IACUC Guidance:	**TAMU-G-008**	Title:	**Guidelines on Working with Zebrafish**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	10/20/2022	04/30/2025
Houston	N/A	N/A

1. PURPOSE

1.1. This document is intended to assist researchers working with Zebrafish in determining when Institutional Animal Care and Use Committee (IACUC) review is required, and to inform researchers of IACUC approved methods of anesthesia and euthanasia.

2. SCOPE

2.1. Applies to zebrafish used in research, teaching, testing or other purposes at Texas A&M University.

2.2. Does not describe analgesia. See TAMU-G-035, Appendix A.

2.3. Does not describe working with MS222, see TAMU-G-021.

2.4. Does not describe sanitation or evaluation of sanitation in depth, see TAMU-G-026.

3. RESPONSIBILITY

3.1. The PI is responsible for:

3.1.1. Completely describing the proposed animal activities in the animal use protocol for zebrafish three days post fertilization.

3.1.2. Ensuring all personnel are properly trained and documenting this training, according to TAMU-G-029.

3.2. The IACUC is responsible for:

3.2.1. Reviewing the stated experience and qualification of protocol participants and identifying any needed additional training requirements.

3.2.2. Inspecting and approving all surgical areas and procedure rooms (where surgery will occur) prior to use.

3.2.3. The PI and the AV share responsibility for ensuring that postsurgical care is appropriate.

3.2.4. Principal Investigator and Surgeon: Responsible to ensure appropriate surgical preparation, technique and monitoring for each animal as well as to oversee the animal's post-operative recovery period.

4. DEFINITIONS AND/OR ACRONYMS

4.1. **AAALAC:** AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

4.2. **AUP**: Animal Use Protocol. Document submitted by the PI indicating the housing and procedures involving animals.

4.3. **AV**: Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.

4.4. **AWAR/AWR**: Animal Welfare Act Regulations. Also known as Animal Welfare Regulations. Regulations administered by the United States Department of Agriculture (USDA). USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations.

4.5. **AWO:** Animal Welfare Office. Supports the IACUC administratively.

4.6. **DPF**: Days post fertilization

4.7. **HHS:** U.S. Department of Health & Human Services. Includes various agencies and offices, including the NIH (see list).

4.8. **HPF**: hours post fertilization

4.9. **IACUC**: Institutional Animal Care and Use Committee. Institutional body responsible for ensuring adherence to federal regulation and institutional policy relating to the care and use of animals in teaching, testing and research. Appointed by the Institutional Official.

4.10. **Environmental Enrichment**: The deliberate, variable, and scheduled additions to an animal's environment with which it can interact. The goal is to allow animals to express a range of species-typical behaviors which may enhance their well-being.
4.11. **(The) *Guide***: *The Guide for the Care and Use of Laboratory Animals.* Primary reference document for meeting the needs and requirements of animals used in biomedical research. The PHS Policy requires institutions to use the Guide as a basis for developing and implementing an institutional program for activities involving animals.
4.12. **Macro-environment:** The physical environment of the secondary enclosure or space outside of the immediate animal enclosure (e.g., room, barn, outdoor enclosure, etc.)
4.13. **Micro-environment:** The immediate physical environment surrounding the animal (i.e., the environment in the primary enclosure such as the aquaria, pan, cage, pen, run, stall or tank).
4.14. **NASA:** The National Aeronautics and Space Administration
4.15. **NSF:** National Science Foundation
4.16. **OLAW**: Office of Laboratory Animal Welfare. Provides guidance and interpretation of the Public Health Service (PHS) Policy on the Humane Care and Use of Laboratory Animals (Policy) for PHS-funded research and monitors compliance with the Policy by Assured Institutions.
4.17. **PHS:** Public Health Service. Federal agency under the United States Department of Health and Human Services responsible for oversight of the PHS Policy.
4.18. **PHS Policy**: PHS Policy on Humane Care and Use of Laboratory Animals. Federal policy established by the Health Research Extension Act of 1985 and administered by OLAW.
4.19. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
4.20. **Stocking Density**: The number of fish per volume of water.
4.21. **USDA**: United States Department of Agriculture. USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations (AWAR).
4.22. **Zebrafish Embryos**: Fish at 0-3 dpf. Although hatching occurs over a wider range of times, an arbitrary end to the embryonic period is defined by the attainment of the protruding mouth stage (~72 hpf at 28.5 °C).
4.23. **Zebrafish Larval Stage**: An individual that is no longer an embryo but has yet to become a juvenile (72 hours until the onset of metamorphosis).

