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The Experts Speak

Views on Four Key Questions About Zebrafish Research

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Prior to the Zebrafish Development and Genetics 2004 meeting in Madison, Wisconsin, the editors of *Zebrafish* asked the zebrafish community four questions: 1. *What is the most significant contribution the zebrafish model system has made to our understanding of basic biology and medicine?* 2. *What piscine model systems best compliment the zebrafish as a model system for basic biology and medicine by providing capabilities the zebrafish cannot?* 3. *Are there areas of research in which fish models are not currently being used but you feel will play a significant role in the future?* 4. *What is the next up-and-coming piscine species to become a significant contributor to broad areas of biological and biomedical sciences, following in the footsteps of zebrafish and medaka?* For such questions, there is of course no one "right" answer. I present a brief commentary on Question 1, followed by the individual responses to all the questions, presented alphabetically by contributor.

The answers to the second through fourth questions reflect a diversity of creative thinking in the zebrafish community, and are presented below "as is." However, the first question deserves separate discussion. The written answers to Question 1 most commonly referred, directly or indirectly, to the fact that the zebrafish is the only vertebrate organism in which it has been practical (though difficult) to perform mutant screens (forward genetics) entirely within the confines of a single laboratory. Many also noted the power of reverse genetics in the zebrafish, by which morpholinos can be used to knock down the function of virtually any gene.[1] Specific contributions from the zebrafish community reflected both the expertise of individual responders and the pioneering nature of much work in the field.

A verbal survey: Upon receipt of the replies, I noted that a number of senior members of the zebrafish community did not respond to the survey. Out of curiosity, I made some informal, verbal inquiries during the course of the meeting in Madison. The inquiries confirmed my suspicion: Although the first question is interesting, some of us in the field were concerned that our answers to the question may be misrepresented as a means of evaluating the value of the zebrafish as a model system before its potential has been more fully explored. This concern became more acute with a common perception of the zebrafish community that the NIH is considering weakening support for the field at an important point in its development. The wide range of work presented by 950 meeting attendees made it clear that important contributions can be expected from an inspiring number of new zebrafish investigators interested in a wide range of human diseases.

The zebrafish will continue, for the foreseeable future, to contribute to the elucidation of a wide range of biological mechanisms. The zebrafish is a key model system for adding detail to our knowledge of developmental processes. The contributions of zebrafish genetics and reverse genetics have not nearly reached saturation. Mary Mullins (U. Penn.), who was centrally involved in one of the two large screens that launched the field, and is well-versed in the power of genetic analysis in Drosophila, said it well. She noted that, even though there are areas of extensive understanding, we still do not fully understand how things work. Indeed, for virtually any signal transduction or biochemical pathway, we lack a comprehensive knowledge of the function in the context of the whole organism's anatomy and lifespan. The rate of new contributions to such knowledge will be greatly accelerated as the sequencing of the zebrafish genome is completed (about a third completed, as reported at the Madison zebrafish meeting) and the lifespan atlas is established (see announcement in this issue).

There remain many unfunded novel approaches and discoveries that utilize the zebrafish. The second reason for discomfort with this first question is that anything short of a miraculous response may be erroneously taken by some as evidence of insufficient progress. A recent example of the misuse of scientific information was a statement made in the last Senate race by one candidate to the effect that it is wasteful to fund research using fruit flies. Could zebrafish be far behind? Exciting work in non-traditional (non-developmental) areas for zebrafish such as cancer, drug discovery, clotting, and aging have yet to reach maturity. For example, cancer research in the zebrafish is just reaching an exciting phase of development. For just one example, zebrafish somatic genomic instability mutants generated in the mid 1990s were recently found to show a strong cancer susceptibility phenotype.[2] The Look lab has generated a beautiful zebrafish model for hematopoietic malignancy[3] that will facilitate drug screens, as well as suppressor/enhancer screens. Collaborative work between the Lees and Hopkins labs resulted in an extraordinary set of cancer susceptibility phenotypes among carriers of multiple ribosomal gene mutations[4] among the famous Hopkins lab insertional mutants;[5] their work may constitute the discovery of a new genetic mechanism contributing to cancer. Other exciting cancer models that include drug screening are emanating from the Zon lab.[6] A variety of exciting work is being pursued in the areas of vasculogenesis, heart development/physiology, and genomics. The breadth of contributions to a broad range of scientific questions, including those relating to human health, continues to expand in a very exciting way.

What is on the horizon? We might ask whether we have yet seen the best opportunities for contribution of the zebrafish community for the benefit of science and man. The resounding answer is that the best is yet to come. Since appropriate review of zebrafish grants cannot occur without involvement of knowledgeable investigators, facilitations of fair review for zebrafish grants must continue. Of clear relevance to the missions of NSF and NIH is the fact that continuing support for the zebrafish community represents a particularly smart investment towards the future of science and benefit to human health.

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1. ***What is the most significant contribution the zebrafish model system has made to our understanding of basic biology and medicine?***

Without doubt, the biggest contribution of the zebrafish system has been in the delineation of principles underlying early vertebrate embryonic development. This has been made possible, in large part, to the amenability of zebrafish to mutagenesis screens similar to those performed in fruit flies. In addition, the zebrafish lends itself to novel kinds of mutagenesis screens that seem to be restricted only by the investigators' imaginations (and, to some degree, budget). With the availability of the genome sequence and better analysis tools (e.g., microarrays, expression databases, morpholinos, GFP lines) the body of knowledge emerging from zebrafish will grow exponentially in the next few years.

