Centre universitaire de santé McGill
Institut de recherche
McGill University Health Centre
Research Institute

McGill

# LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

## Introduction to the Laboratory Zebrafish

## Table of Contents

# 1. ANIMALS USED IN RESEARCH

At McGill, the use of animals is subjected to scientific/pedagogical merit and ethical review to ensure that animals are used only when necessary and under humane and appropriate conditions. McGill University is committed to conducting the highest-quality research and to providing animals with the best care. The use of animals in research, teaching and testing is a privilege governed by public concerns, federal and provincial laws and regulations, the Canadian Council on Animal Care (CCAC) guidelines and policies, and McGill University policies, procedures and guidelines.

The CCAC is the national peer-review organization responsible for setting and maintaining standards for the ethical use and care of animals used in science throughout Canada. McGill's Animal Care and Use Program is certified by the CCAC based on institutional compliance with CCAC standards. This certification is required for receiving federal Tri-Agency and other research funds.

McGill University's Policy on the Study and Care of Animals outlines the basic principles for the care of animals involved in research, teaching or testing at McGill University and affiliated institutions.

The privilege of using animals can be withdrawn for individuals who, by their negligence or deliberate actions, establish non-compliance with CCAC guidelines, McGill Policies and SOPs, and the approved animal use protocol. These individuals might face additional disciplinary measures, including reporting the non-compliance to other instances.

## 1.1. The Animal Use Protocol

All procedures involving the use of animals in research, teaching and testing must be described in an Animal Use Protocol (AUP).

All animal-based protocols comply with CCAC and McGill University policies and guidelines; are peer-reviewed for scientific or pedagogical merit; are approved by the local Facility Animal Care Committee (FACC) before animals are purchased and used; and are performed in a facility which ensures the safety of the staff and students while maintaining the health and welfare of animals through high standards of animal care and facility management.

AUPs also contain detailed information on:

- All research, teaching, testing and husbandry procedures including euthanasia and the use of potentially hazardous agents.
- The numbers of animals to be used in a given year including alternatives for replacement and reduction of animal use.
- Anticipated signs of morbidity and monitoring frequency.
- Endpoints, which are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal.

McGill University has created over 100 Standard Operating Procedures (SOPs) that provide a detailed description of commonly used procedures including analgesia, anesthesia, surgical and experimental procedures, euthanasia, etc.

SOPs establish best practices and offer investigators an alternative to writing detailed procedures in their AUP.

The Quality Assistance Program (also known as the Post-Approval Monitoring) ensures animal wellbeing, is a resource and assistance to the FACC and to the research community and ensures adherence to approved procedures. Adherence to Animal Use Protocols is achieved by assessment visits and procedure observations.

### 1.2. Education and Training

All individuals involved in the care and use of animals must receive appropriate training, both theoretical and practical, and adequate preparation before undertaking a procedure or using and caring for a species. Having a good working knowledge of the AUP is essential.

Individuals using or caring for animals at McGill or its affiliated institutions have a responsibility for the proper stewardship of animals under their care, this includes adhering to protocols, policies, procedures and guidelines. Furthermore, each participant in the Animal Care and Use Program is accountable for reporting animal welfare and compliance concerns. The Guidelines for Animal Welfare and Compliance Concerns describes participants' responsibilities.

Failure to adhere to McGill policies, procedures and guidelines may result in access to the animal facility being revoked.

## 2. OCCUPATIONAL HEALTH PROGRAM

The Occupational Health Program (OHP) for Animal Related Activities addresses the health risks which may result from working with animals. Participation in the OHP is voluntary for personnel in contact with aquatic species. However, individuals who are exposed to animals, tissues, body fluids, wastes, or equipment involved in the care and use of animals are strongly encouraged to participate in the OHP.

## 3. VETERINARY CARE

Husbandry of laboratory animals is the responsibility of the Animal Care team; they make sure animals have clean tanks, food and clean water and that the facilities are well maintained. Attendants also observe cages and tanks on a daily basis and report any animal that appear ill or injured to the Veterinary Care team.

The Veterinary Care team is composed of animal health technicians and veterinarians; their role is to provide medical and preventive care by evaluating clinical cases, providing treatment and monitoring animals. They also provide training and technical services to the research community, make recommendations and share expertise, monitor the overall health of the colonies, and work to improve the general welfare for animals used in research,. The veterinarians have the authority and responsibility to make determinations concerning animal wellbeing and to assure that this is appropriately monitored and promoted.

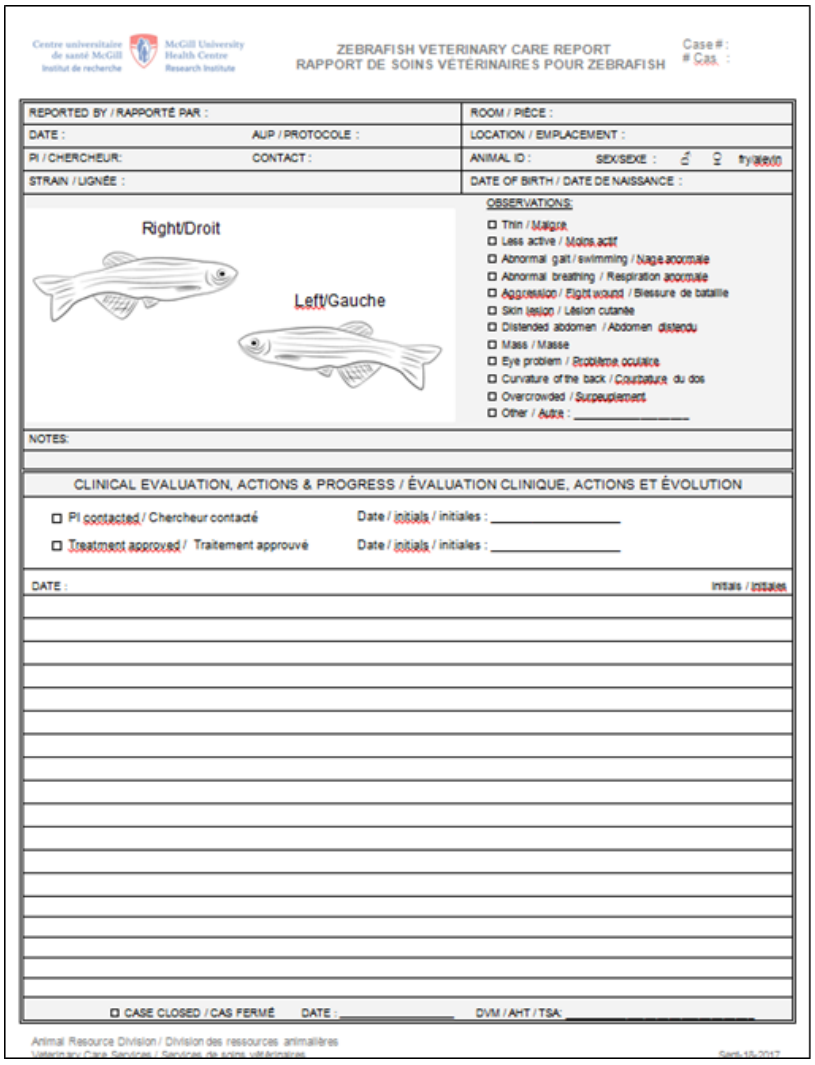
You can contact the Veterinary Care team if you have any questions concerning animal health and wellbeing.

