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Office of Research Infrastructure Programs

Cryopreservation and Other Preservation Approaches for Animal Models Workshop

Session I. Invertebrate Models in Biomedical Research

September 6, 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. These sessions addressed the following topics related to cryopreservation and other preservation methods, including, but not limited to—

- The needs and scientific status of cryopreservation and other preservation of gametes (e.g., sperm, oocytes, and embryos), reproductive tissues, larvae, and whole animals and their production of live offspring after revival.

- Emerging cryopreservation and other preservation methods and technologies, as well as how to optimize and implement them.

- 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.

- The sharing of technologies, including hands-on training for cryopreservation best practices, and training of next-generation scientists.

- The preservation and management of samples from collection to utilization.

Session I addressed topics related to cryopreservation and other long-term preservation methods of invertebrate models commonly used in biomedical research, including *Drosophila melanogaster*, *Caenorhabditis elegans*, and sea urchin. The session presentations focused on current approaches and limitations in preserving these organisms and lessons learned from preserving other invertebrate organisms, such as tardigrades, mosquitoes, and black soldier flies. The session included discussions of the potential for desiccation and diapause to serve as preservation methods. The participants discussed gaps and challenges in the field, including training activities required to accurately disseminate protocols; the benefits of specialized versus generalized protocol development; strains and organisms that are challenging to propagate and cryopreserve; and issues related to storage space, sample curation, and quality control.

Session Chair
Joseph Rinehart, Ph.D., U.S. Department of Agriculture (USDA)

Presenters
John Bischof, Ph.D., University of Minnesota
Christopher Fang-Yen, Ph.D., The Ohio State University
Daryl Gohl, Ph.D., University of Minnesota
Jonathan Hibshman, Ph.D., Southern Methodist University
Estefania Paredes, Ph.D., Vigo University, Spain
Arun Rajamohan, Ph.D., USDA
Ann Rougvie, Ph.D., University of Minnesota
Rebecca D. Sandlin, Ph.D., Massachusetts General Hospital and Harvard Medical School
Nicholas Teets, Ph.D., University of Kentucky
Jeffery Tomberlin, Ph.D., Texas A&M University
Cale Whitworth, Ph.D., Indiana University

Session I Organizing Committee

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Jonathan Hibshman, Ph.D., Southern Methodist University
Veronica Frances Hinman, Ph.D., Carnegie Mellon University
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Yogesh Wairkar, Ph.D., National Institute of General Medical Sciences, NIH
Sige Zou, Ph.D., ORIP, DPCPSI, OD, NIH

Workshop Report

Opening Remarks

Joseph Rinehart, Ph.D., U.S. Department of Agriculture (USDA)
Franziska Grieder, D.V.M., Ph.D., Director, 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)
Sige Zou, Ph.D., Workshop Coordinator, Program Official, ORIP, DPCPSI, OD, NIH

Dr. Joseph Rinehart, Session I Chair, and Dr. Franziska Grieder, Director, ORIP, welcomed the attendees to the workshop. In her opening remarks, Dr. Grieder highlighted ORIP's 2021–2025 Strategic Plan, which commits that ORIP will assess the contribution of its resources to improving scientific rigor and reproducibility and will make strategic investments in methods and infrastructure tools for enhancing the rigor and reproducibility of animal models and related biomaterials. She noted that ORIP is currently developing its 2026–2030 Strategic Plan.

ORIP comprises two divisions: the Division of Comparative Medicine (which supports research centers and resources, research project grants, and training programs for veterinary scientists) and the Division of Construction and Instruments (which sustains research infrastructure through construction and instrumentation awards and an equipment program). ORIP also supports small businesses through Small Business Innovation Research and Small Business Technology Transfer programs.

Dr. Grieder noted that ORIP's mission is "Infrastructure for Innovation." She highlighted challenges in supporting animal resources. Diverse animal models—which include worms, flies, aquatic animals, rodents, pigs, and nonhuman primates—require different strategies for maintenance and distribution. These animal models must be maintained constantly and efficiently for experimental rigor and reproducibility, and reliable animal welfare coverage must be ensured. The rapid growth of animal resource development (due to genome editing breakthroughs) has increased costs for animal resource centers' physical infrastructure and personnel, but the NIH budget has remained relatively flat. Dr. Grieder pointed out that reliable cryopreservation was one way to address many of these challenges. She noted that over the past 20 years, ORIP (formerly the National Center for Research Resources) has invested more than $106 million in cryopreservation activities, which does not include funds for cryopreservation incorporated into research center awards. Research centers and resources have access to off-site cryopreservation storage that also is supported by funding from ORIP.

Dr. Grieder introduced Dr. Sige Zou, Workshop Coordinator, who thanked Dr. Rinehart and the rest of the Workshop Organizing Committee for planning the meeting. He explained that this session is one of six days of the workshop that will take place over the next few months. Future workshop topics include aquatic models, technologies and resources, rodent models, and nonhuman primate and other large animal models. Dr. Zou emphasized that the purpose of Session I is to consider the role of cryopreservation and other preservation approaches in addressing the challenges facing invertebrate animal research. Participants also should discuss the current scientific state, gaps, and emerging technologies in the field of cryopreservation and other preservation methods for invertebrate models.

Established Models

Moderators: John Bischof, Ph.D., University of Minnesota, and Yogesh Wairkar, Ph.D., National Institute of General Medical Sciences (NIGMS), NIH

Dr. Yogesh Wairkar introduced the keynote speaker, Dr. John Bischof, and thanked the session's presenters, who shared updates about cryopreservation in established invertebrate animal models.

Keynote Presentation: Cryopreservation of* Drosophila *Embryos
John Bischof, Ph.D., University of Minnesota

Dr. Bischof introduced the U.S. National Science Foundation Engineering Research Center (ERC) for Advanced Technologies for the Preservation of Biological Systems (ATP-Bio), which aims to "stop biological time" by advancing biological preservation technologies. ATP-Bio has assembled experts in biology, medicine, and engineering to address the overarching challenges involved in cryopreservation—which include excessive ice formation, cryoprotectant agent (CPA) toxicity, and cellular cracks caused by thermal stresses—via target areas that focus on biological engineering, the multiscale thermodynamics of water, and rapid and uniform warming in several model organisms and other systems. One important outcome of ATP-Bio was the assembly of an adjacent NIH R24-funded group at the University of Minnesota focused more narrowly on *Drosophila* embryo cryopreservation, which is relevant to ORIP's mission.

Metabolic suppression increases as storage temperature is reduced; evidence indicates that "suspended animation" for many years is possible for samples in a vitrified, cryopreserved state. Vitrification is the process whereby liquids solidify without crystallization and directly transform from a liquid state to an amorphous, solid state without ice formation. Although successful vitrification has been achieved, rewarming remains a challenge. To prevent devitrification (i.e., ice crystallization during rewarming), the warming rate for a sample must be orders of magnitude higher than the cooling rate. Moreover, the rewarming needs to be uniform; otherwise, the thermal stress caused by the temperature gradient will lead to cracks during rewarming. To avoid toxicities associated with CPAs, the lowest possible CPA concentration is used. Notably, the lower the concentration of CPA in a sample, the higher rate required for achieving vitrification during cooling and avoiding devitrification upon rewarming. Increased CPA volume is used as sample sizes increase; however, this can reduce the ability to quickly cool and rewarm during cryopreservation. For instance, a traditional straw of bovine oocytes with a volume of approximately 250 microliters (µl) can be cooled at a rate of 2,500 degrees Celsius per minute (°C/min) and warmed at a rate of 2,700°C/min. Samples with much smaller volumes (e.g., cryo-loops, cryo-tops, quartz microcapillaries) allow even faster rates that can exceed 200,000°C/min. The fastest approach to rewarm vitrified systems on a cryo-top currently is laser nanowarming, which can reach up to 13,000,000°C/min, whereas another approach using joule (electrical pulse) heating of metal substrates can rewarm up to 600,000,000°C/min for smaller systems (i.e., approximately 10 microns). With these parameters, a landscape of various cryopreservation approaches for zebrafish, *Drosophila*, and other model systems can be established.

