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The Effects of Osmolality, Cryoprotectant and Equilibration Time on Striped Bass *Morone saxatilis* Sperm Motility

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Abstract

Four experiments were designed to evaluate the effects of osmolality, cryoprotectant, and equilibration time on striped bass sperm motility. In the first experiment, solutions of NaCl or KCl with osmolalities ranging from 0 to 700 mmol/kg were tested on sperm activation. Over 60% of the sperm were activated by isotonic NaCl and KCl solutions with a treatment osmolality of 350 mmol/kg. Sperm remained motile until osmolality increased to 600 mmol/kg. In the second and third experiments, Extenders 1, 2 and 3 with osmolalities of 350, 500, and 600 mmol/kg, respectively, were tested. Sperm samples stored in Extender 2 showed significantly higher ($P < 0.01$) sperm motility after 10 min of exposure as well as greater ($P < 0.01$) post-thaw motility when compared to samples stored in Extenders 1 and 3. In the fourth experiment, two trials were carried out to evaluate the effects of cryoprotectant and equilibration time. In the first trial, methanol with a concentration of 5% and 10% yielded the highest ($P < 0.05$) sperm motility prior to freezing at all equilibration times examined. However, 5% DMSO yielded the highest ($P < 0.01$) post-thaw motility (38 ± 3.6%). DMSO with concentrations of 10% and 15% resulted in 17 ± 2.3% and 6 ± 1.0% post-thaw motility, respectively. Both methanol and DMA, at all concentrations tested, resulted in less than 10% post-thaw motility. In the second trial, four DMSO concentrations with three different equilibration times were examined. We observed a significant ($P < 0.001$) interaction effect between DMSO concentration and equilibration time. Post-thaw motility was significantly greater ($P < 0.01$) with a concentration of 5% DMSO at all equilibration times examined, compared to 1.25, 2.5, and 10% DMSO. An average post-thaw motility of 40 ± 2.9% was achieved after 10 min equilibration using 5% DMSO.

The striped bass *Morone saxatilis* is an important commercial and recreational resource finfish species in the United States. Hybrid striped bass production increased almost tenfold from 1986 to 1995 (Striped Bass Growers Association 1998) and is now the fourth finfish species by value in the U.S. (Carlberg et al. 2000). However, rapid growth remains constrained by continued reliance on wild broodstock for seedstock, which poses significant risks to the industry (Harrell et al. 1990; Leffler 1999). Cryopreservation of fish spermatozoa is one approach that could potentially help solve this problem. Numerous studies that have examined cryopreservation of fish spermatozoa, especially the salmonids, carps and tilapias, have found that effective techniques often vary greatly between different species even within the same family or genus (Scott and Baynes 1980; Chao et al. 1987; Gwo et al. 1991; Linhart et al. 1993; Piironen 1993; Lahnsteiner 1996; Yao et al. 2000). While there has been some published research describing the cryopreservation of striped bass spermatozoa (Kerby 1983, 1985; Brown and Brown 2000; Jenkins-Keeran et al. 2001; Jenkins-Keeran and Woods 2002a, 2002b), there is still no commercially viable protocol available.

It is important that sperm are not activated prior to freezing, since motility exhausts limited cell energy. To keep sperm immotile, it is essential to understand what triggers sperm motility. Changes in the ionic and osmotic environment of the sperm cells have been identified as two critical factors that may be responsible for initiat-

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ing motility in fish spermatozoa (Morisawa 1994). Sperm motility of rainbow trout *Oncorhynchus mykiss* can be easily controlled by K^+ concentration regardless of osmolality, and a K^+ concentration of 40 mM effectively inhibits motility (Morisawa 1983a). The sperm of freshwater cyprinids, such as goldfish *Carassius auratus* (Morisawa and Suzuki 1980), carp *Cyprinus carpio* (Morisawa et al. 1983b; Perchec et al. 1997), and marine teleosts, such as puffer *Takifugu niphobles* (Oda and Morisawa 1993) and summer whiting *Sillago ciliata* (Goodall et al. 1989), have all been shown to be regulated by osmolality. Sperm were quiescent in solutions when osmolality was isotonic to that of the seminal plasma, but became motile when osmolality decreased for freshwater teleosts and increased for marine teleosts. Some anadromous species have been studied with regard to their mechanisms of sperm activation. Atlantic salmon *Salmo salar* (Stoss and Reftsie 1983) sperm motility was regulated by external K^+ that is normally lower when compared to the concentration found in the seminal plasma, the same as rainbow trout. For the catadromous Japanese eel *Anguilla japonica*, sperm were immotile in isotonic solutions and the motility was initiated by hyper-osmotic solutions (Ohta and Izawa 1996).