## 3.1. Veterinary Care program

The Veterinary Care program aims to detect and diagnose sick or injured animals thus preventing unnecessary pain and distress.

You may come across a Veterinary Care cage card on one of your tanks. This indicates that an illness/injury report has been submitted for one or more animals in that cage. Many of the cases tend to be common conditions such as egg bound female or damaged scales/fin. A member of the Veterinary Care staff will contact you if they find a case that requires your attention.

## 3.2. Illness/Injury Card and Report


ZEBRAFISH VETERINARY CARE REPORT
RAPPORT DE SOINS VÉTÉRINAIRES POUR ZEBRAFISH

Case # :
# Cas :

| | |
|---|---|
| REPORTED BY / RAPPORTÉ PAR : | ROOM / PIÈCE : |
| DATE : AUP / PROTOCOLE : | LOCATION / EMPLACEMENT : |
| PI / CHERCHEUR: CONTACT : | ANIMAL ID : SEX/SEXE : ♂ ♀ ☿ ◐ |
| STRAIN / LIGNÉE : | DATE OF BIRTH / DATE DE NAISSANCE : |

Right/Droit

Left/Gauche

OBSERVATIONS:

- ☐ Thin / Maigre
- ☐ Less active / Moins actif
- ☐ Abnormal gait / swimming / Nage anormale
- ☐ Abnormal breathing / Respiration anormale
- ☐ Aggression / Fight wound / Blessure de bataille
- ☐ Skin lesion / Lésion cutanée
- ☐ Distended abdomen / Abdomen distendu
- ☐ Mass / Masse
- ☐ Eye problem / Problème oculaire
- ☐ Curvature of the back / Courbature du dos
- ☐ Overcrowded / Surpeuplement
- ☐ Other / Autre : _____

NOTES:

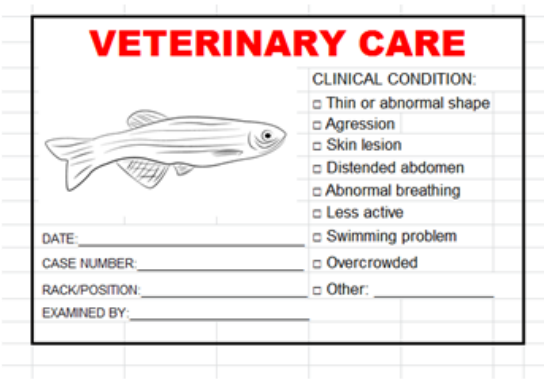
CLINICAL EVALUATION, ACTIONS & PROGRESS / ÉVALUATION CLINIQUE, ACTIONS ET ÉVOLUTION

- ☐ PI contacted / Chercheur contacté — Date / initials / initiales : _____
- ☐ Treatment approved / Traitement approuvé — Date / initials / initiales : _____

| DATE : | Initials / Initiales |
|---|---|
| | |

☐ CASE CLOSED / CAS FERMÉ DATE : _____ DVM / AHT / TSA: _____

Animal Resource Division / Division des ressources animalières
Veterinary Care Services / Services de soins vétérinaires



# 4. THE LABORATORY ZEBRAFISH

The zebrafisH, *Danio rerio*, is a species of freshwater fish of the family Cyprinidae. They are the most common small, warm-water fish held in institutions in Canada for scientific purposes. In their natural habitat, zebrafish are typically found in standing or slow-moving bodies of water, such as pools, ponds, lakes, ditches or rice paddies. They are native to South Asia.

## 4.1. General biology and physiological data

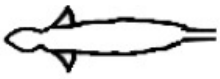
- Similar genetic structure to humans, sharing 70% of genes.
- Possess all of the classes of senses: touch, balance, hearing, smell, vision and taste.
- Circadian pattern of daytime activity and night-time rest- 14 hours light:10 hours dark cycle.
- Zebrafish can display shoal cohesion.
- Use olfactory cues to distinguish between kin and non-kin. Important for feeding and reproduction.
- Swim bladder: a gas filled organ that lines the dorsal portion of the abdominal cavity on the ventral side of the kidneys- helps the fish have stability and buoyancy.
- Adults are usually less than 5cm in length and weigh on average of 1g.
- Sexual maturity: 2-6 months or around 23 mm in size.
- Breed ~ every 10 days and can produce as many as 50 to 1000 eggs at one time.
- Embryo: Post-fertilization to hatching (usually 48-72h post fertilization.
- Larvae**:** Post-hatching to <30 dpf.
- Juvenile: Fish >30 dpf up till 3 months of age.
- Adult: Zebrafish aging 3+ months of age.
- Average lifespan of 3½ years.
- In laboratory: It is generally not recommended to keep zebrafish older than 18 months old as they can accumulate pathogens with age.

## 4.2. Body condition (BC) scoring system

Scoring the body condition of rodents is a non-invasive method for assessing health and establishing endpoints where body weight is not a viable monitoring tool, such as with tumor models, ascites production, pregnancy, or in young growing animals.

Body condition scores (BCS) range from 1 (emaciation) to 5 (obesity).

Scores are determined by frequent visual and hands-on examination of each animal. There is a recent published BC scoring system for the zebrafish (J Am Assoc Lab Anim Sci. 2018 Nov; 57(6): 698–702.)

| Adult Zebrafish BCS | | |
|---|---|---|
| | Lateral View | Dorsal View |
| BCS 1:<br>• Head larger than body (big head)<br>• Lateral- concave ventral surface between head and abdomen (narrow abdomen)<br>• Dorsal- body is more narrow than head and linear<br>• **Fish is thin (emaciated)** |  |  |
| BCS 2:<br>• Head and body equal size<br>• Lateral- flat ventral surface between head and abdomen<br>• Dorsal- head and width of abdomen are equal<br>• **Fish is underconditioned** |  |  |
| BCS 3:<br>• Body larger than head<br>• Lateral- slight convex ventral surface<br>• Dorsal- head is slight smaller to a fusiform body<br>• **Fish is well-conditioned** |  |  |
| BCS 4:<br>• Body significantly larger than head<br>• Lateral- body moderately convex ventral surface<br>• Lateral- Symmetrical ventral surface<br>• Dorsal- head visually smaller to a moderately distended abdomen<br>• **Fish is over-conditioned** |  |  |
| BCS 5:<br>• Body significantly larger than head<br>• Lateral- body significantly convex ventral surface<br>• Lateral- Symmetrical or asymmetrical ventral surface<br>• Dorsal- head visually smaller to a significantly distended abdomen<br>• **Fish is obese (large)** |  |  |

# 5. HUSBANDRY

## 5.1 Daily Care and Maintenance

Daily care and maintenance of aquatic equipment is essential to fish welfare. Environmental parameters and maintenance checks should be verified and recorded in the daily maintenance log located in the aquatic holding room by the Animal Care Team. Refer to McGill SOP 519 or RI-MUHC OP-07.01 for complete details.