Several members of ATP-Bio were the first to develop a cryopreservation technique for teleost fish embryos. The method, which was pioneered in zebrafish, involves an injection of CPA and plasmonic gold into the yolk of the fertilized egg, followed by immersion in a precooling bath. The embryos are rapidly cooled in liquid nitrogen before being stored and then are recovered via laser nanowarming (i.e., conversion of laser light into heat by plasmonic gold). Success rates are low, but the fish that survive the process spawn normally. Improved delivery of the CPA and plasmonic gold with automated microinjections has increased the technique's success rate. Other members of ATP-Bio have used NIH R21 and R24 funding to develop and continue to refine a protocol for cryopreserving *Drosophila* embryos. *Drosophila* stocks must be maintained through tedious and costly labor involving frequent fresh food transfer. Preliminary techniques for freezing *Drosophila* embryos have been developed, but embryo staging issues and other barriers to reproducibility remain. The affiliated R24 group has developed a method for addressing these challenges and improving survival during cryopreservation (e.g., collecting eggs at a specific time and temperature, permeabilizing with gentler reagents, ensuring that embryos are rehydrated properly after dehydration and before CPA loading, reducing the Leidenfrost effect during freezing, warming frozen embryos in a sucrose solution, feeding newly thawed embryos an optimal diet to support their reanimation). Recent improvements to the method will be published shortly and

disseminated widely for both individual laboratory and scaled-up stock-center use. Furthermore, in-person training on the protocol will be available at the University of Minnesota, and a website for sharing relevant resources, such as videos and publications, has been developed.

Discussion

- A participant asked how ATP-Bio researchers label each cryo-mesh. Dr. Bischof explained that supports for the mesh are 3D-printed and can be labeled. He can be contacted at bischof@umn.edu for more information about directly labeling cryo-mesh.

Cryopreservation of* Caenorhabditis elegans *Stocks
Ann Rougvie, Ph.D., University of Minnesota

Dr. Ann Rougvie provided an overview of *C. elegans* cryopreservation at the *Caenorhabditis* Genetics Center (CGC). The nematode *C. elegans* has a rapid life cycle and is easy and inexpensive to grow on bacteria-coated agar plates. The tiny worm was adopted as a model metazoan by Dr. Sydney Brenner in the 1960s. *C. elegans* have an invariant cell lineage for developmental studies, are transparent for optical analysis, and reproduce via self-fertilization and crossing for genetic analysis. Sequencing the *C. elegans* genome revealed that approximately 50% of worm genes have clear human homologs and 40% of described human disease genes have clear worm homologs. Techniques for cryopreserving *C. elegans* were established early during the adoption of this model organism.

The CGC was established as a community resource in 1979 to acquire, maintain, and distribute nematode strains throughout the scientific community. During the past 30 years, the number of strains in the CGC collection has multiplied tenfold. Currently, the CGC distributes approximately 30,000 strains annually. The CGC's aims related to cryopreservation include the following: (1) preserve critical strains for long-term use by scientists, (2) minimize genetic drift, (3) develop high-throughput workflows, scalable for up to 100 strains per day, and (4) employ methods that ideally apply to all worm strains. The ultimate goals of the CGC are to enhance reproducibility, standardize reagent use, maximize access to strains, and remove the burden of strain distribution from individual laboratories. The CGC cryopreservation workflow typically involves receiving new strains growing on agar plates. The worms are then cleaned, expanded, and frozen in seven aliquots using glycerol as a cryoprotectant; one vial is thawed to verify the viability of the stocks, four cryovials are prepared for long-term storage in liquid nitrogen, and two cryovials are prepared for working stocks, all of which are stored temporarily at −80°C. Both types of samples are frozen slowly in Styrofoam at the rate of approximately 1°C/hour. The long-term stocks are split and stored in liquid nitrogen in two different local buildings, and one cryovial is shipped to the USDA for storage. Limited access is granted to these stocks. The working stocks comprise a suspension of worms in soft agar, which can be accessed up to five times per vial and dispensed onto fresh plates for thawing when a strain is requested. If the last aliquot of the final cryovial is used, the strain is re-expanded on fresh plates, starved, and frozen to replenish the frozen stocks.

Wild-type worms can survive more than 30 years of storage in liquid nitrogen. Young worms (i.e., starved, early larval–stage worms) are most likely to survive thawing after cryopreservation and storage at −80°C or in liquid nitrogen. Thousands of worms can be grown and frozen easily, so precise survival rates are not critical. Essentially, all worm strains can be cryopreserved using this technique. For mutations that are hard to grow or are sensitive to freezing, genetic balancers are bred into the strains so that they can be frozen as heterozygotes. Other CPAs used by the *C. elegans* community include a dimethylsulfoxide (DMSO) solution and a combined trehalose–DMSO solution. Challenges faced by the CGC (and the wider *C. elegans* research community) include a lack of storage space, issues with freezer durability, and the large carbon footprint associated with storing worms.

Discussion

- In response to a query from Dr. Bischof, Dr. Rougvie responded that backup samples are shipped to the USDA's National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado.

- Dr. Abdul-Rashid Iddi asked about plans to expand the CGC to other continents. Dr. Rougvie remarked that the CGC is working with the logistics industry to increase the ease of international shipping. Funding is not yet available to expand overseas, but this issue has been recognized and is a topic of discussion at the CGC.

Preservation of* Caenorhabditis elegans *by Desiccation
Christopher Fang-Yen, Ph.D., The Ohio State University

Dr. Christopher Fang-Yen presented a novel method for *C. elegans* preservation. Ultracold preservation is widely used for long-term storage of *C. elegans* strains. However, strains are vulnerable to refrigeration failures due to power outages and other emergencies. Conventionally frozen *C. elegans* (i.e., via the glycerol protocol, soft agar protocol, or DMSO protocol) lose viability after a single freeze–thaw cycle. Additionally, once thawed, strains must be subcultured before refreezing. Osmotic dehydration is being used to improve *C. elegans* dehydration. Dehydration can inhibit intracellular ice formation, but the process itself can damage cells and tissues. Xeroprotectants are agents that protect organisms from damage during desiccation. Exogenous trehalose and other compounds were evaluated for their performance as xeroprotectants during *C. elegans* preservation. Worm suspensions were prepared in control, trehalose, glycerol, or DMSO solutions and incubated for 48 hours in uncapped cryotubes in an airtight container with anhydrous calcium sulfate as a desiccant. Exogenous trehalose and glycerol (but not DMSO) improved worm survival after desiccation, especially that of dauer-phase worms. This effect was observed after repeated freeze–thaw cycles, with trehalose being a more effective xeroprotectant than glycerol.

Desiccation has some drawbacks, however. Dauer-defective worms do not survive the procedure, and the viability of dehydrated strains depends on storage temperature. Desiccated worms can be stored for a week at room temperature, for a month at 4°C, for a year at −20°C, and indefinitely at −80°C. Desiccation of a small volume of worms is inefficient and allows only a single recovery per tube. A method for dehydrating larger volumes of worms (that can be subsampled when a strain is recovered) was developed. *C. elegans* suspensions were mixed with a granular medium (i.e., cornmeal) before desiccation. Upon dehydration, worms adhere to the granules, and recovery can be performed using a very small volume of the granules in the tube. Ongoing efforts—including optimizing dehydration conditions to maximize survival and testing the dehydration method with many diverse strains—aim to improve the technique.

Discussion

- Mr. Xiaojun Xing asked about the size of the cryotubes used for dehydration. Dr. Fang-Yen explained that standard cryotubes with a volume of 1.2 milliliters are used. He added that the technique's success does not depend on the tube volume.

