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ARTICLE

On-Site Capabilities of a Mobile Laboratory for Aquatic Germplasm Cryopreservation

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Abstract

The cryopreservation of genetic material can be an important tool for researchers and others involved with imperiled fishes, wild fisheries, aquaculture, and biomedical research. The standardization and reliable collection of diverse, high-quality samples are persistent challenges to the successful cryopreservation of aquatic species. The overall goal of this study was to work with different user groups, cryopreserving sperm on-site at their facilities to evaluate the uses and challenges of a mobile laboratory with high-throughput and quality control capabilities comparable to those of a specialized central facility. The objectives were to demonstrate the collection and cryopreservation of sperm from (1) large-bodied freshwater Blue Catfish *Ictalurus furcatus* for aquaculture; (2) small-bodied freshwater swordtails and platyfishes *Xiphophorus* spp. for biomedical research and repository development for gametes from imperiled species; and (3) saltwater Red Snapper *Lutjanus campechanus* for wild fisheries research. Over the course of this project, the mobile laboratory traveled more than 4,000 km, collecting germplasm from more than 650 male fish. A total of 137 Blue Catfish were processed in 2015 and 2016, yielding 6,146 0.5-mL French straws. A total of 521 males from 11 different species in the genus *Xiphophorus* were processed over 4 d in 2015, yielding 488 0.25-mL French straws. Lastly, a total of 17 Red Snapper males were processed during 2015, yielding 316 0.5-mL French straws. This study documents the development of a mobile cryopreservation laboratory with high-throughput capability for aquatic species. If mobile laboratories prove to be effective, user groups will no longer be limited to germplasm resources that can be shipped as samples or transported as live animals to a central cryopreservation facility. Mobile laboratories would thus create opportunities to collect higher-quality germplasm and provide access to new species. Also, they would enable direct cooperation, including training, among a wide variety of user groups for numerous applications.

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Research on the cryopreservation of genetic resources of aquatic species is currently restricted to producing data for publication. Small-scale applications can produce working material (samples), with low to medium throughput, but the samples typically lack quality assessment. Commercial cryopreservation applications could produce products, potentially numbering in the millions, with high standards of quality management (Torres et al. 2016). These are qualitatively different levels of activity, and research does not equal application or necessarily lead to it. Transitioning cryopreservation processes for aquatic species across these levels requires establishing new laboratory capabilities. Cryopreservation within aquatic species has predominantly remained in the research phase for the past 70 years, but it has begun the transition to small-scale use, primarily at stock center repositories that have been developed to support specific research communities, such as the Zebrafish International Resource Center (www.zebrafish.org) for biomedical research. The primary approach at this level involves centralized facilities, which requires that samples or animals be transported to a specialized facility with expensive equipment and specifically trained personnel.

A second approach to obtaining material for repositories is mobile laboratories (Childress et al. 2018). However, this approach is still at the research level, and little analysis has been conducted to expand it for use with small-scale applications. The centralized approach has constrained the development of germplasm repositories for aquatic species, as it is often difficult to transfer animals or samples and transfer can result in the loss of gamete quality, yielding poor source material at the start of the cryopreservation process (Rurangwa et al. 2004). Therefore, more work is needed to evaluate the mobile laboratory concept with respect to its capabilities and requirements and to bring it into reality for routine applications. Such work would include evaluating the feasibility of mobile cryopreservation laboratories for scalable medium- to high-throughput applications, including their performance with respect to the biosecurity and quality management components that encompass quality control and quality assurance. The mobile laboratory approach should include sufficient flexibility and adaptability to accommodate a wide range of species, environments, and host facilities.

As part of early efforts to extend the utility of sperm cryopreservation beyond central facilities, field techniques for multiple species have been developed, wherein technicians use shipping dewars to transport samples (Wayman 1996; Harvey et al. 1998; Tiersch et al. 1998). The World Fisheries Trust began developing field cryopreservation methods in the 1990s to freeze sperm from a variety of warmwater and coldwater species (Harvey 2011). To accomplish this, the Trust traveled with a field kit that contained various-sized straws, cryoprotectant solutions, field notebooks, and shipping dewars. Sample collection and cryopreservation took place exclusively in the field, and the sperm was frozen immediately at the site of collection. Using this method in 1995 and 1996, they cryopreserved 2,000 samples. However, quality control and biosecurity activities were essentially excluded from these efforts (Harvey et al. 1998). Similar studies were performed on-site with endangered and marine species in the mid 1990s (Mongkonpunya et al. 1995; Wayman et al. 1996, 1997), but these efforts had the same shortcomings and were characterized by low throughput (tens of samples).

For sperm cryopreservation, quality control can include repeated motility and cell concentration estimations, membrane integrity assessment, and disease screening. These activities help to increase the repeatability and consistency of operations among laboratories and institutions. Consistency is a critical component of cryopreservation processes for the development of germplasm repositories (Torres et al. 2016). Biosecurity programs for cryopreservation, especially for aquatic species, have to account for disease transmission, the potential introduction of exotic species, and the potential genetic consequences in the present and into the future when the samples are used (Tiersch and Jenkins 2003). Improper sample storage in the field or during transportation to and from working locations can increase the possibility of negative outcomes. Also, because most fish release sperm and eggs directly into the water, biosecurity efforts should address the potential effects of laboratory procedures on surrounding environments, such as the release of endemic or nonendemic pathogens (Tiersch and Jenkins 2003).

Although the cryopreservation of aquatic and livestock germplasm started around the same time in the 1950s, the collection and distribution of livestock germplasm has become a billion-dollar global industry, while the commercial use of aquatic cryopreservation has not yet been realized. This lag behind the livestock industry can be attributed to a lack of standardization in methods and reporting and the lack of general acceptance of cryopreservation as a useful tool with economic benefits (Torres et al. 2016). For livestock, practical sperm concentrations and freezing protocols have long been standardized and automated equipment has been developed. This type of equipment includes automated filling, sealing, labeling, and freezing systems. In efforts to extend the utility of sperm cryopreservation, protocols and equipment that are used in the livestock industry have been adopted for use with aquatic species (Roppolo 2000; Lang et al. 2003; Hu et al. 2016). The use of high-throughput (e.g., hundreds to thousands of samples) cryopreservation equipment provides consistent products with predictable output by maintaining quality control (Hu et al. 2013). However, high-throughput cryopreservation efforts have only been

applied at central laboratories, with no true examinations of a mobile high-throughput application. If cryopreservation is to succeed at a commercial scale or in repository development, other approaches to the collection and cryopreservation of samples are needed to expand the scope of cryopreservation services to additional users and species of interest. These approaches need to include the same biosecurity and quality control procedures that are used in a central facility.

