# University of Maine Institutional Animal Care and Use Committee (IACUC) Guidelines for Use of Zebrafish in Research, Teaching, and Testing

## General Guidelines

This document is intended to assist researchers/instructors working with Zebrafish in determining when Institutional Animal Care and Use Committee (IACUC) review is required. These guidelines were adapted from the “Guidelines for Use of Zebrafish in the National Institutes of Health (NIH) Intramural Research Program.”

Current Office of Laboratory Animal Welfare (OLAW) interpretation of Public Health Service (PHS) policy considers aquatic species as "live, vertebrate animals" at hatching (as does the University of Maine’s Policies and Procedures for the Humane Care and Use of Animals). Although this is an imprecise stage for Zebrafish, it can be approximated at 72 hours post fertilization. The IACUC has agreed on the following guidelines for all research, teaching, or testing activities involving Zebrafish:

* 0-3 days post fertilization (dpf), IACUC protocol submission for approval ***is not***required; however, euthanasia guidelines must be followed (see below).
* 4+ dpf, IACUC protocol submission for approval ***is*** required. Since early stages (4-7dpf) do not feel pain or distress, the researcher/instructor may indicate Class C for the pain when working at those 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.
* If proposed studies involve fish at both stages 1 and 2 above, the protocol should mention the use of Zebrafish at 0-3 dpf, but only descriptions of procedures at the 4+ dpf stage are required.

## Scientific Background

These guidelines are predicated on the need to minimize suffering and distress in Zebrafish. Suffering requires that the animal have both the neural apparatus for detecting noxious stimuli as well as the mental ability to interpret such stimuli as aversive (1). Many studies have demonstrated that adult Zebrafish show evidence of higher order cognition, being responsive to a variety of learning protocols (e.g., 2, 3, 4, 5), including learning to avoid aversive stimuli (6, 7, 8, 9). Thus while the ability of adult fish to experience suffering remains controversial in the scientific literature [for recent reviews reaching conflicting opinions see (10) and (11)], there is sufficient evidence to take a cautious approach in adult Zebrafish by instituting guidelines that ensure rapid euthanasia.

In contrast, there is no evidence of higher order cognition in Zebrafish during the first week of development although this may change as research techniques in pain perception science improve (12). Developmental studies examining learning (13), reward (14), social (15, 16) and fright (17) behaviors have found that these functions become operational only in older fish. During the first week of development, embryonic movements are simple reflexes that do not provide evidence for a capacity for suffering. Thus during the first week, Zebrafish larvae can respond to simple stimuli but are assumed not to have reached the point in brain development where stimuli can be experienced as aversive.

Zebrafish larvae during the first week resemble early mouse embryos in that they are chiefly sustained by nutrients derived from the yolk. The criterion of nutritional independence for developmentally immature animals is subject to empirical verification and has found support in international regulations for the welfare of immature vertebrates (18). While the capacity for suffering is the primary criterion for establishing a threshold for 8 days post fertilization (dpf) for euthanasia in Zebrafish, the criterion of independent feeding also supports this age.

Hatching occurs at approximately 72 hours (which would be at the end of day 3 post fertilization), although hatching is not an accepted staging index in Zebrafish (19). Zebrafish larvae are not able to feed upon hatching and are sustained by nutrients derived from the yolk, which is not depleted until 7 dpf (20). Only after 7 dpf do Zebrafish larvae manifest signs of ill health in the absence of external feeding (21). Active feeding cannot commence at hatching because brain structures required for detecting and catching prey have not developed and the mouth and gut are occluded. At hatching, larvae lack taste buds (22, 23), have poor visual acuity (15), and cannot swim effectively as they lack a swim bladder and have deficient motor control (24, 25). Therefore, in Zebrafish the period between hatching and nutritional independence at 8 dpf is essentially an extension of the early embryonic stage during which the fish continues to develop sensory and motor functions required for the independent larval stage.

## Euthanasia Guidelines

The acceptable method of euthanasia of Zebrafish at all stages is by overdose of tricaine methane sulfonate (MS222, 200-300 mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. A request for an exception to use any other method must be submitted to the IACUC for review/approval.

Zebrafish carcasses should be disposed of as according to University policies.