- Dr. Bischof asked whether the volume of water left in the samples after dehydration had been determined. He suggested that differential survival at different temperatures was affected by the amount of water retained in each sample. Dr. Fang-Yen remarked that his group had not measured the residual water in the dehydrated samples. He estimated that only a small percentage of the original water volume remained, adding that worm samples that were too dehydrated exhibited poor survival.

- Dr. Iddi suggested experimenting with variable cooling rates using different storage materials.

- In response to a question from Dr. Bischof about the dauer-defective worm mutant, Dr. Fang-Yen explained that the mutated gene is a transcription factor linked to many downstream effector proteins, including heat shock proteins (HSPs).

Long-Term Maintenance of* Drosophila *Stocks by Live Cultures
Cale Whitworth, Ph.D., Indiana University

Dr. Cale Whitworth described the maintenance of *Drosophila melanogaster* stocks at the Bloomington *Drosophila* Stock Center (BDSC). The BDSC collection comprises more than 92,000 genetically defined *Drosophila* strains maintained as independent, duplicate copies. The fly's life cycle spans about 12 days; for maintenance, flies from each stock are examined for health and transferred to fresh food vials every 2 weeks. All stocks at the BDSC collection are maintained by hand by 87 stockkeepers, most of whom are part-time workers. The BDSC collection requires approximately 60,000 effort hours per year just for maintenance. This massive effort is still less expensive than the cryopreservation of *Drosophila*, which would cost approximately $165 for a single freeze–thaw cycle of a sample (compared to $17.32 per year to maintain living cultures of both copies of a given stock). The cumulative cost of live maintenance of *Drosophila* stocks exceeds that of cryopreservation only after 10 years. Additionally, the BDSC ships approximately 3,500 samples each week. Most stocks are ordered within a 3-year window, and the cost of $15 per ordered stock likely would not cover the cost of freezing and thawing a sample.

If the cryopreservation of *Drosophila* stocks were more economically feasible, it would be incredibly beneficial to the BDSC and *Drosophila* researchers. Frozen stocks would help with emergency preparedness, eliminate the potential for error associated with live culture, and mitigate genetic drift. Certain strains are extremely valuable and cannot be regenerated. Cryopreservation would help to preserve rarely used but potentially useful stocks. Cryopreservation has advantages but cannot replace live culture for routine maintenance and distribution activities. For the moment, live culture and cryopreservation are complementary methods that address unique needs for cost-effective *Drosophila* stock maintenance.

Discussion

- Drs. Bischof and Fang-Yen asked why *Drosophila* cryopreservation is so costly. Dr. Whitworth answered that the precise cost of cryopreservation is difficult to calculate. Community members are hired and trained to perform live culture maintenance. The assumption behind the increased cost is that this technique will require more laboratory experience and a concomitant increase in hourly wages. Dr. Bischof offered to share lessons learned from his institution's experience with training cryobiologists.

- Dr. Nicholas Teets asked whether rearing stocks at lower temperatures could extend the fly life cycle. Dr. Whitworth noted that the fly life cycle extends to almost 20 days when grown at 18°C. However, bacterial and fungal contamination are more likely to occur at 18°C than at the standard 22°C. Dr. Whitworth mentioned a publication demonstrating that fly generation times can be extended significantly with maintenance regimes involving fluctuating temperatures from 6°C to 22°C.

- Drs. Whitworth and Bischof discussed efforts to examine potential mutagenic effects of the freeze–thaw process on *Drosophila* embryos. The second and fourth chromosomes from 10 different lines from the BDSC collection were isogenized, and their genomes were sequenced. Their genomes will be resampled after multiple freeze–thaw cycles to identify mutations caused by cryopreservation.

Cryopreservation of Sea Urchin
Estefania Paredes, Ph.D., Vigo University, Spain

Dr. Estefania Paredes presented an overview of sea urchin cryopreservation. Cryopreservation studies involving oocytes, sperm, embryos, and larvae have been performed on 16 of approximately 950 species of sea urchins. Sea urchin sperm can be frozen in a 7% to 10% (volume/volume) solution of DMSO and seawater. Samples in straws are equilibrated for 8 minutes in liquid nitrogen vapor in a Styrofoam box, stored in liquid nitrogen, and thawed for 6 seconds at 35°C, with the stepwise addition of seawater to reactivate the sperm. After a freeze–thaw cycle, approximately 50% of sperm cells are motile in *Paracentrotus lividus*. Notably, even sperm that are not motile are capable of fertilization. Embryos and larvae also can be cryopreserved. Approximately 50% of cryopreserved sea urchin embryos (blastula stage) can develop into juveniles successfully after thawing. Sea urchins can be cryopreserved at the four-armed pluteus larval–stage, but some post-thaw arm damage has been observed. Experiments are planned to determine whether this damage affects development to the metamorphosis stage.

Successful cryopreservation of sea urchin oocytes has not yet been achieved. Oocyte cell size (approximately 100-micron diameter), water content, and slightly low membrane permeability are not the main barriers to the development of cryopreservation protocols. The egg cells are extremely sensitive to toxicity, dehydration, and chilling. Exposure to CPAs without freezing is sufficient to damage the oocyte membrane and internal vesicles. CPA cocktails that minimize toxicity are being evaluated.

Discussion

- A participant asked for clarification on the ideal cooling rate for freezing sea urchin sperm in liquid nitrogen vapor. Dr. Paredes explained that the cooling rate can be regulated by placing samples closer to or further away from the liquid nitrogen. The maximum survival rate of 50% is observed when sperm are cooled at an approximate rate of 8°C per minute.

- In response to a question from Dr. Bischof, Dr. Paredes noted that these protocols are geared toward the aquaculture industry and must be inexpensive and user-friendly.

- Dr. Veronica Hinman asked about the earliest embryonic stage tested for cryopreservation and wondered whether embryo size plays a factor in cryopreservation. Dr. Paredes remarked that the last embryo stage (i.e., blastula stage) is the earliest that has been tested and successfully cryopreserved.

- Mr. Gerardo Reyes asked whether better survival rates could be obtained by freezing whole gonad tissues. Dr. Paredes commented that this possibility has been considered. However, *in vitro* culture of marine organisms is underdeveloped. Oocytes extracted from tissues cannot yet be matured under laboratory conditions to be capable of fertilization, which will be necessary to test viability after cryopreservation.

- In response to a question about differences between aquaculture gametes and those of agricultural species (e.g., pig, cow, sheep), Dr. Paredes noted that sea urchin fertilization involves increased cell numbers. Unlike in mammals, millions of eggs can be collected from a single sea urchin gonad.

- In response to a query from Dr. Bischof about differences in membrane and cell composition between sea urchin and mammalian oocytes, Dr. Paredes responded that sea urchin oocytes have higher water content and more lipid droplets than mammalian egg cells, which appears to inhibit diffusion of CPAs throughout the cell. Sea urchin oocytes grow in seawater and are sensitive to ionic balance, which can be disrupted by the addition of CPAs. Dr. Bischof suggested that

calorimetry or spectroscopy be performed on cooling oocytes to determine whether a lipid phase change occurs.

Emerging Models

Moderators: Arun Rajamohan, Ph.D., USDA, and Mahua Mukhopadhyay, Ph.D., Eunice Kennedy Shriver *National Institute of Child Health and Human Development (NICHD), NIH*

Dr. Rinehart introduced the keynote speaker, Dr. Arun Rajamohan, who highlighted insect cryopreservation activities at the USDA's Edward T. Schafer Agricultural Research Center (ETSARC) and then moderated the session on the cryopreservation of emerging model organisms with Dr. Mahua Mukhopadhyay.

Keynote Presentation: Insect Germplasm Cryopreservation—To Automate or Not to Automate, That Is the Question

Arun Rajamohan, Ph.D., USDA

Dr. Rajamohan presented on the role of automation in the cryopreservation of insect germplasm. Germplasm refers to organ systems involved in germ cell production and their cellular products: testes, semen, ovaries, and eggs. Cryopreservation of insect germplasm is becoming critical as species are threatened by habitat degradation and loss, intensive farming practices, pesticides, diseases, and the effects of climate change. Cryopreservation also is used by facilities that maintain insect populations for sterile insect technique programs, perform research, and aim to preserve pollinator diversity and important genotypes and phenotypes. For example, honeybee strains have been bred to be cold-resistant and exhibit traits that improve resistance to infection with the *Varroa destructor* mite.

