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National Institutes of Health
Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs

Cryopreservation and Other Preservation Approaches for Animal Models Workshop

Session II. Development of Sustainable Germplasm Repositories for Aquatic Biomedical Models

September 9–10, 2024
Virtual Meeting

Final Report

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Executive Summary

The Cryopreservation and Other Preservation Approaches for Animal Models Workshop was held in six sessions to address topics related to cryopreservation and other preservation methods, including, but not limited to, (1) the needs and scientific status of cryopreservation and other preservation of gametes (sperm, oocytes, and embryos), reproductive tissues, larvae, and whole animals and their production of live offspring after revival; (2) emerging cryopreservation and other preservation methods and technologies, as well as how to optimize and implement them; (3) methods, technologies, and infrastructure to assess the impact of intrinsic and extrinsic factors on the quality, efficiency, and success of cryopreservation and other preservation protocols and revival, including scalability and reproducibility; (4) the sharing of technologies, including hands-on training for cryopreservation best practices, and training of next-generation scientists; and (5) the preservation and management of samples, from collection to utilization.

Session II addressed topics related to cryopreservation of aquatic species used for biomedical research and conservation efforts. Each session focused on the layers of activity and engagement involved in biobanking critical model species—building from basic research to repository applications to incorporating repositories into resource centers and to wider networks with user communities and other resources. The sessions, which featured short presentations by 39 panelists and presenters followed by a moderated discussion, focused on approaches currently used by shared genetic resources and common challenges experienced in the field. The workshop participants discussed challenges associated with funding and training, the need for standardized protocols, and the benefits that universal data management systems and training hubs would provide.

Panelists and Presenters

Caroline Albertin, Ph.D., M.S., Marine Biological Laboratory (MBL), The University of Chicago
Claudia Alvarez, National *Aplysia* Resource (NAR), University of Miami
Carl Anderson, National *Xenopus* Resource (NXR), MBL, The University of Chicago
Harvey Blackburn, Ph.D., U.S. Department of Agriculture (USDA)
Sarah Bodenstein, Ph.D., M.S., Louisiana Sea Grant, Louisiana State University
Ingo Braasch, Ph.D., Michigan State University
Christopher Brownlee, Ph.D., Stony Brook University
Raissa Cecil, *Ambystoma* Genetic Stock Center (AGSC), University of Kentucky
Michael Chang, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Jose Cibelli, Ph.D., Michigan State University
Jonathan Daly, Ph.D., Reef Restoration and Adaptation Program, University of New South Wales
April Freeman, Zebrafish International Resource Center (ZIRC), University of Oregon
Stephanie Grainger, Ph.D., Van Andel Institute
Zongqi Guo, Ph.D., M.Eng., University of South Florida
Amro Hamdoun, Ph.D., University of California, San Diego
Marko Horb, Ph.D., NXR, The University of Chicago
Jill Jenkins, Ph.D., U.S. Geological Survey
Jack Koch, Ph.D., Aquatic Germplasm and Genetic Resources Center (AGGRC), Louisiana State University Agricultural Center
Andrew Kouba, Ph.D., M.S., Mississippi State University
Yue Liu, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center
Mike Lomas, Ph.D., National Center for Marine Algae, Bigelow Laboratory for Ocean Sciences
Yuan Lu, Ph.D., *Xiphophorus* Genetic Stock Center (XGSC),[1] Texas State University

[1] The *Xiphophorus* Genetic Stock Center is now the Texas State University Institute for Molecular Life Sciences.

Daniella McDonald, Ph.D., NAR, University of Miami
Brian Mitchell, Ph.D., Northwestern University
Theodore Morgan, Ph.D., U.S. National Science Foundation (NSF)
Daniel Powell, Ph.D., Louisiana State University
Phil Purdy, Ph.D., USDA
Caird Rexroad, Ph.D., USDA
Lindsey Sanchez, XGSC, Texas State University
Michael Schmale, Ph.D., M.S., NAR, University of Miami
Patricia Schneider, Ph.D., Louisiana State University
Shannon Tessier, Ph.D., Harvard Medical School and Massachusetts General Hospital
Summer Thyme, Ph.D., UMass Chan Medical School
Terrence Tiersch, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center
Zoltan Varga, Ph.D., ZIRC, University of Oregon
S. Randal Voss, Ph.D., AGSC, University of Kentucky
Stephen Watts, Ph.D., M.S., The University of Alabama at Birmingham
William Wayman, Ph.D., M.S., U.S. Fish and Wildlife Service
Nikolas Zuchowicz, M.S., University of Minnesota

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Workshop Report

Day 1: Monday, September 9, 2024

Session 1: Introduction and Overview
Moderator: Yue Liu, Ph.D., M.S., Aquatic Germplasm and Genetic Resources Center (AGGRC), Louisiana State University Agricultural Center

Greetings and Workshop Preview
Yue Liu, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center

Dr. Yue Liu welcomed the attendees to the workshop. He explained that the current workshop is a follow-up to the 2017 Cryopreservation of Aquatic Biomedical Models Workshop convened in Birmingham, Alabama, by the National Institutes of Health (NIH) Office of Research Infrastructure Programs (ORIP). That workshop aimed to identify gaps, opportunities, and emerging technologies relevant to germplasm cryopreservation in aquatic models. Dr. Liu noted that the current workshop seeks to identify ways to incorporate fundamental cryopreservation techniques and protocols into practical, sustainable repositories that can manage and distribute genetic resources for the scientific community. He emphasized the workshop's focus on cryopreservation advancement needs for supporting biomedical research, evidenced by the number of invited speakers in early stages of their careers. Dr. Liu noted that input from the meeting would be compiled to identify the needs of investigators and research institutes, as well as to inform federal agencies of the current and future challenges and opportunities. In response to a Slido poll, approximately 40% of attendees answered that they were staff members, technicians, or managers; 38% were faculty members; 9% were graduate students; 8% were postdoctoral researchers; and 11% answered "Other." A second Slido poll asked participants to share the single word that represents the most pressing need for germplasm preservation in their work. The most popular answers included "community," "efficiency," "funding," "protocols," "scalability," "standardization," "support," and "training."

Advancing Biomedical Research: ORIP's Strategic Investment in Cryopreservation of Aquatic Models
Michael Chang, Ph.D., Deputy Director, ORIP, Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), NIH

Dr. Michael Chang provided a historical overview of ORIP's efforts to support the preservation of aquatic models. Since its early days, ORIP's Division of Comparative Medicine has supported numerous research centers and resources, including the Zebrafish International Resource Center (ZIRC), National *Aplysia* Resource (NAR), and National *Xenopus* Resource (NXR), as well as a variety of research resources for other aquatic species (e.g., swordtail fish of the genus *Xiphophorus*, medaka, salamander, sea urchin). ORIP also has funded R01 awards focused on cryopreservation and has supported the aquatic models of human disease (AQMHD) research community, which has been instrumental in advocating for resources, conferences, and workshops to promote the use of aquatic models and technical advancements in the field. The proceedings of every AQMHD conference, which have been held since 2000, are published in comparative biology and physiology journals.

Dr. Chang reviewed aquatic cryopreservation gaps that were highlighted during various AQMHD conferences. In 2003, the lack of a reliable method for zebrafish sperm cryopreservation was noted, and in 2005, the need for further development and standardization of cryopreservation was emphasized again. In response to the needs described at AQMHD conferences, ORIP issued P40 and R24 awards to Dr. Ronald Walter in 2003 to establish technology enabling the cryopreservation of *Xiphophorus* sperm samples and initiate the establishment of a sperm repository for many of the *Xiphophorus* Genetic Stock Center (XGSC) stocks. In 2008, ORIP awarded Dr. Terrence Tiersch an R24 grant to lead a collaborative effort to develop high-throughput cryopreservation methods for zebrafish and medaka sperm for research and

stock center applications. Subsequent improvements in the field included developing a new extender and cryoprotective medium, introducing quality assessment to the cryopreservation process, determining the optimal cooling rate for various samples, and improving post-thaw *in vitro* fertilization (IVF) processes. These improvements increased the post-thaw fertilization rate from approximately 15% to almost 70%.

