Universal Healthcare for Zebrafish

Zoltán M. Varga,¹ Christian Lawrence,² Stephen C. Ekker,³ and Judith S. Eisen⁴

THE SUCCESS OF zebrafish as a model vertebrate has probably greatly surpassed even the wildest expectations of its earliest proponents. Although zebrafish have been studied since the 1950s, they really hit the big time in 1981 with an article from George Streisinger showing that this small, popular, tropical fish was easily reared in the laboratory, could be cloned, and was amenable to genetic analysis.¹ Since that time, more than 26,700 articles on zebrafish have been indexed in PubMed, and the animal is studied in nearly 1000 laboratories worldwide. A number of important scientific advances have been made based on research conducted using zebrafish, and the prospects for its continued significance as a premier vertebrate experimental model are extremely positive. Further expansion will not only be driven by the development of new technologies that researchers can leverage in zebrafish but also by improvements in management of the environment and health of these animals in laboratory settings.

Indeed, the continued growth and maturation of zebrafish as a model organism will now depend on widespread adoption of data-driven strategies for animal husbandry and health programs. These advances will enable the definition and eventual standardization of environmental conditions across a wide range of different types of experimental paradigms. The present general lack of consistency and the few community-wide standards available for zebrafish husbandry and health could potentially have adverse effects on research outcomes, some of which may be difficult to discern because the necessary information is currently not even available.^{2–4}

To this end, we are excited to present this special issue of *Zebrafish* devoted to husbandry and health. It is both a celebration of the extraordinary growth of the model and an initiation of a discussion on ways to capitalize on what has been learned about zebrafish husbandry and health so that zebrafish can continue to be an outstanding model, whether one has a single small tank or a gigantic facility. This collection of 23 articles submitted by contributors from around the world addresses a wide range of issues ranging from management of many types and sizes of zebrafish research facilities,^{5–10} strategies for monitoring colony health,^{11–14} case reports of pathogen outbreaks in zebrafish colonies,^{15,16} results of studies of endemic and exotic pathogens,^{17–19} testing of disinfection

protocols,²⁰ establishing regulations for health surveillance for zebrafish importation,²¹ to standardization of reporting on health status,^{8,22–24} nomenclature,²⁵ and nutrition.²⁶ We view this as the beginning of a community-wide dialogue that will promote zebrafish health and thus enhance research productivity and validity of results. Going forward, we welcome additional submissions to *Zebrafish* that address these and other issues related to fish husbandry and health, which will add to this important and growing body of literature.

To begin to understand the range of husbandry and health surveillance methodologies used throughout the zebrafish research community, we conducted a survey of 19 established facilities in North America, Europe, Asia, and Australia. Clearly, this is not exhaustive by any means, but the results do provide some baseline information that is instructive for establishing scalable, standardized parameters. We present a brief glimpse of some data from these surveys in the accompanying Survey Synopsis article. Even though only 19 laboratories and zebrafish facilities participated in this survey, the data are rich and complex and will require a more thorough analysis than we present here. However, two clear themes emerged. First, husbandry and health management strategies are often informal and difficult to quantify. Second, the variation and range of methodologies and protocols used in different zebrafish facilities are considerable.

The articles presented in this special issue of *Zebrafish* reflect a similar dynamic. While there are some common management practices used in many zebrafish facilities, the overwhelming trend is that the various components that constitute the environment of zebrafish as an experimental subject—whether it is water quality, physical characteristics of housing, nutrition, or pathogen status—are highly variable, not well-characterized and almost universally underreported. All of these factors impact and ultimately define the health of the fish utilized for research, and by extension, the quality, reproducibility, and integrity of the results of that research.

At the same time, the articles presented here also represent an excellent step toward protocol standardization, where appropriate, and disclosure of husbandry practices and health status. The diversity of contributions included in this issue is representative of the vibrant, innovative, and collaborative

¹Zebrafish International Resource Center, University of Oregon, Eugene, Oregon.

²Aquatic Resources Program, Boston Children's Hospital, Boston, Massachusetts.

³Department of Biochemistry and Molecular Biology, Mayo Clinic Cancer Center, Rochester, Minnesota.