ETSARC researchers have successfully extracted sperm cells from the seminal vesicles of bumblebees and the seminal duplex of monarch butterflies and cryopreserved the samples. A whole mosquito testis also has been cryopreserved, and the sperm have been revived. Reliable insect embryo cryopreservation protocols have been reported in approximately 15 insect species, and more than half of these protocols have been developed at ETSARC. A typical vitrification procedure involves removal of the chorion, permeabilization, and CPA loading before dehydration and freezing on a polycarbonate filter membrane. Protocol templates for different cell types are customized for different species, which exhibit variable permeability and sensitivity to CPA toxicity. Samples that cannot be permeabilized are stored using alternative approaches (e.g., cold storage). The optimal developmental stage for cryopreservation must be determined because the insect cuticle—which develops as the larva grows—inhibits permeabilization. Obtaining samples from most insect species involves careful dissection and remains a challenge.

Portable, reliable, and user-friendly automation for cryopreservation would benefit the field. A system for automated embryo permeabilization and loading has been developed and deployed at Comisión Panamá Estados Unidos para la Erradicación y Prevención del Gusano Barrenador del Ganado—a program to eradicate screwworm flies—in Pacora, Panama, and the International Atomic Energy Agency (IAEA) laboratory in Seibersdorf, Austria. The IAEA has reported better results with robotic processing than with manual processing. In Panama, the automated protocol has been used to freeze more than 1 million embryos. The cryopreserved embryos have been used to reinvigorate colonies of factory flies and screwworms. Currently, only the preprocessing steps of cryopreservation are automated. Thawing is an important component of the procedure and has a strong effect on embryonic viability. Automated thawing procedures are being developed in various fields.

Discussion

- A meeting participant asked about the volume of cryostraws used at ETSARC and how they are labeled. Dr. Rajamohan explained that embryonic samples are preserved on polycarbonate membranes. Spermatozoa are frozen in 250-µl straws, which are stored in labeled cryovials.

- In response to a question about disseminating the automated cryopreservation technology, Dr. Rajamohan noted that the robot prototype and protocol have not yet been published. He added that the USDA develops open-source technology as part of its strategic plan.

- Dr. Bischof asked Dr. Rajamohan to comment in further detail on barriers to the cryopreservation of honeybee embryos. Dr. Rajamohan emphasized that honeybee embryos naturally lack extensive protection from dehydration because they are well-protected in the milieu of the beehive (32°–35°C and 50% humidity). Water removal and CPA loading can be achieved easily, but the embryos are sensitive to physical pressure and can collapse during handling. Microfluidic technology is a possible approach for handling these sensitive embryos.

Lessons Learned from Tardigrades for Long-Term Preservation of Invertebrates
Jonathan Hibshman, Ph.D., Southern Methodist University

Dr. Jonathan Hibshman reviewed strategies used by tardigrades to survive extreme environments. Tardigrades can survive extreme conditions, including desiccation, exposure to space in low-Earth orbit, dosing with ionizing radiation, and freezing. Researchers are interested in leveraging biochemical strategies for surviving desiccation for use in long-term storage modalities, transporting sensitive reagents without refrigeration, and mitigating damage due to cold-chain failures. Desiccation stresses the animal, leading to cellular damage, including DNA damage and protein aggregation. Identifying tardigrade protectants that contribute to cellular stability and survival is of general interest. Some examples of these discoveries include abundant heat-soluble proteins (e.g., cytoplasmic abundant heat-soluble proteins, mitochondrial abundant heat-soluble proteins, and secreted abundant heat-soluble proteins), the DNA-binding damage suppressor (or Dsup) protein, and other transcriptional responses to protect against the stress associated with dehydration.

Dr. Hibshman shared a new strategy to identify protectants. First, a cDNA library of tardigrade genes was expressed in bacteria, which were subsequently desiccated. Plasmids from bacteria that survived desiccation were sequenced to reveal genes that improved desiccation tolerance. A mitochondrial single-stranded DNA-binding (mtSSB) gene was one of the most enriched when the experiment was performed using RNA extracted from two different tardigrade species. Notably, mtSSBs bind to single-stranded DNA. Evidence suggests that DNA binding is essential for mtSSBs to promote bacterial desiccation tolerance. Small heat shock proteins (sHSPs) are known to prevent protein aggregation, a cellular stress induced by desiccation. The genome of the tardigrade *Hypsibius exemplaris* encodes nine sHSPs, several of which can improve desiccation tolerance when expressed in bacteria. Tardigrade sHSPs can also limit desiccation-induced protein aggregation and loss of enzyme activity in an *in vitro* assay. Future studies of various aspects of desiccation tolerance (e.g., water replacement, glass transition, molecular shielding, metabolic restructuring) will provide a more comprehensive understanding of how tardigrades survive extreme conditions and will reveal mechanisms that can be leveraged for other applications, including the long-term storage of biological materials.

Discussion

- Dr. Bischof asked Dr. Hibshman to comment on the differences between naturally occurring CPAs and the standard CPAs used for cryopreservation. Dr. Hibshman remarked that trehalose is a great example of a CPA that is highly effective and often endogenously upregulated in the

context of desiccation. He noted the likelihood of overlap between CPAs and agents that are protective during desiccation; both processes involve vitrification and management of cellular water content. Naturally occurring and synthetic protective agents are two approaches to achieving the same goal.

- Dr. Paredes asked about the cryoprotective role of bovine serum albumin (BSA), which was used as a control in the protein aggregation assay. Dr. Hibshman commented that BSA is an excipient that stabilizes proteins and is protective during desiccation. He explained that he is less familiar with the role of BSA as a cryoprotectant.

Long-Term Preservation of Mosquitoes
Rebecca D. Sandlin, Ph.D., Massachusetts General Hospital and Harvard Medical School

Dr. Rebecca D. Sandlin discussed the development of vitrification methods for preserving *Anopheles* mosquitoes. Vector-controlled methods to address diseases carried by mosquitoes use genetically modified, sterile mosquito strains to compete with endemic mosquito populations. Without preservation techniques, continuous maintenance of mosquito stocks is necessary. Continuous laboratory rearing involves an increased risk of cross-contamination and genetic drift and the possible catastrophic loss of mosquito colonies. Mosquito cryopreservation is associated with several challenges, including low or no CPA permeabilization through the exoskeleton, the inability to characterize CPA uptake using standard methods, and the formation of ice crystals during cooling and upon rewarming.

Mosquitoes are cryopreserved via vitrification during the L1 larval stage. Samples are cooled quickly through the glass transition to prevent ice formation. To address low CPA diffusion across the exoskeleton, CPAs were spiked directly into the rearing solution for uptake through the midgut. However, mosquitoes ceased feeding after prolonged exposure to CPAs and in response to cold. To address CPA-uptake kinetics, low-toxicity CPA solutions were developed based on the CPA median lethal dose (LD_{50}), and CPA cocktails were assessed for their ability to reduce toxicity during vitrification. Raman microspectroscopy was used to estimate CPA diffusion. Estimated CPA distribution was able to explain patterns of ice crystallization in certain samples: inadequate CPA equilibration leads to suboptimal CPA concentrations in certain areas of the sample, leading to ice crystallization in those areas upon cooling. Current approaches to mosquito vitrification involve increased CPA loading using an external osmotic gradient followed by desiccation. Larvae are thawed in water to remove the CPA and reared in a recovery solution. Under these conditions, reanimation is observed in the form of twitching, full mobility, and/or feeding. However, survival to adulthood following vitrification remains a challenge.