Besides the specific ions that comprise an extender, as well as its osmolality, sperm motility may also be correlated with cryoprotectant and equilibration time prior to freezing. Dimethyl sulfoxide (DMSO), glycerol, methanol, dimethylacetamide (DMA), and propylene glycol are commonly used cryoprotectants for fish sperm cryopreservation. DMSO has been used successfully as a cryoprotectant to freeze sperm from Atlantic croaker *Micropogonias undulatus* (Gwo et al. 1991), yellowfin seabream *Acanthopagrus latus* (Gwo 1994), European catfish *Silurus glanis* (Linhart et al. 1993), channel catfish *Ictalurus punctatus* (Tiersch et al. 1994), and rainbow trout (Conget et al. 1996; Lahnsteiner 1996). Methanol has proven effective for cryopreserving milt from Razorback sucker *Xyrauchen texanus* (Tiersch et al. 1998) and tilapia *Oreochromis spp.* (Chao et al. 1987; Rana et al. 1990). DMA was used successfully to cryoprotect sperm from European catfish (Ogier de Baulny et al. 1999). Horvath and Urbanyi (2000) reported that both DMA and DMSO were more suitable for cryopreserving African catfish *Clarias gariepinus* sperm, compared to glycerol, methanol, ethylene glycol, or propylene glycol. Kerby (1983) evaluated glycerol, ethylene glycol, propylene glycol, and DMSO for potential use as cryoprotectants for striped bass sperm. He found that DMSO was the only cryoprotectant that yielded any post-thaw motility or fertilization. Jenkins-Keeran and Woods (2002a) reported as high as 23% post-thaw motility and a 21% fertilization rate with 5% DMSO at −40 C /min freezing rate. Equilibration time also plays a major role in the success of cryopreservation. Time is required for cryoprotectants to permeate into sperm cells and become balanced between intracellular and extracellular environments. However since most cryoprotectants are toxic to cells (Leung 1991), excessive equilibration time itself may cause cell damage or death.

This study was conducted as part of our ongoing efforts to establish a commercially viable protocol for cryopreservation of striped bass sperm. We examined the effects of osmolality, cryoprotectant, and equilibration time on striped bass sperm motility.

Materials and Methods

Three-year-old striped bass males were randomly selected from a population maintained under the previously described (Woods et al. 1990, 1995) ambient photothermal conditions of the flow-through, 6-m diameter tank system at the University of Maryland's Crane Aquaculture Facility. Water temperature ranged from 5–30 C during the year. In the spring, males (N = 20) were moved into a 4-m diameter tank, part of a recirculating water system, and held at

15 ± 1 C for the remainder of the 5-wk study. Each fish was given a cholesterol cellulose implant (Sherwood et al. 1988) containing 150 μg of mammalian gonadotropin-releasing hormone (Hodson and Sullivan 1993), inserted into the dorsal lymphatic sinus, as previously reported for striped bass (Woods and Sullivan 1993). Three days after administering the implant, the fish were anesthetized in a 70 mg/L quinaldine bath (Woods et al. 1992) and urine was removed by applying gentle pressure around the urogenital vent. Milt was expressed directly into 50 mL sterile conical tubes and placed immediately on ice. In each experiment, striped bass sperm samples exhibiting motility ≥ 85% were used.

Experiment 1

The activation of fresh sperm was examined using solutions of NaCl and KCl at osmolalities of 0, 100, 200, 300, 350, 400, 500, 600, and 700 mmol/kg. Osmolality was measured using a vapor pressure osmometer (Wescor Model 5400). To estimate the percentage of motile sperm, aliquots of fresh milt were placed into a Makler counting chamber and 10-μL activation solution was added and quickly mixed. The loaded Makler chamber was immediately placed under a compound microscope (Zeiss model D-7082) at 400×. The activation of each sample was recorded on videotape using a Hitachi Model KP-140 video camera. The percentage of motile sperm was determined from the videotapes by dividing the number of motile sperm by the total number of sperm in four Makler grid frames in the visual field on the monitor screen. The number of sperm cells in each frame ranged from 50 to 200. The percentage of motile sperm was estimated for all samples by the same observer to avoid bias. Spermatozoa that simply vibrated or did not show progressive forward movement were not considered motile.

