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Simulation modeling of high-throughput cryopreservation of aquatic germplasm: a case study of blue catfish sperm processing

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Abstract

Emerging commercial-level technology for aquatic sperm cryopreservation has not been modeled by computer simulation. Commercially available software (ARENA, Rockwell Automation, Inc. Milwaukee, WI) was applied to simulate high-throughput sperm cryopreservation of blue catfish (*Ictalurus furcatus*) based on existing processing capabilities. The goal was to develop a simulation model suitable for production planning and decision making. The objectives were to: 1) predict the maximum output for 8-hr workday; 2) analyze the bottlenecks within the process, and 3) estimate operational costs when run for daily maximum output. High-throughput cryopreservation was divided into six major steps modeled with time, resources and logic structures. The modeled production processed 18 fish and produced 1164 ± 33 (mean $\pm$ SD) 0.5-ml straws containing one billion cryopreserved sperm. Two such production lines could support all hybrid catfish production in the US and 15 such lines could support the entire channel catfish industry if it were to adopt artificial spawning techniques. Evaluations were made to improve efficiency, such as increasing scale, optimizing resources, and eliminating underutilized equipment. This model can serve as a template for other aquatic species and assist decision making in industrial application of aquatic germplasm in aquaculture, stock enhancement, conservation, and biomedical model fishes.

Keywords

process simulation; high-throughput; sperm cryopreservation; blue catfish

Introduction

Computer simulation has been widely used to mimic system behavior (Kelton et al., 2007). Within aquaculture, simulations have been applied to many areas including structural design, heat transfer, chemical distribution, and farm-level production (Table 1). Those

models assisted aquaculture production by reducing experimentation time and costs, minimizing animal welfare problems, identifying uncontrollable factors, and providing off-site monitoring of systems (Halachmi, 2006; Lee, 2000). With the continued development of computer technology, object-oriented simulation has brought these models ever closer to representing reality (Bolte et al., 2000), and visual interactive simulations can provide straightforward user-friendly interfaces (Porter, 1991). ARENA (Rockwell Automation, Inc. Milwaukee, WI) is one of the most popular simulation software packages for discrete event simulation (Halachmi, 2006; Halachmi et al., 2005), and its visual interaction and fast processing speed have made it a comprehensive tool for use with production processes of interest such as the growing of ornamental fish in aquaculture recirculating systems (Halachmi, 2006).

As a newly refined aquaculture technology, germplasm cryopreservation preserves valuable genetic material (Hu & Tiersch, 2011), and can assist breeding programs and genetic selection. However, compared to the multi-billion dollar global industry that exists for bull semen (NAAB-CSS, 2011), the industrialization of aquatic germplasm is still at the beginning stage. The only current application reported in a large-scale commercial farm has been for hybrid catfish production (Hu et al., 2011b). The industrial high-throughput application of aquatic germplasm will likely be achieved by establishment of facilities capable of processing multiple species during different seasons (Hu & Tiersch, 2011).

Currently, there is no process modeling of cryopreservation in any animal, as such this is the first work of its kind. To model high-throughput cryopreservation, the process pathway needs to be separated into functional components, and associated models, which can then be reintegrated (Ernst et al., 2000). The activities in a comprehensive aquatic germplasm facility would share equipment and supplies, but would produce different products from different materials. For example, the germplasm may come from: fish or shellfish; different batch sizes, (e.g., different aged or sized fish can change the batch size dramatically); different schedules, (e.g., some fish spawn in spring, some in summer); different processing, (e.g., catfish testes need to be dissected, salmon can be stripped), and as with other agricultural commodities, the incoming sample quality would typically be inconsistent (Parthanadee & Buddhakulsomsiri, 2010). All of these factors provide challenges for process engineering, and to address them all simultaneously has not previously been practical (Lee, 1995).