Discussion

- Dr. Bischof commented on similar challenges experienced during attempts to cryopreserve shrimp. Shrimp larvae survive vitrification but do not develop into the L2 stage. He wondered whether the two animals shared a common factor. Dr. Sandlin noted that CPA controls during the procedure demonstrate that CPA treatment alone is insufficient to prevent pupation in mosquito larvae. Ice crystal formation during cooling or rewarming likely causes a small amount of damage that is not immediately lethal but prevents development into adulthood.

- In response to a question about techniques to assess CPA diffusion, Dr. Sandlin remarked that, after optimization, Raman spectroscopy would be a reliable approach. Using the technique on whole organisms remains challenging.

Long-Term Maintenance of Black Soldier Flies (Diapause)
Jeffery Tomberlin, Ph.D., Texas A&M University

Dr. Jeffery Tomberlin presented on the long-term maintenance of the larval black soldier flies, *Hermetia illucens*. Sustainable agriculture practices are becoming increasingly important as the world population increases. *H. illucens* is endemic to the American South and Central and South America. The black soldier fly historically was viewed as a pest but increasingly is being recognized for its ability to recycle organic waste and serve as an ingredient in animal feed. Black soldier flies eat animal feed, fish, fruits and vegetables, kitchen waste, organ meat, and manure. Facilities currently use black soldier flies to digest 100 tons of kitchen waste—which makes up almost half of U.S. food waste—each day into frass, a material that can be used as fertilizer. During digestion, black soldier flies kill *Escherichia coli* and *Salmonella*, removing these pathogens from a given ecosystem. The flies also digest antibiotics, mycotoxins, and antibiotic-resistant bacteria. Enhanced feeding and bioconversion rates can be achieved via bacterial supplementation. The fly also can be used as feed for aquaculture, poultry, swine, and domestic pets. Industry and government institutions are showing increasing interest in advancing black soldier fly agriculture.

Long-term storage of the black soldier fly is achieved by incubating first-instar larvae under the correct conditions (e.g., density and feed amount) to convert them into a hibernating state. Hibernating larvae can be stored at room temperature for 6 months. Flies are recovered by adding food, and they complete larval feeding in 7 days. Future studies will explore how to extend the storage life of this important insect.

Discussion

- When asked about omics analyses of the fly, Dr. Tomberlin responded that the black soldier fly's genome and microbiome have been studied carefully. Gene editing approaches currently are being used to potentially enhance the fly's ability to recycle waste.

- Dr. Bischof asked how industrialization is affecting the genetic background of the fly. Dr. Tomberlin noted concerns in the community about a pathogen outbreak potentially devastating the global fly population. He directed Dr. Bischof to research by Dr. Chris Jiggins at the University of Cambridge that assessed black soldier fly genomics and used artificial selection to drive trait improvement in the species. In response to a follow-up question from Dr. Bischof, Dr. Tomberlin shared that an Israeli company has developed a cryopreservation method that, unlike diapause, would preserve the flies' genomes during long-term storage.

- Dr. Iddi suggested a collaboration with the Institute of Material Science at Leibniz Universität Hannover, and Dr. Tomberlin requested relevant contact information.

Lightning Round Presentations by NIH-Supported Grantees
Moderators: Arun Rajamohan, Ph.D., USDA, and Mahua Mukhopadhyay, Ph.D., NICHD, NIH

Dr. Rajamohan introduced Drs. Daryl Gohl and Teets, who presented lighting talks on NIH-supported cryopreservation research.

Development of a Novel Method for Cryopreservation of **Drosophila melanogaster**
Daryl Gohl, Ph.D., University of Minnesota

Previous studies have shown that primordial germ cells can be isolated from flies and injected into an agametic background to regenerate the original stock. A related approach is being developed for long-term storage of *Drosophila* involving the cryopreservation of embryonic nuclei and regeneration of stocks via embryonic nuclear transportation (ENT). Osmolyte solutions for nuclear cryopreservation have been

optimized, and Raman spectroscopy has shown that CPAs sequester frozen nuclei from areas of ice crystal formation. Previous studies indicated that *Drosophila* clones can be generated by injecting nuclei into embryos derived from a male-sterile mutant stock with defects in the earliest stages of embryonic development. In current attempts to generate clones from injected nuclei, a small number of embryos expressing donor markers from cryopreserved nuclei are obtained but do not yet survive to adulthood. Alternative approaches, including experimenting with other genetic backgrounds, are being explored, and machine learning is being used to automate microinjection of *Drosophila* embryos.

Discussion

- In response to a request from Dr. Bischof for more detail regarding strain recovery after ENT of cryopreserved embryos, Dr. Gohl explained that fertile adults have not yet been recovered. So far, a very small percentage of embryos develop to early larval stages.

***Development of Long-Term Preservation and Revival Protocols for* Drosophila**
Nicholas Teets, Ph.D., University of Kentucky

Long-term preservation and revival protocols for *Drosophila* that address current challenges are being developed. The impermeability of fly egg membranes can be addressed using dietary loading with proline and trehalose or sonoporation, a method using ultrasonic frequencies in conjunction with lipid microbubbles to generate small pores in cells and drive uptake of solutes. Osmotic stress during desiccation can be addressed via exogenous expression of late embryogenesis abundant (or LEA) proteins from desiccation-tolerant organisms.

Discussion

- Dr. Rajamohan asked about the stage of the embryos used in the study. Dr. Teets responded that early-stage embryos were used to maximize permeability before cellularization. The reagents being studied, however, are capable of being loaded into more mature embryos.

- Dr. Sandlin asked whether sonoporation disrupts the yolk. Dr. Teets noted that *Drosophila* embryos appear to be highly resistant to damage associated with sonoporation.

Group Discussion and Summary
Moderators: Joseph Rinehart, Ph.D., USDA

Dr. Rinehart opened the discussion and requested that the meeting participants consider the objectives of the meeting when presenting their comments.

- Dr. Tomberlin asked about the evolutionary history of traits associated with survival under extreme conditions that, for example, are associated with tardigrades. Dr. Hibshman noted that these traits appear to have evolved or been lost several independent times. The basic machinery for surviving desiccation is present in many organisms, and some seem to have been able to capitalize on the presence of these pathways. He highlighted the association between desiccation tolerance and parthenogenic or hermaphroditic reproduction in many species, which likely is connected to the ability to spread to new ecological niches and populate them upon rehydration. In response to a follow-up question from Dr. Tomberlin, Dr. Hibshman noted that little is known about how the microbiome changes during rounds of desiccation; in general, the tardigrade microbiome is understudied. Dr. Teets remarked that the African sleeping midge, *Polypedilum vanderplanki*, is the only known insect to incorporate genes for desiccation tolerance via horizontal gene transfer from bacteria.

- Dr. Hibshman asked Dr. Fang-Yen whether attempts had been made to extend the survival of desiccated *C. elegans* at room temperature beyond several days. Dr. Fang-Yen explained that his group has not yet attempted these experiments. He speculated that the protocol currently involves 48 hours of drying at room temperature; speeding up the drying process (e.g., with a vacuum desiccator) or performing the procedure at a colder temperature might improve the viability of the desiccated worms.

- Dr. Paredes commented that different model organisms face similar challenges related to cryopreservation. She noted that a cross-species approach to CPA toxicity and dehydration stresses might be a more effective way to address these gaps. Dr. Bischof noted recent efforts at the ERC to address the biophysics of CPAs during vitrification and high-throughput capabilities being developed by Dr. Sandlin's group that could be applied to studying biological stresses. Dr. Sandlin described her group's approach to rapidly assessing CPA toxicity, which involves determining the compound's LD_{50} and using these values to understand toxicity thresholds when developing CPA cocktails. Such heuristics as differential evolution and Taguchi methods also can be incorporated into the experimental design.

- In response to a query from Dr. Kevin Cook, Dr. Bischof noted that the ERC has training programs to disseminate the *Drosophila* cryopreservation protocol and plans to expand these training activities to encompass protocols for additional species.

