RECIRCULATING SYSTEMS FOR ZEBRAFISH

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In the past several years, zebrafish [*Danio rerio*] have emerged as a very important model for embryological, neurological, and genetics research. Scientists are using this tiny tropical freshwater fish as a biological model because of its quick generation time, high fecundity, and transparent eggs. The emergence of zebrafish in a growing number of research laboratories has brought about a new breed of aquatic systems to house them. This article will discuss basic zebrafish system design criteria, water quality management, and general considerations when deciding on the right system for your research laboratory.

RECIRCULATING SYSTEM DESIGN CRITERIA

The objective of any zebrafish system manufacturer is to build a system that allows researchers to concentrate on research instead of maintaining water quality, cleaning filters, or preventing disasters. A properly designed recirculating system will provide adequate filtration to maintain excellent water quality for the fish at the highest biological loading capacity expected. Redundancy and proper engineering of the system should prevent any failures that may occur during a power outage or pump failure. Piping should be sized correctly for the designed flow rate to allow the system to balance properly.

A typical recirculating system for zebrafish is comprised of a series of components to accomplish four main objectives. These objectives include mechanical removal of solids, sterilization, biofiltration, and oxygenation. In order to achieve maximum energy efficiency and use the fewest number of pumps, components should be placed in series and flow patterns should allow water to feed by gravity whenever possible. Modular systems, which have filtration systems integrated under each individual rack, can be used for small laboratories (1-5 rack systems). However, larger laboratories with many racks should consider centralized filtration systems in order to maximize flexibility and minimize maintenance required.

Mechanical Filtration

A typical zebrafish system uses a mechanical filter to remove uneaten food, fecal matter, and other organic solids. There are several different types of mechanical filters that can be used for this application. When choosing the appropriate mechanical filter, you must consider the maximum flow rate, the smallest sized particle to be removed, and the filter that requires the least amount of maintenance.

Mechanical filters range from simple mechanisms such as pre-filter pad material placed in the sump tank to capture larger solids, to pressurized vessels with pre-determined porosities such as pleated cartridge filters and pressurized bag filters. For larger laboratories with central filtration systems, propeller-washed floating bead filters are used. Bead filters can be equipped with automatic backwash systems to minimize maintenance.

Combinations of filters are usually placed in series to obtain stepped particle filtration >15 - 25 microns. Capturing larger particles with one filter and finer particles with another tends to optimize the performance of each component and reduce the required maintenance. All of these filters vary in their ability to capture certain sized particles, the amount of water discharged for backwashing, flow rate capacity, and frequency of maintenance. The following chart helps organize the types of applications for each filter:

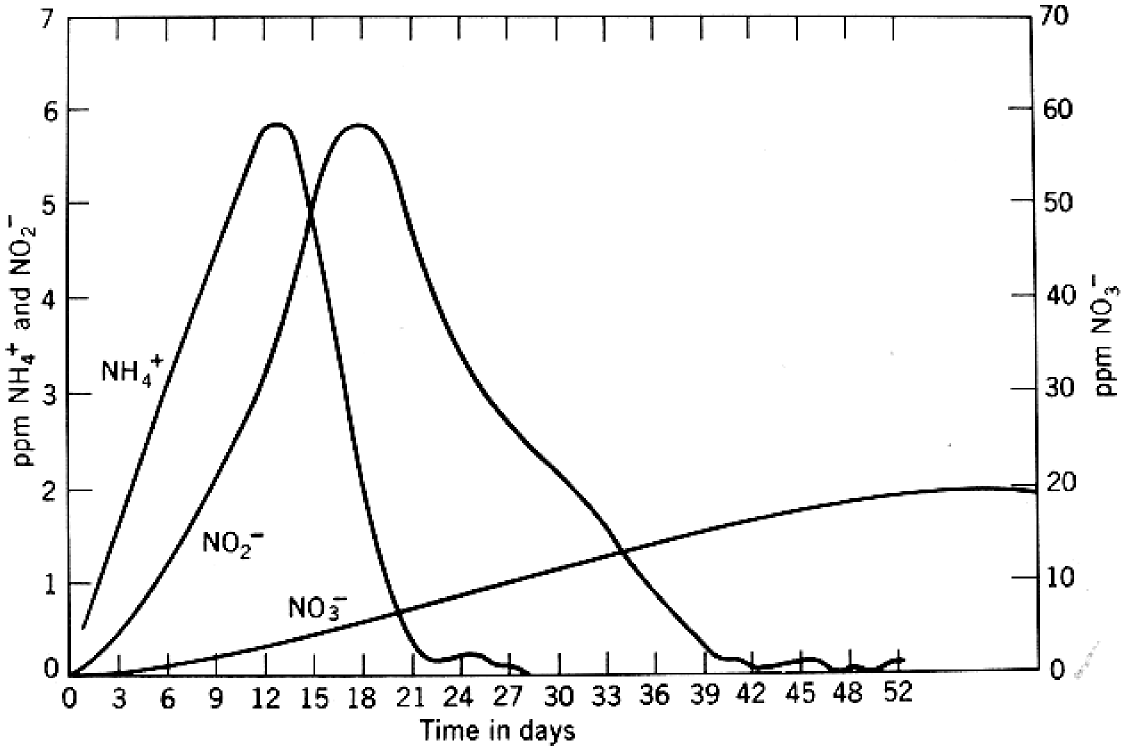
MECHANICAL FILTER COMPARISON

	Particle Size	**Capacity**	**Discharge**	**Maintenance**
Pre-filter pad	>75 microns	Low-Med	0 gallons	every 1-3 weeks
Cartridge Filter	>16 microns	Low-Med	0 gallons	weekly
Bag Filter	> 0.5m -150m	Low-Med	0 gallons	weekly
Bead Filter	> 25 microns	High	10-50 gal/bw	every 1-3 days

Biological Filtration

Biological filtration is required to remove ammonia and nitrites from the water, which are toxic byproducts of fish waste and can adversely affect fish health and growth. Biofilters essentially provide a large surface area for nitrifying autotrophic bacteria to establish. Therefore the ability of a biofilter to process a given amount of ammonia is a function of the amount of surface area provided by the filter and the availability of sufficient oxygen to drive the conversion of ammonia (NH_4+) to relatively harmless nitrate (NO_3-).

Biofilters require a period of 4-8 weeks to establish nitrifying bacteria necessary for nitrification. When ammonia first becomes available in the system, a group of bacteria of the genus *Nitrosomonas* begins to develop which converts ammonia (NH_4+) to nitrite (NO_2-). As nitrite becomes available in the system, a second group of bacteria of the genus *Nitrobacter* establishes, which begins to convert nitrite to nitrate (NO_3-). The following graph illustrates this process, which can be monitored through water quality testing.



There are several different biological filters available for zebrafish systems. The most commonly used types are fluidized bed biofilters, trickle filters, and submerged thin-film bioreactors. Fluidized beds keep fine silica sand in suspension and provide the greatest specific surface area per unit volume. Fluidized beds are very effective at removing even trace amounts of ammonia in lightly loaded systems, however they become oxygen limited in heavily loaded systems. They must also be carefully engineered to restart automatically in case of a shut down or power failure, otherwise they may become anaerobic and the bacteria population can die.

Trickle filters distribute water over commercially available biofilter materials with low to medium specific surface area per unit volume. They are very effective nitrifiers because they take advantage of excellent oxygen exchange essential for nitrification. The advantages to using trickle filters are their reliability, heavy bioloading capacity, and the fact that they yield a net oxygen gain after processing wastewater. The disadvantage to trickle filters is that the relatively low surface area limits their ability to remove trace amounts of ammonia < 0.1 ppm in one pass.

Submerged thin-film bioreactors keep biomedia with low to medium specific surface area submerged in the sump tank. The advantage to these filters is their ability to keep nitrifying bacteria established even during a prolonged system shut down. This is due to the fact that they remain submerged and have adequate food and oxygen available to survive for prolonged periods.

The disadvantage is the low nitrification rates that are achieved with low surface area and little oxygen transfer.

Sterilization

An ultraviolet sterilizer is recommended to disinfect water and prevent the spread of disease in recirculating zebrafish systems. It is important to remove solids >15 microns in size before flowing water through the unit in order to maximize the UV light intensity used to disinfect harmful microorganisms. The UV light intensity required to kill specific microorganisms varies widely. UV sterilizer manufacturers typically recommend dosages of 30,000 - 45,000 μwattsec/cm^2 for the disinfection of bacteria, fungi, some viruses, parasites, and protozoa. It should also be noted that UV sterilizers should be turned off during the initial bacterial establishment period in the biofilter, which can last up to 2 months.

Oxygenation

An adequate oxygen supply and stripping of excess CO_2 is essential in aquatic systems. Individual zebrafish tanks and sump tanks should be provided with aeration from a central device such as a blower. Individual tanks should be aerated mainly as a precautionary measure to prevent oxygen levels from dropping to dangerous levels during a system shut down or failure.

Since fluidized bed biofilters are closed vessels, they require a packed column or trickle filter placed at the exit of the filter to re-aerate oxygen deficient water and strip excess CO_2. This is due to the oxygen demand of 2 ppm of oxygen for every 1 ppm of ammonia removed in the biofilter and residual accumulation of CO_2. Submerged thin-film reactors require aeration to mix water in the tank and provide enough oxygen to be effective. Trickle filters are the only biofilters that yield a net gain of oxygen and do not require additional aeration.