Experiment 2

Three extenders (Table 1) were evaluated on the basis of sperm motility after a short-term storage interval (30 min). Osmolalities of Extender 1, 2, or 3 were adjusted to 350, 500, or 600 mmol/kg using NaCl. To examine the effects of extenders on sperm motility for short-term storage, immediately after collection, 1 mL of fresh milt from each fish was diluted with Extender 1 or 2 in a 1:1 ratio of milt:extender. Extended sperm were stored at 4 C and activated using deionized water at time 0 (control), 5, 10, 15, 20, 25, and 30 min after sperm were diluted. Motility was estimated using methods previously described in Experiment 1.

TABLE 1. *The components of the sperm extenders.*

	Extenders		
	1	2	3
NaCl (mg)	890	1,400	1,740
KCl (mg)	40	40	40
$NaHCO_3$	200	200	200
Glucose (mg)	100	100	100
Water[a] (mL)	100	100	100
pH[b]	7.6	7.6	7.6
Osmolality (mmol/kg)	350	500	600

[a] Deionized ultra-filtered water.
[b] pH was adjusted with HCl.

Experiment 3

Based on the results and conclusions of previous research (Kerby 1983; Jenkins-Keeran and Woods 2002a), DMSO with a concentration of 5% was used as a cryoprotectant to examine the effect of extenders on post-thaw motility. Fresh milt was diluted in a two-step process. A 3-mL sample of fresh milt was collected from each fish and kept at 4 C. Milt (1 mL) was diluted with Extender 1, 2, or 3 in a 1:1 ratio. Then after 15 min storage at 4 C, the extended samples were subsequently diluted 1:1 with either Extender 1, 2, or 3 each containing 10% DMSO. The final dilution ratio was 1:3 (milt:cryomedia) and the final DMSO concentration was 5%. This final sperm mixture was quickly pipetted into 500-μL cryo-straws (TS Scientific Inc.) in aliquots of 150 μL. Six cryo-straws were filled and sealed for each extender and each

fish. The total time that the extended milt and cryoprotectant were allowed to equilibrate before freezing was 20 min. Eighteen cryo-straws containing sperm samples from each fish were frozen together using a programmable freezer (Planer Kryosave-Model KS30) with a selected freezing rate: −40 C/min until −120 C (Jenkins-Keeran and Woods 2002a). The samples were then immediately placed into liquid nitrogen. Three days later, samples were thawed in a 35-C water bath for 8 sec. The thawed milt mixture was immediately poured out of each straw into a Makler counting chamber, activated with deionized water, and recorded as described above.

Experiment 4

To test the effects of different cryoprotectants, equilibration times, as well as their interaction on sperm motility, aliquots of 2-mL fresh milt from each striped bass was diluted (1:1) with Extender 2. After 15-min storage at 4 C, the extended milt was subsequently diluted (1:1) with Extender 2 containing cryoprotecant to make one of the following treatments (final concentration): DMSO (1.25, 2.5, 5, 10, or 15%), methanol (5, 10, or 15%), or DMA (5, 10, or 15%). The final dilution was 1:3 (milt: cryomedia). To examine pre-freezing sperm motility, sperm were activated by deionized water at 10, 20 or 30 min after milt was diluted with a specified cryomedia. To examine post-thaw sperm motility, three 500-μL straws per treatment were immediately filled with 150-μL aliquots of extended sperm and sealed for each fish. The sperm samples were frozen after 10, 20 or 30 min equilibration. The methods used to freeze and thaw sperm samples were identical to those previously described in Experiment 3.

Statistical Analysis

To detect significant changes in sperm motility, data were subjected to analysis of variance (ANOVA) for mixed-model procedures. When time was involved, a repeated measurement design was used. In each experiment, sperm samples from five male striped bass were used. In Experiment 3 and 4, the post-thaw motility for each male was averaged from six and three straws respectively. The P value was preset as 0.05. Data were shown as means ± standard error of the mean (SEM). All statistical procedures were run using SAS 8.0 software system (SAS Institute Inc.).

