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Simulation modelling 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 modelled 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-h workday; (2) analyse 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 modelled with time, resources, and logic structures. The modelled production line processed 18 fish and produced 1164 ± 33 (mean ± SD) 0.5-mL straws containing one billion cryopreserved sperm. Two such production lines could support all hybrid catfish production in the United States 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 fish.

Keywords: process simulation, high-throughput, sperm cryopreservation, blue catfish

Introduction

Computer simulation has been widely used to mimic system behaviour (Kelton, Sadowski & Sturrock 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, Nath & Ernst 2000), and visual interactive simulations can provide straightforward user-friendly interfaces (Porter 1991). ARENA (Rockwell Automation, Inc. Milwaukee, WI, USA) is one of the most popular simulation software packages for discrete event simulation (Halachmi 2006; Halachmi, Simon, Guetta & Hallerman 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 programmes and genetic selection. However, compared with the multi-billion dollar global industry that exists for bull semen (NAAB-CSS 2011), the industrialization of aquatic

Table 1 Examples of previous uses of computer simulation in aquaculture applications

Culture type	Literature citation
Cage culture	Kim, Yang, Hwang, Jang & Hur 2011
	DeCew, *et al.*, 2010
	Zhao *et al.*, 2010
	Lee, Kim, Lee, Choe, Lee & Koo 2008
	Huang, Tang & Liu 2006
	Fredriksson, Swift, Irish, Tsukrov & Celikkol 2003
Pond culture	Gutiérrez-Estrada, Pulido-Calvo, de la Rosa & Marchini 2012
	Bolte *et al.* 2000
	Jamu & Piedrahita 1998
	Hargreaves 1997
	Gao & Merrick 1996
	Leung & Shang 1989
	Brooks & Kimball 1981
Raceway systems	Li, Willits, Browdy, Timmons & Losordo 2009
Recirculating systems	Halachmi 2006
	Halachmi *et al.* 2005
	Weatherley *et al.*, 1993

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, Yang & Tiersch 2011). The industrial high-throughput application of aquatic germplasm will likely be achieved by the establishment of facilities capable of processing multiple species during different seasons (Hu & Tiersch 2011).

Currently, there is no process modelling 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, Bolte & Nath 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, Yang *et al.* 2011), the existing high-throughput cryopreservation process for the sperm of blue catfish (*Ictalurus furcatus*) can serve as a model for other species, although ictalurid catfish 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-h workday; (2) analyse 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, Hu & Tiersch 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.

Dissection

Blue catfish were held in recirculating systems overnight before processing. A single technician brought a single 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.

Sample preparation

While the dissector continued dissecting fish, another technician cleaned the testes and extracted sperm into extender solution (Hanks'

balanced salt solution (Tiersch, Goudie & Carmichael 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-um mesh). Samples sometimes were placed in a queue if labour or tools were not immediately available for filtering. The final filtered sperm suspensions were held in loosely capped 50-mL centrifuge tubes.

Inspection and organization

Technicians gathered all filtered sperm suspensions as a group before analysis. The motilities were tested using darkfield microscopy (Olympus CX41RF, Tokyo, Japan) at 200-× magnification. For each sample, a 1-μL drop of sample was activated by the addition of 20 μL of water on a glass slide on the microscope (Hu, Yang *et al.* 2011). 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, USA). For each sample, 2 μL was used for measurement, and each sample was tested three 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, Yang *et al.* 2011). 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).

Equilibration and 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, USA) 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, Yang *et al.* 2011). During this time, all mixtures were loaded into straws, heat-sealed at both ends, labelled with alphanumeric and barcode sample information using 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).

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 5°C during loading, and after the chamber temperature had stabilized, the freezing programme was initiated. The programmed cooling rate was 5°C /min from 5°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.

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, USA) 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 five to seven 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 modelling. To mimic real-life conditions, the maximum process time was confined to within a single 8-h day. The settings within the simulation model included resources (Table 2) and processes (Table 3).

