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ZEBRAFISH ENVIRONMENTAL SUMMARY  
as of 31 Dec 2021

STOWERS INSTITUTE FOR MEDICAL RESEARCH

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# Preface

The following is an environmental summary of the Zebrafish Facility at the Stowers Institute for Medical Research for the period indicated. It is a general summary only, not a complete description of all aspects of the program. Specific projects and research needs are not described here. Materials and methods are subject to change. Any descriptions of procedures or methods, or references to products or services, do not constitute an endorsement, recommendation, or advice.

# Macroenvironment

## Main Colony - Room A

### Lighting

Photoperiod: 14 hours light : 10 hours dark with dusk/dawn dimming starting at 0545h.

Lamps: Fluorescent - 6500K color temperature with a Color Rendering Index (CRI) of 90 replaced on a two-year rotating schedule.

## Quarantine - Room B

### Lighting

Photoperiod: 14 hours light : 10 hours dark with dusk/dawn dimming provided by fluorescent lamps starting at 0730h.

Lamps: Fluorescent - 6500K color temperature with a Color Rendering Index (CRI) of 90 replaced on a two-year rotating schedule.

# Microenvironment Water Quality

## Acceptable Water Quality

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Ideal** | **Acceptable Range** | **Monitoring Frequency** |
| Temperature | 28.5°C | 27.0 – 29.5°C | Daily |
| Salinity[[1]](#footnote-2) | 0.2 ppt | 0.2 - 0.4 ppt | Daily |
| Specific Conductance[[2]](#footnote-3) | 500 µS/cm | 400 – 700 µS/cm | Daily |
| pH | 7.6 | 7.2 - 7.8 | Daily |
| Dissolved Oxygen[[3]](#footnote-4) | 100% | 85 - 101% | Daily |
| Total Gas Pressure[[4]](#footnote-5) | 100% | 98 - 101% | Daily |
| Total Ammonia Nitrogen | 0 mg/L | < 0.5 mg/L | Weekly |
| Nitrite | 0 mg/L | < 0.5 mg/L | Weekly |
| Nitrate | 0 mg/L | < 40 mg/L | Weekly |
| Alkalinity | in range | 10 – 120 mg/L as CaCO3 | Weekly |
| General Hardness | in range | 20 – 150 mg/L as CaCO3 | Weekly |
| Copper | 0 mg/L | 0 mg/L | Monthly |
| Free Chlorine | 0 mg/L | 0 mg/L | Monthly |
| Total Chlorine | 0 mg/L | 0 mg/L | Monthly |
| Carbon Dioxide | in range | < 1 mg/L | As Needed |

## Online Monitoring and Control[[5]](#footnote-6)

|  |  |
| --- | --- |
| **Parameter** | **Setpoint** |
| pH | 7.6 |
| Specific Conductance | 500 µS/cm |
| Temperature | 28.5°C |
| TGP – Total Gas Pressure | System shutdown when exceeds 103%. |

## 

## Biannual Monitoring[[6]](#footnote-7)

|  |  |
| --- | --- |
| **Parameters** | **Method** |
| Pesticides | EPA 8081B |
| Organophosphorus Pesticides | EPA 8141B |
| Chlorinated Herbicides | EPA 8151A |
| Metals, Total | EPA 200.7 |
| Mercury | EPA 245.1 |
| Semivolatile Organics | EPA 625.1 |
| Volatile Organics | EPA 624.1 |

Analytes reported may not reflect the entirety of the method.

## Chemical Dosing Stock Solutions[[7]](#footnote-8)

pH: 16 g/L Sodium Bicarbonate USP No. 1 Powdered (Church & Dwight Co., Inc.)

Conductivity: 50 g/L Instant Ocean sea salt (Spectrum Brands, Inc.)

# Life Support Systems (LSS)

Aquaculture Type: Recirculating, Group Housing

Manufacturer: Pentair Aquatic Eco-Systems, Inc.

Standard 1X Stocking Density: 12 fish per 1.5 L tank; 25 fish per 3 L tank

Tanks: Clear polycarbonate tank with blue tinted polycarbonate baffle and lid. 1.5 L, 3 L

Tank Rotation Frequency: 4 months

## Zebrafish System 1

Location: Main Colony - Room A

In-service: 2008

### System Size

# Racks: 15 with 6 shelves per rack

Tank Capacity: 20 – 1.5 L tanks per shelf or 12 – 3 L tanks per shelf

600 – 1.5 L tanks and 720 – 3 L tanks in Base Configuration

Fish Capacity: up to 25,200 in Base Configuration not including pre-filtration sentinels

Volume: ~7,689 L in Base Configuration not including pre-filtration sentinels

### Filtration

#### Mechanical

Coarse and 100 µm fine polyester prefilter pads replaced as needed.