- Dr. Rinehart commented on the difficulty of disseminating new protocols throughout the wider scientific community. Robust protocols are not adopted, and even when they are, challenges like high staff turnover prevent the knowledge from being established in new laboratories. He noted that robots and automation are possible solutions to this issue. Dr. Paredes added that training is critical to disseminating standardized protocols. In-person workshops are the best option, but educational videos also can be helpful. Published protocols cannot necessarily be easily understood and applied without additional training.

- Dr. Zou remarked that NIH has several mechanisms to support training efforts, including T32 and T35 training awards and funds embedded in larger awards (e.g., P40 grants) and R24 awards that can support training and educational activities.

- Dr. Gohl asked whether approaches to develop cryopreserved stocks that can be resampled over time would address gaps and challenges discussed during the presentations. Dr. Bischof noted that the suggested approach could be tailored to species that are frozen on cryomesh or require larger volumes to re-establish a line. He remarked that it might be challenging with stocks that are difficult to recover after cryopreservation.

- Dr. Jack Koch asked how people develop genetic resource protection pathways based on community needs. Dr. Rinehart responded that as a federal institution, the USDA receives and responds to input from the wider community about its needs.

- Dr. Terrence Tiersch asked about the appropriate point in a research project to consider scalability for application within a germplasm repository. Dr. Rajamohan noted his group standardizes protocols before considering scalability.

- Dr. Cook and Dr. Aric Daul discussed the issue of embryo collection in mutants with low viability or egg production. This issue might be addressed through specialized protocols and applications. Drs. Cook and Rajamohan agreed that strains with low productivity are a bottleneck for many species. Dr. Rajamohan noted that only 14 of 21 strains of screwworms

tested by his group have been cryopreserved successfully. Painstaking labor to hand-select healthy embryos can overcome this challenge; approaches combining large-particle sorting, automation, image analysis, or machine learning might be beneficial for overcoming this barrier.

- Dr. Rajamohan asked about approaches to secure stored samples. Dr. Daul answered that CGC stocks are stored at −80°C and in liquid nitrogen in multiple locations. Large dewars that hold 42,000 vials are used; the larger volume of liquid nitrogen takes longer to evaporate to a critical level. Equipment is monitored electronically by building resources, and liquid carbon dioxide backups are installed in all ultra-low freezers. Sufficient chest freezer space is available to accommodate the contents of an ultra-low freezer in the event of a failure. Other participants agreed that alarms, automated systems, designated locations for valuable samples, and multiple backups were beneficial for securing stored samples.

- Dr. Rinehart asked whether it was worthwhile to investigate diapause states or fluctuating thermal regimes as strategies for short- or long-term sample storage. Drs. Hibshman and Teets agreed that these strategies might be simple and cost-effective, especially in species with a naturally occurring diapause state. Upper limits for this type of storage, as well as the associated tissue damage, should be investigated.

- Dr. Daul explained that research groups within the *C. elegans* community store samples in their own collections, which are too large to be stored within the CGC when the laboratory is retired. Sample curation and storage space increasingly have become an issue. Dr. Whitworth emphasized that sample curation and quality control are critical for scientific rigor and reproducibility.

Dr. Rinehart briefly summarized the discussion, which included such topics as training activities, protocol development, challenging strains and organisms, and curation and quality control. Dr. Zou thanked the moderators, speakers, and participants for their engagement and adjourned the meeting.

Appendix A: Meeting Agenda

Session I. Cryopreservation and Other Preservation of Invertebrate Models in Biomedical Research

Virtual Meeting
September 6, 2024

10:00–10:10 a.m. **Opening Remarks**
Joseph Rinehart, Ph.D., USDA
Franziska Grieder, D.V.M., Ph.D., Director, ORIP, DPCPSI, OD, NIH
Sige Zou, Ph.D., Workshop Coordinator, Program Official, ORIP, DPCPSI, OD, NIH

10:10 a.m.–12:10 p.m. **Established Models**

Keynote Presentation: Cryopreservation of *Drosophila* Embryos
John Bischof, Ph.D., University of Minnesota

Cryopreservation of *C. elegans* Stocks
Ann Rougvie, Ph.D., University of Minnesota

Preservation of *C. elegans* by Desiccation
Christopher Fang-Yen, Ph.D., The Ohio State University

Long-Term Maintenance of *Drosophila* Stocks by Live Cultures
Cale Whitworth, Ph.D., Indiana University

Cryopreservation of Sea Urchin
Estefania Paredes, Ph.D., Vigo University, Spain

12:10–1:00 p.m. **Lunch Break**

1:00–3:00 p.m. **Emerging Models**

Keynote Presentation: Insect Germplasm Cryopreservation—To Automate or Not to Automate, That Is the Question
Arun Rajamohan, Ph.D., USDA

Lessons Learned from Tardigrades for Long-Term Preservation of Invertebrates
Jonathan Hibshman, Ph.D., Southern Methodist University

Long-Term Preservation of Mosquitoes
Rebecca D. Sandlin, Ph.D., Massachusetts General Hospital and Harvard Medical School

Long-Term Preservation of Black Soldier Flies (Diapause)
Jeffery Tomberlin, Ph.D., Texas A&M University

Lightning Round Presentations by NIH-Supported Grantees

Development of a Novel Method for Cryopreservation of *Drosophila melanogaster*
Daryl Gohl, Ph.D., University of Minnesota

Development of Long-Term Preservation and Revival Protocols for *Drosophila*
Nicholas Teets, Ph.D., University of Kentucky

3:00–4:00 p.m. **Group Discussion and Summary**

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

Appendix B: Speaker Biographies (In Order of Appearance)

Session I. Cryopreservation and Other Preservation of Invertebrate Models in Biomedical Research

Virtual Meeting
September 6, 2024

Dr. Joseph Rinehart is a Research Leader for insect studies for the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) in Fargo, North Dakota, where his team focuses on the cold physiology of a variety of insects, including the cryopreservation of agriculturally and ecologically important species. He has more than 25 years of experience in insect cold physiology. His research has ranged from studies on the stress physiology of pollinators and on the molecular characterization of diapause in flesh flies and mosquitoes to field studies on the stress physiology of an Antarctic midge (for which he was awarded the United States Antarctic Service Medal). Dr. Rinehart also is an adjunct faculty member at North Dakota State University. He enjoys hosting numerous undergraduate and graduate students in his lab, including participants of the annual summer Research Experience for Undergraduates program.

Dr. John Bischof obtained a B.S. in bioengineering from the University of California, Berkeley (UCB); an M.S. in biomedical engineering from UCB and the University of California, San Francisco; and a Ph.D. in mechanical engineering from UCB. After a postdoctoral fellowship at Harvard's Center for Engineering in Medicine & Surgery, he joined the faculty of the University of Minnesota (UMN) in 1993. Dr. Bischof is now a Distinguished McKnight University Professor, the Medtronic-Bakken Endowed Chair for Engineering in Medicine, Director of the Institute for Engineering in Medicine at UMN, and Director of the National Science Foundation (NSF) Engineering Research Center (ERC) for Advanced Technologies for the Preservation of Biological Systems (ATP-Bio). Dr. Bischof works in the area of thermal bioengineering with a focus on cryopreservation, thermal therapy, and nanomedicine. In 2019, he became the Director of UMN's Institute for Engineering in Medicine (IEM). One of the centers that IEM helped launch is Bischof's own $26-million, 5-year renewable NSF ERC for ATP-Bio, which he directs in the cryopreservation space.

