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Design and Cost Analysis of a Self-contained Mobile Laboratory for Commercial-scale Aquatic Species Cryopreservation

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Abstract

Although aquatic species cryopreservation protocols have been studied around the world over the past 60 yr., germplasm repository development efforts and commercialization have begun only recently. The goal of this project was to develop a self-contained mobile laboratory for on-site high-throughput cryopreservation of aquatic species. The objectives of this study were to: (1) identify how a mobile laboratory would function in different operational scenarios, (2) customize an enclosed cargo trailer to function as a mobile laboratory, (3) evaluate the laboratory layout and ability of cryopreservation equipment to operate from generator power, and (4) document the investment costs for private and public groups to integrate a mobile laboratory into an existing cryopreservation facility at three levels of automation and estimate the total cost per trip based on hypothetical assumptions for two scenarios (aquaculture production and repository development). There were three operational designs identified for the mobile laboratory: (1) self-contained work inside the unit using generator power, (2) work inside the unit using external facility power, and (3) using the equipment inside of a host facility. The investment costs for a base-level mobile laboratory ranged between US$5670 and US$5787 for private groups and between US$5208 and US$5315 for public groups. With the addition of a range of automated processing equipment, total investment costs ranged from US$13,616 to US$103,529 for private groups and US$12,494 to US$94,891 for public groups. The total cost per trip to cryopreserve sperm of 59 blue catfish, *Ictalurus furcatus*, males to produce 6300 0.5-mL French straws was estimated to range from US$6089 to US$14,633 for private and between US$5703 and US$16,938 for public groups depending on the level of automation. Total cost per trip to cryopreserve sperm of 500 males of five different species in the genus *Xiphophorus* to produce 641 0.25-mL French straws was estimated to range from US$6653 to US$7640 for private and US$7582 to US$8088 for public groups depending on level of automation. Overall, a commercial-scale mobile laboratory was developed that can assist current germplasm activities and support future repository and industry development, and the layout information provided can help others to design and build comparable units.

KEYWORDS

aquatic species, cryopreservation, mobile laboratory, repository development

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In recent years, cryopreservation has been increasingly recognized as an important tool for aquatic species in aquaculture, biomedical research, wild fisheries, and imperiled species (Tiersch et al. 2007; Cabrita et al. 2010). Most published cryopreservation studies have been performed in specialized laboratories located kilometers from a river, ocean, or fish hatchery. When working with fish farms, hatcheries, or stock centers, fish usually are either transported live to a cryopreservation facility and held until they are ready to be processed, or sperm is collected and shipped to the cryopreservation facility. Physiological stress to the animals during transport can increase the possibility of a disease outbreak or spreading of pathogens from a source facility to the cryopreservation facility and can reduce gamete quality (Hagedorn et al. 2009). When collecting samples in the field, time constraints or remote locations often limit the ability to ship these samples, and if they are shipped, quality can be degraded, affecting cryopreservation or causing the samples to be discarded.

On-site cryopreservation of germplasm in nitrogen-vapor shipping dewars has been used for more than 35 yr to address these problems (Gwo 1994; Wayman et al. 1996). Shipping dewars contain an adsorbent material that is designed to hold several liters of liquid nitrogen without the risk of spilling if the dewar is tipped over. These dewars typically have a narrow neck (diameter between 51 and 216 mm) and an overall height of between 470 and 671 mm. In usual practice, French straws or other containers are placed in an upper or lower plastic goblet attached to aluminum canes, which are placed into a metal canister that is positioned within the shipping dewar for freezing. Differential placement of the goblet on the cane provides the ability to adjust the cooling rate. From 1990 to 2006, the World Fisheries Trust (WFT) focused exclusively on field cryopreservation, collecting sperm from wild stocks of salmonids, *Oncorhynchus nerka*, *Oncorhynchus tshawytscha*, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, and *Salmo salar*, and black cod, *Anopoploma fimbria*, in North America and the conservation of migratory fish species of the genera *Salminus*, *Leporinus*, *Piaractus*, *Brycon*, *Pseudoplatystoma*, and *Prochilodus* in South America (Harvey 2011). In 1995 and 1996, the WFT cryopreserved samples from 750 sockeye salmon that totaled 2000 French straws representing 15 stocks by use of a field cryopreservation kit containing basically cryoprotectant solutions, straws, shipping dewars, and field notebooks (Harvey et al. 1998; Harvey 2011). Despite the previous application of this method, there are still many limitations to on-site cryopreservation with shipping dewars. Due to the size of shipping dewars, these studies are small scale and can produce only tens of samples per day (Blundell and Ricketson 1979). The freezing temperatures that can be maintained and achieved are also affected by the size of the dewar, the number of samples being frozen (due to the cumulative heat load), and the amount of liquid nitrogen remaining in the dewar (Wayman and Tiersch 2011).

Over the past 15 yr there have been successful adoptions of equipment and processes used in high-throughput livestock cryopreservation for aquatic species (Roppolo 2000; Lang et al. 2003; Hu et al. 2011). This equipment can include automated straw packagers and computer-controlled freezers. Automated packagers are able to fill and seal 0.25- or 0.5-mL French straws at a rate of as many as approximately 15,000 per hour depending on the model (e.g., MPP Quattro, www.minitube.com). Programmable freezers are suited to handle the large output of straws from the automated packagers and can automatically control the temperature inside the cooling chamber, yielding uniform freezing. Other equipment used during cryopreservation can include spectrophotometers to estimate sperm concentration and flow cytometers to analyze membrane integrity. However, these instruments are delicate, expensive, and not easily transportable.

Since beginning in 2000, the US Department of Agriculture (USDA) Agricultural Research Service (ARS) National Animal Germplasm Program in Fort Collins, Colorado, holds 99,683 aquatic species samples in inventory, but those samples only represent 48 species of the estimated 33,000 finfish species, with six of those

48 species being marine (Animal-GRIN, https://www.ars-grin.gov/). For a germplasm repository to be effective, there must be proper representation of species and lines. Those samples (tens to hundreds of thousands) also need to be of high quality. If on-site cryopreservation is going to be an effective tool for multiple users, a customizable approach needs to be developed for the use of high-throughput and specialized equipment in the field with the same quality control as a central facility.

The goal of this study was to develop a self-contained mobile laboratory for on-site high-throughput cryopreservation of aquatic species. The objectives of this study were to: (1) identify how a mobile laboratory would function in different operational scenarios, (2) customize an enclosed cargo trailer to function as a mobile laboratory, (3) evaluate the laboratory layout and ability of cryopreservation equipment to operate from generator power, and (4) document the investment cost for private and public groups to integrate a mobile laboratory into an existing cryopreservation facility at three levels of automation and estimate the total cost per trip based on hypothetical assumptions for two scenarios (aquaculture production with large-bodied fishes and repository development with aquarium fishes). In this study, the mobile laboratory that was developed could address three different operational scenarios, was completely self-contained, and produced samples with quality control equivalent to a central facility. The capability developed as part of this research is the first example of integrating high-throughput cryopreservation equipment with mobile capability to process aquatic species germplasm on site and opens the door for commercialization and outreach aimed at assisting new users in the field.

Materials and Methods

Operational Setup Scenarios

Three operational scenarios were identified for the mobile aquatic cryopreservation laboratory to address: (1) using space in an existing facility, (2) using the mobile laboratory with on-site power, and (3) using the mobile laboratory with generator power. Within Scenario 1, equipment and supplies were brought into the on-site facility and unpacked for processing and freezing. Within Scenario 2, the mobile laboratory was connected to on-site power and did not rely on a generator. Depending on location, some processing or freezing could be performed inside an on-site facility or in a portable structure. Within Scenario 3, power was provided exclusively by a generator and all processing and freezing were performed inside the mobile laboratory or outside under a portable structure. A fourth scenario, "backpack," was identified that excluded the use of the trailer. Although this scenario was not covered in the project, this has been a traditional approach used in previous studies for on-site cryopreservation. For this scenario, only essential equipment and supplies would be brought to the location and all processing and freezing would be performed outside.

