



ANIMAL WELFARE ASSESSMENT CONTEST 2019



Zebrafish Scenario

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NOTES

- 1. THESE ARE FICTITIOUS FACILITIES.** They have been created using pictures and information collected from multiple sites, as well as realistic, yet fabricated information. ***Neither facility actually exists as presented here.***
- 2. Please use the images and videos provided throughout the presentation as well as text when making your assessment.**

Overview

Facility Z



- 2-year old core facility for zebrafish used in research across the university
 - Research spans areas ranging from neurobiology and cardiology to environmental toxicology and aquatic biology
 - The facility is AALAC accredited
- ~100,000 adult fish are typically housed in the facility
 - Population consists of a wide variety of wild type, mutant and transgenic lines
- 6 rooms are used to house fish
 - One room is a dedicated nursery
 - Separate rooms are dedicated to imaging and screening of zebrafish
 - An additional room is reserved for quarantine

Facility F



- 15-year old facility is located in a departmental building that contains faculty offices, classrooms, and bench laboratories
 - Research conducted on embryological development
 - The facility is AALAC accredited
- 2,000 adult fish are typically housed in the facility
 - Population is mainly wild type zebrafish
 - A small number of tanks of a few transgenic and mutant lines are maintained for specific research projects
- 1 room houses the fish
 - Separate areas on the lab benches in the room are dedicated as nursery and quarantine areas

Facility Z



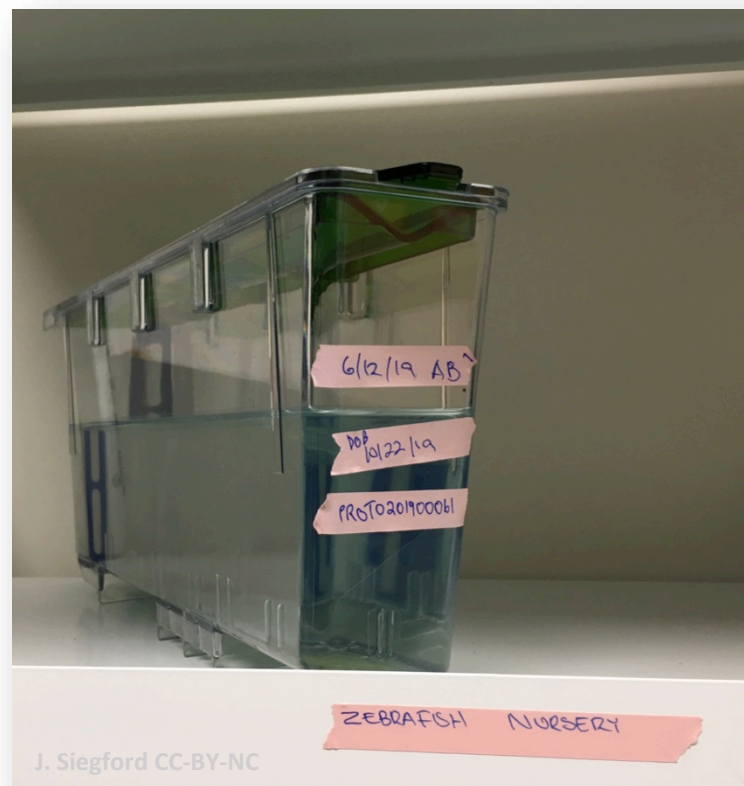
Facility F



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Personnel

Facility Z

- Overseen by university director of animal care services
 - >25 years zebrafish experience
 - Internationally acknowledged zebrafish research expert
- Facility has a dedicated attending veterinarian
 - Board certified in laboratory animal medicine
 - Has worked with zebrafish for 20 years
- An animal facility manager oversees daily operations
- 4 full-time technicians provide care to fish
- All personnel completed general animal care and use training and specific training related to care of zebrafish
 - All are encouraged to participate in continuing education related to use of animals in research and do annual refreshers
- Director and attending veterinarian participate in zebrafish research
 - Attend and present at zebrafish care and research meetings
 - Subscribe to Zebrafish journal and are part of Zebrafish Information Network