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

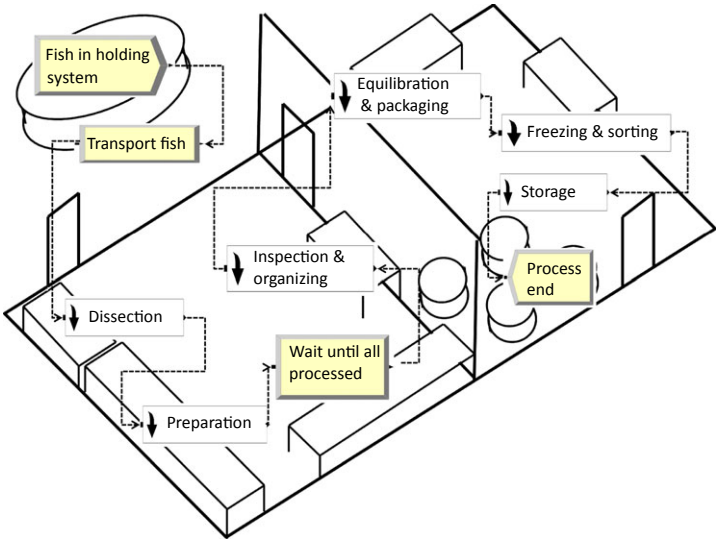

Figure 1 Simulation model structure with facility layout. The entities (fish) began from 'Fish in holding system', went through the processes (shaded outlines) and submodels (rectangles with black arrows), and finished at 'Process end'. Each submodel represented one step in the high-throughput cryopreservation process. Time spent on relocating samples within the facility was insignificant compared with processing time, and therefore it was not included in the model.

A simulation run represented a single day of work by five people. Each simulation run was performed 50 times with the same settings to produce sufficient data for statistics.

Simulation modelling 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. Six components of conceptual logic were 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 modelling element) represented the holding system, and each entity that was 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 one fish to carry to the dissection table (Fig. 2).

The second logic component was for minimizing labour use. In the ARENA model, labour is considered as a resource. The resource capacity of labour is a constant throughout the process, so it is hard to identify part-time labour. 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 was 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 were 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 modelling, instead of assigning random straw numbers for each suspension, estimations were made on the average number of suspensions that

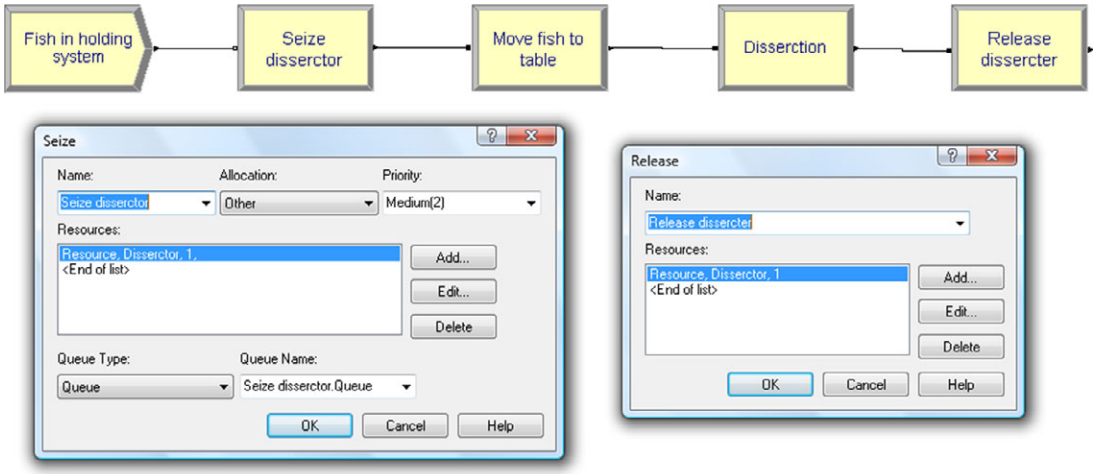

Figure 2 Logic for minimizing the queue on the dissection table. The Seize Model-Object named 'seize dissector' forced entity (fish) to associate with the available dissector only (left setting window). As long as the entity was not dissected, the dissector could not capture another fish. Once each fish was processed, the dissector was free again for other entities by 'release dissector' (right setting window).

