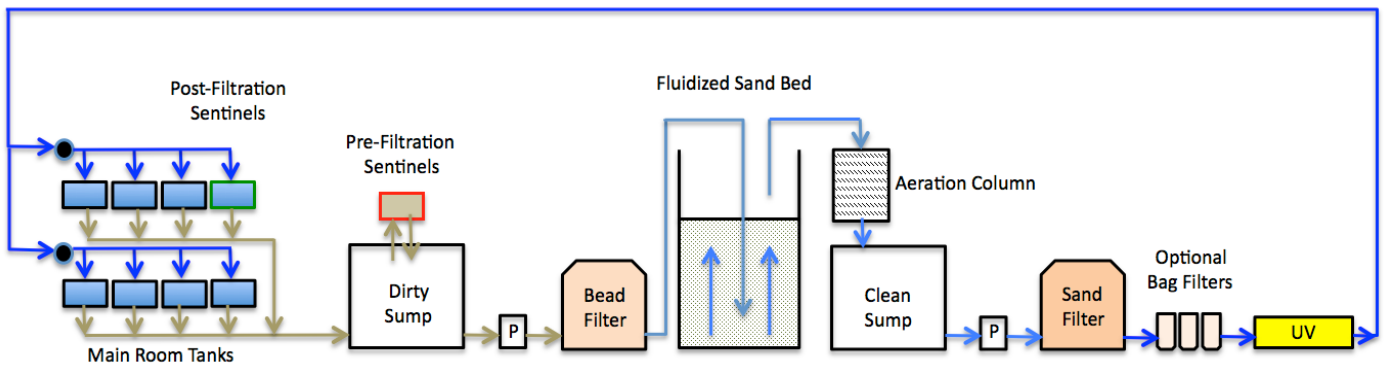




The Animal Health Report provides an overview of health monitoring, diagnostic sampling, and test results for zebrafish reared at ZIRC. The ZIRC rears zebrafish for in-house use and for shipment to customers. Some embryos shipped to customers are generated by in vitro fertilization using eggs from AB 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

Description of water system: Three recirculating water systems supply the Main Fish Room. The room is divided into two sides. Water from two systems is intermingled and feeds side A. A separate water system feeds side B.



Water source is reverse osmosis treated municipal water with added salt and aragonite. 10-12% water exchanged per day.

Bead filter – mechanical and biological filtration

Fluidized sand bed – biological filtration

Sand filter – pressurized sand filter for fine particle filtration

UV sterilizer – minimum UV dose 132,000 $\mu\text{Wsec}/\text{cm}^2$

P = pump

New fish strains: Only surface-sanitized embryos enter the Main Fish Room. The majority of 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. A subset of all 8-month wild-type stocks is submitted for histopathology or PCR for *P. neurophilia*.
3. A subset of retired stocks is submitted for histopathology or PCR for *P. neurophilia*.
4. A subset of fish from the sentinel source tank is screened for *P. neurophilia* by histology or PCR.



5. 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.
6. *Mycobacterium* species is identified by qPCR on frozen fish.

Sentinel fish results:

Sample Date	October 2020			
	Pre-	Pre-	Post-	Post-
Location of Fish Relative to Filtration and UV				
Sample Information	10 Fish	10 Fish	10 Fish	10 Fish
Time in Sentinel Tank	6 mos.	1 year	6 mos.	1 year
GROSS PATHOLOGY	Normal	Normal	Normal	Normal
HISTOPATHOLOGY				
Cestode larvae	0	0	0	0
Encysted metacercariae (digenetic trematodes)	0	0	0	0
Fungal organisms	0	0	0	0
Gram-negative bacteria	0	0	0	0
<i>Edwardsiella ictaluri</i>	0	0	0	0
<i>Ichthyophthirius multifiliis</i>	0	0	0	0
<i>Mycobacterium</i> spp.*	1	0	0	0
<i>Myxidium streisingeri</i> n. sp. (myxozoa)	0	0	0	0
<i>Piscinoodinium</i> sp.	0	0	0	0
<i>Pseudocapillaria tomentosa</i> (nematode)	0	0	0	0
<i>Pleistophora hypheobryconis</i> (microsporidia)	0	0	0	0
<i>Pseudoloma neurophilia</i> (microsporidia)	1	5	0	0
<i>Tetrahymena</i>	0	0	0	0

Noninfectious pathologies observed in sentinel fish include hepatic megalocytosis, egg-associated inflammation, nephrocalcinosis, enteritis, hepatobiliary duct hyperplasia, and ultimobranchial adenoma.

****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 (all fish sampled):

In last 3 months: *Mycobacterium chelonae*, *Pseudoloma neurophilia*

In last 12 months: *Mycobacterium chelonae*, *Pseudoloma neurophilia*

In last 36 months: *Mycobacterium chelonae*, *Pseudoloma neurophilia*