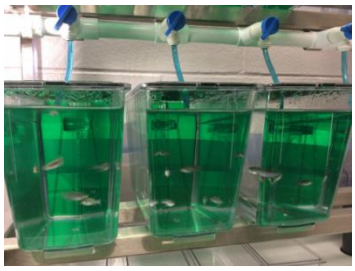
## 5.2 Housing

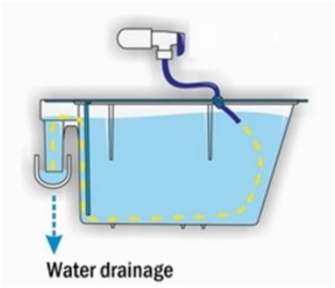
### 5.2.1. Water Parameters

- Water temperature: 24-29°C
- pH: 6.8-8.5
- Conductivity: 300-1,500 µS
- Ammonia: 0 ppm
- Nitrite (NO2): 0 ppm
- Nitrate (NO3): <200 ppm
- Hardness (TH): 0-200 ppm

### 5.2.2. Water Flow

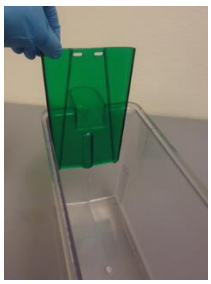
Clean water travels from system hose inserted into a hole on the tank cover. The water flow direction travels from the hose down towards the front of the tank then towards the back where the baffle is placed and gets suctioned out through the top into the tank's overflow drain.



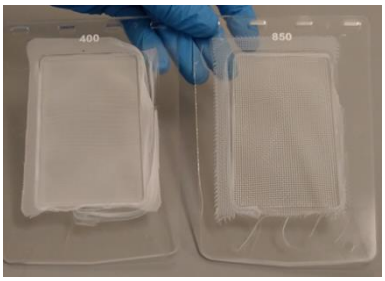



### 5.2.3. Tank Components

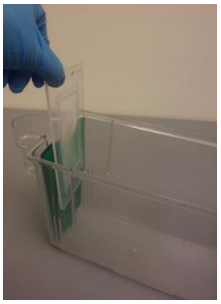
Tank



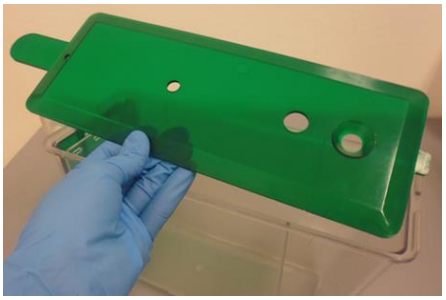
Baffle




Larvae screen 400, 850 µm



Baffle and larvae screen



Lid

Complete tank

## 5.3 Density

Many different tank sizes are used for fish. The tank density depends on amount of fish per liters of water.

The following tanks are used the most:

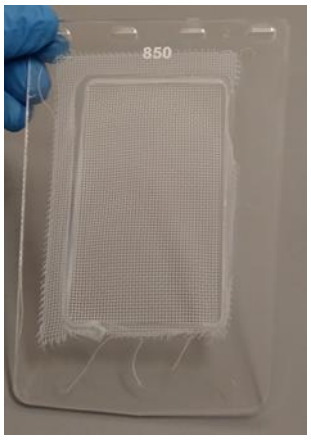
2.8 L tank- can hold up to 15 adult fish or 45 fry or juveniles.

6 L tank- can hold up to 30 adult fish or approximately 100 larvae or juveniles.

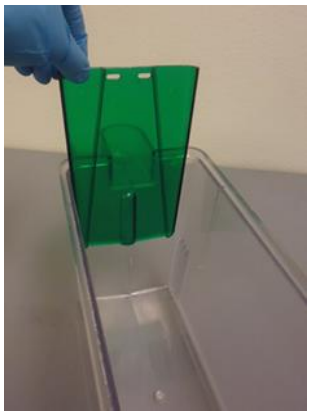
## 5.4 Larvae Screen Schedule

Due to the small size of the larvae, a fry screen is required to ensure fish do not get suctioned into the tank's overflow drain. As the fish grow the fry screens are changed as well. The two main fry screens used are the 400 and the 850µm screens.


400µm larvae screen is needed for up to 4 weeks of age




850µm fry screen is needed for up to 8 weeks of age



By 2 months of age there shouldn't be any need for a larvae screen and a regular baffle should be placed.

## 5.5 Environmental enrichment

Consideration should be given to provide zebrafish with environmental enrichment suited to the housing conditions. The most important form of enrichment remains group housing.

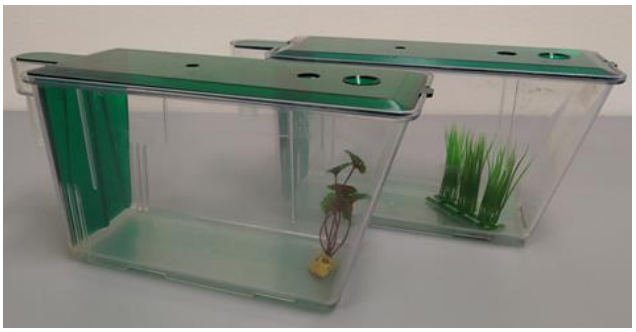
Enrichment can come in a variety of ideas:

- Lily pads
- Plants
- Floating grass
- Adhesive plants
- Substrate pictures

## 5.6 Enrichment Type and Quantity Per Tank Size

The enrichment should provide some hiding spaces for the fish, but too much enrichment can be obstructive for the daily health checks. Considering there is no standard for zebrafish enrichment, we recommend the following:

### 2.8 L tanks- 1 small plant



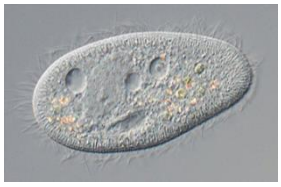
### 6 L tanks – 1 large plant and 1 small plant

All tanks – Placed on an aquarium rock poster of correct size

## 5.7 Feeding

Zebrafish are omnivorous in nature, primarily eating zooplankton, phytoplankton, insects and insect larvae, although they can eat a variety of other foods, such as worms and small crustaceans, if their preferred food sources are not readily available. In research, adult zebrafish are often fed with processed food, supplemented sometimes with live diets.

### 5.7.1. Live diets



#### Paramecium:

Single cell protists (100-250 µm). Suitable as an initial food source for developing zebrafish larvae.

#### Rotifer:

small ciliated protozoan (150-300 µm). Suitable as an initial food source for developing zebrafish larvae.

#### Artemia (brine shrimp):

Small aquatic crustaceans (size depends on culture time). Suitable as an initial food source for later stage larvae, juveniles or adult zebrafish.

| Advantages | Disadvantages |
|---|---|
| Balanced nutritional profiles | Can introduce environmental contaminants and pathogens into the system |
| Highly palatable and easily digestible | Extra labor, equipment and costs |
| Can help increase growth rate of larvae | |
| Can be considered a source of enrichment | |

### 5.7.2. Processed diets

Zebrafish should be fed 5% of body weight daily of processed food, can be split into multiple times a day. Amount fed should be consumed in 5 mins, leftovers should be removed to avoid a decrease in water quality. Gemma micro processed diet is commonly used in laboaratory zebrafish colonies.

- Designed to replace live diets, formulated using biological materials
- Cost effective
- Greater control over the nutritional state
- Reduces the risk of introducing pathogens or toxins via the diet

Gemma micro 75 ZF: given to zebrafish < 30 days post-fertilization (dpf)
Pellet size: 50-100µm

Gemma micro 300 ZF: given to fish > 30 days post-fertilization (dpf)
Pellet size: 200-500µm

**Procedure:**

A food dispenser can be used to feed the adult zebrafish population.