Chris T. Amemiya
Benaroya Research Institute at Virginia Mason
Seattle, WA

The zebrafish has made significant contributions to the study of developmental genetics, and I am not sure there is one contribution that surpasses the others. By far, the most important is the ability to complete forward genetic screens, which as generated a host of mutations that are involved in basic developmental processes. Most of the mutations that have been molecularly identified thus far have confirmed gene function in signaling cascades that have been described in other model organisms. However, there are some that have elucidated novel and important roles of specific genes, such as Mib/ubiquitin ligase in neurogenesis and Oep/Cripto in endoderm formation. At the same time, the zebrafish system has been used for a model to study regeneration (fin and heart) and many of the mutations can be used as models for human disease states. The future will hold many more exciting contributions.

Kristin Bruk Artinger
University of Colorado Health Sciences Center
Denver, CO

This has been the confirmation that human genetic disease can be usefully approached via large-scale random mutagenesis in a vertebrate. The relevance of the mutations isolated so far to disease as well as embryogenesis has been a huge reward.

David Bassett
Western General Hospital
Edinburgh, United Kingdom

A significant contribution of the zebrafish model is that it has provided science and medicine with a model that truly integrates across the scientific disciplines of genetics, developmental biology, neuroscience, medicine, and psychology. A common model provides scientists with a uniform framework to which each area can contribute a significant piece of the big picture. This vertebrate animal has become one of the models of choice in the field of genetics, partly because of its ability to breed so rapidly. Similarly, in developmental biology, the zebrafish's rapid development and transparent chorion make it a remarkable model for developmental studies. Perhaps there is no better example of the impact that this animal model has made in integrating the sciences than a visit to the Zebrafish Information Network (ZFIN)[1] website of the University of Oregon. This is a website for anyone who

[1]ZFIN website: zfin.org.

has any interest in zebrafish research. It not only is a valuable source for learning about research in one's specific area, but it also provides an opportunity to learn about the research of scientists using this model in other disciplines. By bringing scientists from different disciplines together to use a common model, the advances made can be astounding. Just take a look at the advances made with the zebrafish model in the short time it has become a viable animal model.

Joseph Bilotta
Western Kentucky University
Bowling Green, KY

Already asking what the most significant contribution zebrafish has made to our understanding of biology and medicine is akin to asking what contribution a 15-year old prodigy has made to humanity. Like the prodigy, zebrafish has been wonderful, but its best years are yet to come. The danger of the question is that if future funding depends solely upon the contributions made to date, the prodigy may never make its most important contributions if stunted by starvation.

There are two contributions made by this model that make it a landmark organism in the history of biology. First is that we now know that a multitude of biological functions can be dissected using forward genetics (mutant screens). The initial screens provided proof of principle for developmental biology. Other screens have shown utility in the areas of cancer, cell differentiation, clotting, and aging, whose story is not yet told. The second most important advance made possible by the zebrafish is the ability to knock down the function of virtually any gene using morpholinos, combined with the ability to determine the tissue localization of your gene of interest. These approaches are being applied across a large number of important biological questions, and a much more meaningful answer to the question will be forthcoming in the coming decades.

Keith C. Cheng
Penn State College of Medicine
Hershey, PA

The ability to obtain a stable cell line of normal spermatogonia from the adult testis without immortalization or transformation and to produce motile test-tube sperm from such spermatogonial cell line represents one significant contribution of the fish models. Spermatogenesis proceeds throughout the adult life that produces sperm to transmit genetic information between generations. Sperm originate from the male germ stem cell spermatogonia that are segregated as primordial germ cells from the somatic lineage. In the adult testis, spermatogonia self-renew to maintain the stem cell pool or differentiate through meiosis to generate spermatids that undergo dramatic changes in morphology, gene expression, and genetic remodeling, eventually giving rise to motile sperm. Efforts have long been made to derive mammalian spermatogenic cell lines and sperm in vitro. In mammals, however, only after immortalization or transformation can spermatogenic cell lines be obtained. Also motile sperm have never been obtained in culture, because the recapitulation of sperm production cannot proceed beyond the spermatid stage. Is it possible to obtain normal spermatogenic cell lines and motile test-tube sperm from them in mammals? Most recently, medaka has provided the answer. We have succeeded in the establishment of a normal medaka spermatogonial cell line capable of sperm production in vitro. Currently, we are using this cell line as an in vitro model to study gene regulation in germ cells and during different stages of spermatogenesis, and to explore the efficient, full recapitulation of sperm production as an attractive approach for germline transmission by in vitro fertilization.

Hong Yunhan
National University of Singapore
Singapore

I have been fascinated by the finding of a larger number of genes in fish genomes and the concept of global and local genome duplications. The selective advantage of the retention of these duplicated genes in earlier vertebrates compared to mammals remains to be understood. Among the duplicated genes we found factor VIIi, an inhibitor for coagulation. It is ironic that man has designed a therapeutic agent without knowing fish have already invented this and retained this agent as one of the duplicated genes. Probably such gene retention is helping them to avoid thrombotic disease. Adding back such genes to mammalian species may provide insights into the function of these genes. Such a provocative approach may receive extreme criticism. However, mutagenesis methods may unravel novel pathways unique to vertebrate biology and I am excited to see the advances in this field.