5. GUIDELINES OR PROCEDURE

5.1. Regulation:

5.1.1. Zebrafish are not subject to regulations promulgated by the USDA under the Animal Welfare Act (AWA).
5.1.2. As an institution receiving HHS, PHS, NSF or NASA funding and/or holding AAALAC accreditation, zebrafish research must adhere to the PHS Policy and the general policies described in the *Guide*.

5.2. AUP Inclusion of Embryonic and Larval Stage Zebrafish:

5.2.1. Zebrafish embryos are not considered live vertebrate animals and do not need to be included in your AUP. However, a description of their use may be necessary as part of a complete description of experimental protocols involving adult breeding zebrafish.
5.2.2. Zebrafish larvae (> 3 dpf) are considered live vertebrate animals and must be included in the AUP.
5.2.3. There is no evidence to suggest the presence of higher order cognition in zebrafish during the first week of development (<8 dpf). The researcher/instructor may indicate the absence of pain perception in the AUP when working with these stages. The pain and distress categorization of the ≥8dpf fish should be determined by the investigator based on the specific procedures described in the protocol.
5.2.4. The number of animals used may need to be provided as an estimate, particularly with young larvae, considering their size and normal housing conditions.

5.3. Arrival and Identification

5.3.1. Upon arrival, live fish should be housed in a quiet, stress-free area of quarantine while they acclimate to the new water, new husbandry protocols, and new feed.

5.3.2. Tank labels are the most basic identification tools used in a zebrafish facility. Tank labels should be water-resistant, fit properly on the front of the tanks, be legible, and be printed so that the information does not fade.

5.3.3. Zebrafish are identified in terms of the specific genetic makeup of the strain. A complete description of the convention for naming zebrafish based on guidelines established by The Zebrafish Nomenclature Committee (ZNC) can be found on the Zebrafish Information Network (ZFIN) website.

5.3.4. See TAMU-G-014 for information on identification requirements.

5.4. Husbandry

5.4.1. Water Quality

5.4.1.1. Source water needs to be safe for fish and may need to be treated, filtered, and conditioned before entering the housing system.

5.4.1.2. Maintaining good water quality is essential. This maintenance depends upon the water flow system and may include: mechanical, biological, or chemical filtration, oxygenation and sterilization.

5.4.1.3. Development of facility-specific standard operating procedures (SOPs) for maintaining good water quality is recommended and should be made available to the IACUC upon request. See sample SOPs TAMU-F-025 and TAMU-F-027.

5.4.1.3.1. SOPs should describe cleaning and sanitation of the micro- and macro-environments and provide a description of the type and frequency of monitoring performed to maintain water quality specific to the type of housing utilized, e.g.: temperature, pH, alkalinity, nitrogen waste products (ammonia, nitrite and nitrate), conductivity/salinity, hardness (osmolality/dissolved minerals). Light cycle should also be described.

5.4.2. Nutrition

5.4.2.1. The natural diet of zebrafish consists of zooplankton, insects, algae, and invertebrate eggs. An appropriate diet, to be determined by the PI, should be provided to meet nutritional requirements.

5.4.2.2. Diet should be stored as described in TAMU-G-019.

5.4.2.3. Enrichment, such as algae or a fatty acid blend, should be stored as per vendor recommendations.

5.4.2.4. Food delivery methods should ensure that all animals are able to access food for a sufficient period of time while minimizing feeding aggression and nutrient loss (*Guide*, p. 84).