Dr. Ann Rougvie obtained a B.S. in biochemistry from Iowa State University. She earned her doctorate from Cornell University, where she worked with Dr. John Lis and discovered that the promoter-proximal RNA polymerase II present on uninduced *Drosophila* heat shock genes was transcriptionally engaged but stalled, revealing a post-initiation, rate-limiting step in gene expression. Following the completion of her degree, Dr. Rougvie began studying developmental timing in the nematode *Caenorhabditis elegans* as a Postdoctoral Fellow working with Dr. Victor Ambros at Harvard University. She continued those studies after establishing her own laboratory at UMN in 1992 and deciphered the mechanisms that time developmental events in animals with high precision while maintaining the flexibility to deal with variable environmental conditions. Dr. Rougvie is in her 17th year as Director of the NIH-funded *Caenorhabditis* Genetics Center (CGC), the international repository and distribution center for *C. elegans* strains. Her tenure has seen a nearly sevenfold increase in usage, and the CGC now ships approximately 30,000 samples per year to support scientists worldwide.

Dr. Christopher Fang-Yen completed a B.S. in physics and mathematics at Stanford University and a Ph.D. in physics at the Massachusetts Institute of Technology (MIT). Following postdoctoral appointments at MIT and Harvard University, he joined the faculty of the University of Pennsylvania as an Assistant Professor and then as an Associate Professor in the Department of Bioengineering and the Department of Neuroscience. Since 2023, Dr. Fang-Yen has been a Professor of Biomedical Engineering

at The Ohio State University. He and his research group develop innovative technologies and apply them to address questions in biology, using the roundworm *C. elegans* as a model.

Dr. Cale Whitworth is Co-director of the Bloomington *Drosophila* Stock Center (BDSC), which is internationally recognized as the primary source of *Drosophila melanogaster* strains used for research. He obtained his doctorate in cell, molecular, and developmental biology from Johns Hopkins University, where he examined mechanisms of human disorders of sexual development using *D. melanogaster* as a model system. He continued his work on sexual development as a Postdoctoral Fellow in the National Institute of Diabetes and Digestive and Kidney Diseases within NIH. As Co-director of the BDSC, Dr. Whitworth is a Principal Investigator (PI) on grant P40OD018537, which is funded by the Office of Research Infrastructure Programs, the National Institute of Neurological Disorders and Stroke, and National Institute of General Medical Sciences. He is involved in all aspects of BDSC administration, including operations, finance, advocacy, research activities, and, most importantly, ensuring the collection meets the ever-changing needs of the research community.

Dr. Estefania Paredes holds a bachelor's degree and a doctoral degree in oceanography from the University of Vigo in Spain, where she is a Ramón y Cajal Professor. She is the President of the Spanish Society of Cryobiology and Adjunct Professor at the United Nations Educational, Scientific, and Cultural Organization Chair of Cryobiology based in Ukraine. She is an elected member of the Board of Governors of the Society for Cryobiology and the Board of the Association of Galician Oceanographers. Dr. Paredes' research focuses on marine cryobiology and developing protocols for the preservation of marine organisms and cells with applications for model organisms' conservation, biobanking, aquaculture, and biodiversity conservation.

Dr. Arun Rajamohan is a Research Entomologist and the Lead Scientist for the Weed and Insect Biology Research Unit at the USDA ARS in Fargo, North Dakota. He has a Master of Philosophy and a doctorate in zoology from the University of Madras in Chennai, India. For his doctoral research, he studied the cryopreservation of marine shrimp larvae. Over the past 20 years, Dr. Rajamohan has concentrated his research on the preservation of insect embryos and germplasm. As the Cryobiologist at the Fargo USDA ARS unit, he is responsible for all cryopreservation efforts. More specifically, he develops original cryopreservation storage technologies for such pollinators as honeybees and bumble bees and such pest species as tephritid fruit flies and the New World screwworm, as well as other insects of importance, such as the monarch butterfly and the endangered Sacramento Mountains checkerspot butterfly. Dr. Rajamohan also develops quality control methods for cryopreserved and cold-stored insects to ensure physiologically normal post-storage development of the embryos to reproducing adults.

Dr. Jon Hibshman is an Assistant Professor in the Department of Biological Sciences at Southern Methodist University. He graduated from Gettysburg College with a double major in philosophy and biochemistry and molecular biology. He then obtained his doctorate in genetics and genomics at Duke University, working to better understand both immediate and intergenerational responses to starvation and dietary restriction in *C. elegans*. As an NIH F32-funded Postdoctoral Fellow at The University of North Carolina at Chapel Hill, he developed a research program that seeks to reveal mechanisms of extreme stress tolerance, with a particular focus on desiccation. His laboratory at Southern Methodist University uses a panel of model organisms—including tardigrades, *C. elegans*, and bacteria—and implements biochemical, genetic, cell biological, and molecular methods to discover new protectants and decipher the mechanisms by which they function to promote survival of extremes.

Dr. Rebecca D. Sandlin is an Assistant Professor at the Center for Engineering in Medicine & Surgery at Harvard Medical School and Massachusetts General Hospital. She received a doctorate in chemistry from Vanderbilt University, where she studied biocrystallization, malaria parasite biology, and high-throughput screening. During her postdoctoral training, she transitioned to the field of cryobiology and developed techniques to preserve whole blood and circulating tumor cells for clinical and research applications. As a

faculty member, Dr. Sandlin's laboratory focuses on the development of biostabilization methods for a range of specimens, including parasites, human primary cells, and whole organisms. To overcome obstacles associated with the preservation of delicate specimens, her laboratory has developed approaches to efficient experimental design for screening cryoprotective agents and specialized specimen containers to achieve rapid heat transfer.

Dr. Jeffery Tomberlin is a Professor, AgriLife Research Fellow, and Presidential Impact Fellow in the Department of Entomology at Texas A&M University and a Fellow of the Entomological Society of America. He is the PI of the Forensic Laboratory for Investigative Entomological Sciences Facility at Texas A&M University, and he is the Director of the NSF Center for Environmental Sustainability through Insect Farming. Dr. Tomberlin's research efforts for the past 27 years have involved developing methods to produce alternate protein sources for use as livestock, poultry, and aquaculture feed from these resources. These efforts have been accomplished predominantly through his research with the black soldier fly. To date, he has edited eight books, published 28 book chapters, and published more than 260 research articles. Dr. Tomberlin has worked with companies around the world, including, but not limited to, Malaysia, China, and Australia.

Dr. Daryl Gohl leads the UMN Genomics Center's Innovation Lab and is a Research Assistant Professor in the UMN Department of Genetics, Cell Biology, and Development. He received his doctorate in molecular biology from Princeton University and completed postdoctoral training in the Department of Neurobiology at Stanford University. Dr. Gohl's work has focused on developing new tools for genetic manipulation of *Drosophila* and other organisms and on developing new techniques for genomics-based measurements. In addition to his academic work, he is a Co-founder of two biotechnology companies, CoreBiome Inc. and Objective Biotechnology.

Dr. Nicholas Teets is an Associate Professor in the Department of Entomology at the University of Kentucky. His research primarily focuses on the mechanisms by which insects survive extreme environmental conditions. This fundamental research includes comparative physiology and the genomics of Antarctic insects, the evolutionary genetics of thermal tolerance, and the molecular mechanisms of overwintering dormancy. More recently, the laboratory began exploring applications of this work, including applying lessons from nature to improve insect cryopreservation. The laboratory has received funding for this work from NIH, NSF, and USDA. Dr. Teets also holds a teaching appointment and teaches a general entomology course and an advanced molecular genetics course. His laboratory is active in K–12 outreach, including designing and leading a week-long genetics "bootcamp" for local high school students.

