Introduction

Welcome to the Working with Fish in Research at UAB (AU_FI) Course Material. The goal of this course is introducing you to working with fish in research at UAB.

Objectives

At the conclusion, participants should be able to:

1. Care for and monitor fish used in research.
2. Comprehend the physiological and environmental issues when working with fish.
3. Identify who to contact for guidance if there are questions.

Used in Research

Because fish represent the “simplest” vertebrates, scientists (including medical researchers) can study many of the same systems as are found in humans but in a much less complicated organism.

Other appealing attributes of fish as research models are:

- Replacement of mammalian models
- Good breeders
- Easy to maintain
- Size allows for efficient housing space

Zebrafish are the most common fish model at UAB. They are used for a variety of research projects involving development, disease prevention and treatment, and genetic disorders.
Types

Fish are the most numerous group of vertebrates with approximately 30,000 known species. They belong to the phylum Chordata, subphylum Vertebrata. Traditional classification divides fish into three classes.

- **Jawless Fish (Class Agnatha)** – It does not have jaws or paired fins but does have a cartilaginous skeleton. This class includes Lampreys and Hagfish.

- **Cartilaginous Fish (Class Chondrichthyes)** – They have skeletons made of cartilage and usually have tooth rows that are replaceable. Sharks and Sting Rays are in this class of fish.

- **Bony Fish (Class Osteichthyes)** – They have a true bony skeleton and fixed teeth fused with the jaw. This class includes ray-finned and fleshy-finned fish.
Care and Husbandry

Housing

Fish generally fall into three categories with some fish changing salinity preferences as they develop.

Water Recommendations

The ARP Staff monitors the following for the Zebrafish at UAB. If you have questions, contact the UAB ARP Veterinary Staff.

- Water Type: Only non-chlorinated water
- Temperature:
  - 70°-90°F is recommended for zebrafish
  - Varies by species
  - Warmer water holds less oxygen
- pH Levels:
  - 6.5-8 is the recommendation
  - Carbonates, Bicarbonates, Aeration, and Decomposition increases pH
  - Nitrification, CO₂, and feeding decreases pH
- Other Monitoring
  - Dissolved oxygen
  - Waste product (nitrate, ammonia) levels
  - Conductivity (500-2500 microsiemens)
- Adding salt increases conductivity

### Light and Density

Recommendations for fish at UAB:

- Use low light levels
- Fish can be affected by variations in the photoperiod.
- Monitor housing density because it affects growth, maturation, and sex determination.

If you have questions about the light levels or density, contact the UAB ARP Veterinary Staff.

### Types of Holding Systems

There are typically three types of systems used for housing fish.

**Static:** Water is stationary and needs to be replaced or replenished regularly.

**Flow-Through:** Water is constantly replaced.

**Recirculating:** UAB primarily uses recirculating systems. Be aware of the volume of water being removed from the system (e.g., setting up breeding tanks) so that the capacity of the overall system is not reduced.
Acclimation & Quarantine

Acclimation

Acclimation is the process of becoming adjusted to a new environment or situation and is necessary due to factors like stress from transportation. This stress could alter various blood parameters, immune cell function, and animal behavior. Upon arrival at UAB, fish must have an acclimation period of at least 48 hours before use in research studies. It may be desirable to extend the acclimation period beyond 48 hours. If possible, one week of acclimation is best.

Quarantine

The goal of quarantining and separating animals is preventing the transmission of diseases between new animals and animals already present in established colonies. ARP coordinates all animal acquisitions ensuring animals from new or unknown sources undergo the required quarantine period. Animals from approved and pathogen-free sources do not require quarantine.

A quarantine period is vital for avoiding loss of investigator time and valuable research data due to the introduction of disease, and the cost of controlling or eliminating infections escaping into established colonies.

Acclimation and quarantine periods can run at the same time even though they serve different purposes. For more information about acclimation and quarantining your fish, contact the UAB ARP Veterinarians.
**Health Procedures**

Procedures are in place ensuring the research colonies at UAB are free of specific diseases and pathogens.

- Every quarter, fish from the colony are collected and euthanized for analysis in the Comparative Pathology Lab (CPL).
- Histopathology analyses are conducted to determine if there are bacterial or fungal contaminants present.
- Sick or abnormal fish may also be collected for health surveillance testing as needed.

**Animal Identification**

There are several acceptable methods for identifying fish:

1. Dyes or chemical markings (elemental or fluorescent)
2. Microchips or radio telemetry
3. Natural markings or coloration
4. Fin clips
5. Tags (internal or external)
Enrichment promotes an animal’s natural behaviors by providing a stimulating and interactive environment. Environmental enrichment leads to improved health in your animal colony.