5.4.3. Housing

5.4.3.1. Zebrafish are usually kept in rectangular, transparent tanks or aquaria. The interior surface of the tank should be smooth, sealed, and inert. Tanks should be covered to prevent fish from jumping out.

5.4.3.2. Sanitation should be performed to allow viewing of fish in the tank or aquaria. See TAMU-G-026 for more information on sanitation.

5.4.3.3. Stocking density profoundly effects fish health, welfare, and productivity. A typical stocking density is 10 adult fish per liter of water.

5.4.3.4. Space requirements are affected by water quality, size, age, and feeding regimen and therefore may require optimization in each facility.

5.4.3.5. Additional Housing Recommendations (see REFERENCES, 7.1.11):

Zebrafish Characteristics	Housing Recommendations

Highly social species	• Group housing. Avoid isolation and housing in pairs but when unavoidable provide enrichment (e.g. silicone* or plastic plant) and visual and olfactory cues of conspecifics • Larval and juvenile fish should be housed separately from adults • House in mixed-sex groups to prevent egg over-retention in females
Shoal in small, mixed-sex schools	Tank size and stocking density should allow shoaling behavior where individuals can swim in a cohesive manner
Dominance hierarchies form via aggressive interactions	Avoid holding fish in small groups where aggression becomes problematic
Natural environment has abundant aquatic vegetation for spawning and cover from predation	Provide silicone* or plastic plants and an area or resource (e.g. plastic* tubes or large pebbles) for shelter
Naturally forage on zooplankton and insects	Should provide live food at least once a week
Olfaction and vision important sensory systems	Use of gravel, image of gravel or dark substrate. Fish housed in isolation should have olfactory and visual cues of other fish to reduce stress
Diurnal	Use of dusk and dawn lighting to avoid sudden bright light. 14:10 light:dark regime.
Preference for structural complexity within tank	Use of substrate (e.g. gravel, pebbles) and silicone* or plastic plants
Use of whole water column and constant swimming	• Tank design should allow swimming both vertically and horizontally. • Control water flow based on the age of fish (low to no flow for larval stages and increased rates of flow for adults)
*Use of silicone may assist in the avoidance of leached phthalates and other plasticizers from plastics. PIs should select the material which best supports their individual needs.	

5.4.4. Enrichment

5.4.4.1. Can affect several aspects of the biology of captive fish, for example, aggression, stress, energy expenditure, injury and disease susceptibility.

5.4.4.1.1. Enrichment reduces anxiety (see REFERENCES, 7.1.15)

5.4.4.2. Enrichment of fish may include:

5.4.4.2.1. Social enrichment with direct or indirect contact with conspecifics or humans. Indirect contact may imply visual, olfactory or auditory cues.

5.4.4.2.2. Occupational enrichment which can encompass psychological devices that provide animals with control or challenges as well as enrichment encouraging exercise such as mechanical devices.

5.4.4.2.3. Physical enrichment which can imply an alteration of the size or the complexity of the animal's enclosure. This includes the addition of objects, substrate etc.

5.4.4.2.3.1. Zebrafish females prefer to release eggs over a substrate (plants, rocks, marbles, etc.).

5.4.4.2.4. Sensory enrichment which could include visual, auditory, olfactory, tactile or taste stimuli.

5.4.4.2.5. Nutritional enrichment involving the type and delivery of food. The type of food can be varied or novel, while the delivery of food may imply the variation in frequency or presentation of food.

5.4.4.3. For zebrafish, the combination of both enrichment (silicone/plastic plants and gravel substrate) and exercise (swimming zones water flow set at 14 m/s) may be more important than either factor alone.

5.4.4.4. Performance standards should be applied taking into consideration the health, welfare and species-typical behavior.

