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# Mayo Clinic Zebrafish Facility Overview

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## Abstract

The zebrafish (*Danio rerio*) is a premier nonmammalian vertebrate model organism. This small aquatic fish is utilized in multiple disciplines in the Mayo Clinic community and by many laboratories around the world because of its biological similarity to humans, its advanced molecular genetics, the elucidation of its genome sequence, and the ever-expanding and outstanding new biological tools now available to the zebrafish researcher. The Mayo Clinic Zebrafish Facility (MCZF) houses ~2,000 tanks annotated using an in-house, Internet cloud-based bar-coding system tied to our established zfishbook.org web infrastructure. *Paramecia* are the primary food source for larval fish rearing, using a simplified culture protocol described herein. The MCZF supports the specific ongoing research in a variety of laboratories, while also serving as a local hub for new scientists as they learn to tap into the potential of this model system for understanding normal development, disease, and as models of health.

## Introduction

The Mayo Clinic Zebrafish Facility (MCZF; Rochester, MN) was established in 2007 and supports the specific ongoing research in a variety of laboratories from cancer biology, addiction science, regenerative medicine, individualized medicine, angiogenesis, heart disease, muscle regeneration, mitochondrial biology, polycystic kidney disease, and other human health and basic science applications. The MCZF also serves as a critical hub for new scientists as they learn to tap into the potential of this model system for understanding normal development, disease progression, and as models of health. Through the use of advanced zebrafish genetic tools such as protein trap transposons,[1] TALENs,[2] and other tools such as CRISPR/Cas9 (as reviewed in Peng *et al.*[3]), the MCZF houses more than 500 living distinct genetic strains while maintaining a rich archive through sperm cryopreservation.

## Administrative Oversight

The MCZF has a faculty oversight committee that helps with strategic planning, equipment design, and cost recovery. Tank charges are developed from a 3-year expenditure average and have been implemented on 3-year financial cycles designed to cover direct costs associated with running this facility. The MCZF has achieved nearly complete paid occupancy from externally funded research projects. The primary source of this support is from the National Institutes of Health, but some projects are funded by external grants from private foundations, and two projects are supported by a benefactor's gift to the Mayo Clinic.

## MCZF Aquatic Housing Facility

At any given time, the MCZF has ~2,100 active fish tanks with about 53,000 fish in an ~1030-square feet room. Typical usage distribution that can vary includes 231 small (1.4 L), 1680 medium (2.8 L), and 171 large (9.5 L) capacities. Fish are maintained at a target of ≤8 adults/l density. The nominal room temperature is 28°C and is actively regulated through supplemental heaters in the ceiling to address the wide range of temperature and humidity in the four-season local environment in Minnesota, USA. The ambient air temperature is used to control the water temperature, which day to day remains stable, but over the course of a year the water temperature ranges from 26°C to 30°C.

Access to the MCZF main housing room is controlled by a magnetic ID card scanner system. To gain access to the MCZF, individuals are required to complete a regular full institutional IACUC training module that must be refreshed every 3 years, be on an approved IACUC protocol, and to have completed MCZF training provided by MCZF staff. The latter training covers the location of materials, use of the zfishbook database, proper procedures, and where to access Standard Operating Procedure (SOP) descriptions, and tank owner expectations and responsibilities. There are also informational ''Zig-Zag'' zebrafish interest group science meetings held every 2 weeks with formal presentations by researchers conducting their experiments using the zebrafish model system.

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### Water Systems

The MCZF uses two separate and parallel recirculating water systems (numbers 1 and 2) housed in an adjacent room to reduce noise in the main housing area. The source water is from a reverse osmosis filtered well water system capable of producing up to 1000 L/day. The system water chemistry is monitored and recorded daily for pH, conductivity, and temperature. In the past 8 years, the water system has been extraordinarily stable for pH, with only modest variances from a stable pH around 7.9. Conductivity can range between 450 and 1000 $\mu$S but is usually about 630 $\mu$S. A SCADA 3000 monitors the feed from the system sump probes and can activate chemical pumps as necessary to make adjustments for pH and conductivity. The nursery system is split between the two main systems (see hereunder for larval rearing protocol).

The water recovery path on the main systems involves water flow through the fish tanks down into a gutter and then down a downspout through a gravity flow arrangement. A distributing rod at the base of the downspout spreads the water out over the surface of a fabric prefilter through which the water percolates and collects into rack sumps. These rack sumps are all connected through pipes that then enable the water to be actively drawn out. A pump sends the water to the bottom of a fluidized bed biofilter (FBB) where bacteria convert ammonia to nitrite and nitrate. The surface water of the FBB is then diverted into a system sump where the water chemistry is monitored. A different pump draws this water from the system sump and pumps it through sand filters and then through two 200-watt UV sterilizers before the water is distributed back to the racks. Flow to the tanks is maintained at $\sim$0.14–0.32 L/min.

### Quarantine

All adult animals from outside sources are required to be housed in the designated room-within-a-room quarantine area with separately marked fish housing (tanks) and supplies (i.e., mating tanks and nets). These two freestanding quarantine systems recirculate the water within their ''bookcase'' units and receive water (one-way only) from the main water system (number 2). Only bleached embryos from outside facilities or the adults mentioned are then moved to start strains on the main water system.