50 µm polyester sock filter replaced as needed.

#### Chemical

10 L Seachem MatrixCarbon (Seachem Laboratories) replaced every three months.

#### Biological

Moving bed biofilm reactor using Kaldnes K1 Media (Evolution Aqua Ltd.). Aerated by Sweetwater regenerative blower model S-41 (Pentair Aquatic Eco-Systems, Inc.).

#### Ultraviolet

Two model 03AS20 TrojanUVLogic Series units in series (Trojan Technologies Group ULC); fluence minimum 200 mJ/cm2 at end of lamp life.

### Water Quality

Source Water: Municipal source water purified by reverse osmosis and deionization.

Water Exchange: 15 - 20% by volume per day

#### Water Quality Equipment

YSI 5200A (YSI Incorporated) for primary dosing control of pH, conductivity (specific conductance), and temperature.

Heaters: Two - Process Technology controller DRA302-X with 3500 watt immersion heater (Process Technology). Controlled by YSI 5200A permissive signal.

### Sentinel Housing

Up to three 3 L tanks can be added to the pre-filtration segment of the system (prior to the principal mechanical, chemical, and UV filtration) to house purpose-bred fish for health diagnostic purposes.

## Zebrafish System 2

Location: Main Colony - Room A

In-service: 2011 (originally with 9 racks)

### System Size

# Racks: 24 with 6 shelves per rack

Tank Capacity: 20 – 1.5 L tanks per shelf or 12 – 3 L tanks per shelf

960 – 1.5 L tanks and 1152 – 3 L tanks in Base Configuration

Fish Capacity: up to 40,320 in Base Configuration not including pre-filtration sentinels

Volume: ~10,059 L in Base Configuration not including pre-filtration sentinels

### Filtration

#### Mechanical

FAIVRE 60 Series drum filters with HDPE tanks (FAIVRE Ets.), 63 µm screen drum filter followed by a 36 µm screen drum filter.

#### Chemical

Carbon filter side loop used as needed. Contains up to ~170 L Seachem MatrixCarbon (Seachem Laboratories, Inc.) replaced biannually when in service. Housed in Legacy Fiberglass Sand Filter model 36CCFG (Neptune Benson – Evoqua Water Technologies LLC) with ~57 liters ¼” x 1/8” filter gravel bed. Automatic backwash three times weekly with Aquastar Comfort 6000 (Praher Plastics Austria GmbH) automated backwash valve when in service.

#### Biological

Moving bed biofilm reactor using Kaldnes K1 Media (Evolution Aqua Ltd.). Aerated by Sweetwater regenerative blower model S-41 (Pentair Aquatic Eco-Systems, Inc.).

#### Ultraviolet

Two model 06AS20 TrojanUVLogic Series units in series (Trojan Technologies Group ULC); fluence minimum 200 mJ/cm2 at end of lamp life.

### Water Quality

Source Water: Municipal source water purified by reverse osmosis and deionization then pre-dosed to ~7.1 pH and ~400 µS/cm specific conductance.

Water Exchange: 30% by volume per day

#### Water Quality Equipment

Vendor customized Programmable Logic Controller (PLC) for monitoring and control of pH, conductivity (specific conductance), and temperature.

Heaters: Two - Process Technology controller DRA302-0513 with 5000 watt immersion heater (Process Technology). Controlled by PLC permissive signal.

Chiller: Aqua Logic Multi Temp MT-5 Air Cooled Chiller (Aqua Logic Incorporated) on side loop. Controlled by PLC permissive signal.

### Sentinel Housing

Up to nine 3 L tanks can be added to the pre-filtration segment of the system to house purpose-bred fish for health diagnostic purposes.

## Quarantine Systems

Location: Quarantine - Room B

Two stand-alone LSSs (Quarantine System A and Quarantine System B). The description below applies to each system individually.

### System Size

# Racks: 1 with 5 shelves per rack

Tank Capacity: 20 – 1.5 L tanks per shelf or 12 – 3 L tanks per shelf

40 – 1.5 L tanks and 36 – 3 L tanks in Base Configuration

Fish Capacity: up to 1,380 fish in Base Configuration

Volume: ~243 L in Base Configuration

### Filtration

#### Mechanical

Coarse and 100 µm fine polyester prefilter pads replaced as needed.

50 µm Harmsco 801-50 pleated cartridge filter (Harmsco, Inc.) replaced at least monthly.

#### Chemical

~800 mL Seachem MatrixCarbon (Seachem Laboratories) replaced bimonthly.

#### Biological

Moving bed biofilm reactor using Kaldnes K1 Media (Evolution Aqua Ltd.). Aerated by Sweetwater SL22 linear piston air pump (Pentair Aquatic Eco-Systems, Inc.).