While fish may not require complex programs like many mammalian models, there are subtle environmental qualities to consider:

- Group housing for schooling fish
- Changing the photoperiod (the time fish are exposed fish to light per day)
- Using different food to encourage natural feeding behaviors
- Adjusting water flow and allow interaction with a diffuser when appropriate
- Placing aquatic plants in the tank

Breeding

Fish species can be Oviparous (egg-laying), Viviparous (live bearing) or, Ovoviviparous (embryos develop inside eggs that are retained within the mother’s body until they are ready to hatch).

Zebrasfish (Danio rerio) are synchronous egg-layers that reach sexual maturity at 3-4 months of age and maximum embryo production between 7-10 months of age. Zebrasfish most commonly spawn (lay eggs) during the first few hours of daylight. Therefore, photoperiod is very important for them.
Handling Eggs

1. To avoid a loss of eggs through the filter system, place males and females in static breeding tanks.
2. A gridded insert or layer of marbles is placed on the floor of the tank to prevent the parents from consuming the eggs.
3. Collect eggs for incubation or use in experiments.

Biological Features

Basic Structure

Most fish have streamlined bodies with scales covering their skin, use their fins for locomotion, and are vertebrates.

Gills

Gills are the respiratory organ most aquatic animals use to extract oxygen from water and to excrete carbon dioxide.

Water is pulled in through the mouth and pumped over the gill surface where oxygen is absorbed into the capillary beds before the water is then forced out through the side of the pharynx. A bony covering protects the external gill opening called the operculum. While all fish have gills, some fish can gulp air directly from the surface.
Fish have highly developed sense organs. Sensory nerve cells or sense organs used for smell or taste are called chemoreceptors. These respond to chemical stimuli. The lateral line detects currents, vibrations, and nearby movements in the water.

**MALT**

The Mucus Skin Barrier or Mucosa-Associated Lymphoid Tissue (MALT) is an important component of the fish immune system. Since aquatic environments contain microorganisms, the mucosal immune response is significant in fighting off disease and illness in fish. The mucus layer prevents attachment of parasites, acts as a bandage for wounds to prevent infection and helps prevent desiccation (drying).

**Eye**

A fish has eyes like those of humans and birds. Most fish can see in color and see ultraviolet or polarized light.

**Metabolism**

Most fish are ectotherms meaning they rely primarily on their environment for regulation of body temperature. The metabolic rate for fish varies greatly depending on size and activity levels but is strongly dependent on its environment. Water temperature, oxygen levels, food availability, and salinity, can affect growth and reproduction rates.
Behavior

Fish behavior can vary widely by species.

- **Social**: can range from territorial to a dominance hierarchy.
- **Defensive Behaviors**: Species exhibit different defensive behaviors such as:
  - **Prey Species**: These usually appear in schools; others excrete toxic liquids or shoot barbs.
  - **Predators**: These are most often solitary creatures who can be very aggressive.
- **Activity**: Vary per species. Some are active at night (nocturnal), and others are active during the day (diurnal)
- **Feeding**: Range from meat-eaters to small cell organisms. Examples:
  - **Meat eaters (carnivores)**
  - **Plant eaters (herbivores)**
  - **Plant and meat eaters (omnivores)**
  - **Primarily algae and microorganisms eaters (liminivores)**

Handling

Only handle fish when necessary since handling will cause stress. A brief stressful event affects blood chemistry for up to 24 hours in zebrafish.

Clean nets and small containers properly before use. Cleaning detergents, hand lotions, and other common chemicals are toxic to fish. Be careful when selecting, handling, and washing this equipment.

Restraint

Restraint methods vary by procedure and size of the fish. Avoid physical restraint of unsedated fish to prevent injury. However, if you must handle fish, use gentle restraint to protect the mucus-skin barrier. If you have questions or need assistance, contact the [UAB ARP Veterinarian Staff](mailto:UABARPVet@uab.edu).
Detecting Pain and Distress

Common Stressors

Everyday stressors for fish are poor water quality, overcrowding, handling, the introduction of new fish, and transportation.

Examples:

1. Dropsy: Abdominal swelling caused by deteriorating health
2. Fin Rot: Bacterial or fungal infection characterized by frayed or discolored fins
3. Mycobacteriosis: Caused by Mycobacterium sp.
   Symptoms include wasting, lesions, raised scales, and frayed fins
4. Microsporidiosis: Intracellular parasite affecting the nervous system and commonly spread through cannibalism. Symptoms include emaciation and curvature of the spine.

