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Cost Analysis for Integrating Cryopreservation into an Existing Fish Hatchery

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Abstract

Fish sperm cryopreservation is documented by a large body of technical research. However, there are no reports of the economic requirements for using this technology in aquatic species. This study establishes a generic analysis of the investment and operating costs required to integrate sperm cryopreservation into existing fish hatcheries and can serve as a template for implementation of cryopreservation programs. Equipment and supplies were identified in a species-independent description of sperm cryopreservation, and capital and operating costs were documented for private and public hatcheries at three production capacities (3,000, 6,000, and 9,000 0.5-mL straws). Compared to public hatcheries, investment costs were found to be 70% higher, and operating costs 20% higher for private hatcheries due primarily to interest on borrowed capital. Equipment costs were dependent on the scope of work. Investment in required equipment incurred costs of $5,460 to $10,458 (public) or $9,497 to $18,190 (private), depending on production level. Purchase of optional research equipment increased initial investment by 300% without increasing output. Per unit costs decreased at higher production levels for all scenarios, but greater economies of scale were associated with private research hatcheries. Production costs ranged between $6.13 and $1.86 per straw (private), to $1.59 to $1.18 per straw (public). Increased commercialization is expected to occur as research protocols for fish sperm cryopreservation are applied in the private sector and markets for cryopreserved sperm are established.

Cryopreservation of fish sperm has been researched for the last 40 yr, with the majority of studies reported during the present decade. To date, over 200 reports exist for cryopreservation research in more than 65 species of fish (Fiegel and Tiersch 1997). Much of this research was based on potential applications in aquaculture, fisheries management, and genetic conservation. While cryopreservation techniques continue to emerge for fish sperm, most studies are characterized by a purely technical approach and there are currently no published reports that address the economic requirements of these applications.

One possible explanation for the lack of economic analysis is the perceived tremendous variation in cryopreservation protocols, among and within species. Despite the lack of standardization, most protocols employ similar equipment and share procedures. This study identifies these general characteristics and documents those costs associated with integrating a basic sperm cryopreservation program into existing fish hatcheries. Our specific objectives included: 1) describing generic activities of fish sperm cryopreservation; 2) depicting a range of integration scenarios for model fish hatcheries; 3) documenting costs for capital outlay and operating expenditures; and 4) demonstrating how such costs would vary between public and private hatcheries at various production levels. The resulting analysis constitutes the first cost projections documented for the application of cyropreservation technology with aquatic species.

Materials and Methods

Generic Activities

Generic activities of fish sperm cryopreservation (Fig. 1) were identified by a re-

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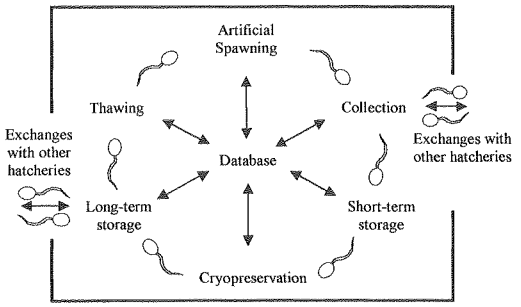

FIGURE 1. *Generic activities of fish sperm cryopreservation. Consecutive components are delineated by a clockwise flow of sperm and two-way arrows are used to indicate maintenance of a centralized database for information on motility, fertilization and inventory.*

view of current practices. To begin, sperm is collected on-site during artificial spawning by manual stripping or surgical gonad removal. Collection of sperm may also include field sampling and exchange of fresh sperm between hatcheries (disease screening could be added, but was not included in the present analysis). Collected sperm is typically diluted with salt solutions or "extenders" at an appropriate osmolality to prolong cell viability during short-term storage (Graybill and Horton 1969; Bates et al. 1996). Prior to cryopreservation, chemical cryoprotectants are added to preserve cell integrity during freezing and thawing. Freezing usually involves loading diluted (extended) sperm into plastic straws of 0.5 mL to 5.0 mL in volume. Samples are typically cryopreserved using one of three methods: 1) placement of straws on dry ice; 2) placement of straws into vessels containing cryogenic nitrogen vapor or dry ice; or 3) by use of a controlled-rate freezer. After cryopreservation, samples are placed in dewars that contain liquid nitrogen (LN) for long-term storage. Cryopreserved sperm is thawed to evaluate cell viability or motility and for use in artificial spawning. Finally, information collected during each activity would be stored in a centralized database and used for refining subsequent protocols (the costs for the maintenance of the database were not included in this analysis).