Results

Experiment 1

The solutions with 350 mmol/kg, isotonic to striped bass seminal plasma, activated more than 60% of the sperm cells regardless of whether NaCl or KCl was used (Fig. 1). The percentage of motile sperm in both solutions with osmolalities at 100, 200, and 300 mmol/kg was not significantly ($P > 0.05$) different from 0 mmol/kg (deionized water). Only 5% of the sperm were activated when the osmolality was 500 mmol/kg, and sperm motility was completely blocked when osmolality was higher than 600 mmol/kg.

Experiment 2

Based on the results of Experiment 1, Extenders 1, 2, and 3 with osmolalities of 350, 500, or 600 mmol/kg, respectively, were tested on sperm motility after a short-term storage interval. After 5 min, sperm motility decreased significantly ($P < 0.01$) in all three extenders compared to fresh sperm (0 min) activated with deionized water (Fig. 2). However, sperm in Extender 2 was observed to "recover" motility after 5 min, and 70% of the motile sperm could be activated after 15 min, which was significantly ($P < 0.01$) higher than the estimated sperm motility after only 5 min. In contrast, no significant ($P > 0.05$) motility recovery was observed after 5 min in either Extender 1 or 3. Only 40 and 50% of the sperm extended in Extender 1 and 3, respectively, could be activated after 15 min (Fig. 2).

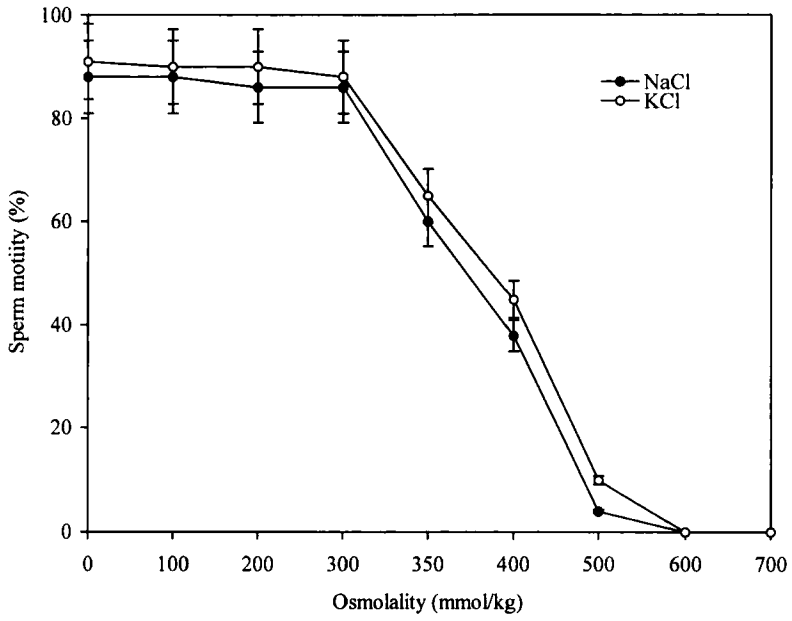

FIGURE 1. *The percentage of motile sperm (mean ± standard error). Fresh sperm from five striped bass male were activated by NaCl or KCl solution with various osmolalities.*