5.5. Handling and Restraint

5.5.1. All forms of handling are stressful for zebrafish and should be minimized as much as possible.

5.5.2. Proper handling procedures will minimize trauma to the "slime layer", a protective coating of mucus which covers the skin and provides a barrier to infection. Use of soft nylon nets for capturing fish will minimize trauma. Using gloves when handling fish and placing fish on moist surfaces will help protect the slime layer.

5.5.3. Darkness or dim light seems to decrease the stress impact to fish that require handling. It is advisable to minimize loud noises and vibrations during manipulations to decrease stress to fish.

5.5.4. Fish should not be kept out of water for more than 90 seconds to prevent drying of the gills and skin. If fish must be handled repeatedly, it is advisable to allow time for recovery between events.

5.5.5. An adult zebrafish can be restrained by supporting it with a sponge or piece of Styrofoam with a V-shaped groove cut into it. The material of the restrainer should be clean, water compatible, non-abrasive and non-adhesive. Care should be given to the amount of pressure applied on the fish as well as to avoid direct contact with the eyes and gills. Chamois cloths are soft and protect the skin of the fish during handling.

5.6. Pain and Distress

5.6.1. It is highly likely that fish experience pain (see REFERENCES, 7.1.17). Because pain perception in fish is still under investigation, the use of analgesics should be considered (unless withholding is scientifically justified) whenever comparable procedures in other vertebrates would be perceived as painful.

5.6.2. Symptoms of Distress in Fish May Include:

Inappetence	Appetite can be an indicator of well-being in fish. Appetite may decrease when fish are stressed and energy reserves may be mobilized. Fish are less resistant to stress when deprived of food. Healthy zebrafish will feed actively when presented with palatable food items
Lethargy	Decreased swimming; inactivity they may be staying at the bottom or toward the very top of the tank.
Isolation	Zebrafish are schooling animals and stay together. A fish that remains separate from others may have an underlying problem.
Abnormal Swimming Pattern	Whirling or spiraling swimming often indicates neurological damage; often caused by ammonia toxicity.
Surface breathing	Fish "piping" for air at the surface of the water is often due to low dissolved oxygen in the water or supersaturation of dissolved oxygen.
Clamped fins	"Sticky" fins that cannot spread out, making it difficult for the fish to swim; caused by stress or disease.
Flashing	Quick twisting swimming motions, fish turning on their sides, or rubbing on the sides of the tank--commonly seen with ectoparasite infection.
Skin Lesions	External bacterial infections often cause skin lesions; signs of septicemia may be exopthalmia (pop-eye), abdominal swelling, ascites (dropsy) and disease of the

	internal organs. Chronic irritants such as external parasites or toxins in the water may result in excess mucus on the skin.
Body Color Changes	Zebrafish change their skin color in response to environmental clues, social subordination, and physiological stress such as crowding, excess light, dominance hierarchies, poor water quality or disease.
Petechiation or hemorrhage	A number of different bacterial infections can result in skin ulcerations, hemorrhage, and petechiation.
Improper buoyancy	Buoyancy control allows fish to swim in a controlled and purposeful manner. A fish should be able to maintain its position in the water column. It should not sink or float. Swimming behavior, swimming pattern, speed, and position in the water column are affected by factors such as stress and health.
Skeletal deformity	Nutritional deficiencies, microsporidian infections, genetics, and advanced age all contribute to skeletal deformities in zebrafish.
Respiratory rate	Abnormal opercular movement may indicate respiratory disease such as gill damage; respiratory rate assessed by observing and counting opercular movements; increased demand caused by physical movement.
Growth rate	Growth rate is affected by genetics, environmental parameters, stocking density, chronic stress, and nutrition. Zebrafish can grow even after sexual maturity. By establishing conditions for optimal growth, the rate of growth can be used as an indicator of health. Straightforward to measure using body length, mass and body condition.
Body Condition	Body shape can be assessed for changes that may be related to reproduction (egg bound) or illnesses due to infectious or non-infectious causes (water quality). *Pseudoloma neurophilia* can lead to body curvature. Mycobacteriosis can lead to emaciation.
External morphology	Non-specific changes such as frayed fins, discolored gills, protruding eyes may indicate chronic problems associated with poor water quality. Eye color and size should be bilaterally symmetrical. Exophthamos is the forward protrusion of an eye and is usually caused by sepsis.
Reproductive performance	Many factors influence reproductive performance of fish including stress, poor water quality, pheromones, nutritional imbalance, hormone imbalance, and age. Straightforward to measure using clutch size, egg viability, spawning frequency.