Scenarios for Integration

Certain activities of fish sperm cryopreservation require specialized equipment and supplies. However, much of the equipment is not exclusive to cryopreservation and may be found at a fish hatchery depending on range of effort. Additionally, methods for cryopreservation can range from inexpensive and simple to costly and complex. Therefore, the cost of investing in cryopreservation ultimately depends on the scope of a hatchery and the level of sophistication desired for a cryopreservation program (e.g., from pure production to pure research). Costs within this range may be further delineated by whether the hatchery is privately owned or publicly operated.

Few private fish hatcheries utilize sperm cryopreservation in the United States; however, commercial application should increase as protocols are refined. Adoption of the technology could occur among the large-scale suppliers of fry and fingerlings to the sport-fishing and aquaculture industries. These commercial operations could eventually utilize and market frozen sperm and provide cryopreservation-based services such as collection and storage. Such operations already exist in the dairy and pork industries where producers routinely rely on cryopreserved sperm as a source of improved germplasm, and the distribution of cryopreserved sperm is a large, multimillion dollar industry by itself. These commercial operations evolved from breeder's clubs and state-run cooperatives that provided artificial insemination (Herman 1980). A similar industry for fish sperm could develop along a comparable trajectory. Such development might see sperm products and services originally provided by public hatcheries with increasing commercialization resulting as protocols are applied and refined and markets for frozen fish sperm are developed.

The decision criteria for investing in

cryopreservation are different between private and public hatcheries. Public hatcheries are often financed by state or federal budgets and usually have more freedom to invest in projects with unknown technical and economic feasibility. Public hatcheries thus comprise a broader range of effort, ranging from small state-run hatcheries servicing put-and-take fisheries, to large-scale research hatcheries such as the Regional Fisheries Technology Centers operated by the U. S. Fish and Wildlife Service.

Cost Analysis Assumptions

The costs of equipment and supplies for fish sperm cryopreservation can be estimated using a modified cost analysis. Such analyses are traditionally used to evaluate the capital and operating expenditures required for specific changes in management or investments in technology (Shang 1990). However, fish sperm cryopreservation is currently non-commercial, and information is limited to the cost data generated by public research institutions. In this study we focus only on capital and operating costs and considering how these costs vary between public and private hatcheries. Capital and operational costs were generated for each generic activity (defined in Fig. 1) and expressed for public and private hatcheries at three levels of production.

The generic character of the analysis precludes estimation of fertilization units. Fertilization units are defined by species-specific parameters such as egg-to-sperm ratios, sperm concentrations, and dilution ratios for extenders and cryoprotectants. Instead, a "production unit" was used in this analysis and was defined as a single 0.5-mL plastic straw. Straws of this size are commonly used in fish sperm cryopreservation and with the sperm of other animals such as cattle. Maximum production capacity in the analysis was dictated by storage space defined as 3,000 production units per storage dewar and was calculated for 1, 2, and 3 dewars. At each production level, an additional storage dewar was budgeted for back-up purposes but was not included in the calculation of production capacity.

