**Thomas More University Institutional Animal Care**

**and Use Committee Protocol**

**Section I. General information:**

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| Date of application: | 3/11/2021 |
| Principal Investigator: | Hannah Abshire, Madison McVey, Animal Bx Lab Students |
| Department: | Psychology |
| E-mail: | [pughw@thomasmore.edu](mailto:pughw@thomasmore.edu) |
| Phone: | 859-344-3405 |
| Faculty supervisor: | Dr. Whitney Pugh |

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| Title of research project: | Learning & Memory in Fish |
| Protocol (select from pull-down menu): |  |

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| Course (dept. and number): | Animal Behavior Bio325/PSY325 |
| Anticipated start date: | **4/5/2021** |
| Anticipated completion date: | **4/16/2021** |

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| **Vertebrate study species** (use rows 2-4 if more than one species used) | | Number used |
| Common name | Latin name |
| **1.** Zebrafish | *Danio rerio* | 48-60 |
| **2.** |  |  |
| **3.** |  |  |
| **4.** |  |  |

Section II. Experiment description:

**A. Abstract:** This experiment will evaluate associative learning and operant learning abilities in zebrafish (*Danio rerio*), as well as the retention of the learned behavior. The experiment will use a visual cue (signal) for the presence or absence of food, and observe the behavior of the zebrafish in relation to this cue.

**B. Experimental design:** 48-60 zebrafish, approximately 1.5-2 inches in length, will be divided among five to six aquaria, maintaining 12 fish per tank. All fish will be purchased from local pet stores such as World of Pets or Petco. The experiment will occur in the tank in order to reduce stress that occurs from handling and relocation. Waterproof LED lights will be attached to either end of the aquaria. These LED lights are capable of changing color and will serve as the discriminative stimuli (cues) for the appropriate behavioral response. The colors selected will be either green or red, properly counterbalanced amongst the experimental groups. This will ensure our ability to detect and/or prevent any color preference amongst the fish.

For the experimental conditions, when the S+ (either green or red LED lights) is presented, food will be delivered. The S- (the opposite color) will be presented at the opposing side of the tank, and no food will be presented on the respected side of the aquarium. For the control conditions, the fish will be fed when the LED lights come on, but not in accordance with any specific color light. In other words, the reinforced color selected for that trial will be at random for each trial.

Training will occur on day one and should conclude on day four. Every 10th trial will be a probe trial to assess the rate of learning, in which the stimuli are presented without the consequence (food). The behavior will be measured based on latency to travel to the side of the aquarium with the discriminative stimulus. On Day 4 of training, a learning criterion will be evaluated. The learning criterion will be based on the number of fish that exhibit intentional movement to the discriminative stimulus (S+). A minimum number of 9 fish (75%) is the criterion. If the tank does not meet the learning criterion, and additional training day will be added. the fish demonstrating they have acquired the association between the consequence (food). All dietary needs will be met during the training trials on Training Day 1 through Training Day 4.

The fish will be tested 7 days after the last training trial for retention of the learned behavior. This retention test will assess the memory capabilities of the zebrafish. The 7-day retention test will have a single presentation of the two stimuli, and the behavior of the fish will be recorded. During the retention interval (Days five through 6), the fish will be fed the appropriate amount to sustain proper dietary conditions.

**C. Chemical application:** A non-toxic and odorless nail polish will be administered on various fins of the fish. See section IV(A) for more details.

**D. Invasive procedures:** No invasive procedures will occur.

**E. Behavioral apparatus and study:** The fish will remain in their home aquaria during the entirety of the experiment. Please see section VI(B) for more details.

# Section III. Surgical procedure (if applicable):

**A. Nature of any surgical procedure:**  No.**Nical procedure:** No.

**C. Behavioral and surgical effects**: No.

**D. Euthanasia:** No.

# Section IV. Assessment of pain and distress:

**A. Will the procedures of your studies cause animal subjects to experience momentary or slight pain or distress?**

Prior to the start of the experiment, all fish will need to be identifiable. This may cause a temporary and momentary stressor for the zebrafish, but is necessary for the experiment. The fish tails will need to be out of the water long enough to be marked prior to the experiment. No physical harm will occur to the fish.