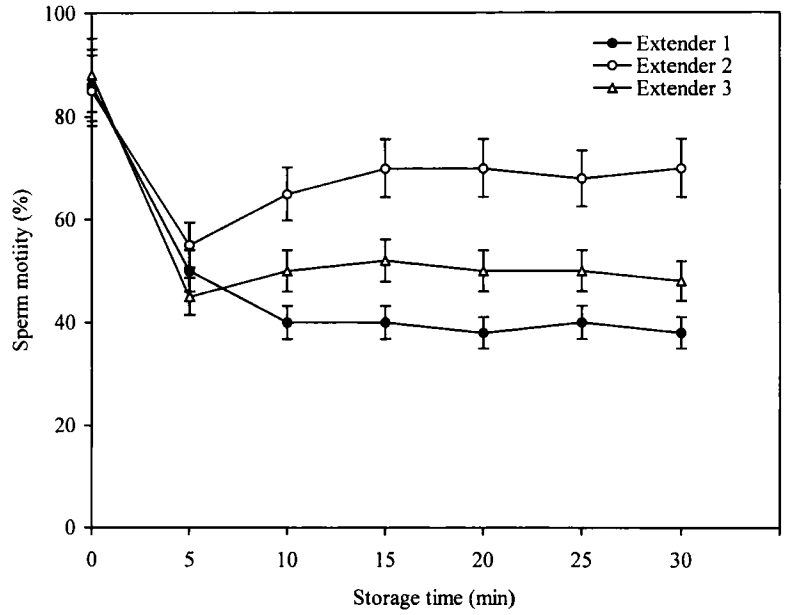

FIGURE 2. *The percentage of motile sperm (mean ± standard error). Fresh sperm from five striped bass male were mixed with Extender 1 (350 mmol/kg), Extender 2 (500 mmol/kg), or Extender 3 (600 mmol/kg) in a ratio of 1:2 (milt : extender). Sperm were activated at time 0 (control), 5, 10, 15, 20, 25, or 30 min after being mixed with the extenders.*

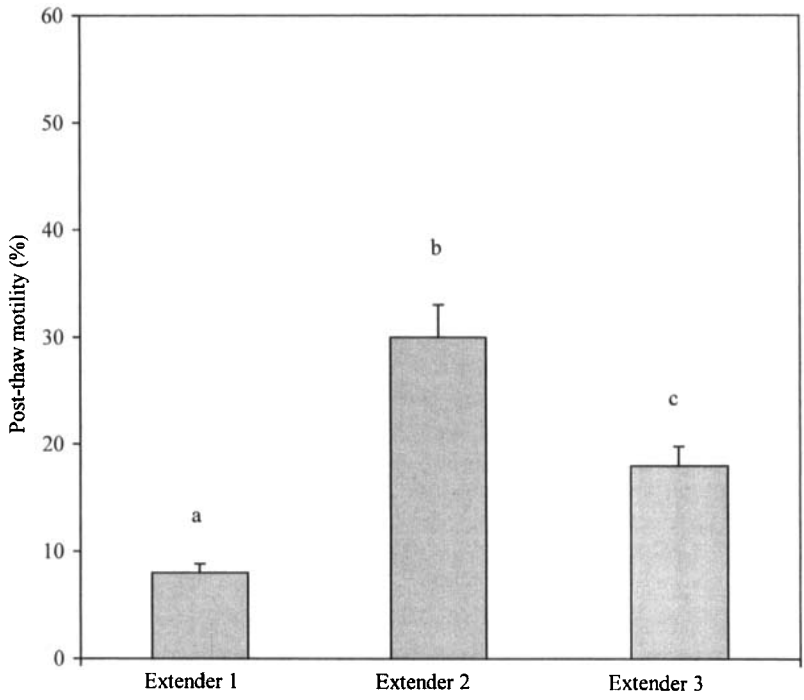

FIGURE 3. *Post-thaw motility (mean ± standard error). Fresh sperm from five striped bass male were extended by Extender 1 (350 mmol/kg), Extender 2 (500 mmol/kg), or Extender 3 (600 mmol/kg). Extended sperm were cryopreserved with 5% DMSO after 20 min equilibration. Bars with different letters are significantly different at 5% level by LSD.*

Experiment 3

The percentage of motile sperm after thawing was also examined with Extender 1, 2 and 3 (Fig. 3). Extender 2 demonstrated significantly higher ($P < 0.01$) post-thaw motility and was chosen to use in the freezing trials of Experiment 3.

Experiment 4

In trial 1, DMSO, methanol, and DMA were evaluated for their effects on sperm motility prior to freezing. Methanol resulted in a higher percentage of motile sperm prior to freezing than both the DMSO and DMA (Table 2). Sperm exposed to the 5% and 10% methanol treatments were 70% motile after exposure for up to 30 min. However, very low post-thaw motilities (~ 10%) were achieved by 5% and 10% methanol respectively after 10-min equilibration (Fig. 4). DMA reduced sperm motility prior to freezing to 10% (Table 2) even at the lowest concentration (5%) and with the shortest equilibration time examined (10 min). Sperm preserved with 5% and 10% DMA had less than 2% post-thaw motility. No post-thaw sperm motility was observed when 15% DMA was used (Fig. 4). Sperm motility prior to freezing was significantly ($P < 0.01$) decreased with increased DMSO concentrations (5, 10, and 15%). Drastic reductions in motility were also observed with increased equilibration time (10, 20, and 30 min) when DMSO was used (Table 2). Striped bass sperm mixed with 5% DMSO yielded the highest ($P < 0.01$) post-thaw motility (38 ± 3.6%). Only 17 ± 2.3% and 6 ± 1.0% post-thaw motilities were gained using 10 and 15% DMSO, respectively (Fig. 4).