Make-up Water Filtration

Recirculating systems require the addition of some makeup water to replace that lost due to evaporation, backwashing filters, and for water exchanges to prevent the buildup of nitrates. Incoming water used both to initially fill the tanks and to make up water loss should be properly filtered to avoid changing water chemistry or quality. Some commonly used filters for this purpose are reverse osmosis systems and carbon filters. Reverse osmosis systems use extremely fine membranes to remove approximately 98% of all salts, most hard water minerals, and other dissolved and suspended solids. Activated carbon filters are very effective chemical filters and can be used to remove 99.5% of the chlorine present in tap water. Chlorine must be removed prior to entering the biofilter, since it can be lethal to the nitrifying bacterial colonies.

Tanks, Racks, and Plumbing

Zebrafish tanks come in three standard sizes from most manufacturers (1 liter, 5 liter, 10 liter). The smallest tanks are usually used to isolate a mating pair or individual zebrafish without taking up too much tank space. The 5 and 10 liter sizes are used to house larger numbers of fish. Zebrafish tanks are typically made of polycarbonate plastic and have taken on several design features to promote self-cleaning. Fry and juvenile tanks also require the addition of fine mesh screens to prevent the escape of fish from the tanks.

The support racks should be designed to maximize the tank space and plumbed to allow researchers the maximum operating flexibility . For example, the system should allow each individual rack, shelf, and tank to be shut down without affecting the rest of the system. Drainage lines and gutters should also be readily accessible and removable for easy cleaning or replacement. The racks themselves should be made of non-corrosive materials such as stainless steel since they are housed in very humid environments. Zebrafish racks and filtration systems should be pre-engineered, plumbed, and installed by factory certified personnel to ensure the system works to its design specification.

WATER QUALITY MANAGEMENT

Zebrafish are hardy freshwater tropical fish that are tolerant to a relatively wide range of temperatures. Based on testing at the University of Oregon zebrafish center, the ideal temperature for breeding zebra fish is 28.5°C. Some zebrafish laboratories run lower temperatures (23 - 27°C) both for employee comfort and to extend fish life span.

Dissolved oxygen (DO) is one of the most important water quality parameters in aquaculture systems. Levels should be maintained near saturation, which is 7.7 ppm at 28.5°C.

When using water treated by reverse osmosis (r/o) to fill tanks and make up water loss, trace amounts of salts and minerals must be added to the zebra fish system to replenish those lost during the r/o filtration process. The total dissolved solids (TDS) can be monitored with a conductivity probe in the system sump tank. The delivery of a pre-mixed salt solution can be either manually added in the sump or automatically controlled by a computer monitoring and control system. The ideal range for conductivity in a zebra fish system is between 500 - 1000 ppm.

The pH in the system will tend to slowly decrease over time as the buffering capacity of the water decreases. Low alkalinity levels or high CO_2 are factors that can contribute to the drop in pH. A solution of sodium bicarbonate (baking soda) and water can be added to the system to increase pH. The ideal range for pH in zebra fish systems is typically between 6.8 - 7.2.

It is important to test total ammonia (NH_4) and nitrite (NO_2^-), about once a week using a water quality test kit. The water sample should be taken from the wastewater sump tank, before the water has been processed by the biofilter.

Ammonia and nitrite levels should be below 0.3 ppm. Nitrate (NO_3^-) is relatively harmless to aquatic animals in low levels. There is no data that indicates any water quality problems with nitrate levels < 200 ppm. Weekly testing of nitrates is suggested, to maintain levels < 150 ppm. If ammonia, nitrites or nitrates reach the upper limits, a water exchange of 10% of the water volume at a time is recommended.

Computer Monitoring and Control

Computer monitoring and control systems are excellent tools to help monitor water quality, log data on the computer, set alarms, and control water quality parameters such as pH and conductivity. If any parameters rise above or dip below programmed set points, the alarm system may trigger an audible alarm, call a pager, send a fax, or e-mail a message to responsible personnel. It is important that the set points are carefully selected to ensure the highest health, reproductive success, and safety of the animals in the system.

RECOMMENDED WATER QUALITY SETPOINTS

Parameter	**Desired Setpoint**	**High Alarm**	**Low Alarm**
Temperature	28.5° C	30° C	25° C
Dissolved Oxygen	99% saturation	N/A	6 ppm
Conductivity	500 ppm	1500 ppm	200 ppm
pH	6.8	7.4	6.5

CONCLUSION

A properly designed recirculating zebrafish system will require low maintenance, have sufficient filtration capacity to maintain good water quality, and also be engineered to prevent any failures in the system that may cause fish mortality. The manufacturer must meet the individual needs of the researcher such as available budget and space limitations. With the purchase of a zebrafish system, researchers must also be trained how to properly manage the system and maintain good water quality, which will ultimately improve fish health, reproduction, and growth.

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