U.S. Department of Health and Human Services
National Institutes of Health
Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs
Division of Comparative Medicine

# Validation of Animal Models and Tools for Biomedical Research
# Session VII. Validation of Non-Zebrafish Aquatic Models for Preclinical Research

Thursday, December 17, 2020
Virtual Meeting

## Workshop Report

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# Executive Summary

The seventh of 10 sessions of the Virtual Workshop on Validation of Animal Models and Tools for Biomedical Research was held on December 17, 2020. This workshop is intended as a venue to discuss the status of and needs for the validation of animal models used in biomedical research. Session VII focused on the validation of non-zebrafish aquatic models for preclinical research. Highlighted models included *Xiphophorus*, threespine stickleback, cavefish, *Xenopus*, axolotl salamander, and *Aplysia.* Additionally, deep phylogenetic mapping was discussed. Several participants emphasized the importance of increased support for nontraditional model systems that take advantage of extreme phenotypes in nature for uncovering new principles of biological variation. They also expressed interest in strengthening connections between and across model communities. Participants voiced support for increased representation and dissemination of information across National Institutes of Health (NIH) study sections. Several attendees discussed the need for coordination of databases across organisms. The following needs for validation were identified: tools for phenotyping (e.g., definition of stages, description, course of phenotype changes, disease trajectories, morphology, histology, molecular phenotyping, premalignant stages, detection of new disease phenotypes), tools for functional validation (e.g., transgenesis, genome modification, *in vitro* validation cell culture lines, antibodies for protein function), reagents (e.g., antibodies to differentiate between cell types across populations, primer sets for specific biomarkers), datasets and databases (e.g., genomics, transcriptomics, proteomics, metabolomics, microbiomics), an atlas of developmental stages (e.g., single cell through adult, several representative populations), archive repositories (e.g., microbes, whole animals, tissues), stock centers (e.g., pathogen-free animals, knockout animals), gnotobiotic foods (e.g., live, dry), microscopy tools (e.g., imaging of live animals and gut through development), establishment of the spatial context of gene expression (e.g., single-molecule fluorescent *in situ* hybridization, spatial transcriptomics, antibody validation), development of resources for functional interrogation of genomics data (e.g., GAL4/UAS), concerted programs for diversity sampling, comparative genomics programs for integrated disease modeling (e.g., resequencing and transcriptomic approaches), transgenic animals (e.g., development of viral vectors, electroporation of newly laid eggs), improved gene annotation and resources for accessing genomic and transcriptomic data (i.e., akin to Xenbase, FlyBase, Zebrafish International Network), cell lines, and cryopreservation of stocks.

**Session Co-Chairs**
John Postlethwait, Ph.D., University of Oregon
Crystal Rogers, Ph.D., University of California, Davis

**Presenters**
Lynne Fieber, Ph.D., University of Miami
Matthew Harris, Ph.D., Boston Children's Hospital
Carole LaBonne, Ph.D., Northwestern University
Kathryn Milligan-Myhre, Ph.D., University of Connecticut
Nicolas Rohner, Ph.D., Stowers Institute for Medical Research
Manfred Schartl, Dr. rer. nat., Texas State University, San Marcos
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Sige Zou, Ph.D., Coordinator, Program Official, Office of Research Infrastructure Programs (ORIP)

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# Workshop Report

**Opening Remarks**
*Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, ORIP*
*Sige Zou, Ph.D., Coordinator, Program Official, ORIP*

Drs. Stephanie Murphy, Director, Division of Comparative Medicine, ORIP, and Sige Zou, Coordinator, Program Official, ORIP, welcomed the participants and offered thanks to the Organizing Committee and Session Co-Chairs for their efforts in organizing the event. Dr. Murphy explained that the meeting is the seventh in a series of 10 sessions. Drs. Murphy and Zou also acknowledged the support of several NIH Institutes: the National Heart, Lung, and Blood Institute (NHLBI); National Institute on Aging (NIA); National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); National Institute of General Medical Sciences (NIGMS); and National Institute of Neurological Disorders and Stroke (NINDS). Dr. Murphy reminded the participants that validation of animal models and tools is a critical part of ORIP's trans-NIH efforts. She expressed appreciation for the participants' input. Dr. Crystal Rogers, Co-Chair, introduced the speakers.