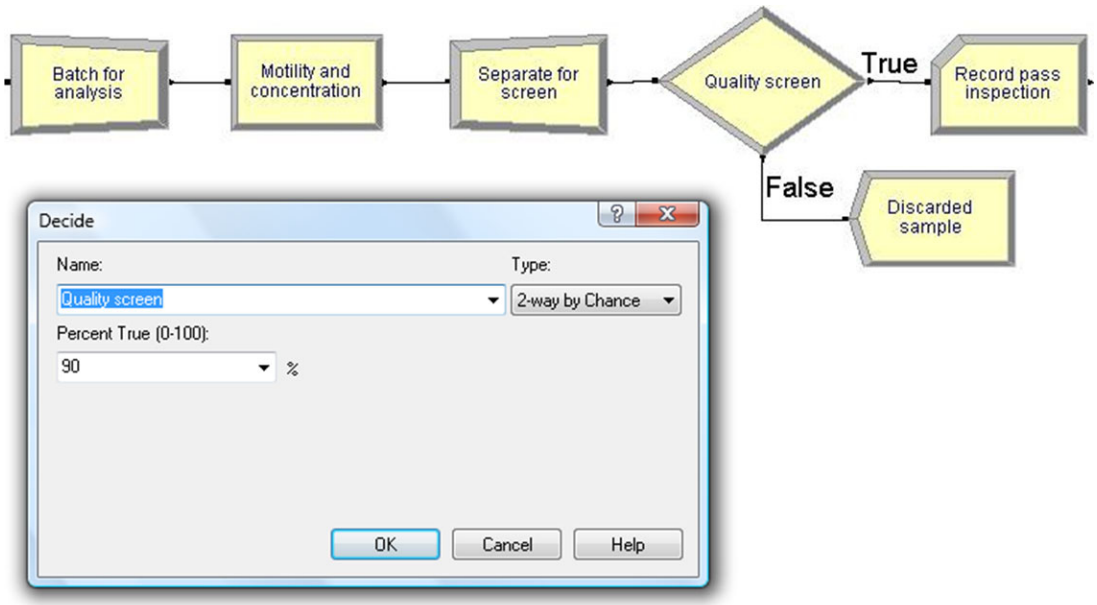

Figure 3 Logic for inspecting and discarding sperm suspensions. All suspensions after filtering were gathered by a Batch Model-Object named 'batch for analysis', and went through inspection (motility and concentration testing) at once. A Separate Model-Object named 'separate for screen' broke the batch 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).

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 two 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.