Larvae fish (<30dpf) are fed a very small amount. An entire tank of fish will consume an amount less than ½ a gram. Food should be consumed within five minutes. Any uneaten food which sinks to the bottom of the tank should be pipetted out to avoid algae blooms from growing and leaving larvae vulnerable to becoming trapped in the biofilm. A metal spatula can be used to feed the fry.

Overfeeding is never recommended for fish at any age. Fish do not have stomachs but rather an intestinal bulb which means their digestive system cannot handle too much food at a time. It is preferable to feed small portions in multiple feeding intervals.

# FISH FEEDING GUIDELINES – AN EXAMPLE

## Food Labels:

**75 ZF** - Larvae < 30 days post-fertilization
**300 ZF** – Juveniles/Adults > 30 days post-fertilization

## Juveniles and Adults:

Using metal dispenser:
Feed **300 ZF (2x/day – AM and PM)**

**(Full Pressure)**

**(1/2 Pressure)**

**Food Quantity- 2x/day**

| # Adult Fish (approx.) | Feeder Pressure |
|---|---|
| 5 | 1/2 |
| 10 | 1 Full |
| 15 | 1 Full + 1/2 |
| 30 | 3 Full |
| Juveniles | 1/2 |

## Fry fish:

Using metal spatula:
Feed **75 ZF** (**2x/day- AM and PM**).
The **Animal Health Technician** will **feed a 3rd time** at ~11:00-12:00PM daily.

**(Arrow shows quantity of food to feed- ~ 0.02 µg)**

# 6. HANDLING AND RESTRAINT

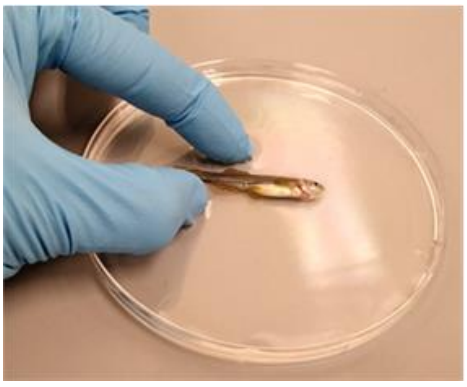
## 6.1. Manual restraint

- Fish should be handled only when necessary, and the number of handling episodes should be minimized.
- Measurements such as weight and length, which involve hands-on manipulation, should be conducted quickly and in a manner that is minimally stressful. Procedures that involve more than momentary restraint or require that large numbers of fish be handled should be conducted under sedation.
- The length of time that fish are held out of water should be minimized, as it is a stressful event for fish. In general, fish should be exposed to air for the shortest time possible, and no longer than a few seconds. If fish are held out of water for more than a few seconds, efforts should be made to wet the fish and the gills and operculum should be kept moist.
- Before opening the tank observe the fish within. Nervous or startled fish can jump out very quickly and escape.

## 6.2. Restraint devices

There are different size nets for capturing fish from tanks. A small plastic container can also be used to safely remove fish from the tank and avoid fins from being injured.

When manual manipulation is required, fish are anesthetized and placed in a dish filled with a sufficient amount of water to keep fish wet and the gills and operculum moist.



# 7. SEX DETERMINATION

- Sexing of fish is based on body appearance.
- Males are slender and faster, darker and redder in color. Females have larger bellies where eggs are stored, slower and paler in color.
- An opposite sex comparison is advisable. Compare animals of similar age.

**A = Male**

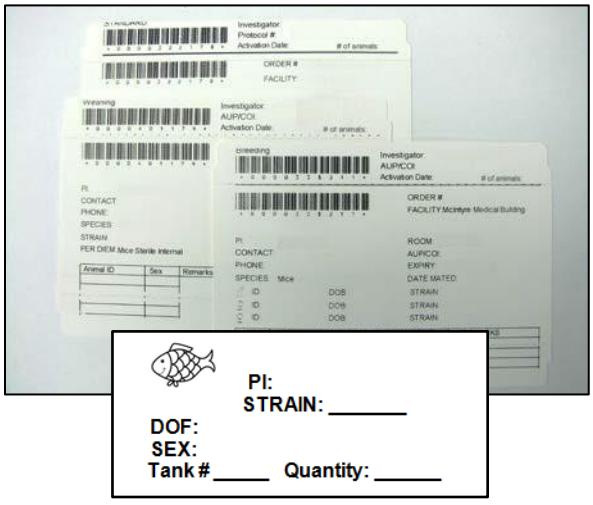

**B= Female**


https://www.jove.com/video/4196/regular-care-maintenance-zebrafish-danio-rerio-laboratory-an

# 8. IDENTIFICATION

## 8.1. Cage cards

- All tanks must have an identification label and a Darwin cage card. All sections of label and card must be completed.
- Cage cards should be kept in the animal holding room or near the racks
- Individual tank labels should identify the tank with the following:
  - Principal Investigator (PI)
  - Strain
  - Date of birth (DOB)
  - Sex (can be mixed sexes or separated by sex)
  - Quantity (can be an approximate).



## 8.2. Physical Identification

Careful consideration must be given to whether physical identification (marking) of individual fish is necessary. If so, the least invasive method should be used.

If marking individual fish is necessary, the method of identification must:

- Cause minimal suffering or impact on the animal, both during the marking process and subsequently
- Last an appropriate time (dependent upon the duration of the study)
- Be reasonably quick and simple to apply; and be easy and quick to read/identify.

Current evidence suggests fish should be assumed to perceive pain in a way analogous to mammals and therefore anesthetics should be considered for methods that may cause pain.

The table below lists possible identification methods. The optimal method will depend on the experimental conditions and outcomes. The available methods should be reviewed by the investigator and approved by the FACC.

| METHOD | POINTS TO CONSIDER |
|---|---|
| Subcutaneous dye injection | • Appropriate in clear or light-colored fish.<br>• Marks can be distinguished for approximately 30 days.<br>• Does not appear to affect social interactions, such as shoaling behaviour.<br>• Fish must be anesthetised during the procedure. |
| Elastomer marking | • An elastomer material containing pigment is injected in liquid form beneath an area of translucent skin.<br>• Over a short period this becomes a pliable solid.<br>• Fish must be anesthetised during the procedure. |
| Fin clipping | • Small clip(s) from different fins or at different positions can be used to identify individuals within tanks.<br>• Fins can regrow quite rapidly.<br>• Fins are innervated so could be painful. Fish must be anesthetised during the procedure. |
| Radio frequency identification (RFID) microtags | • Where necessary, microtags may be used but less invasive methods are preferred.<br>• There is the potential for fish mortality, loss of microtags and the inability to read chips.<br>• Intracoelomic implantation of microtags (1 mm diameter, 6 mm length, ~10 mg mass) in juvenile zebrafish has been shown to result in 82% survival rate and microtag loss of 11% after 5.5 months, with no negative effects on growth or behaviour.<br>• Success rate for reading microtags was 73% for zebrafish 350–450 mg (26 mm).<br>• Fish must be anesthetised during the procedure. |

# 9. TISSUE SAMPLING FOR GENOTYPING

The most common biopsy technique used for zebrafish is to cut off a small part of the caudal fin or tail using a sterile razor blade, scalpel or surgical scissors. Fish should be anaesthetized during this procedure. Only the minimum amount of tissue necessary (2-3 mm is sufficient) should be taken, as the caudal fin is innervated and clearly important for locomotion.