Based on the first report of commercial application of cryopreserved aquatic sperm (Hu et al., 2011b), the existing high-throughput cryopreservation process for sperm of blue catfish (*Ictalurus furcatus*) can serve as a model for other species, although, ictalurid catfishes require dissection of testis (Steeby & Avery, 2005) instead of stripping of sperm. The same thinking used for knowledge-based control systems (Wu & Joseph, 1992) can be adapted to build a simulation model. Thus, the goal of this study was to develop a simulation model useful for production planning and decision making. More specifically our objectives were to: 1) predict the maximum processing output for a typical 8-hr workday; 2) analyze the bottlenecks within the process, and 3) estimate operational costs when the process was run for maximum output.

Methods

The high-throughput cryopreservation of blue catfish sperm can be categorized into six primary steps: 1) dissection, 2) sample preparation, 3) inspection and organization, 4) equilibration and packaging, 5) freezing and sorting, and 6) storage (Liao et al., 2012). From Steps 1 to 3, fish are processed individually. However, from Step 4 onward, individual samples can be grouped to maximize the utility of system capacity (Liao et al., 2012). The details of each step are described below.

1) Dissection: Blue catfish were held in recirculating systems overnight before processing. A single technician brought one male fish from the system to the dissection table. The male was killed by a sharp blow to the head according to IACUC approved procedures. The body cavity was opened by cutting from alongside the anal fin to the pectoral fin (to avoid cutting of the intestine and release of its contents), and testes were extracted by carefully separating attached tissues.

2) Sample Preparation: While the dissector continued dissecting fish, another technician cleaned the testes and extracted sperm into extender solution (Hanks' balanced salt solution (Tiersch et al., 1994) at an osmolality of 300 mOsmol/kg). If the testis weight was below 3 g, the extracted sperm were considered to be insufficient for collection. Therefore, any testes 3 g or smaller were discarded. After extraction, the sperm were filtered through three layers of screens (1-mm, 0.5-mm, and 200- μ mesh). Samples sometimes were placed in a queue if labor or tools were not immediately available for filtering. The final filtered sperm suspensions were held in loosely capped 50-ml centrifuge tubes.

3) Inspection and organization: Technicians gathered all filtered sperm suspensions as a group before analysis. The motilities were tested using darkfield microscopy (Olympus CX41RF, Japan) at 200 $\times$ magnification. For each sample, a 1- μ l drop of sample was activated by the addition of 20 μ l of water on a glass slide on the microscope (Hu et al., 2011b). Estimation was made based on the percentage of swimming (progressively motile) sperm in relation to all visible sperm. Concentrations were estimated using a microspectrophotometer (NanoDrop 1000, Thermo scientific, Wilmington, DE). For each sample, 2 μ l were used for measurement, and each sample was tested 3 times. To ensure the quality of cryopreserved sperm, two quality specifications were used for inspection: a minimum motility of 40%, and a minimum sperm concentration of 1×10^9 /ml (Hu et al., 2011b). The volume of the sample was specified by the weight of testis. After rejecting the suspensions that failed inspection, the remaining suspensions were adjusted to the same working concentration 2×10^8 /ml (Hu et al., 2011b). A grouping computation was performed for these suspensions due to the large difference in capacities between the automated packaging system and freezer. This calculation was used to determine the ordering of suspensions in terms of which freezing batch they would be assigned to based on estimated straw production of each suspension, such that each freezing batch would minimize waste of freezer space and packaging materials (Liao et al., 2012).

4) Equilibration & packaging: The operator of the automated packaging system (MAPI, CryoBioSystem, Paris, France) gathered each of the freezing batches based on computation

results from Step 3, and mixed the suspensions with 10% methanol (reference number: MX0488-6, EMD, Billerica, MA) to initiate equilibration. The time between addition of cryoprotectant and when chamber temperature dropped below 5°C (defined as equilibration time) was strictly maintained at 30 min (Hu et al., 2011b). During this time, all mixtures were loaded into straws, heat-sealed at both ends, labeled with alphanumeric and barcode sample information by the MAPI system, and all straws were placed on horizontal metal racks (reference number: 007119, IMV, Maple Grove, MN) in the freezer (Micro-Digitcool, CBS™, Paris, France).