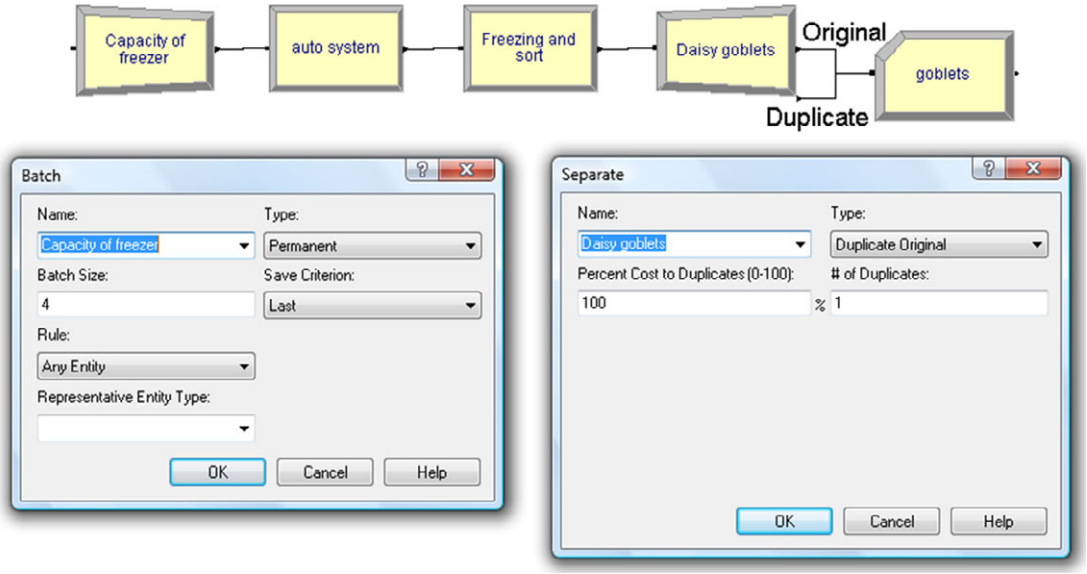

Figure 4 The logic of determining freezer loading size by the number of fish. According to the capacities of the automatic packaging system and freezer, the entities (sperm suspensions) were grouped using a Batch Model-Object named 'Capacity of freezer' (left setting window). In this case, an average of four entities made one batch for freezing. After freezing and sorting, the batched entity was separated using 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).

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 h 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 *et al.*, 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 h. According to the space, equipment, labour 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 analysed 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).

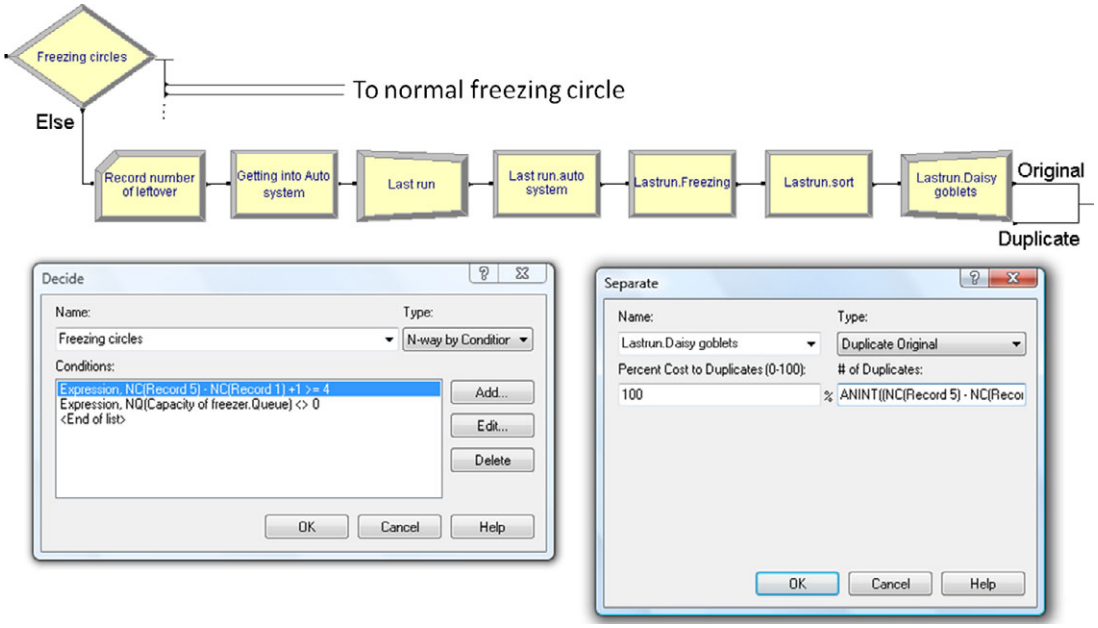

Figure 5 The logic use to ensure that no remainders existed in the system. A Decision Model-Object named 'freezing batches' was set before entering freezing batches (left setting window). Two conditions were used: remainder in the system was greater than one regular batch size; and there were some entities 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 using 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.