Appendix C: Participants

Session I. Cryopreservation and Other Preservation of Invertebrate Models in Biomedical Research

Virtual Meeting
September 6, 2024

Jessica Bell, Stowers Institute for Medical Research
Thomas Bell, National Disease Research Interchange
Abby Bernardini, University of Oklahoma Health Sciences Center
John Bischof, University of Minnesota
Haddy Bittaye, Max Planck Institute
Jorge Blanco Mendana, University of Minnesota
Jeri Brandom, The University of Alabama at Birmingham
Tobias Braun, University of Veterinary Medicine Hannover
John Buchanan, Center for Aquaculture Technologies
Bettina Buhring, ORIP, DPCPSI, OD, NIH
Melanie Burns, University of Minnesota
Isabel Campos, Champalimaud Foundation
Juganaru Carmen, Ghent University
Samantha Carrillo Rosas, Instituto Tecnológico y de Estudios Superiores de Monterrey
Jasper Chan, University of Hong Kong
Susan Chandran, ORIP, DPCPSI, OD, NIH
Brooke Chang, University of California, Berkeley
Rajan Chaudhary, Agroforestry Polytechnic Institute
Ken Chen, Albert Einstein College of Medicine
Yong Chen, ORIP, DPCPSI, OD, NIH
Chi Kei Chow, University of Hong Kong
Jonathan Clayton, University of Nebraska
Ana Clementin, PanTHERA CryoSolutions
Autumn Cole, University of California, Davis
Miguel Contreras, ORIP, DPCPSI, OD, NIH
Kevin Cook, Indiana University
Rachel Cox, Uniformed Services University of the Health Sciences
Aric Daul, University of Minnesota
Céline Daviaud, Institut Pasteur
Korie DeBardlabon, USDA
Daina Domahidi, University of Toronto
Veronica Dominguez, Centro de Biología Molecular Severo Ochoa
Sheri Dorsam, USDA
Erin Ducharme, University of New England
Michael Durnin, Stowers Institute for Medical Research
Charles Elder, University of Louisville
Bradley Ellis, Massachusetts General Hospital
Christopher Fang-Yen, The Ohio State University
Al Feinberg, Rutgers, the State University of New Jersey
Madison Floden, North Dakota State University
Thales Franca, Universitat Politecnica de Valencia
Madison Francoeur, Brown University
Martin Fray, The Mary Lyon Centre at Medical Research Council Harwell

Sabrina Gacem, Valencia University
Lakshya Gangwar, University of Minnesota
Alexia Giannakopoulou, Heidelberg University
Victoria Gibbs, The University of Alabama at Birmingham
Victoria Gilbert, University of California, Davis
Birgit Glasmacher, Leibniz Universität Hannover
Daryl Gohl, University of Minnesota
Magdalena Góra, International Institute of Molecular and Cell Biology
Linda Gower, Vanderbilt University
Franziska Grieder, ORIP, DPCPSI, OD, NIH
Rodgee Mae Guden, Ghent University
Audra Guikema, Van Andel Research Institute
Maria Teresa Gutierrez-Wing, Louisiana State University
Jenna Hakkesteeg, University College London
David Hall, Albert Einstein School of Medicine
Richard Hall, Stowers Institute for Medical Research
Zonghu Han, University of Minnesota
Dorit Hanein, University of California, Santa Barbara
Tom Hays, University of Minnesota
Mike Heinrich, University of Minnesota
Nikki Hernandez, Baylor College of Medicine
Jonathan Hibshman, Southern Methodist University
María Jesús del Hierro, Centro Nacional de Biotecnología
Veronica Hinman, Carnegie Mellon University
Juliette Horwood, The Francis Crick Institute
Allison Hubel, University of Minnesota
Oleksandra Hubenia, Leibniz Universität Hannover
Abdul-Rashid Iddi, Leibniz Universität Hannover
Mayumi Isaka, Regeneron Pharmaceuticals
Nicholas Jean, University of California, Berkeley
Katie Johnson, Boise State University
Purva Joshi, Massachusetts General Hospital and Harvard Medical School
Thomas Kaufman, Indiana University
Mehakpreet Kaur, North Dakota State University
Natasha Kiel, Stowers Institute for Medical Research
Jeffrey Kim, University of Louisville
Yongdeok Kim, University of California, Berkeley
Jack Koch, Louisiana State University
Xiangbo Kong, University of Michigan
Malgorzata Korzeniowska, Instytut Medycyny Doświadczalnej i Klinicznej
Elizabeth Kotus, University of Michigan
Peter Koulen, University of Missouri-Kansas City
Kathy Krentz, University of Wisconsin
Katrina Kulesh, Brown University
Maureen Lamb, BDSC, Indiana University
DoYoung Lim, Mayo Clinic
Jhony Lisboa Benato, Universidade Federal do Rio Grande do Sul
Leo Lou, University of California, Berkeley
Evyn Loucks, University of Oregon
Troy Louwagie, University of Minnesota Twin Cities
Andreia Joana Miguel Madalena, Institute of Science and Technology Austria

Saumya Mathew, Baylor College of Medicine
Stephanie Mauthner, BDSC, Indiana University
Maura McGrail, Iowa State University
Rebecca Mercier, PanTHERA CryoSolutions
Shane Miller, Stowers Institute
Oleg Mirochnitchenko, ORIP, DPCPSI, OD, NIH
Anna Morgunowicz, PORT Polish Centre for Technology Development
Mahua Mukhopadhyay, NICHD, NIH
Mckenzie Mungai, Eckerd College
Amanda Neisch, University of Minnesota
Bruce Newell, Deakin University
Mylinh Nguyen, The University of Texas Southwestern Medical Center
Rada Norinsky, The Rockefeller University
Lindsey O'Brien, University of Minnesota
Harriette Oldenhof, University of Veterinary Medicine Hannover
Armedia O'Neill-Blair, University of Missouri
Nathalie Oulhen, Brown University
Ilkka Paatero, University of Turku
Estafania Paredes, Vigo University
Annette Parks, BDSC, Indiana University
David Pasnik, USDA
Leon Peshkin, Harvard University
Cornelia Peterson, Tufts University
Michael Pettigrew, Archive Sciences, Inc.
Sukumal Prukudom, Kasetsart University
Paulina Pyrek, Norwegian University of Life Sciences
Hamid Ur Rahman, Hazara University Mansehra
Arun Rajamohan, USDA
Laurel Rawls, University of California, Davis
Morgan Reade, Louisiana State University
Hannah Reich, State University of New York College of Environmental Science and Forestry
Gerardo Reyes, Brown University
Joseph Rinehart, USDA
Samson Rokkarukala, National Institute of Oceanography, Goa, India
Isabel Clara Rollan Delgado, European Molecular Biology Laboratory
Corinna Ross, Texas Biomedical Research Institution
Ann Rougvie, University of Minnesota
Niloofar Sadeghi, Texas Biomedical Research Institute
Susan Sanchez, The University of Georgia
Rebecca Sandlin, Massachusetts General Hospital and Harvard Medical School
Julio Aurelio Sarabia Alonso, University of California, Riverside
Dora Schade, Technische Universität Dresden
Michèle Schaffner, Eidgenössische Technische Hochschule Phenomics Center
Manfred Schartl, Texas State University
Jacqueline Schlamp, Northwestern State University
Michael Schmale, University of Miami
Christine Schnitzler, University of Florida
Soaleha Shams, Mayo Clinic
Nataliia Shapovalova, University of Zurich
Karen Siu, University of Hong Kong
Hannah Skaggs, University of Louisville

Stephanie Slater, Seattle Children's Research Institute
Tina St Laurent, Van Andel Institute
Joyce Stuckey, Rutgers, the State University of New Jersey
Yongjun Sui, National Cancer Institute, NIH
Tsung-Chang Sung, The Salk Institute
Robert Taft, The Jackson Laboratory
Toru Takeo, Kumamoto University
Akiko Takizawa, Medical College of Wisconsin
Ritesh Tandon, ORIP, DPCPSI, OD, NIH
Robyn Tanny, Johns Hopkins University
Nicholas Teets, University of Kentucky
Nathalia Teixeira, Universidade Federal do Rio Grande do Sul
Riley Thompson, Colorado State University
Biao Tian, ORIP, DPCPSI, OD, NIH
Terrence Tiersch, Louisiana State University
Melanie Tischleder, North Dakota State University
Jeffery Tomberlin, Texas A&M University
Juliana Torriani Maciel, Stiftung Tierärztlich Hochschule Hannover
Reiko Toyama, NICHD, NIH
Sy Traore, Medical University of South Carolina
Simon Tröder, University of Cologne
Walter Tsark, City of Hope
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