Signs of Pain and Distress

Signs of pain and distress include:

- Opercular flaring
- Flashing (swimming on its side in a circular motion)
- Inappetence (loss of appetite)
- Changes in coloration
- Increased respiration
- Changes in activity level
- Fins clamped close to the body
- Loss of buoyancy control
Procedures for Injections and Blood Collection

Injection Sites

Intramuscular Injections (IM) are commonly made into the large dorsal epaxial and the abdominal muscles (which avoids the lateral line and the ventral blood vessels).

Blood Collection

There are three common sites for blood collection in fish:

- Cardiac puncture – through the ventral side or operculum
- Venous puncture – tail vessels running along the vertebrae
- Caudal bleeding – terminal collection where the caudal fin is removed

Before performing these procedures, check with the UAB ARP Veterinarian Staff to make sure that you are adequately trained.
Analgesics, Sedatives, and Anesthetics

You should check with the UAB ARP Veterinary Staff for assistance in determining a dose rate appropriate for use in fish for analgesics and anesthetics.

### Analgesics

An analgesic is a medical term used for pain medicine or those drugs that help to control pain. Choose the analgesic regimen to match the expected duration of pain or discomfort caused by the procedure. If you have any questions, contact the UAB ARP Veterinary Staff.

### Anesthetics

Tricaine Methane Sulfonate (MS-222) and Benzocaine are the most common anesthetics for fish. When using these for anesthesia, be sure to monitor opercular motion to assess the depth of anesthesia. After the procedure, the fish should be allowed to recover in a tank of fresh, clean water.

### Surgery Techniques and Requirements

**Procedures**

The most common surgical procedures performed in research fish are fin clipping, tagging, or telemetry implants; however, other surgeries may be required as part of veterinary care (e.g., laceration repair, mass removal).

Rinse the skin surface but don’t disinfect. Disinfectants and surgical scrubs damage the protective mucus coating on the skin.
Sterile gloves, instruments, and equipment are required for surgery. For other procedures, clean all instruments and equipment. Plastic drapes help prevent desiccation (drying).

**Long Procedures**

For lengthy procedures, it may be necessary to bathe the gills in the anesthetic water to keep the skin moist, allow oxygenation, and maintain the anesthesia level.

**Euthanasia**

UAB requires two methods of euthanasia listed on your approved IACUC Protocol. If you have not been trained to euthanize fish, please contact one of the UAB ARP Veterinary Staff before attempting any procedures.

**Primary Methods**

Primary methods of euthanasia for fish can include an overdose of anesthetic (MS-222) or rapid chilling by submersion in ice water. When using the rapid chilling method, use a 50:50 mixture of ice and water. The fish must not be allowed to contact the ice directly.

**Secondary Methods**

To ensure death, you must follow primary euthanasia with a secondary physical method such as decapitation or maceration. Immersion in a 1.05% bleach solution may be used to euthanize embryos that are three days old or younger.

**Carcass Disposal**

After confirming euthanasia, place the carcasses, fry, and embryos in black carcass bags. Then put the black bags in the appropriate bin in the animal facility morgue.

There may be special disposal instructions for your fish or your housing facility, so follow any special instructions you are given.
Occupational Health Issues

Handwashing

Handwashing is the most important safety measure for the protection of both personnel and fish. Wash your hands:

- Before entering the room
- Between rooms or tank systems (to avoid contamination)
- Before directly handling the fish and leaving the room

Other Safety Issues

There are some precautions you should take when working with fish.

- Avoid tripping & electrical shock by proper handling and storage of electrical equipment & cords (keep electrical cords off of the floors).
- Slips and trips are common due to water on the floor. Watch your step.
- Eye protection is recommended whenever a splash hazard is present (e.g., cleaning tanks and equipment).
- Some fish have large or sharp teeth that cause severe injury if not handled properly (Piranhas).
- Some species of fish may pose a hazard due to venom excreted from the fins (Lionfish).

Allergens and Disease

Risk of transmission of zoonotic disease between fish and people is low. However, fish can carry a variety of bacteria and protozoa that can infect people (e.g., *Mycobacterium*, *Aeromonas*, *E. coli*, *Streptococcus*, *Staphylococcus*, *Cryptosporidium*). For more information contact EHS at (205) 934-2487.
Conclusion

This section concludes the Working with Fish in Research at UAB (AU_FI) Course Material. You should take the assessment now. The passing score is 80% or higher. For a glossary, reference guides, and other information visit the IACUC website.

Other Required Training

If you are working with any animal at UAB, you must complete Using Animals for Teaching, Testing, and Research at UAB (AU_UA).