Focus on Fish 2024

Abstracts

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An adaptable, user-friendly score sheet to monitor welfare in experimental fish

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Fish are increasingly used as experimental animals across research fields, yet welfare assessments for experimental fish are in their infancy compared to rodents. This lack of guidelines and tools presents a challenge for researchers (particularly, for newcomers), for ethics committees, and for implementing refinement measures.

Here, we present an adaptable, user-friendly score sheet for fish. The parameters contained in the excel tool are based on a literature review, have been validated by expert interviews, and evaluated by a fish pathologist. The tool allows to score individuals as well as groups, calculates summary scores and visualizes trends. We provide the underlying literature, give use examples and provide instructions on the adaptation and use of the score sheet.

We hope that this tool will empower researchers to include welfare assessment in their routines, foster discussions on fish welfare parameters among scientists, facilitate interactions with ethics committees, and most importantly, enable the refinement of fish experiments.

Guidance for checking of laboratory fishes

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To safeguard the welfare of laboratory animals, routine checks of the animals, their housing systems and environmental conditions must be performed. For animals undergoing regulated procedures, animal checks must additionally assess adverse effects to enable actual severity assessment, humane interventions to limit suffering and ensure authorised severity limits are not exceeded. Furthermore, records of adverse effects often represent scientific data, integral to analysis of treatment effects. Laboratory fishes are not amenable to routine physical examination or non-invasive physiological measures, so animal checks are limited to observable clinical signs, i.e. health and behaviour indicators that are visible in situ. The quality of the checks is therefore determined by the performance of personnel. Despite the importance of such checks, published guidance is limited. Guidance is needed to train and confirm competence, and to standardise the scientific data gathered within and between establishments. Here we summarise practice at an establishment with experience of a variety of fish species.

FELASA-AALAS Recommendations for Monitoring and Reporting of Laboratory Fish Diseases and Health Status, with an Emphasis on Zebrafish

J-P Mocho

DanioVet

Fish may be exposed to pathogens (e.g. bacteria, fungi, parasites, and viruses) and adverse husbandry conditions capable of affecting both experimental results and fish and personnel health and welfare. To help identify such issues and support the safe exchange of fish between research laboratories, FELASA and AALAS established a joint working group with the task to advise on good practices for fish health monitoring and biosecurity. In a first manuscript, the working group guides the reader towards disease literature, makes recommendations for fish health monitoring (e.g. type and number of samples, screening pattern) and proposes templates to describe fish facilities and report health status. The second manuscript focuses more on biosecurity. Management of zoonotic hazards are discussed. Then, the working group provides recommendations to optimise barriers, staff and animal flows, water treatment, sanitation and personnel engagement with the biosecurity programme, including quarantine and import policy. A previous survey identified the latter concepts as in particular need of improvement in fish laboratories. The manuscripts take zebrafish (*Danio rerio*) as a lead example to illustrate the concepts through various scenarios and expand to the case of multi-species facilities.

Applying the 3Rs principles in wild fish research

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The ethical framework encompassing the 3Rs principles (Replacement, Reduction, and Refinement) has long guided research involving animals to ensure humane and responsible scientific practices. However, applying these principles in the context of wild fish research presents unique challenges and opportunities, given the complexities of aquatic ecosystems and the inherent difficulties in studying fish in their natural habitats. This talk will explore innovative approaches to integrate the 3Rs into wild fish research, emphasizing strategies that minimize welfare impact while maximizing scientific and conservation outcomes.

Guess who! The use of pattern recognition for individual ID

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AI and facial recognition has been an increasing technology with mass capabilities. The same concept has been applied to species for individual identification, using spot patterns to identify individual sharks for example. Pattern recognition through photographs cannot only save the user time when conducting experiments, it can also reduce stress on individuals, improving welfare. Here, we discuss the use of spot pattern recognition software in controlled and wild environments on a range of fish and amphibian species. We discuss the barriers and potential uses on further species, and other methods of recognition which reduce handling and impact welfare.