**Introduction to the Validation of Aquatic Models in Research**
*John Postlethwait, Ph.D., University of Oregon*

Dr. John Postlethwait, Co-Chair, presented an overview of the use of aquatic models in biomedical research. First, he presented a broad overview on the use of evolutionary mutant models for human disease. Genetic mechanisms of development and function are widely conserved, and phenotypes in mutant model organisms clarify gene functions. He compared forward genetic screening (i.e., randomly mutagenize, select a phenotype, identify the gene) and reverse genetic screening (i.e., mutagenize a specific gene, study the phenotype). Dr. Postlethwait noted that phenotypes in forward genetic screening often appear early in development, but early phenotypes can mask later pleiotropic effects. Additionally, the mutations often are recessive and affect protein-coding regions; in human diseases, however, many mutations are dominant and play regulatory roles. Evolutionary genetic screens use nature to select mutant genotypes that are likely to be useful in a particular context. In evolutionary screens, the phenotypes mimic human diseases, and the mutations often are regulatory. The validation of evolutionary models involves face, construct, and predictive validity.

***Xiphophorus*, an Evolutionary Model for Epistatic Interactions in Disease**
*Manfred Schartl, Dr. rer. nat., Texas State University, San Marcos*

Dr. Manfred Schartl discussed the use of *Xiphophorus* as a model for epistatic interactions in disease, including malignant melanoma. He explained that melanoma is prevalent in the United States. Only a small fraction of cancer patients has benefited from the research community's incomplete knowledge of cancer-initiating tumor driver genes. Because tumors evolve and different background effects are present, patients with the same mutation can exhibit differing courses of disease. Tumor modifier genes play determining roles in the course of disease. These genes are challenging to detect because they are pleiotropic, have individual-specific effects, provide partial contributions to disease phenotype, and can easily escape detection in cancer genome sequencing projects. Non-zebrafish aquatic models, such as *Xiphophorus,* can be used for disease gene detection, characterization, and validation. *Xiphophorus* models are evolutionary; they can be used for the identification of disease drivers, disease modifiers, new disease genes, and drug targets. Additionally, evolutionary models offer such technical advantages as ease of production, availability of inbred lines, genotypic and phenotypic diversity from wild populations, and extensive resources and tools. Dr. Schartl employs three approaches for studies using *Xiphophorus*: (1) targeted crosses that combine Mendelian genetics with genomics and *in vitro* biochemistry, (2) use of

genotypic variation and population genomics for the creation of natural hybrids, and (3) use of hybrid incompatibilities and epistatic gene interactions to expand the *Xiphophorus* model to a wide variety of diseases. The model satisfies face, construct, and predictive validity. It replicates melanoma clinical findings in humans (e.g., tumor cell localization, disease progression stages); predicts pathways, interferon resistance mechanisms, and osteopontin's role in tumor growth; and exhibits molecular drivers, pathways, and disease signatures that are largely consistent with human disease. Current areas for development of validation tools include phenotyping (e.g., definition of stages, description, course of phenotype changes, disease trajectories, morphology, histology, molecular phenotyping, premalignant stages, detection of new disease phenotypes) and functional validation (e.g., transgenesis, genome modification, *in vitro* validation of cell culture lines, antibodies for protein function).