During 2015 and 2016, a high-throughput mobile cryopreservation laboratory was developed and deployed into the field from the Aquatic Germplasm and Genetic Resources Center (AGGRC) of the Louisiana State University Agricultural Center in Baton Rouge. The mobile laboratory was designed to address three common working scenarios: (1) processing and freezing inside an on-site facility; (2) processing and freezing inside the mobile laboratory using on-site power; and (3) processing and freezing inside the mobile laboratory using generator power (Childress et al. 2018). The overall goal of this project was to work with different user groups, cryopreserving sperm on-site at their facilities to evaluate the uses and challenges of a mobile laboratory platform in real-world situations while maintaining high-throughput and quality control capabilities comparable to those of a centralized facility. The study objectives were to demonstrate the collection and cryopreservation of sperm from (1) large-bodied freshwater Blue Catfish *Ictalurus furcatus* for aquaculture; (2) small-bodied freshwater swordtails and platyfishes *Xiphophorus* spp. for biomedical research and repository development for gametes from imperiled species; and (3) saltwater Red Snapper *Lutjanus campechanus* for wild fisheries research. To our knowledge this is the first published account of the development of a comprehensive, fully enclosed mobile laboratory with the capability of traveling to facilities to cryopreserve aquatic germplasm on a commercial scale with full quality control and biosecurity.

METHODS

Mobile laboratory.— The mobile laboratory used during this project consisted of a custom-built, fully enclosed cargo trailer equipped with a workbench, folding table, and shelving unit (Childress et al. 2018). When technicians were working inside of the mobile laboratory, the samples were processed and assessed at the workbench, packaged at the folding table, frozen using a programmable freezer on the shelving unit, and stored in a dewar (not pictured) (Figure 1).

Because the work was done at three different locations, the Institutional Animal Care and Use Committee protocols used were determined by the host institution for the specific animals being collected. Mobile Laboratory Site Assessment and New Species Cryopreservation Suitability forms (see the Appendix) were filled out prior to each trip. The Mobile Laboratory Site Assessment form was used to determine the resources available at the site (e.g., whether there was space inside a building to set up the laboratory equipment, whether there was access to power and water outside of the building, and what existing laboratory equipment was on site and available for use).

The New Species Cryopreservation Suitability form was used to establish basic information for each species in three categories (husbandry and life cycle, spawning and early life history, and gametes and cryopreservation) to identify the specific needs of each species of interest. The main laboratory equipment components for every trip included a microscope with dark-field capability, an automated straw packager and sealer, a programmable freezer, a liquid nitrogen tank, and a high-capacity shipping dewar. Other equipment components, such as a flow cytometer or stereo microscope, were included if they were needed. Specific laboratory equipment and supplies were loaded into the mobile laboratory based on the information provided from the forms for each trip and then brought to the on-site facility. The information that was collected on these forms was also used to determine how many technicians were needed for each trip.

Blue Catfish.— Single 3- to 4-d trips were made each year in May of 2015 and 2016 to the U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS) Warmwater Aquaculture Research Unit in Stoneville, Mississippi, to cryopreserve sperm from males of four strains of Blue Catfish (D&B, Mississippi River [MS], Rio Grande, and Texas). The fish were killed by a blow to the head, weighed, and measured. The testes of each fish were removed by dissection, and the anterior portion of each testis was placed into a tared weighing boat with Hanks' balanced salt solution at an osmolality of 300 mosmol/kg (HBSS300; Hu et al. 2011) to create a suspension. The testes were weighed and HBSS300 was added at two times the testis mass (g). Then the testes were placed between a folded piece of 15.2- × 15.2-cm standard gray window screen (Phifer, Tuscaloosa, Alabama), which was placed back in the weighing boat (812 × 812 × 25.4 mm; Fisherbrand, reference number 08-732-114, Waltham, Massachusetts) with the salt solution and crushed. This suspension was filtered through a 200-μm mesh screen into a 50-mL centrifuge tube (Fisherbrand, reference number 06-443-18).

The cell concentration and motility for the samples were measured using a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) at 200× magnification (Olympus CX41RF, Japan). For each sample, sperm motility was measured at three points in the cryopreservation process: (1) before the cryoprotectants were added ("initial motility" [<1 h after collection]); (2) at the time of initiation of cooling after the cryoprotectants were added

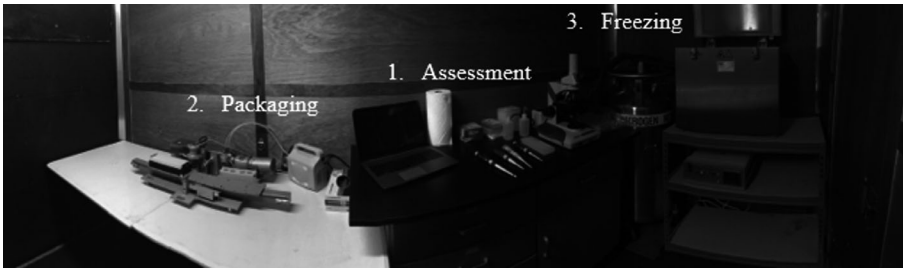

FIGURE 1. Panoramic photograph of the three main workstations inside the mobile laboratory: (1) assessment, (2) packaging, and (3) freezing. Overall, the working space was $3.8 \times 1.7 \times 2.2$ m (length × width × height). All of the work could be performed inside of the mobile laboratory, or samples could be collected outside and frozen inside.

("equilibration motility" [within 15 min of adding the cryoprotectant]); and (3) after the samples were thawed ("post-thaw motility" [at least 7 d after freezing]). A 1:19 (volume basis) dilution was created for each male by adding 15 μL of sperm to 285 μL of HBSS300 in a 1.5-mL centrifuge tube (Fisherbrand, reference number 05-408-129). To estimate the sperm concentration for each sample, technicians placed 1 μL of this dilution onto the counting chamber, counted the number of sperm cells in three random squares, and applied the following equation:

$$\text{Sperm concentration (millions per mL)} = \text{average of the 3 squares counted} \times \text{dilution factor} \times 10^6.$$

To estimate the sperm motility for each sample, 19 μL of filtered deionized distilled water was placed onto the counting chamber to activate 1 μL of sperm. The sample was observed in three random squares to count the number of motile sperm (those showing progressive movement) in a single square as well as the number of nonmotile sperm (those showing nonprogressive movement, such as vibration in place) in the same square. This measurement was collected for each of the three squares to calculate the average number of motile (the total number of motile sperm divided by 3), the average number of nonmotile (the total number of nonmotile sperm divided by 3), and the total number of sperm for all three squares (motile sperm plus nonmotile sperm). A percentage of motility was estimated by applying the following equation:

$$\text{Percent motility} = (\text{average number of motile sperm} \div \text{total number of sperm}) \times 100.$$

If the samples were at a concentration that was higher than 2×10^9 cells/mL, the proper amount of HBSS300 was added to reach this concentration. If it was below this level, the concentration was kept as it was.

The samples were frozen following an established protocol, with minor modifications to the equilibration time and cooling rate (Hu et al. 2011). For each male, ≤15 mL of sperm suspension was added to a new 50-mL centrifuge tube, followed by an equal volume of premade 20% methanol, creating a final cryoprotectant concentration of 10% and a sperm concentration of 1×10^9 cells/mL (or lower depending on the original concentration). After mixing, the samples were loaded into preprinted 0.5-mL French straws (IMV Technologies, Paris) and sealed using an automated straw filling and sealing machine (MRS1; IMV Technologies). The straws were placed onto freezing racks and loaded into a computer-controlled freezing chamber (in 2015: Micro-Digitcool, IMV Technologies; in 2016: IceCube 14M, SY-LAB, Neupurkersdor, Austria).