Zebrafish are very similar in appearance, therefore, observing the schools of fish for humans to determine one fish from another is increasingly challenging. To identify single fish, a colored mark will be placed on the caudal fin, dorsal fin, or anal fin. The mark will be made using a nail polish that is non-toxic and odorless. Piggy Paint is a water-based nail polish that does not contain formaldehyde, toluene, acetone, Bisphenol A, and ethyl acetate. Nail polish such as Piggy Paint has been used to tag marine animals such as Sea Urchins, *Paracentrotuslividus* (see Cipriano, Burnell, Culloty, & Long, 2014).

**B. Will the procedures of your studies cause animal subjects to experience more than momentary or slight pain or distress?**

 No.

**C. Does the project involve the use of painful procedures or paralytic drugs without the benefit of anesthetics or analgesics?**

 No. 

**D. Is death (without euthanasia) an endpoint of the study?**

No.

# Section V. Animal care after the study:

**A. If euthanasia is required to complete the study, explain and justify.**

N/A

**B. If euthanasia is not required, what will happen to the animals after you complete the experiment?**

All surviving zebrafish will remain in the care of the Animal Behavior professor, Dr. Whitney Pugh. They will be housed in Dr. Pugh’s office for future studies of a similar nature.

 Section VI. Animal maintenance:

**A. Qualifications:** List name and qualifications (include education, training and experience) of individual(s) who will perform any procedures listed in sections II-III. List by procedure, including individual(s) who will monitor post-operative recovery, surgery, and euthanasia.

 Supervised by Dr. Pugh, PhD in Cognitive and Behavioral Sciences

**B. Housing:**

During the experiment, the fish will be housed in the green house room on the metal tables underneath the UVB lighting. All aquaria are 10-gallon tanks of tempered glass. There will be 12 fish per aquarium. Along the bottom of the tank is Aquarium gravel, in shades of blue. Each tank will have at least one decorative feature in which the fish can use to hide. Each tank will be fitted with an Aqua-Tech filter suited for tanks 5-15 gallons.

**C. Will animals stay outside of a Thomas More University lab or animal colony at any time?**

No. They will remain on campus immediately after purchase.

IACUC members reserves the right to inspect facilities if research animals are housed off campus.

**D. Supply:** Animal supplier (if known) and source of research funds:

World of Pets pet store.

Funds are provided by Thomas More University for the animal behavior laboratory. All funding was collected by the laboratory fees.

# Section VII. Investigator's assurance:

Federal regulations hold this University responsible for the conduct of animal research on this campus and specific associated facilities. In response to this requirement, the University has established the following procedures:

A. Approval of an Institutional Animal Care and Use Committee Protocol, by the Thomas More University Animal Research Committee, is required for all vertebrate animal use.

B. Any significant change in personnel, species usage, animal procedures, anesthesia, post-operative care or biohazards procedures must be reported in writing as appropriate. Committee approval of the proposed changes is required prior to proceeding with the revised animal procedures under all but emergency conditions.

C. Unannounced inspections and observations of animal quarters and/or experimental procedures may be performed by the IACUC members. Where procedures are causing severe distress to an animal and the pain cannot be relieved, veterinarians are authorized to humanely euthanize that animal. When possible, institutional veterinarians will always make a concerted effort to discuss such situations with investigators prior to initiating such drastic action.)

D. Investigator must ensure that the health and care of animal subjects be maintained throughout the entire course of the experiment. This includes that arrangements be made for appropriate future care of animals at the end of the experiment. Note that this applies if the investigator leaves campus (e.g., for a weekend or school break) during the course of study.

I HAVE READ THE ABOVE STATEMENTS AND AGREE TO ABIDE BY THE INSTITUTIONAL POLICIES GOVERNING USE OF VERTEBRATE ANIMALS. I FURTHER CERTIFY THAT THE PROPOSED WORK DOES NOT UNNECESSARILY DUPLICATE PREVIOUS EXPERIMENTS.

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| Date: | 3/12/2021 |
| Name: | Ehmet Thornton-Ayres |

NOTE:  Electronic transmission of this document from your e-mail account to your Faculty Supervisor will be interpreted as submission of a signed document

References

Cipriano, A., Burnell, G., Culloty, S., & Long, S. (2014) Evaluation of 3 tagging methods in marking sea urchin, *Paracentrotuslividus*, Populations under Both Laboratory and Field Conditions*.* J Aquac Res Development 5: 276 doi:10.4172/2155-9546.1000276