5) Freezing and sorting: The straws prepared for a single freezing batch were frozen at the same time. The freezer (Microdigitcool, CryoBioSystem, Paris, France) had a capacity of 280 straws. The chamber was held at 4°C during loading, and after the chamber temperature had stabilized, the freezing program was initiated. The programmed cooling rate was 5°C/min from 4°C to -80°C (based on chamber temperature). After the freezer chamber reached -80°C, the products were held inside for another 5 min before unloading. The operator oversaw the cooling progress and removed the frozen straws for sorting into daisy goblets (reference number: 015144, Cryo Bio System, Paris, France) under liquid nitrogen. Each daisy goblet contained 12 compartments. Each compartment had a capacity of 12 frozen straws. The grouped straws from each sample were held together under liquid nitrogen. During sorting, all straws from each sample were sorted into a single goblet in close proximity to each other. During this time, the freezer chamber was warmed automatically for the next freezing batch.

6) Storage: After the freezing of all batches was completed (based on computation results from Step 3), the daisy goblets were recorded in daily logs and moved into a storage dewar (MVE 800 series-190, Chart®, Garfield Heights, OH) for long-term storage. The operator managed this process and finished cleaning as needed to complete the daily work.

Timing of the process

During the processing of blue catfish sperm, the elapsed time for each step was recorded 5 to 7 times with digital timers. The recorded times were applied to the ARENA Input Analyzer to generate distributions, and simulations were performed with process times randomly generated from the respective distributions. Based on the data collected, nine distributions were tested and those with residual error were selected to represent the data. Among the data on processing times, three types of distributions were applied in the simulation model: normal (NORM) and triangle (TRIA) for dissection and testes processing time; and uniform (UNIF) for automated packaging and freezing (Kelton et al. 2007).

Simulation conditions and parameters

The ARENA software package (Version 13.50.000000) was used for simulation modeling. To mimic real-life conditions, the maximum process time was confined to within a single 8-hr day. The settings within the simulation model included resources (Table 2) and processes (Table 3). A simulation run represented a single day of work by 5 people. Each simulation run was performed 50 times with the same settings to produce sufficient data for statistics.

Simulation modeling structure

The simulation model strictly followed the structure of each process step (Fig. 1). The facility was based on a two-room design: one “dirty” for fish processing and another “clean” for sperm handling and processing. There were six components of conceptual logic used to improve model accuracy.

The first component was minimizing the queue on the dissection table. Fish suffered less stress in the holding system than on the floor by the dissection table. In the ARENA model, the Entity Create Model-Object (the basic modeling element) represented the holding system, and each entity that produced by the Entity Create Model-Object represented a single fish. To prevent the Entity Create Model-Object from sending entities constantly regardless of the availability of the next process, a Seize Model-Object was applied (Kelton et al., 2007). To prevent the dissector from removing too many fish at once, a logical rule was established that allowed only an available dissector to capture fish to carry to the dissection table (Fig. 2).

The second logic component was for minimizing labor use. In the ARENA model, labor is considered as a resource. The resource capacity of labor is a constant throughout the process, so it is hard to identify part-time labor. To address this issue, non-paid idle time was applied, such that although the resource capacity remained the same, there was no cost for non-working technicians (Table 2). For example, the dissector position would exist until there were no additional fish in the holding system, and only the time involved with capturing, transporting, and dissection were considered to be paid. In real life, this technician would then be available to assume a new position in the processing pathway.

The third logic component was inspecting and selecting suspensions. Not all suspensions could pass inspection and all rejected suspensions were discarded to ensure overall product quality. Instead of estimating the suspension population each time after inspection, the simulation model performed random selection on each suspension (Fig. 3). For example, if the acceptance rate was 90%, each entity that went through the selection process had a 10% chance of being rejected. This design better represented the real condition during processing than estimation of a selected population because if there was a small number of suspensions in the process, the overall acceptance rate was more likely to be different from 90% due to randomness.