Capital expenditures ranged from the minimal investment for required equipment to the maximum investment for required and optional equipment. This range of expenditures provided hatcheries with a high and low investment boundary at each level of production capacity. It should be noted that existing hatcheries may possess some or all of the optional equipment. Miscellaneous costs were defined in investment and operating budgets as 5% of budgeted items. For this analysis, private hatcheries were assumed to finance their initial investment with a 5-yr intermediate loan at a 10% annual percentage rate (APR) and a charge of 12% APR for operating capital. Private hatcheries were also assumed to pay an 8% local sales tax on all purchases. Equipment depreciation was listed as "Facility Maintenance," and was calculated using a straight-line method at 10% per year with no salvage value. All prices (reported in $US) represent the mean of three commercial estimates collected from equipment and supply vendors in 1999 (i.e., Parsons Airgas, Southland Cryogenics, Tech Air, Sigma, Scientific Products, Curtis Matheson Scientific, and VWR Scientific). The resulting cost analysis is generic and independent of species, location, and application.

Results

Capital Outlay

Capital costs were classified as being required or optional to the cryopreservation process (Table 1). Required equipment included only those items used during cryopreservation, storage, and transport of fish sperm. The category consisted primarily of LN dewars and associated equipment. Although dewars are primarily used for long-term storage of sperm or for transport of frozen sperm, storage and shipping dewars can also be utilized for freezing of fish sperm. The basic program described in this

TABLE 1. *Capital costs for integrating cryopreservation into existing fish hatcheries.*

Item	Unit price	Storage capacity (0.5-mL straws) 3,000	6,000	9,000
Required equipment				
Storage dewar (35 L, high capacity)	$945	$1,890	$2,835	$3,780
Roller base for storage dewars	160	320	480	640
Low-level alarms (storage dewars, 115v)	435	870	1,305	1,740
Shipping dewar (4.3 L, spill proof)	565	1,130	1,695	2,260
Cases for shipping dewars	275	550	825	1,100
LN transfer hose and phase separator	190	190	190	190
Thermometer (digital, hand-held, ± 100 C)	250	250	250	250
Subtotals		$5,200	$7,580	$9,960
Miscellaneous (5%)		260	379	498
Sales tax (8%)		437	637	837
Interest on capital (10%)		3,600	5,248	6,895
Total investment (required equipment only)	Public	$5,460	$7,959	$10,458
	Private	$9,497	$13,843	$18,190
Optional equipment				
Pipettor (1–10 μL)	227	227	227	227
Pipettor (10–100 μL)	227	227	227	227
Pipettor (100–1,000 μL)	227	227	227	227
Water bath (8–16 L, temperature to 90 C)	1,212	1,212	1,212	1,212
Analytical balance (0.01 g readability, 1,500 g max)	1,249	1,249	1,249	1,249
Data logger (hand-held, 5 inputs)	1,350	1,350	1,350	1,350
Distilled water source (2L/h)	1,460	1,460	1,460	1,460
Vapor pressure osmometer (0–2,000 mOsmol/kg)	4,681	4,681	4,681	4,681
Laboratory microscope (dark field 200×)	7,181	7,181	7,181	7,181
Controlled-rate freezer	12,500	12,500	12,500	12,500
Subtotals		35,514	37,894	40,274
Miscellaneous (5%)		1,776	1,895	2,014
Sales tax (8%)		2,983	3,183	3,383
Interest on capital (10%)		22,766	24,291	25,817
Total investment (required and optional equipment)	Public	$37,290	$39,789	$42,288
	Private	$63,039	$67,263	$71,488

study purchased two high-capacity storage dewars (35-L capacity) mounted on rollers for ease of handling. One additional dewar was added at each additional level of production capacity. Each storage dewar was fitted with an audible alarm for detection of low LN levels. Two smaller shipping dewars (~5 L) were budgeted initially with one additional shipping dewar added at subsequent levels of production capacity. Shipping dewars were assumed to be used exclusively for field collection, freezing, and transport of frozen sperm and were not included in the calculations of storage and production capacity. Total investment for the required equipment ranged between $5,460 and $10,458 (public), and between $9,497 to $18,190 (private). The lower end of this cost range was defined as 3,000-unit production capacity and the upper end of the range represented a 9,000-unit production capacity.