Pudur Jagadeeswaran
University of Texas Health Science Center
San Antonio, TX

I feel that there has not been any one specific significant contribution, and I would hate to be put on the spot to have to identify "one" at the expense of the others. I would categorically state there have been several significant contributions, and the obvious attributes of the embryo and the genetic manipulations have and will continue to allow us to make inroads toward better understanding basic biology and human genetic diseases. All of these reports have permitted us to go into areas that are unavailable to those utilizing nonvertebrate model systems. Towards that end, the genetic screens have played an instrumental role as have the genome sequencing projects. I think anyone worldwide who operates a zebrafish lab and has published papers using the model can be proud to think of him- or herself as making a significant contribution to the field.

Gregory M. Kelly
University of Western Ontario
London, Ontario, Canada

Forward genetic screens in zebrafish have successfully generated numerous mutants, which are very useful for both basic biological research and drug discovery. Leveraging the transparency and external development of the zebrafish embryo, researchers have been conducting both basic scientific investigations and industrial drug screening. Research using the zebrafish model has produced important insights in many areas of biology and medicine, particularly in cardiovascular diseases and neurodegeneration. The zebrafish model has also contributed significantly to toxicological studies.

Chaoyong Ma
Phylonix Pharmaceuticals, Inc.
Cambridge, MA

Probably most significant has been the identification of genes and the multiple roles they play in the Nodal signal transduction pathway that is important in a number of different developmental processes in vertebrates.

Ferenc Müller
Institute of Toxicology and Genetics
Karlsruhe, Germany

Technically, I think the major contribution is the demonstration that forward genetic screens in a vertebrate are feasible, which has opened huge possibilities for analyzing complex biological processes in an organism quite similar to ourselves. Even where such processes have been previously analyzed in other organisms, genetic screens in zebrafish have clarified and added to the information. Analysis of the Nodal and BMP signaling pathways are great examples of this.

Hazel Sive
Massachusetts Institute of Technology
Cambridge, MA

So far the zebrafish has mainly contributed to our understanding of developmental mechanisms (and mostly restricted to gene identification). There is a huge opportunity for the zebrafish and medaka to contribute to our understanding of human diseases, from cardiovascular diseases to cancer.

Didier Stainier
University of California, San Francisco
San Francisco, CA

The most important contributions of the zebrafish are in the fields for which the zebrafish system was initially chosen, the genetic analysis of development and neurobiology. The big advantage of the zebrafish is the fact that it allows forward genetics as known from *Drosophila* or *C. elegans* but with the important difference of being a vertebrate. This classical genetic approach identifies genes by function and is particularly useful to sort genes into pathways. This is reflected in the characterization of entire biochemical pathways controlling pattern formation in the early embryo such as the Nodal or BMP signaling systems.

Uwe Strähle
University of Heidelberg
Institute of Toxicology and Genetics
Forschungszentrum Karlsruhe
Germany

It's difficult to say. Alex Schier's (NYU, NY) work[1,2] on the long-range action of morphogens in support of gradient ideas has been important. Len Zon's (Children's Hospital of Boston, MA) work[3] on blood mutants with iron uptake/metabolism problems could have important medical implications.

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Brant M. Weinstein
National Institutes of Health
Bethesda, MD

Perhaps the most significant contribution of zebrafish has been to re-focus attention of a broad spectrum of researchers on the value of alternative, nonmammalian models in basic and biomedical research. Zebrafish have been particularly valuable in developmental biology and in highlighting the value of fish as models.

Richard N. Winn
The University of Georgia
Athens, GA

Restricting comments to my field (neurodevelopment/neurobiology), I see major progress coming from the zebrafish neurogenetic model in the elucidation of early induction and determination processes of the neuroectoderm that lead to the formation of the neural plate and its further elaboration into basic divisions of the central and peripheral nervous systems. Examples are the early induction of the forebrain[1] (e.g., Wilson lab [University College, London, UK]) or the establishment and role of the midbrain–hindbrain boundary[2] (e.g., Brand lab [Max-Planck Institute, Dresden, Germany]).

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Mario F. Wullimann
Institute of Neurobiology A. Fessard
Gif sur Yvette Cedex, France

The most significant contribution of the zebrafish model system should be insights being gained from large-scale mutation screening for early embryo development and organogenesis of vertebrates.

Rongjia Zhou
Wuhan University
Wuhan, People's Republic of China

The zebrafish has made central contributions to the understanding of organogenesis. For instance, in the heart field, we now know a few new genes that are involved in migration from bilateral heart primordia to form the structure of the heart. I also believe that the relation of zebrafish to human disease is just beginning to be explored. There have been several examples now of zebrafish modeling human disease. In two cases, a human disease was discovered because of a fish model. The crypto receptor is known to be involved in holoprocencephaly and the ferroportin gene is involved in hemochromatosis. I believe this will happen over and over again.