**Adapting the Evolutionary Model Organism, Threespine Stickleback, for Host–Microbe Interaction Studies**
*Kathryn Milligan-Myhre, Ph.D., University of Connecticut*

Dr. Kathryn Milligan-Myhre presented on the threespine stickleback as a model for host–microbe interactions. The microbiota plays a critical role in host development, and Dr. Milligan-Myhre is analyzing the contribution of the host genetic background on complex traits. Because threespine stickleback (*Gasterosteus aculeatus*) populations are abundant in the northern hemisphere, they have been used widely in biomedical studies. Dr. Milligan-Myhre is interested in identifying stickleback from freshwater, anadromous, and marine populations in Alaska that display genetic variants representing human diseases. Juvenile sticklebacks are optically transparent, allowing researchers to observe organ and tissue development. Additionally, they share numerous biological pathways with humans, such as bone development, immune responses, cell type development, and variation in innate immune response to microbiota. Their evolution and development have been described thoroughly, and experimental genetic manipulation and generational crosses are well established in the laboratory. Dr. Milligan-Myhre's group performs gnotobiotic studies to identify genes and gene combinations involved in complex traits. Current needs include reagents (e.g., antibodies to differentiate between cell types across populations, primer sets for specific biomarkers), datasets and databases (e.g., transcriptomics, proteomics, metabolomics, microbiomics), an atlas of developmental stages (e.g., single cell through adult, several representative populations), archive repositories (e.g., microbes, whole animals, tissues), stock centers (e.g., pathogen-free fish, knockout fish), gnotobiotic foods (e.g., live, dry), and microscopy tools (e.g., imaging of live fish and gut through development).

**Cavefish as a Model for Natural Resilience to Metabolic Disease**
*Nicolas Rohner, Ph.D., Stowers Institute for Medical Research*

Dr. Nicolas Rohner highlighted cavefish as models for metabolic diseases. Cavefish are highly abundant; many researchers study the Mexican tetra, *Astyanax mexicanus*, because it contains two distinct but closely related populations—a blind cave form and a sighted surface form. *A. mexicanus* has been used in analyses of retinal degeneration, albinism, sleep loss, and heart regeneration. Dr. Rohner's group is studying *A. mexicanus* as a model for metabolic resilience. The cave and surface fishes live in distinct metabolic environments; the cavefish have adapted to a "feast and famine" metabolic cycle. They overeat when food is available and store excess energy as body fat (i.e., hypertrophic visceral adipocytes). Cavefish exhibit traits that reflect their adaptation (e.g., high blood sugar, glucose intolerance, insulin resistance); however, they live long, healthy lives and do not develop inflammation or advanced glycated end-products. Dr. Rohner emphasized that cavefish are a model for health and resilience, not a disease model. Advantages of the cavefish model include closely related populations (i.e., genetic mapping, complementation analysis, comparative genomics), independently derived populations with similar traits (i.e., multiple hits to the same pathway, alternative approaches), and an established research system

(i.e., tools and resources). Current tools include genome projects, transgenic lines, established husbandry practices, and brain atlases. Current needs include improved genome resources, comprehensive identification of major cell types (e.g., single-cell RNA sequencing, proteomics, metabolomics), establishment of the spatial context of gene expression (e.g., single-molecule fluorescent *in situ* hybridization, spatial transcriptomics, antibody validation), development of resources for functional interrogation of genomics data (e.g., GAL4/UAS), and facilitation of access to defined populations to maintain genetic variation (e.g., stock centers). Dr. Rohner emphasized the importance of increasing support for nontraditional model systems that take advantage of extreme phenotypes in nature for uncovering new principles of biological variation.