After an equilibration time of 15 min (the time allowed for the cryoprotectant to interact with the sample), the straws were cooled from 4°C to −80°C at a rate of 10°C/min, held for 5 min, and then transferred into an open vessel that was filled with liquid nitrogen. The straws were sorted into 12-compartment daisy goblets (IMV Technologies, reference number 015152) and stored in a 7-canister shipping dewar (MVE CryoMoover, Chart Industries, Ball Ground, Georgia). After the samples were returned to the AGGRC, two straws from each male were thawed in a water bath at 40°C for 8 s. Post-thaw motility was assessed as described above. Two samples from each straw were stained with SYBR-14 and propidium iodide (PI) (LIVE/DEAD Sperm Viability Kit, catalog number L-7011; Molecular Probes, Eugene, Oregon) for flow cytometric analysis of cell membrane integrity (Daly and Tiersch 2011).

Xiphophorus *spp*.— One 4-d trip was made in July of 2015 to the *Xiphophorus* Genetic Stock Center at Texas State University in San Marcos to cryopreserve sperm from 11 species within the genus *Xiphophorus*. Based on the goal for this trip and the species being processed, a flow cytometer (Accuri C6; Accuri Cytometers, Ann Arbor, Michigan) to measure membrane integrity and

stereo microscopes (10–40× zoom, Motic, British Columbia) for completing dissection were included on the equipment list. The fish were euthanized by rapid chilling, placed onto a paper towel, and blotted dry. While viewing each fish with a stereo microscope, technicians recorded the standard length (mouth to the base of the tail), body weight, and gonopodium length and removed the testes. The testes were placed into tared 5-mL centrifuge tubes (Argos Technologies, reference number T2076A, Elgin, Illinois) containing 100 μL of HBSS300 for weighing. Testes from males of the same species, strain, and phenotype (if applicable) were pooled into single 5-mL centrifuge tubes (the sample sizes ranged from 5 to 67 males per tube). The largest group of males (Southern Platyfish *Xiphophorus maculatus* JP163B) was pooled and cryopreserved in 3 batches (58, 36, and 44 males per batch, respectively) to minimize sample use during the quality control evaluation. After all of the fish from each pooling group were dissected, HBSS300 was added at a volume (μL) corresponding to approximately 30 times the total testis weight (mg) and the testes were crushed with forceps to release the sperm. After the testes were crushed, the remaining large tissue pieces were removed with forceps.

Sperm motility and cell concentration for each sample were measured simultaneously using a dark-field microscope with a charge-coupled device camera (Sony XC-ST50, Japan) at 200× magnification. A 5-μL sperm sample was placed onto a Makler counting chamber, and the measurements were recorded as described above. The percentage for sperm motility was estimated by applying the following equation:

$$\text{Percent motility} = (\text{average motile sperm} \div \text{total number of sperm}) \times 100.$$

Cell concentration was estimated by apply the equation:

$$\text{Sperm concentration (millions per mL)} = (\text{the average of the 3 squares counted} \times 10^6).$$

The samples were adjusted to a sperm cell concentration of 2×10^8 cell/mL. A 10-μL sample from each pooled group was stained with PI–SYBR-14 for flow cytometric analysis of cell membrane integrity.

The samples were frozen following an established protocol (Yang et al. 2007). The sperm suspensions from each pooling were mixed with equal volumes of 28% glycerol in HBSS300 to achieve a final cryoprotectant concentration of 14% and a sperm cell concentration of 1×10^8 cell/mL. The samples were loaded into 0.25-mL French straws (IMV Technologies) using a 1-mL syringe, sealed with an ultrasonic sealer (Ultraseal 21; Minitube of America, Verona, Wisconsin), and placed into a computer-controlled freezing chamber (Micro-Digitcool) at 4°C. After an equilibration time of 15 min, the straws were cooled from 4°C to –80°C at a rate of 20°C/min, held for 5 min, and then transferred to an open vessel that was filled with liquid nitrogen. The straws were sorted into 12-compartment daisy goblets and stored in a shipping dewar (MVE Cryo-Moover). After the samples were returned to the AGGRC, one straw from each pooled batch was thawed in a water bath at 40°C for 8 s. The post-thaw motility for each sample was assessed as described above. Two samples (10 μL each) from each straw were stained with PI–SYBR-14 for flow cytometric analysis of the cell membrane integrity for each sample.

Red Snapper.— Two 3-d trips were made in June and July of 2015 to the Louisiana Universities Marine Consortium (LUMCON) facility in Chauvin to collect Red Snapper during the natural spawning season. During this study, sperm samples were collected from fish that were caught on a broodstock collection trip and from a recreational fishing boat off the coast of Louisiana. The fish that were caught on the recreational fishing boat were placed on ice after they were captured, and they were sampled within 6 h at Coco Marina (Cocodrie, Louisiana). The fish that were caught on the broodstock collection trip were placed in a 450-L oxygenated live well that was filled with ~35‰ seawater at the collection site and brought back to LUMCON. Upon return to LUMCON, each fish was injected intramuscularly with a 500-IU/kg priming dose of human chorionic gonadotropin (Chorulon; Intervet, Millsboro, Delaware; Riley et al. 2004). The fish were then placed in a recirculating tank and held at 25–27°C and 30–35‰ salinity. At ~18 h after injection, sex was identified and all of the males were killed with an overdose of the anesthetic tricaine methanesulfonate (MS-222). To collect the sperm samples, the testes from each fish were dissected, placed into 946-mL Ziploc bags (S. C. Johnson and Son, Racine, Wisconsin), and held on ice until all of the fish were processed (<1 h). Then, each sample of testes was removed from the bag, blotted dry with a paper towel, weighed, and sliced to release the sperm. The sperm samples were collected into 50-mL centrifuge tubes and diluted with calcium-free Hanks' balanced salt solution at an osmolality of 200 mosmol/kg (C-F HBSS200) at a 1:3 (volume basis) ratio (Riley et al. 2008).

The samples were frozen following an established protocol, with modifications to the cooling rate (Riley et al. 2004). For each sample, the sperm cell concentration and motility were measured using a dark-field microscope at 200× magnification, as described above. The samples were adjusted to a sperm cell concentration of 2×10^9 cells/mL. A 20% dimethyl sulfoxide solution was mixed at a 1:1 (volume basis) ratio for each sample to achieve a final dimethyl sulfoxide solution concentration of 10% and a sperm cell concentration of 1×10^9 cell/mL. While they

were equilibrating for 20 min, an automated packager (MRS1) was used to package and seal the samples in 0.5-mL French straws. After equilibration, the straws were frozen in a Micro-Digitcool at 40°C/min. The straws were then sorted into 12-compartment daisy goblets and stored in a shipping dewar (MVE CryoMoover). After the samples were returned to the AGGRC, two straws from each male were thawed in a water bath at 40°C for 8 s. The post-thaw motility for each sample was assessed as described above.

