

10th European Zebrafish Meeting 2017, Budapest: Husbandry Workshop Summary

Jana Oltová,^{1,*} Carrie Barton,^{2,*} Ana Catarina Certal,^{3,*} Francesco Argenton,^{4,*} and Zoltán M. Varga⁵

Abstract

A husbandry workshop on July 3, 2017, at the 10th European Zebrafish Meeting in Budapest, Hungary (July 3–July 7, 2017), focused on the standardization, optimization, and streamlining of fish facility procedures. Standardization can be achieved for example by developing novel software and hardware tools, such as a fish facility database for husbandry and environmental facility management (Zebrabase, Oltova), or a hand-held, air-pressurized fish feeder for consistent food distribution (Blowfish, Argenton). Streamlining is achieved when work hours are reduced, as with the standardized fish feeder, or by limiting the number and types of fish diets and observing the effect on animal welfare and performance (Barton). Testing the characteristics of new fish diets and observing whether they produce better experimental outcomes (Certal) optimizes diets and improves fish productivity. Collectively, the workshop presentations emphasized how consistency and harmonization of husbandry procedures within and across aquatic facilities yield reproducible scientific outcomes.

Keywords: euthanasia, facility database, hand-held feeder, fish diet, food, feeding

Introduction

A 1-H HUSBANDRY WORKSHOP with ~150 attendees was held on July 3, 2017 during the 10th European Zebrafish Meeting in Budapest, Hungary (July 3–July 7, 2017).

Institutional or departmental animal facilities are tasked with providing fish to a variety of research laboratories, often with very diverse research goals. Concurrently, animal caretakers must provide efficient, standardized, high-quality animal care. Owing to differing expectations, the boundaries between standardized animal care and optimization for specific research outcomes (i.e., reproductive performance) are often fluid. In this workshop, tools were presented that address both the standardization of animal care, for example, a database and a feeder, and optimization for specific research needs, for example, streamlining with a single food and the reproductive performance of animals fed with a particular diet. Please note the summary of presentations is in the order of the workshop program, whereas the authors contributed equally to the workshop and this report.

Presentations

Zebrabase: an intuitive zebrafish tracking database solution

Jana Oltova (jana.oltova@img.cas.cz) reported on the Zebrabase database development project, which develops an animal tracking database solution at the Institute of Molecular Genetics in Prague (CZ). An interdisciplinary team of zebrafish researchers, programmers, and web designers was initially tasked with the development of an application for their own zebrafish facility use, but this solution has now been made available to fish facilities worldwide.

Zebrabase is a scalable, cross-platform, web-based, animal tracking database developed specifically for aquatic research facilities. It has been built around three key concepts—comprehensive animal tracking, interactive breeding history, and advanced management features, including requesting and experiment planning. Importantly, Zebrabase is suitable both for small or budget-conscious facilities (there is a free plan for up to 150 tanks or 3 racks) and for large facilities

¹Department of Cell Differentiation, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.

²Department of Environmental and Molecular Toxicology, Sinnhuber Aquatic Research Laboratory (SARL), Oregon State University, Corvallis, Oregon.

³Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon, Portugal.

⁴Department of Biology, Università Degli Studi di Padova, Padova, Italy.

⁵Zebrafish International Resource Center, University of Oregon, Eugene, Oregon.

*These authors contributed equally to this workshop.

aiming to improve workflow, facility management, or extending animal reporting capabilities (e.g., fish census or usage reports). An additional aim is to provide an intuitive, robust, and secure tracking solution for diverse research projects. Therefore—each user (facility, laboratory, etc.) can create an individualized ZebraBase environment that includes facility layout and customized configurations. These customized databases can be hosted on a virtual server using Secure Sockets Layer-encrypted communication. Data are backed up daily and archived for up to 6 months. Every 30 min, recent activities are copied onto a separate secondary cluster to ensure maximum availability of the service in case the primary cluster becomes unavailable.

ZebraBase supports both desktop and mobile use on Mac, Linux, Windows, Android, and iOS and has a built-in QR code generator and QR code reader functionality that provide instant access to all the fish stock information and related actions on a mobile device. A demo database is available for testing (<https://demo.zebrabase.org/>) and additional information is updated on the ZebraBase webpage (<https://zebrabase.org/>).

Successful elimination of live feeds in a high-production zebrafish facility

Carrie L. Barton (Carrie.Barton@oregonstate.edu) from the Sinnhuber Aquatic Research Laboratory (SARL) at Oregon State University, Corvallis, Oregon (US) presented data from a diet study, in which a combination of traditional commercial dry and live feed (*Artemia nauplii*) was compared with a new commercial formula in the absence of any live supplementation. Different protocols were administered to three cohorts of fish and the long-term effects of the diets were assessed. Animals were split into two groups and fed either (1) a commonly used blend of Zeigler feeds: AP100 at the larval stages, transitioning to Aquatox at 90 days post-fertilization (dpf), Golden Pearls (Brine Shrimp Direct), and Cyclop-eeze (Argent) additionally supplemented with live *Artemia*, or (2) Gemma Micro (Skretting), a commercial diet that based on the manufacturer's recommendations did not require the use of live feed supplementation. Feeds were size matched to the life stage of the fish (75–300 μ). The number of embryos produced by adults and the survival of the larvae and juveniles between 5 and 90 dpf were the performance endpoints. Results demonstrated that adult zebrafish thrived and produced large numbers of embryos in the absence of live feeds. Fish on the Gemma Micro diet began spawning earlier and produced more embryos over their lifetime than animals on the Zeigler-based feed supplemented with *Artemia*. This study demonstrates the practicality of eliminating live feeds in high-production zebrafish facilities with the advantages of reduced labor costs and minimized diet-dependent research variability.

