

Aquatics Labs: Five Questions You Don't Want to Have to Ask

You've made it through the first part. Your equipment is set up, the facility is approved, you have animals in the system, and they're happy. Things are going great; in fact, they couldn't be better! And then something happens.

Mark Francis

Something changes. You don't know why it changed, and you don't know how to change it back. What do you do before the integrity of the system is compromised? This article answers five very common questions that arise in the operation of an aquatics lab.

My ammonia levels are too high. What should I do?

Ammonia is a waste byproduct of living things and it may cause problems for your animals. The commercial aquatic holding systems available today have filtration designed to control ammonia levels, but sometimes ammonia levels are high.

The first thing to do when ammonia levels are high is to remember that we are part of a scientific community. One must accurately assess the situation prior to making changes, and the first step towards doing that is taking a step back and looking at the water. Does it have a yellow tinge? Do the fish look OK? Do the tanks look reasonably clean? Have the filter pads been changed recently? Next, check the pH to make sure it is between 6.8 and 7.8 and then measure the nitrites and nitrates. These are important preliminary steps in finding the cause of an ammonia problem.

One of the most common causes of ammonia spikes is overfeeding. Overfeeding leads to excess food buildup in the tanks and filter pads. This can create a localized high ammonia level in that particular tank. Additionally, this excess food can exceed the capacity of the biofilter, especially on older systems. Filter pads that have gross amounts of excess food and are overflowing, can also create high levels of ammonia. It's important to remember that if the filter pad isn't changed, the food and waste that it has captured will continue to break down and produce ammonia. Scheduled filter pad changes (optimally two to three hours after feeding) will remove this waste from the system, and the overall impact on the biofilter will be reduced, resulting in lower overall ammonia level.

Another problem could be that the biological filter is not operating properly. On new systems, the filter must first be acclimated in order for the bacteria to function. This process occurs naturally over about six to eight weeks. An easy test to determine whether or not the biological filter is working is to test the levels of nitrites and nitrates. If ammonia levels are high (greater than 1.0 ppm) and nitrites and nitrites are very low (less than 2.0 ppm nitrites and less than 10-15 ppm nitrates), the filter could either not be acclimated or needs to be re-acclimated. Additionally, if the pH is below 6.5, the nitrifying bacteria stop converting ammonia to non-toxic nitrate.

This leads to another common, related concern: fluctuations in the pH levels in the system water. Different species prefer different pH levels to be happy, and wide variations can seriously affect eating, breeding, and ultimately, survival of your animals. Monitoring these levels is an integral part of lab procedure, yet still the

question often arises.

My pH readings are too low/high. What should I do?

pH is an important factor in both animal health and the operation of the biological filter. Fish prosper with a pH between 6.0 and 9.0. For breeding Zebrafish, most labs operate between a pH of 6.8 and 7.8, but good results have been obtained with a pH as high as 8.5. Often the biggest problem on a system level is that when the system is heavily loaded with fish, the CO₂ produced will drive the pH down. Sodium bicarbonate can be dosed into the water to maintain the desired level.

Other problems with pH are related either to maintenance of the pH controller or incoming water. When troubleshooting pH, one must be careful to approach it systematically. The first thing to check is whether or not the instrumentation is working properly. If the pH probe is not regularly cleaned per manufacturer's recommendations, it can read a higher level than really exists. Furthermore, pH monitors can occasionally malfunction. Before assuming your pH is out of range, check the monitoring system. Doing a manual pH test is a good first step; however, it's best to insert the probe into new 4.0 and 7.0 calibration solutions. If the monitor reads accurately when the probe is placed in the new solutions, you can assume the monitor is working properly. If it does not read accurately, the probe needs to be recalibrated. pH probes typically last one to two years before requiring replacement, although if they are carefully maintained they will last longer. Be sure to follow the manufacturer's maintenance instructions for the care of your pH electrode. It is possible that the probe has simply outlived its usefulness and needs to be replaced.

Once it is established that the pH monitor is working, activated carbon can be considered as a possible cause of high pH levels. Activated carbon is used in many recirculating systems to "polish" the water; high quality systems use only acid-washed, activated carbon. Other carbons have been known to raise the pH in a system to 9.0 in a matter of minutes. Although fish can survive this, it creates undue stress for lab managers. Activated carbon is also used as the primary method for removing chlorine and chloramines from incoming water to the system. This might be out of the lab manager's control if a house reverse osmosis (RO) system is used. This is when it is imperative to have accurate, reproducible pH measurements that can persuade others that the equipment is not working properly.

The RO Water supply isn't working. What should I do?

Most protocols dictate that Zebrafish labs use RO water for their makeup water, although it is important to note that some labs, and most commercial hatcheries, have excellent results with municipal water with only the chlorine and chloramines removed. It is not uncommon to hear questions about RO water supply.

The amount of makeup water (the new, incoming water exchanged for system water) is generally 5 to 10% of system volume per day. Although Zebrafish don't require anywhere near this much, in research it is thought that a

higher exchange rate will keep trace chemicals and toxins from accumulating, as well as decrease how often the tanks need to be washed. RO water makers don't remove chemicals, so they must have carbon pre-treatment to remove chlorine and chloramines. Many systems have dedicated RO water makers, which results in the troubleshooting and maintenance by the lab technicians.