Laboratory Structure and Design Considerations

A custom-built $3.8 \times 1.7 \times 2.2$ m ($L \times W \times H$) singe-axle (1590 kg capacity), fully enclosed cargo trailer was purchased in 2013 (Pro Pull Trailers, Baton Rouge, LA, USA) to provide the structure for the mobile cryopreservation laboratory (Fig. 1). Cargo trailers are usually available from local vendors and can be customized with readily available over-the-counter components. Simple construction allows for low cost of maintenance and repairs if needed. The trailers can also be towed and maneuvered using a towing-capable vehicle and be disconnected at the working location for safe parking. The mobile cryopreservation laboratory had 6.58 m^2 of floor space and was cooled by a rooftop air conditioner (Airxcel, Inc., Wichita, KS, USA). A breaker box containing 15- and 20-amp breakers was used to distribute power. The 15-amp breaker controlled a standard 120-V US wall outlet and a 1.2-m fluorescent light. The 20-amp breaker controlled the air conditioner. Because the floor and walls were made of wood, the entire inside was coated in a waterproofing wood protector (Thompson's water seal®; Thompson's Company, Cleveland, OH, USA) to prevent water damage. The trailer

FIGURE 1. *The enclosed cargo trailer (3.8 × 1.7 × 2.2 m; L × W × H) showing the side door, rear fold-down door, and rooftop air conditioner.*

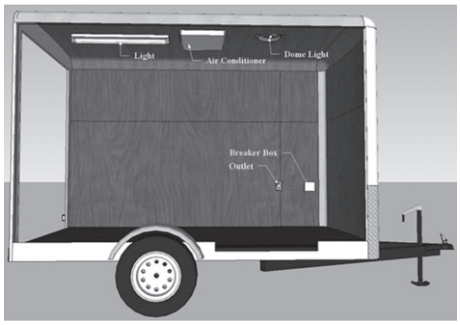

FIGURE 2. *SketchUp three-dimensional rendering of the empty enclosed trailer with internal electrical components. This computer-assisted design model can be scaled and rotated to evaluate various layout configurations.*

came equipped with a standard 0.8 × 1.9 m ($W \times H$) door located on the right wall (hinged toward the front) and a rear 1.6 × 2 m ($W \times$ H) bottom-hinged spring-assisted ramp door. The ramp door allowed equipment to be easily loaded and unloaded from the trailer, and the side door allowed for access during operation. A pair of support legs located at the rear of the trailer added stability when equipment was loaded and unloaded, and was used to level the trailer when parked on sloped ground.

Several factors were considered during the design process for the inside layout of the mobile laboratory. The most important factor was floor space. A laboratory workbench and other storage options were needed to process and freeze inside. There also needed to be sufficient storage space for samples to enable high-throughput production. In addition, enough room was needed for two or more technicians to work comfortably. During transportation to and from working locations, the laboratory had to serve as a cargo trailer, securing all equipment and supplies and protecting them from vibration. Because there was a wide range of available amenities (e.g., power, water) at every working location, the mobile laboratory needed to be flexible and, if necessary, completely self-contained. For freezing, a portable liquid nitrogen cylinder needed to be transported to each location. Safety concerns necessitated provision of the same working environment of a central laboratory, including ventilation of nitrogen gas and an oxygen meter. Proper protective measures would be required when transporting expensive and delicate equipment. To assist design, the trailer was rendered to scale using computer-aided design software (SketchUp, 2015, version 15.0.9351; Trimble Navigation, Sunnyvale, CA, USA; Fig. 2). Various components (e.g., workbench, shelves, and table) were modeled into the available trailer space to evaluate different layouts.

Laboratory Evaluation

The mobile laboratory was initially tested in the parking lot of the Aquatic Germplasm and Genetic Resources Center and electricity was provided by a 5.5-kW portable generator (Generac GP5500; Generac Power Systems, Inc., Waukesha, WI, USA). A variety of electrical components were used to test the generator power: (1) 1.2-m florescent light, (2) roof-top air conditioner, (3) dark-field microscope (Optiphot-2, Nikon, Garden City, NY, USA), (4) automated straw packager (MRS1, reference number: 020072; IMV Technologies, Paris, France), (5) a 1.6-amp vacuum pump (Air Admiral, Cole-Parmar, Vernon Hills, IL, USA), and (6) a commercial-scale programmable freezer (Micro Digitcool, reference number: 007261; IMV Technologies). For the duration of the trial, the fluorescent light, air conditioner, and microscope were continuously operated to simulate field operation. The dome light was not used. The automated straw packager, vacuum pump, and programmable freezer were turned on and off as needed. Because fresh sperm was unavailable at the time of testing, common carp, *Cyprinus carpio*, sperm frozen in 0.5-μL

French straws (reference number: 005569; IMV Technologies) were thawed for 8 sec in a water bath (Model 1141; VWR, Radnor, PA, USA) at 40 C for testing of microscopy (thawing was not performed inside the mobile laboratory). With all equipment running, 1 μL of unactivated sperm was placed onto a counting chamber (Makler®; Sefi-Medical Instruments, Haifa, Israel) and examined using the dark-field microscope at 200× magnification to determine if vibration was occurring that could affect motility estimates. A common sperm diluent, Hanks' balanced salt solution (HBSS) at an osmolality of 300 mOsmol/kg (HBSS300: 0.137 M NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, and 5.55 mM glucose, pH 7.2) was packaged using the automated straw packager and frozen in the programmable freezer at a cooling rate of 20 C/min to simulate the packaging and freezing of sperm.

Cost Analysis Assumptions

To date, there have only been two studies that examine the cost of integrating central cryopreservation activities into an existing fish hatchery (Caffey and Tiersch 2000; Caffey and Tiersch 2011), although economic models have been used in the aquaculture industry for decades (Shang 1990; Landau 1992). In a previous study, a modified cost analysis model was created to help estimate how capital costs (items purchased once) and operating costs (items purchased often) would change at different levels of cryopreservation production for public and private fish hatcheries (Caffey and Tiersch 2000). In the present study, a modified cost analysis model was created to estimate what the capital costs would be for an existing cryopreservation facility to construct a mobile laboratory at different levels of automation and to estimate the total cost per trip based on hypothetical assumptions for public and private groups.

Three production scenarios (manual, semi-automated, and fully automated) were created based on the level of automated equipment utilized. The manual scenario assumed that no automated high-throughput equipment was utilized. French straws were hand filled and sealed by two operators, and 30 straws were frozen at a time in liquid nitrogen vapor using a polystyrene box and a three-dimensional floating platform (Hu et al. 2017). The semi-automated scenario assumed that operators were hand filling and sealing straws, but a programmable freezer was utilized that could freeze as many as 2760 samples per freeze. The fully automated scenario assumed that an automated straw packager (capable of filing and sealing 3600 straws per hour) and a programmable freezer were utilized. Capital costs were generated for construction of the mobile laboratory and for the items needed to achieve these different levels of automation. The assumption was made that the cryopreservation facility already possessed some standard cryopreservation equipment that did not need to be bought for the mobile laboratory (e.g., microscope, pipettes, and others). Miscellaneous costs (unexpected costs during operation) were calculated as 5% of budgeted items. It was assumed that private groups would finance their initial investment with a 10-yr loan at a 12% annual percentage rate (APR). There is not yet an established commercial industry in the United States for cryopreservation of aquatic germplasm; therefore, a higher APR was chosen because of the higher-risk investment. Private groups would also pay 10% local sales tax on all purchases. The straight-line method was used to calculate the depreciation for capital investment items and was set between 5 and 10 yr (10–20% per year) with no salvage value depending on the estimated useful life (Landau 1992). For all costs, a mean price was calculated, when possible, from as many as three price quotes from different equipment and supply vendors (i.e., VWR Scientific, Radnor, PA, USA; Grainger, Lake Forest, IL, USA; Thermo Fisher Scientific, Waltham, MA, USA; USA Scientific, Ocala, FL, USA) in 2016 and 2017 (Table 1).

Two general lists of assumptions were developed to estimate the total variable costs for a single hypothetical cryopreservation trip for aquaculture production (Table 2) and a trip for repository development (Table 3). For aquaculture production, channel catfish × blue catfish hybrids were chosen as the target application and the desired annual production was set to

TABLE 1. *Supplies and equipment (>US$1000) used for cryopreservation processing.*[a]