RESULTS

Blue Catfish

A total of 78 Blue Catfish (20 D&B, 19 MS, 20 Rio Grande, and 19 Texas) were processed during 2015, and 59 Blue Catfish (20 D&B, 20 MS, 18 Rio Grande, and 1 Texas) were processed during 2016 (Table 1). Day 1 included traveling to the USDA–ARS Warmwater Aquaculture Research Unit (~460 km one way), and completing equipment setup. All of the equipment was ready for processing within 1 h of arrival. Two of the fish were processed on day 1 to test the high-throughput equipment. During 2015, 38 fish were processed on day 2, 25 fish were processed on day 3, and 13 fish were processed on day 4. The males had a standard length of 758 ± 105 mm (mean $\pm$ SD), body weight of 6 ± 2 kg, and testis weight of 14 ± 6 g. The sperm volume collected per fish after the final dilution of the sample was 28 ± 12 mL, resulting in the production of 3,642 0.5-mL French straws. The final sperm cell concentration per milliliter was $5.7 \times 10^8 \pm 1.96 \times 10^8$. The initial motility of the fresh sperm from all of the strains was $34 \pm 12\%$. Post-thaw, the samples demonstrated a motility of $8 \pm 4\%$. During 2016, 56 fish were processed on day 2. The males had an average standard length of 794 ± 74 mm, body weight of 7 ± 2 kg, and testis weight of 15 ± 6 g. The sperm volume collected per fish was 29 ± 12 mL, resulting in the production of 2,504 French straws. The final average sperm cell concentration was $7.1 \times 10^8 \pm 3.61 \times 10^8$ cells/mL. The initial motility of the fresh sperm from all of the strains was $41 \pm 17\%$. Post-thaw, the sperm samples demonstrated a motility of $12 \pm 8\%$. Four technicians were needed for each trip.

Xiphophorus spp.

A combination of 11 species created a total sample of 521 males, which were processed in 4 d (Table 2). Day 1 included traveling to the *Xiphophorus* Genetic Stock Center (~697 km) and setting up all of the equipment inside the center (Figure 2). Processing started on day 2 with 211 fish, followed by 245 fish on day 3 and 65 fish on day 4. Samples from Cuatro Ciénegas Platyfish and Pánuco Swordtails could not be cryopreserved because they showed poor initial sperm quality (<5% motility). The males had a standard length of 26 ± 5 mm, body wet weight of 355 ± 143 mg, and testis weight of 4 ± 2 mg. The volume of sperm collected per fish after the final dilution was 2 ± 3 mL, resulting in the production of 488 0.25-mL French straws. The percentage of initial motility of the sperm samples for all of the species combined was $76 \pm 12\%$, with a post-thaw motility of $55 \pm 22\%$. Five technicians were used.

Red Snapper

A total of 17 male Red Snapper were processed using high-throughput equipment during both trips (~200 km), producing a total of 361 0.5-mL French straws. Twelve of the fish were processed, and the sperm was frozen inside the mobile laboratory. The other five fish were processed in an existing laboratory inside LUMCON. The fish had a standard length of 504 ± 98 mm, body weight of 2 ± 2 kg, and testis weight of 50 ± 21 g. The sperm volume per fish was approximately 50 mL. The percentage of initial motility of the sperm samples from the fish that had been frozen using the mobile laboratory was $61 \pm 17\%$, with a post-thaw motility of $17 \pm 13\%$. The percentage of initial motility of the sperm samples from fish that had been frozen in an existing laboratory was $57 \pm 16\%$, with a post-thaw motility of $16 \pm 14\%$ (Table 3). One technician was used for both trips.

DISCUSSION

Typically, interactions between user groups and cryopreservation facilities are limited to the transport of fish or sperm samples. Mobile cryopreservation capabilities (i.e., cryopreserving aquatic germplasm on site at user group facilities) could offer multiple benefits related to the production and preservation of aquatic species, such as germplasm repository development. Higher-quality samples could be collected on-site, thereby avoiding the negative effects of transporting fish or samples to a central facility on the subsequent quality of the samples (Rurangwa et al. 2004). Genetic (i.e., blood or tissue), phenotypic, and environmental (e.g., water quality or location) samples and data could be systematically collected on site. Also, methods and terminology could be standardized between user groups and cryopreservation personnel. The exchange of information and exposure to the technology on site would encourage user groups to initiate standardized and harmonized cryopreservation procedures for their own samples, contribute meaningfully to repository development, and establish germplasm user communities based on shared genetic resources, technologies, or applications.

With enough user groups contributing, repositories could be supplied in aggregate with thousands of standardized samples, establishing much larger and more

TABLE 1. The number of males that were processed, percentage of sperm motility (mean ± SD), and number of French straws produced for each strain of Blue Catfish. The initial motility is the motility of the sperm suspension before the addition of the cryoprotectant. The equilibration motility is the motility of the sperm suspension after the 15-min equilibration time. The post-thaw motility is the motility of the sperm suspension after thawing at 40°C for 8 s.

Year	Strain	Males processed	Initial motility (%)	Equilibration motility (%)	Post-thaw motility (%)	Post-thaw membrane integrity[a] (%)	Straws produced
2015	D&B	20	28 ± 9	22 ± 9	6 ± 3		923
	Rio Grand	20	27 ± 10	21 ± 8	7 ± 3		954
	Mississippi River	19	43 ± 9	35 ± 8	11 ± 5		915
	Texas	19	39 ± 11	30 ± 11	8 ± 4		850
2016	D&B	20	33 ± 12	29 ± 10	8 ± 4	20 ± 7	706
	Rio Grande	18	34 ± 13	29 ± 10	12 ± 5	23 ± 6	749
	Mississippi River	20	57 ± 14	46 ± 12	14 ± 10	25 ± 9	1,018
	Texas	1	25	37	7 ± 2	53 ± 15	31

[a]Not measured in 2015.

TABLE 2. The number of males, percentage of sperm motility, membrane viability (mean ± SD), and the number of straws produced for each species, strain, and phenotype (if applicable) for the genus *Xiphophorus*. Initial sperm motility was assessed before the addition of the cryoprotectant. Equilibration motility is the motility of the sperm suspension after the 20-min exposure prior to freezing. Post-thaw motility is the motility of the sperm suspension after thawing at 40°C for 8 s.