The problem can generally be broken down into two areas: water supply and mechanical. Water supply problems will often shut the machine down due to low pressure, which might appear to be a mechanical problem. Most RO machines require a feed water supply minimum pressure of 40 to 50 psi. This must be maintained while the machine is operating since although the line pressure might be 60 to 70 psi; once the machine is turned on, pressure drops can reduce this to a much lower level. Often, the building water supply cannot keep up and a booster pump is necessary. However, the normal culprit is the RO machine's prefilters needing replacement. It is important that when the RO water maker is installed, pressure gauges are installed before and after the prefilters to assist in determining when the filters require changing.

Another important point to remember is that many municipalities change their water supplies each fall and spring. Therefore, the RO machine may be operating fine until the switch is made to a different water supply, when all of a sudden the filters start plugging up frequently. Check with the local water district to see if they have semi-annual operating changes so you can anticipate these kinds of problems.

Once feed pressure and pre-filters have been determined to be operating correctly, use the RO machine's operator manual to troubleshoot mechanical problems. Remember, in a pinch, the fish can go without any water changes for several days, and Zebrafish will do just fine on carbon-filtered water to remove the chlorine and chloramines.

My fish have stopped laying eggs and I can't figure out why.

One of the primary activities of many aquatics labs is breeding the animals and raising the babies to various stages of development. So, it's a big concern when the eggs stop coming.

Zebrafish are quite hardy, and normally eager to lay eggs as long as the water quality parameters are in the middle of the suggested ranges. They often continue to reproduce even if one of the parameters is pushed

to the high or low limits of the range. However, one should be careful that all parameters are not pushed to the maximum or minimum values at once. If the pH is between 7.0 and 8.0, the ammonia and nitrites are undetectable (less than 0.1 ppm), and the water temperature is between 24 and 28 C, a female that is less than a year old should lay eggs every two weeks. If this isn't occurring, other causes should be investigated.

Frequently, nutrition is the culprit. Many investigators have strong opinions about the proper feed and feeding protocols for Zebrafish. Unfortunately, the food bought at local pet shops is generally not dated and the shelf life and expiration date are unknown. It is imperative that a quality, dated diet specifically developed for Zebrafish is used. A poor diet - especially a diet low in fish meal - can lead to egg-bound females (egg-bound females will appear very rotund, but do not lay their eggs). Sometimes if egg-laying drops off, the tendency is to feed the fish more in the hope that increased food will increase the fish health and thus lead to egg-laying, when in reality that is the worst thing to do. Changing the food as advised above, not overfeeding, and adding live food such as Artemia will enhance egg production.

Once a female has become egg-bound or has stopped laying eggs for a long period of time, it is difficult to get her to start laying eggs again. If the particular fish are important, sometimes the female is squeezed to remove the excess eggs, but in general, it makes sense to improve the nutrition for new females added into the system and encourage them to breed on a regular basis, whether the eggs are required every week for current research or not. One of the fastest ways to assure that your fish won't lay eggs is to leave the lights on all the time. More than one researcher in the course of trying to find out why the eggs weren't coming, discovered that their room light timer had been changed without their knowledge, and the lights were staying on all night. Most labs find that a schedule of 12 hours on, 12 hours off results in productive females. Once the nutrient level, schedule, and water parameters are established and maintained, egg laying should continue successfully.

How often do I need to clean the tanks?

More important than monitoring, feeding, and egg-gathering, the animal care staff must visually inspect the fish daily. Tanks have varying degrees of visibility over time. Cleaning protocols for aquatic tanks are much less stringent than those for rodent cages. It is important that animal care staff remember that fish require a "clean" environment, but not the "sterile" environment that is common for rodents. In aquatics, a healthy biofilm on the walls of the tanks is generally accepted as beneficial, since this will discourage unwanted bacteria from getting a foothold in the tank. Obviously, uneaten food on the bottom of the tanks and algae growth that is too thick to allow viewing of the fish is not acceptable.

Although all modern Zebrafish systems utilize polycarbonate tanks, most labs do not autoclave them. If a cage washer is used to clean polycarbonate tanks, they should be thoroughly rinsed and have careful attention given to any chemicals used, since detergents are toxic to the animals.

Factors that determine when a tank needs to be cleaned include feeding, water quality, and lighting. Overfeeding is most likely the biggest cause of waste on the bottom of the tanks since most designs have "self-cleaning" features that help the waste wash out of the tank. If excess waste accumulates on the bottom of the tank, this can allow both parasites and bacteria to grow and be eaten by the fish. As mentioned before, frequent water exchanges keep the nitrogen levels down and help discourage algae growth. Properly located lighting in the aisles, not over the racks, also reduces the algae growth and cleaning frequency.

Operating an aquatics lab is an exercise in adapting to change. With experience, procedures can be established to maximize research efficiency and minimize downtime. Some questions arise frequently enough that even if it isn't a problem for your facility now, the odds are good that you may find yourself asking one of the above questions in the future. However, with a little foresight and preparation, you can take the actions necessary to avoid being in the position of asking our five questions.

Mark Francis is President of Aquaneering, Inc. For over 30 years he has been instrumental in developing and improving filtration systems for commercial aquaculture as well as aquatic research facilities. **Aquaneering, Inc.** San Diego, CA. 858-578-2028 www.aquaneering.com



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AQUANEERING, INC., 7960 Stromesa Court, San Diego, CA 92126 Tel., 858-578-2028