For more information: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4116811/

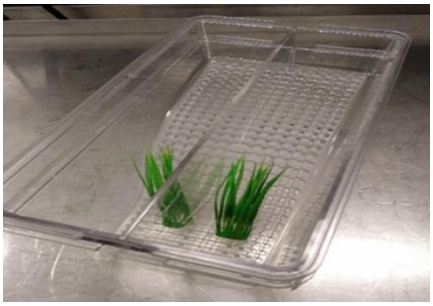
# 10. BREEDING

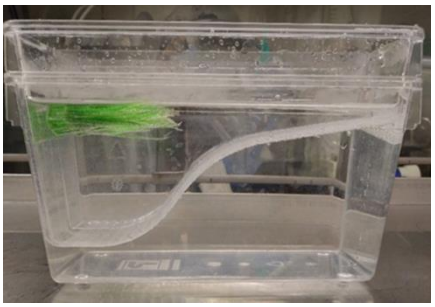
Mating behavior of zebrafish is influenced by the exposure of mating partners to one another during the 24 hours before spawning begins (at sunrise), with males stimulated to perform courtship behavior by the detection of female pheromones in the water and luring females to a shallow water area for eggs to be released. Eggs are fertilized and developed outside of the female's body. Good quality eggs have a translucent egg yolk.

Refer to RI-MUHC SOP on Zebrafish Breeding for more details.

## 10.1. Day prior to fertilization (Day -1)

- Select fish to be bred. It is ideal to pair males and females from different tanks, that are healthy, around 23 mm in size and around 3-6 months for best results.
- In the evening, after having been fed (ideally an hour before), males and females are placed in a slope breeding tank (to mimic a shallow water area as in the wild) at a male to female ratio of 1:1, 1:2 or 2:3 (if smaller fish) and separated by a clear plastic separator.



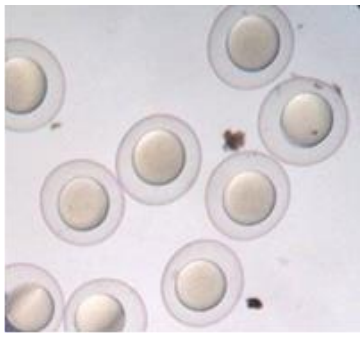
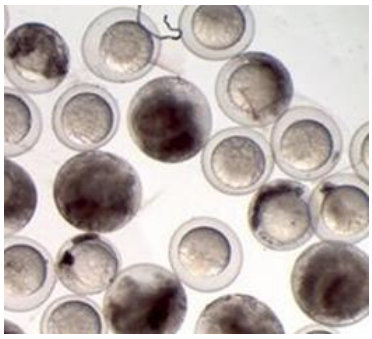


## 10.2. Day of fertilization (Day 0)

- When the lights turn on in the morning, the separator is removed from the breeding tank to allow males and females to interact.
- Egg production usually occurs within 15 minutes, but fish can be kept together for 2-3 hours.
- Carefully collect the embryos from the bottom of the breeding tank using a 400 µm fry screen as a strainer.

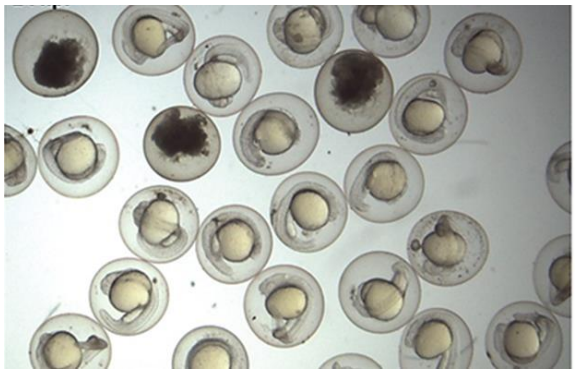
- Using a freshly prepared bottle of 0.00002% methylene blue (MB), cleanse all surfaces of the tank to remove any remaining embryos possibly adhered to tank walls or floor. Tank water should be used for preparation of the MB solution in order to maintain the same water conditions (0.1ml of MB 0.1% to 500ml of tank water).


- Under a microscope, use a short pipette to remove all debris, feces and non-viable embryos. Non-viable embryos are opaque/cloudy/white. Label all petri dishes with strain and date of fertilization (DOF) and place in incubator at 28.5°C. Make sure that the light timer is turned on to ensure embryos are kept at 14:10 light cycle, similar to holding room. Place a regular fish tank with 1/3 of sterile water in the back of the incubator to keep humidity inside while avoiding bacteria formation. Make sure to place the breeders back in their original tanks and to feed them.

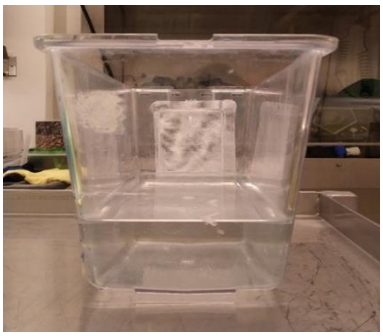
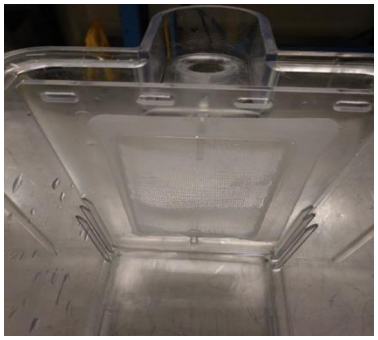
## 10.3. Day 1 post-fertilization (Day 1)

- Freshly prepared MB water is pre-warmed at least 30 minutes inside the incubator to attain the same temperature. Under a microscope, verify the viability of the embryos and remove non-viable embryos and debris. If there is high mortality ≥ 50%, consider using other fish breeders for future embryo collection.



## 10.4. Day 2 post-fertilization (Day 2)

- Remove all non-viable embryos. Remove the larvae with a full swim bladder and transfer them to a tank and feed them with 75 ZF diet. Place a 400 µm fry screen in the tank and fill with water only ¼ full. Do not add any water flow. This gives fish time to strengthen their swimming and be able to reach water surface to feed.