The fourth logic component was determining freezer loading size by the number of fish. The actual straw productivity of suspensions varied due to biological and technical factors. To simplify the modeling, instead of assigning random straw numbers for each suspension, estimations were made on the average number of suspensions that filled a single freezer chamber (Fig. 4). For example, in this study, four suspensions provided sufficient straws to fill the chamber of the Micro-Digitcool programmable freezer. The ratio between a full chamber of straws and the number of daisy goblets was one chamber to 2 daisy goblets (maximum capacity of 144 straws each). Therefore, after freezing and sorting, the materials from four sperm suspensions were transferred into two daisy goblets.

The fifth logic component was to make sure that there were no remainders in the system. Sample remainder was an essential issue for the process simulated. Ideally, this remainder could be held until the next simulation, resulting in continuous processing. However, the blue catfish sperm process did not tolerate a remainder in reality due to the biological characteristics of samples (they lose quality with extended storage time). If there was not enough suspension for a single freezing batch, the remainder was programmed to be frozen at once (Fig. 5). Therefore, the freezing batch for this remainder would not be performed at full capacity. Therefore, the output from the last freezing batch would change according to the actual batch size.

The sixth logic component was recording of late working hours. Instead of pausing the process at 8 hr and calculating the waste in the system, the efficiency was evaluated by the outputs and consideration of additional working hours because of the quality considerations for biological samples. The timing object in the model would record the current time when a product went through. The time of the final product was considered as the operation time.

Model verification

Data used in the model were collected in the Aquatic Germplasm and Genetic Resources Laboratory of the Aquaculture Research Station of the Louisiana State University Agricultural Center during the years 2009–2011. Steps for model verifications as related to aquaculture have been provided previously (Halachmi, 2005). Those steps were used in the verification of the present model as follows.

Experiment 1: prediction of the maximum output of the process within 8 hr

According to the space, equipment, labor, and management of the existing facility, the containment system could hold 30 fish maximum. The simulation model would test for 10 to 30 fish in the containment system and compare the results of 50 model runs.

Experiment 2: analysis of bottlenecks in the process

Based on the selected total entities number from Experiment 1, each process in the model was analyzed for accumulated time (the sum of time spent on this process from each entity) and queue (the entities that waited in line to be processed).

Experiment 3: estimation of operational costs when the process was run for maximum output

Based on the selected total entities number from Experiment 1, each resource in the model was analyzed for cost according to the values from the Settings Table (Table 2).

Results

Process capability

After testing different total entities from 10 to 30, 18 entities had the process times closest to 8 hr (Table 4). Each daisy goblet contained 144 straws, and thus 18 entities produced 1164 ± 33 (mean $\pm$ SD) straws. A linear regression was developed for processing time as follows:

$$\text{Process time (min)} = 0.3671 * \text{Total entities} + 1.2045; R^2 = 0.9975, P = 0.00.$$

For each increased entity, 0.3671 hr (22 min) was added to the processing time.

Bottlenecks within the operation

The freezing and sorting step consumed the most accumulated total time and accumulated waiting time (Fig. 6). Other than the freezing and sorting step, the testis processing (3.77 ± 0.11 hr, mean $\pm$ SD) and dissection (1.86 ± 0.05 hr, mean $\pm$ SD) steps required the longest accumulated total time. Accumulated waiting time and queue time indicated that the testis processing step (0.0002 hr) and filtering step (0.002 hr) presented only brief delays in the entire process.

Cost of operation

In the case of 18 entities, the operational cost was based on resource usage (Table 5). Overall, the single-day processing cost was USD 310.67, including a 70% working cost, 19% usage cost, and 11% idle cost (Fig. 7). Each daisy goblet cost USD 38.45, and each straw cost USD 0.27 (144 straws per daisy goblet).