Items	Price 1	Price 2	Price 3	Avg.
Ingredients for HBSS (ACS grade, 500 g each)				
NaCl	$23	$38	$25	$29
KCL	$40	$32	$46	$40
$CaCl_2 \cdot 2H_2O$	$60	$32	$33	$41
$MgSo_4 \cdot 7H_2O$	$29	$72	$83	$61
Na_2HPO_4	$57	$50	$43	$50
KH_2PO_4	$38	$44	$37	$39
Na_2HCO_3	$19	$23	$19	$21
$C_6H_{12}O_6$	$32	$20	$39	$30
Cryoprotectants (500 mL each)				
Dimethyl sulfoxide (DMSO)	$32	$50	$37	$40
Methanol	$11	$20	$11	$14
Glycerol	$41	$39	$52	$44
12″ Tweezers	$8	$8	$11	$9
Air vacuum (20' Hg)	$205	$248	$346	$266
Automated packager (1 straw)	$40,000	$32,980	–[b]	$36,490
Automated packager (4 straws)	$60,000	$57,161	$104,161	$73,774
Centrifuge tubes (15 mL, case of 500)	$179	$123	$143	$148
Centrifuge tubes (50 mL, case of 500)	$204	$175	$382	$253
Controlled-rate freezer	$36,490	$47,796	$19,879	$34,722
Cryo-gloves	$202	$183	$216	$200
Data logger (hand-held, 5 inputs)	$161	$151	$161	$157
Exam gloves (100 gloves per box)	$28	$23	$25	$25
Generator (5500 watts or more)	$689	$485	$579	$584
High capacity shipping dewar (35 L)	$1430	$1651	$1670	$1584
Kimwipe (60 boxes, 280 wipes per box)	$127	$210	$202	$180
Laboratory table	$396	$502	$523	$474
Liquid nitrogen freezing tank (120 L)	$2512	$3366	$3595	$3158
Liquid nitrogen transfer hose	$284	$245	$284	$271
Microcentrifuge tubes (1.5 mL, pack of 500)	$18	$14	$26	$19
Microcentrifuge tubes (5 mL, pack of 250)	$63	$77	$71	$70
Moving dolly (454 kg)	$16	$20	$20	$19
MRS1 0.25-mL disposable needle (box of 100)	$60	–[b]	–[b]	$60
MRS1 0.5-mL disposable needle (box of 60)	$50	–[b]	–[b]	$50
Nalgene bottles (1 L, case of 24)	$214	$199	$199	$204
Pipettor (100–1000)	$290	$389	$421	$367
Pipettor (10–100)	$290	$389	$421	$367
Pipettor (1–10)	$290	$389	$421	$367
Pipettor tips (100-1000 µL, pack of 1000)	$19	$37	$25	$27
Pipettor tips (1-10 µL, pack of 1000)	$19	$37	$43	$33
Pipettor tips (20-200 µL, pack of 1000)	$19	$14	$37	$23
Portable balance (4000 g max, 0.1 g readability)	$582	$582	$835	$666
Roller base for storage dewars	$240	$240	$245	$242
Safety goggles	$12	$13	$16	$14
Serological pipet (1 mL, pack of 1000)	$305	$148	$200	$218
Serological pipet (10 mL, pack of 500)	$252	$279	$165	$232
Serological pipet (25 mL, pack of 200)	$136	$257	$253	$215
Serological pipet (5 mL, pack of 500)	$171	$236	$242	$216
Serological pipetting device (10 mL)	$45	$49	$44	$46
Serological pipetting device (25 mL)	$61	$59	$46	$55
Shelving unit (0.9 × 0.5 × 0.9 m)	$60	$50	$60	$57
Sterile filters (0.22 µm, pack of 50)	$178	$168	$168	$171
Supply case	$59	$90	$100	$83
Syringe (1 mL – 100, pack of 100)	$56	$62	$60	$59
Syringe (3 mL – 100, pack of 100)	$37	$28	$33	$33
Table (3 m)	$65	$65	$97	$76
Tent (2.4 ×2.4 m)	$102	$115	$80	$99
Thermometer (digital, hand-held, −73 to 260 C)	$252	$260	$263	$258
Trailer	$3500	$2500	$2800	$2933
Type-T thermocouple (5 pack)	$59	$54	–[b]	$56
Utility cart (1 × 0.7 m)	$194	$129	$105	$143
Ziploc storage bags (quart, box of 48)	$4.46	$4.46	$4.00	$4.31

[a]The average (avg.) unit price is the mean of one to three price quotes from different vendors and equipment suppliers in 2016–2017. The equipment and supplies can be customized (i.e., automated packaging and freezing system) for cryopreservation depending on scale and level of sophistication. Items in this list were sorted alphabetically after the listing of Hanks' balanced salt solution (HBSS) ingredients and cryoprotectants.

[b]Additional prices not available.

5,000,000 sac fry for the 3-mo spawning period (May–July). Based on conservative assumptions of a 50% neurulation rate and 50% hatching rate, it was backward extrapolated that 20,000,000 eggs would be required (Hu et al. 2011). To determine the number of eggs produced from a single female, it was assumed that the average female weighed 1.2 kg and produced 8800 eggs per kilogram of body weight (Tucker and Robinson 1990). Therefore, a single female could produce 10,560 eggs, and it would require 1894 females to produce the required number of eggs. To determine the number of 0.5-mL French straws required for fertilization, we assumed that the sperm-to-egg ratio was 1.5×10^5 and the desired sperm concentration per milliliter was 1.0×10^9 (Hu et al. 2011). Using these ratios, one straw could fertilize 3333 eggs and 3.17 straws would be needed to fertilize the eggs of a single female, resulting in 6000 total straws required (Hu 2012). To estimate the number of males required, it was assumed the average male weighed 6 kg and had a testis weight of 14 g (our unpublished data). For blue catfish, the testes are crushed with HBSS at an osmolality of 300 mOsmol/kg (Hu et al. 2011). The ratio of HBSS300 (mL) to anterior testis (g) (Sneed and Clemens 1963) is 2:1, resulting in a total sperm suspension of approximately 28 mL. The desired sperm concentration before the addition of cryoprotectant was set at 2.0×10^9 and it was assumed that all samples were at this concentration (Hu et al. 2011). A methanol solution at 20% is mixed with each sperm sample at a 1:1 (v : v) ratio to yield a final volume of 56 mL, cryoprotectant concentration of 10%, and concentration of 1.0×10^9 cells/mL. It was assumed that there was a 5% waste during packaging, resulting in a total of 106 0.5-mL French straw per male. A 5% waste was also assumed during fertilization of eggs with cryopreserved straws, resulting in the need for a total of 60 males to produce 6300 straws.

For repository development, the genus *Xiphophorus* was chosen as the target organism based on our experience with these fishes and their value as a biomedical model (Yang and Tiersch 2009). To begin, it was assumed that 100 males from five different species would be processed during a trip and males would be pooled in various batches. Males were assumed to have an average body weight of 0.3 g and testis weight of 4.5 mg (Yang et al. 2006, 2009). For *Xiphophorus*, the testes are crushed in HBSS at an osmolality of 300 mOsmol/kg using a volume of 30 times the testis weight (Yang et al. 2007). This results in a sperm suspension of approximately 135 µL per male. The desired sperm concentration before the addition of cryoprotectant was set at 2.0×10^8 cells/mL. It was assumed that the average sperm concentration was 2.5×10^8, resulting in the need for an additional 34 µL to create a final volume of

TABLE 2. *Hypothetical assumptions for cryopreservation of blue catfish sperm for the aquaculture production of hybrid catfish (channel catfish female × blue catfish male).*

Description	Assumption
Target organism	Hybrid catfish
Target size (e.g., fry, fingerling, adult)	Sac fry
Annual production (number of fish)	5,000,000
Hatching rate (%)	50%
Neurulation rate (%)	50%
Total number of eggs required	20,000,000
Avg size female (kg)	1.2
Average eggs per kg of body weight	8800
Average spawn per fish (number of eggs)	10,560
Number of females needed	1894
Egg-to-sperm ratio	1:150,000
Desired sperm concentration per milliliter	1.0×10^9
Number of eggs fertilized per straw	3333
Number of 0.5-mL straws needed	6000
Number of 0.5-mL straws per female	3
Average size male (kg)	6
Average testis weight (g)	14
Extender-to-testis weight ratio (mL : g)	2:1
HBSS osmolality (mOsmol/kg)	300
Extended sperm volume/male (mL)	28
Desired sperm concentration	2.0×10^9
Average sperm concentration	2.0×10^9
Additional HBSS needed (mL)	0
Total adjusted sperm volume/male (mL)	28
Cryoprotectant (type)	Methanol
Cryoprotectant (active %)	10
Cryoprotectant-to-extended sperm ratio (v : v)	1:1
Packaging waste (%)	5
Total final sample volume per male (mL)	53
Straw size (mL)	0.5
Number of straws per male	106
Usage waste (%)	5
Number of males	59
Total number of 0.5-mL straws	6300

HBSS = Hanks' balanced salt solution.

TABLE 3. *Hypothetical assumptions for cryopreservation of multiple species in the genus* Xiphophorus *for repository development.*

Description	Assumption
Target species	*Xiphophorus* spp.
Number of males	500
Number of males pooled	Variable
Average size male (g)	0.3
Average testis weight (mg)	4.5
HBSS osmolality (mOsmol/kg)	300
Extender-to-testis weight ratio (µL:mg)	30:1
Extended sperm volume/male (µl)	135
Desired sperm concentration	2.0×10^8
Average sperm concentration	2.5×10^8
Additional HBSS needed for concentration adjustment (µL)	34
Total adjusted sperm volume/male (µL)	169
Cryoprotectant (type)	Glycerol
Cryoprotectant (active %)	14
Cryoprotectant ratio to extended sperm	1:1
Processing waste (%)	5
Straw size (mL)	0.25
Number of straws per male	1.3
Total number of 0.25-mL straws	641

HBSS = Hanks' balanced salt solution.

187 µL. A glycerol solution at 28% is mixed with each sperm pooling at a 1:1 (v : v) ratio to yield a final cell concentration of 1.0×10^8 and cryoprotectant concentration of 14% (Yang et al. 2007). After assuming a 5% processing waste, each male was estimated to create a total volume of 321 µL, resulting in 1.3 French straws (0.25 mL) on average per male.