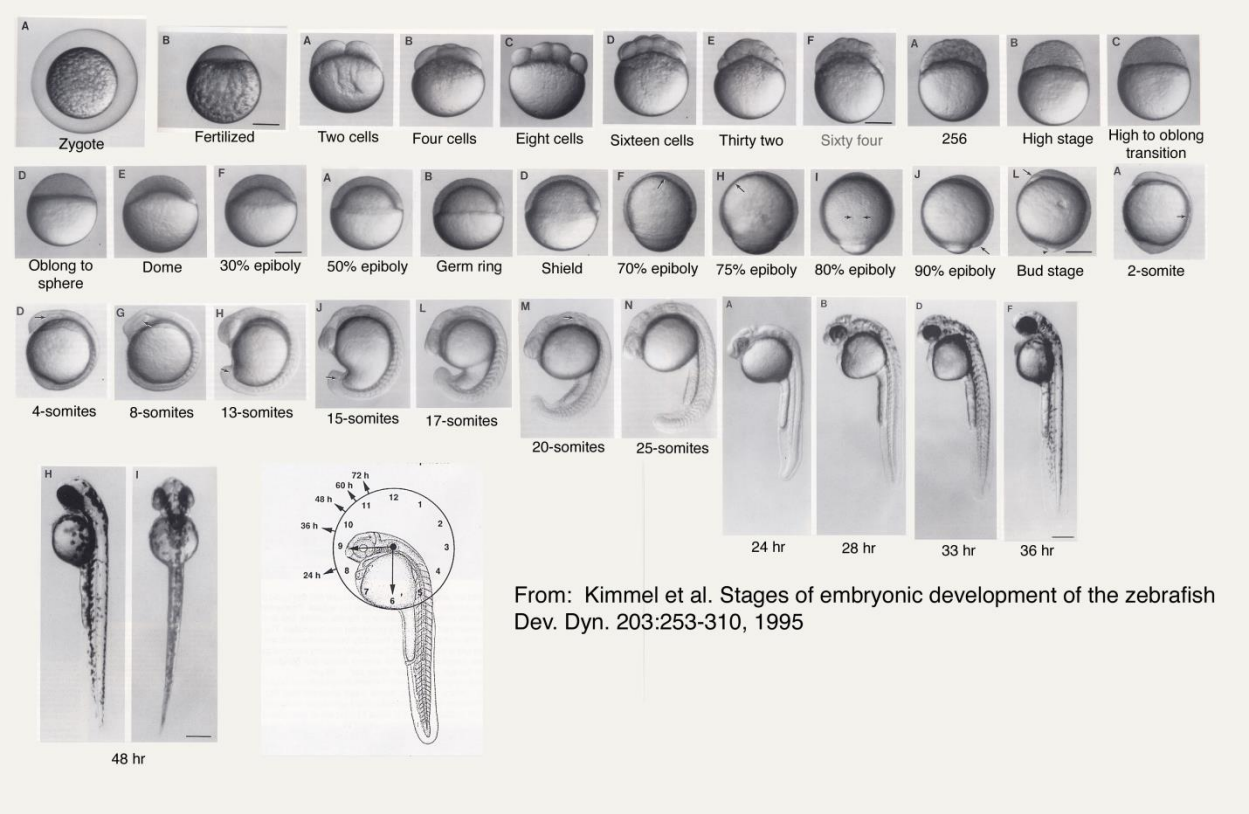



- The embryos that have not hatched yet can be placed back in the incubator for an extra day

## 10.5. Day 3 and 4 post-fertilization (Day 3 and 4)

- Transfer remaining larvae from incubator to tank and feed with 75 ZF.
- At Day 4, add a water flow at a rate of 1 drop/second and then increase gradually over the next 3 days until the flow is equal to the flow rate of all other tanks. This allows fish to acclimate to a water current.

## 10.5. Embryo development




From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

# 11. HUMANE INTERVENTION POINTS

Humane intervention points are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal. Intervention points should be balanced with the experimental endpoints to ensure that animals can be kept on study humanely while they reach the scientific endpoint.

It is the responsibility of the research staff to monitor animals according to the frequency indicated in the Animal Use Protocol (AUP). The frequency of monitoring should be increased as health status declines or as the endpoints are approaching.

Humane interventions are clearly defined in the AUP and are defined as actions or instructions including, but not limited to, the following:

- Adequate veterinary treatment, analgesia and/or supportive therapy to the animal(s)
- Termination of painful procedures
- Removal of the animal(s) from the study
- Modification of the experimental procedures to minimize the discomfort to the animal(s)
- Increasing the frequency of animal observations
- Modification to the housing and husbandry practices to improve the comfort of the animal(s)
- Euthanasia

Note that death is never an acceptable endpoint. Intervention points must be selected to avoid animal death.

General intervention points for fish include:

- Weight loss exceeding 20% of baseline bodyweight. For young animals, failure to maintain normal weight gain within 15% of age-matched controls animals.
- Body condition score (BCS) less than 2.
- Impaired mobility.
- No or weak response to external stimuli.
- Respiratory distress: labored breathing, increased or decreased respiratory rate, cyanosis
- Abnormal posture in water column and lethargy.
- Incoordination, buoyancy issue and paralysis
- Uncontrolled hemorrhaging
- Specific organ failure assessed by physical examination and, where possible, diagnostic tests.

# 12. EUTHANASIA

Zebrafish can be euthanized in a variety of acceptable, effective and humane methods; these methods can be either chemical or physical. Only the approved euthanasia method described in the Animal Use Protocol can be used. Refer to SOP 303 for more details.

## 12.1. Larva (<7 dpf), juvenile or adult zebrafish euthanasia non-physical methods

### 12.1.1. MS-222 (Tricaine methanesulfonate) immersion

- Prior to using MS-222, contact local environmental health and safety officer to proper handling and disposal.
- MS-222 is acidic and in concentrations >500 mg/L, it should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5.
- Place fish in a solution of MS222 dissolved in water (minimum concentration of 250 mg/L).
- Wait until loss of equilibrium and operculum movement ceases. Leave this fish in the solution for an additional 2 minutes.
- Follow by a physical method of euthanasia, such as decapitation, is required on your animals before disposal to ensure that they have been correctly euthanized.

### 12.1.2. Clove oil immersion

- Prior to using MS-222, contact local environmental health and safety officer to proper handling and disposal.
- Mix 1-3 ml Clove Oil in 10 ml of Ethanol
- Mix 10 ml of this solution to 1L of water
- Wait until loss of equilibrium and operculum movement ceases. Leave this fish in the solution for an additional 2 minutes.
- Follow by a physical method of euthanasia, such as decapitation, is required on your animals before disposal to ensure that they have been correctly euthanized.

### 12.1.2. Benzocaine hydrochloride

- Place fish into a bath of benzocaine hydrochloride solution of >250 mg/L.
- Wait until loss of equilibrium and operculum movement ceases. Leave this fish in the solution for an additional 2 minutes.
- Follow by a physical method of euthanasia, such as decapitation, is required on your animals before disposal to ensure that they have been correctly euthanized.

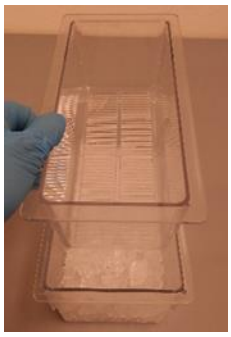
### 12.1.3. Rapid cooling (hypothermia)

- Prepare a tank containing approximately equal amounts of ice and water to achieve a temperature of 2 to 4 °C.
- Submerge the fish and wait until loss of equilibrium and opercular movement ceases. Leave the fish in the ice water bath for an additional 2 minutes minimum.

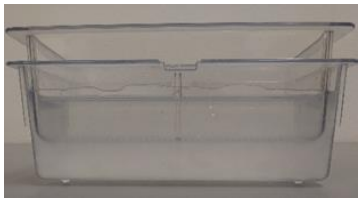
- Follow by a physical method of euthanasia, such as decapitation, is required on your animals before disposal to ensure that they have been correctly euthanized.

Obtain outer and inner tank

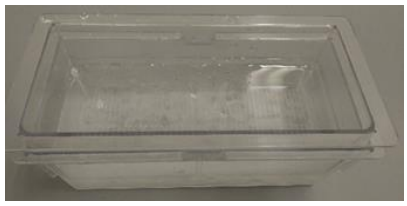
Fill ice in outer tank ½ full



Place inner tank in outer tank



Fill with cold water to submerge ice



Allow ice to melt to obtain an ice bath

Place fish in ice bath and wait for movement and breathing to cease (~ 5 sec.)

## 12.2. Physical method

Physical methods of euthanasia are also an appropriate means to assure death after euthanasia with a non-physical method. Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.