Discussion

The existing high-throughput pathway could process testes from as many as 18 blue catfish per day with a single dissector, three cleaners, and one operator (in reality only four people), and could produce approximately eight daisy goblets (1,152 straws) of quality product. With the linear relationship between total fish processed and process time, production volume could be increased by adding additional hours or shifts to the work schedule. Based on the observed fertility of 5×10^8 sperm per cryopreserved straw (Hu et al., 2011a), two straws can produce 1,402 hybrid fry (with an egg quality sufficient to produce a typical 66% hatch rate using fresh sperm). Thus, the 1,152 straws processed in a single day could produce 807,552 hybrid fry. In 2011, 15% of 732 million total catfish fry (about 111 million) produced in the industry were hybrid catfish (Chatakondi, 2012). Thus, by simple calculation, the existing demonstration cryopreservation line could process sperm sufficient for all hybrid catfish production in 138 d of operation. Therefore, two high-throughput lines could satisfy the sperm processing necessary for current hybrid catfish demands and supply all small-scale channel catfish artificial spawning (currently only research efforts) during the natural spawning season (April to June in the southeastern US). Moreover, 15 such lines could satisfy the entire US catfish industry if it was to convert to artificial spawning instead of relying on pond-based spawning. With thermal control of ponds, it is possible to extend the period of spawning condition in catfish broodstock by about 3 months (starting in January) (Hall et al., 2002; Pawiroredjo et al., 2008), and as such the sperm processing demand could be met by even fewer production lines during a longer operational period. Although it is not recommended that the industry switch completely to hybrid catfish for catfish production it could in the future utilize artificial spawning to speed genetic improvement programs. The ultimate approach for genetic improvement should be to focus on channel catfish breeding and selection (rather than hybridization between species), and cryopreservation can greatly assist this process to produce improved fish populations and to transport, maintain and archive germplasm (Silverstein, 2011; Tiersch, 2011c).

To further expand capacity, improvements can be made by increasing the scale of operations. The most time-consuming steps were freezing and sorting, and straw packaging. However, each step had constant processing times that were determined experimentally (Hu et al., 2011b). To reduce the negative effect on time arrangement, scale could be increased by adapting equipment with larger capacities or by operating multiple parallel production lines. Scale change involves other considerations such as equipment investment, position setup, and operational layout. The ARENA software has the capability to test such scenarios and provide evaluations.

Meanwhile, improvements can be made in the existing processing steps. The cumulative time for the process could be shortened by optimizing the number of resources available. With additional workers and tools for each step, more entities could be processed within the same time. Therefore, overall time could be shortened. In this study, testis processing and dissection required the second-highest amount of time. If there were two dissectors and two sets of dissection supplies available, fish processing would be doubled. Furthermore, the time required by the process could be reduced by improving the quality of resources. An experienced technician could prepare one testis in 10 min, compared to 12.73 min of average cleaning time for a novice cleaner. In real-life production, any improvements in technique could provide significant positive effects. Future modeling will evaluate these changes with a focus on optimization of resources to maximize production. This is necessary, for example, to avoid bottlenecks that would negate rate improvements in earlier steps.

In addition to bottleneck identification, the cost analysis also provided important information. There was an 11% idle cost that provided no value to the final products. Although some idle cost was unavoidable, such as during freezing and automated system operation, redundant equipment such as separate computers for organizing and storage could be combined. In addition, within the 70% busy cost, more than 90% came from labor costs (Lee, 1995). Therefore, increases or decreases in labor costs would affect overall cost more significantly than would changes in other parameters. The labor costs used in these simulation was set at USD18/hr. Real-life costs could be different depending on specific staffing decisions.

Overall, the results proved the value and efficiency of simulation modeling to optimize a process such as high-throughput cryopreservation (Leung, 1986), and indicated options for further improvement of the existing process: 1) to increase production, additional automated systems and freezers could be obtained in the facility; 2) to speed production, fish and testis processing could be optimized; and 3) to reduce costs when developing a new facility, equipment such as multiple computers used for organizing and inventory could be combined. In addition, to reevaluate the changes (Wu & Joseph, 1992), an expert system approach (Alexander, 1987) could be adapted for further analysis.