Dr. Chang reviewed the major challenges currently associated with supporting aquatic model resources. Resources must efficiently and reliably maintain and share aquatic models while facing increasing infrastructure and personnel costs. With advances in genetics, the growth of aquatic resources has expanded rapidly, but reliable fish germplasm cryopreservation methods are still lacking. Individual laboratories are experiencing the increasing burden of maintaining and sharing their own aquatic models. To address these challenges, ORIP currently supports an interagency agreement with the National Laboratory for Genetic Resources Preservation within the Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), providing funding for cryotanks, liquid nitrogen, maintenance, labor, and databasing of ORIP resource centers' backup collections. ORIP also supports efforts to safeguard genetic resources by ZIRC, NXR, NAR, and the *Ambystoma* Genetic Stock Center (AGSC); establish an integrated platform for diploid germplasm conservation in zebrafish; and modernize 3D-printing capabilities at AGGRC. Since 2004, when it was known as the National Center for Research Resources, ORIP has dedicated more than $106 million in awards and administrative supplements to cryopreservation efforts led by resource centers. More than $25 million of that significant investment has supported activities related to aquatic models.

Previous ORIP-sponsored workshops on cryopreservation have included the 2007 Achieving High-Throughput Repositories for Biomedical Germplasm Preservation and the previously mentioned 2017 Cryopreservation of Aquatic Biomedical Models Workshop, during which aquatic cryopreservation hubs were established, a call for technical advancements was developed, and mechanisms were implemented to allow stock centers to increase their planning, personnel, and ability to secure genetic resources. Dr. Chang noted that the objectives of the current workshop comprise (1) evaluating current needs and advancements, (2) advancing and disseminating emerging methods and technologies, (3) optimizing protocols for quality and efficiency, (4) facilitating knowledge transfer and capacity building, and (5) strengthening sample preservation and management.

Advancements and Impact Since the 2017 Workshop in Birmingham, Alabama
Zoltan Varga, Ph.D., ZIRC, University of Oregon

Dr. Zoltan Varga reviewed developments since the 2017 Cryopreservation of Aquatic Biomedical Models Workshop. The overall goals of the meeting were to assess the status of germplasm cryopreservation in various biomedical aquatic models and allow the scientific community to develop and prioritize a consensus of actionable needs to advance the field of aquatic resource cryopreservation. Final recommendations included (1) establishing a centralized aquatic cryobanking technology "Hub," (2) developing mechanisms to support innovative technical advancements, and (3) assisting the various aquatic model stock centers with increasing planning efforts, as well as promoting interaction within a network of aquatic model species repositories. The Hub was established at AGGRC to serve as a central resource, working with other resource centers to establish their germplasm repository capabilities with high-throughput production pathways, quality management programs, and resource-sharing platforms. AGGRC disseminates cryopreservation technology, training, and information and offers support to other centers in the NIH Aquatic Biomedical Germplasm Repository Network, including AGSC, NAR, NXR, XGSC, and ZIRC. The resource centers share resources, cryopreservation services, technologies, and training within their communities.

The Hub and the network have achieved many successes. NXR led efforts to enhance *Xenopus* cryopreservation, sperm collection, and fertilization rates; introduced standardized quality assays using

computer-assisted sperm analysis (CASA); and provided training and infrastructure upgrades. AGSC worked with AGGRC to develop the first *Ambystoma* cryopreservation protocol (achieving a 50% fertilization rate and producing hatched larvae with thawed sperm used for fertilization), developed assays for sperm quality, and supported repository setup and staff training. NAR has achieved the survival of *Aplysia* larvae using vitrification, developed algae culture pathways, created image-based assessments and electrical sensing for cryogenic monitoring, and designed efficient, low-cost devices for egg-mass processing. The AGGRC Hub has enhanced communication with resource centers, formed the NIH Aquatic Biomedical Germplasm Repository Network for repository development, engaged in educational outreach, and advised student projects.

Methodological advances in aquatic cryopreservation are ongoing. For example, as shown by the Bischof group (University of Minnesota), zebrafish embryo vitrification and ultra-fast thawing are now feasible, although technically challenging. The Cibelli research group is working with AGGRC and ZIRC to develop somatic cell nuclear transfer (SCNT) techniques for preserving diploid zebrafish cells. ZIRC is engaging with the scientific community to establish pathways and routine operations for biobanking zebrafish.

Visions of the Need in the Future of Genetic Resources of Aquatic Species
Terrence Tiersch, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center

Dr. Liu introduced Dr. Tiersch, who highlighted the importance of protecting aquatic genetic resources. For context, Dr. Tiersch indicated that thousands of researchers developed a cryopreservation industry for dairy cattle, a single species, while the ratio of researchers to aquatic species studied is 1 to 100. Therefore, researchers and other user groups will need to collaborate, communicate, and cooperate to address the challenges associated with preserving aquatic genetic resources efficiently.

Dr. Tiersch noted that cryopreservation is part of a larger scheme of operations and capabilities involved in a repository, which itself is a small component of broader research communities and networks. ORIP and other agencies have been instrumental in developing a network of regional resources for aquatic models across the United States, including the USDA, AGGRC, and NIH Aquatic Biomedical Germplasm Repository Network. Dr. Tiersch reviewed AGGRC's mission, which is to support the development of germplasm repositories for aquatic genetic resources through interdisciplinary collaboration. AGGRC aims to support stock centers across the nation, which can then share resources and expertise within their respective communities. Dr. Tiersch highlighted the USDA ARS National Animal Germplasm Program (NAGP), which has the mission to collect, assess, and preserve animal genetic resources for the United States. NAGP operates a gene bank for livestock, aquatic, poultry, and insect genetic resources and has an agreement with ORIP to store germplasm of biomedical models.

Looking ahead, the key challenge is to find ways to collaborate across aquatic species—including cross-model research—to enhance standardization and efficiency across the field. Innovative thinking will be necessary to further these efforts. For example, open hardware and comprehensive kits can be shared with the communities, providing inexpensive and standardized capabilities. Dr. Tiersch explained that, rather than being educational, this workshop is intended to stimulate discussion among participants at every level of the field. Input from the meeting will be compiled and presented to ORIP as a series of priorities related to the current and future needs of the aquatic resource community.

Session 2: From Cryopreservation Research to Repository Application

Panel I: Advancement in Technologies and Research for Germplasm Repository Development
Moderator: Jose Cibelli, D.V.M., Ph.D., Michigan State University
Speakers: Jose Cibelli, D.V.M., Ph.D., Michigan State University; Zongqi Guo, Ph.D., M.Eng., University of South Florida; Jill Jenkins, Ph.D., U.S. Geological Survey (USGS); Phil Purdy, Ph.D., USDA; Shannon Tessier, Ph.D., Harvard Medical School and Massachusetts General Hospital; and Nikolas Zuchowicz, M.S., University of Minnesota

Dr. Jose Cibelli introduced the session on research and technological advancements for germplasm repository development. He discussed his collaboration with AGGRC and ZIRC, which aims to achieve diploid germplasm conservation in zebrafish using SCNT. Embryonic cells can be isolated 24 hours after fertilization and frozen for storage. Nuclei from the thawed cells can be transferred into enucleated oocytes to create a cloned animal that can be reared and mated successfully. Dr. Cibelli noted that ZIRC has been especially helpful with the most challenging aspect of the project—streamlining the process. He requested that panelists and discussion participants focus on the main technical challenges of the field.