Optional equipment (Table 1) included items that were not exclusive to the cryopreservation process. Investors could purchase some or all of the optional equipment depending on the level of sophistication desired. Purchase of optional equipment increased production quality but had no effect on production quantity. Optional items included three pipettors with volumes of 1 to 10 μL, 10 to 100 μL, and 100 to 1,000 μL. In conjunction with an analytical balance of 0.01 g readability, these pipettors provide

the precision necessary to accurately measure extender constituents. An alternative (although less flexible) means of producing extenders is available through the purchase of pre-mixed materials. A vapor pressure osmometer (Wescor 5500 or equivalent) is an accurate method for adjusting the extender to an osmolality required for maintaining sperm cells in an inactive state. Deionized water for use in sperm extenders can be purchased, or provided by laboratory water purification equipment. Existing hatcheries may possess an adequate microscope for viewing sperm samples; however, a laboratory-quality, dark-field microscope would be useful for evaluation of large numbers of samples. Hand-held data loggers with multiple inputs would be useful for temperature monitoring during freezing or storage. A temperature-controlled water bath is useful for consistent thawing of sperm samples, but is not absolutely necessary depending on the species and number of samples. The most expensive piece of optional equipment was a controlled-rate freezer. These allow precise control of cooling rates and offer the greatest benefit to research, but would be useful anywhere quality control is required. Costs for these start at $10,000 and increase to ~$15,000 based on available options. It is assumed that only the larger research hatcheries would invest in a controlled-rate freezer.

The total investment for all required and optional equipment ranged from $37,290 to $42,288 (public), and $63,039 to $71,488 (private) for production capacities of 3,000 to 9,000 straws. Compared to public hatcheries, the additional costs of taxes, depreciation, and interest resulted in 70% higher investment costs for private hatcheries at all three production levels. Most of this increase was due to interest, accounting for approximately 50% of total investment costs for private hatcheries.

Operating Expenditures

Annual costs were calculated for operating a cryopreservation program at an existing hatchery (Table 2). Supplies were budgeted for maximum production capacities of 3,000, 6,000, or 9,000 straws per yr. Five 0.5-mL straws are held in a standard 9-mm cylindrical plastic container called a goblet; two goblets can be mounted on an aluminum frame called a cane. Canes provide additional storage by utilizing vertical space in the dewar. Polyvinylchloride (PVC) powder was budgeted for sealing of straws. The quantity of supplies required to support each production level was estimated using a 5 to 1 to 0.5 ratio of straws to goblets to canes.

Chemical supply prices were based on 33%, 67%, or 100% of the price obtained for the bulk quantities described for each item. This fractioning allowed budgeting for volume discounts on items with a shelf-life of 3 or more years. For the extenders, reagent-grade chemicals were budgeted to provide ingredients sufficient for mixing of 50 L of Hanks' balanced salt solution (HBSS). Variations of HBSS have been used successfully with the sperm of multiple fish species (Tiersch et al. 1997) and these same ingredients can be used to formulate most other extenders used for fish sperm. Four commonly used cryoprotectants were budgeted: 1) dimethyl sulfoxide (DMSO); 2) dimethyl acetamide (DMA); 3) methanol, and 4) glycerol. Extracellular cryoprotectants such as egg yolk and milk are also commonly used but their costs were negligible and were not included.

Labor costs were based on the use of a part-time technician at $10 per hour. Labor costs reflected manual filling and freezing of individual straws; however, these costs may eventually decline with automation. Automated straw fillers and freezers have been developed for 0.5-mL straws and are used extensively for the cryopreservation of bovine sperm, but were not included in this analysis.

Annual operating costs ranged from $4,768 to $10,608 (public), and $5,768 to $12,831 (private) for straw capacities of 3,000 to 9,000. Private hatcheries incurred

TABLE 2. *Annual operating costs for integrating cryopreservation into existing fish hatcheries.*