**Strength in Diversity: Deep Phylogenetic Mapping to Understand the Causes and Compensation of Disease-Related Phenotypes**
*Matthew Harris, Ph.D., Boston Children's Hospital*

Dr. Matthew Harris discussed deep phylogenetic mapping to study the underlying causes of disease phenotypes. Dr. Harris' group uses forward genetics to establish models for disease (e.g., skeletal dysplasia, aging, craniofacial development). The group is studying genetic background effects of expressivity and penetrance of phenotypes. For example, they characterize genetic variations in the presentation of osteogenesis imperfecta. Strategies include quantitative trait analysis, modified forward genetic screens and epistasis, and evolutionary analysis (e.g., forward genetics, PhyloMap). Dr. Harris outlined the PhyloMap approach, explaining that sampling of small population pools allows analysis of variance within and between species and identification of unique variants that potentially underlie character change within a clade. This approach allows researchers to determine evolutionary divergence and replicate comparisons within a clade. Dr. Harris highlighted the Antarctic notothenioid fishes—which display a wide array of phenotypic variation that reflects their unique environment—as an evolutionary case study for model development. His group used this phylogenetic structure to define genetic pathways associated with disease-related phenotypes in notothenioids; they observed progressive accumulation of mutations (e.g., bone loss, anemia) that became fixed over time. In future studies, deep taxonomic profiling might provide a unique approach to resolve such complex phenotypes as regulation of aging. Necessary resources to support validation include concerted programs for diversity sampling; support for existing experimental models; comparative genomics programs for integrated disease modeling (e.g., resequencing and transcriptomic approaches); and tools to address convergence, network analysis, and selection.

***Xenopus*, a Powerful Vertebrate Model for Biomedical Research and Modeling Human Disease**
*Carole LaBonne, Ph.D., Northwestern University*

Dr. Carole LaBonne discussed the importance of *Xenopus* as a model for human disease and congenital defects. Advantages of this model include synchronously developing embryos; validated fate maps and powerful lineage tracing; lateralized injections and CRISPR mutagenesis; high genomic conservation; relevant organs (e.g., septated heart ventricles, mucociliary epidermis, limbs, lungs); ease of tissue explants, transplants, and organ culture; tissue regeneration; and rapid, inexpensive validation of mutations implicated in human diseases. Dr. LaBonne's group is interested in the development and evolution of the vertebrate neural crest. The group is sampling the transcriptome during the process of cell differentiation to gain mechanistic insight on the process. Xenbase provides a critical resource for compiling searchable, functional *Xenopus* data (e.g., genomic, transcriptomic, phenotypic) through a standardized pipeline. The National Xenopus Resource, which maintains and distributes more than 250 lines of transgenic, mutant, and inbred frogs, is another essential resource. Dr. LaBonne highlighted examples of studies using *Xenopus* to model human disease genes; she emphasized the breadth of work being performed in this area. Additionally, vertical integration has been established previously using this

model organism to screen for genetic variants of interest. Technologies needed for validation include knock-in technologies for protein tagging in frame, improved CRISPR-based generation of disease alleles, validated antibodies, transgenic and mutant lines, and large-scale protein interaction data. Dr. LaBonne emphasized that continued support of *Xenopus* databases is essential to the research community. She also underscored the importance of supporting a diverse set of research organisms, educating study sections on the need for and advantages of these organisms, and applying rapid and inexpensive approaches more effectively to maximize benefits for patients.

**Genomics and Phenomics of Complex Tissue Regeneration Using Salamander Model Systems**
*Elly Tanaka, Ph.D., Research Institute of Molecular Pathology, Austria*