5.7. Anesthesia

5.7.1. To decrease stress and avoid trauma, fish should be anesthetized for any procedure beyond transfer from tank to tank:
- 5.7.1.1. Examination, weighing, measuring
- 5.7.1.2. Fin clipping for genotyping
  - 5.7.1.2.1. Only the minimum amount of tissue necessary (2-3mm is sufficient) should be taken from the distal margin of the upper or lower lobe of the caudal fin.
  - 5.7.1.2.2. Fins will completely grow back in 10-14 days.
- 5.7.1.3. Placement of chips, tags, etc.
- 5.7.1.4. Manual gamete removal
- 5.7.1.5. Surgical procedures

5.7.2. MS222 (Tricaine Methanesulfonate) is the agent used for anesthesia in zebrafish. See TAMU-G-021.

5.7.3. Hypothermia: Cooling the water slowly by adding ice will result in immobility of fish, but is not known to block pain receptors and is **not approved** for use when performing painful procedures.

5.8. Surgery

5.8.1. Skin mucus and scales act as important barriers against infection. Disinfection of the surgical site with chlorhexidine solution, iodine solution, or sterile saline with a cotton swab is a good practice to minimize contamination.
5.8.2. The skin should be kept moist during the procedure. Using a saturated sponge for restraint and positioning aids in hydration during manipulations.
5.8.3. Surgical sites can be closed using non-reactive, non-capillary suture material. Monofilament suture material such as nylon, polydioxanone (PDS), and polyglyconate (Maxon) are preferred.
5.8.4. Skin sutures should be removed in 10-14 days.

5.9. Post-Operative Care

5.9.1. Following surgery, zebrafish should be housed individually and monitored for signs of infection or abnormal behavior.
5.9.2. Care should be taken to maintain a clean tank to prevent opportunistic infections until the surgical site has healed.
5.9.3. Post-operative analgesia is an active area of research. Consult with the AV, or designee for current recommendations.
5.9.4. Analgesia in individual zebrafish (7.1.11):

Drug Class	Drug	Effective Dose
Local Anesthetic	Lidocaine	5 mg/l
Opioid	Morphine	48 mg/l 8 mg/kg IP 5 mg/kg IP 2.5-5 mg/kg IM
NSAIDs	Acetylsalicylic acid	2.5 mg/l
	Diclofenac	40 mg/kg IP
	Flunixin	8 mg/l
	Indomethacin	5-20 µl IP with 200 mg/l

5.10. Methods of Euthanasia of Zebrafish include but are not limited to:

5.10.1. MS222
5.10.1.1. Immersion of adult zebrafish in MS222 for **30 minutes** is recommended as a precautionary measure until research is available to demonstrate immersion times needed to reliably cause irreversible death in zebrafish.
5.10.1.2. A secondary (physical) method of euthanasia is recommended to ensure death.
5.10.1.3. MS222 alone is not effective for euthanasia of zebrafish larvae (< 14 days old), and other methods should be used for these life stages.
5.10.2. Sodium and Calcium Hypochlorite
5.10.2.1. Sodium hypochlorite (bleach) and solutions made from calcium hypochlorite granules act as solvents and oxidants in tissue, resulting in saponification of fatty acids, denaturation of proteins, and derangement of cellular processes.
5.10.2.2. Hypochlorite has been used to euthanize unhatched and hatched zebrafish up to 7 days after fertilization, after which time larvae are considered developed beyond an embryonic form and capable of experiencing distress or pain.
5.10.3. Rapid Chilling of Zebrafish
5.10.3.1. To ensure optimal hypothermal shock (i.e., rapid killing), transfer of fish into ice water must be completed as quickly as possible. This means rapid transition from acclimatization temperature to 2° to 4°C must be achieved. This can be accomplished by using minimal water volume to transfer fish (i.e., using a net to place fish in chilled water).