Item	Unit price	Storage capacity (0.5-mL straws)		
		3,000	6,000	9,000
Straws (0.5 mL)	0.06	180.00	360.00	540.00
Goblets	0.26	156.00	312.00	468.00
Canes	0.21	16.38	32.76	49.14
Sealing powder (PVC)	42.00	13.99	28.14	42.00
Ingredients for HBSS (ACS grade) 500 g each				
NaCl	23.38	7.72	15.66	23.38
KCl	23.13	7.63	15.50	23.13
$CaCl_2 \cdot 2H_2O$	48.50	16.01	32.50	48.50
$MgSo_4 \cdot 7H_2O$	45.24	14.93	30.31	45.24
Na_2HPO_4	38.65	12.75	25.90	38.65
KH_2PO_4	35.81	11.82	23.99	35.81
Na_2HCO_3	16.32	5.39	10.93	16.32
$C_6H_{12}O_6$	21.74	7.17	14.57	21.74
Cryoprotectants 500 mL each				
Dimethyl sulfoxide (DMSO)	50.00	16.50	33.50	50.00
n,n-dimethyl acetamide (DMA)	50.00	16.50	33.50	50.00
Methanol	50.00	16.50	33.50	50.00
Glycerol	50.00	16.50	33.50	50.00
Liquid nitrogen	116.00	459.36	932.64	1,392.00
Tank rental	37.33	37.33	37.33	37.33
Cryovials (1.2 mL) case of 500	185.00	61.05	123.95	185.00
Centrifuge tubes (15 mL) case of 500	148.33	48.95	99.38	148.33
Centrifuge tubes (50 mL) case of 500	195.67	64.57	131.10	195.67
Microcentrifuge tubes (1.5 mL) pack of 1,000	40.37	13.32	27.05	40.37
Pipettor tips (small) pack of 1,000	47.07	15.53	31.54	47.07
Pipettor tips (large) pack of 1,000	56.00	18.48	37.52	56.00
Sterile filters (0.22 μm) case of 12	56.80	18.74	38.06	56.80
Disposable sterile bottles (500 mL) case of 100	56.20	18.55	37.65	56.20
Type T thermocouple	31.67	63.34	126.68	190.02
Cryo gloves	100.67	201.34	201.34	201.34
Safety goggles	7.67	15.34	15.34	15.34
Labor				
Technician (per hour)	10.00	2,400.00	3,600.00	4,800.00
Facility maintenance	0.10	589.68	859.57	1,129.46
Subtotals		4,541.37	7,335.40	10,102.84
Contingency (5%)	0.05	227.07	366.77	505.14
Sales tax (8%)	0.08	381.47	616.13	848.64
Interest on operating capital (12%)	0.12	617.99	998.13	1,374.80
Annual operating costs	Public	$4,768.43	$7,701.65	$10,607.99
	Private	$5,767.90	$9,315.91	$12,831.42

the additional costs of sales tax and interest resulting in a 20% average increase over the operating cost of public hatcheries.

Per Unit Analysis

Per unit analysis is useful for isolating the effects of production volumes on costs. In general, as production capacity increases, per unit costs decrease until production is maximized for a given level of technology. A per unit analysis can also be used to identify economies of size for various production capacities. For example, Tisdell et al. (1993) used per unit analyses to identify cost economies for seed production of the giant clam *Tridacna gigas*.

Per unit costs for production capacities of 3,000, 6,000, and 9,000 straws were expressed for the total cost of private hatcheries investing in required equipment (iden-

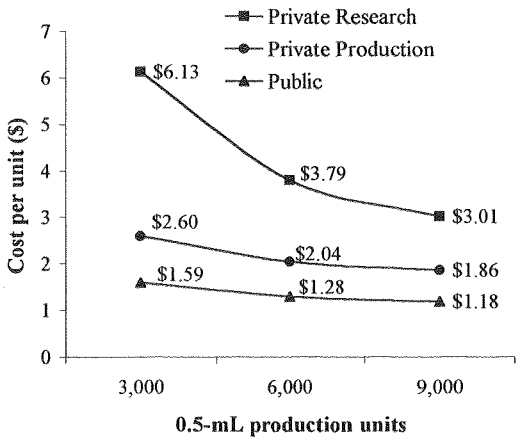

FIGURE 2. *Effective costs per unit of production for cryopreserved fish sperm.*

tified as private production), private hatcheries investing in required and optional equipment (private research), and for operating costs at public hatcheries (public) (Fig. 2). In each case, negatively sloped cost curves indicated economies of scale at increased levels of production.

