Introduction to the Special Issue on Zebrafish in Research and Education

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The zebrafish (*Danio rerio*) is a small freshwater fish native to South Asia that belongs to the minnow family. They can reach 2–5 cm in length and are a popular aquarium fish as they are robust and relatively easy to care for. In the 1970s, George Streisinger began to explore using zebrafish as a vertebrate model system at the University of Oregon. He recognized that this small fish had many characteristics of an attractive model system, particularly for the study of embryonic development and gene function. Zebrafish tend to spawn near daybreak, thus, in a controlled laboratory environment where the light cycle can be regulated, embryos can be obtained in a predictable fashion. The eggs and embryos are relatively large and optically clear and also develop externally from the mother. The eggs are approximately 1 mm in diameter featuring a single cell sitting upon a large yolk, all encased in a clear chorion. The fertilized egg develops from a single cell to a pharyngula in approximately 24 hours (Kimmel et al. 1995). Furthermore, pigmentation isn't observed until the pharyngula period, meaning the embryos are completely transparent until this time, allowing the observation of the developing tissues and organs. Because of these features, one can fairly easily observe early development from a single cell, through cleavage, gastrulation, and segmentation (or somitogenesis) to a distinctly vertebrate pharyngula in the space of a day and through a simple compound microscope. Indeed, for years it was virtually impossible to read a manuscript that made use of zebrafish that didn't quote and refer to these characteristics at some point in the introduction.

A group of researchers, many with roots in the University of Oregon, began to establish a collaborative community which led to a desire to gather in a more structured setting. This led to the first open meeting of zebrafish researchers in 1994 at Cold Spring Harbor for the *Meeting on Zebrafish Development and Genetics*. This community has steadily grown in numbers and scope as has this conference which has evolved into a biennial meeting of zebrafish researchers from around the world. During the era of the early 1990's, an infrastructure for using the model system also began to be developed including a guidebook for common laboratory techniques known as *The Zebrafish Book* (Westerfield 1993) now in its 5th edition and an embryonic staging series (Kimmel et al. 1995). The establishment of the Zebrafish Information Network (ZFIN, https://zfin.org/), a database that provides an organized storehouse of zebrafish research data, and the Zebrafish International Resource Center (ZIRC, https://zebrafish.org), which acts as a stock center as well as a resource for zebrafish health services further cemented the place of zebrafish as an important model system. The seminal 1996 special issue of Development (volume 123) described the remarkable first large-scale zebrafish mutagenesis screens and reported on 372 genes defined by the mutants generated from them by the Nusslein-Volhard, Driever and Bonhoeffer labs. This forward genetic approach firmly cemented zebrafish as a true vertebrate genetic model system that often led to comparisons with *Drosophila melanogaster*. In following years, the genome was sequenced and targeted gene disruption protocols became available. These protocols, which took a path through using transposases, morpholinos, zinc-finger nucleases (ZFNs),

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transcription activator-like effector nucleases (TALENs) and most recently CRISPR/Cas9. The ability to perform reverse genetics in a reliable and straight forward manner increased the utility of this model system even further. Over the years the field has continued to evolve and expand in scope and zebrafish have been adapted for studies that touch many subfields of Biology including behavior and neural activity (e.g., Huang et al. 2020); using them as hosts for human xenografts with an eye towards utilizing them for personal medicine approaches (reviewed in Neff 2020); regeneration (reviewed in Gemberling et al. 2013); bacterial pathogenesis (Torraca and Mostowy 2018); ecotoxicology (Dai et al. 2014); and also as a teaching tool (Wilk et al. 2018).

This special issue of the *Eastern Biologist* (now *eBio*) on zebrafish sprang from the Zebrafish Symposium held as part of the Association of Southeastern Biologists (ASB) annual meeting. I organized the first symposium at the 2016 ASB annual meeting held in Concord NC with the help of funding from the North Carolina Biotechnology Center. It was immediately clear that this symposium was a success and also, importantly, a much-appreciated outlet for zebrafish researchers within ASB's membership from primarily undergraduate as well as master's degree-granting institutions. Zebrafish scientists at these institutions desired a place for them and their students to be able to present their research to peers from similar settings. In addition, I feel there was a desire to be part of a community of researchers at similar institutions with shared concerns, challenges and also opportunities. Because of the success of this first symposium, a second Zebrafish Symposium was organized for the 2017 ASB annual meeting in Montgomery AL and again at the 2019 ASB annual meeting in Memphis TN with a goal of maintaining this as a biennial event. At the Montgomery meeting, conversations began about the possibility of developing the symposium into this special issue. It was clear that just as this group of scientists desired a place to be part of a community of researchers from similar institutions to share their science and strategies for advancing their research, they also wanted to have an avenue to publish their research in a peer reviewed journal. The establishment of the *Eastern Biologist* (now *eBio*), an official journal of ASB, provided the perfect landing place for these manuscripts.