⁴Institute of Neuroscience, University of Oregon, Eugene, Oregon.

state of the zebrafish research community. In short, there are solutions to the myriad management challenges faced by facilities that vary widely in scale, and sharing these solutions with the entire zebrafish research community will promote better utilization of our limited time and resources.

Effective and reproducible management of the environment experienced by research subjects is critical to the success of the scientific results emanating from that research. It is also the most basic determinant of subject health. Zebrafish are clearly tolerant of a wide range of conditions, and many of the articles in this issue provide ample demonstration of this point. For example, there are few defined standards for nutrition; many facilities feed a mixture of live and processed diets with little or no description of actual quantities of feed delivered to zebrafish at different life stages. Water quality is also a variable. Some of the tested parameters vary from facility to facility, and in some cases, water quality parameters are not well tracked or reported. There is little consistent information on flow rates, tank turnover times, or even physical configuration of housing tanks. There is also a lack of consensus with regard to optimal holding densities.

An important step toward understanding how these various parameters affect zebrafish health is to glean the information from a variety of facilities and make it available, so that the various parameters can be experimentally tested. Simply put, the various components of zebrafish husbandry and management are all potential variables for experiments and thus should be monitored and included in publications. This special issue of *Zebrafish* brings together in one place more published information on water quality, diet, and other husbandry conditions than what has previously been reported in the literature. In particular, articles by Martins *et al.* and Borges *et al.* provide an excellent level of detail on a variety of environmental and husbandry parameters.^{11,13} The articles describing management strategies at the International, European, Chinese, and Taiwanese Stock Centers are also instructive in terms of the information they collect and report.^{7–10}

The articles presented here also reveal beginning steps in the sophistication of certain husbandry methods, especially as they relate to the evaluation and quantification of common practices. For example, live feeds are thought to be important in zebrafish health and environmental enrichment,^{2,27–30} and many facilities feed some form of live zooplankton to larval fish or to encourage breeding.^{28,31} In their case report of a *Mycobacterium marinum* outbreak, Mason *et al.* discuss both the potential of live feeds such as *Paramecium* and rotifers to become a vector for pathogen transmission and the value of screening live feeds on routine basis as part of a health monitoring program.¹⁶ For this reason, Barton *et al.* have developed a facility without live feed and describe the health benefits.⁵ In their review on the relationship between fish health and nutrition, Watts *et al.* discuss the challenges associated with both the utilization of live feeds in the diet of research fish and using processed diets without sufficient knowledge of fish nutrient requirements.²⁶

Not only do the articles in this special issue bring together a wealth of information on zebrafish husbandry and management strategies, they also bring together the most comprehensive collection of information on zebrafish health monitoring. Why monitor fish health? The article by Kent *et al.* shows that subclinical infection by *M. chelonae* does not affect the number of eggs or viability.¹⁸ In contrast, the article by

Mason *et al.* shows that *M. marinum* can have serious consequences for a facility that negatively impact research, but on a positive note that outbreaks such as this can be ameliorated through training, vigilance, and surveillance.¹⁶ As with other aspects of facility management, health monitoring varies from facility to facility. Articles by Geisler *et al.*, Legendre *et al.*, Murray *et al.*, Collymore *et al.*, and Borges *et al.* provide nice descriptions of health monitoring programs.^{7,8,12,13,23} Several articles also point out how simple strategies, for example, using brightly colored markers or different room colors, can simplify monitoring by making health status of particular fish available at a glance.^{5,15,16}

The articles in this issue and the results of our survey reveal that most facilities have quarantine space so that zebrafish imported from other facilities are monitored before being moved into main facility housing. This is important to prevent spread of disease between facilities. For example, fish pathogens from the hobbyist trade can spread to other species, including zebrafish.¹⁹ Although quarantine is important, quarantine space comes in many different configurations, ranging from a single rack within a facility, to a separate room within a facility, to a room or set of rooms that are completely separated from the main housing area. A commonality among all of the different quarantine arrangements is to further the aim of ensuring that both zebrafish and personnel within a facility are protected from pathogens that may be carried by imported fish.^{6,7,9–11,13} Larger facilities or those that aspire to be specifically pathogen free may consider more elaborate, multistage quarantine that can afford increased health surveillance opportunities and thus contribute to improved health monitoring that will benefit not only those facilities but also the community at large.^{5,8,15,16}