Anesthesia is necessary prior to physical methods of euthanasia, unless described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

### 12.2.3. Decapitation

- Use sharp scissors to ensure that the head is quickly separated from the body rapidly and completely.

## 12.3. For embryos or larva > 7 dpf

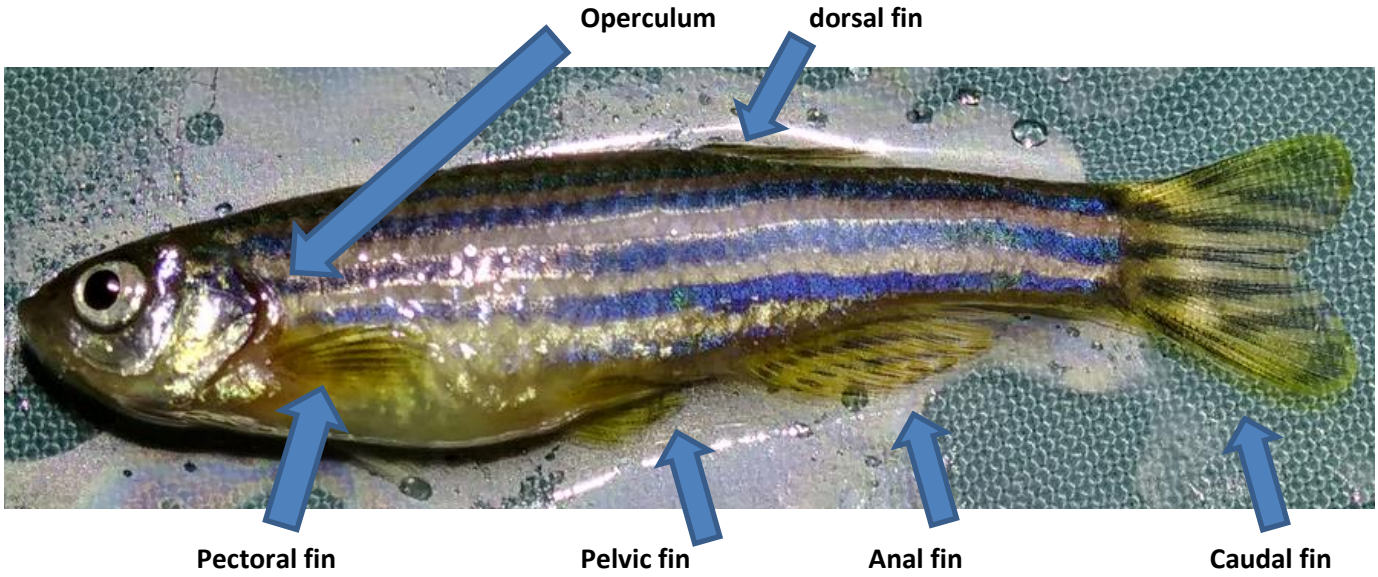
- Place embryos and larva in 1/6 diluted bleach12h or overnight.

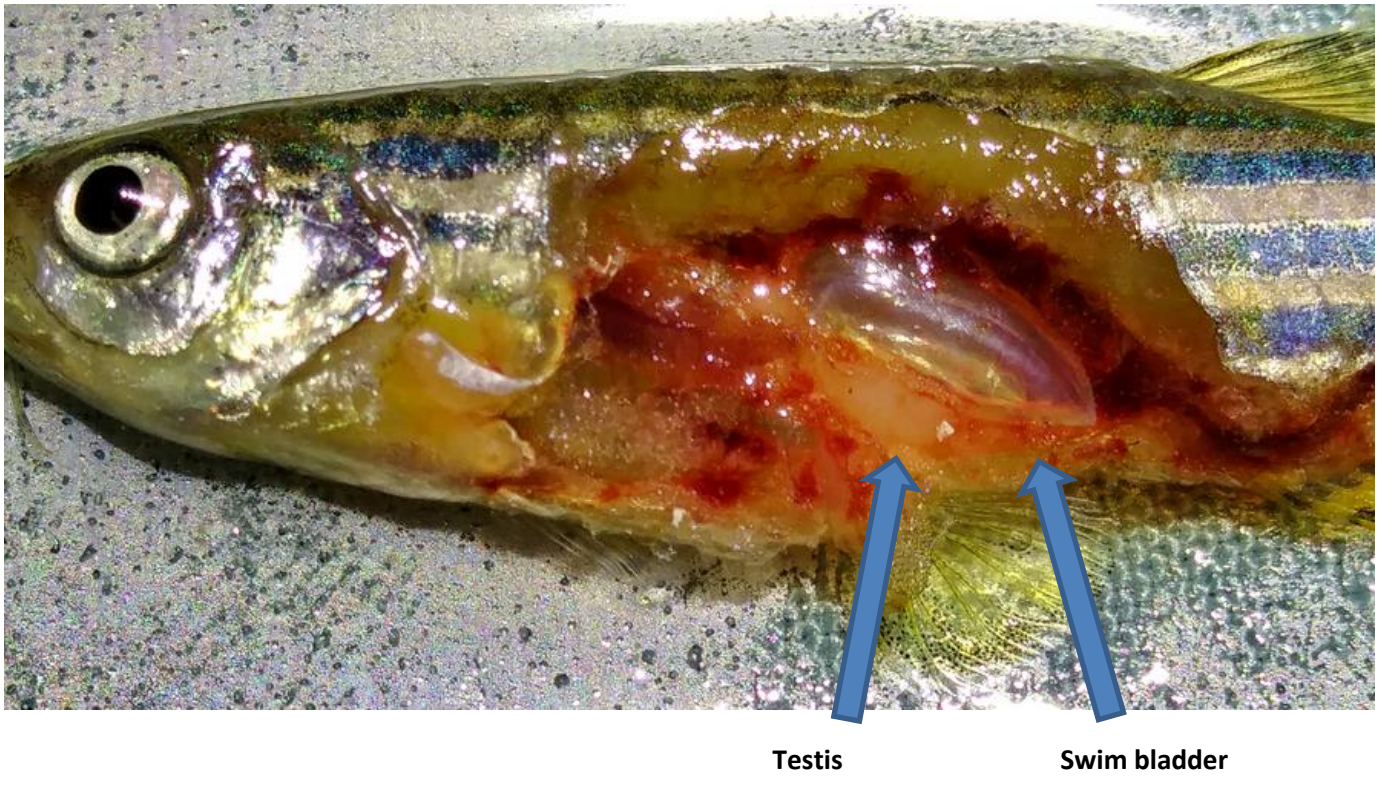
# ZEBRAFISH EUTHANASIA

| METHODS OF EUTHANASIA | NON-PHYSICAL | PHYSICAL |
|---|---|---|
|  <br>For larva > 7 dpf, juvenile or adult | • Rapid cooling (hypothermia)<br>• Eugenol (clove oil)<br>• MS-222 (Tricaine methanesulfonate)<br>• Benzocaine hydrochloride | • Decapitation using sharp scissors (only after a non-physical method of euthanasia or under anesthesia unless approved by the FACC) |
|  <br>For embryos or larva > 7 dpf | • 1/6 Diluted Bleach in water (12h or overnight) | • N/A |