Scientific name	Strain	Phenotype	Males processed	Initial motility (%)	Equilibration motility (%)	Post-thaw motility (%)	Post-thaw membrane viability (%)	Straws produced
X. maculatus	JpYlr	[a]	27	77	65	56	14 ± 2	56
	YSdSr	[a]	38	83	76	42	15 ± 1	26
	JP163B	[a]	138	84	75	81	14 ± 0.6	86
	JpYlrBr	[a]	13	79	59	76	13 ± 1	4
	Ysp	[a]	13	82	88	43	9 ± 1	8
X. xiphidium	SC	ct ct	22	63	47	44	25 ± 1	24
X. variatus	Encino	X+Y+	28	82	70	47	23 ± 2	8
X. couchianus	Xc	[a]	40	62	62	27	14 ± 0.7	13
X. andersi	andC	[a]	67	80	67	73	23 ± 0.6	26
X. helleri	Bel	[a]	15	77	82	68	60 ± 3	10
X. pygmaeus	pyglll	Ty	21	34	[b]	0	2 ± 0.4	5
	pyglll	+	11	77	59	24	9 ± 0.1	1
X. birchmanni	birchll	cam	17	84	67	60	18 ± 0.3	7
	birchll	+	9	77	74	83	33 ± 4	9
X. montezumae	OjoCal	[a]	13	82	84	68	52 ± 0.6	3
X. milleri	mil82	X-Srf Y-Gnl	7	84	80	66	45 ± 3	1
	mil82	X+ Y-Gnl	7	85	88	68	46 ± 3	1
	mil82	SplyArGnD	12	79	67	60	34 ± 2	9
X. gordoni	gordoni		5	<5	[c]	[c]	[c]	0
X. nigrensis	nigrn	Cb	18	<5	[c]	[c]	[c]	0

[a]No phenotype.
[b]Equilibration motility not recorded.
[c]Samples not processed.

diverse repositories than are currently possible with the few available centralized cryopreservation facilities (Hu et al. 2017). Moreover, user community development can lead to the exchange of ideas and the development of new techniques and devices produced by emerging technologies, such as three-dimensional printing, that could further

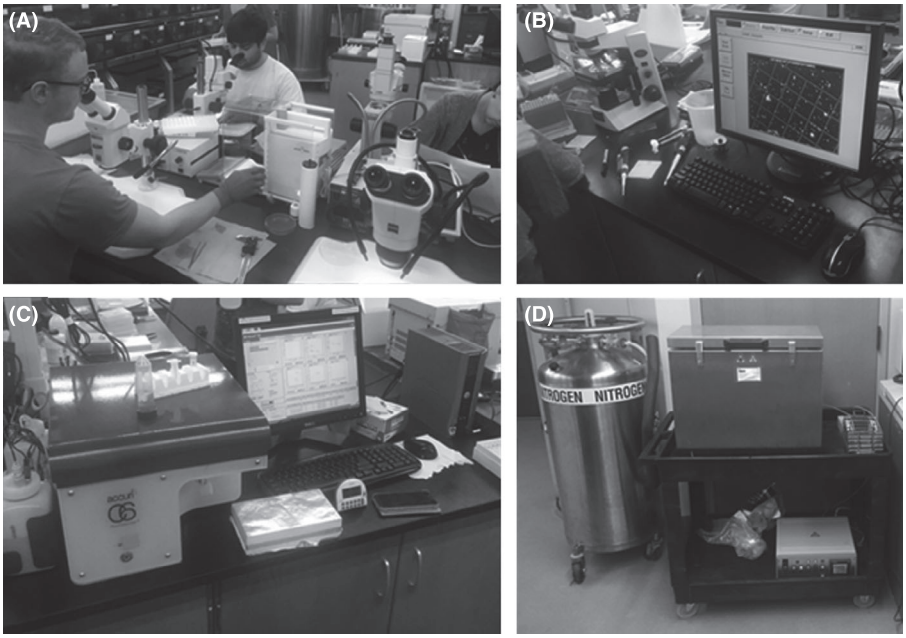

FIGURE 2. Setup of the major processing stations at the *Xiphophorus* Genetic Stock Center: (**A**) dissection with stereo microscopes, (**B**) motility and concentration estimates with dark-field microscopy, (**C**) membrane integrity using flow cytometry, and (**D**) freezing using a programmable freezer.

TABLE 3. The number of males, percentage of sperm motility, and the number of straws produced for Red Snapper at the mobile and central laboratories. The percentage of initial sperm motility was assessed before the addition of cryoprotectant. Equilibration motility is the percentage of motility of the sperm suspension after the 20-min exposure prior to freezing. Post-thaw motility is the percentage of motility of the sperm suspension after thawing at 40°C for 8 s.

Processing location	Males processed	Initial motility (%)	Post-thaw motility (%)	Straws produced
Mobile laboratory	12	61 ± 17	17 ± 13	236
On-site (central) laboratory	5	57 ± 16	16 ± 14	125

increase standardization and harmonization throughout user groups and applications. In this demonstration, three different groups of fishes based on user group, habitat, reproductive mode, relationship between sperm activation and osmolality, sample collection method, and size were chosen to represent the diversity of user groups for which a mobile cryopreservation laboratory could conceivably work in the field (Table 4).

The focus of this study was the utility and high-throughput capability of the mobile laboratory and not the testing or development of specific cryopreservation protocols (e.g., cryoprotectants or cooling rates). The protocols were considered independently of the performance of the mobile laboratory, as they did not dictate the utility or high-throughput capability of the laboratory and they can be customized as desired by individual users. Over the course of this demonstration, more than 6,500 French straws were produced, with the same standards of quality control and biosecurity as are present at a centralized facility (e.g., the AGGRC). The use of a mobile laboratory allowed fresh samples to be collected and frozen during the same day instead of being stored for ≥ 24 h while they are transported to a centralized facility. Estimates of percent motility and cell concentration could be obtained within 1 h of collecting sperm samples, along with membrane integrity analysis by flow cytometry when needed. The fish did not have to be transported and held before they were processed, and the sperm samples were not shipped overnight, which would risk a loss of quality (Tiersch 2011). Without the limitations in the numbers of fish that can be transported or sperm samples that can be shipped when users depend on a centralized facility, larger numbers of samples could be processed. Cryopreserving sperm samples on site would also provide the opportunity to conduct fertilization trials when eggs are available, such as at a hatchery.

Along with packing the appropriate equipment and sufficient supplies, determining the number of technicians needed for a trip is an important planning factor. The basic central cryopreservation pathway for collecting

TABLE 4. Comparison of user group, habitat, reproductive mode, sperm activation model, collection method, and average body length, body weight, testis weight, and sperm volume collected per male for the model species used in this project. These species represent large and small-bodied; freshwater and marine; and aquaculture, wild fisheries, imperiled, and biomedical model fishes.

Species	User group	Habitat	Reproductive mode	Sperm activation	Collection method	Body length (mm)	Body weight (kg)	Testis weight (g)	Sperm volume[a] (mL)
Blue Catfish	Aquaculture	Freshwater	External	Hypotonic	Dissection	768	6	14	27
Xiphophorus spp.	Biomedical/ imperiled	Freshwater	Internal	Isotonic	Dissection/ stripping	26	0.00035	0.004	2
Red Snapper	Wild fisheries	Marine	External	Hypertonic	Dissection/ stripping	504	2	50	50

[a]Sperm volume available after final dilution.

sperm and processing, packaging and equilibrating, freezing, and storing the samples typically required two technicians (Figure 3). This assumes that all of the activities are performed in the same building and that less than 10 fish are being processed in a day. For the Blue Catfish, two extra technicians were needed to assist with the planned activities. One extra technician was needed for handling the fish (i.e., retrieving the fish from a holding pond), and another technician was needed to complete the dissections, as more than 10 fish were being processed in a day. To process the *Xiphophorus* spp., three extra technicians were required. Two technicians were needed to help dissect the fish (more than 500 males), and another technician was needed to help perform the quality assessment and quality control (i.e., flow cytometry) procedures. For Red Snapper, only one technician was used, but if an additional technician had been available, the total processing time could have been reduced from 3 d to 2 d.

