

European Zebrafish Meeting 2015 Husbandry Session Report

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Abstract

A workshop to address husbandry and animal welfare was held during the 9th European Zebrafish Meeting in Oslo, Norway, from June 28 to July 2, 2015. The husbandry workshop took place on Monday, June 29, and was well attended by ~100 audience members. It highlighted problems arising from the diversity of current husbandry practices and included presentations on recent efforts to find common husbandry and animal welfare standards from a variety of international contributors, from Norway, Portugal, the United Kingdom, as well as the United States and Japan. Presentations included zebrafish and medaka as representatives of aquatic species used in biomedical research and addressed a diverse range of topics such as proposed European guidelines for zebrafish husbandry, general fish facility health and husbandry standards, cryopreservation, publication standards, and feeding strategies. The workshop highlighted the desire to develop common husbandry standards for the aquatic research community across the world.

Introduction

THE HUSBANDRY SESSION was held during the 9th European Zebrafish Meeting in Oslo, 2015 (Fig. 1). A full meeting report has been published in a previous issue of Zebrafish by Gareth *et al.*, Zebrafish 2016; 13:132–137; (<http://online.liebertpub.com/doi/full/10.1089/zeb.2015.1212>).

Despite their popularity, standardized husbandry guidelines have not been established for small aquarium fish in biomedical sciences. Research communities in different countries, with varying animal welfare regulations and cultural sensitivities, still struggle to find a common denominator for husbandry standards. However, such common standards are crucial to ensure reproducibility of research results among different laboratories and scientific disciplines.

Overall, the presentations at this husbandry workshop highlighted that the zebrafish research community should adopt a larger view by (1) including as many international communities as possible and by (2) expanding focus on welfare beyond zebrafish to include other small aquarium fish for biomedical research.

Presentations

Guidelines proposed for zebrafish housing, husbandry, and health monitoring recommendations

A FELASA–EUFishBioMed joint workgroup prepared a set of proposed zebrafish guidelines for husbandry routines to

ensure that zebrafish animal welfare is based on scientific knowledge and consistent with Directive 2010/63/EU on the protection of animals used for scientific purposes. P.A. presented the status of the current guideline draft. Key principles will include the biology of the species and the three “R”s: Replacement, Reduction, and Refinement. However, because zebrafish have been maintained in captivity and were purpose-bred for many generations in laboratories, husbandry research must address the species-specific needs for domesticated fish and how relevant our evolving knowledge is of the zebrafish’s biology in its natural environment and how that translates to laboratory maintenance.

Comment on the current draft version of FELASA—EUFishBioMed zebrafish husbandry guidelines from a facility management perspective

C.W. pointed out that despite its popularity as a laboratory research animal, diverse husbandry practices are being followed in zebrafish facilities and laboratories. As the manager of a large fish facility, she commented on the current draft of FELASA–EUFishBioMed Zebrafish Husbandry guidelines, which aim to standardize zebrafish husbandry practices. C.W. pointed out that the new guidelines must balance both animal welfare and the goals of scientific programs. New guidelines must address ethical considerations, which can be culturally different between countries/research communities—whereas the practical, technical, and working environment of a zebrafish facility also must take into account the

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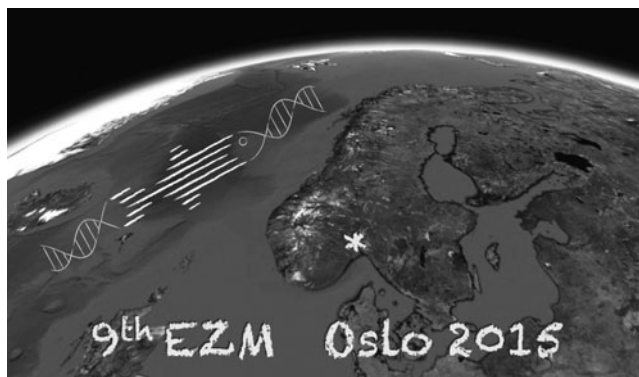


FIG. 1. A detail from the 9th EZM 2015 Meeting Program Booklet and meeting website front page.

various scientific disciplines and their diverging research goals. Optimized and standardized husbandry guidelines will have to address both sides of this equation.