Cryopreservation has been achieved in several hundred aquatic species (Tiersch, 2011a). Essentially all of this work has been performed at a laboratory scale that is not applicable for high-throughput commercial-scale application. For example, almost all laboratory studies involve results based entirely from production of tens (rarely hundreds) of straws (or other

containers) performed during one to several days of effort. This usually involves hand filling of containers and freezing in small batches not useful for commercial-scale activities. With increasing demand, adoption of high-throughput cryopreservation techniques will play a more important role in aquaculture. The simulation model in this study can serve as a template that can be easily adapted to other species or applications. In fact, although there were multiple aspects directly involving cryopreservation (Leibo, 2011), from the process level, only three factors will cause major changes in the model (Table 6): refrigerated storage, shipment, and sperm quantity from each animal.

Refrigerated storage is essential to cryopreservation, because a suitable extender solution will protect sperm cells and prolong the handling time (Christensen & Tiersch, 2007; Wayman et al., 2011; Cloud, 2011; Billard et al., 2004). If fish processing is geographically distant from the cryopreservation facility, shipping would be required. The receiving facility would not perform the sample collection, but an additional quality inspection step would be required upon receipt. Because, much like refrigerated storage, shipping conditions directly affect sample quality (Huszar et al., 2004; Tiersch, 2011b), shipping with sub-optimal conditions can cause quality reduction or loss of entire collections (Dong et al., 2005).

The definition of “sufficient sperm” varied according to the capacity of equipment and package design, but generally, large-bodied fishes would be more likely to provide sufficient sperm at the individual level. For small-bodied fishes such as the biomedical research models, zebrafish *Danio rerio* or *Xiphophorus* species, pooling of samples would be recommended if possible for high-throughput processing (Tiersch et al., 2011). Other species-dependent details such as equilibration time and loading concentration, and type of cryoprotectant, can be modified by changing values in the Settings Tables without other logic or structural modifications.

Conclusions

This study represents the first simulation modeling of high-throughput cryopreservation. The results from this model provided valuable information on existing processes and supports future decision making. The model provides the capability for planning and expanding the scale of high-throughput cryopreservation production. As such, future research on related economic and engineering topics can be performed in this “virtual facility”, and the significant investment in construction and management could be examined rationally, and hypothesis testing could be accelerated. More specifically, whether it is in developed countries where automation is applied broadly, or in developing countries where trained labor plays a major role in facilities, the simulation model can provide solutions for determination of feasible production and time management. With the development of tools such as simulation models, the use of aquatic germplasm could more quickly become a valuable tool for aquaculture, conservation biology, fisheries management, and genetic research.

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Figure 1.

Simulation model structure with facility layout. The entities (fish) began from “Fish in holding system”, went through the processes (shaded outlines) and submodels (rectangles with black arrows), and finished at “Process end”. Each submodel represented one step in the high-throughput cryopreservation process. Time spent on relocating samples within the facility was insignificant compared to processing time, and therefore it was not included in the model.

Figure 2.

Logic for minimizing the queue on the dissection table. The Seize Model-Object named “seize dissector” forced entity (fish) to associate with the available dissector only (left setting window). As long as the entity was not dissected, the dissector could not capture another fish. Once each fish was processed, the dissector was free again for other entities by “release dissector” (right setting window).

Figure 3.

Logic for inspecting and discarding sperm suspensions. All suspensions after filtering were gathered by a Batch Model-Object named “batch for analysis”, and went through inspection (motility and concentration testing) at once. A Separate Model-Object named “separate for screen” broke the batch into original entities (suspensions), and went through a Decision Model-Object named “quality screen”. The number of suspensions that entered next step was determined according to the previously established sample acceptance rate (90% in the setting window).

Figure 4.

The logic of determining freezer loading size by number of fish. According to the capacities of the automatic packaging system and freezer, the entities (sperm suspensions) were grouped by a Batch Model-Object named “Capacity of freezer” (left setting window). In this case, an average of 4 entities made one batch for freezing. After freezing and sorting, the batched entity was separated by a Separate Model-Object named “daisy goblets”, because all straws were sorted into daisy goblets for long-term storage. Each freezing could create two daisy goblets, so in Separate Model-Object, there was 100% chances for each entity (batched freezing) to yield two (daisy goblets) (right setting window).

Figure 5.