Dr. Shannon Tessier shared how her early studies of suspended animation informed later translational work in cryobiology, preservation, and transplantation. Her group's approach to addressing cryopreservation of zebrafish embryos—interrupted cooling—was inspired by freeze-tolerant wood frogs (*Rana sylvatica* or *Lithobates sylvaticus*). The method is a two-step process that involves inducing embryos into a non-injurious frozen state at sub-zero temperatures (−5°C to −22°C) followed by long-term cryogenic storage in liquid nitrogen. The first step's parameters (e.g., cryoprotective agent [CPA] loading temperature, embryonic stage, incubation time) currently are being optimized, and the method can achieve up to 85% survival.

Dr. Zongqi Guo noted his interest in cryopreservation, biopreservation, heat transfer, and microfabrication to support scalable resource preservation for health care research and protection of biodiversity. His group has used principles of thermal conductivity to develop scalable cryo-mesh systems with improved cooling rates that can be used to cryopreserve a variety of species with improved viability. Future work will focus on CPA design and optimization, automation, and a model system for cryopreservation platform analysis.

Mr. Nikolas Zuchowicz described conservation biobanking's mid-scale problem—conservationists require specialized equipment and protocols that are field-hardy, inexpensive, and manageable at different scales, but most cryopreservation equipment is meant for use at commercial and industrial scales, with commensurate price points. Mr. Zuchowicz noted that scalable, open-technology solutions will be indispensable for addressing this challenge. He shared the example of an open-source, 3D-printed cryopreservation device costing $80 that can replace expensive controlled-rate freezers.

Dr. Jill Jenkins explained that the USGS Wetland and Aquatic Research Center (WARC) conducts research needed to conserve and restore aquatic ecosystems throughout the nation. WARC is involved in conserving gametes for use in the U.S. Fish and Wildlife Service (FWS) hatchery system. WARC could assist with research to delineate sperm quality parameters and other beneficial knowledge for conservation species. Grants or contracts could be jointly developed with or provided to WARC for such investigations.

Dr. Phil Purdy introduced himself as a cryobiologist and reproductive biologist with the USDA ARS NAGP, which aims to capture genetic diversity via germplasm preservation and maintains a collection of more than 1.3 million samples. USDA ARS research focuses on sperm activation and cryopreservation, repository quality control and process optimization, rooster and turkey sperm cryopreservation and artificial insemination, CASA applications in bull and boar fertility evaluation, and chicken primordial

germ cell collection, preservation, and utilization. The overarching needs of NAGP include techniques to preserve female germplasm and enhance quality-control methods.

Panel Discussion

- Dr. S. Randal Voss asked Dr. Tessier whether wood frog embryos are like adults in terms of tolerating freezing. She cited a study showing that the embryos tolerated supercooling at high subzero temperatures but not freezing. As a follow-up, Dr. Cibelli asked whether the wood frog offers additional lessons for cryopreserving other species. Dr. Tessier noted that studying wood frog embryos, which are far less sensitive to cold than other species, might reveal mechanisms associated with low-temperature or freezing tolerance.

- In response to a question from Dr. Stephanie Grainger about access to the Cryorack design, Mr. Zuchowicz shared a link to the files needed to 3D print and assemble the device. He noted that the original model was designed for the cryopreservation of coral sperm and cools at a rate of 20°C per minute. Newer versions are being designed, with the goal being a Cryorack that functions as a controlled-rate freezer.

- Dr. Cibelli asked the panelists to share their thoughts on potential innovations in cryopreservation over the next 10 years. He expressed an interest in the development of microfluidic devices that can select high-quality zebrafish oocytes for storage. Mr. Zuchowicz remarked that novel CPAs will be needed to freeze tropical species—which are highly sensitive to cold—for conservation. Dr. Tessier commented that, if funds were not limited, her future goal would involve a network of living biorepositories with a universal approach, including standardized protocols and CPAs that work across species and tissues. Dr. Purdy remarked that such a goal was feasible; the biggest challenge is the tedious work of streamlining and codifying the relevant information. Mr. Zuchowicz added that robust and standardized training should be included as a future goal. A unified textbook and courses would be beneficial in this area.

- In response to a question from Dr. Amro Hamdoun about genetic modifications that improve performance in cryopreservation, Dr. Purdy noted that researchers have begun exploring the genetic changes that have occurred as the agricultural industry has selected for resistance to freezing in bull sperm for many years.

- Dr. Liu asked how a gold-standard cryopreservation protocol emerges for a species when multiple successful approaches exist. Dr. Tessier noted that settling too early on a single method might impede innovation and cause important discoveries to be overlooked. Dr. Estefania Paredes commented that the most user-friendly techniques are the methods most commonly adopted by a community. Researchers will sacrifice a certain amount of viability for protocols that are inexpensive, reliable, and easy to learn.

Panel II: Challenges in Research and Technology Development for Emerging Models
Moderator: Ingo Braasch, Ph.D., Michigan State University
Speakers: Caroline Albertin, Ph.D., M.S., Marine Biological Laboratory (MBL), The University of Chicago; Harvey Blackburn, Ph.D., USDA; Ingo Braasch, Ph.D., Michigan State University; Amro Hamdoun, Ph.D., University of California, San Diego; Patricia Schneider, Ph.D., Louisiana State University; and Stephen Watts, Ph.D., M.S., The University of Alabama at Birmingham

Dr. Ingo Braasch introduced the session on challenges associated with emerging model systems. He described his research on major morphological and genomic transitions in vertebrate evolution. Teleost fish are derived from a lineage that underwent a genome duplication that can hinder comparisons to the human genome. Dr. Braasch uses the spotted gar (*Lepisosteus oculatus*) as a bridge organism between

teleost fish and tetrapods (e.g., reptiles, birds, mammals). The gar's genome is more similar to tetrapods, and its biology is more similar to teleost fishes. Studies in the gar have revealed deep homology of vertebrate development across such features as synovial joints, bilateral vision, fin/limb regeneration, and genome maintenance. The animals are housed at Michigan State University's Ancient Fish Facility, which aims to become a stock center for the emerging model organism in partnership with AGGRC. Challenges faced by gar investigators include the species' underexplored reproductive biology, poorly defined genetic backgrounds, and lack of cryopreservation methods.

Dr. Patricia Schneider explained that four known fish species have undergone eye duplication; three are native to the Galapagos region, and the fourth, the largescale four-eyes (*Anableps anableps*), is found across Central and South America. Dr. Schneider has adopted *Anableps* as a model system to study the evolutionary and developmental biology of the visual system. Germplasm repositories for *Anableps* would enable easier and faster access to embryonic and larval stages, which will facilitate studies of the regulatory elements that control the evolution of the eye. Dr. Schneider has established an *Anableps* colony at Louisiana State University and is tailoring resources and tools to this species.

Dr. Harvey Blackburn shared an overview of progress by the USDA ARS NAGP from its inception in 1999 to the present. Repository samples have been used to improve dairy production by $400 million annually, support research into the Holstein cow's lost Y chromosome, and regenerate populations of interest to the dairy industry. Future NAGP efforts will focus on developing improved techniques and additional collections and repositories.

Dr. Caroline Albertin provided an overview of her efforts to develop cephalopod models for biomedical research. Cephalopods (e.g., cuttlefish, nautilus, octopus, squid) possess the largest invertebrate brains and exhibit complex behaviors. The genetic tractability, sequenced genome, and robust multigenerational culture of the hummingbird bobtail squid (*Euprymna berryi*) are reasons Dr. Albertin chose to develop this species as a model cephalopod. Current *Euprymna* resources include robust laboratory culture and CRISPR-mediated genome editing. The ability to cryopreserve germplasm would eliminate a portion of the laborious effort to maintain wild-type and knockout *Euprymna* strains.