For each hypothetical trip, total variable costs were calculated using a spreadsheet model by estimating labor, travel, and supplies (Table 4) for each production scenario (manual, semi-automated, and fullyautomated). Parameters were established based on current cryopreservation methods used in the laboratory and previous research. Labor costs comprised hourly wages and fringe benefits for research associates and technicians, meal *per diem* (public group only), and lodging. It was assumed that hourly wages would be the same regardless of private and public, but fringe benefits would differ. Fringe benefits were based on the average compensation for public and private industry workers in 2017 according to the Bureau of Labor Statistics (BLS 2017). Technicians were defined as permanent employees with or without a bachelor's degree. Technicians help with daily tasks and experiments within the laboratory. Researchers were defined as permanent employees with at least a bachelor's degree but typically holding a master of science or doctoral degree. Researchers supervise the technicians and lead projects or research grants. For the aquaculture production scenario, it was assumed that the staff of the aquaculture facility were responsible for dissecting the males and giving the sperm samples to the cryopreservation crew, which consisted of one research associate responsible for quality control and overall management and two technicians responsible for filling, freezing, and sorting straws. All cryopreservation activities were assumed to take place inside the mobile laboratory using generator power. For the repository development scenario, the cryopreservation crew was responsible for also dissecting males. The crew for this scenario consisted of one research associate for quality control and overall management and three technicians. Two technicians were responsible for dissection only and one technician dissected fish and assisted the research associate during the freezing process as needed. All cryopreservation activities were assumed to take place inside an existing laboratory or stock center. It was assumed that each individual had their own hotel room. For the aquaculture production, it was estimated that it would take 14 d to process 60 blue catfish using the manual scenario, 5 d for semi-automated, and 3 d for fully automated. For repository development, it was estimated that it would take 5 d to process 500 *Xiphophorus* for all levels of automation, but more male pooling would be needed for the manual scenario as only 30 straws could be frozen at a time compared to using a computer-controlled freezer. All estimations assumed a total of 2 d for round-trip travel. For travel, the round-trips were set randomly at 500 miles with a vehicle *per diem* of 51 US cents per mile. The cost of labor for initial gathering and loading of equipment and supplies into the mobile laboratory and the final unloading and cleaning were also included in this analysis. It was assumed one technician would take 1 d for gathering and loading of equipment and supplies

TABLE 4. *Spreadsheet model assumptions for total variable costs associated with individual hypothetical cryopreservation trips.*

Variable description	Mean value	Source/Justification
Research associate labor cost ($/h)	$20.00	Hourly wage for research associates
Technician labor cost ($/h)	$13.00	Hourly wage for technician
Private fringe benefit (%)	30.4%	Average private 2017 fringe
Public fringe benefit (%)	37.3%	Average public 2017 fringe
Labor days	Variable	Based on trip length
Labor hours per day	8	Common workday
Meal *per diem* ($/day/meals)	$56.00	LSU travel guide (PM-13)
Lodging ($/night)	$100.00	Variable
Lodging nights (number)	Variable	Based on trip length
Travel distance (miles round trip)	500	Proximity of the destination
Vehicle *per diem* (cents/mile)	$0.51	LSU travel guide (PM-13)
Generator fuel use (g/h)	0.72	Generator specifications
Daily hours of generator use	Variable	Based on electricity availability
Generator fuel ($/gallon)	$2.21	Average 2016 gas price
Straws ($/straw)	$0.06	Table 1
Daisy goblet ($/unit)	$8.73	Table 1
HBSS-300 mOsmol/kg ($/L)	$0.61	Table 1
CF-HBSS-200 mOsmol/kg ($/L)	$0.39	Table 1
CF-HBSS-1000 mOsmol/kg ($/L)	$2.01	Table 1
DMSO ($/mL)	$0.08	Table 1
Methanol ($/mL)	$0.03	Table 1
Glycerol ($/mL)	$0.09	Table 1
Pipettor tips (1–10 µL) ($/unit)	$0.03	Table 1
Pipettor tips (20–200 µL) ($/unit)	$0.02	Table 1
Pipettor tips (100–1000 µL) ($/unit)	$0.03	Table 1
Centrifuge tubes (15 mL) ($/unit)	$0.30	Table 1
Centrifuge tubes (50 mL) ($/unit)	$0.51	Table 1
Microcentrifuge tubes (1.5 mL) ($/unit)	$0.04	Table 1
Microcentrifuge tubes (5 mL) ($/unit)	$0.28	Table 1
Serological pipet (1 mL) ($/unit)	$0.22	Table 1
Serological pipet (5 mL) ($/unit)	$0.43	Table 1
Serological pipet (10 mL) ($/unit)	$0.46	Table 1
Serological pipet (25 mL) ($/unit)	$1.08	Table 1
Nalgene bottle ($/unit)	$8.50	Table 1
Thermocouple ($/unit)	$11.27	Table 1
Safety goggles ($/unit)	$13.57	Table 1
12″ Tweezers ($/unit)	$9.00	Table 1
Syringe (1 mL) ($/unit)	$0.59	Table 1
Syringe (3 mL) ($/unit)	$0.33	Table 1
Sterile filters ($/unit)	$3.42	Table 1
Exam gloves ($/unit)	$0.25	Table 1
Kimwipes ($/unit)	$0.01	Table 1
MRS1 0.5-mL disposable needle ($/unit)	$0.84	Table 1
MRS1 0.25-mL disposable needle ($/unit)	$0.60	Table 1
Ziploc storage bags (quart) ($/unit)	$0.09	Table 1

DMSO = Dimethyl sulfoxide; HBSS = Hanks' balanced salt solution.

and 1 d for final unloading and cleaning. These are conservative estimates as an experienced technician could perform these duties in less time. Supply costs included gasoline for the generator if used, French straws, chemicals, plastic-ware items, and other miscellaneous items needed for cryopreservation (Table 4). As above, a mean price was calculated, when possible, from as many as three price quotes from different supply vendors in 2016 and 2017. Private groups were assumed to pay a 10% tax on supply and hotel purchases. Both private

and public groups had a contingency (incidental expense) set at 10%.

The total cost per trip was estimated by adding the total variable cost per trip to the total fixed cost. The total fixed cost for the private group was based on the required loan amount as described above. The total fixed cost for a public group was based on the depreciation value of the capital investment items. Allocating fixed costs was difficult because of the unknown number of trips that would be taken annually. For simplicity, it was assumed that 12 cryopreservation trips would be made in a year, resulting in 1/12 of the annual fixed cost allocated per trip. If a different number of trips were taken per year, proportional fixed costs would have to be allocated per trip. Other estimations, such as breakeven price and gross revenue were also calculated. Breakeven price is the cost-per-straw needed to cover the total cost per trip and was estimated by dividing the total cost per trip by the number of straws produced. Gross revenue is the total revenue received before any deductions (e.g., cost per trip) and was estimated by multiplying the final cost per straw after a 50% nominal profit margin (percent markup of the breakeven cost-per-straw) was added by the total number of straws produced.

Results

Operational Procedures

A schematic flow chart was created to aid in the decision-making process of choosing to perform cryopreservation at the central facility or on site (Fig. 3). To determine if it would be more cost and quality efficient to ship or pick up, three factors were considered: (1) is the location ≥3 h away; (2) if shipped, would there be a ≥50% motility rate; and (3) how many males are being processed. If on-site cryopreservation was chosen, additional questions about the availability of liquid nitrogen and on-site capabilities would be used to determine which supplies were needed and to select the best operational plan.

Layout and Operation

In preparation for a trip, a Mobile Laboratory Client and Site Assessment form would be filled out providing information about the purpose of the trip (e.g., repository development, aquaculture production) and the on-site facilities available. A master checklist would be used to gather equipment and supplies based on the objectives identified (e.g., organism, number of males) and characteristics of the cryopreservation site (e.g., availability of electric power, laboratory space, and equipment available) and package them inside custom-sized plastic transport cases (Pelican 1660; Pelican Products, Torrance, CA, USA; reference number: 184011099 and 109,099,399; Tractor Supply, Co., Brentwood, TN, USA) or polystyrene (styrofoam) boxes. The day before departure, all items would be loaded into the trailer and secured using wall and floor tie downs (Fig. 4). A push cart (Uline, Pleasant Prairie, WI, USA) and 454-kg capacity moving dolly (Haul Master; Harbor Freight Tools, Calabasas, CA, USA) would be used to load and unload large items. Upon arrival, all equipment and supplies would be unpacked and assembled inside the trailer or inside an on-site facility. The number of technicians utilized for each trip would vary based on the number of fish being processed and who would be responsible for collecting the sperm samples. Some hatcheries would use their own technicians to collect sperm samples, requiring only two or three technicians for cryopreservation. During freezing, the doors to the trailer would always be open and a low-oxygen alarm ($MaxO_2{}^+A$; Maxtec, Salt Lake City, UT, USA) would be utilized. After all samples were processed, equipment and supplies would be reloaded into the mobile laboratory and transported back to the central facility where they would be unloaded, cleaned, and stored to await the next trip.