The logic use to ensure that no remainders existed in the system. A Decision Model-Object named "freezing batches" was set before entering freezing batches (left setting window). Two conditions were used: remainder in the system was greater than one regular batch size; there were some entities waiting to be batched in normal freezing batches. All entities that failed both conditions went to last run ("Else" pathway). The total number of remainders was recorded by the Record Model-Object named "Record number of remainder". Therefore, the batch size of Batch Model-Object named "Last run" and the duplication number in the Separate Model-Object named "Lastrun.daisy goblets" (right setting window) were changed accordingly.

Figure 6.

Accumulated time of each step for simulations of processing a total of 18 blue catfish in a single 8-hr day (ranked from largest to smallest). The black bars represent accumulated total time, and the white bars represent accumulated waiting time. The error bars present the standard deviations generated based on data from 50 simulations.

3:41:55PM

Category Overview

June 25, 2012

*Values Across All Replications***Unnamed Project**

Replications: 50

Time Units: Hours

Key Performance Indicators**All Entities**

	Average
Non-Value Added Cost	0
Other Cost	11
Transfer Cost	0
Value Added Cost	264
Wait Cost	0
Total Cost	275

All Resources

	Average
Busy Cost	216 *
Idle Cost	36
Usage Cost	59 *

Total Cost 311

* These costs are included in Entity Costs above.

System

	Average
Total Cost	311
Number Out	10

50 / 50 (24.0000 Hours) Tuesday, June 26, 2012

Figure 7.

Overview page of ARENA summary report. The report was generated based on 50 simulations of processing a total of 18 blue catfish in a single 8-hr day.

Table 1

Examples of previous uses of computer simulation in aquaculture applications.

Culture type	Literature citation
Cage culture	Kim et al., 2011
	DeCew et al., 2010
	Zhao et al., 2010
	Lee et al., 2008
	Huang et al., 2006
	Fredriksson et al., 2003
Pond culture	Gutiérrez-Estrada et al., 2012
	Bolte et al., 2000
	Jamu & Piedrahita, 1998
	Hargreaves, 1997
	Gao & Merrick, 1996
	Leung & Shang, 1989
	Brooks Jr & Kimball, 1981
Raceway systems	Li et al., 2009
Recirculating systems	Halachmi, 2006
	Halachmi et al., 2005
	Weatherley et al., 1993

Table 2

Settings Table for Resources used in the ARENA simulation model. Capacity represents the availability of resources. “\$ busy/hour” was the hourly cost during operation. “\$ idle/hour” was the hourly cost when the resources were not occupied. “\$ per use” was the cost of disposable supplies during operation. Properties define the function of resources. Values in this table were estimated based on actual operation in the Aquatic Germplasm and Genetic Resources Laboratory of the Aquaculture Research Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana, during 2009–2011. \$: USD.

Resource	Capacity	\$ Busy/hour	\$ Idle/hour	\$ Per use	Properties
Dissector	1	18	0	0	Worker
Dissection table	1	0	0	0.25	Tool (one fish at a time)
Cleaner	3	18	0	0	Worker
Cleaning kit	3	0	0	0.15	Tool (one testis at a time)
Filter	1	0	0	0.15	Tool
Operator	1	18	0	0	Worker (multiple tasks)
Inspection tool	1	0.1	0.1	4	Tool
Grouping calculator	1	0.05	0.05	0	Tool (grouping)
Automated system	1	0.4	0.4	5	Tool (straw packaging)
Freezer	1	2	1	0.25	Tool
Sorting tank	1	0	0	5	Tool (straws sorting)
Inventory computer	1	0.05	0.05	0	Tool

Table 3

Settings Table for Processes used in the ARENA simulation model. The process names starting with “Lastrun” were part of the control logic to eliminate remainders in the system. Each process employed a worker and tools. The exception was the Dissection process because of the logic for minimizing the queue on the dissection table. The delay values represented process time which was presented as distributions or expressions. All values in this table were measured from the existing process at the Aquatic Germplasm and Genetic Resources Laboratory of the Aquaculture Research Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana, during 2009–2011. NORM: normal distribution; TRIA: triangle distribution; UNIF: uniform distribution.