In trial 2, DMSO concentration had a significant interaction ($P < 0.001$) with equilibration time (Fig. 5). Post-thaw motility was significantly higher ($P < 0.01$) with

TABLE 2. *Percentage of motile sperm (mean ± standard error) prior to freezing obtained with different cryoprotectants (final concentration) and equilibration times. Values with different superscript letters are significantly different at 5% level by LSD within one column. Values with different superscript numbers are significantly different at 5% level by LSD within one row.*

	Equilibration time		
	10 min	20 min	30 min
DMSO			
5%	$63 \pm 5.4^{b,1}$	$54 \pm 6.4^{c,2}$	$48 \pm 3.2^{b,3}$
10%	$26 \pm 5.2^{c,1}$	$14 \pm 3.0^{e,2}$	$9 \pm 1.8^{d,2}$
15%	$2 \pm 0.6^{d,1}$	$0^{f,2}$	$0^{e,2}$
Methanol			
5%	$78 \pm 1.7^{a,1}$	$77 \pm 1.8^{a,1}$	$71 \pm 2.1^{a,1}$
10%	$77 \pm 2.4^{a,1}$	$72 \pm 1.2^{b,12}$	$67 \pm 4.4^{a,2}$
15%	$68 \pm 3.8^{b,1}$	$38 \pm 1.5^{d,2}$	$23 \pm 1.7^{c,3}$
DMA			
5%	$9 \pm 0.7^{d,1}$	$5 \pm 0.6^{f,2}$	$5 \pm 0.3^{df,2}$
10%	$6 \pm 0.3^{d,1}$	$2 \pm 0.3^{f,2}$	$2 \pm 0.3^{ef,2}$
15%	$2 \pm 0.3^{d,1}$	$0^{f,2}$	$0^{e,2}$

5% DMSO at all equilibration times examined compared to 1.25, 2.5, and 10% DMSO. DMSO with a concentration of 5% with 10-min equilibration time obtained a high post-thaw motility (40 ± 2.9%), similar to our results obtained in trial 1.

Discussion

The extender solution isotonic to striped bass seminal plasma failed to inhibit sperm motility. Striped bass sperm were activated by extenders until the osmolality reached at least 600 mmol/kg. This characteristic of striped bass sperm is different from that reported for most other published teleost species. One exception is the freshwater fish pejerrey *Odontesthes bonariensis*. Strussmann et al. (1994) reported that sperm of pejerrey were activated when diluted with solutions having osmolalities up to 551 mmol/kg, even though the osmolality of its seminal plasma was 330 mmol/kg. Both hypo-osmotic and hyper-osmotic solutions activated sperm of striped bass and pejerrey. Similar to rainbow trout, striped bass have been found to have a higher K^+ concentration (51.6 ± 5.6 mM) in seminal plasma compared with the concentration in the blood (2.7 ± 0.1 mM) (Jenkins 1999). However, our data indicated that potassium in the extender as high as 110 mM (300 mmol/kg) failed to inhibit sperm motility. These results suggested that the initiation mechanism of striped bass sperm was different than that reported for trout and carp. Some other factor(s) besides osmolality and K^+ concentration may be involved in striped bass sperm initiation. However, the effects of such factors on sperm motility initiation were negated by hyper-osmotic solutions (≥ 600 mmol/kg).