Different impact of dry feeds on zebrafish growth and reproductive performance

During the third presentation, Ana C. Certal reported on the impact of dry feeds on zebrafish growth and reproductive performance. The study was conducted at the Champalimaud Center for the unknown in Lisbon (PT) based on a previously published study where three commercial feeds were compared and one of them stood out as the best performing feed¹

(presented at the EZM 2015 in Oslo). Ana Catarina Certal reported on Zebrafeed (Sparos), a new dry feed, which presented a better alternative to the current feed used in her facility (Gemma Micro, Skretting).

Breeding fish were raised either only on the new Zebrafeed or on the current feed until 60 dpf and were then switched to the new Zebrafeed. The latter group showed a statistically significant increase in embryo viability as compared with the first group, showing that the new Zebrafeed diet supported reproduction of zebrafish better. The study validated both feeds for optimal zebrafish rearing and for breeding programs for which redundancy in feed suppliers is an important aspect of facility management. Thus, Zebrafeed is an optimal feed for “goal-directed” feeding protocols, for example, when a large number of progeny or fast growth rates are expected by facility users.

A pneumatic dispenser for fast delivery of fish food

In the last presentation, Francesco Argenton from the Department of Biology, Università degli Studi di Padova (IT), reported on a novel pneumatic dispenser for fast delivery of fish food. Zebrafish are usually fed several times per day by rotating students and technicians that need to be well trained for accurate food dosing and delivery. Overfeeding leads to the accumulation of excess food at the bottom of the tank and degrading food impacts water filters negatively, leading to fish health problems. In addition, food can accumulate on aquarium lids, near the feeding hole, leading to mold. Feeding too little, in contrast, impairs fish growth and reproductive fitness. Hence, the amount of food provided must be adjusted according to the number of fish, their age, weight, and function. Despite the importance of delivering the correct dose per tank, the amount of food delivered even by well-trained operators has a significant intra-, and inter-individual variance.

To overcome the variability intrinsic to the routine feeding of a varying number and ages of fish in a facility, a pneumatic device for fast and precise delivery of food was developed. A reservoir containing 80 g dry food of 100 μ m diameter and larger was mounted on a portable pneumatic gun at low pressure (1.5 atm). A trigger is pressed to blow food through a short polypropylene tube into fish tanks. Because the amount of food released at each pulse is consistent (independent from the length of the pulse), it can be regulated by tightening or releasing a ring valve. The device has been successfully used in two fish rooms with 1500 fish tanks at the University of Padua since 2015, reducing the feeding time from 90 min (manual feeding) to 30 min with the pneumatic dispenser. Thus, the pneumatic feeder saved food and time, produced no waste around the feeding hole, and more time became available for other husbandry-related tasks, for example, for cleaning tanks or filters. Finally, by controlling the amount of delivered food, and improving facility hygiene, the use of this device supports animal health and the Replacement, Reduction, and Refinement (3Rs) Principles of animal welfare. The device is self-cleaning, lacks moving parts, and is low maintenance. A video is provided here: (www.bio.unipd.it/development/BLOWFISHVOICE.mov).

The feeder, manuals, and instructions can be obtained from the University of Padua with an Material Transfer Agreement (email: Francesco.argenton@unipd.it).

Zebrafish Euthanasia

Robert Geisler from the European Zebrafish Resource Center (EZRC, www.ezrc.kit.edu/), at the Karlsruhe Institute of Technology (KIT) in Germany, provided a brief report and update about the outcomes of an international Workshop entitled *Euthanasia for Zebrafish—A Matter of Welfare and Science*, which had been hosted by EZRC on March 9, 2017, at the KIT (sponsored by EuFishBioMed; www.eufishbiomed.kit.edu/). In brief, because the directive 2010/63/EU does not address adequately the euthanasia of small tropical aquarium fish that are used in biomedical science, various methods for euthanasia of zebrafish had been discussed and proposed at this workshop with the goal to identify methods that consider the physiology of zebrafish and its use as a key biomedical research organism. Importantly, the euthanasia methods discussed were to follow the 3Rs Principles in animal experimentation and focus on animal welfare during anesthesia and euthanasia. A report of this workshop has been published in this journal.²

Disclosure Statement

No competing financial interests exist.

References

1. Farias M, Certal AC. Different feeds and feeding regimens have an impact on zebrafish larval rearing and breeding performance. *Int J Marine Biol Res* 2016;1:1–8.
2. Köhler A, Collymore C, Finger-Baier K, Geisler R, Kaufmann L, Pounder KC, *et al.* Report of workshop on euthanasia for zebrafish—a matter of welfare and science. *Zebrafish* 2017; 14:547–551.

Address correspondence to:
Zoltán M. Varga, PhD
Zebrafish International Resource Center
University of Oregon
Eugene, OR 97403

E-mail: zoltan@zebrafish.org