Facility F

- Overseen by university attending veterinarian
 - Board certified in laboratory animal medicine
 - Has particular expertise with avian species
 - Joined the university 5 years ago
- Facility principle investigator (PI) has used zebrafish as a model for > 15 years
- A technician cares for the fish room, a mouse room as well as conducts experiments in the PI's lab
 - Technician was trained by the PI
 - ~4 years of zebrafish experience
- A postdoc and graduate student assist with fish care
 - Postdoc was trained in a previous lab, 3 total years of fish experience
 - Graduate student was trained by technician, 3 months of experience
- All personnel completed general animal care and use training as well as a fish care module
 - Annual refresher training is not required

Lighting & Temperature

Facility Z

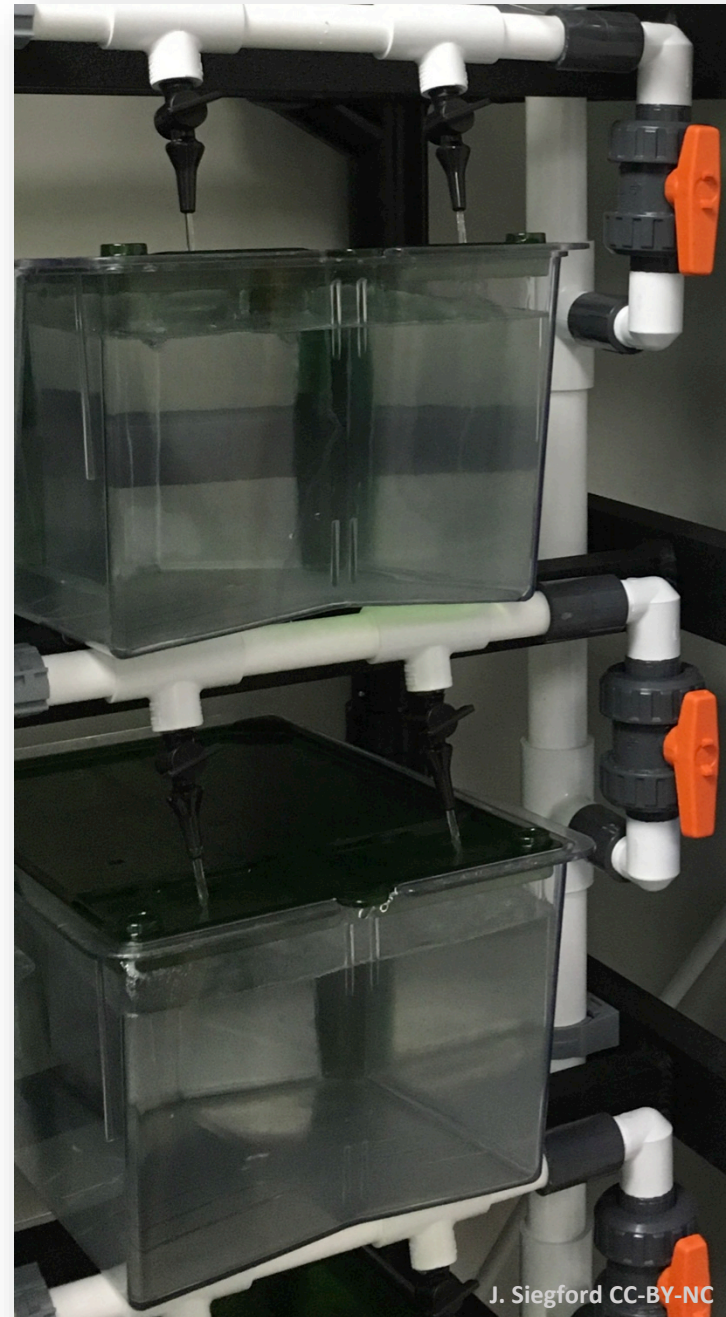
- 14 hours of light/day starting at 8:00am
 - Light source = multiple overhead LED lights, one in front of each rack of tanks
 - Light intensity ranges from 300 lux at back of lower tanks to 500 at front of upper tanks
 - Digital timer controls lights
 - Lights gradually brighten in morning and dim in evening over 30 minute periods
 - System sends alerts to technicians' cell phones if electricity, timer or lights fail
- Average tank temperature = 28.5C
 - Temperature is maintained by a combination of room temperature and heater in reservoir
 - Temperatures is monitored continuously using sensors in reservoir and in lines leading to tanks

Facility F

- 14 hours of light/day starting at 9:30am
 - Light source = one bank of overhead fluorescent lights in center of room
 - Light intensity ranges from 135 lux at back of lower tanks to 300 in front of upper tanks
 - Analog timer turns lights on and off
 - On at least 2 recent occasions, lights have been off in the morning when the technician has come in to check and feed fish
- Average tank temperature = 27C
 - Temperature is maintained by a combination of room temperature and heater in reservoir
 - Temperature is monitored using thermometers in tanks and reservoir