#### Ultraviolet

Two 50 watt Emperor Aquatics SMART UV High-Output Sterilizers (Pentair Aquatic Eco-Systems, Inc.) in parallel; fluence estimated to be 170-196 mJ/cm2 at end of lamp life.

### Water Quality

Source Water: Zebrafish System 1 or Zebrafish System 2

Water Exchange: >1X volume per day

#### Water Quality Controls

Heater: Local controller with 700 watt immersion heater (Process Technology).

#### Water Quality Parameters

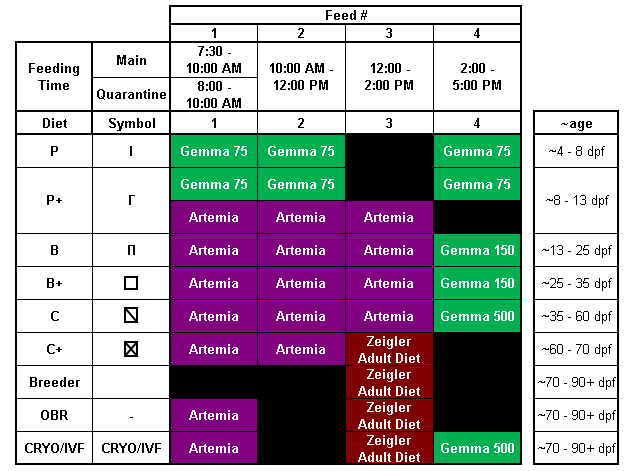
Water quality provided through high water exchange rate. Water quality target is same as the source system.

##### Online Control

|  |  |
| --- | --- |
| **Parameter** | **Setpoint** |
| Temperature | 28°C |

# Feeding

## Feeding Chart



## Feed Information

### Brine Shrimp Direct Premium Grade Brine Shrimp Eggs

<https://www.brineshrimpdirect.com/brine-shrimp-eggs/premium-grade-brine-shrimp-eggs/> (accessed 10 Oct 2021)

This product is decapsulated on-site and stored in a hypersaline solution prior to hatching. The Artemia is hatched and fed out at Instar I.

Nutrient Profile from the manufacturer:

<https://www.brineshrimpdirect.com/about-us/frequently-asked-questions/do-you-have-any-analysis-information-brine-shrimp/> (accessed 10 Oct 2021)

### Skretting Gemma Micro

<https://zebrafish.skrettingusa.com/collections/all> (accessed 10 Oct 2021)

Sizes: 75, 150, and 500 microns

### Zeigler Adult Zebrafish Diet

<https://www.zeiglerfeed.com/research-diets/adult-zebrafish-diet/> (accessed 10 Oct 2021)

<https://www.zeiglerfeed.com/Literature/Adult%20Zebrafish%20Diet.pdf> (accessed 10 Oct 2021)

# Breeding

## Natural Breeding

Fish are setup in breeder cages with fish system water, generally in the early to mid-afternoon, the day before embryos are needed. A divider may be used to separate females and males if spawning needs to be timed. The next morning, any dividers are pulled and cages without an internal gradient are tilted to provide a gradient to facilitate breeding.

## In Vitro Fertilization (IVF)

For priming, female fish are setup in breeder cages with male fish separated by a divider the day before ova are needed. For male fish, males may be held alone or primed with females and separated by a divider. The next day, fish are anesthetized to collect gametes within the first few hours of lights on in the facility.

## Breeder Cage Equipment

2-liter breeder cage (Aquaneering, Inc., ZHCT200)

2-liter breeder cage (Pentair Aquatic Eco-Systems, Inc., BREEDER TANK-2)

1-liter breeder cage with internal gradient (Pentair Aquatic Eco-Systems, Inc., SBTANK)

Tecniplast 1.7-liter Breeding Tank – Beach Style Design (with internal gradient) (Tecniplast USA)

# Embryo Care

Embryos are generally held in single-use 100 mm x 15 mm plastic Petri dishes with 0.5 X E2 Embryo Media without methylene blue. (Zebrafish International Resource Center, <https://zebrafish.org/wiki/_media/protocols/nursery/e2_solution.pdf> accessed 10 Oct 2021). Use of methylene blue is optional.

Embryo dishes are cleaned at 1 dpf and 3 dpf (days post fertilization). Fry rearing on-system typically begins at 5 dpf but may be as early as 4 dpf or as late as 8 dpf depending upon the research project.

## Main Room Use Incubators

Embryos are incubated at 28.5°C in diurnal incubators (Sanyo Electric Co. Ltd., MIR-554) with a photoperiod of 14 hours light : 10 hours dark by fluorescent or LED lamps starting at 0600h each day.