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 analysed 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 h (Table 4). Each daisy goblet contained 144 straws, and thus 18 entities produced 1164 ± 33 (mean ± 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 h (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 h,

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 ± SD for the process time and number of daisy goblets produced

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

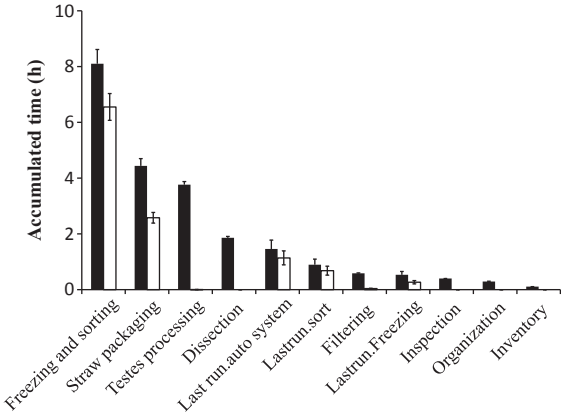

Figure 6 Accumulated time of each step for simulations of processing a total of 18 blue catfish in a single 8-h day (ranked from largest to smallest). The black bars represent accumulated total time, and the white bars represent accumulated waiting time. The error bars present the standard deviations generated based on data from 50 simulations.

mean ± SD) and dissection (1.86 ± 0.05 h, mean ± SD) steps required the longest accumulated total time. Accumulated waiting time and queue time indicated that the testis processing step (0.0002 h) and filtering step (0.002 h) 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).

Table 5 Resource costs during the processing of 18 male blue catfish. The ARENA model of high-throughput sperm cryopreservation performed 50 simulations and presented the mean ± 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 ± 0.86	0	0
Dissection table	0	0	4.50 ± 0.00
Cleaner	77.58 ± 1.88	0	0
Cleaning kit	0	0	2.70 ± 0.00
Filter	0	0	2.70 ± 0.00
Operator	90.24 ± 2.93	0	0
Inspection tool	0.04 ± 0.00	2.36 ± 0.00	4.00 ± 0.00
Organizing computer	0.01 ± 0.00	1.19 ± 0.00	0
Automated system	0.87 ± 0.03	8.73 ± 0.03	21.80 ± 0.69
Freezer	3.63 ± 0.12	22.18 ± 0.06	1.09 ± 0.03
Sorting tank	0	0	21.80 ± 0.69
Inventory computer	0.01 ± 0.00	1.19 ± 0.00	0

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 (1152 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, Baxter & Tiersch 2011), two straws can produce 1402 hybrid fry (with an egg quality sufficient to produce a typical 66% hatch rate using fresh sperm). Thus, the 1152 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 days 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 south-eastern United States). Moreover, 15 such lines could satisfy the entire US catfish industry if it was to convert to artificial spawning