Dr. Hamdoun discussed his efforts to develop sea urchin resources. The purple sea urchin (*Strongylocentrotus purpuratus*) is a classic animal model that has been stymied by the lack of appropriate tools and techniques. The supplies of wild animals are unpredictable, and their use has introduced genetic variability into sea urchin studies. Dr. Hamdoun uses a related species, the painted sea urchin (*Lytechinus pictus*), to create and share genetic lines and reproducibly study single mutants. *L. pictus* has a shorter generation time than *S. purpuratus*, and its eggs are larger. Dr. Hamdoun's group is developing genetic tools for *L. pictus*—including methods for sperm cryopreservation and stable germline transgenesis—and is attempting to address challenges related to scaling, efficiency, cost reduction, and increased throughput.

Dr. Stephen Watts is the director of the Lab Animal Nutrition Core, which was established to study laboratory diets for animal models with translation to human health. Dr. Watts noted that certain dietary formulations might increase cellular integrity and result in more successful cryopreservation and recovery.

Panel Discussion

- Dr. Liu emphasized three challenges being addressed by Dr. Hamdoun's group: (1) how to track animals through the production cycle, (2) how to establish efficient germplasm repositories for transgenic animals, and (3) how to disseminate animals and lines efficiently, support end users, and track impact.

- Dr. Braasch asked the panelists to discuss issues related to reproductive biology that they have encountered. Dr. Schneider responded that because *Anableps* is a live-bearing animal, following its internal fertilization and development process has presented significant challenges.

- Dr. Albertin remarked that building *Euprymna* resources from nothing will require significant amounts of research and community support. Dr. Braasch noted that investigators who spearhead new model organisms must collaborate with expert reproductive biologists or become experts themselves.

- Dr. Braasch and the panelists discussed how to prioritize populations for cryopreservation. Dr. Blackburn cautioned against self-imposed limitations that prevent growth in the future.

- Dr. Blackburn described the challenges of testing published protocols for freezing shrimp. He emphasized the need for community-level, standardizable guidelines for defining effective cryopreservation. In response to a follow-up question from Dr. Braasch, Dr. Blackburn responded that emerging model systems should be incorporated into existing repositories and biobanks for increased efficiency and sustainability. Successful frameworks for model organisms exist and should be disseminated thoroughly throughout the classic and emerging model communities.

- Drs. Braasch and Hamdoun agreed that plans to develop resources for emerging animal models (especially funding to advance maintenance and preservation efforts) should be included from the early stages of adoption.

Session 3: Operations of Repositories at Resource Centers

Panel III: What Have We Learned from Translating Protocols to Germplasm Repositories?
Moderator: Jonathan Daly, Ph.D., Reef Restoration and Adaptation Program, University of New South Wales
Speakers: Claudia Alvarez, NAR, University of Miami; Carl Anderson, NXR, MBL, The University of Chicago; Raissa Cecil, M.S., AGSC, University of Kentucky; Jonathan Daly, Ph.D., Reef Restoration and Adaptation Program, University of New South Wales; April Freeman, ZIRC, University of Oregon; and Lindsey Sanchez, XGSC, Texas State University

Dr. Jonathan Daly welcomed the participants to the session on translating resource protocols into germplasm repositories. He described his efforts to adapt cryopreservation methods to coral, with the aim of developing a repository of coral native to the Great Barrier Reef. The Reef Restoration and Adaptation Program currently maintains banked samples from more than 30 coral species. Advanced methods for coral sperm cryopreservation have been developed, while protocols for cryopreservation of larvae and symbionts are under development. Basic cryobiology studies and protocols and adult tissue storage protocols remain in preliminary stages.

Mr. Carl Anderson provided an overview of NXR, which stocks approximately 400 genetically modified lines of the African clawed frog (*Xenopus laevis*) and the western clawed frog (*Xenopus tropicalis*). Cryopreservation is essential for *Xenopus* aquaculture, which is threatened by disease, system failures, and human error. Recently, NXR and AGGRC have collaboratively developed new methodologies and resources—including machine learning, high-throughput processing, and quality management programs—that have improved sample quality, increased yields, and enabled efficient storage of lines.

Ms. Raissa Cecil described AGSC efforts to maintain axolotl (*Ambystoma mexicanum*) breeding colonies, distribute animals, curate information about AGSC stocks, and develop expertise in axolotl

cryopreservation. The ability to cryopreserve axolotl sperm would reduce husbandry loads and allow banking of genetically modified lines. Animals have been generated in the AGSC facility via IVF using sperm cryopreserved by AGGRC staff. Live and motile sperm were observed after thawing samples previously frozen at AGSC. Future efforts will aim to achieve reliable and consistent post-thaw motility and fertilization outcomes.

Ms. Claudia Alvarez introduced NAR, which has been funded through P40 awards for 29 years to support maintenance of California sea hare (*Aplysia californica*) colonies. The facility, which houses between 15,000 and 20,000 animals at any given time, is the only resource in the world to replicate the complex *Aplysia* life cycle in the laboratory. Outbred stocks are maintained because the animal does not respond well to inbreeding. The unusual life cycle—featuring internal fertilization, a dense egg capsule required for larval development, and an extended, fragile larval stage—poses significant challenges to effective cryopreservation and establishing a germplasm repository for *Aplysia*.

Ms. Lindsey Sanchez emphasized the importance of germplasm cryopreservation resources for *Xiphophorus* species. Cryopreservation enables maintenance of genetic lines and preservation of species extinct in the wild (e.g., *X. couchianus*, *X. meyeri*) or endangered (e.g., *X. gordoni*, *X. andersi*). XGSC has worked with AGGRC to secure more than 4,500 cryopreserved *Xiphophorus* samples from 24 of 26 known species, which are archived with the USDA ARS NAGP. The facility has a success rate of up to 25% when reviving live young fish. Future needs include standardization of artificial insemination procedures, onsite cryopreservation capabilities, and community-accessible germplasm samples.

Ms. April Freeman described ZIRC's transition from maintenance of live zebrafish stocks to a cryopreservation-based program. This evolution has reduced the need for husbandry staff and infrastructure, decreased risks associated with pathogens and disease, and resulted in faster import and export times. One of the disadvantages of the change is that in a few cases, adult zebrafish may be available only after a delay of 4–6 months. This happens when the quality or quantity of sperm imported directly from submitters requires raising and preserving a new generation of genetic carriers to reliably maintain the specific line in the repository. ZIRC invested effort into an amended protocol for increased cryopreservation efficiency, as well as significant improvements to quality control, husbandry, infrastructure, and outreach. Future goals include intermediate-term sperm storage and shipment methods at room temperature, a cryopreservation toolkit, and expanded research into egg quality. ZIRC currently is evaluating whether to offer cryopreservation services to the zebrafish community.

Panel Discussion

- Dr. Daly asked the panelists to discuss challenges encountered when adopting cryopreservation protocols with their facilities. Mr. Anderson described issues stemming from users employing different sample storage methods than those used at NXR. While NXR cryopreservation protocols are optimized for storage in liquid nitrogen, many users experienced poor results after storing samples in −80°C freezers.

- Ms. Freeman remarked that cryopreservation was essential for ZIRC's ability to supply zebrafish to the scientific community. She noted that time is a critical factor in repository operations and that developing reliable protocols early greatly enhances efficiency. ZIRC has also encountered challenges with egg quality and maintaining biosecurity.

- In response to a question from Dr. Daly about implementing cryopreservation in a facility, Ms. Alvarez and Ms. Cecil identified space, labor, and cost as the primary obstacles. Dr. Daly and Ms. Cecil noted that having time to develop robust cryopreservation techniques before the scientific community urgently needs frozen samples is a significant advantage.