Working on-site eliminated the risk of introducing nonnative fish to different regions (Zajicek et al. 2009) and lowered the risk of introducing diseases and pathogens to the central cryopreservation facility (Watson et al. 2010; Wynne and Wurts 2011) or spreading them to nonendemic regions. Biosecurity programs for mobile laboratories can be developed that are tailored to user specifications to protect samples while they are being transported from the cryopreservation site to a user group's central facility or to other third-party users. For example, the movement of frozen samples between dewars should be limited and carefully recorded. Only properly sealed and labeled samples should be stored, and all others should be discarded properly (Jenkins 2011). Precautions should be taken to decrease the potential for pathogen transfer, such as completely washing the mobile laboratory at a separate facility (e.g., a commercial carwash) before traveling between distant areas and after each trip. This should include cleaning the inside and outside of the trailer and truck. If possible, the laboratory equipment and supplies should be stored at the central facility away from healthy fish populations, and when working inside of an on-site facility the equipment should be cleaned before it is packed into the mobile laboratory.

Safety is an important consideration when technicians are traveling and working with liquid nitrogen. Precautions should be the same as when working in a centralized

FIGURE 3. The basic central cryopreservation pathway (contained within the long rectangle) with additional activities used to plan the number of technicians needed for a trip. The squares represent activities that are incorporated into the central pathway due to an increase in scale (e.g., increasing the number of males being processed) or the addition of activities (e.g., a quality assessment). The circle represents an activity that is incorporated due to an expanded physical area for work (e.g., retrieving fish from a pond).

facility (Wayman and Tiersch 2011). The doors to the mobile laboratory should always be held open during its operation to prevent the accumulation of nitrogen gas. The gas is not toxic (it composes ~78% of Earth's atmosphere), but it can displace oxygen, so the use of a nitrogen or an oxygen meter is suggested. It would be even better to perform the freezing process outside of the trailer, if possible. When transporting a liquid nitrogen cylinder, the cylinder should be secured by floor and wall fasteners to prevent it from inadvertently tipping. Also, users should be aware of any laws or regulations for the highway transportation of liquid nitrogen. Sperm samples should only be stored and transported in dewars that are designed specifically for shipping. The back-and-forth motion of a standard storage dewar during transportation can cause the neck tube to crack and lose its vacuum (Nebel 2006).

Integration of Mobile Laboratories into Existing Programs

Currently, there are more than 300 national and state fish hatcheries in the United States (Figure 4) and there are more than 3,000 wild fish species, with more than 200 of these designated as vulnerable, endangered, or critically endangered (iucnredlist.org). At present, there are only four facilities in the United States that have central processing capability and a long-standing focus on aquatic cryopreservation: (1) the USDA–ARS National Animal Germplasm Program in Fort Collins, Colorado; (2) the U.S. Fish and Wildlife Service's Warm Springs Fish Technology Center in Warm Springs, Georgia; (3) the AGGRC at the Louisiana State University Agricultural Center in Baton Rouge; and (4) the Zebrafish International Research Center at the University of Oregon in Eugene. With this limited number of cryopreservation facilities, it is difficult to gather germplasm from hatcheries and wild populations, and it would cost millions of dollars to build, outfit, and staff new cryopreservation facilities across the United States.

In addition to building new facilities, a program could be developed to provide a fleet of mobile cryopreservation laboratories that could to be deployed across the United States to gather germplasm for repository development. The facilities listed above, or others, could initially act as stations from which the mobile units are deployed, and such facilities could temporarily hold samples before they are entered into a secure repository network, perhaps anchored by the USDA–ARS National Animal Germplasm program in Fort Collins, which hosts a powerful databasing capability (Animal-GRIN; https://www.ars-grin.gov/). Each mobile unit would have fully automated processing capabilities, and the units could be operated by two technicians for most work (Childress et al. 2018).

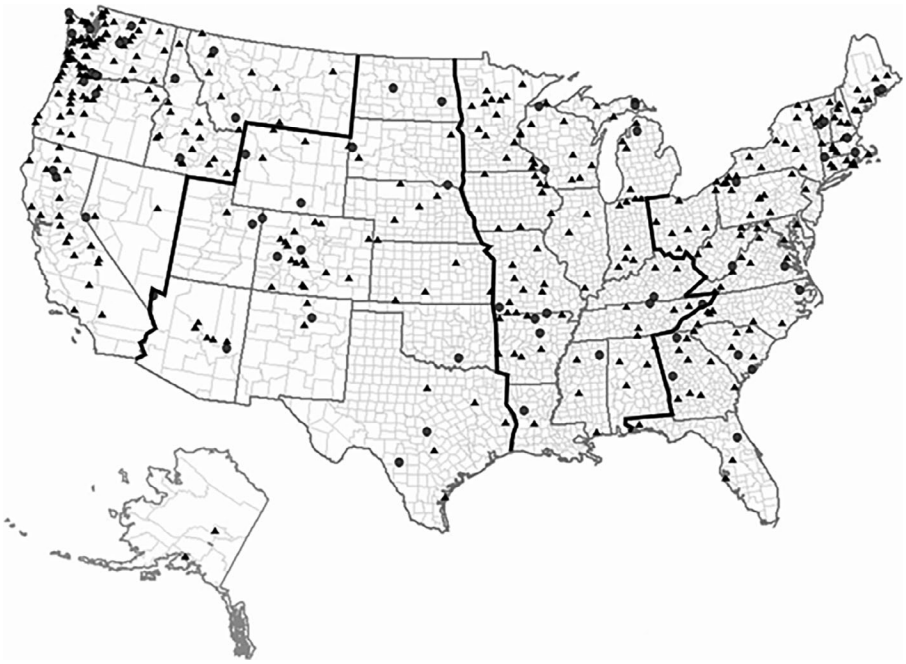

FIGURE 4. Representative distribution of the federal (circles) and state (triangles) hatcheries and the four existing high-throughput cryopreservation facilities (squares) in the United States. The hatchery addresses were obtained from state parks' and fisheries' Web sites and that of the U.S. Fish and Wildlife Service. This distribution shows the opportunity for mobile laboratories to interact with hatcheries to protect aquatic genetic resources across the United States.

Annual production cycle calendars could be developed for each unit based on the spawning periods of species in the region to help plan cryopreservation activities throughout the year. The assembly and operation costs for mobile laboratories for aquatic species based on different levels of throughput and different application scenarios have been calculated (Childress et al. 2018) and range from US$13,616 to $103,529 for private groups and from $12,494 to $94,891 for public groups.