The trailer layout was chosen to maximize countertop and floor space (Fig. 5). A wooden laboratory workbench ($1.2 \times 0.5 \times 0.9$ m; $W \times D \times H$) with cabinet storage and drawers was permanently secured to the left wall at 0.7 m from the front of the trailer. This workbench had a $1.5 \times 0.6 \times 3$ cm countertop. A three-tiered metal shelving unit ($0.9 \times 0.5 \times 0.9$ m) was positioned at the front of the trailer to provide additional storage space. Adhesive magnetic locks (Dreambaby, Sydney, Australia) were

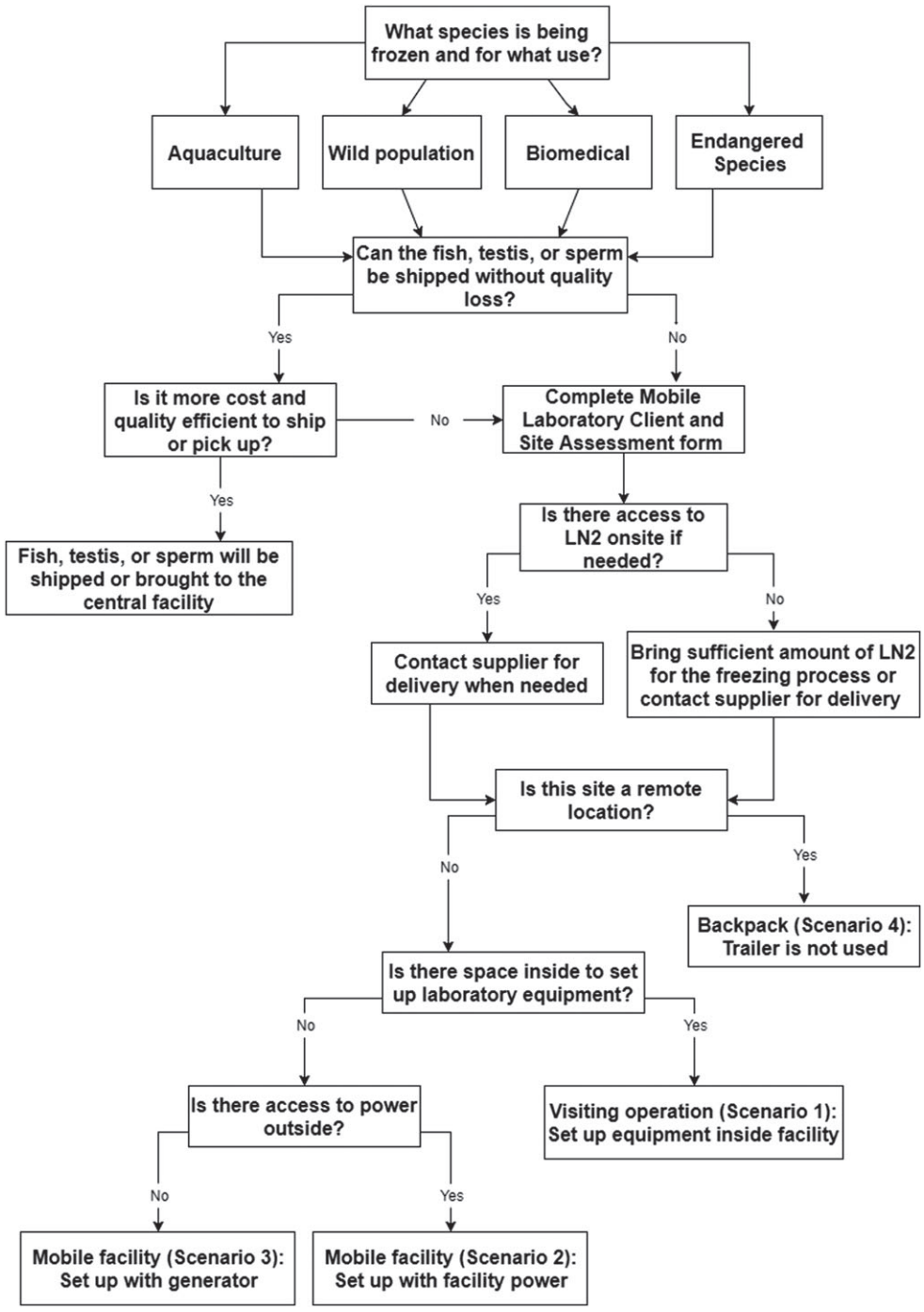

FIGURE 3. *Steps in the decision-making process used to compare cryopreservation at a central facility or on site. The chart begins with identification of the organism being cryopreserved and the user group. Next, it is determined if the species or sperm samples can be shipped or transported to the central facility and if it would benefit quality and cost efficiency to do so. If not, questions about capabilities of the on-site facilities are used to subsequently decide which operational scenario would be most appropriate. LN2 = liquid nitrogen.*

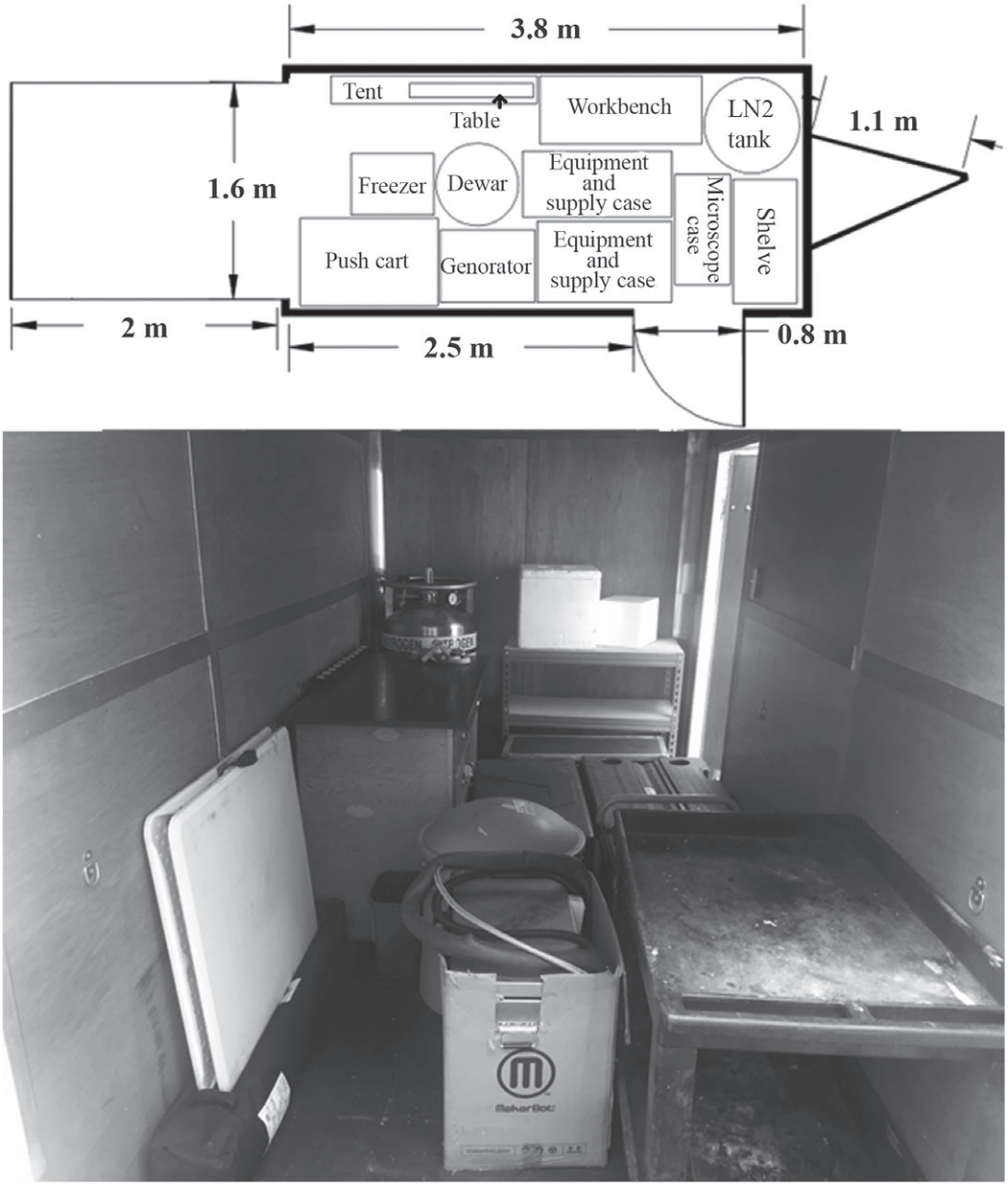

FIGURE 4. *Blueprint (top view) and photograph (taken from the rear door) of equipment and supplies loaded inside the trailer for transportation. Wall and floor hooks were used to secure down items with ratchet straps. Heavy items were loaded in the front of the trailer for proper weight distribution.*

added to the workbench to keep the cabinet and drawers from opening while driving. A 1.8-m folding table (Rubbermaid, Atlanta, GA, USA) was used when needed to provide work space along the wall with the laboratory workbench. Use of a folding table provided floor space during transportation to and from working locations. A first-aid kit and eye-wash station were attached to the wall for emergencies. A fire extinguisher was stored in the cabinet of the