- Dr. Blackburn asked about needs related to comprehensive database development (e.g., sample management, genotyping, phenotyping) for different species. Ms. Freeman shared that ZIRC's impressive electronic database was developed internally with NIH funding. Ms. Sanchez stated that XGSC currently uses physical, handwritten forms to track samples. While new software is in development, full implementation will take time. Mr. Anderson noted similar challenges at NXR, where records are managed using spreadsheets.

- Dr. Hamdoun noted that a universally adaptable tracking system would benefit model organism repositories across all species. Dr. Tiersch highlighted the USDA ARS Animal Germplasm Resources Information Network (A-GRIN), which tracks animal species. Ms. Freeman noted that in the absence of other resources, ZIRC benefitted greatly from the expertise of The Jackson Laboratory, which primarily works with mice.

Panel IV: Lessons from Combining Germplasm Repositories with Resource Center Operation
Moderator: Sarah Bodenstein, Ph.D., M.S., Louisiana Sea Grant, Louisiana State University
Speakers: Sarah Bodenstein, Ph.D., M.S., Louisiana Sea Grant, Louisiana State University; Marko Horb, Ph.D., NXR, The University of Chicago; Yuan Lu, Ph.D., XGSC, Texas State University; Danielle McDonald, Ph.D., NAR, University of Miami; Michael Schmale, Ph.D., M.S., NAR, University of Miami; Zoltan Varga, Ph.D., ZIRC, University of Oregon; and S. Randal Voss, Ph.D., AGSC, University of Kentucky

Dr. Sarah Bodenstein introduced the session on integrating germplasm repository and resource center operations at the community level. She noted that cryopreservation is just one of many activities (e.g., sample thawing, line amplification, data management) involved in operating a resource center. Resource centers must consider needs related to equipment, space, time, personnel, training, production scale, data storage, and the wider research community when a facility intends to incorporate cryopreservation or a germplasm repository into its repertoire.

Dr. Varga reiterated Ms. Freeman's comments on how cryopreservation has transformed operations at ZIRC. The shift required reconfiguring the ZIRC database, expanding the facility, and developing a production pathway. ZIRC is interested in engaging with the zebrafish community to encourage increased biobanking and access to genetic resources.

Dr. Marko Horb discussed cryopreservation at NXR. *Xenopus* researchers use cryopreserved sperm to generate injectable embryos for developmental studies. Frozen sperm stocks often are ordered from NXR because of space limitations within individual laboratories. Dr. Horb noted that the facility is experiencing challenges with end-user adoption of straws, which are more efficient than cryovials but not well acknowledged by researchers. NXR also is experiencing challenges with the personnel and funding for cryopreservation at the facility.

Dr. Voss noted that AGSC aims to become a community germplasm repository. The main challenge faced by AGSC is how to integrate germplasm management into the budget and workflow while existing as the sole axolotl stock center in the world. The facility also is interested in approaches for educating and involving the aquatic model community.

Dr. Michael Schmale introduced **Dr. Danielle McDonald,** the new scientific director of NAR. He reviewed barriers to the development of cryopreservation techniques in *Aplysia*. One challenge is rearing the larval stage after thawing. Dr. Schmale highlighted techniques developed in partnership with AGGRC that involve vitrification of *Aplysia* egg strands that have enabled larvae to be frozen and thawed successfully.

Dr. Yuan Lu explained that a significant challenge with *Xiphophorus* is maintaining the 24 species housed at the stock center. The genus is known for forming interspecies hybrids. Embryonic cryopreservation techniques are especially important because conservation is a major focus of the facility.

Panel Discussion

- Dr. Bodenstein asked the panelists to share a challenge they have addressed when incorporating repositories into resource centers. Dr. Varga recalled challenges with having adequate space, which were resolved with renovations that expanded the facility's footprint. Dr. Voss noted an issue with axolotl sperm, which are approximately 0.5 millimeters long. Standard CASA systems often cannot be used with such large cells, and alternative approaches to qualifying and quantifying axolotl sperm had to be developed.

- Dr. Tiersch commented that a cost–benefit analysis can be performed on ZIRC's transition to cryopreservation to determine the valuable outcomes resulting from the initial investment.

- Dr. Bodenstein asked for more information about personnel or workforce challenges experienced by the panelists. Dr. Horb noted that funding is a limiting issue with personnel but added that slower workforce growth is more sustainable.

- Dr. Schmale noted reduced availability and health of wild *Aplysia* stock, which is incorporated into the NAR collection at regular intervals.

- In response to a question about whether NIH can help support germplasm repositories, Dr. Schmale recalled Dr. Hamdoun's comments about a universally adaptable database for tracking animals, tissue samples, and stocks. Support from NIH for basic data storage frameworks would be very beneficial and could be customized to individual needs.

- Dr. McDonald discussed the necessary infrastructure (e.g., back-up power and generators) for protecting resources from natural disasters.

Day 2: Tuesday, September 10, 2024

Session 4: Resource Sharing Within User Communities

Panel V: Opportunities and Challenges for User Communities to Interact with Repositories
Moderator: Yue Liu, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center
Speakers: Christopher Brownlee, Ph.D., Stony Brook University; Stephanie Grainger, Ph.D., Van Andel Institute; Yue Liu, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center; Brian Mitchell, Ph.D., Northwestern University; Daniel Powell, Ph.D., Louisiana State University; and Summer Thyme, Ph.D., UMass Chan Medical School

Dr. Liu introduced the session on interactions between users and repositories with a presentation on developing community-driven capabilities in germplasm repositories for aquatic species. As the number of resources in a scientific community rapidly increases, no single repository will be capable of providing all the maintenance and care that the stock needs. Dr. Liu shared a vision for the future, when stock centers and repositories will guide and train their respective communities in sample processing and quality management capabilities. To balance the need for standardization and reproducibility with the need for customization and portability, a line of 3D-printed inexpensive devices is being developed by AGGRC. The devices will be assembled into user-friendly cryopreservation capability packages for distribution within the wider community. Dr. Liu called for biologists to think of creative and

interdisciplinary solutions for addressing challenges currently faced by resource centers. He invited users to share their experiences with repositories.

Dr. Brian Mitchell explained that he studies the ciliated epithelium in a *Xenopus* model. His group does not have enough tank space to maintain the transgenic animals that are essential for tagging the ciliated epithelium during studies. Currently, his group purchases fresh testes from NXR, but these samples last only 1 week, and experiments require careful planning. Dr. Mitchell noted that onsite *Xenopus* cryopreservation capabilities would enable the rapid fertilization of transgenic animals for experimentation.

Dr. Daniel Powell shared an overview of his evolutionary studies of reproductive barriers in *Xiphophorus* species. His research requires many crosses within and across species—access to reliable germplasm would reduce the burden of maintaining so many distinct colonies. Furthermore, many incompatibility phenotypes are embryonic lethal, and observing them requires multiple pregnancies, which also would be enabled by onsite cryopreservation.

Dr. Grainger described her studies of hematopoietic stem cells in zebrafish. Her institution recently adopted cryopreservation capabilities to address the burden of zebrafish breeding and maintenance. Two technicians proficient in mouse cryopreservation were trained at ZIRC and returned to practice and optimize in-house protocols. Successful cryopreservation and IVF were achieved within a year. Dr. Grainger emphasized that retaining skilled technicians is a critical aspect of cryopreservation.

Dr. Summer Thyme explained that she used zebrafish to study neurodevelopmental disorders. Her research involves the generation of numerous zebrafish mutant lines. Cryopreserving lines at ZIRC enabled Dr. Thyme to move her laboratory to another institution. Cryopreservation also conserves lines that are difficult to generate or those with unique expression profiles. Dr. Thyme noted that training videos developed by experts would benefit the community. She highlighted prohibitive costs associated with recovering lines and expressed hope that cryopreservation of zebrafish larvae would one day be achieved.