5.10.3.2. Fish should not be in direct contact with the ice in the water; rather a depression should be formed in the ice slurry to expose the entire surface of the fish to the chilled water. Full contact with cold water ensures optimal exposure and rapid chilling of the fish.

5.10.3.3. Water temperature must not exceed 2° to 4°C. Well-insulated containers, such as coolers, will assist in maintaining the ice slurry and a probe thermometer can be used to confirm water temperature.

5.10.3.4. Zebrafish is rapidly chilled until loss of orientation and cessation of opercular movements. The fish is exposed to additional chilled water for times specific to fish size and age to ensure death.

5.10.3.4.1. Rapid chilling of adult zebrafish has been reported to result in cessation of vital signs 20 times as fast as in the case of MS 222 overdose.

5.10.3.4.2. Adult zebrafish should be exposed for a minimum of 10 additional minutes following the loss of opercular movements.

5.10.3.4.3. Zebrafish larvae 4 to 14 days after fertilization (dpf) should be exposed for at least 20 additional minutes following loss of opercular movements.

5.10.3.5. If necessary to ensure death, rapid chilling may be followed by an approved adjunctive euthanasia method: decapitation, pithing, exsanguination, freezing, and other physical or chemical methods for destroying brain function.

5.10.4. Clove oil and its derivatives (isoeugenol, and eugenol)

5.10.4.1. Acceptable agents of euthanasia for fish.

5.10.4.2. Clove oil and its derivatives are not FDA approved for use as an agent of euthanasia. See TAMU-G-010 for guidance on nonpharmaceutical drug use.