Leonard I. Zon
Howard Hughes Medical Institute
Boston, MA

2. ***What piscine model systems best complement the zebrafish as a model system for basic biology and medicine by providing capabilities the zebrafish cannot?***

Carp and goldfish are closely related to zebrafish and are considerably larger in size; thus, they are much more tractable to initiation of cell cultures, something still highly problematic with zebrafish. Likewise, the channel catfish, a very important aquacultural species whose hematopoietic cells can be cultured, is a nice model system for studies on both the innate and adaptive immune systems. These other systems, (carp, goldfish, and catfish) can be used to effectively complement studies in zebrafish.

Chris T. Amemiya
Benaroya Research Institute at Virginia Mason
Seattle, WA

I believe the medaka system holds the most promise for complimenting the zebrafish as a model system. Medaka is similar in its biology and development, including the ability to do reverse genetic screens. Undoubtedly, these mutations uncovered in the medaka system are certain to compliment zebrafish mutations. Further, the genomic resources have grown exponentially in medaka, and thus the ability to clone mutant genes and create transgenic lines is already ongoing. The most exciting methodology that is yet to be described in zebrafish is the ability to culture medaka stem cells over a longer period of time such that gene targeting can be attempted in a fish model. The ability to do forward genetics in the fish model will certainly increase our knowledge of gene function.

Kristin Bruk Artinger
University of Colorado Health Sciences Center
Denver, CO

The fugu as a genomic model for its lack of junk DNA and facilitation of sequence comparison, and medaka for providing complimentary mutations so far.

David Bassett
Western General Hospital
Edinburgh, United Kingdom

The answer to this question depends upon the scientific question being asked. A primary bioinformatic partner to zebrafish, though not an experimental model, is the pufferfish (fugu), whose value lies in its relatedness to zebrafish, significant conservation of gene order, and little "junk" DNA. The medaka is likely to provide sets of mutants that complement those found in zebrafish. Experiments requiring larger amounts of tissue will require larger fish species such as goldfish, tilapia, and carp. Increasing environmental contamination makes it imperative for civilization to establish effective environmental monitoring. Zebrafish will play an important role as a toxicological model, but must be complemented by feral species (piscine and nonpiscine) that will be more appropriate for "in situ" environmental monitoring. The monitoring species used will depend upon the geography of the waters and the environment being monitored. Finally, complementation of zebrafish research by work with other genetic model systems will be highly informative. The nonpiscine vertebrate, *Xenopus tropicalis*, should be considered for development as a complementary vertebrate genetic model system.

Keith C. Cheng
Penn State College of Medicine
Hershey, PA

Medaka is my favorite. Compared to zebrafish, medaka provides additional advantageous features. Medaka is peaceful; a single female lays eggs every day; egg laying takes place within 30 minutes after light switches on; there are many inbred strains, populations that provide genetic diversity for phenotypic analyses. The speed of embryonic development can easily be slowed down by decreasing temperature, allowing many embryos to be micro-injected with cells for chimera formation on a single day. Cell culture is a powerful tool in mammals but has not been popularly used in fish. Zebrafish appears to be resistant for cell culture derivation. This is reflected by the availability of few cell lines. In this regard, medaka seems better, as cell cultures are easy to obtain from embryos and adult tissues.

Hong Yunhan
National University of Singapore
Singapore

Zebrafish is a one-inch capsule for studying the genetics of vertebrate biology. Since there are approximately 30,000 genes in mammals and most of them are present in fish, it is virtually possible to understand the functions of almost all the genes using zebrafish model system. However, because genes related to lungs, prostate, and breast mostly function in mammals, related research cannot be attempted with zebrafish even though some of the genes expressed in these tissues are present in zebrafish. Furthermore, since zebrafish are long lived, questions of longevity cannot be addressed that easily. Thus, other fish models that could complement the above programs are needed. For example, annual fish could provide information on age-related genes. Unfortunately, while lungfish conceptually could help in issues with lung development, it may not become a laboratory genetic model due to their size and seasonal breeding.

Pudur Jagadeeswaran
University of Texas Health Science Center
San Antonio, TX

The pufferfish (*Fugu rubripes*) has the potential to provide important insights in gene function, due to its compact genome, with about the same number of genes as the human genome packed into only one-eighth of genome size. Other teleosts, in particular medaka, have been used in specific research areas (such as environmental toxicity testing) for a longer time than zebrafish, and researchers have developed specific protocols and databases with those teleost models that are not readily replaceable by zebrafish. However, these models lack the comprehensive resources that are being generated for zebrafish, and in most aspects are less favorable than zebrafish for research in basic biology and medicine. However, there is a huge number of other teleost species that have not been studied, and it is possible that more favorable models can be identified in the future, especially for certain areas of research.

Chaoyong Ma
Phylonix Pharmaceuticals, Inc.
Cambridge, MA

At the moment it is medaka and pufferfish. Medaka is a good "all-rounder" model with advanced genetics and developmental biology and emerging genomics.

Ferenc Müller
Institute of Toxicology and Genetics
Karlsruhe, Germany

I would choose an amphibian rather than another fish. *Xenopus tropicalis* is being actively developed as a genetic system, and while *X. tropicalis* genetics and genomics tools are nowhere near the level of zebrafish sophistication, the system is very promising. In particular, *Xenopus* offers early embryos that are in many ways much easier to analyze than fish counterparts. Although zebrafish embryos are beautiful, they are more difficult to micromanipulate than *Xenopus* embryos—for example, it is possible to easily separate the germ layers in a *Xenopus* gastrula, not so in zebrafish. Another fantastic tool in *Xenopus* is the easy ability to generate stable transgenic animals. These can be used in promoter analysis, for example, where such analyses are very difficult to do well in the zebrafish at present. Finally, comparison of similar processes in fish and frogs is a wonderful tool to define evolutionarily conserved (or nonconserved) gene function.