Housing—Facility Z

- Fish are housed in 2 L or 4 L polycarbonate tanks
 - Transparent green lids, no screens
 - Tank bottom is clear
 - Water flows in via slots in front of lid
 - Flow controlled at level of tank and row
 - Water flows out via channel in tank back
 - Channel is covered with perforated insert
- Stocked with 10 fish/L
- Fish are housed in single-sex groups, unless research dictates otherwise



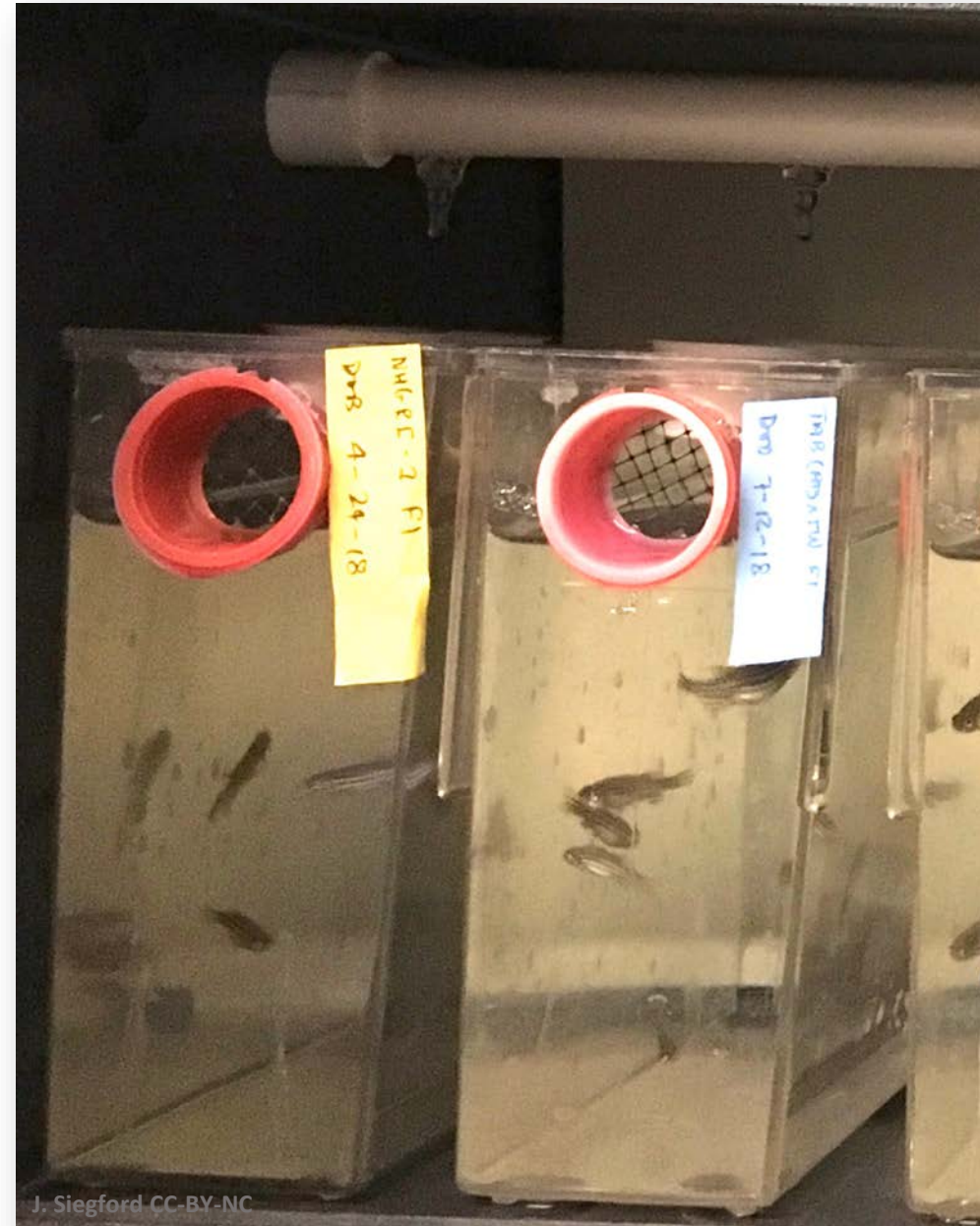
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Housing—Facility F