Dr. Christopher Brownlee discussed modeling diseases like ciliopathies and microcephaly using mutant *Xenopus* embryos. Cryopreserved lines decrease the costs and increase the efficiency of such studies. The main issue his group has encountered is the quality of preserved sperm samples, whose viability generally varies from 20% to 50%.

Panel Discussion

- Dr. Liu asked the panelists to describe difficulties experienced when they received cryopreserved samples from resource centers. Dr. Mitchell shared his experiences with low fertilization efficiency. Dr. Brownlee noted that he has worked with NXR to troubleshoot the issue, which appears to be occurring at NXR before shipment. Dr. Thyme added that training laboratory technicians in IVF procedures is a hurdle.

- The panelists discussed the challenge of adjusting reliable procedures to research groups with varying resources. For example, certain samples must be stored in liquid nitrogen. Laboratories that store such samples in dry ice or a −80°C freezer because they do not have access to liquid nitrogen will experience significantly poorer outcomes.

- Dr. Powell commented that IVF of *Xiphophorus* fish, which are live bearing, rarely results in embryos capable of development. He described efforts to develop a 3D-printed artificial insemination device to replace outdated and inefficient mouth-pipetting techniques.

- Dr. Thyme noted that researchers need more support to be trained in cryopreservation or need organizations that will provide cryopreservation services.

Session 5: Networks of Aquatic Biomedical Genetic Resources

Panel VI: Opportunities for Developing National Networks and Shared Genetic Resources of Aquatic Species
Moderator: Jack Koch, Ph.D., AGGRC, Louisiana State University Agricultural Center
Speakers: Jack Koch, Ph.D., AGGRC, Louisiana State University Agricultural Center; Andrew Kouba, Ph.D., M.S., Mississippi State University; Mike Lomas, Ph.D., National Center for Marine Algae, Bigelow Laboratory for Ocean Sciences; Theodore Morgan, Ph.D., U.S. National Science Foundation (NSF); Caird Rexroad, Ph.D., USDA; and William Wayman, Ph.D., FWS

Dr. Jack Koch introduced the session on developing national networks of aquatic resources. He explained that he studies *Aplysia*, one of more than 500,000 known species of aquatic invertebrates. His group has integrated 3D-printed technology into *Aplysia* cryopreservation techniques. Dr. Koch spoke about the power of connecting different communities and resources for more effective progress in conservation, aquaculture, and biomedical research.

Dr. Caird Rexroad discussed aquaculture research at USDA ARS and its relevance to cryopreservation. Investing in genetic improvement is a significant focus of ARS Aquatic Genetic Resources. To this end, germplasm preservation is important for protecting stocks from catastrophic events, responding to changing priorities, conserving genetic diversity, providing access to researchers, and preserving lines with special genotypes and phenotypes. USDA is interested in partnering with other federal groups that share a mutual interest in germplasm characterization, preservation, and utilization.

Dr. William Wayman described cryopreservation efforts at the FWS Warm Springs Fish Technology Center, which coordinates efforts with 70 national hatcheries to preserve the biodiversity of threatened species and certain sport fish. The center stores samples of 27 species. Most species are divided between two locations for security; USDA ARS NAGP is the main backup location.

Dr. Andrew Kouba explained that 40% of amphibians are threatened with extinction. Captive assurance colonies are established for these species to assist with conservation strategies, but poor reproduction in some of these colonies necessitates assistive technologies. Assistive reproductive technology for amphibians comprises hormone therapy, ultrasound analysis to select animals with healthy gametes, IVF, and cryopreservation to manage the genetics of the species. The National Amphibian Genome Bank contains approximately 3,000 biobanked straws from 21 species. The organization has partnered with zoos in the Midwest, South, and Mid-Atlantic to establish the Amphibian Conservation and Biobanking Network. Current goals include sustaining and growing the network.

Dr. Mike Lomas noted his research interests in the role of microalgae in the cycling of carbon, nitrogen, and phosphorus in the ocean, as well as the integration of microalgae into aquaculture systems. He also leads the National Center for Marine Algae and Microbiota (NCMA), which has been cryopreserving microalgae since 1992. NCMA has cryopreserved and reanimated more than 1,600 unique strains of algae using tailored protocols for different species. Algae are becoming genetic models, and new technologies are being used to create many more mutant strains. In response to these changes, NCMA is interested in standardizing its cryopreservation methods and developing high-throughput capacity. NCMA's current needs are to network with communities addressing similar challenges and to translate technology into operational procedures.

Dr. Theodore Morgan described NSF's Enabling Discovery through Genomics (EDGE) program, a partnership with the National Human Genome Research Institute to better understand the relationship between genotype and phenotype in diverse species.

Panel Discussion

- In response to a question about which networks to prioritize, Dr. Lomas pointed out that microalgae metabolites are a novel source of biomedicines. Connecting centers focused on algae with biomedical researchers would be a first step in exploring this research. Dr. Morgan noted that interagency collaboration through the EDGE program has been beneficial. Interdisciplinary coordination also might prevent duplicative efforts. Dr. Kouba noted that connecting novel species to biomedical research would enable access to a larger funding pool.

- Dr. Morgan informed the meeting participants about a 2022 executive order that the White House Office of Science and Technology Policy issued on Advancing Biotechnology and Biomanufacturing Innovation for a Sustainable, Safe, and Secure American Bioeconomy, which called for a coordinated federal effort to support biotechnology.

- When asked what is needed to support network formation and continuity, Dr. Morgan noted that funding is always needed. He shared information about NSF's Research Coordination Network awards that support groups of investigators in communicating and coordinating their research, training, and educational activities.

- Dr. Wayman responded that communication among various groups is a huge need. Dr. Lomas emphasized that communication between funding agencies and user communities is especially important. Dr. Kouba suggested that network coordinators with expertise in communication be included in grant applications.

Session 6: Summary Discussion
Moderator: Jack Koch, Ph.D., AGGRC, Louisiana State University Agricultural Center

Summary of Challenges, Opportunities, and Needs

In response to a repeat of the Day 1 Slido poll, approximately 50% of attendees answered that they were faculty members; 29% were staff members, technicians, or managers; 5% were graduate students; 2% were postdoctoral researchers; and 19% answered "Other." A final Slido poll invited participants to share the single word that represents the most pressing need in genetic resource preservation for the aquatic species community. The most popular answers included "collaboration," "communication," "consistency," "embryo," "funding," "interaction," "protocols," "repositories," "scalability," "standardization," "support," and "training." Dr. Koch opened the discussion and requested that the meeting participants share any actionable needs of the community.

- Dr. Lomas commented that **quality management** is a key need, especially given frequent staff turnover. User-friendly quality control metrics are needed to generate high-quality samples.

- Dr. Varga noted that quality control metrics are especially important when results are inconsistent.

- Dr. Tiersch highlighted the difference between quality control (i.e., a product-based assessment) and quality assurance (i.e., an assessment of the conditions of the process). He added that training and sustainability form a third pillar of quality management.

- Dr. Wayman commented that quality management is necessary for retaining users.

- Dr. Purdy remarked that infrastructure development is needed for quality management. Products and processes must all be documented.

- Dr. Lomas added that **support for stakeholder networks** is critical for the community.

- Dr. Liu asked about ways to develop and share **data management strategies and systems**. Dr. Purdy noted that individual users cannot use A-GRIN to manage their sample data. Dr. Blackburn explained that the GRIN system cannot be exported to outside facilities; centers access the system to use it as their database. The approach provides increased security. Certain features would have to be modified or incorporated to make the system more flexible. He suggested building on A-GRIN's existing framework and adding features for describing species and lines.

- Dr. Lomas noted that the community must decide on core metadata before multiple databases can be integrated.

- In response to a prompt from Dr. Koch about **sample tracking**, Dr. Varga remarked that samples should be tracked like a static inventory. Over the years, ZIRC has developed an inventory database.