Outreach and Training Support

When they are not being used to collect germplasm, mobile cryopreservation laboratories could be used to provide outreach, training, and support to new or current user groups. As new user group communities are formed, the application of standardized protocols for sample preparation, cryopreservation, and quality control could be uniformly adopted for a wide range of purposes. Recently, three-dimensional printing has provided a means of fabricating low-cost cryopreservation devices (Tiersch and Monroe 2016; Hu et al. 2017; Tiersch et al. 2018). These devices could easily be incorporated into training programs, facilitating community development and allowing smaller user groups to develop cryopreservation programs.

With more user groups following methods that are standardized (i.e., using the same protocols) or at least harmonized (i.e., using different methods but obtaining the same target results) (Torres and Tiersch 2018), the samples from various groups could be combined for quality-controlled accessions in a germplasm repository. For example, creating a repository for imperiled species could help prevent the loss of genetic resources (Thorpe et al. 1995) and help to maintain genetic diversity. A germplasm repository for model organisms is vital for the advancement of biomedical research, as these organisms could be instrumental in developing cures for cancer or other diseases and maintaining frozen samples is far less expensive and less risky than maintaining these genetic lines as live populations. Cryopreservation has great potential for increasing, protecting, and distributing the genetic gains achieved by selective breeding programs in aquaculture species, and the technology is already an important component of the catfish breeding program at the USDA–ARS Warmwater Aquaculture Research Unit. Repositories for aquaculture species can protect investments made in genetic research, genome mapping, and selective breeding. In all of these examples, cryopreservation allows for the maintenance of original reference populations, which will become increasingly valuable in the future.

In the present study, we demonstrated the potential of a single customizable mobile laboratory with high-throughput capability to produce large quantities of quality-controlled samples from on-site cryopreservation of Blue Catfish, 11 species within the genus *Xiphophorus*, and Red Snapper to demonstrate the feasibility of this approach across a range of user groups. Based on the results, the on-site cryopreservation processes that were completed in a mobile laboratory demonstrated the capabilities that are necessary to support high-throughput sample production, with biosecurity and quality control standards that are similar to, if not superior to, those of most centralized facilities. If spermatozoa can be collected and frozen within hours on site (i.e., where the fish or shellfish are located), cryopreservation can be more beneficial to the user. Research efforts have been directed at adapting high-throughput technologies and equipment from the livestock industry to meet the demands for cryopreservation of aquatic sperm, but currently there are no examples of automated equipment developed specifically for aquatic species.

The future growth of aquatic cryopreservation in commercialization and repository development requires changes in conventional thinking (Hu and Tiersch 2011). A mobile approach such as that presented in this study could (1) support the research and management of freshwater and saltwater shellfish and finfish; (2) provide support in repository development for user groups that are currently unable to use cryopreservation; (3) provide outreach and support to user groups that are interested in expanding cryopreservation programs and repositories; and (4) serve as a platform for industry development—offering custom collection services to interested user groups to support the development of lucrative markets for genetic resources—similar to the multibillion-dollar global markets currently operating for livestock semen.

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REFERENCES

Childress, W. C., R. H. Caffey, and T. R. Tiersch. 2018. Design and cost analysis of a self-contained mobile laboratory for aquatic species cryopreservation. Journal of the World Aquaculture Society 49:805–826.

Daly, J., and T. R. Tiersch. 2011. Flow cytometry for the assessment of sperm quality in aquatic species. Pages 201–207 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Harvey, B. 2011. The application of cryopreservation in fish genetic conservation in North and South America. Pages 783–788 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic apecies, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Harvey, B., C. Ross, D. Greer, and J. Carolsfeld. 1998. Action before extinction: an international conference on conservation of fish genetic diversity. World Fisheries Trust, Vancouver.

Hu, E., W. Childress, and T. R. Tiersch. 2017. 3-D printing provides a novel approach for standardization and reproducibility of freezing devices. Cryobiology 76:34–40.

Hu, E., R. Cuevas-Uribe, H. Yang, R. Sanderson, A. O. Gill, H. Daniels, and T. R. Tiersch. 2016. High-throughput cryopreservation of sperm from sex-reversed Southern Flounder, *Paralichthys lethostigma*. Journal of the World Aquaculture Society 47:555–565.

Hu, E., T. Liao, and T. Tiersch. 2013. A quality assurance initiative for commercial-scale production in high-throughput cryopreservation of Blue Catfish sperm. Cryobiology 67:214–224.

Hu, E., and T. R. Tiersch. 2011. Development of high-throughput cryopreservation for aquatic species. Pages 672–683 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Hu, E., H. Yang, and T. R. Tiersch. 2011. High-throughput cryopreservation of spermatozoa of Blue Catfish (*Ictalurus furcatus*): establishment of an approach for commercial-scale processing. Cryobiology 62:74–82.

Jenkins, J. A. 2011. Minimizing microbial contamination of sperm samples. Pages 684–687 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Lang, P. R., K. L. Riley, J. R. Chandler, and T. R. Tiersch. 2003. The use of dairy protocols for sperm cryopreservation of Blue Catfish (*Ictalurus furcatus*). Journal of the World Aquaculture Society 34:66–75.

Mongkonpunya, K., T. Pupipat, N. Chairak, and T. R. Tiersch. 1995. Cryopreservation of Mekong Giant Catfish sperm. Asian Fisheries Science 8:211–222.

Nebel, R. L. 2006. Techniques for artificial insemination of cattle with frozen–thawed semen. Pages 253–258 *in* R. S. Youngquist and W. R. Threlfall, editors. Current therapy in large animal theriogenology, 2nd edition. Saunder Elsvier, St. Louis, Missouri.

Riley, K. L., E. J. Chesney, and T. R. Tiersch. 2008. Field collection, handling, and refrigerated storage of sperm of Red Snapper and Gray Snapper. North American Journal of Aquaculture 70:356–364.

Riley, K. L., C. G. Holladay, E. J. Chesney, and T. R. Tiersch. 2004. Cryopreservation of sperm of Red Snapper (*Lutjanus campechanus*). Aquaculture 238:183–194.

Roppolo, G. S. 2000. Techniques for the commercial-scale production of cryopreserved sperm from aquatic species. Louisiana State University, Baton Rouge.

Rurangwa, E., D. E. Kime, F. Ollevier, and J. P. Nash. 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture 234(1–4):1–28.

Thorpe, J. E., G. A. E. Gall, J. E. Lannan, C. E. Nash, and B. Ballachey. 1995. The conservation of aquatic resources through management of genetic risks. Pages 33–46 *in* J. E. Thorpe, G. A. E. Gall, J. E. Lannan, and C. E. Nash, editors. Conservation of fish and shellfish resources: managing diversity. Academic Press, San Diego, California.

Tiersch, T. R. 2011. Shipping of refrigerated samples. Pages 690–691 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Tiersch, N. J., W. M. Childress, and T. R. Tiersch. 2018. Three-dimensional printing of vitrification loop prototypes for aquatic species. Zebrafish 16:252–261.

Tiersch, T. R., C. R. Figiel, W. R. Wayman, J. H. Williamson, G. J. Carmichael, and O. T. Gorman. 1998. Cryopreservation of sperm of the endangered Razorback Sucker. Transactions of the American Fisheries Society 127:95–104.

