

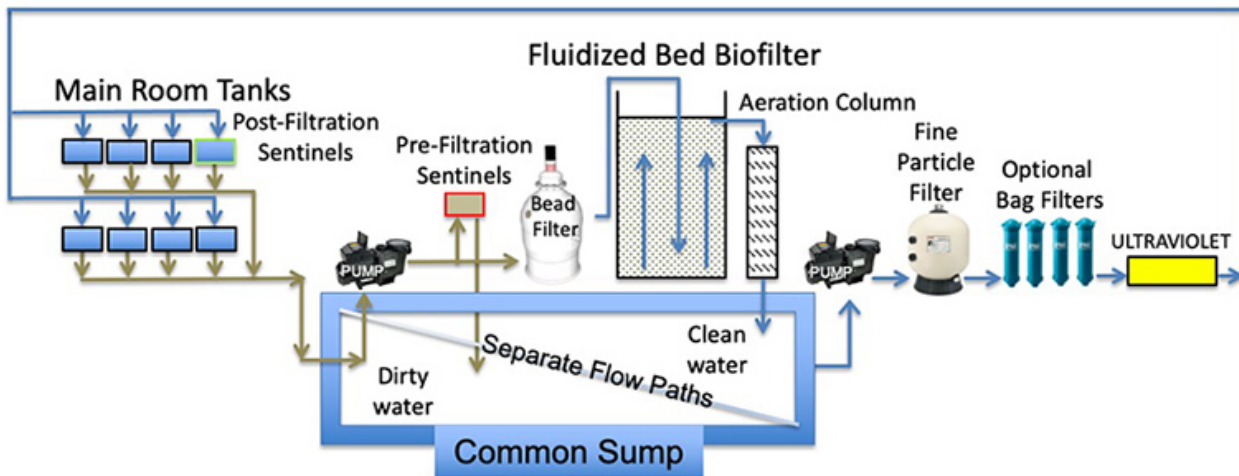


The Animal Health Report provides an overview of health monitoring, diagnostic sampling, and test results for zebrafish reared at ZIRC. The ZIRC rears zebrafish for in-house use and for shipment to customers. Some embryos shipped to customers are generated by in vitro fertilization using eggs from AB fish reared at ZIRC and cryopreserved sperm from males not reared at ZIRC. The health status of the males contributing the sperm was not evaluated and neither the embryos nor paternal stocks have been on the ZIRC water system. The ZIRC recommends use of strict quarantine practices for all imported fish; adults and embryos.

Location: ZIRC Main Fish Room

| Quarter | Average population | Average weekly morbidity rate | Average weekly mortality rate |
|---------------|--------------------|-------------------------------|-------------------------------|
| July-Dec 2024 | 33,765 | 0.009% | 0.007% |

Description of water system: From 2006 - 2022, the two sides of the ZIRC fish room received water from separate recirculating systems. As part of a facility remodel that will eventually entail installation of 3 or 4 new water systems, the two water systems were removed and the room receives water from a single new recirculating system since July 2022. The new water system is diagramed below. Water source is reverse osmosis treated municipal water with added salt and aragonite. Water exchange is 10-12% per day. The UV sterilizer delivers a minimum UV dose of 132,000 $\mu\text{Wsec}/\text{cm}^2$.



New fish strains: Only surface-sanitized embryos enter the Main Fish Room. Most new introductions are generated by in vitro fertilization using cryopreserved sperm and eggs from AB females. Occasionally adult fish in the Quarantine Room are spawned and their surface-sanitized embryos moved to the Main Fish Room.

Embryo surface sanitization: All embryos are surface sanitized by immersion in 30 ppm sodium hypochlorite for 10 minutes.

Diagnostic testing:

1. The majority of moribund fish are submitted for histopathology.



2. Wild-type stocks are screened for disease at 8 months of age; a subset of fish is submitted for histopathology or tanks water is tested for *P. neurophilia* by dPCR.
3. A subset of fish from the sentinel source tank is screened for *P. neurophilia* by histopathology or water is tested by dPCR before the stock is used to populate sentinel tanks.
4. Pre and post-filtration sentinel fish are submitted quarterly for histopathology. Sentinel samples represent 6 months and 1 year of exposure to system parameters. One-year-exposure sentinels are sampled every 6 months.
5. The species of *Mycobacterium* is identified by qPCR on frozen fish.

Sentinel fish results:

| Sample Date | July 2025 | |
|--|-----------|---------|
| | Pre- | Post- |
| Location of Fish Relative to Filtration and UV | | |
| Sample Information | 10 Fish | 10 Fish |
| Time in Sentinel Tank | 6 mos. | 6 mos. |
| GROSS PATHOLOGY | Normal | Normal |
| HISTOPATHOLOGY | | |
| Cestode larvae | 0 | 0 |
| Encysted metacercariae (digenetic trematodes) | 0 | 0 |
| Fungal organisms | 0 | 0 |
| Gram-negative bacteria | 0 | 0 |
| <i>Edwardsiella ictaluri</i> | 0 | 0 |
| <i>Ichthyophthirius multifiliis</i> | 0 | 0 |
| <i>Mycobacterium</i> spp.* | 0 | 0 |
| <i>Myxidium streisingeri</i> n. sp. (myxozoa) | 0 | 0 |
| <i>Piscinoodinium</i> sp. | 0 | 0 |
| <i>Pseudocapillaria tomentosa</i> (nematode) | 0 | 0 |
| <i>Pleistophora hypnessobryconis</i> (microsporidia) | 0 | 0 |
| <i>Pseudoloma neurophilia</i> (microsporidia) | 0 | 0 |
| <i>Tetrahymena</i> | 0 | 0 |

Other pathologies observed in this group of sentinel fish include distal gut enteritis and egg-associated inflammation.

****Mycobacterium* spp.**

A single species of *Mycobacterium*, *M. chelonae*, has been identified from zebrafish and biofilms sampled from the ZIRC aquaculture facility (Whipps et al., 2008). We continue to periodically test fish by qPCR for *Mycobacterium*. *M. chelonae* is the only species that has been identified in fish reared in the ZIRC Main Fish Room.

Pathogens detected in fish and water samples:

In last 3 months: *Mycobacterium chelonae*

In last 12 months: *Mycobacterium chelonae*

In last 36 months: *Mycobacterium chelonae*, *Pseudoloma neurophilia* (last detected May 2022)