Dr. Elly Tanaka presented on the use of salamanders as models for complex tissue regeneration. The democratization of model organisms through genome sequencing and the application of CRISPR to phenotypes have created new opportunities in the life sciences to obtain unique access and insights to disease-relevant biology. Axolotl salamanders display high degrees of tissue regeneration in both embryos and adults. Additionally, large clutch sizes enhance capabilities for researchers. Dr. Tanaka provided an overview of available molecular resources and capabilities, which include the Ambystoma Genetic Stock Center, chromosome-scale genome assembly, efficient transgenesis, CRISPR-mediated mutation and gene knock-in, and viral systems for somatic expression. Other routes to functional testing in salamanders include pharmacological accessibility, transparency for imaging, and expression cloning (i.e., *ex vivo* to *in vivo* assays). Fibroblast phenotyping represents a strategy toward regenerative medicine. In nonregenerative vertebrate organisms, fibroblasts form scar tissue; in the axolotl, fibroblasts serve as a multipotent limb progenitor. Molecular discoveries, as well as studies of regenerative and nonregenerative organisms, are needed for a full understanding of the regeneration process. Previous studies have demonstrated that nerves and fibroblasts from opposite locations can induce the regeneration of full limbs. Dr. Tanaka stated that large-scale genomic and phenomic studies are needed to prevent scarring and promote regeneration in fibroblasts and epithelia. These studies include molecular phenotyping by single-cell sequencing of fibroblasts in regenerating and nonregenerating conditions; *ex vivo* assay of intracellular and extracellular factors with systems biology analysis; and *in vivo* validation and screening for phenotypes, allowing diversion of fibroblasts from scarring and toward regeneration. Resource development needs include unigene axolotl cDNA panels, antibody and nanobody production for key antigens (e.g., cell surface molecules), an extended community web resource, and a frozen sperm resource of key transgenic stocks.

**More Alike Than Not: Exploring Neural Models of Human Aging and Neurodegenerative Diseases in *Aplysia***
*Lynne Fieber, Ph.D., University of Miami*

Dr. Lynne Fieber discussed neural *Aplysia* models for human aging and neurodegenerative diseases. *Aplysia* is a well-validated model with a large user community. The National Resource for Aplysia breeds, rears, and ships about 10,000 organisms per year at all life stages to laboratories in the United States and worldwide. Notable *Aplysia* discoveries related to learning and memory include synaptic plasticity in long-term memory, cell-intrinsic memory, DNA methylation, and memory transfer via long noncoding RNA. Dr. Fieber emphasized that cellular-level studies of memory are highly challenging. Model validation for aging in *Aplysia* includes assays for reflex behaviors (e.g., tail withdrawal), nerve conduction velocity, and inhibition of sensitization (e.g., response to electric shock). Dr. Fieber's group has identified molecular correlates of aging using these approaches; these findings can be applied to human orthologs in Alzheimer's disease. Dr. Fieber outlined resource needs: transgenic animals (e.g., development of viral vectors, electroporation of newly laid eggs), improved gene annotation and resources for accessing genomic and transcriptomic data (i.e., akin to Xenbase, FlyBase, Zebrafish

International Network), cell lines, and cryopreservation of larvae. She noted that a project to address larval cryopreservation has been funded and is being undertaken by Dr. Terrence Tiersch's group at Louisiana State University.

**Group Discussion**
*John Postlethwait, Ph.D., University of Oregon*
*Crystal Rogers, Ph.D., University of California, Davis*

Dr. Tanaka highlighted the importance of supporting evolutionary models for resilience to disease. Dr. Postlethwait added that the models often represent phenotypes of disease in humans, even though the organisms of interest are healthy in their native environments. These models could be used for the discovery of novel therapeutics. Dr. Spencer Nyholm added that many established and emerging models (e.g., host–microbiota interaction models) can address the mechanism of resilience and healthy state. He voiced his support for continued support of emerging models.

Dr. Schartl stated that many model organism communities are small and tight-knit. He suggested centralized platforms to serve different communities' needs. Dr. LaBonne added that wiki websites (e.g., xenbase.org/reagents/antibody.do) are useful for sharing information on antibody validation within communities. Dr. Rogers emphasized the importance of communication. Dr. Joshua Currie wondered whether ORIP provides funding support for sabbaticals and scientific exchange among investigators. Dr. Murphy replied that the NIH has a program to support sabbaticals (Ruth L. Kirschstein National Research Service Awards for Senior Fellows, F33).

Dr. Tanaka expressed that many investigators are wary of purchasing "risky" antibodies. She noted that she has had success in protein fragment screening for the identification of antibodies. Dr. Dominique Alfandari noted that recent technological advancements have enabled new capabilities for model development. New antibodies and nanobodies will be needed. He also stated that BenchSci (benchsci.com) is a useful resource for antibody validation. Dr. Katia Del Rio-Tsonis added that cell lines are needed.