Refining the use of general anaesthesia in embryo larval zebrafish: effective and tolerated concentrations of six commonly used fish anaesthetics

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Zebrafish (*Danio rerio*) are one of the most widely used laboratory animals in scientific research worldwide. Most experimental procedures are undertaken in the embryo-larval life stages, (typically <7 days-post-fertilisation or dpf), and many involve the use of anaesthesia, either as the first step towards euthanasia, or for procedures such as sedation for microscopic evaluation. Despite this, there is very little guidance available for humane and appropriate anaesthetic choices for fish, particularly for embryo-larvae. This knowledge gap has considerable implications both for the welfare of these animals, and for experimental design more widely. Our study used 4.5 dpf zebrafish embryos to explore the sedative and aversive characteristics of six of the most widely used fish anaesthetic agents: tricaine methanesulfonate, benzocaine, isoeugenol, 2-phenoxyethanol, quinaldine sulphate, and etomidate. For each agent, the fully anaesthetic concentration was established, and non-recoverable concentrations were established for application during euthanasia. Next, the aversive properties of each anaesthetic were assessed at a low (the highest concentration not causing anaesthesia) and a high (the highest concentration not eliminating heartbeat at recovery) concentration, using a novel behavioural avoidance test, combined with automated video analysis. Collectively, these studies revealed pronounced differences between the anaesthetic agents tested, both in their effectiveness and speed of induction, as well as in terms of their aversive properties. Our findings indicate that certain anaesthetics are more effective and/or less aversive to zebrafish embryo-larvae and may, therefore, offer more ethical and scientifically appropriate alternatives in experimental procedures involving these widely used laboratory animals.

A systematic review of the impact of environmental enrichment in zebrafish

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Environmental enrichment (EE) consists of a series of interventions carried out in the home environment to promote greater exposure to sensory stimuli and mimic the natural habitat of laboratory-housed animals, providing environments closer to those found in nature. Some studies have shown the positive effects of EE in zebrafish housed in a laboratory environment. However, this evidence is still recent and accompanied by contradictory results. Furthermore, there is great variability in the protocols applied and in the conditions of the tests, tanks and materials used to generate an enriched environment. This substantial variability can bring many uncertainties to the development of future studies and hinder the reproducibility and replicability of research. Here, in this context, we carried out a systematic review of the literature, aiming to provide an overview of the EE protocols used in zebrafish research. The literature search was performed in PubMed, Scopus and Web of Science and the studies were selected on the basis of predefined inclusion/exclusion criteria. A total of 901 articles were identified in the databases, and 27 of those studies were included in this review. We conducted data extraction and risk-of-bias analysis in the included studies. Among these studies, the effect of EE was evaluated in two different ways: (1) for animal welfare and (2) as an intervention to prevent behavioral, biochemical, molecular, developmental and breeding dysfunctions. Although the EE protocols in zebrafish presented a series of experimental differences, the results showed that the benefits of the EE for zebrafish were consistent. According to the results described here, the use of EE in the zebrafish home tank improves welfare and may reduce sources of bias in scientific research. However, it is still necessary to develop standardized protocols to improve the application of EE in scientific studies using zebrafish.

Improving zebrafish maintenance conditions and handling procedures with ethology and ecology in mind

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Over 100,000 scientists employ zebrafish around the world to answer a wide variety of basic questions about vertebrate biology and/or to model human diseases using sophisticated research methodology. Unfortunately, however, not all aspects of zebrafish science kept up with the rapid rise of this species in science. A fundamental question of how we should maintain these fish in our laboratories remains mainly unstudied, which may explain the diverse practices and the board range of environmental parameters employed in zebrafish facilities. Also, several zebrafish studies require some form of human handling of the fish, yet little attention has been paid to the potential stressful effects of this handling. In this talk, I provide a few examples for laboratory maintenance parameters, practices and handling procedures that may be problematic. I will briefly discuss a theoretical model to explain why keeping zebrafish under unnatural conditions may lead to increased error variation and thus reduced replicability. I call for systematic studies that could establish what may be the optimal maintenance conditions & handling procedures for zebrafish. And I will briefly discuss why an ethological perspective, i.e., knowing more about the natural behaviour and ecology of this species, could be an excellent guiding principle in these investigations.

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