Hazel Sive
Massachusetts Institute of Technology
Cambridge, MA

Carp and catfish. They provide larger size and the ability to do more physiology and anatomy and get larger amounts of tissue for experiments on adults compared to zebrafish, but are (especially for carp) genetically similar enough that one can cross back into zebrafish using findings from these other fish.

Fugu, with its more compact genome, also makes a useful complementary model organism for studies of genome organization and structure, gene regulation, and positional cloning, among other things.

Brant M. Weinstein
National Institutes of Health
Bethesda, MD

Medaka has a variety of important attributes not available in the zebrafish that, at the very least, provide opportunities for expanded experimentation. Among these features include genetic distance (possibly equivalent to the chicken and the human), a smaller genome, tolerance of a wide range of temperature (~1°C to 40°C) and other environmental variables (e.g., salinity), XY sex determination, apparent lower incidence of chronic infectious diseases, and precocious young that feed almost immediately upon hatching. And don't forget, similar to the zebrafish, medaka has transparent embryos!

Richard N. Winn
The University of Georgia
Athens, GA

The second significant piscine neurogenetic model, the medaka fish, offers a particular chance for a developmental comparison of different phenotypes in relatively closely related models (as opposed to widely divergent other existing models). This comparison might be of use for the understanding of how certain alterations in developmental pathways effect morphogenetic outcome, and, ultimately, results in the evolution of different morphotypes.

Mario F. Wullimann
Institute of Neurobiology A. Fessard
Gif sur Yvette Cedex, France

Zebrafish as a fish model system has disadvantages, for example, in sex determination, in vivo fertilization, fertilized egg implantation, gestation, and parturition. Other fish model systems, for example, both the rice field eel (sex reversal) and medaka (Y chromosome) can be complementary for sex determination, and both mosquitofish and guppy for developing embryos in the mother.

Rongjia Zhou
Wuhan University
Wuhan, People's Republic of China

The medaka species is very closely related in terms of a model system. There are large-scale screens going on in the medaka system with a very significant interest in organogenesis and embryonic patterning. It is possible that novel mutants will be obtained and this will complement the zebrafish activity. The fugu species has also been invaluable for understanding genome structure based on chromosomal synteny and for positional cloning approaches in the zebrafish system.

Leonard I. Zon
Howard Hughes Medical Institute
Boston, MA

3. *Are there areas of research in which fish models are not currently being used but you feel will play a significant role in the future?*

Two areas that have not really been exploited in fishes are: (1) the use of fish as models for gene(ome) duplications to understand subfunctions of genes in tetrapods (humans); and (2) the use of fish for drug and toxicological screening assays. Regarding (1), due to retained functions of certain duplicated genes in teleost fishes, natural experiments exist in which gene functions have been subdivided among retentates, thus allowing better dissection of the individual components of nonduplicated homologs in tetrapods. This certainly applies to disease causing genes in humans, and several examples are coming to light with the availability of the zebrafish genome sequence. Regarding (2), small fishes such as zebrafish would seem to be ideal for studying the *in vivo* effects of small molecules and toxicants.

Chris T. Amemiya
Benaroya Research Institute at Virginia Mason
Seattle, WA

An area of research in the fish model that is not being pursued much is neuroscience research, more specifically in creating models for neurodegenerative diseases, behavior, and repair. Perhaps one of the reasons that this area has not been pursued is the basic difference between behaviors in the fish versus human. However, it is certain that the same molecular cascades that are used in the human is conserved in the fish and thus a clearer understanding of gene function can be obtained using this model.

Kristin Bruk Artinger
University of Colorado Health Sciences Center
Denver, CO

Pharmacology: there will be more testing of drug effects and side effects using fish.

David Bassett
Western General Hospital
Edinburgh, United Kingdom

Fish models, particularly zebrafish, have played an important role in our understanding the mechanisms of genetics, physiology, and developmental biology. The advances made in these areas based on work from a variety of fish species are remarkable. However, presently, there have been few advances made in using these species in behavioral neuroscience and the behavioral sciences in general. This, of course, is due to the limited use of paradigms available to study behavioral measures in fish species. Fortunately, these paradigms are being developed and are beginning to be used to address questions regarding the role of genetics and physiology in behavior. It will soon be the case that the wealth of genetic information obtained with the zebrafish will be used in conjunction with behavioral measures to examine the role of genetics in both simple and complex learning abilities. In addition, integrating the knowledge from developmental biology and neuroscience with behavioral measures will advance not only the scientific field but the field of medicine as well. This integration will help propel the zebrafish to become a useful model in understanding problems such as drug abuse, fetal alcohol syndrome, and the impact of prenatal factors on development.

Joseph Bilotta
Western Kentucky University
Bowling Green, KY

The types of investigations that can be pursued in the zebrafish are limited only by the imagination of the geneticist. One of the most important reasons for continued strong support for the zebrafish community is that the application of our collective imaginations has only just begun.