Surface disinfection of fish embryos, typically by sodium hypochlorite (bleach),^{32–34} is another essential component of fish health management. Surveys indicate a bewildering number of variations of bleaching protocols, many of which vary in dosage and exposure times. While a number of previously published studies document the efficacy of various disinfecting agents on different zebrafish pathogens,^{35,36} little work has been done to determine whether or not pathogens are actually being removed by existing disinfection protocols. The article by Chang *et al.* in this issue provides a systematic assessment of another reagent—povidone-iodine (PVPI)—in disinfecting eggs exposed to *Mycobacterium* species.²⁰

At the time the special issue was planned, the editors reached out to several laboratories working with other small aquarium fish in biomedical science, such as medaka, stickleback, fugu, and swordtails/platyfish. The submitted contributions in this issue focus mainly on zebrafish, but we want to point out that at least some fundamental species-specific differences and husbandry requirements exist between zebrafish and other aquatic organisms that are used in research. Although zebrafish has become an excellent model organism for many human conditions and diseases, probably not all lessons described in this special issue can be applied to other species. Animal welfare policy dictates that researchers, institutional compliance officers, and lawmakers remain cognizant of the similarities between aquatic species, while ensuring that species-specific requirements are considered, particularly in facilities that house multiple aquatic and nonaquatic species.

We hope that this special issue will find interest beyond the obvious and direct stakeholders in zebrafish health: researchers and animal caretakers. It is important to reach out to all participants who play a role in shaping the conversation around animal health and research reproducibility. This list should include institutional compliance officers, veterinarians, animal ethics committees, institutional animal care and use committees, as well as legislators and government agencies. To bring these constituents into the conversation, the editors reached out to the Canadian Food Inspection Agency (CFIA) and the Office of Laboratory Animal welfare (OLAW) and invited responses to two contributions in this special issue (Hanwell *et al.*²¹ and Moulder²⁴). Another attempt to bridge divides is to establish a common, standardized language, centered on zebrafish well-being and health. This idea is supported by establishing a centralized, web-based glossary for welfare terms, as outlined in the article by Goodwin *et al.* establishing a wiki that is expected to receive continued input and refinement from the community, thereby shaping our common language and ultimately resulting in an ontology for welfare terms that can be used by all stakeholders.²⁵

In conclusion, we intend this special issue of *Zebrafish* on husbandry and health to mark the beginning of a conversation, propose key standards derived from our current knowledge, reveal areas in which key research is lacking, and establish a baseline for tracking progress in achieving the goals of making zebrafish husbandry and health information a standard aspect of zebrafish research. The four specific health program standards we propose for zebrafish research facilities include the following: (1) *Quarantine*: Limiting risk of accidental pathogen import. (2) *Monitoring*: Regular evaluation of fish health and well-being. (3) *Pathogen Control*: Strategies for eliminating or reducing already existing pathogens in our facilities to mitigate the risk of disease outbreaks.⁴ (4) *Disclosure*: communication of colony health status between facilities exchanging fish lines.

Of course, all of these measures need to be scalable so that they can be applied in some form to small facilities with only a few tanks as well as to large facilities or resource centers, without undue hardship on available resources. We hope that these health program components, but more importantly the combined and individual contributions in this special issue, stimulate a dialogue in the community that includes researchers, animal caretakers, principal investigators, institutional compliance officers, and the federal institutions who entrust all stakeholders to uphold best practices and high standards for the well-being of the animals we study. We express our hope that this dialogue will be fruitful and that future surveys and special editions to this topic will track the progress we have made in improving the health of our colonies and maintaining biosecurity between our laboratories.

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Address correspondence to:
Judith S. Eisen, PhD
Institute of Neuroscience
1254 University of Oregon
Eugene, OR 97403

E-mail: eisen@uoneuro.uoregon.edu