- Fish are housed in 2 L polycarbonate tanks
 - Polycarbonate covers with mesh screens
 - Tanks sit on black shelves
 - Water flows in through openings in cover
 - Flow controlled at level of row
 - Water flows out via port in tank front
 - Port covered by mesh screen
 - Tanks tested for BPA leaching (none)
- Maximum of 8 fish per tank
- Fish are kept in mixed sex groups (roughly 1:1)



Nutrition & Feeding—Facility Z

- Fed various combinations of dry salmon starter, *Spirulina* and live brine shrimp (*Artemia*) at each meal
- On weekdays, fish are fed 2x a day (8:30am and 5:00pm)
 - *Artemia* only in the morning
 - Salmon starter and *Spirulina* in evening
- On weekends, fish are fed 1x a day
 - Fish receive a mix of salmon starter, *Spirulina* and *Artemia*
 - Fish are given roughly twice as much as they would receive in a single weekday feeding
- Feed is poured onto front of tank lid
 - It trickles slowly into tank, carried by water flowing into tank by the same route
- Fish are observed to see if they move to the top half of the tank to start feeding

Cones used to grow *Artemia*



Spirulina powder



Salmon starter (magnified)

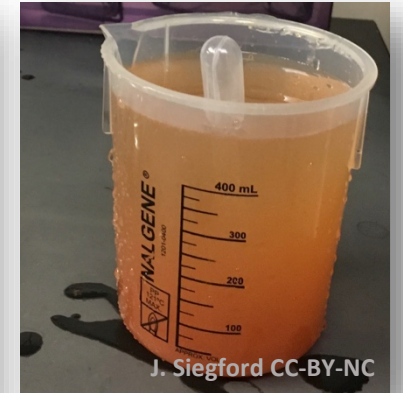
Nutrition & Feeding—Facility F

- Fish are fed exclusively on brine shrimp (*Artemia*)
- On weekdays, fish are fed 2x a day (10:00am and 6:30pm)
- On weekends, fish are fed 1x a day
 - Fish are given roughly twice as much as they would receive in a single weekday feeding
- Feed is squirted into tank via outflow port using disposable pipettes
 - Fish are observed to see if they move towards front of tank to eat



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Cone used to grow *Artemia*



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Observation

Facility Z

- During the week, technicians check the fish 2x/day during feedings
- On the weekend, checks are done 1x/day
 - Technicians rotate weekend duty
- Personnel work through the banks of tanks in each room as they feed fish
 - Examine tanks and lines for leaks
 - Check water flow into tanks
 - Check fish for signs of abnormal movement or appearance
- Dead fish are removed and recorded
- Fish that are obviously ill are removed, euthanized and recorded

Facility F

- During the week, the technician, postdoc or graduate student checks fish 2x/day during feedings
- On weekends, checks are done 1x/day
 - Graduate student usually does the checks
- Personnel work slowly through the bank of tanks in a systematic way
 - Examine tanks for signs of algal build up or left over food, check outlets and mesh for debris/fish trapped
 - Examine fish in tanks for mortalities, signs of abnormal behavior (i.e., weak swimming, floating near surface, tilted swimming) or injury (i.e., tail bites due to aggression) or illness (i.e., signs of fin rot or egg binding)
- Dead fish are removed and recorded
- Fish exhibiting poor health (injury, weakness, etc.) are removed from the system, euthanized and recorded

Facility Z—Fish during observation



Facility F—Fish during feeding



Veterinary Care & Oversight

Facility Z

- Facility veterinarian routinely visits the fish rooms with technicians
- Animal care team meets weekly to discuss colony status and plan for projects
- Twice a year, fish from each bank of tanks are randomly culled and sent for pathological screening
 - Fish are routinely screened for *mycobacterium* and *P. neurophilia* and a set of other common zebrafish infectious diseases

Facility F

- Attending veterinarian is contacted in emergency situations where a large number of fish are exhibiting problems
 - Has not occurred in the past 3 years
- PI and animal care team meets regularly to discuss fish and research in the lab
- Fish mortalities or biopsies are not routinely sent for screening