Integration of data related to gene function is an area that we have begun to develop, and are extremely excited about. The potential for that integration was inspired by the thousands of whole mount *in situ* images generated by the Thisse and Dawid labs, will be enhanced by a growing collection of transgenics generated by the zebrafish community in which tissue-specific fluorescence occurs, and which will be more completely enabled by the high-resolution virtual life-span atlas we have just begun. As will be described in detail, we are proposing that the zebrafish be used as a "hub" organism for a new level of genomic analysis dedicated to the comprehensive description of genes with regard to space (tissue, cell, subcellular localization), life-span, and function (mutants, knock-downs, disease). We are calling this area of investigation, "Systems Morphogenetics," for which a FASEB Symposium has been organized for 2005 and for which collaborative work and other meetings are being planned.

Keith C. Cheng
Penn State College of Medicine
Hershey, PA

Stemness is an active research field. Such studies largely rely on *in vitro* experiments using mammalian stem cells. Theoretically, early developing embryos are ideal for stemness, because the decision between stem cell renewal and differentiation takes place at early stages of embryogenesis. However, mammalian embryos of critical stages (e.g., gastrulation) are inaccessible. In contrast, both zebrafish and medaka provide a large number of eggs/embryos every day under robust conditions, and the embryology is easy, external, transparent, and accessible for observation/manipulation at the single cell level. This unique feature is ideal for studying stem cell biology. Furthermore, the fish models as the most distantly related vertebrate provide an important reference for comparative approaches to define stemness.

Gene targeting is widely utilized in mouse genetics but has not been fully developed in fish. The key here is cell-mediated germline transmission. Our ability to derive chimera-competent embryonic stem (ES) cell lines from medaka and Collodi's recent report on germline chimeras from zebrafish embryo cells after weeks of culture are important steps towards ES cell technology.

Hong Yunhan
National University of Singapore
Singapore

Currently, there have been only a few reports regarding the utilization of annual fish for aging research. Certainly, the annual fish are easy to breed and have many strains available. Particularly noteworthy species seems to be *Nothobranchius*. Thus, future research in identifying genetic markers and appropriate strains for genetic studies are needed for the utility of this model. I am confident that the *Nothobranchius* model will undoubtedly contribute rapidly to the progress of vertebrate longevity.

Pudur Jagadeeswaran
University of Texas Health Science Center
San Antonio, TX

Zebrafish are undoubtedly not going to replace Atlantic salmon or rainbow trout as the next food fish in your local supermarket. In the future, however, identifying alternative fish species that are easy to maintain, grow fast under a variety of differing and often inhospitable environmental conditions, and can be produced in large enough quantities for human consumption whilst remaining cost effective will be a necessity to meet growing food demands. Fish that meet these criteria will only be discovered after conducting intensive field studies following genetic manipulations and analyses performed in the lab. I do not have a crystal ball to predict how we should initiate these studies, but I do hope that the many lessons we have learned from zebrafish in the lab can be translated to these "newly identified" food-fish species in the "field"—so to speak!

Gregory M. Kelly
University of Western Ontario
London, Ontario, Canada

Although tumorigenesis has been investigated in zebrafish, a good zebrafish tumor model has not been established. I think this is a very promising area, considering the similar histology of neoplasms in fish and humans, and the experimental tractability of zebrafish that enables both thorough mechanistic studies and high throughput *in vivo* drug screening.

Chaoyong Ma
Phylonix Pharmaceuticals, Inc.
Cambridge, MA

Physiology is up and coming, especially physio-genomics where fish that occupy such a diversity of habitats and environmental conditions with remarkable plasticity in adaptation will almost certainly have a large impact.

Toxicology is another field where the use of fish and fish embryos in particular will have an important complementing role to *in vitro* techniques and will replace higher vertebrate models in countries where law and public opposition concerning animal rights have restricted the use of other vertebrate models. Aspects of neurobiology may be another area, including genetics and cell biology of behavioral science.

Ferenc Müller
Institute of Toxicology and Genetics
Karlsruhe, Germany

I believe analysis of older fish to address adult-onset or nonlethal phenotypes will become very important. This opens the door to analyzing a large spectrum of disease-related phenotypes, including neurodegenerative diseases such as multiple sclerosis. Behavioral screens are currently being performed, but I believe emphasis on such screens will increase, including those that relate to human mental health disorders.

Hazel Sive
Massachusetts Institute of Technology
Cambridge, MA

Zebrafish were mostly used as models for early developmental processes. Relatively little effort has so far been put into identification of mutations that affect adult body homeostasis and could thus serve as human disease models. Even though we lack so far tools like conditional knock-out, systematic searches for adult phenotypes, even with the current zebrafish technologies, may identify mutations that affect body homeostasis and behaviour. These models could be used in systematic compound screens to identify novel pharmaceuticals with relevance for the human condition.

Uwe Strähle
University of Heidelberg
Institute of Toxicology and Genetics
Karlsruhe, Germany

Evolutionary biology, behavioral genetics, drug testing, and pharmacogenetics. Although there is little current use for these areas of research, they could play a bigger role in the future.

Brant M. Weinstein
National Institutes of Health
Bethesda, MD

Fish are being embraced in such a wide range of research areas, and at such a rapid pace, that it is becoming difficult to describe an area in which fish are NOT being applied. However, possibly the most promising, and most challenging task remains in developing and testing the efficacy of fish models that exhibit various human diseases. In addition, small aquarium fish models, due to short generation times and prolific capacity for reproduction, can be invaluable in deciphering the roles of the environment and genetics in transgenerational diseases.