Dr. Jesse Webber stated that repeatability has been emphasized by the NIH in recent years; this effort is challenging in small communities. He suggested a funding mechanism to support standardized approaches for repeatability. Additionally, annual strategic meetings would help foster efforts and prioritization within communities. Dr. Jeramiah Smith emphasized the importance of engagement among representatives from different model organism communities.

Dr. Laura Borodinsky inquired about strategies to promote nontraditional models for research. Several participants voiced support for increased representation across NIH study sections. Dr. Keith Cheng noted that this point has been raised in previous workshop sessions. He suggested continuing the discussion with NIH representatives. Dr. Hugo Bellen suggested that applicants recommend reviewers from their model organism communities.

Dr. Whited suggested emphasizing the potential application of knowledge from nontraditional models to improve traditional models (e.g., mice). Dr. Del Rio-Tsonis suggested engaging with NIH advisory panels to raise awareness of nontraditional models. Dr. Postlethwait highlighted the importance of emphasizing model validity in grant applications. Additionally, Dr. LaBonne suggested publishing an editorial article to highlight this issue.

Dr. Harris conveyed that the Aquatic Models of Human Disease (AQMHD) Conference will be held October 7–11, 2021, at the Marine Biological Laboratory in Woods Hole, Massachusetts. The conference will include a workshop on genomic tools for aquatic models and will facilitate in-person discussions on

shared research organism database infrastructures. More information can be found at mbl.edu/aqmhd. Dr. Ingo Braasch thanked the NIH for its support and encouraged the participants to contact the meeting organizers by email (aqmhd@mbl.edu) and follow updates through Twitter (@AquaticModels). A participant suggested incorporating workshops on resource sharing and the use of aquatic organisms in biomedical research.

Dr. Rogers commented on the need for funding for personnel to manage and curate databases. Dr. Bellen agreed, noting that these resources are costly to maintain. Dr. Schartl suggested that the Zebrafish International Network host data on other aquatic organisms. Dr. Postlethwait added that this effort would enable data coordination across species but noted that additional funding would be needed.

Dr. Bellen stated that data availability would enable bioinformatic analyses for integration across studies. Dr. Schartl inquired about efforts for automated literature screening. Dr. Bellen noted that efforts in this area are ongoing, but a uniform system for manual curation is needed. Dr. Rogers added that Session VIII will address technologies, phenotyping, and data science.

**Additional Comments**

In the Zoom chat, Dr. Smith suggested considering additional categories for validation beyond face, construct, and predictive validity (e.g., regeneration, novel regulatory pathways). Dr. Tanaka agreed. Dr. Prayag Murawala noted that the axolotl community is interested in forming a singular database; support from the NIH is needed. Several attendees discussed the need for coordination of databases across organisms. Dr. Duygu Özpolat noted that *Platynereis* researchers are developing a community resource and have been discussing this topic. She suggested a wiki template. Dr. Nyholm added that a database for *Euprymna* is under development; common platforms for comparisons would be valuable. Dr. Karen Echeverri highlighted ongoing efforts in database development at alliancegenome.org. Dr. LaBonne stated, however, that support for existing local sites should be continued. Dr. Cheng agreed and noted the importance of maintaining customizations for individual groups. He added that Session VIII will address questions regarding computational infrastructure. An artificial intelligence (AI) expert will be in attendance; Dr. Cheng suggested collecting questions about data analysis for this session.