## Quarantine Incubator

Embryos are incubated at 28.5°C (Jeio Tech, ILP-02). Photoperiod is provided by the animal room lights by leaving the outer door open and the inner, transparent door closed.

# Health

## Daily Health Observations

All fish are observed at least once daily for health. The following table is used as a guide to identify behavioral or physical abnormalities that may indicate a health issue.

|  |  |
| --- | --- |
| **Behavioral Abnormalities** | **Physical Abnormalities** |
| * Lethargy (drifting with the current; lying on bottom; sluggish movements) * Remaining at water’s surface or water inlet * Decreased appetite * Abnormal equilibrium (loss of buoyancy control; swimming with either head or tail pointed down sharply) * Circling, twirling, or spinning * Abnormal respiration (unusually rapid, slow, or erratic opercular movements) * Piping (‘gasping for air’ at the water’s surface) * Flashing (rubbing and/or bumping body on tank surfaces) * Darting (lack of schooling/shoaling behavior; a single fish moving quickly and/or erratically) | * Color change or paleness * Weight loss or abnormal weight gain * Slow growth rate (e.g., not keeping up with cohorts) * Exophthalmia (bulging eye) * Distended abdomen * Skeletal deformity * Visible mass or swelling * Redness, bruising, or bleeding * Discharge * Gas bubbles on/in the skin * Bristling (protruding scales) * Fin erosion or lesion (ragged fins or holes in fins) * Skin ulceration or lesion (open sores; missing scales) * Excess mucus (thickening or discoloration of mucus layer of skin) * Foreign organism |

## 

## Sentinel Health Monitoring

Purpose-bred fish are housed in the pre- and post-filtration segments of the main room LSSs to monitor for pathogens in the colony. Fish are sent to a diagnostic lab for PCR and histological evaluation at 9 months of age.

# Cage Wash

## Hand Wash - Bleach Method

1. Scrub to remove gross detritus (e.g., algae, biofilm).
2. Minimum 5-minute soak in bleach solution: 44.78 mL/L bleach (6% sodium hypochlorite) - yields 0.27% sodium hypochlorite working solution.
3. Dip or soak in neutralizer solution (3.674 mM sodium thiosulfate).
4. Rinse: Flood rinse in reverse osmosis deionized water, 1X volume turnover.

Bleach Chemical Source: Pure Bright Germicidal Ultra Bleach (6% sodium hypochlorite) (KIK International LLC)

Neutralizer Chemical Source: Sodium thiosulfate (anhydrous or pentahydrate) (Wintersun Chemical)

## Tecniplast Calypso Aquatic Cabinet Washer

Model: Calypso 9CALY (Tecniplast USA)

### Breeder Cages – No Chemical Wash Recipe

1. 120 second pre-wash with soft tap water or the previous cycle’s final rinse reverse osmosis deionized water.
2. 600 second wash with soft tap water at 75°C.
3. 60 second rinse with soft tap water.
4. 60 second final rinse with reverse osmosis deionized water.

### Tanks and Other Items – Chemical Wash Recipe

1. 120 second pre-wash with soft tap water or the previous cycle’s final rinse reverse osmosis deionized water.
2. 900 second wash with 0.9% working solution CLOUT-AQUA (Pharmacal Research Laboratories, Inc.) and 0.7% working solution OXY-AQUA (Pharmacal Research Laboratories, Inc.) in soft tap water at 75°C.
3. 60 second neutralizer wash with 0.05% URID-AQUA (Pharmacal Research Laboratories, Inc.) in soft tap water.
4. 60 second rinse with soft tap water.
5. 60 second final rinse with reverse osmosis deionized water.

## Microbiological Testing

Monthly validation with FireFly 2 and PocketSwab Plus (Charm Sciences, Inc.) ATP (adenosine triphosphate) assay swabs.

Pass: < 500 RLU (relative light units)

Fail: ≥500 RLU (relative light units); item will be swabbed for culturing on LB plate (35°C for 5 days)

1. Salinity may be derived from specific conductance instead of direct monitoring. [↑](#footnote-ref-2)
2. Specific conductance is derived from conductivity temperature compensated to 25°C. [↑](#footnote-ref-3)
3. Dissolved oxygen is monitored only in Quarantine LSSs. [↑](#footnote-ref-4)
4. Total Gas Pressure is not monitored in Quarantine LSSs. [↑](#footnote-ref-5)
5. Not applicable to Quarantine LSSs. [↑](#footnote-ref-6)
6. Not directly applicable to Quarantine LSSs. [↑](#footnote-ref-7)
7. Not directly applicable to Quarantine LSSs. [↑](#footnote-ref-8)