Private hatcheries purchasing required and optional equipment could realize significant per unit cost reductions by expanding production and storage capacity. At 3,000 units, costs for these hatcheries were relatively expensive at $6.13 per straw. Per unit costs decreased as each additional storage dewar was added, resulting in a cost of $3.01 per straw for 9,000 units. As previously mentioned, this scenario represented a complete investment in all required and optional equipment (listed in Table 1). Hatcheries investing only in the required equipment had lower initial costs and exhibited only a $0.74 per straw reduction as capacity was expanded to 9,000 units. These two scenarios of research and production represent the upper and lower cost boundaries for commercial hatcheries. Such hatcheries considering a cryopreservation program would have per unit costs somewhere within this range depending on the scope of the program. Additionally, commercial hatcheries could expect costs to decrease considerably after debt retirement in year 5. However, the per unit cost for cryopreservation at commercial hatcheries would ultimately depend on a production level determined by sales and in-hatchery usage of frozen sperm. Because public hatcheries would likely pay off the initial investment in 1 yr, per unit costs for year 2 and beyond were calculated using only operating expenditures. Estimated production costs for cryopreserved sperm at public hatcheries were therefore substantially lower, ranging between $1.59 and $1.18 per straw.

Discussion

Four decades of research on the cryopreservation of fish sperm have yielded a growing body of technical reports with potential applications ranging from genetic conservation to commercial aquaculture. Application of this technology is hampered by a number of problems, however, including a lack of economic analyses. This study addresses the lack of economic information on the costs required to implement cryopreservation programs at existing fish hatcheries and also provides a list of required and optional equipment and supplies.

Generic activities of a fish sperm cryopreservation program were identified as sperm collection, short term storage, cryopreservation, long-term storage, thawing, and artificial spawning. Capital and operating expenditures were described for each activity at a range of production capacities for private and public hatcheries. A commercial hatchery implementing a cryopreservation program could expect to spend as much as 70% more on initial investment and 20% more on annual operating costs compared to a public hatchery. The increased costs were associated with sales tax and interest, which accounted for over 50% of the final investment of private hatcheries.

As production capacity was expanded from 3,000 to 9,000 units, public and private hatcheries had a 100% increase in required equipment expenditures. Investment

in optional equipment increased the costs by as much as 300%, but yielded no additional output. Within the boundaries of pure production and pure research, individual fish hatcheries implementing a cryopreservation program would incur costs specific to the scope of their facility. For example, small-scale commercial fish hatcheries are often production-oriented, placing no effort in research. Cryopreservation programs established at these hatcheries would probably utilize required equipment only. Conversely, large state or federal hatcheries interested in developing a research-oriented cryopreservation program might have much of the optional equipment on hand, and thus a research program could be established for a lower investment.

Economies of size were identified for production increases in all scenarios with the greatest advantages for increased production coming from private research hatcheries. As production increased from 3,000 to 9,000 units of sperm, per unit costs fell by 50% at private research hatcheries, 30% at private production hatcheries, and 25% at public hatcheries. Increased application of cryopreserved fish sperm within private hatcheries could be expected as public hatcheries refine protocols and apply them in the private sector. Commercial markets will develop as cryopreserved sperm becomes more cost-effective compared to traditional spawning methods. Such preliminary estimates of relevant production costs constitute the initial half of the data required for a budget analysis of fish sperm cryopreservation.

Further economic analyses would require species-specific data on parameters including broodstock collection and holding costs, sperm production rates, sperm-to-egg ratios, fertilization rates for fresh vs. cryopreserved sperm, percent viability of cryopreserved sperm, and dilution ratios for extenders and cryoprotectants. Such information coupled with estimates of potential genetic gain for specific production traits and the conservation value of genetic resources will ultimately define profitability and usage of cryopreservation.

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