Process Name	Resources occupied	Delay Value (min)
Dissection	Dissection table	NORM(6.12, 2.09)
Testes processing	Cleaner, cleaning kit	NORM(12.73, 4.94)
Filtering	Cleaner, filter	TRIA(1.57, 1.78, 2.12)
Inspection	Operator, inspection tool	3.67+1.13 *filtered samples
Organization	Operator, organizing computer	1.5 + 1 *number of acceptance
Straw packaging	Operator, automated system	30
Freezing and sorting	Operator, freezer, sorting tank	25
Lastrun.auto system	Operator, automated system	30
Lastrun.Freezing	Operator, freezer	25
Lastrun.sort	Operator, sorting tank	UNIF(15, 25)
Inventory	Operator, inventory computer	0.5+0.75 *number of goblets

Table 4

Process time and output for different inputs of incoming materials (total entities) for high-throughput cryopreservation of blue catfish sperm. The ARENA model simulated each scenario 50 times and presented the mean $\pm$ SD for the process time and number of daisy goblets produced.

Total entities	Process time (hr)	Daisy goblets produced
10	4.97 $\pm$ 0.15	4.78 $\pm$ 0.13
15	6.75 $\pm$ 0.15	6.68 $\pm$ 0.19
16	6.89 $\pm$ 0.13	7.04 $\pm$ 0.24
17	7.35 $\pm$ 0.17	7.60 $\pm$ 0.26
18	7.73 $\pm$ 0.16	8.08 $\pm$ 0.23
19	8.26 $\pm$ 0.16	8.48 $\pm$ 0.23
20	8.63 $\pm$ 0.13	8.90 $\pm$ 0.16
25	10.50 $\pm$ 0.10	11.12 $\pm$ 0.20
30	12.16 $\pm$ 0.18	13.22 $\pm$ 0.25

Table 5

Resource costs during the processing of 18 blue catfish males. The ARENA model of high-throughput sperm cryopreservation performed 50 simulations and presented the mean $\pm$ SD for three types of costs. “Busy cost” represented the cost when resources were occupied; “idle cost” represented the cost when resources were not in use; “usage cost” represented the cost of supplies that were used each time resources were operated. Unit: USD.

Resource	Busy cost	Idle cost	Usage cost
Dissector	44.04 $\pm$ 0.86	0	0
Dissection table	0	0	4.50 $\pm$ 0.00
Cleaner	77.58 $\pm$ 1.88	0	0
Cleaning kit	0	0	2.70 $\pm$ 0.00
Filter	0	0	2.70 $\pm$ 0.00
Operator	90.24 $\pm$ 2.93	0	0
Inspection tool	0.04 $\pm$ 0.00	2.36 $\pm$ 0.00	4.00 $\pm$ 0.00
Organizing computer	0.01 $\pm$ 0.00	1.19 $\pm$ 0.00	0
Automated system	0.87 $\pm$ 0.03	8.73 $\pm$ 0.03	21.80 $\pm$ 0.69
Freezer	3.63 $\pm$ 0.12	22.18 $\pm$ 0.06	1.09 $\pm$ 0.03
Sorting tank	0	0	21.80 $\pm$ 0.69
Inventory computer	0.01 $\pm$ 0.00	1.19 $\pm$ 0.00	0

Table 6

Binary identification table for adapting the simulation model from this study to other species. For each condition (left column), logic or structural changes have to be made to the model with specific considerations (right column).

Condition	Change needed	Considerations
1.Capable of refrigerated storage	Logic	Hold samples until cryopreservation
a. Processing on site	Structural	Add processer and steps
b. Processing off site (shipping)	Structural	Add inspection on arrival
2.Not capable of refrigerated storage	Logic	Continuous processing
a.Processing on site	Structural	Add processer and steps
i.Sufficient sperm available	Logic	Freeze as individuals
ii.Insufficient sperm available	Logic	Freeze as pooled samples
b. Processing off site (shipping)	N/A *	Contingency plan for low quality samples

*
not applicable