### Health/Pathology

The tanks are visually examined daily for dead fish. Any fish deaths are reported through zfishbook that generates individualized emails to the corresponding tank owners. If an excessive number of dead fish is reported, automated emails are sent alerting MCZF staff. As deemed necessary by MCZF staff or users, specimens are sent out to ZIRC or our local, on-site health-monitoring laboratory for pathogen testing.

### Feeding Overview

The larval fish are moved from incubators to the nursery system between 4 and 6 days postfertilization (dpf) and are fed *Paramecium* sp. at a rate of 1 mL per larva ($\sim$300–600 paramicia/mL) twice a day during the week and once a day on weekends through 14 dpf. Beginning on 10 dpf, the larvae are also fed *Artemia* sp. twice a day every day to provide a 4-day transition period. At 14 dpf, the water flow to the baby tank is turned on for the first time by drip. The baby zebrafish are transferred from the nursery section to the main system between 6 and 8 weeks postfertilization.

Juvenile and adult fish (up to 18 months of age): The feeding regime consists of the 15 dpf and older fish being fed hatched *Artemia* sp. twice a day every day. During the week, these 15 dpf and older fish are also given supplemental feeding once a day rotating between Tetra flakes and Ziegler diet. Under these conditions, the fish typically mature between 3 and 4 months, but the fish density within the tank can influence the maturation period. To make the fish more robust for regular embryo production, some principal investigators also choose to have an additional supplemental feeding that uses the Ziegler diet and is conducted during the regular work week for fish in their designated tanks. Normally, the fish are on the main system until 18 months post-fertilization, unless the institutional animal care committee has approved an older maintenance age based on specific protocol needs.

### Facility and Animal Inventory Management

zfishbook.org (Clark *et al.*[4] describes the initial platform) is an Internet-based ''cloud'' solution for most fish strain inventory maintenance and for MCZF husbandry and regulatory requirements. The zfishbook database automatically alerts tank owners when their fish are ready to be taken off the nursery or whether their fish are more than 18 months old. The database uses a barcode system on both tanks and rack locations to track and record information on tank location, fish mortality, and to split or merge tanks. Real-time mobile access to the database is provided through a professional barcode scanner connected to a local Mac in the MCZF, or from an iOS app that can run on either a personal iOS device or a MCZF-provided iPod Touch. The iOS application is able to interpret the rack and tank barcode information from a captured image directly on the mobile device; the interpreted barcode number is the only information stored on the iOS device as this is necessary to perform the user's desired function. This central database is also used for automated monthly billing for cost recovery.

### Sperm Cryopreservation

The MCZF has a full archive of cryopreserved sperm, with the majority of inventory from protein trap lines.[1] Sperm samples were initially collected using a modified version of the large-scale protocol for N-ethyl-N-nitrosourea mutant archiving,[5] adding a 2D-barcoded cryo tube and reader for integration with zfishbook.org. Recently, the MCZF has switched to a new protocol (Jen Matthews and Zoltan Varga, personal communication; available at http://zebrafish.org/documents/protocols.php) that uses reagents with a more stable shelf life. Efficacy data from post-thaw fertilization testing show good or higher and seemingly more consistent sperm cryopreservation success rates than the earlier protocol, although long-term testing is still forthcoming.

### Summary of Larval Rearing Materials and Methods

*Paramecia* are raised in media composed of boiled water from the main fish system, protozoan pellets (# 132360; Carolina Biological), and wheat seeds (# 132425; Carolina

Biological). Multiple containers of these media are made daily and allowed to cool overnight before being inoculated the following day. Once inoculated, the media containers are set aside to culture for a week. A week later, samples of the cultures are then examined under a microscope, and the best culture of *Paramecia* from that day is used to inoculate the next batch of media. The remaining containers of cultured *Paramecia* that are not used to inoculate media and show good counts are set aside until needed. The cultures are rechecked visually under a dissecting scope for quantity and quality of any contamination such as coleps before being harvested for feeding. The harvested cultures are fed to larval zebrafish between 4 and 14 dpf at a rate of 1 mL per larva as noted earlier. The example SOP protocol for MCZF paramecia culture protocol is included in Supplementary Data (Supplementary Data are available online at www.liebertpub.com/zeb).

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## Disclosure Statement

No competing financial interests exist.

## References

1. Clark KJ, Balciunas D, Pogoda HM, Ding Y, Westcot SE, Bedell VM, *et al.* In vivo protein trapping produces a functional expression codex of the vertebrate proteome. Nat Methods 2011;8:506–515.
2. Bedell VM, Wang Y, Campbell JM, Poshusta TL, Starker CG, Krug RG, 2nd, *et al.* In vivo genome editing using a high-efficiency TALEN system. Nature 2012;491:114–118.
3. Peng Y, Clark KJ, Campbell JM, Panetta MR, Guo Y, Ekker SC. Making designer mutants in model organisms. Development 2014;141:4042–4054.
4. Clark KJ, Argue DP, Petzold AM, Ekker SC. zfishbook: connecting you to a world of zebrafish revertible mutants. Nucleic Acids Res 2012;40:D907–D911.
5. Draper BW, Moens CB. A high-throughput method for zebrafish sperm cryopreservation and in vitro fertilization. J Vis Exp 2009:pii: 1395.

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