Our results indicated that sperm motility significantly decreased in all three extenders examined after 5 min. When exposure to hyper-osmotic Extenders 2 and 3, sperm cells lost intercellular water with a subsequent decrease in motility. However, sperm in Extender 2 were observed to "recover" motility after 5 min and could keep, on average, 70% of sperm viable for activation for up to 30 min. This length of time was sufficient to complete the remaining activities of the cryopreservation process. Sperm in Extender 3 did not show significant motility recovery, presumably due to its higher osmolality compared to Extender 2. However, since Extender 1 vigorously activated sperm and excessive energy was expended,

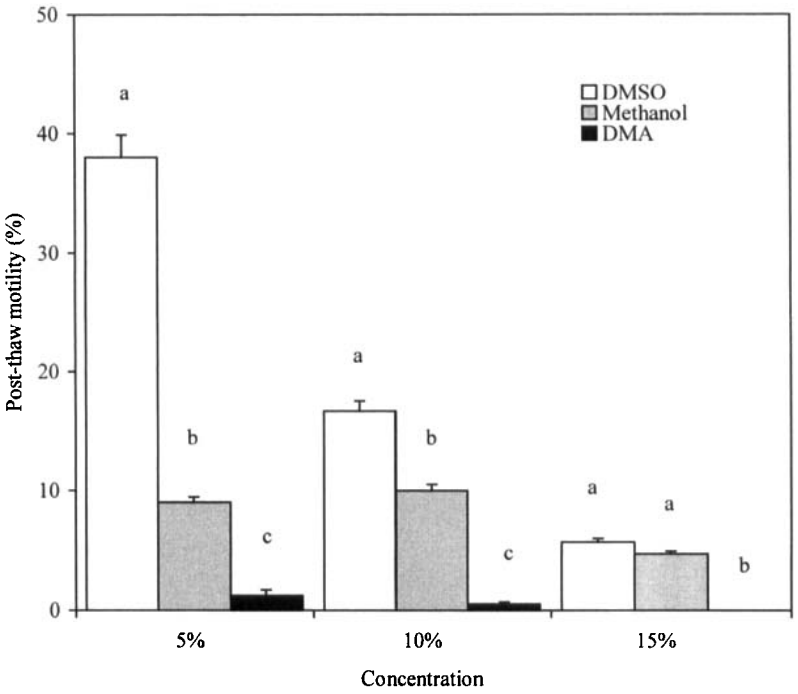

FIGURE 4. *Post-thaw motility (mean ± standard error). Fresh sperm from five striped bass male were extended by Extender 2. Extended sperm were cryopreserved with DMSO, methanol, or DMA after 10-min equilibration. Bars with different letters are significantly different within each concentration at 5% level by LSD.*

no sperm motility recovery was observed in Extender 1. Perchec et al. (1995) reported that fish sperm could not produce enough energy to make up for that lost during premature activation, which resulted in a shorter duration of sperm motility. The duration of striped bass sperm motility only lasts 30–60 sec normally (Jenkins-Keeran and Woods 2002a, 2002b), so available energy becomes one of the critical factors to initiate and keep sperm motile.

In our experiment, DMSO proved to be the best cryoprotectant for cryopreservation of striped bass spermatozoa when compared to methanol and DMA. DMSO with a concentration of 5% yielded significantly higher ($P < 0.01$) post-thaw sperm motility than 1.25, 2.5, 10, and 15% DMSO. Our results support previous published reports (Kerby 1983; Jenkins-Keeran and Woods 2002a) where they examined DMSO concentrations ≥ 5%. Our results indicated that methanol maintained higher sperm motility prior to freezing. This may be due to the lower osmolality of methanol compared to that of both DMSO and DMA. Our findings, however, demonstrated that DMSO provided better cryoprotection than methanol for the striped bass spermatozoa during freezing and post-thaw conditions. Very low post-thaw motility was achieved with DMA and is not recommended for use with striped bass.

Equilibration time played an important role in the success for cryopreservation of striped bass milt when using DMSO as a cryoprotectant. The shortest equilibration time examined, 10 min, showed both the best pre-freezing and post-thaw motilities. This was consistent with a recent study on African catfish (Horvath and Urbanyi 2000). In their study, 10 min also yielded the highest pre-freezing and post-thaw motility compared to other equilibration times ranging from 2 min to 30 min when DMSO was used. Equilibration times shorter than