- Dr. Kouba asked whether any participants had uploaded their databases onto open-source software platforms. Dr. Lomas responded that NCMA is investigating the feasibility of using open-source software. Dr. Kouba added that such databases would be public-facing and easy to search.

- In response to a prompt about how to provide **repository support** to the community, Dr. Watts expressed interest in learning about cryopreservation and repository development from more established biomedical models.

- Dr. Braasch stated that online **training**—as well as local, in-person training and resources—would be helpful. A sustainable central resource platform—such as a hub or center—for research, training, technology development, and community support is needed.

- Dr. Schmale emphasized the benefits that a flexible data-management system would provide to smaller emerging resource centers. The community could collectively determine which software to use and whether the system should function as a central database.

- Dr. Tiersch called for **standardization** of data collection methods. He noted, for example, that most people measure cooling rates differently. A simple standard measurement of time and temperature would enable comparisons across samples for improved quality control. He suggested that with streamlined procedures, a machine-learning approach could be used to determine the highest-quality samples that should be saved and stored. This would be greatly facilitated by community adoption of open-hardware approaches, especially when distributed in the form of comprehensive kits.

- Dr. Liu proposed compiling and sharing a list of all data management software used by resource centers with a company specializing in custom software development. The community also could establish a customizable and sustainable open-source software system that can be generalized for multiple resource centers. Dr. Liu mentioned collaborating with a small company that provides fully customizable database systems using virtual servers that can be transferred to the home institution, requiring no coding for further customization.

- In response to a prompt about methods for **sharing capabilities,** Mr. Zuchowicz remarked that hands-on, in-person training with different pieces of equipment is extremely valuable.

- Dr. Braasch suggested that cryopreservation courses be offered at a central facility with faculty-led instruction. Additional course modules can be developed to address more specific needs. Dr. Watts noted that when his institution experienced difficulties with zebrafish husbandry, a workshop was held during which the attendees performed every component of zebrafish maintenance, and the problems were resolved. He added that most of the training was conducted by staff members.

- Dr. Bodenstein noted that **educating the community** about cryopreservation dynamics at the cellular level will enable people to troubleshoot issues with their samples.

- Dr. Kouba added that a repository of cryopreservation knowledge (e.g., trainings, webinars, videos, tips) should be developed. He noted that grant funding could support the development of these materials and that extension faculty are valuable for education and training activities.

White Paper Preparation

Dr. Liu remarked that the white paper developed from the workshop has the potential to have a sustained impact on the future of the aquatic resources community. He noted that the structure of the white paper would be parallel to the levels addressed by the workshop sessions. The Panel moderators of each session will lead the writing teams for the portions of the report that address their respective levels. Panelists will review other sections of the report.

- Dr. Blackburn noted that the white paper might be a place to address concerns about the use of animals in scientific research. He also suggested including opportunities for interactions between resource centers and federal agencies. For example, the role that genetic resources will play during climate change could be a potential area of overlap.

- Dr. Kouba agreed with Dr. Blackburn and emphasized the importance of building connections between conservation biologists and biomedical researchers and enabling them to share expertise and insights.

- Mr. Zuchowicz proposed that the paper include a table with biomedical model organism information, such as cryopreservation parameters, to facilitate cross-species comparisons. Dr. Liu suggested an additional table comparing the usage of animal models and associated research outcomes.

- Dr. Tiersch recommended focusing on common infrastructures and overlapping features of aquatic animal models.

- Dr. Varga noted that a society for cryobanking would be a driving force for advocating for advancement in the field.

- Dr. Kouba suggested including a section on top needs related to distributing materials and expanding educational capabilities for the community.

- Dr. Albertin recommended including suggestions for developing a community-wide or community-specific data management system.

Appendix A: Meeting Agenda

Session II. Development of Sustainable Germplasm Repositories for Aquatic Biomedical Models

Virtual Meeting
September 9–10, 2024

Day 1 (September 9, 2024), Moderated by Dr. Yue Liu

Session 1: Introduction and Overview

1:00–1:10 p.m. **Greetings and Workshop Preview**
Yue Liu, Ph.D., M.S., Aquatic Germplasm and Genetic Resources Center (AGGRC), Louisiana State University Agricultural Center

1:10–1:25 p.m. **Advancing Biomedical Research: ORIP's Strategic Investment in Cryopreservation of Aquatic Models**
Michael Chang, Ph.D., Deputy Director, Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives, National Institutes of Health

1:25–1:40 p.m. **Advancements and Impact Since the 2017 Workshop in Birmingham, Alabama**
Zoltan Varga, Ph.D., Zebrafish International Resource Center (ZIRC), University of Oregon

1:40–1:55 p.m. **Visions of the Need in the Future of Genetic Resources of Aquatic Species**
Terrence Tiersch, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center

1:55–2:00 p.m. *Discussion*

Session 2: From Cryopreservation Research to Repository Application

2:00–2:15 p.m. **Panel I: Advancement in Technologies and Research for Germplasm Repository Development**

Presentations by Panelists:
Jose Cibelli, D.V.M., Ph.D., Michigan State University (Panel Moderator)
Shannon Tessier, Ph.D., Harvard Medical School and Massachusetts General Hospital
Zongqi Guo, Ph.D., M.Eng., University of South Florida
Nikolas Zuchowicz, M.S., University of Minnesota
Jill Jenkins, Ph.D., U.S. Geological Survey
Phil Purdy, Ph.D., U.S. Department of Agriculture (USDA)

2:15–2:30 p.m. *Discussion*

2:30–2:45 p.m. **Panel II: Challenges in Research and Technology Development for Emerging Models**

Presentations by Panelists:
Ingo Braasch, Ph.D., Michigan State University (Panel Moderator)
Patricia Schneider, Ph.D., Louisiana State University

Harvey Blackburn, Ph.D., USDA
Caroline Albertin, Ph.D., M.S., Marine Biological Laboratory (MBL), The University of Chicago
Amro Hamdoun, Ph.D., University of California, San Diego
Stephen Watts, Ph.D., M.S., The University of Alabama at Birmingham

2:45–2:30 p.m. *Discussion*

Session 3: Operations of Repositories at Resource Centers

3:00–3:15 p.m. **Panel III: What Have We Learned from Translating Protocols to Germplasm Repositories?**

Presentations by Panelists:
Jonathan Daly, Ph.D., Reef Restoration and Adaptation Program, University of New South Wales (Panel Moderator)
Carl Anderson, National Xenopus *Resource (NXR), MBL, The University of Chicago*
Raissa Cecil, Ambystoma *Genetic Stock Center (AGSC), University of Kentucky*
Claudia Alvarez, National Aplysia *Resource (NAR), University of Miami*
Lindsey Sanchez, Xiphophorus *Genetic Stock Center (XGSC), Texas State University*
April Freeman, ZIRC, University of Oregon

3:15–3:30 p.m. *Discussion*

3:30–3:45 p.m. **Panel IV: Lessons from Combining Germplasm Repositories with Resource Center Operation**

Presentations by Panelists:
Sarah Bodenstein, Ph.D., M.S., Louisiana Sea Grant, Louisiana State University (Panel Moderator)
Zoltan Varga, Ph.D., ZIRC, University of Oregon
Marko Horb, Ph.D., NXR, The University of Chicago
S. Randal Voss, Ph.D., AGSC, University of Kentucky
Michael Schmale, Ph.D., M.S., NAR, University of Miami
Yuan Lu, Ph.D., XGSC, Texas State University

3:45–4:00 p.m. *Discussion*

4:00 p.m. **Day 1 Adjournment**

Day 2 (September 10, 2024), Moderated by Dr. Terrence Tiersch

Session 4: Resource Sharing Within User Communities

1:00–1:15 p.m. **Panel V: Opportunities and Challenges for User Communities to Interact with Repositories**