Dr. Braasch emphasized the value of common ontologies, curated gene orthologies, and single-cell comparisons across systems. Dr. Harris cautioned against extending homology statements in ontologies. Dr. Smith suggested the templates and pipelines for Xenbase be applied across model systems. He shared a resource for axolotl genome data: ambystoma.uky.edu. Dr. Rogers suggested support mechanisms for descriptive studies to generate or analyze validated antibodies for tissue and cell type research. Dr. Özpolat asked whether the NIH supports funding mechanisms for sperm cryopreservation techniques; Dr. Marko Horb explained that Dr. Tiersch is funded by ORIP to perform work in this area. Dr. Whited noted that the Marine Biological Laboratory provides education of techniques specific to *Xenopus*. She suggested developing a similar resource for the axolotl community. Drs. Tanaka, LaBonne, and Alfandari agreed to converse via email to share protocols and optimization techniques for identification of antibodies.

Several participants emphasized the need for support of nontraditional models and to strengthen connections among and within model communities. Dr. Amro Hamdoun suggested including scientists from traditional models in relevant conversations. Several participants emphasized the value of in-person meetings to foster discussions on this topic. In response to a question from Dr. Andrea Wills, Dr. Miguel Contreras confirmed that ORIP program officers attend the AQMHD conference. Dr. Michael Schmale suggested hosting conferences in the Washington, D.C., area to maximize the potential for NIH representation. Dr. Scott Fraser noted that a hybrid meeting format might be beneficial for ensuring this representation. Dr. Peggy Biga suggested establishing additional organized structure (e.g., formal

societies) to connect communities on a regular basis. Dr. Schmale expressed concern about the administrative burden related to these efforts.

**Summary and Suggestions**

Non-zebrafish aquatic models have emerged as strong research organisms to identify and model mechanisms of human disease. This session addressed several aspects of validation, including face, construct, and predictive validity. Although the community of aquatic medical models is diverse with specialized needs, several unifying themes emerged during the session. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Creation of platforms to support horizontal and vertical integration of information (e.g., genomic, transcriptomic, proteomic, metabolomic, phenomic, anatomical nomenclature) among laboratories and research institutes for increased transparency, collaboration, and expeditious discovery
- Creation and maintenance of annotated genomes (i.e., informatics) for non-zebrafish aquatic models tied to existing members of the Alliance of Genome Resources
- Expansion of current resource and/or stock centers to include additional species, ensuring access to various aquatic models
- Research conferences to bring together participants using nontraditional and unique aquatic models for human disease
- Mechanisms for the creation and validation of such molecular tools as transgenic animals, verified antibodies, and vectors (e.g., similar to CRISPR-mediated integration cassette in flies) that can be utilized within and across species for further vertical integration and validation

# Appendix A: Meeting Agenda

**Session VII. Validation of Non-Zebrafish Aquatic Models for Preclinical Research**
**2:00–4:00 p.m. EST**
**December 17, 2020**

**Chairs**
John Postlethwait, Ph.D., University of Oregon
Crystal Rogers, Ph.D., University of California, Davis

2:00–2:05 p.m. **Opening Remarks**

Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, Office of Research Infrastructure Programs (ORIP)
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

2:05–3:30 p.m. **Presentations**

John Postlethwait, Ph.D., University of Oregon
*Introduction to the Validation of Aquatic Models in Research*

Manfred Schartl, Dr. rer. nat., Texas State University, San Marcos
Xiphophorus*, an Evolutionary Model for Epistatic Interactions in Disease*

Kathryn Milligan-Myhre, Ph.D., University of Connecticut
*Adapting the Evolutionary Model Organism, Threespine Stickleback, for Host–Microbe Interaction Studies*

Nicolas Rohner, Ph.D., Stowers Institute for Medical Research
*Cavefish as a Model for Natural Resilience to Metabolic Disease*

Matthew Harris, Ph.D., Boston Children's Hospital
*Strength in Diversity: Deep Phylogenetic Mapping to Understand the Causes and Compensation of Disease-Related Phenotypes*

Carole LaBonne, Ph.D., Northwestern University
Xenopus*, a Powerful Vertebrate Model for Biomedical Research and Modeling Human Disease*

