RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Zebrafish Environmental Enrichment		
SOP Category: Enrichment	**CMR SOP #: 6.09**	**Page: 1 of 2**
Effective Date: 10/02/2024	**Approval:** Roseann Kehoe	
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SCOPE:

This document describes the procedures to be followed when providing enrichment to zebrafish. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to summarize zebrafish environmental enrichment. This SOP is concordant with the Rutgers Environmental Enrichment Program, which states that all laboratory animal species used at Rutgers **must** be provided with species-specific environmental enrichment unless scientifically justified.

DEFINITIONS:
Natural Behavior: The Zebrafish (*Danio rerio*) is a tropical freshwater fish belonging to the minnow family. Zebrafish are native to the slow-moving waters of the Ganges River, located in the Himalayan region of Southern Asia. Zebrafish exhibit shoaling behavior in the wild, and are found in shallow well vegetated, clear pools of water with silty substrate/beds. Zebrafish rely on eyesight, water clarity and light to hunt live prey. Shoaling fish group together for social reasons and do not swim in a uniform group.

Abnormal Behavior:
Abnormal behaviors in zebrafish include:

1. Excessive aggression
2. Agitated/ abnormal shoal movement
    a. Lingering at the bottom of the tank
    b. Darting through the water
3. Decreased breeding

When these behaviors are observed, VS will evaluate the need for additional environmental enrichment.

PROCEDURES:

1. Social Enrichment
    - Zebrafish must be housed in social groups whenever possible; the size of the group is dependent on the size of the tank. The negative behaviors listed above may be displayed due to improper tank density.
    - Exceptions to group housing may be made due to signs of aggression and during mating.

2. Physical Enrichment
    - A laminated picture of natural rocks or a solid color (red, green, or blue) may be placed under the clear tank to mimic natural substrate.
    - Artificial plants will be placed in tanks of single housed fish. Artificial plants may also be added to tanks of group housed fish if abnormal behaviors are identified.

Figure 1: Examples of Artificial Plants and an Enrichment Runner for zebrafish tanks

3. Activity/food
    - Food enrichment options such as slow-release containers or other options may be considered if the study permits.
4. Sanitation of Enrichment Items
    - To prevent biofilm buildup on enrichment items placed in tanks (eg: plastic plants), these items should be removed from the tank and sanitized either when visibly dirty or when the tank is changed, whichever action comes first.

Considerations:

- Cost, availability of materials, safety issues for the animals and the staff, study constraints, and sanitation requirements must be evaluated for each idea.

- For novel devices or strategies, a group of animals may be selected for a pilot study to evaluate any behavioral changes, extra time needed in animal rooms, and additional steps necessary for sanitation of the devices.
- ACS assist in the environmental enrichment program by placing environmental enrichment devices in cages as directed by supervisor, investigators and veterinary staff. VS, ACS or designated RS within a facility are responsible for removing and replacing damaged or soiled devices, and sanitizing or disposing of devices as appropriate.

References:

Stevens CH, Reed BT, Hawkins P. Enrichment for Laboratory Zebrafish-A Review of the Evidence and the Challenges. Animals (Basel). 2021 Mar 5;11(3):698. doi: 10.3390/ani11030698. PMID: 33807683; PMCID: PMC8001412.