Water Monitoring & Management

Facility Z

- A closed, recirculating system is used to provide conditioned water to fish
 - Each room has a separate system
- Ammonia, salinity, and pH are monitored constantly by inline system
- Twice a week pH, ammonia, nitrates and nitrites are manually tested
 - Information is recorded in log book
- Chlorine is tested monthly
- Each day, 15% of water is replaced

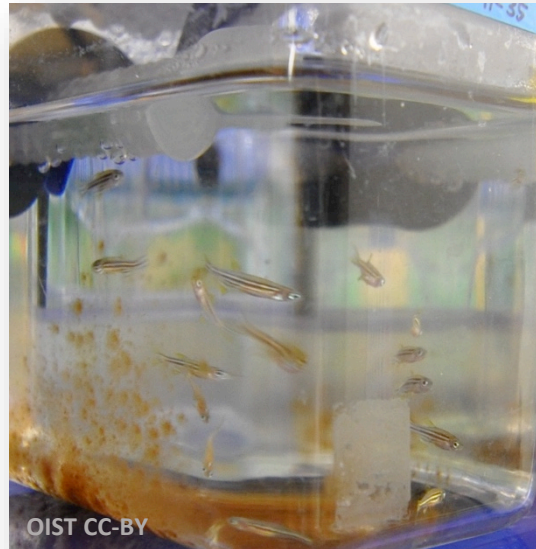
Facility F

- A closed, recirculating system is used to provide conditioned water to fish
- Ammonia, salinity, nitrates and pH are manually tested twice daily
 - Information is recorded in log book
- Each day, 10% of water is replaced and particulate filters cleaned
- Each week the entire system and all filters are cleaned and checked

Cleaning

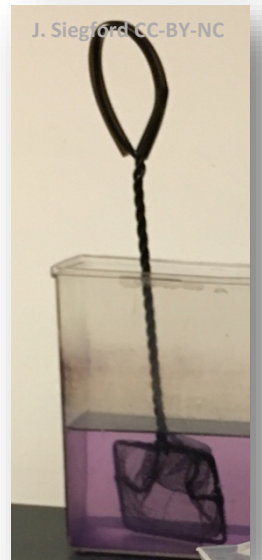
Facility Z

- Each week, tank tops are cleaned to remove food debris and water outlets checked for blockage
- Every 2 weeks, debris is siphoned out of tanks
- Tanks are rotated out every 3 months
 - Scrubbed and sanitized using a bleach bath
- Nets are rinsed with tap water then soaked in a commercial net disinfectant
 - Disinfectant solution changed every 2 weeks



Facility F

- Cleanliness of tanks, lids, and screens is checked each morning
 - Any tank with visibly dirty sides/bottom is removed for cleaning
 - Floating debris is removed using pipette
- Tanks are rotated out 1x/month
 - Scrubbed then sanitized via autoclave
- Nets are stored in a commercial net disinfectant solution on the bench
 - Different nets are used with different lines of fish
 - Disinfectant solution changed every week
 - Nets are also autoclaved 1x/month



Water Parameters

	Facility Z	Facility F
Temperature	28.5C	26.3C
pH	7.35	7
Ammonia	0.25ppm	0 ppm
Nitrites	<0.1 mg/L	0 mg/L
Nitrates	0.5 mg/L	0.2 mg/L
Conductivity	602 μ S	1505 μ S
Dissolved Oxygen	6.0 mg/L	
Problems of note	When setting up the facility, ammonia levels fluctuated. Spike caused fish to die (only small # in residence during this water conditioning phase)	If nitrates or ammonia are >0, then 10% water changes are done every 2 h until readings return to 0

Handling & Procedures

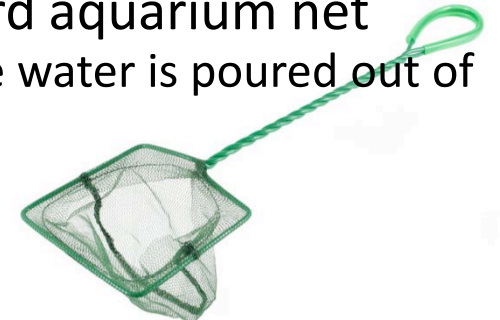
Facility Z

- Technicians are responsible for handling related to routine care and breeding
 - Egg collection and fin clipping is most common reason technicians handle fish
- Research personnel from specific labs carry out research protocols involving the fish, including surgeries and special matings
 - Cardiac and neurosurgery are most invasive
- Nitrile gloves worn when working with fish
 - Gloves are changed between rooms
- Fish are moved to new tanks or breeding containers using a net shaped to fit the geometry of the tanks