## References

1. Sneddon LU, Braithwaite VA, & Gentle MJ (2003) Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc. R. Soc. Lond. B* 270(1520):1115-1121.
2. Darland T & Dowling JE (2001) Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc Natl Acad Sci U S A* 98(20):11691-11696.
3. Colwill RM, Raymond MP, Ferreira L, & Escudero H (2005) Visual discrimination learning in zebrafish (Danio rerio). *Behavioural Processes* 70(1):19-31.
4. Risner ML, Lemerise E, Vukmanic EV, & Moore A (2006) Behavioral spectral sensitivity of the zebrafish (Danio rerio). *Vision Res* 46(17):2625-2635.
5. Braubach OR, Wood HD, Gadbois S, Fine A, & Croll RP (2008) Olfactory conditioning in the zebrafish (Danio rerio). *Behav Brain Res* doi:10.1016.
6. Pradel G, Schachner M, & Schmidt R (1999) Inhibition of Memory Consolidation by Antibodies against Cell Adhesion Molecules after Active Avoidance Conditioning in Zebrafish. *Journal of Neurobiology* 39(2):197-206.
7. Pradel G, Schmidt R, & Schachner M (2000) Involvement of L 1. 1 in memory consolidation after active avoidance conditioning in zebrafish. *Journal of Neurobiology* 43(4):389-403.
8. Rawashdeh O, de Borsetti NH, Roman G, & Cahill GM (2007) Melatonin Suppresses Nighttime Memory Formation in Zebrafish. *Science* 318(5853):1144.
9. Shcherbakov D*, et al.* (2005) Magnetosensation in zebrafish. *Current Biology* 15(5):161-162.
10. Braithwaite VA & Boulcott P (2007) Pain perception, aversion and fear in fish. *Diseases of Aquatic Organisms* 75(2):131.
11. Rose JD (2007) Anthropomorphism and'mental welfare' of fishes. *Proc. R. Soc. Lond. B* 75(2):139-154.
12. Braithwaite, VA (2010) [Do Fish Feel Pain?](http://www.oupcanada.com/catalog/9780199551200.html) *Oxford University Press*. [ISBN 9780199551200](http://en.wikipedia.org/wiki/Special:BookSources/9780199551200)
13. Williams FE, White D, & Messer WS (2002) A simple spatial alternation task for assessing memory function in zebrafish. *Behavioural Processes* 58(3):125-132.
14. Bretaud S*, et al.* (2007) A choice behavior for morphine reveals experience-dependent drug preference and underlying neural substrates in developing larval zebrafish. *Neuroscience* 146(3):1109-1116.
15. Clark DT (1981) Visual Responses in Developing Zebrafish (Brachydanio rerio). Ph.D. (Oregon, Eugene).
16. Engeszer RE, Da Barbiano LA, Ryan MJ, & Parichy DM (2007) Timing and plasticity of shoaling behaviour in the zebrafish, Danio rerio. *Anim Behav* 74(5):1269-1275.
17. Whitlock KE (2006) The Sense of Scents: Olfactory Behaviors in the Zebrafish. *Zebrafish* 3(2):203-213.
18. United Kingdom (1986) Guidance on the Operation of the Animals (Scientific Procedures) Act.
19. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, & Schilling TF (1995) Stages of embryonic development of the zebrafish. *American Journal of Anatomy* 203(3):253–310.
20. Jardine D & Litvak MK (2003) Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. *Journal of Fish Biology* 63(2):388-397.
21. Goolish E & Okutake K (1999) Lack of gas bladder inflation by the larvae of zebrafish in the absence of an air-water interface. *Journal of Fish Biology* 55(5):1054-1063.
22. Kotrschal K, Krautgartner WD, & Hansen A (1997) Ontogeny of the Solitary Chemosensory Cells in the Zebrafish, Danio rerio. *Chem Senses* 22(2):111-118.
23. Lindsay SM & Vogt RG (2004) Behavioral Responses of Newly Hatched Zebrafish (Danio rerio) to Amino Acid Chemostimulants. *Chem Senses* 29(2):93-100.
24. Robertson GN, McGee CA, Dumbarton TC, Croll RP, & Smith FM (2007) Development of the swimbladder and its innervation in the zebrafish, Danio rerio. *Journal of Morphology* 268(11):967.
25. Muller U & van Leeuwen J (2004) Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development. *Journal of Experimental Biology* 207(5):853-868.

Approved 06/27/2011