Tiersch, T. R., and J. A. Jenkins. 2003. Biosecurity considerations for cryopreserved gametes and early life stages of aquatic species. Pages 171–198 *in* C.-S. Lee and P. J. O'Bryen, editors. Biosecurity in aquaculture production systems: exclusion of pathogens and other undesirables. World Aquaculture Society, Baton Rouge, Louisiana.

Tiersch, T. R., and W. T. Monroe. 2016. Three-dimensional printing with polylactic acid (PLA) thermoplasic offers new opportunities for cryobiology. Cryobiology 73:396–398.

Torres, L., E. Hu, and T. R. Tiersch. 2016. Cryopreservation in fish: current status and pathways to quality assurance and quality control in repository development. Reproduction, Fertility and Development 28:1105–1115.

Torres, L., and T. R. Tiersch. 2018. Addressing reproducibility in cryopreservation, and considerations necessary for commercialization and community development in support of genetic resources of aquatic species. Journal of the World Aquaculture Society 49:644–663.

Watson, C. A., K. H. Kilgore, and C. V. Martinez. 2010. Shipping fish in boxes. Southern Regional Aquaculture Center, Publication 3903, Stoneville, Mississippi.

Wayman, W. 1996. Refrigerated storage and cryopreservation of sperm from fishes of the family Scianidae. Louisiana State University, Baton Rouge.

Wayman, W. R., R. G. Thomas, and T. R. Tiersch. 1996. Cryopreservation of sperm of Spotted Seatrout (*Cynoscion nebulosus*). Gulf Research Reports 9:183–188.

Wayman, W. R., R. G. Thomas, and T. R. Tiersch. 1997. Refrigerated storage and cryopreservation of Black Drum (*Pogonias cromis*) spermatozoa. Theriogenology 47:1519–1529.

Wayman, W. R., and T. R. Tiersch. 2011. Research methods for cryopreservation of sperm. Pages 672–683 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Wynne, F., and W. A. Wurts. 2011. Transportation of warmwater fish: equipment and guidelines. Southern Regional Aquaculture Center, Publication 390, Stoneville, Mississippi.

Yang, H., L. Hazlewood, S. J. Heater, P. A. Guerrero, R. B. Walter, and T. R. Tiersch. 2007. Production of F_1 interspecies hybrid offspring with cryopreserved sperm from a live-bearing fish, the Swordtail *Xiphophorus helleri*. Biology of Reproduction 76:401–406.

Zajicek, P. W., J. E. Hill, N. Stone, H. Thomforde, C. Ohs, D. Cooper, G. Flimlin, B. McLane, and W. D. Anderson. 2009. Preventing hitchhiking nonindigenous species in live shipments. Southern Regional Aquaculture Center, Publication 3902, Stoneville, Mississippi.

Appendix: AGGRC Mobile Laboratory Site Assessment Form

AGGRC Mobile Laboratory Site Assessment Form

Name: _____ Title: _____

Address: _____ Email:_____

City: _____ State/Zip: _____ Phone:_____

Type of work: ☐ Aquaculture ☐ Research ☐ Biomedical ☐ Endangered Species ☐ Other _____

Species: _____

Freezing Purpose: _____

Please see the FAQ on the back to help fill out the following.

1. Number of males available for collection? _____ ☐ Unknown

2. Will eggs be fertilized with the cryopreserved material? ☐ Yes ☐ No ☐ Unknown
   A. Dates of fertilization? _____ ☐ Unknown
   B. What is the expected percent fertilization? ☐ 0%-25% ☐ 25%-50% ☐ 50%-75% ☐ >75%

3. Who will be responsible for processing the fish? ☐ AGGRC ☐ Facility personal ☐ Unknown
4. Is there space inside a building to set up laboratory equipment? ☐ Yes ☐ No ☐ Unknown
   A. If yes, would you allow us to set up laboratory equipment inside? ☐ Yes ☐ No ☐ Unknown

5. Is there access to power outside the building? ☐ Yes ☐ No
   A. If yes, would you allow us to use your facility`s power? ☐ Yes ☐ No

6. Is there access to a 220 volt outlet? ☐ Inside ☐ Outside ☐ Neither

7. Is there access to water outside the building? ☐ Yes ☐ No
   A. If yes, would you allow us to use your facility`s water? ☐ Yes ☐ No

8. Do you have the ability to order liquid nitrogen? ☐ Yes ☐ No ☐ Unknown

9. Do you have space and equipment to store the cryopreserved material? ☐ Yes ☐ No ☐ Unknown

10. Do you have or have access to any of the following?

	Yes	No
1) Osmometer		
2) Spectrophotometer		
3) Microscope		
4) 20x objective (200x total)		
5) Dark field		
6) Counting chamber		
7) Flow cytometer		
8) Liquid nitrogen		
9) Dry ice		
10) Water bath		
11) Dewar		
12) Computer-controlled freezer		
13) Data logger with type t thermocouple		

Any additional information:

_____

_____

_____

New Species Cryopreservation Suitability Survey Questions

Name: _____
Institution: _____
Email: _____

Type of work: ☐ Aquaculture ☐ Research ☐ Biomedical ☐ Endangered Species ☐ Other _____
Type of Tissues: ☐ Sperm ☐ Blood ☐ Eggs ☐ Embryos ☐ Larvae ☐ Other _____
Species: _____

Section 1 – Husbandry and Life Cycle

1) What is the size of your animal population and do you routinely kill animals? _____
_____

2) What is the average body size of adult animals? _____

3) What size tanks are adult animals housed in? _____

4) What is the salinity of the water used to hold and breed the organism? _____

5) What is the temperature range of the organism throughout the life cycle? _____

6) What is the feeding regimen for adults? _____

7) Do you work with the entire life cycle? _____

8) How long is the life cycle? _____

Section 2 – Spawning and Early Life History

1) Does the organism spawn seasonally? If so, what is the spawning season? _____
_____

2) What is the spawning temperature of the organism? _____

3) Do you perform early life stage culture? _____

4) What are the major stages and duration of larval development? _____
_____
_____

5) What is the temperature ranges for early life stages? _____

6) What is the feeding regimen for early life stages? _____

7) What is the size of embryos? _____

8) What is the size of larvae? _____

9) Do you reproduce the animals by natural spawning? If no, how? _____
_____

Section 3 – Gametes and Cryopreservation

1) Can you collect gametes? _____

2) Can you perform in vitro fertilization? _____

3) What is the volume of sperm collected per male? _____

4) What is the sperm concentration per male? _____

5) How many eggs can be collected per female? _____

6) What is the average size per egg? _____

7) What is the number of eggs usually assigned per mating? _____

8) Can gametes be stored after collection and before fertilization? For how long? _____

9) Can gametes be shipped? If so, how are they shipped? _____
_____

10) Can mature animals be shipped? _____

11) Has any cryopreservation research been performed on this organism? _____
_____

12) Do any cryopreservation protocols exist for this organism? _____

13) Do you use cryopreservation at your facility? If no, why? _____
_____

14) What container do you use to freeze? _____

15) Can you collect blood? _____