is from fish



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food, and leaching from activated carbon. Avoid using other products that contain phosphate.

5.5 Maintaining Water Quality

Maintaining proper water quality is done through 3 types of filtration: mechanical, chemical, and biological. Frequency of maintenance on these filtration types depends on the size of the fish system, its biological load, and the efficiency of the particular type of filtration. For specific details of existing fish systems filtration types and maintenance see **Chapter 6**.

Mechanical Filtration

Mechanical filtration is the straining of solid particles from the water. Most common mechanical filter media do not remove bacteria, ammonia or algae from the water. The simplest method is to vacuum debris directly out the fish tank, but it is impossible to remove all of the solid particles in a large recirculating water system by vacuuming.

The four most popular mechanical filtration media are sponges, paper cartridges, loose and bonded polystrand, media that are reusable to different degrees. All 4 types of mechanical filtration exist in the fish facilities it depends on the particular fish system and so does the frequency of its maintenance.

Chemical Filtration

Chemical filtration in short, is the removal of dissolved wastes from water. Dissolved wastes exist in the water at a molecular level, and fall into two general categories, polar and nonpolar. The most common chemical filtration method involves filtering the water through gas activated carbon, which works best on the nonpolar wastes but also removes some polar wastes.

Granular activated carbon (GAC) is manufactured from carbon, typically coal, heated in the presence of steam at very high heat. This process causes the carbon to develop huge numbers of tiny pores, which trap nonpolar wastes at the molecular levels by means of adsorption and ion exchange, and removes heavy metals and organic molecules, which are the source of undesirable colors and odors, through a process known as molecular sieving.

GAC has one draw back it can be a source of phosphate. Purchasing only high quality acid washed carbon from a reputable company such as Kent Marine can minimize this problem. Soaking the carbon in water for several days can also mitigate this affect. The phosphate in GAC stems from the fact that activated carbon is manufactured from coal, which was once living plant matter. All living matter is high in phosphates. The leaching of phosphate from GAC is known to be high initially and to decrease over time.

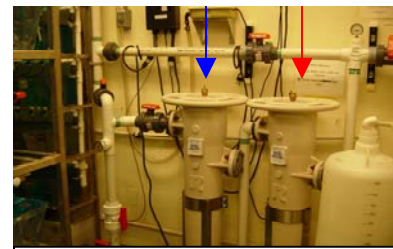


Figure 5.1 Blue arrow: A mechanical bag filter. Red arrow: A chemical carbon filter.



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Biological Filters

Advances in the design of biological filters and better understanding of the nitrification process are one of the main reason why recirculating water system can support so many fish. The biological filter is actually a rather simple filter in that all it contains is substrate for the nitrifying bacteria to live on, then all you need to do is deliver them food-nitrogen and oxygen. Sand, glass beads, porcelain makes nice bacterial substrate. Generally you want something small but has a large surface area for bacteria to grow. The total amount of surface area for bacterial growth in the biofilter will determine the filters capacity.

The substrate must be kept wet and have continual flow of nutrient rich water passing through them. The bacteria can survive for some time with out water flow, but they will die quickly out of the water. The *Nitrosomonas* and *Nitrobacter* bacteria are constantly starved in fish system. Once the biofilter is adequately adapted the bacteria can quickly respond to increasing loads of fish, but when load are decreased drastically you can starve and kill part of the biological filter. Evidence of this is a white bloom from the biofilter-dead bacteria. One should always moderate loading of fish system because of this.

Nitrosomonas the bacteria that breakdown ammonia, grow quickly but it takes time for them to oxidate ammonia compared to *Nitrobacter* the genus of bacteria that oxidizes nitrite, they grow slowly but quickly convert nitrite to nitrate. This can be seen by monitoring NH_3 and NO_2 levels during the acclimation of a new biofilter. Ammonia levels will remain high until the *Nitrosomonas* population grows large enough to decrease the levels. This will lead to an increase in the amount of nitrite and subsequently a jump in the *Nitrobacter* population. The populations of both bacteria will autoregulate themselves according to the amounts of NH_3 and NO_2 in the water.



Figure 5.2 Red arrows indicate 3 different types of biofilters found in fish facility. A. Is a sand biofilter B. Is a polystrand pad biofilter C. Is an hour-glass bead filter.



5.6 Acclimating Biofilters

There is 3 main ways to acclimate a new biofilter: chemical, inoculate-chemical and gradual. The choice of which method to use depends on how quickly you want to establish the filter and what resources you have. Start your new biofilter with system water, see section 5.3

Chemical method is to simply add ammonium chloride and sodium nitrite to a new recirculating system at concentrations of 3 mg/l and 1 mg/l respectively. This is cheap and easy, but it will take the longest time of all the methods before you can start to put fish on the system i.e. longest time for ammonia and nitrite levels to reach safe levels.

Inoculate and chemical is an accelerated startup. First you need to “seed” the filter first. Seeding simply means add the bacteria *Nitrosomonas* and *Nitrobacter*. There are many commercial products available for this such as proline and bio-start. If your starting up a biofilter in a facility that already has a fish system you can simply transplant some of the existing biofilter media to the new filter. The later method is preferred because biofilters can support many types of bacteria. Adding bacteria from the outside (commercial source) may introduce bacteria that your fish themselves are not acclimated to. This is the quickest way to acclimate a biofilter, but you still have to wait to add fish to the system. Timing with this method and the chemical method can be tricky. Once nitrogen-ammonia and nitrogen-nitrite levels reach safe levels you will need to fill the system with fish to maintain the filter. You may have to endure another nitrogen spike or a bacterial die off if you do not load the system properly.