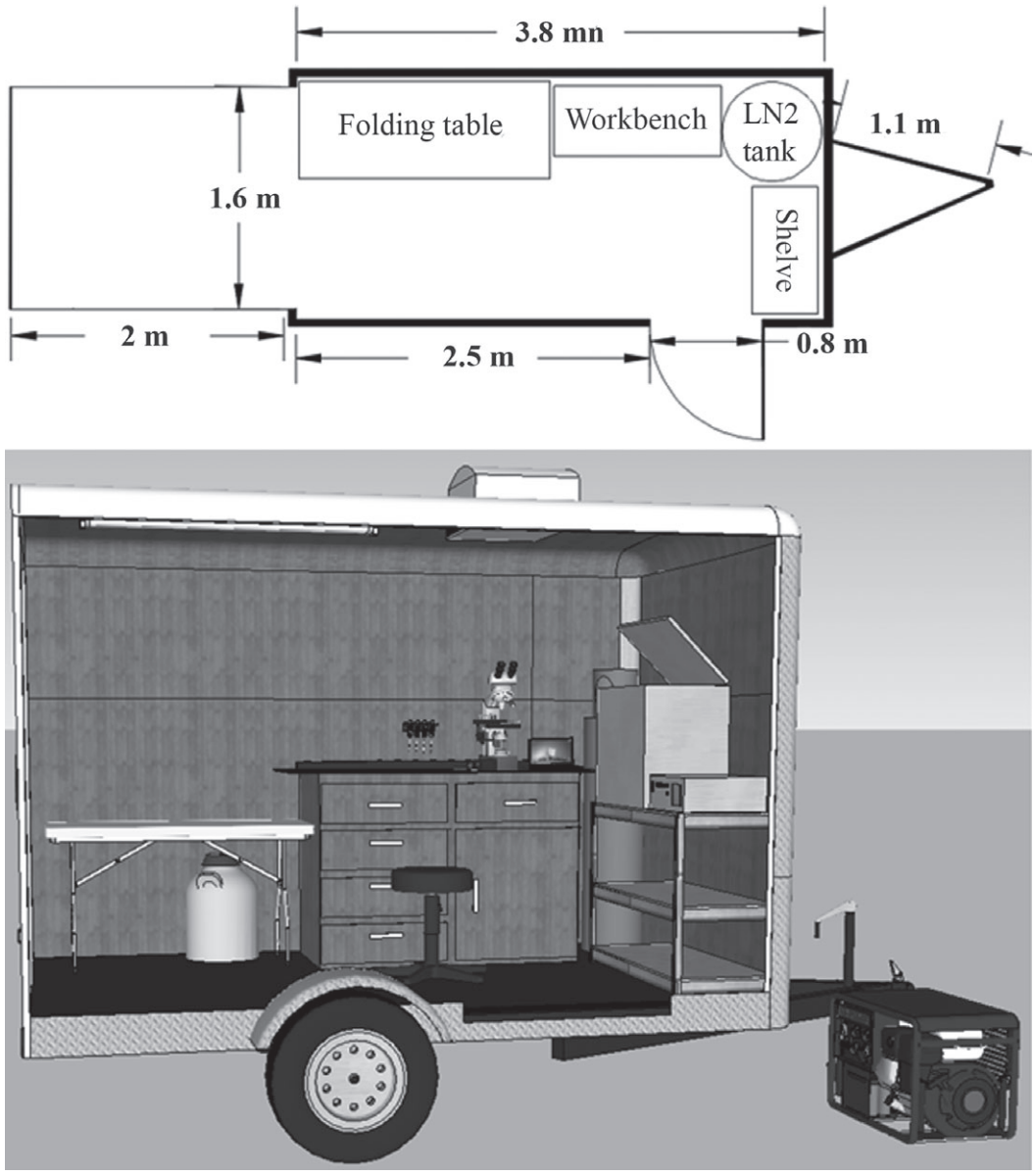

FIGURE 5. *Blueprint (top view) and SketchUp rendering of the layout inside the mobile laboratory when used for processing. This layout provided the most efficient use of floor space. Supplies were stored inside the workbench or on the metal shelf. Two or three people could work comfortably inside simultaneously.*

laboratory workbench and another was stored in the rear seat of the towing vehicle. Cryopreservation processing steps were followed at the remote sites as they would have been at a central facility (Fig. 6). A large liquid nitrogen cylinder (120-L Cryo-Cyl; Chart Industries, Inc., Ball Ground, GA, USA) was used to provide liquid nitrogen on site. This tank was maneuverable by one person and could supply enough liquid nitrogen for approximately 1 d or more depending on the usage level of the programmable freezer (Micro Digitcool, IMV Technologies, L' Aigle, France or IceCube 14M, Sy-Lab Cryobiology, Neupurkersdorf, Austria). Nitrogen-vapor

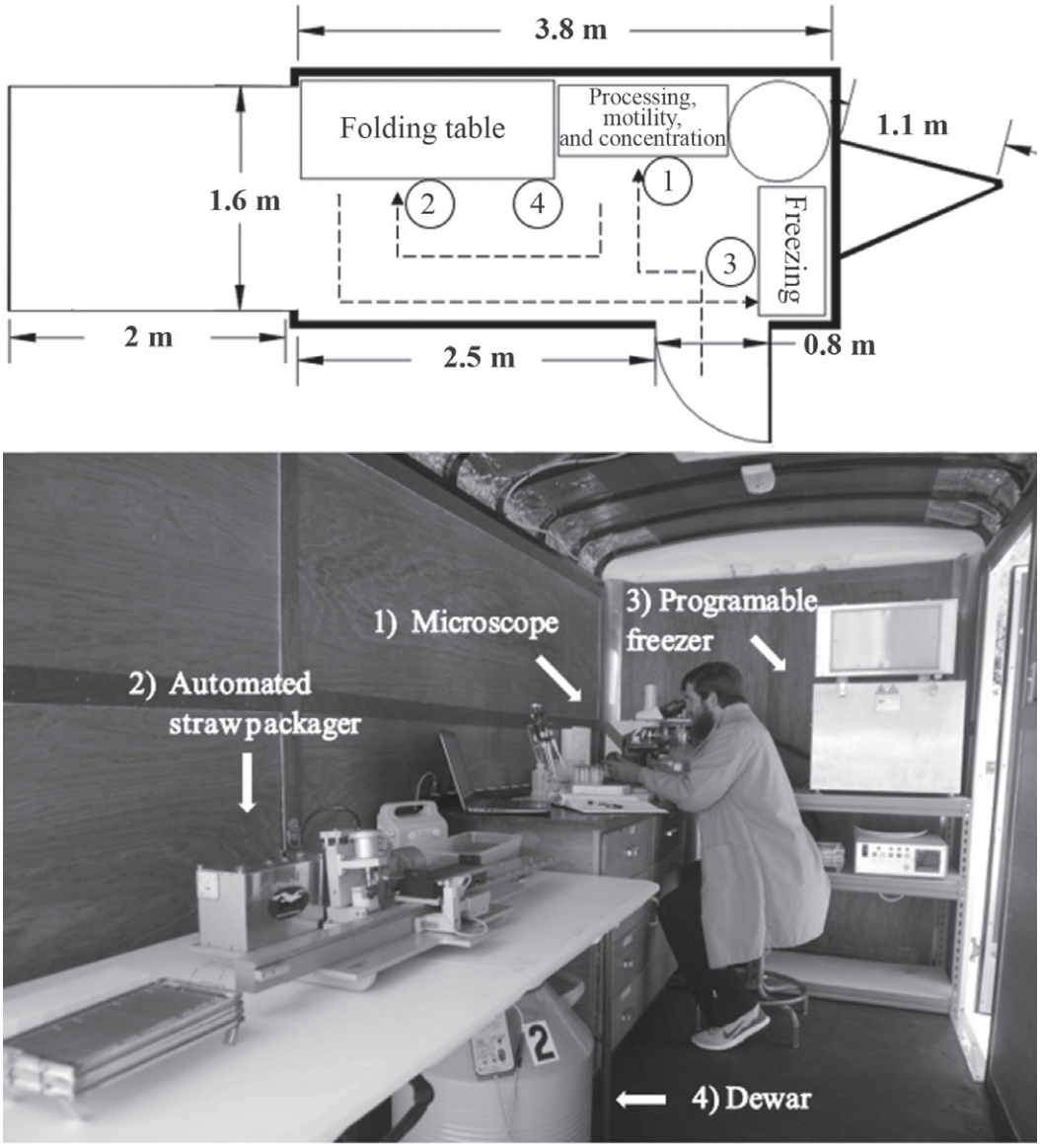

FIGURE 6. *Blueprint (top view) of the cryopreservation work stations and photograph of a technician with cryopreservation equipment. Samples entered the laboratory where they were (1) processed and assessed at the workbench, (2) equilibrated and packaged at the folding table, (3) frozen by cooling in the programmable freezer on the shelving unit, and (4) stored in the dewar.*

shipping dewars (MVE Cryomoo-ver; Chart Industries, Inc.) were used to safely store and transport samples back to the central facility. The shipping dewars could store as many as 14 full daisy goblets each (reference number: 015152; IMV Technologies), allowing a total of 3360 0.5-mL French straws or 6720 0.25-mL French straws to be stored. A 2.4×2.4 m ($L \times W$) canopy tent (E-Z UP, Norco, CA, USA) was available for working outside the trailer.

