University of Colorado Denver (CU Denver)

Subject: **Zebrafish Overcrowding**	
Source: Institutional Animal Care and Use Committee (IACUC) Effective Date: 03/12/2018 Replaces: New Applies to: Personnel involved in research or teaching studies involving animals Reference: PHS Policy on Humane Care & Use of Laboratory Animals; Guide for the Care & Use of Laboratory Animals	IACUC ~:~ *03/12/2018*
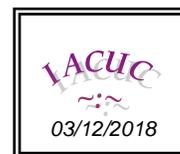
Introduction

The Institutional Animal Care and Use Committee (IACUC) maintains oversight of federally mandated rules and regulations of animal research for the University of Colorado Denver (CU Denver).

Policy Statement

This policy addresses zebrafish overcrowding, which can negatively affect animal welfare. It is a violation of federal regulations, the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) accreditation guidelines, our Public Health Service (PHS) Assurance and CU Denver policy to permit overcrowding.   A tank is overcrowded when the number allowed per tank exceeds the CU Denver IACUC maximum tank density for the species which are based on the *Guide for the Care and Use of Laboratory Animals* (8th Edition) and recent scientific publications on zebrafish welfare. Only the IACUC may grant exemptions to this policy based on scientific justification. For any exemptions, it is the responsibility of the Principal Investigator (PI) or their staff to inform the Office of Laboratory Animal Resources (OLAR). OLAR will verify that the approval has been granted and assist the investigator in the proper labeling of the tank or rack where the tanks are held.

Responsibilities

- PIs and researchers:
    - MUST ensure that the number of fish per tank is acceptable per the regulations, AAALAC standards, as well as CU Denver IACUC policy.
    - MUST monitor their colony frequently enough to prevent overcrowding.
    - MUST perform responsible colony management by ensuring that fish are housed appropriately and at the proper housing density based on age and size of fish. Any deviations from this policy can impact animal welfare and potentially be reported to the IACUC and OLAW.
    - MUST ensure that there is sufficient space to house animals in the room. If adequate vacant space is not available in the housing room, the investigator must discuss this with OLAR.
- OLAR staff:
    - Will monitor the number of animals per cage during daily health checks. Will identify improperly housed animals and provide notice at tank level.
    - Will provide research staff until 11 AM of the second business day after being found to correct issues.
    - Will separate tanks that require correction at the due date/time and charge $50 for the service.

Numbers per Tank

In order to provide the best welfare for the research animals and to allow investigators the opportunity to perform research without undue restrictions, several tank sizes are available. The investigator should choose the best tank size for their research and for the age of fish that they are working with. Fish are a schooling species and when housed at low densities aggression has been noted. Therefore, group housing fish (>3 fish in a tank) is required unless there is a justification in the IACUC protocol. In-tank enrichment objects (e.g. non-toxic artificial aquarium plants) may also be used to provide a visual barrier to reduce aggression at low densities.[1]
Responsible and active colony management is required.   Any noncompliance with this policy that is an animal welfare issue, as determined by the veterinary staff, will require immediate corrective action by OLAR staff. Any separation performed by OLAR will result in a $50 charge to the PI.

The Guide recommends a stocking density of 5 adult zebrafish per liter of water, though it notes that this number may change as our knowledge about zebrafish welfare advances.[2-5] More recent publications have not found significant differences in growth and/or breeding performance in zebrafish housed in modern systems at higher densities, suggesting these may also be appropriate.[6,7] Hence, the CU Denver IACUC recommends a housing density of 5-7 adult zebrafish per liter, but up to 12 adult zebrafish per liter may be permitted as long as no animal welfare issues are identified.

Tank Size	Maximum number of adult fish
1.4 Liter	16
1.8 Liter	21
3 Liter	36
6 Liter	72
9 Liter	108

Procedures

- Separations performed by OLAR for overcrowded tanks will incur a $50 charge to the PI.
- Tank flags will be the only notification to the PIs for overcrowded tanks. OLAR will not send email notifications regarding overcrowded tanks.
- Animal care providers will place a completed "Overcrowded Notice" flag on the tank with the date that the overcrowding must be corrected by. The PI will have until 11 AM on the second business day to correct the situation or OLAR staff will separate the animals.
- Severely overcrowded tanks
  - o Tanks that have more than twice the maximum number of animals permitted are reported to a veterinarian as an animal welfare concern. They will be separated immediately if the PI cannot be contacted. Ultimately, concern for animal welfare overrides investigator notification.
- Placing tank flags
  - o Per the Animal Identification IACUC policy, the research staff must immediately label tanks with appropriate identifying information including the birthdate of the animal and the PI name and ensure that the number of animals 3 days old and older are appropriately documented for deduction from the protocol. If tanks are not identified OLAR staff will contact the PI and they will have 72 hours to appropriately identify the tank or the PI will be charged $5 per tank for OLAR staff to label the tanks. All tanks must be placed on rows that contain a cage card. If a tank is placed on a row without a cage card, the Zebrafish facility manager must be notified within 72 hours of tank placement so that a new card can be ordered or the PI will incur a $5 per cage card.

Exceptions to this policy will be considered by the IACUC on a case by case basis on presentation of adequate scientific justification.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at CU Denver might be temporarily suspended or even completely revoked.

References

1. Keck VA, Edgerton DS, Hajizadeh S, et al.(2015) Effects of Habitat Complexity on Pair-Housed Zebrafish. Journal of the American Association for Laboratory Animal Science : JAALAS. 54(4):378-383.
2. Institute for Laboratory Animal Research (2011). Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press
3. Brand, M., Granato, M. & Nüsslein-Volhard, C. (2002) 'Keeping and raising zebrafish' in NüssleinVolhard & Dahm (2002) Zebrafish - A Practical Approach ; Oxford University Press, Oxford, UK.
4. Matthews, M., Trevarrow, B. & Matthews, J. (2002) 'A virtual tour of the Guide for zebrafish users' Lab Animal 31 (3), p34-40
5. Vargesson, N.A. (2007) 'Zebrafish' in Manual of Animal Technology (ed. S. Barnett) Blackwell Publishing Ltd: Oxford, UK.

6. Castranova, D., et al. (2011). The Effect of Stocking Densities on Reproductive Performance in Laboratory Zebrafish (Danio rerio). Zebrafish, 8(3), 141-146. doi:10.1089/zeb.2011.0688
7. Rabbane G., Rahman M., and Hossain A. (2016) Effects of stocking density on growth of zebrafish *(Danio rer*io, Hamilton, 1822). Bangladesh J. of Zool. 44(2): 209-218.