

ZEBRAFISH AND OTHER SMALL, WARM-WATER LABORATORY FISH

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These frequently asked questions (FAQs) are intended to assist investigators, instructors, and members of animal care committees in the implementation of the *CCAC guidelines: Zebrafish and other small, warm-water laboratory fish* (CCAC, 2020). FAQs provide general responses to comments and questions received by the CCAC during the external reviews of this guidelines document.

If you do not find the answer to your question here, do not hesitate to [contact the CCAC](#) and we will be pleased to provide assistance.

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1. When is it safe to add fish to a new tank?

Regardless of the system being used, it is critical that all tanks are commissioned before fish are introduced. This involves running the full battery of water quality tests described in the guidelines over a 24 hour period. Once the water quality of the tank meets the specifications listed in the guidelines, a small number of fish can be added, with the number of fish gradually increased, to minimize any potential negative impacts. During this process, all fish should receive the care and attention outlined in the guidelines, including the first few that are added.

2. Why do the guidelines support the addition of substrate to tanks when it can affect water quality and have detrimental effects on the health of the fish?

Water quality is recognized to be of critical importance to the health of zebrafish, with the following two guidelines statements dedicated to this:

Guideline 2: Facilities must ensure an adequate amount of water of suitable quality is provided at all times for the species and life stage of the fish being housed.

Guideline 3: Water quality variables must be routinely monitored to permit predictive management of water quality. Contingency plans must be in place to deal with deviations from acceptable limits for the species being held.

The welfare of zebrafish is also important and can be improved by paying attention to the natural behaviours of the species. Zebrafish have been shown to prefer environments with substrate. In situations where the addition of substrate impedes cleaning and may affect water quality, positioning photographs of natural surroundings under the tanks is recommended as an alternative.

3. CCAC guidelines documents now put greater emphasis on welfare assessment. What is expected of those working with zebrafish and what tools are available?

Welfare assessment is a necessary component of all animal-based studies to ensure a good quality of life for the animals within the constraints of the study, and for the quality of scientific data. The upcoming *CCAC guidelines: Animal welfare assessment* (in prep.) details the general requirements for all animals, and the *CCAC guidelines: Zebrafish and other small, warm-water laboratory fish* (CCAC, 2020) builds on this foundation to provide indicators that can be used in developing welfare assessments for zebrafish. The zebrafish guidelines list a number of health indicators. In terms of behavioural indicators, the document provides some indicators of negative welfare (escape behaviour, frantic movements, significant reduction in activity, increased respiration, blanching of colour, and occupying lower locations in the water column), but notes that fish do not always display a response to adverse conditions and an absence of these behaviours does not necessarily mean that the fish have good welfare. It is important to keep up-to-date with further work in this area.

4. Given the health and welfare concerns of transporting adult zebrafish, why do the guidelines not specify that they can only be transported as fertilized eggs or early larvae?

The guidelines state that zebrafish should be shipped as embryos, and that embryos should be surface sanitized using a process that has been documented as effective in reducing the spread of pathogens. The subcommittee recognized that some facilities only use adult zebrafish and do not have the capacity to handle eggs or early larvae. However, the use of the term “should” requires the decision to ship adult fish to be fully justified to the animal care committee

5. There has been an ongoing debate in the scientific literature as to whether fish experience pain. How has this been addressed in these guidelines?

As in the *CCAC guidelines on: the care and use of fish in research, teaching and testing* (CCAC, 2005), the zebrafish guidelines recognize that the literature concerning pain in fish is contentious and cite a more recent literature review. Given this continued debate, the zebrafish guidelines follow the precautionary principle: fish should be assumed to perceive pain in a way analogous to mammals, and therefore anesthetics and analgesics should be considered for methods that may cause pain.

6. When are anesthetics and analgesics required for work with zebrafish?

The guidelines state that anesthetics must be used in procedures where there is expected to be noxious stimuli, and in experiments entailing extensive handling or manipulation with a reasonable expectation of trauma and physiological insult to the fish. There is considerable evidence for the use of a number of anesthetics on fish, and this is detailed in section 10.4.1, “Anesthesia”, along with a note that researchers should ensure they stay informed of latest scientific literature on appropriate anesthetic regimes for fish.

There is currently less evidence available for the use of analgesics in fish; however, zebrafish welfare is a rapidly growing field and more information is expected to be available in the near future. To acknowledge this situation while ensuring zebrafish receive optimal care, the guideline in section 10.4.2, “Analgesia”, states “Following the precautionary principle, fish should be provided with analgesia for procedures that are likely to be painful, based on the best available scientific evidence”.