Richard N. Winn
The University of Georgia
Athens, GA

The use of the zebrafish in biomedical applications, for example in the context of neurodegenerative disorders, has been limited and its potential remains yet to be fully taken advantage of. One main reason is the poor neurobiological understanding and even identification of the relevant zebrafish systems affected in potentially similar neurological disorders as seen in mammals. Perhaps studies on the dopaminergic system are most progressed (e.g., Driever lab [University of Freiburg, Germany])[1] where a complexity of subsystems, (including a potential nigrostriatal system) and their developmental control has been revealed. In contrast, the zebrafish brain cholinergic system is practically unknown presently; the definition of basal forebrain cholinergic neurons being critical before their eventual role in a "zebrafish Alzheimer model" may be studied.

1. Holzschuh JS, Hauptmann G, Driever W. Genetic analysis of the roles of Hh, FGF8, and nodal signaling during catecholaminergic system development in the zebrafish brain. J Neurosci 2003:23:5507–5519.

Mario F. Wullimann
Institute of Neurobiology A. Fessard
Gif sur Yvette Cedex, France

Teleosts are the most numerous of vertebrates and display remarkable diversity in morphological and physiological adaptation. Many different fish species have unique characteristics. Fish model systems will play a significant role in research on global environmental change and in space life sciences.

Rongjia Zhou
Wuhan University
Wuhan, People's Republic of China

The zebrafish could be invaluable for this approach in the basic circuitry of behavioral responses. There are a few instances of zebrafish models of cancer and this will ramp up significantly over the next 5 years. The ability to make transgenic models as well as to create tumor suppressor gene knock-outs by TILLING should enhance our understanding of cancer in the vertebrate.

Leonard I. Zon
Howard Hughes Medical Institute
Boston, MA

4. ***What is the next up-and-coming piscine species to become a significant contributor to broad areas of biological and biomedical sciences, following in the footsteps of zebra-fish and medaka?***

I believe there will be two species that will make large contributions: (1) Nile tilapia; and (2) sticklebacks. The tilapia represents a large and diverse group of fishes (cichlids) that has undergone extreme speciation and morphological divergence in certain populations (such as in the African Rift Lakes). Sticklebacks are also highly variable with regard to morphological divergence but, at least in the case of three-spine sticklebacks in North America, this variation has occurred very recently and the morphotypes may represent populations on the verge of becoming species. Both tilapia and sticklebacks are culturable in the laboratory, and genome projects have been initiated on the respective species. It will be fascinating to begin to understand the role that the genome plays in the evolution of adaptations and adaptive change, and how these principles may apply to human evolution.

Chris T. Amemiya
Benaroya Research Institute at Virginia Mason
Seattle, WA

Sticklebacks and cichlids for evolution.

David Bassett
Western General Hospital
Edinburgh, United Kingdom

Use of African cichlids to study speciation and evolution. Recently two fish species have been completely sequenced (*Takifugu rubipes*, *Tetraodon nigroviridis*) and two others, zebrafish and medaka, are on their way to being annotated. Lately, the Cichlid Genome Consortium (http://hcgs.unh.edu/cichlid) has proposed to sequence the African cichlids, Tilapia and Haplochromine. The sequencing of these two African fish genomes will help solve a number of questions related to evolution and speciation. One of the most exciting insights that could be gained with the help of cichlid genomes is the study of genome evolution in two closely related species. There is less genomic diversity between two African cichlids from Lake Victoria than there is between two humans from different continents. Despite their high levels of genome conservation, African cichlids are highly polymorphic in terms of morphology, color and behavior. This leads us to other interesting questions, such as how a mutation changing the color of a fish (mutation in the promoter region or in the protein coding gene) or color pattern can affect the reproductive success of the fish? Last but not least, in contrast to Fugu, African cichlids are easy to breed. Moreover, some cichlids are quite small and can be raised in large numbers in aquariums. These are just some of the questions that can be answered by the cichlid, a fish that will surely become more and more popular in the near future.

Yann Gibert
University of Konstanz
Konstanz, Germany

The first ones that come to mind are Xiphophorus and goldfish, as there is already a plethora of background information in many areas that complements that in zebrafish. Having said that, I would not exactly call these species "up-and-coming" as they have been used routinely as fish models for years. Their utility is certainly not in question, but their popularity may have suffered amidst the explosive research flurry with zebrafish. My question is: do we really need to make a conscientious effort to identify which species to earmark as a follow-up to zebrafish? Possibly following along my response to Question 3, instead of contemplating which of the current species do we consider as a successor, rather make a concerted drive to start from the ground floor and identify a "new" species, creating the spreadsheet of pros and cons that in the end best fits the need of the scientific community, not to mention the public and their needs.

Gregory M. Kelly
University of Western Ontario
London, Ontario, Canada

As I mentioned in my opinion to Question number 2, I think the Fugu can complement the power of zebrafish and medaka.