The gradual method allows you to add some fish right away and begin feeding them. Add roughly 1/3 of the system fish capacity every two weeks. Perform 10% water exchanges weekly. This will acclimate the filter gradually, and you may never experience a spike in the nitrogen-ammonia and nitrogen-nitrite levels. This process can be enhanced by lightly “seeding” the biofilter. This is the preferred method of the fish facility because it is the safest.

For all 3 methods monitor pH, temperature, DO and conductivity daily and ammonia, nitrite, nitrate, and alkalinity weekly. Remember that at anytime simply performing a water exchange can reduce nitrogen levels and when using the gradual method reducing feed and/or the amount of fish. Keep the water warm ~28° C. Biofilters consume large amounts of bicarbonate so maintain your alkalinity levels. Since ammonia and nitrite is being oxidated make sure DO levels remain high 5-7 ppm.



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5.7 Water Quality Parameters

Table 5.1 illustrates the optimal water quality standards, and the range of maintained levels. Since optimal conditions are very difficult to achieve for long periods of time the maintain at values are more realistic. To adjust values see water treatments 5.9.

Additional each fish system has an information card attached to it explaining how to adjust some water quality parameters. Do not adjust water quality parameters unless you have been authorized to do so.

Table 5.1 Optimal water quality measurements, and an acceptable range of measurements. For zebrafish recirculating water systems.	pH	Alkalinity	Hardness	DO₂	Conductivity
Optimal	7.0	100 mg/l	175 mg/l	7.7 mg/l	1500 uS
Maintain at:	6.9-7.4	50-100 mg/l	150-250 mg/l	5-7.7 mg/l	1400-1600 uS
	NH₃	NO₂	NO₃	Temperature	Chlorine
Optimal	0	0	0	28.5 °C	0
Maintain at:	Less than 0.02 mg/l	Less than 1.0 mg/l	Less than 50 mg/l	27-29 °C	0

5.8 Water Quality Testing

Water temperature and pH are measured twice a day, conductivity once, alkalinity hardness, DO₂, NH₃, are measured weekly, and chlorine and NO₃ monthly. Of course, any of these measurements can be taken at any time if a problem is suspected. Temperature, conductivity and pH are taken with meters that are calibrated monthly (see equipment notebook for calibration procedures) and recorded on clipboards located on each fish system. The rest of the tests are performed using test kits (mainly Hach) follow test kit instructions.

For test kit measurements collect water samples from the sumps of individual fish systems in labeled 500 ml cap bottles. Remove the cap off the bottle and fill and dump the bottle 3 times keep the 4th sample and cap it. Never put testing equipment directly in to the water. Water samples collected must be tested with in a few hours.

Record water quality on the animal care log (ACL) water supplemental sheet on the clipboard in the H225 laboratory. There is also an electronic database that needs to be filled out on H225 computer. Be sure to include the date of the testing and the testers initials. This is the same electronic database where maintenance records are kept.



5.9 Water treatments

Specific instructions for water treatment on individual systems are given in Chapter 6.

Always add water treatments to the fish system sumps never directly to a fish tank.

Decreasing pH

To decrease pH on a fish system dilute it with system water, which generally has a pH of 7. Never adjust the pH by more than 0.2 units in an hour. Start by filling the sump with system water and place the pH meter cell in to the sump and turn on the meter. Once the pH level has reached the desired level let the system circulate for 30 minutes and measure the pH again. If need be repeat this procedure the next day. If levels remain high 1 M HCl can be used but contact facility manager first.

Increasing pH

First check to see if the system is due for maintenance. Declining pH is often a sign that maintenance is needed. pH is increased on a fish by the addition of 1 M sodium bicarbonate. Follow the green water quality card attached to the fish system for quantities. 1 M sodium bicarbonate can be found on the shelf by the computer in J083b, and on the shelf in H225. See recipes for making sodium bicarbonate solution.

Adjusting Conductivity

Never adjust the conductivity by more than 200 uS in a 24-hour period. To lower conductivity dilute it with RO water. To increase conductivity add salts from the super saturated salt carboy located near the water towers. Follow the green water quality card attached to the fish system for volumes.

Adjusting Hardness

Increase or decrease the amount of coral (calcium carbonate) or if need be conductivity. Calcium hardness can be increased by the following equation:

$$[(V \times C_2) - (V \times C_1)] \times 1.8 = T$$

T = Total weight in milligrams of calcium chloride to add to the system

C₂ = Desired hardness in mg/L

C₁ = Actual hardness in mg/L

V = Total water volume in liters.

Neutralizing Chlorine and Chloramines

Sodium thiosulfate is used to neutralize chlorine. Use the following equation:

$$V \times (6C) = T$$

T = Total weight in milligrams of sodium thiosulfate to add

C = Chlorine concentration in mg/L (ppm)

V = Total water volume in liters.