The subject matter of the ASB Zebrafish Symposia and this special issue captures some of the diversity of research using zebrafish as a model. Presented here are articles that cover subject material ranging from the technical aspects for working with zebrafish in the laboratory and also using them as a teaching tool, to characterizing gene expression and function, to studying how they respond to drugs and pharmaceutical agents and also how they can be used in ecotoxicology studies. Machingo et al characterize the expression of six members of the β1,3 *N*-acetylglucosaminyltransferase family and show the effects on CNS development when these genes are perturbed with morpholinos (Machingo et al. 2019). Two papers add to the knowledge base of working with zebrafish as a model, including cost efficient measures and streamlined techniques to raise zebrafish larvae (Norton et al. 2019) and also efficient mutagenesis using CRISPR/Cas9 (Tennant et al. 2019). Davis et al contribute to the literature of using zebrafish as a teaching tool and describe a detailed set of laboratory exercises they developed using bioinformatics and gene expression analysis that can be used in undergraduate teaching labs (Davis et al. 2019). Several articles also examine adult zebrafish behavior. The Achat-Mendes group examines zebrafish behavior in the context of ethanol and nicotine abuse (Hall-Kim et al. 2018) while the McGrew group describe how zebrafish respond to several biogenic amines in the context of spatial learning and also anxiety (Prasad et al. 2019). The Rhodes group describes the negative effects of analogs of the popular dietary supplement resveratrol on zebrafish development and embryo mortality, adding to the literature that underscores the

need for caution with these types of natural products when pregnant and breastfeeding (Champer et al. 2019). Finally, the Sittaramane group uses zebrafish as part of a study to understand the effect of a chemical spill that occurred in 2014 upstream of Charleston WV. They describe not only the effect of this chemical on zebrafish larvae in terms of the expression of stress response genes and also their behavior but also shifts in the composition of planktonic species that would affect the aquatic food webs in this area to provide a more comprehensive picture of the effects of this spill (Williams et al. 2019).

Individuals at primarily undergraduate / master's-degree granting institutions often face challenges generating sufficient data in a timely manner to allow publication of certain types of research. Science that is never published may as well have never been done and that leads to knowledge that is either lost or duplication of efforts above and beyond duplicating experiments to verify authenticity. It is important to perform basic science and advance our knowledge base regardless of the size of the increments. It is also important to have a mechanism of clear, transparent and rigorous peer review to ensure confidence in these increments. It is our hope that the *Eastern Biologist* (now *eBio*) can provide an outlet for such research, for ASB membership as well as the wider scientific community. As we present this first special issue of the *Eastern Biologist* (now *eBio*), we sincerely hope you will appreciate the reach of the continually expanding field of scientists who use zebrafish as a model system.

Literature Cited

Champer, J., S. Cushion, S. King, C. Rushlow, B. Cenatus, and L.V. Rhodes. 2019. Effects of Resveratrol Analogues on Danio rerio Mortality and Embryonic Development. Eastern Biologist Special Issue 1:90–100.

Dai, Y.J., Y.F. Jia, N. Chen, W.P. Bian, Q.K. Li, Y.B. Ma, Y.L. Chen, and D.S. Pei. 2014. Zebrafish as a model system to study toxicology. Environmental Toxicology and Chemistry 33:11–17.

Davis, A., H. Nguyen, and J. Qian. 2019. Zebrafish Embryos and Bioinformatics: Useful and Marketable Exercises for Students Enrolled in Upper-Level Undergraduate Courses. Eastern Biologist Special Issue 1:47–63.

Gemberling, M., T.J. Bailey, D.R. Hyde, and K.D. Poss. 2013. The zebrafish as a model for complex tissue regeneration. Trends in Genetics 29:611–620.

Hall-Kim, M., M. Granada, S. Fuller, E. Dyle, and C. Achat-Mendes. 2018. Augmented Reinforcing and Anxiolytic Effects of Nicotine and Ethanol in Zebrafish. Eastern Biologist Special Issue 1:1–14.

Huang, K.H., P. Rupprecht, T. Frank, K. Kawakami, T. Bouwmeester, and R.W. Friedrich. 2020. A virtual reality system to analyze neural activity and behavior in adult zebrafish. Nature Methods 17:343–351.

Kimmel, C.B., W.W. Ballard, S.R. Kimmel, B. Ullmann, and T.F. Schilling. 1995. Stages of embryonic development of the zebrafish. Developmental Dynamics 203:253–310.

Machingo, Q.J., A. Seth, M. Adely, L. Wagner, A. Fritz, and B.D. Shur. 2019. Analysis of Genetic Interactions and Developmental Requirements of the β1,3-N-acetylglucosaminyltransferase Family During Zebrafish Development. Eastern Biologist Special Issue 1:15–32.

Neff, E.P. 2020. Building avatar armies with fish and flies. Lab Animal (NY) 49:101–104.

Norton, A., K.F. Franse, T. Daw, L. Gordon, P.F. Vitiello, and M.K. Kinkel. 2019. Larval Rearing Methods for Small-scale Production of Healthy Zebrafish. Eastern Biologist Special Issue 1:33–46.

Prasad, A., A. McCoy, J. Wesley, C. Thornton, and L. McGrew. 2019. Accumulating Evidence of Zebrafish's Response to Biogenic Amines. Eastern Biologist Special Issue 1:75–89.

Tennant, M.D., T. Zerucha, and C.M. Bouldin. 2019. Cost-Efficient and Effective Mutagenesis in Zebrafish with CRISPR/Cas9. Eastern Biologist Special Issue 1:64–74.

Torraca, V., and S. Mostowy. 2018. Zebrafish Infection: From Pathogenesis to Cell Biology. Trends in Cell Biology 28:143–156.

Westerfield, M. 1993. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Brachydanio rerio*). University of Oregon Press, Eugene, OR.

Wilk, R., N. Ali, S.J. England, and K.E Lewis. 2018. Using Zebrafish to Bring Hands-On Laboratory Experiences to Urban Classrooms. Zebrafish 15:156–178.

Williams, A., D. Turner, R. Li, D. Lott, S.F. Riera, A. Wagner, A. Shaikh, R.A Cohen, and V. Sittaramane. 2019. 4-Methylcyclohexanemethanol (4-MCHM) Influences Danio rerio (zebrafish) Larval Behavior and Plankton Community Composition. Eastern Biologist Special Issue 1:101–116.