

Focus on Fish

February 2022

Summary report

In February 2022, the RSPCA hosted the second 'Focus on Fish' meeting, a one-day online conference on practical refinements for fishes in research and testing, which was attended by over 500 delegates from more than 40 countries around the world. This meeting followed on from the first highly successful meeting which was held in 2021.

Fishes continue to be commonly used in scientific procedures around the world, but there remains a pressing need to increase understanding of how to minimise harms and improve welfare. This meeting brought together experts to share knowledge and promote approaches and opportunities to refine and reduce the use of these animals.

The meeting consisted of three sessions. The first session opened with an introduction from the RSPCA, which was followed by a presentation on the **PREPARE guidelines** - a set of guidelines developed by Norecopa, with the support of the RSPCA, aimed at helping researchers plan experiments in order to reduce waste, promote alternatives (all the three Rs), and increase the reproducibility of research and testing. The PREPARE website now includes specific information on fishes to help those people using and caring for fish to quickly identify relevant information. The next talk was entitled '**What zebrafish want: Rearing with preferred environmental enrichment increases survival and accelerates learning**', and discussed how adding environmental enrichment to zebrafish tanks reduces mortality, increases longevity, and improves cognition. The final talk of the session was on the **effects of holding density on zebrafish welfare**, which discussed how holding density is highly variable between different zebrafish facilities, and how the apparent impacts of holding density on welfare seem to vary according to the densities studied and the welfare parameters chosen. The following discussion session addressed a range of topics including where the information gaps lie with respect to the welfare of fishes in research and testing. It was generally agreed that a key issue for fish welfare is the lack of species-specific information and recommendations on good practice.

In sessions two and three, talks covered: the use of score sheets and **humane endpoints** for refinement of fish studies; how different anaesthetics and **anaesthesia protocols** may be appropriate for zebrafish depending on the study; a comparison of the efficacy of different methods of **humane killing** of laboratory fish; the ethical and welfare issues associated with

fish surgical **models of cardiac regeneration**; alternatives to the use of fish in the **testing of bioaccumulation of nanomaterials**; and finally, how understanding of fish mucosal surfaces (such as the skin, gills, gut and olfactory organ) can help advance the application of the 3Rs in research into **fish health**. Participants then discussed topics including the use of anaesthesia for procedures like fin-clipping, alternatives to fin-clipping such as skin-swabbing fish or genotyping larvae, and how to choose the most appropriate anaesthetic for a particular procedure. The meeting closed with a reflection on the continued need to elevate the welfare status of fishes so that they are valued more and given the same level of consideration as other vertebrates.

Action Points

The presentations and discussions raised a number of themes and practical approaches to reducing suffering and improving welfare. These included:

- Different fish species can vary hugely in their needs and responses to stimuli. Wherever possible, refer to species-specific information and guidance when it comes to housing and husbandry, procedures and welfare assessment.
- More species-specific information is needed on aspects of housing, husbandry and care. Reporting as much detailed data as possible can help expand the relevant knowledge base, also allowing data to be compared and meta-analyses to be conducted. Online repositories may be used to report information, such as water quality data, which may be more efficient than only including this information in publications.
- The PREPARE guidelines, developed by Norecopa, can be used to evaluate all the issues which might affect validity, reproducibility and translatability. New sections have been added to the [PREPARE website](#) with resources specific to fish.
- Zebrafish express different degrees of preference for a range of environmental enrichments. Providing them with their preferred conditions improves zebrafish survival and accelerates learning, therefore enrichment should be provided for zebrafish.
- Different holding densities of zebrafish can have effects on parameters which are relevant to welfare, including reproduction, stress and behaviour. The intended outcomes and context should be considered when choosing the appropriate holding density for zebrafish, and the details of animal housing and handling should be reported in detail.
- Score sheets - standardised health and welfare assessment sheets for animal studies - can be useful tools for the application of humane endpoints, and can ensure traceability and transparency in what has been recorded. They should be tailored to specific studies and should regularly be reviewed to ensure they are effective and efficient to use.
- Different anaesthetics induce different levels of anaesthesia in zebrafish - this should be considered when choosing an agent, dose and regime. When the procedure requires

analgesia, MS-222 and propofol/lidocaine protocols may be most appropriate (see [Jorge et al. \(2021\) *Zebrafish*, 18: 330-337](#)).

- A number of factors can influence the efficacy of a given anaesthetic, such as age, strain, overall health and stress levels, repeated anaesthesia and the temperature and pH of the anaesthetic bath. All of these should be considered when choosing a protocol.
- Methods of humane killing of zebrafish differ in appropriateness between larvae and adults. Hypothermic shock appears to work quickly for adults but may cause distress, while electrical stunning devices may work for larvae but need further assessment for adults. The best all-round option for all developmental stages currently appears to be a combination of lidocaine, sodium bicarbonate and ethanol - further guidance is expected to be published by the FELASA Working Group on the Humane Killing of Laboratory Fish in due course.
- The [ARRIVE guidelines](#) are useful when reporting experiments. To adhere to these, papers should include details of the welfare impacts on the animals involved, as well as details of how welfare was monitored and assessed during the study, and the humane intervention points used.

The conference **abstract booklet** can be requested by emailing animalsinscience@rspca.org.uk, and a **full recording** of the event is available to view at www.focusonfish.co.uk. More resources on the housing, husbandry and care of laboratory fishes can be found on the [RSPCA website](#).

The RSPCA would like to thank all of the speakers at the meeting.

This summary report has been produced by the RSPCA Animals in Science Department.

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