

Science Education Collection

Zebrafish Maintenance and Husbandry

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Abstract

The zebrafish (*Danio rerio*) is a powerful vertebrate model system for studying development, modeling disease, and screening for novel therapeutics. Due to their small size, large numbers of zebrafish can be housed in the laboratory at low cost. Although zebrafish are relatively easy to maintain, special consideration must be given to both diet and water quality to in order to optimize fish health and reproductive success.

This video will provide an overview of zebrafish husbandry and maintenance in the lab. After a brief review of the natural zebrafish habitat, techniques essential to recreating this environment in the lab will be discussed, including key elements of fish facility water recirculation systems and the preparation of brine shrimp as part of the zebrafish diet. Additionally, the presentation will include information on how specific zebrafish strains are tracked in a laboratory setting, with specific reference to the collection of tail fin samples for DNA extraction and genotyping. Finally, experimental modifications of the zebrafish environment will be discussed as a means to further our understanding of these fish, and in turn, ourselves.

Transcript

Proper husbandry is essential to the success of experiments performed in zebrafish. Optimal water quality promotes fish health and experimental reproducibility. Additionally, zebrafish egg production is highly dependent on proper nutrition. This video describes how fish are housed and fed in the lab, tips on handling and managing stocks, and a glimpse into how the zebrafish environment is manipulated in biological experiments.

Let's start by going over the basics of the fish habitat.

Wild zebrafish originate from fresh Himalayan waters. In this climate, the fish spend most of their days basking in slow-moving bodies of fresh water.

How is this paradise recreated in the lab? Let's start with the most important part: the water. Although fresh water may seem easy to obtain, tap water is toxic to zebrafish because of chlorine and potential contaminants. Therefore, aquarium water must be passed through a purification system, such as a reverse osmosis unit. Salts and pH buffers are then added back to the purified water to optimize the salinity and pH.

To maximize experimental efficiency, many fish are maintained in a limited amount of water. Waste builds up quickly with all those fish swimming around, making water change essential. This need creates a high water demand, so fish facilities use a recirculation system to minimize water use. Dirty water is filtered and sterilized by UV treatment before flowing back into the system.

Zebrafish are kept in specialized tanks, which are available in multiple sizes. Tanks are covered to reduce evaporation and prevent fish escape. To allow the tank to integrate into the system, the lid has holes through which clean water is constantly flowing. The water level remains stable thanks to an overflow port at the back of the tank, which is covered by a baffle with small holes that allow dirty water, but not fish, to flow out.

Despite the water exchange, algae and solid waste can still build up in the tanks, so they need to be cleaned regularly.

Environmental control in fish facilities is also extremely important. Temperature is maintained near 28 °C, or 80 °F. To maintain the animals' circadian rhythm, lights are controlled on a cycle with 14 hours of light and 10 hours of darkness.

Now that we've gone over zebrafish housing conditions, let's talk about their diet.

In their natural habitat, zebrafish largely consume zooplankton and insects. This diet is recreated in the lab by a combination of dry food and small organisms. Larval and juvenile fish thrive on live microorganisms like paramecia, while adult fish are often fed commercially available powders and small crustaceans known as brine shrimp.

These critters are particularly amenable to laboratory preparation, because their eggs can be stored as dormant cysts at room temperature. To prepare the shrimp for eating, their hard outer shells are removed by treatment with bleach. Next, decapsulated eggs are washed thoroughly. The eggs are then transferred into an aerated tower to allow growth in salt water for about one day. Finally, the hatched shrimp are collected and rinsed in a mesh strainer and then put into bottles for feeding. Fish are generally fed 2 - 3 times per day, alternating live and dry feed, and water flow is turned off during this period so that the food doesn't float away before being eaten.

Now that you know how to house and feed fish, it's time to learn how to work with and keep track of your fish stocks.

Laboratory fish facilities house many different types of zebrafish, from wildtype strains, to fish whose genomes have been modified with disruptive point mutations and inserted transgenes. To keep track of all of these fish, animals with similar genetic backgrounds are kept together in labeled tanks. Tank labels should contain thorough identifying information, including the fish genotype and date of birth.

Zebrafish fertility begins to decline after the first year of life, so stocks should be replenished yearly. Zebrafish do not survive well as fully inbred strains, so genetic diversity must be maintained by collecting progeny from crosses of unrelated fish, or "outcrosses."

When outcrossing transgenic or mutant lines, progeny bearing the desired genetic modification must be identified by fluorophore expression or genotyping. To genotype, first anesthetize the fish in tricaine. Then, cut off a small piece of the tail fin as a source of DNA.

Finally, isolate DNA from the tail sample and use it for PCR analysis to identify specific sequences. While waiting for the results, keep each fish in its own small tank labeled with an identifier.

Now that we have gone over standard fish rearing conditions, let's look at some ways that these standards are manipulated to study biological processes.

The temperature of the system water can profoundly affect zebrafish health. To create a model of diabetes mellitus, these researchers treat fish with the pancreatic toxin, streptozocin, and house them in tanks with reduced water temperature. The diabetic fish show expected phenotypes, such as kidney and eye damage, as well as reduced tail fin regeneration, and can be used to examine the biology of diabetes.

Tank systems can also be modified for specific experimental purposes. Studies of swimming behavior in fish can be used as a readout for anxiety, aggression, or social behaviors. Here, video tracking is used to analyze the difference in swimming behavior between untreated fish and those treated with a neuroactive drug. Studies like these help advance our understanding of neuroscience and can be a tool for drug discovery.

Altered light conditions can also affect zebrafish. Intense light treatment can even be used to ablate the light-sensing cells of the retina. These fish have the capacity to repair damaged retinal tissue by cell proliferation, which is of significant interest to scientists researching retinal degeneration in people.

You've just watched JoVE's video on zebrafish maintenance and husbandry. We've gone over the essentials of how fish are housed, fed, and managed in the laboratory. Thanks for watching!