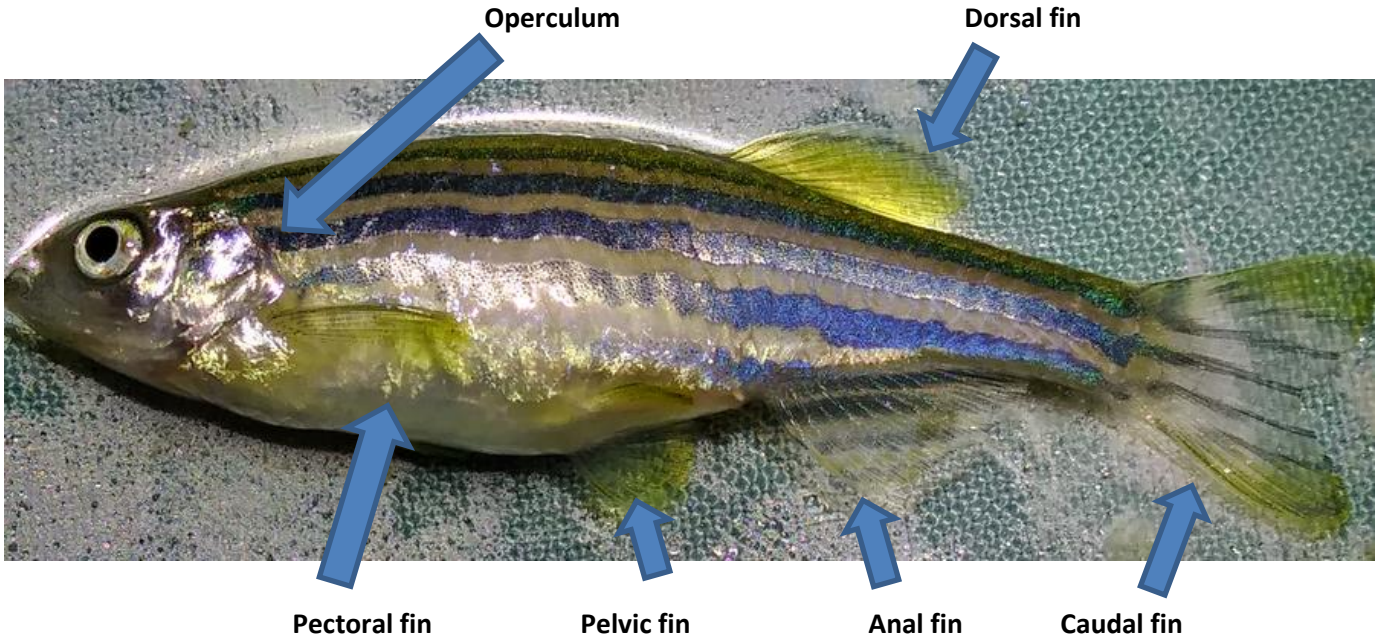
# 13. NECROPSY

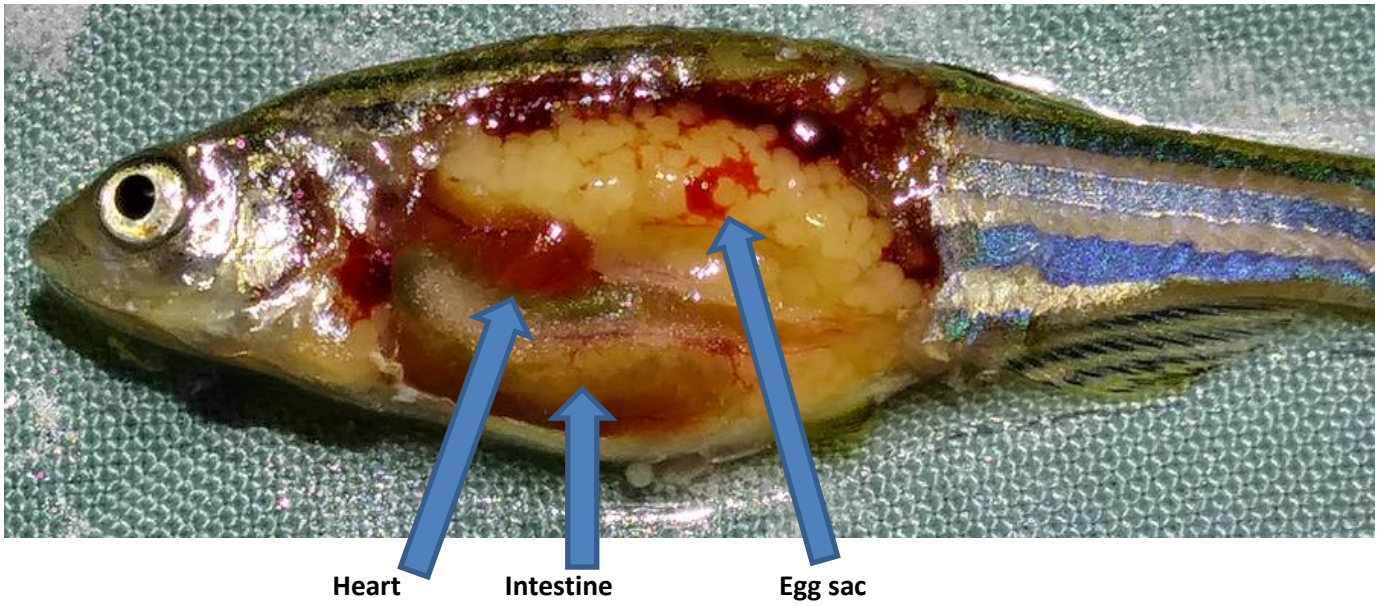
## 13.1 Male anatomy



## 13.2 Female anatomy




# 14. REFERENCES

## 14.1 Animal Resource Division of McGill University Health Centre Research Institute

| | |
|---|---|
| Veterinary Care/ Drug Purchasing and | VetGlen@muhc.mcgill.ca; |
| Technical Services Requests | VetMGH@muhc.mcgill.ca |
| Workshop and Training | ARD.training@muhc.mcgill.ca |
| Equipment Rental/Material | iLab |
| Imports, Transfers and Quarantine | ARD.transfers@muhc.mcgill.ca |
| Quality Assistance | ARD.qualityassistance@muhc.mcgill.ca |
| Animal Use Protocol Questions | FACC.admin@muhc.mcgill.ca |

## 14.2 ARD RI-MUHC Standard Operating Procedures

https://researchportal.muhc.mcgill.ca

## 14.3 Comparative Medicine & Animal Resources Centre

| | |
|---|---|
| CMARC website | www.mcgill.ca/cmarc |
| Veterinary Care | aht.arc@mcgill.ca |
| Technical Services, Equipment rental (anesthetic machines) | rts.arc@mcgill.ca |
| Imports, Transfers and Quarantine | import.cmarc@mcgill.ca |
| Imaging Services | imaging.cmarc@mcgill.ca |
| Irradiator Services | irradiator.cmarc@mcgill.ca |
| Workshop and Training | workshop.cmarc@mcgill.ca |
| Polyclonal Antibody Production | antibodyproduction.cmarc@mcgill.ca |
| Materials and drug sales | drss@mcgill.ca |
| Comparative Pathology | comparative.pathology@mcgill.ca |

## 14.4 McGill Standard Operating Procedures

http://www.mcgill.ca/research/researchers/compliance/animal/sop

**The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre and the Animal Resource Division of the McGill University Health Center Research Institute Animal Health Technicians in preparing this handout.**