Standard operating procedures of medaka husbandry and special reference to see-through medaka

Although the cultural and scientific history of medaka is somewhat different, Shin-Ichi Chisada reminded the audience that medaka is now a similarly well-studied aquatic fish model in the world and is complementary to zebrafish research. The development of consistent guidelines for this small aquarium fish is as important as it is to establish for zebrafish, even if husbandry protocols differ. To this end, Shin-Ichi Chisada highlighted key husbandry requirements for medaka, such as a feeding regimen for reproducible growth curves and appropriate food dosages for 2-week old larvae to 4-month old juveniles. In addition, he also highlighted concerns around the genetic purity of inbred strains (which essentially do not exist in zebrafish), as well as breeding conditions, survival rates, and anesthesia methods for medaka.

A scalable health standard for the zebrafish research community

Because exchange of research animals is a key prerequisite for the reproducibility of research results, Z.V. at the Zebrafish International Resource Center proposed a new international standard for fish health programs to reduce the risk of pathogen transmission between facilities exchanging fish, which can lead to subsequent outbreaks of diseases in the fish community. The new health standard should be scalable, using standardized, consistent strategies and protocols, and should encompass four key elements: (1) Quarantine: limiting risk of accidental pathogen import into fish facilities, (2) Monitoring: regular surveillance of overall fish colony health, (3) Mitigation: strategies for minimizing/eliminating pathogens and the risk of disease outbreaks, and (4) Disclosure: communication of colony health status along with relevant husbandry parameters between facilities exchanging fish lines. Following these biosafety measures will lead to an overall improvement of research and animal welfare by decreasing the variability of research results between laboratories, prevent unnecessary loss of animals due to health problems, and finally, increase the transparency between institutions to minimize zoonotic infection risks to researchers.

Optimized cryopreservation and thawing methods for community and resource center use

Many zebrafish facilities still rely on live maintenance of their fish lines, which is often limited by the capacity of animal facilities to provide appropriate space. Although considerable effort was expended on generating thousands of novel lines in the research community, efforts for efficient exchange, storage, and management of these lines lagged behind, specifically in efforts to reduce the number of live animals to an absolute minimum (one of the three “R’s”). To provide facilities with the capability to back-up and access their genetic research resources in a standardized and efficient manner, ZIRC optimized zebrafish sperm cryopreservation and *in vitro* fertilization methods. The new protocol is scalable and offers more effective methods for several steps of the cryopreservation pathway. Average post-thaw fertilization success rates are significantly higher, up to 66%, thus guaranteeing successful recovery of virtually every sperm sample. The new protocol is available on the ZIRC website for testing.

The importance of accurate reporting of environmental and husbandry conditions for zebrafish studies

Christian Lawrence from Children’s Hospital in Boston made a cursory review of how zebrafish publications show a striking lack of detail in the reporting of environmental and husbandry conditions in many studies. In an overwhelmingly large proportion of cases, authors (1) reference “standard” conditions that do not exist, or (2) include only partial or incomplete descriptions of conditions, or (3) do not include any relevant husbandry/environmental information at all in their materials and methods sections. This presentation outlined the scope of the problem.

Different feeds and feeding regimens have an impact on zebrafish larval rearing and breeding performance

Ana Catarina Certal presented a comparative study using three commercial dry feeds from first feed to 180 days postfertilization in three different feeding regimens. The impact on survival, growth, and age of sexual maturity, fecundity, and embryo viability was assessed in three types of fish strains: transgenic, mutant, and wild type. One variety of food stood out as the best performer in all parameters and strains analyzed, which suggested that it is possible to attain satisfactory performance levels using only dry feed. The results were integrated into currently established feeding protocols where a combination of live feeds and commercially processed feeds is used to attain optimal health, breeding, and developmental and behavioral results for zebrafish research.

Disclosure Statement

No competing financial interests exist.

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