Laboratory Evaluation

The 5500 running watts provided by the generator were sufficient to power all equipment

TABLE 5. *Capital costs for assembly of a mobile laboratory into an existing cryopreservation facility at different levels of automation.*[a]

Item	Unit price	Useful lifetime (yr)	Level of automation		
			Manual	Semi-automated	Fully automated
Mobile laboratory					
Cargo trailer	$2933	7	$2933	$2933	$2933
Laboratory cabinet workbench	474	8	474	474	474
Shelving unit (0.9 × 0.5 × 0.9 m)	57	10	57	57	113
Tent (2.4 × 2.4 m)	99	6	99	99	99
Table (3 m)	76	10	76	76	76
Generator (5500 watts or more)	584	10	584	584	584
Utility cart (1 × 0.7 m)	143	10	143	143	143
Moving dolly (454 kg)	19	10	38	56	75
Subtotals			$4403	$4421	$4497
Miscellaneous (5%)			22	221	225
Depreciation			585	586	594
Sales tax (10%)[b]			462	464	472
Total investment (mobile laboratory only)					
Private			$5670	$5639	$5787
Public			$5208	$5229	$5315

[a]Unit price (US$) is the mean of 2–3 price quotes from various vendors and equipment suppliers in 2016 and 2017.
[b]Only applied to private group.

simultaneously and could operate for 10 h on one tank (27.3 L) of gasoline. There was no vibration detected from the generator when using the microscope inside of the trailer and straws were packaged and frozen properly.

Investment and Trip Costs

Investment costs were expressed as those being required for the mobile laboratory (Table 5) or required for processing (Table 6) at three levels of automation. Mobile laboratory costs were primarily for the furniture-type items needed to provide sufficient storage and workspace capable of processing the required number of straws per day. Investment costs for the mobile laboratory ranged from US$5670 to US$5787 for private groups and US$5208 to US$5315 for public groups. After processing equipment was included, capital investment costs ranged from US$13,616 to US$103,529 for private groups and US$12,494 to US$94,891 for public groups. For the hypothetical aquaculture production scenario, the total variable cost ranged from US$4603 to US$14,438 for private groups and US$4993 to US$16,832 for public groups (Table 7). After the addition of fixed costs, the total cost per trip ranged from US$6089 to US$14,663 for private groups and US$5703 to US$16,938 for public groups. The breakeven cost per straw ranged from US$0.97 to US$2.3 for private groups and US$0.91 to US$2.7 for public groups. The gross revenue ranged from US$9133 to US$21,950 for private groups and US$8554 to US$25,407 for public groups.

The total variable cost for the hypothetical repository development scenario ranged from US$6147 to US$6458 for private groups and US$7372 to US$7475 for public groups (Table 8). With the addition of fixed costs, the total cost per trip ranged from US$6653 to US$7640 for private groups and US$7582 to US$8088 for public groups. For private groups, the breakeven cost per unit ranged from US$10.37 to US$11.91, and for public groups it ranged from US$11.82 to US$12.61. The gross revenue ranged from US$9980 to US$11,459 for private groups and US$11,372 to US$12,132 for public groups.

Discussion

High-throughput processing and automated equipment for livestock sperm cryopreservation have been adopted into protocols for shellfish

TABLE 6. *Capital costs for processing equipment and assembly of a mobile laboratory for integration into an existing cryopreservation facility at different levels of automation.*[a]

Item	Unit price	Useful lifetime (yr)	Level of automation		
			Manual	Semi-automated	Fully automated
Processing equipment					
High-capacity shipping dewar (35 L)	$1584	10	$1584	$1584	$1584
Supply case	83	10	83	166	249
Controlled-rate freezer	34,722	10	–[b]	34,722	34,722
Automated packager	36,490	10	–[b]	–[b]	36,490
Vacuum pump (20' Hg)	266	10	–[b]	–[b]	266
Liquid nitrogen freezing tank (120 L)	3158	10	3158	3158	3158
Liquid nitrogen transfer hose	271	10	271	271	271
Thermometer (digital, hand-held, −73 to 260 C)	258	5	258	258	258
Portable balance (3000–4000 g max, 0.1 g readability)	666	10	666	666	666
Data logger (hand-held, 5 inputs)	157	10	157	–[b]	–[b]
Serological pipetting device (10 mL)	46	10	46	46	46
Serological pipetting device (25 mL)	55	10	55	55	55
Cryo-gloves	200	5	200	401	601
Subtotals			$6278	$40,926	$77,765
Miscellaneous (5%)			314	2046	3888
Depreciation			694	4199	7923
Sales tax (10%)[c]			659	4297	8165
Total investment (trailer and processing)					
Private			$13,616	$57,161	$103,529
Public			$12,494	$52,399	$94,891

[a]Unit price (US$) is the mean of 2–3 price quotes from various vendors and equipment suppliers in 2016 and 2017.
[b]Equipment not needed.
[c]Only applied to private group.

(e.g., Pacific oyster, *Crassostrea gigas*, and Eastern oyster, *Crassostrea virginica*) (Dong et al. 2007; Yang et al. 2012) and multiple fish species (Roppolo 2000; Lang et al. 2003; Hu et al. 2016). With the growing demand for cryopreservation and high-throughput processing of aquatic species germplasm (Tiersch et al. 2012; Asturiano et al. 2016), a self-contained approach is needed that can be scaled to accommodate the needs of specific user groups (e.g., aquaculture, biomedical research, imperiled species, or wild fisheries). Aquaculture groups include private hatcheries, and federal laboratories such as USDA-ARS. Biomedical groups include university research laboratories, and stock centers such as the Zebrafish International Research Center (University of Oregon, Eugene, OR, USA). Imperiled species groups include zoos and aquariums, and federal laboratories such as those within the US Fish and Wildlife Service. Wild fisheries groups include state game and fisheries agencies and the National Oceanic and Atmospheric Administration. Currently, most cryopreservation processing begins by transporting the organism or sperm samples to a centralized facility. During transportation, these organisms can die or be compromised due to poor water quality or disease issues, or sperm samples can be degraded by temperature fluctuations, mishandling, or biological limitations (Watson et al. 2010; Tiersch 2011; Wynne and Wurts 2011).

Recreational charter fishing boats and fishing tournaments have proven to be a viable source for the collection of sperm samples from a variety of saltwater species (Caylor et al. 1994; Roppolo 2000; Riley et al. 2004). Often anglers can provide high-quality samples from a diversity of species by use of traditional icing and storage methods (Riley et al. 2008). The bottlenecks in the processing of such samples have been the location and throughput. Charter boats and tournaments are often located in remote locations and anglers often do not return to the dock until

TABLE 7. *Total variable costs, total fixed costs, and total cost per trip for the hypothetical aquaculture production scenario (for hybrid catfish) at three levels of automation.*

	Level of automation		
Items	Manual	Semi-automated	Fully automated
Labor			
Researchers (private and public)	$2240	$800	$480
Technicians (private and public)	$2928	$1056	$640
Fringe benefits (private)	$1571	$564	$340
Fringe benefits (public)	$1928	$692	$418
Travel			
Vehicle use	$255	$255	$255
Lodging	$3900	$1200	$600
Per diem (public only)	$2352	$840	$504
Supplies (private and public)			
Fuel (generator)	$165	$51	$25
Extender	$4	$4	$4
Cryoprotectant	$9	$9	$9
Straws	$618	$618	$618
Plastic ware	$399	$399	$399
Other supplies	$503	$490	$587
Subtotals			
Labor (private)	$6739	$2420	$1460
Labor (public)	$7096	$2548	$1538
Travel (private)	$4155	$1455	$855
Travel (public)	$6507	$2295	$1359
Supplies (private and public)	$1699	$1571	$1642
Private contingency (10%)	$1259	$545	$396
Public contingency (10%)	$1530	$641	$454
Supply and hotel sales tax (10%)	$585	$303	$250
Total variable cost			
Private	$14,438	$6293	$4603
Public	$16,832	$7055	$4993
Total fixed cost			
Private (loan based)	$195	$820	$1485
Public (depreciation)	$107	$399	$710
Total cost per trip			
Private	$14,633	$7113	$6089
Public	$16,938	$7454	$5703

evening. This can result in samples not being shipped until the day after collection or cryopreservation personnel having to work long hours. After samples arrive at the cryopreservation facility, only small amounts of sperm can be cryopreserved in a day without commercial high-throughput capabilities. The availability of on-site high-throughput cryopreservation removes these constraints and allows for multiple high-quality samples to be processed on the same day the fish or sperm are collected. This work demonstrates that an enclosed cargo trailer can be customized for use as a functional mobile laboratory with the use of routine furniture products (i.e., workbench, tent, shelving unit) from readily available sources, and a generator can be used to power equipment when facility electricity is not available. This provides the ability to operate in three different scenarios that are commonly encountered while working on site.