Elly Tanaka, Ph.D., Research Institute of Molecular Pathology, Austria
*Genomics and Phenomics of Complex Tissue Regeneration Using Salamander Model Systems*

Lynne Fieber, Ph.D., University of Miami
*More Alike Than Not: Exploring Neural Models of Human Aging and Neurodegenerative Diseases in* Aplysia

3:30–4:00 p.m. **Group Discussion**

# Appendix B: Discussants List

**Session VII. Validation of Non-Zebrafish Aquatic Models for Preclinical Research**
**2:00–4:00 p.m. EST**
**December 17, 2020**

Kristin Abraham, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases
Lola Ajayi, Office of Research Infrastructure Programs (ORIP)
Rosie Aldegado, Ph.D., University of Hawaii at Manoa
Dominique Alfandari, Ph.D., University of Massachusetts Amherst
Hugo Bellen, Ph.D., Baylor College of Medicine
Peggy Biga, Ph.D., The University of Alabama at Birmingham
Laura Borodinsky, Ph.D., University of California, Davis
Ingo Braasch, Ph.D., Michigan State University
Keith Cheng, M.D., Ph.D., The Pennsylvania State University
Miguel Contreras, Ph.D., ORIP
Bill Cresko, Ph.D., University of Oregon
Joshua Currie, Ph.D., Wake Forest University
Jacob Daane, Ph.D., Northeastern University
Katia Del Rio-Tsonis, Ph.D., Miami University
Thomas Desvignes, Ph.D., University of Oregon
Karen Echeverri, Ph.D., The University of Chicago
Lynne Fieber, Ph.D., University of Miami Rosenstiel School of Marine and Atmospheric Science
Scott Fraser, Ph.D., University of Southern California
James Godwin, Ph.D., Mount Desert Island Biological Laboratory
Alison Gould, Ph.D., California Academy of Sciences
Amro Hamdoun, Ph.D., University of California, San Diego
Matthew Harris, Ph.D., Boston Children's Hospital
Katrin Henke, Ph.D., Emory University
Marko Horb, Ph.D., Marine Biological Laboratory
Carole LaBonne, Ph.D., Northwestern University
Alex Lin, Ph.D., The Pennsylvania State University College of Medicine
Lisa Schwartz Longacre, Ph.D., National Heart, Lung, and Blood Institute
Yuan Lu, Ph.D., Texas State University
Deirdre Lyons, Ph.D., University of California, San Diego
Catherine (Kate) McCusker, Ph.D., University of Massachusetts Boston
Rachel Miller, Ph.D., McGovern Medical School
Kathryn Milligan-Mhyre, Ph.D., University of Connecticut
James Monaghan, Ph.D., Northeastern University College of Science
Manuel Moro, D.V.M., National Institute on Aging
Prayag Murawala, Ph.D., Mount Desert Island Biological Laboratory
Stephanie Murphy, V.M.D., Ph.D., ORIP
Peter Nghiem, D.V.M., Ph.D., Texas A&M University
Spencer Nyholm, Ph.D., University of Connecticut
Duygu Özpolat, Ph.D., Marine Biological Laboratory
John Postlethwait, Ph.D., University of Oregon
Randall Prather, Ph.D., University of Missouri
Nicolas Rohner, Ph.D., Stowers Institute for Medical Research
Crystal Rogers, Ph.D., University of California, Davis
Rebecca Roof, Ph.D., National Institute of Neurological Disorders and Stroke

Manfred Schartl, Dr. rer. nat., Texas State University, San Marcos
Michael Schmale, Ph.D., University of Miami
Molly Schumer, Ph.D., Stanford University
Clay Small, Ph.D., University of Oregon
Jeramiah Smith, Ph.D., University of Kentucky
Natalie Steinel, Ph.D., University of Massachusetts Lowell
William Talbot, Ph.D., Stanford University
Elly Tanaka, Ph.D., Research Institute of Molecular Pathology
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