5.10.4.3. Animals euthanized with clove oil products are not approved for human consumption.

5.10.4.4. Whenever possible, use products with standardized, known concentrations of essential oils for accurate dosing.

5.11. Recordkeeping

5.11.1. Water quality and environmental parameters should be recorded in a log book if automated system is unavailable.

5.11.2. Surgical and Anesthetic Records

5.11.2.1. See TAMU-G-035 for recordkeeping requirements for fish undergoing surgery.

5.11.2.2. Administration of anesthesia and analgesia and peri-operative monitoring should be recorded.

5.11.3. Records should include:

5.11.3.1. Fish Tracking: Assign unique, sequential stock numbers to track colony population and fish usage

5.11.3.2. Location: Movement of fish within and outside of a facility.

5.11.3.3. Breeding

5.11.3.4. Genetic Management

5.11.3.5. Images: See TAMU-G-042 for additional guidelines

5.11.3.6. Health Status/Daily Care logs

5.11.3.7. Mortality: See TAMU-G-015 for additional guidelines

5.11.3.8. Imports and Exports

6. EXCEPTIONS

6.1. The PI may request an exception to the above standards by describing the departure in the AUP

6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

7.1. References/Resources

7.1.1. AAALAC: ZebrafishReference (aaalac.org)
7.1.2. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
7.1.3. Cartner S, Eisen J, Farmer S, Guillemin K, Kent M, Sanders G, editors. 2019. The zebrafish in biomedical research 1st edition. Cambridge (MA): Academic Press.
7.1.4. CITI Program. Zebrafish: CITI Working with Zebrafish (Danio rerio) in Research Settings
7.1.4.1. Web page: https://about.citiprogram.org/en/homepage/
7.1.4.2. Instructions: https://rcb.tamu.edu/animals/training
7.1.5. DePasquale, Cairsty, et al. "The Impact of Flow and Physical Enrichment On PREFERENCES In Zebrafish." Applied animal behaviour science, v. 215, pp. 77-81. doi: 10.1016/j.applanim.2019.03.015
7.1.6. Felasa > Working Groups: Pain management in zebrafish
7.1.7. Gerber, B et al. Environmental Enrichment and its effects on Welfare in fish 2015.
7.1.8. Guide for the Care and Use of Laboratory Animals
7.1.9. Näslund, Joacim & Johnsson, Jörgen. (2016). Environmental enrichment for fish in captive environments: Effects of physical structures and substrates. Fish and Fisheries. 17. 1-30. 10.1111/faf.12088.
7.1.10. NIH: Guidelines for Use of Zebrafish in the NIH Intramural Research Program.
7.1.11. OLAW Conversations by ICARE: Improving Enrichment and Handling Practices for Laboratory Fish. Feb 24, 2022.
7.1.12. OLAW: Zebrafish 101 for IACUCs: OLAW Webinar 2015
7.1.13. Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. Dev Dyn. 2009;238(12):2975–3015. doi:10.1002/dvdy.22113
7.1.14. Reed, B. and M. Jennings. Guidance on the housing and care of Zebrafish (Danio rerio). 2011
7.1.15. Remy, M. & Gorissen, Marnix & Stokkermans, Mitchel & Zethof, Jan & Ebbesson, Lars & van de vis, Hans & Flik, Gert & Bos, Ruud. (2015). The Effects of Environmental Enrichment and Age-Related Differences on Inhibitory Avoidance in Zebrafish (Danio rerio Hamilton). Zebrafish. 12. 10.1089/zeb.2014.1045.
7.1.16. Singleman C, Holtzman NG. Growth and maturation in the zebrafish, Danio rerio: a staging tool for teaching and research. Zebrafish. 2014;11(4):396–406. doi:10.1089/zeb.2014.0976
7.1.17. Sneddon LU. 2019 Evolution of nociception and pain: evidence from fish models. Phil. Trans. R. Soc. B 374: 20190290. http://dx.doi.org/10.1098/rstb.2019.0290
7.1.18. Zebrafish Information Network (ZFIN)
7.1.19. For more information, please contact:
7.1.19.1. CMP: at (979) 845-7433
7.1.19.2. Sea Life Facility: at (409) 740-4574

7.2. IACUC/AWO Referenced Documents: (requires TAMU NetID authentication)
7.2.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards (not on web - contact AWO for copy)
7.2.2. TAMU-F-025 Housing SOP – Aquatic Species
7.2.3. TAMU-F-027 Housing SOP – Zebrafish Larvae
7.2.4. TAMU-G-010 Guidelines for the Use of Pharmaceutical and Nonpharmaceutical grade Drugs and Compounds
7.2.5. TAMU-G-014 Guidelines for the Identification of Research Animals
7.2.6. TAMU-G-015 Guidelines for Reporting Animal Concerns, Unanticipated or Adverse Events, and Potential Noncompliance
7.2.7. TAMU-G-019 Guidelines on Feed & Bedding Storage
7.2.8. TAMU-G-021 Guidelines for Preparing MS222

7.2.9. TAMU-G-026 IACUC Guidelines for the Evaluation of Sanitation Practices
7.2.10. TAMU-G-029 Guidelines for Animal Use Protocol Participation and Handling
7.2.11. TAMU-G-035 Guidelines on Performing Surgery in Fish
7.2.12. TAMU-G-042 Guidelines on Media Security

8. HISTORY

Effective Date	Version #	Description
06/01/2021	000	College Station/Galveston: New Document
03/24/2022	001	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
05/19/2022	002	College Station/Dallas/Galveston: Renewal; updated scope, definitions, and resources. Added additional housing recommendations. Added individual fish analgesia doses. Added Exceptions section.
10/20/2022	003	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.