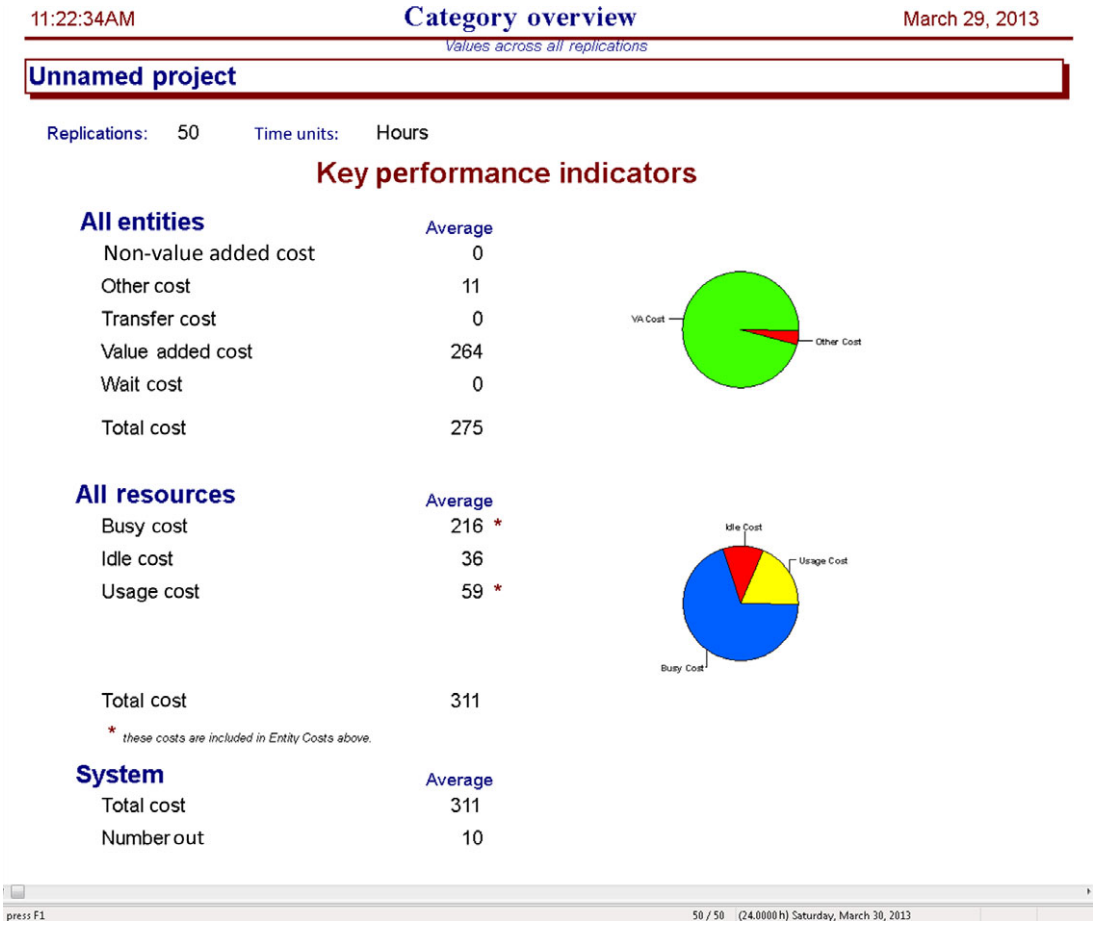

Figure 7 Overview page of ARENA summary report. The report was generated based on 50 simulations of processing a total of 18 blue catfish in a single 8-h day.

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, Finney, Lang & Tiersch 2002; Pawiroredjo, Lamourex, Hall & Tiersch 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 programmes. 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, Yang *et al.* 2011). 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 with 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 modelling 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 labour costs (Lee 1995). Therefore, increases or decreases in labour costs would affect overall cost more significantly than would changes in other parameters. The labour costs used in these simulations were set at USD18/h. Real-life costs could be different depending on specific staffing decisions.

Overall, the results proved the value and efficiency of simulation modelling 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 re-evaluate 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 on the 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, Figiel & Tiersch 2011; Cloud 2011; Billard, Cosson, Noveiri & Pourkazemi 2004). If fish processing is geographically distant from the cryopreservation facility, shipping would be required. The receiving facility would not perform the sample collection,

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

*Not applicable.

but an additional quality inspection step would be required upon receipt. Because, much like refrigerated storage, shipping conditions directly affect sample quality (Huszar, Celik-Ozenci, Cayli, Kovacs, Vigue & Kovanci 2004; Tiersch 2011b), shipping with sub-optimal conditions can cause quality reduction or loss of entire collections (Dong, Eudeline, Huang, Allen & Tiersch 2005).

The definition of 'sufficient sperm' varied according to the capacity of equipment and package design, but generally, large-bodied fish would be more likely to provide sufficient sperm at the individual level. For small-bodied fish 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, Yang & Hu 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 modelling 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 labour 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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