As indicated above, the use of widely available furniture products allows flexibility in assembling mobile laboratories. With an initial investment cost of approximately US$13,616 for private groups and US$12,494 for public groups to construct a mobile laboratory with manual capability, more opportunities can be created to

TABLE 8. *Total variable costs, total fixed costs, and total cost per trip for the hypothetical repository development scenario (for* Xiphophorus *spp.) at three levels of automation.*

Items	Level of automation		
	Manual	Semi-automated	Fully automated
Labor			
Researchers (private and public)	$800	$800	$800
Technicians (private and public)	$1768	$1768	$1768
Fringe benefits (private)	$777	$777	$777
Fringe benefits (public)	$958	$958	$958
Travel			
Vehicle use	$255	$255	$255
Lodging	$1600	$1600	$1600
Per diem (public only)	$1120	$1120	$1120
Supplies (private and public)			
Fuel (generator)	$0	$0	$0
Extender	$1	$1	$1
Cryoprotectant	$3	$2	$2
Straws	$45	$45	$45
Plastic ware	$143	$61	$61
Other supplies	$103	$92	$98
Subtotals			
Labor (private)	$3345	$3345	$3.345
Labor (public)	$3526	$3526	$3526
Travel (private)	$1855	$1855	$1855
Travel (public)	$2975	$2975	$2975
Supplies (private and public)	$295	$201	$207
Private contingency (10%)	$550	$540	$541
Public contingency (10%)	$680	$670	$671
Supply and hotel sales tax (10%)	$215	$206	$206
Total variable cost			
Private	$6260	$6147	$6154
Public	$7475	$7372	$7379
Total fixed cost			
Private (loan based)	$195	$820	$1485
Public (depreciation)	$107	$399	$710
Total cost per trip			
Private	$6455	$6967	$7640
Public	$7582	$7771	$8088

collect germplasm from new sources. Depending on the needs of the user group, automated processing equipment (automated straw packagers and computer-controlled freezers) can be purchased. Incorporating automated equipment will significantly increase the initial capital investment costs (private groups would have a higher loan amount and public groups would have an increase in depreciation costs), but the ability to process more samples in a day can help decrease labor and travel costs per trip. In addition, cost per unit of production decreases as the level of production increases until a certain point where production is maximized and the cost per unit no longer decreases (Caffey and Tiersch 2000). For the aquaculture production scenario, the total variable cost per trip to cryopreserve 60 catfish was estimated to be US$14,438 for private groups and US$16,832 for public groups with manual capability. With fully automated capability, it was estimated to require 3 d compared to 14 d for manual processing, decreasing labor costs from US$6739 to US$1460 for private groups and US$7096 to US$1538 for public groups. Travel costs also decreased from US$4155 to US$855 for private groups and US$6507 to US$1359 for public groups. For the repository development scenario,

the total cost per trip increased from manual operations to fully automated operation for both groups. Total variable cost decreased from manual to fully automated as more straws could be frozen at a time, but the fixed cost also increased, resulting in a higher total trip cost. This indicated that groups working with small-bodied fish producing small volumes of sperm (only one or two straws per male) such as zebrafish, *Danio rerio*, which is utilized in more than 1200 laboratories around the world (ZFIN, www.zfin.org), could restrict investment to a manual, low-throughput mobile laboratory. Another difference between the processing of large fishes and small fishes is the breakeven cost per straw. In the hypothetical processing of 60 blue catfish, 6300 straws were produced with the lowest breakeven of US$0.91 per straw. With a nominal 50% margin, each straw would cost US$1.36 for the consumer. For the processing of 500 *Xiphophorus*, only 641 straws were produced with the lowest breakeven of US$10.37. With the same margin, each straw would cost US$15.55. There are still many unknowns concerning development of markets for the commercialization of aquatic germplasm, such as the willingness of customers to pay for the services or the product (sperm in straws), and what the profit margin should be set at. Instead of pricing by the straw, consumers could be charged by the number of males, the services provided, or other factors relevant to their applications. Livestock cryopreservation has become a multibillion-dollar global industry while aquatic application has remained largely at the research scale despite each being first achieved around the same time more than 60 yr ago (Blaxter 1953; Hu et al. 2011). This success can be attributed to the general acceptance among livestock groups that cryopreserved germplasm can be used for substantial steady genetic gains and the routine availability of custom collection services. It was long ago accepted that the costs for cryopreservation in livestock were small in comparison to the gains in overall profitability (Herman 1981).

Currently, custom collection services are only beginning to be offered for aquatic species. For livestock, these programs offer services to collect, process, store, and ship sperm, and these services provide the basis for the global market. Customers simply drop off their animal (e.g., an elite bull) and the collection facility provides the services necessary to satisfy customer requirements. This includes required health screening for each bull before collection to prevent the spread of diseases. Additional health testing is often required for customers wanting to export material to other countries. Depending on the customer's needs, the sum of these services can be contracted for hundreds to thousands of dollars per bull. Custom collection services are also available for other animals such as bucks (e.g., whitetail, www.greatlakessireservice.com) at similar prices. Once collected, semen from bulls, bucks, or stallions can be purchased by the straw with prices ranging from US$10 to ≥US$10,000. These are considered to be reasonable market-driven expenses offset by the genetic gains that are routinely produced by the use of improved germplasm. There is currently no established basis as to what profit margin should be applied for aquatic cryopreservation services or how to place a value on preserved germplasm. Mobile laboratories could provide an effective platform for development of a commercial industry for aquatic germplasm. A mobile laboratory with high-throughput on-site cryopreservation capabilities could offer the opportunity for custom collection businesses to open and offer cryobanking services for commercial aquaculture or the management of imperiled species (Lang et al. 2003; Asturiano et al. 2016) without the initial need to maintain a large, costly physical facility.

Currently, a number of factors, including lack of standardization, minimal available training, and the perception of high costs continue to prevent cryopreservation from being integrated into new user groups and programs for aquatic species (Torres et al. 2016). Outreach could be a powerful demonstration tool that mobile laboratories can provide to improve technical diffusion into new user groups. Standardized training programs, such as those provided by Certified Semen Services, Inc. (CSS; http://www.naab-css.org, accessed 28 December 2016) in the livestock semen industry, could be developed within aquatic species communities and could

be offered as a traveling service to standardize cryopreservation methods and devices among new and current user groups. Novel user-friendly freezing devices, such as those recently developed with new three-dimensional printing technologies, could be readily incorporated into such training and production programs (Tiersch and Monroe 2016). Such freezing devices are inexpensive, can be standardized across users, and can help new users gain access to the field (Hu et al. 2017). This approach of standardization could help link users and researchers to provide a pathway to assist in aggregate high-throughput production for repository development from within emerging germplasm communities (Torres et al. 2016; Engle 2017).

Conclusions

In this study, a self-contained mobile laboratory was developed to provide high-quality industry-scale processing comparable to a specialized central facility. A cost spreadsheet model was also created as a decision-making tool for the commercial application of aquatic cryopreservation. This tool allows users to input multiple variables related to production assumptions and organism-specific information to help estimate costs of cryopreservation services. The resulting mobile laboratory can be operated at a wide variety of locations, using the same equipment and following the same protocols that are used at a central facility, and can collect high-quality data for sperm (e.g., motility, concentration), blood, or other tissue samples, and environmental conditions (e.g., water quality, location) that central facilities and field cryopreservation studies were previously often unable to collect. As more mobile laboratories are built, a genetic resources community can be created, sharing improvements and helping to standardize or harmonize efficient sample collection and processing that can lead to quality assurance programs and industry-wide quality control. With this mobile platform, samples can be processed and cryopreserved within hours of collection, reducing variability due to effects of storage and transportation (Torres et al. 2016). This approach is also intended to provide a platform to assist repository development and provide access to genetic resources that could not be adequately collected, protected, and distributed using current methods.

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