Facility F

- The technician, postdoc and graduate student carry out all routine handling and research protocols with the fish
 - Transfer to and from new tanks for cleaning and breeding is the most common reason for moving fish
 - Egg collection is the most common reason for handling the fish
 - Sperm collection and fin biopsies are performed infrequently
- Nitrile gloves worn when working with fish
 - Gloves are changed between lines
- Fish are moved to new tanks or breeding containers using a standard aquarium net
 - To assist in capture, some water is poured out of the tank



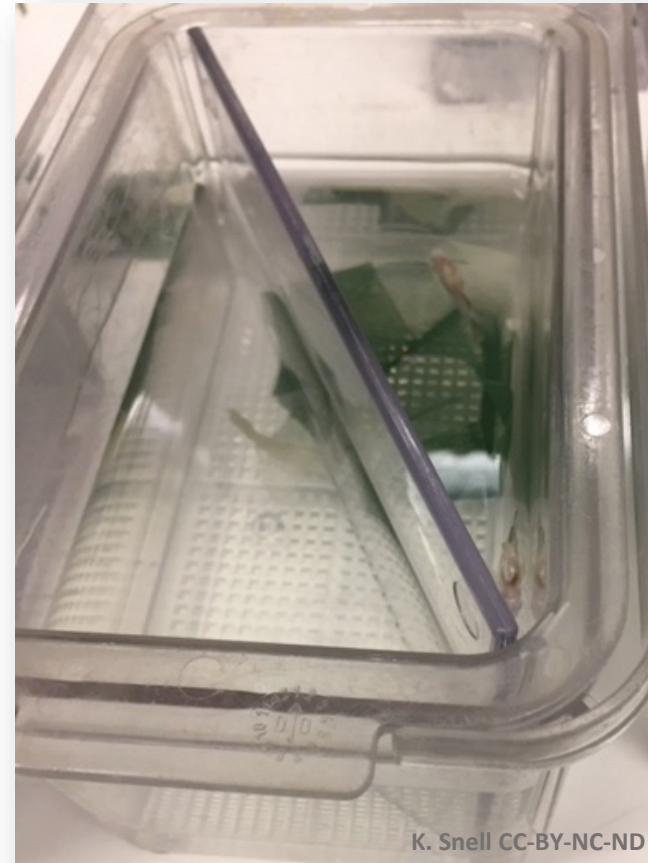
Breeding—Facility Z

- Fish to be used as breeders are isolated into small same-sex groups (~3-4 fish) for 3-7 days before breeding
 - Fish are screened for visible health issues prior to breeding
- The evening (>3pm) before breeding, fish are placed into a breeding box
 - Female is placed below the sieve separator and male(s) above
 - Breeding groups and sex ratios vary depending on the reason behind the mating (1:1 is most common followed by 2-3 males:1 female)
- After lights come on the next morning, the female is netted and placed with the male above the separator
- The pair is kept together for ~4 hours
 - If spawning occurs, the fish are removed and returned to their home tank
- Fish are bred between 6 months and 1.5 years old
- Fish rest for >1 week before being bred again
 - If not needed for research or colony maintenance, females are routinely spawned every 4-5 weeks to maintain health



Breeding—Facility F

- Fish are isolated into small same-sex groups (~3-4 fish) for 10-14 days before breeding
- The afternoon (>6pm) before breeding, one male and one female are placed into a breeding box separated by a barrier
- After lights come on the next morning, the barrier is removed or replaced so both fish are together
- The fish are kept together for ~2 hours
 - If spawning occurs, the pair is removed and returned to their home tank
 - If spawning has not occurred, the pair are separated and will be mated again the next morning
- Fish are typically bred when they are between 6 months to 2 years old
 - Some breeders are > 2.5 years old
- Fish rest for >1 month before being bred again
 - Fish are not routinely spawned if not needed



Fish Responses

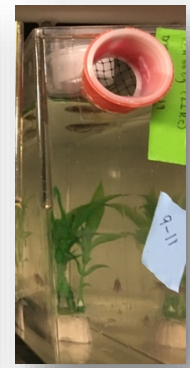
Response to Netting*	Facility Z Wild Type	Facility Z Mutant X	Facility F Wild Type
Freezing (# bouts)	4	3	1
Erratic swimming (# times observed)	19	26	5
Time exploring upper part of tank	35 s	24 s	105 s
Cortisol (ng/g)	0.04	0.05	0.01