Presentations by Panelists:
Yue Liu, Ph.D., M.S., AGGRC, Louisiana State University Agricultural Center (Panel Moderator)
Brian Mitchell, Ph.D., Northwestern University
Daniel Powell, Ph.D., Louisiana State University

Stephanie Grainger, Ph.D., Van Andel Institute
Summer Thyme, Ph.D., UMass Chan Medical School
Christopher Brownlee, Ph.D., Stony Brook University

1:15–1:30 p.m. *Discussion*

Session 5: Networks of Aquatic Biomedical Genetic Resources

1:30–1:45 p.m. **Panel VI: Opportunities for Developing National Networks and Shared Genetic Resources of Aquatic Species**

Presentations by Panelists:
Jack Koch, Ph.D., AGGRC, Louisiana State University Agricultural Center (Panel Moderator)
Caird Rexroad, Ph.D., USDA
William Wayman, Ph.D., U.S. Fish and Wildlife Service
Andrew Kouba, Ph.D., M.S., Mississippi State University
Mike Lomas, Ph.D., National Center for Marine Algae, Bigelow Laboratory for Ocean Sciences
Theodore Morgan, Ph.D., U.S. National Science Foundation

1:45–2:00 p.m. *Discussion*

Session 6: Summary Discussion

2:00–2:40 p.m. Summary of Challenges, Opportunities, and Needs

2:40–3:00 p.m. White Paper Preparation

3:00 p.m. Adjournment

Appendix B: Attendees

Session II. Development of Sustainable Germplasm Repositories for Aquatic Biomedical Models

Virtual Meeting
September 9–10, 2024

Monika Aggarwal, Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Caroline Albertin, Marine Biological Laboratory (MBL), The University of Chicago
Michelle Altemara, The University of North Carolina at Chapel Hill
Claudia Alvarez, National *Aplysia* Resource (NAR), University of Miami
Carl Anderson, National *Xenopus* Resource (NXR), MBL, The University of Chicago
Guilherme Antunes, Champalimaud Foundation
Jhony Lisboa Benato, Universidade Federal do Rio Grande do Sul
Thom Berriman, King's College London
Kevin Bishop, National Human Genome Research Institute (NHGRI), NIH
Harvey Blackburn, U.S. Department of Agriculture (USDA)
Sarah Bodenstein, Louisiana State University
Ingo Braasch, Michigan State University
Jeri Brandom, The University of Alabama at Birmingham
Christopher Brownlee, Louisiana State University
Sara Campos, Universidade de Vigo
Blake Carrington, NHGRI, NIH
Lily Carter, Aquatic Germplasm and Genetic Resources Center (AGGRC), Louisiana State University Agricultural Center
Raissa Cecil, *Ambystoma* Genetic Stock Center (AGSC), University of Kentucky
Susan Chandran, ORIP, DPCPSI, OD, NIH
Michael Chang, ORIP, DPCPSI, OD, NIH
Yong Chen, ORIP, DPCPSI, OD, NIH
Jin Choi, Michigan Technological University
Joseph Christensen, Louisiana State University
Jose Cibelli, Michigan State University
Renee Clark, Zebrafish International Resource Center (ZIRC)
Miguel Contreras, ORIP, DPCPSI, OD, NIH
Nicholas Coxe, Louisiana State University
Jonathan Daly, University of New South Wales
Isabel Clara Rollan Delgado, European Molecular Biology Laboratory
Victoria Denham, Louisiana State University
Sheri Dorsam, USDA
Erin Ducharme, University of New England
Effrosyni Fatira, Universidad de Las Palmas de Gran Canaria
Katie Flaherty, Rutgers, The State University of New Jersey
Thales Franca, Universitat Politècnica de València
Stephen Frederickson, NHGRI, NIH
April Freeman, ZIRC
Thaiza Freitas, Universidade Federal do Rio Grande do Sul
Liezhen Fu, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), NIH

Victoria Gibbs, The University of Alabama at Birmingham
Inês Gonçalves, Champalimaud Foundation
Stephanie Grainger, Van Andel Institute
Franziska Grieder, ORIP, DPCPSI, OD, NIH
Audra Guikema, Van Andel Research Institute
Zongqi Guo, University of South Florida
Maria Teresa Gutierrez-Wing, Louisiana State University
Jenna Hakkesteeg, University College London
Amro Hamdoun, University of California, San Diego
Elise Harmon, AGGRC, Louisiana State University Agricultural Center
Marko Horb, NXR, The University of Chicago
Jen-Jen Hwang-Shum, University of Oregon
Jill Jenkins, U.S. Geological Survey
Jack Koch, AGGRC, Louisiana State University Agricultural Center
Xiangbo Kong, University of Michigan
Andrew Kouba, Mississippi State University
Rachael Labitt, NICHD, NIH
David Lains, ZIRC
Rachel Lenkey, Seattle Children's Research Institute
Yue Liu, AGGRC, Louisiana State University Agricultural Center
Mike Lomas, National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences
Ryan Lopez, Oregon State University
Evyn Loucks, University of Oregon
Yuan Lu, *Xiphophorus* Genetic Stock Center (XGSC), Texas State University
Chethan Magnan, Michigan Technological University
Molly McCoy, Louisiana State University
Danielle McDonald, NAR, University of Miami
Oleg Mirochnitchenko, ORIP, DPCPSI, OD, NIH
Brian Mitchell, Northwestern University
Visila Moiche, BBD BioPhenix S.L.U
Joana Monteiro, Champalimaud Foundation
Theodore Morgan, U.S. National Science Foundation
Mckenzie Mungai, Eckerd College
Stephanie Murphy, ORIP, DPCPSI, OD, NIH
Katy Murray, ZIRC
Estefania Paredes, Vigo University
David Pasnik, USDA
Daniel Powell, Louisiana State University
Phil Purdy, USDA
Brittany Ratliff, AGGRC, Louisiana State University Agricultural Center
Caird Rexroad, USDA
Irene Salinas, University of New Mexico
Lindsey Sanchez, XGSC, Texas State University
Manfred Schartl, Texas State University
Michael Schmale, NAR, University of Miami
Patricia Schneider, Louisiana State University
Nikko-Ideen Shaidani, MBL, The University of Chicago
Soaleha Shams, Mayo Clinic
Stephanie Slater, Seattle Children's Research Institute
Raman Sood, NHGRI, NIH
Dionísio Sousa, Champalimaud Foundation

Tina St. Laurent, Van Andel Institute
Daniel Stutts, The University of North Carolina School of Medicine
Yongjun Sui, National Cancer Institute, NIH
Akiko Takizawa, Medical College of Wisconsin
Ritesh Tandon, ORIP, DPCPSI, OD, NIH
Nathalia Teixeira, Universidade Federal do Rio Grande do Sul
Shannon Tessier, Harvard Medical School and Massachusetts General Hospital
Summer Thyme, UMass Chan Medical School
Biao Tian, ORIP, DPCPSI, OD, NIH
Connor Tiersch, NovelAire
Terrence Tiersch, AGGRC, Louisiana State University Agricultural Center
Karel Torres-Lozano, Universidade Federal do Rio Grande do Sul
Reiko Toyama, NICHD, NIH
Walter Tsark, City of Hope
Gregory Valdivia, Universidade Federal do Rio Grande do Sul
Zoltan Varga, ZIRC, University of Oregon
Desiree von Kollmar, ORIP, DPCPSI, OD, NIH
S. Randal Voss, AGSC, University of Kentucky
Chongmin Wang, NICHD, NIH
Stephen Watts, The University of Alabama at Birmingham
William Wayman, U.S. Fish and Wildlife Service
Brian Will, University of New England
Evan Williams, University of Oregon
Paul Wills, Florida Atlantic University
Heather Wood, Cleveland Clinic
Xiaojun Xing, Yale University
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