Chaoyong Ma
Phylonix Pharmaceuticals, Inc.
Cambridge, MA

The small genome species in the Anabantidae family (*Macropodus* or the siamese fighter fish, *Betta splendens*) have great potential. Both have hardly larger genomes (600 Mb) than pufferfish (350 Mb), but have the great advantage of easy breeding in laboratory conditions. Siamese fighters have been used in the past in laboratory research as this is again a prolific small species with transparent embryos but with the very exciting behavior (nest-building, fighting, etc) which indicates highly developed cognitive functions, potentially making this species a very interesting model for neurosciences including neurodevelopment and behavior. Having a small genome should be great advantage in terms of genetics and genomics in general. I think the advantages of these species do not stop at neurosciences, and can be a nice complement to the already sequenced genomes for comparative analysis.

Ferenc Müller
Institute of Toxicology and Genetics
Karlsruhe, Germany

Again, I would think outside the teleosts here and put my money on *Xenopus tropicalis*.

Hazel Sive
Massachusetts Institute of Technology
Cambridge, MA

The medaka certainly best complements the zebrafish, and the stickleback offers unique opportunities for a number of key questions. However, the effort should be directed at developing the full potential of these systems rather than developing more and more piscine systems.

Didier Stainier
University of California, San Francisco
San Francisco, CA

Sticklebacks (see the recent issue of *Science*[1]).

1. Pennisi E. Changing a fish's bony armor in the wink of a gene. *Science* 2004:304:1736–1739.

Brant M. Weinstein
National Institutes of Health
Bethesda, MD

Fundulus heteroclitus, the Atlantic killifish, is already becoming a useful model in comparative toxicology. Compared to medaka and zebrafish, an additional justification for using *Fundulus* is their environmental relevance. *Fundulus* are found in contaminated environments along the Atlantic coast of the United States, with diseases including cancer and reproductive/developmental deficits. Similar to other laboratory fish, *Fundulus* are easy to maintain in the laboratory because of their small size and hardiness. Adult *Fundulus* are externally sexually dimorphic and are well suited for reproductive and developmental studies. At 2-week intervals, females naturally produce a large number of mature oocytes with a high rate of fertilization success. *Fundulus* have even been suggested as a nonmammalian model for cyclic reproductive activity because their tight follicular sequence of recruitment, maturation, and ovulation is similar to what occurs in the follicular phases of the menstrual and estrous cycles in mammals.[1] Armstrong and Child[2] have described *Fundulus* stages of embryonic differentiation and organogenesis. The early developmental stages (1–20) are marked by rapid cell divisions with little growth. Neural structures begin to form at stage 20 (day 2), and circulation begins about stage 25 (day 3). The remaining stages (27–35) occur over days 4–20 and involve active organodifferentiation and growth followed by hatching. Thus, development from fertilized egg to swimming larvae takes only about 3 weeks; long enough to isolate precise toxicant-mediated developmental deficits, but short enough to be an advantage relative to mammalian developmental toxicity models.

More recently, *Fundulus* is being used as a model for marine toxicology. The genes involved in CYP1 induction including the aryl hydrocarbon receptor (AhR1 and AhR2), the AhR repressor, the Ah nuclear transporter (ARNT2), and several other CYPs from families 1–3 have been cloned. More recently, their antioxidant defense systems have been described. Because *Fundulus* have a relatively small home range, populations of *Fundulus* exist that have been exposed to and tolerated: PAHs (Elizabeth River, VA), PCBs (New Bedford Harbor, NY), dioxins (Newark Bay, NJ), and bleached kraft pulp mill effluent (Miramichi Estuary, NB). Because of the existing understanding of reproduction and development, and the increasing number of toxicological endpoints being characterized, and their environmental relevance, *Fundulus* is an appropriate and useful model for studying mechanisms of environmental contaminant toxicity.

1. Hsiao S-M, Limesand SW, Wallace RA. Semilunar follicular cycle of an intertidal fish: the *Fundulus* model. Biol Reproduct 1996:54:809–818.
2. Armstrong PB, Child JS. Stages in the normal development of *Fundulus heteroclitus*. Biol Bull 1965:128:143–169.

Kristine Willett
University of Mississippi
University, MS

Fundulus heteroclitus can make distinct contributions to broad areas of biological and biomedical sciences. This species has an annotated genetic database that already rivals the other fish species. *Fundulus* may provide unparalleled insight into the role of genetic diversity in disease, particularly the role of the environment on gene expression, and differential susceptibilities to disease.

Richard N. Winn
The University of Georgia
Athens, GA

There is no immediate necessity or desirability for a general additional piscine model in basic developmental biology. However, some fish species are successfully used in particular contexts, for example, nervous control of hormonal regulation of life cycle in salmonids or eels, or neurophysiology of the electric sense and related electromotor behavior in South-American and African weakly electric fish.

Mario F. Wullimann
Institute of Neurobiology A. Fessard
Gif sur Yvette Cedex, France

Several fish species will be powerful model systems for biological and biomedical sciences, for example, the rice field eel and pufferfish following in the footsteps of zebrafish and medaka.

Rongjia Zhou
Wuhan University
Wuhan, People's Republic of China

I think the up and coming model species includes cave fish. Cave fish is a wonderful species for doing quantitative genetics. I think this will nicely complement the zebrafish. Stickleback is clearly a species that has done extremely well with genetic maps and has very interesting evolutionary biology considerations. Lastly, *Xiphophorus* has a tumor suppressor model that has been worked on by a number of investigators and there could have a resurgence based on the increased response to work on cancer in the zebrafish.

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