*Observed for first 5 minutes after being placed in an individual tank

Response to Breeding*	Facility Z 1:1	Facility Z 3:1 [§]	Facility F 1:1
Courtship behavior (# times observed)	17	4	18
Agonistic behavior (# times observed)	13	48	5
Time spent hiding/avoiding chase	47 s	223 s	54 s
% Successful spawns	23%	16%	34%

*Observed for first 5 minutes after being placed with mates. [§]3:1 = 3 males : 1 female

Fish Behavior



Facility Z

- Fish spend about 2 h per day feeding
- Shoaling behavior is regularly observed in home tanks
- Chasing, biting and territorial behaviors have been observed among males when fish are bred in groups

Facility F

- Fish spend about 1.5 h per day feeding
- Chasing and biting have been observed in tanks with fewer fish
 - A plant is now added to tanks with ≤ 3 fewer fish
 - A piece of mesh is now added when fish are bred
- Chasing, biting and territorial behaviors have been observed, particularly in all male groups when fish are separated by gender to prepare for breeding
 - A plant is now added to same sex only groups waiting to be bred

Anesthesia

Facility Z

- Fish are placed in a small container with Tricaine methanesulfanate (MS-222) at 150mg/L
- Personnel monitor the fish closely to ensure the right level of anesthesia
 - Staff uses light anesthesia for egg stripping and fin clipping
 - Researchers performing surgery induce deeper anesthesia
- Fish are placed in a small container with system water to recover
 - Returned to home tank at the end of the day after verifying they have recovered
- Mortalities occur after ~6% of anesthesia uses, typically following deeper sedation/invasive procedures



Facility F

- Fish are placed in a small container with eugenol (clove oil) at 55 mg/L
- Personnel monitor the fish constantly to ensure the right level of anesthesia
 - In general only light anesthesia is used, primarily for egg stripping
- Fish are placed in a small container with system water to recover
 - Observed to ensure they are responsive and swimming normally before returned to their home tank (< 4 h)
- Mortalities occur after <0.5% of anesthesia uses



Euthanasia

Facility Z

- Fish are placed in an ice water (2-4C) bath for 10 minutes
 - Ice is separated from fish by a sieve separator
- Fish removed from bath and disposed of via in-sink garbage disposal (which also results in cranial concussion and decapitation)
- Routine culling to remove fish >1.5 years old and any lines not being used

Facility F

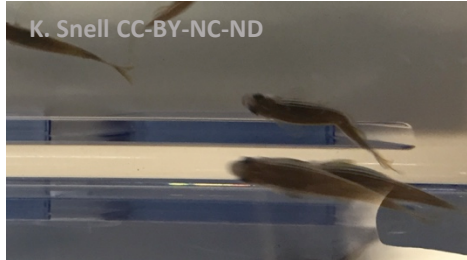
- Fish are placed into a small container containing a Tricaine methanesulfanate (MS-222) solution at ~250mg/L for > 1 hour
- Fish are removed from the container and placed into plastic bag in freezer for later disposal
- The population is not routinely culled

	Facility Z	Facility F
Opercular movement ceases	< 12 s	~143 s
Successful euthanasia (assessed after 10 min)	100%	95%
Behavioral responses during euthanasia	Fish slow swimming and opercular rate gradually. <1% of fish show piping*.	Rapid opercular movement typically observed before death. Fish also show piping* (40%), erratic swimming (5%), and twitching (3%).

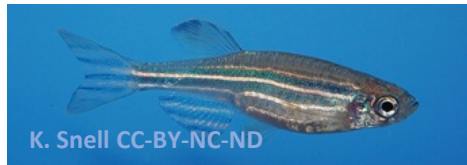
*Piping = gulping of air at the water surface, usually indicative of hypoxia

Morbidity & Mortality

Facility Z Deformity



Facility Z Obese Fish



	Facility Z	Facility F
Yearly mortality rate (excluding culls)	3%	<1%
Yearly mortality rate (including all culls)	50%	4%
Egg bound females	<1%	4%
Fish with physical deformities (including mutant & transgenics)	13%	7%
<i>P. Neurophilia</i> present?	Yes	Yes
<i>Mycobacteriosis</i> present?	Yes	No
<i>P. Tomentosa</i> present?	No	No

Facility F Egg Bound Female



Facility F Mortality