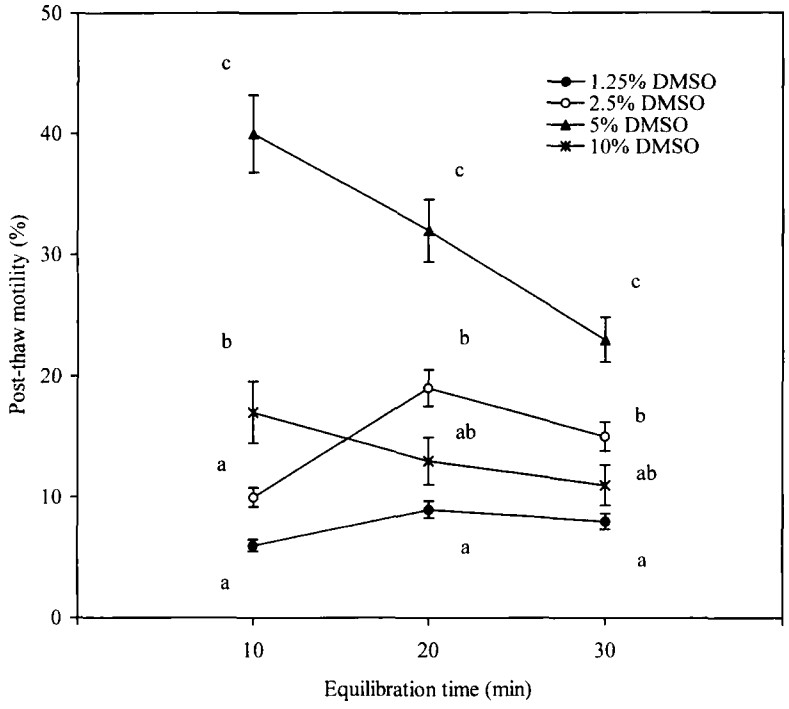

FIGURE 5. *Post-thaw motility (mean ± standard error). Fresh sperm from five striped bass male were extended by Extender 2. Extended sperm were cryopreserved with the final concentration of 1.25, 2.5, 5, or 10% DMSO after 10, 20, or 30 min equilibration. Symbols with different letters are significantly different within each equilibration time at 5% level by LSD.*

10 min were not examined in our experiments, since it's logistically difficult to complete the entire cryopreservation process (filling replicate sperm samples into straws, sealing the straws, placing the straws into the controlled rate freezer) within 10 min before freezing. Examination of shorter equilibration times could be considered if replicates of sperm samples are reduced. The DMSO concentrations we examined had significant interaction effects with equilibration time ($P < 0.001$). This suggested that different equilibration times should be used corresponding to different DMSO concentrations.

The processes prior to freezing play an important role in cryopreservation. It has been demonstrated that damage to cells caused by such processes can be significant (Lahnsteiner et al. 1992; Linhart et al. 1993; Lubzens et al. 1997). How to minimize damage to sperm cells during the processes prior to freezing will be critical to the development of a successful cryopreservation protocol.

In conclusion, our best result, in terms of post-thaw sperm motility, was obtained by using Extender 2 with 5% DMSO, with 10 min of equilibration time, a freezing rate of −40 C/min and thawing of samples in a 35 C water bath for 8 sec. Future research to find additional factors that will keep striped bass sperm immotile, preferably with an isotonic solution may be necessary to improve upon this cryopreservation protocol and to make it commercially viable.

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Literature Cited

Brown, G. G. and L. D. Brown. 2000. Cryopreservation of sperm of striped bass and white bass. Pages 130–137 *in* T. R. Tiersch and P. M. Mazik, editors. Cryopreservation of aquatic species. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Carlberg, J. M., J. C. Van Olst, and M. J. Massingill. 2000. Hybrid striped bass: an important fish in US aquaculture. Aquaculture magazine 26(5): 26–38.

Chao, N. H., W. C. Chao, K. C. Liu, and I. C. Liao. 1987. The properties of tilapia sperm and its cryopreservation. Journal of Fish Biology 30:107–118.

Conget, P., M. Fernandez, G. Herrera, and J. J. Minguell. 1996. Cryopreservation of rainbow trout (*Oncorhynchus mykiss*) spermatozoa using programmable freezing. Aquaculture 143:319–329.

Goodall, J. A., A. W. Blackshaw, and M. F. Capra. 1989. Factors affecting the activation and duration of motility of the spermatozoa of the summer whiting (*Sillago ciliata*). Aquaculture 77:243–250.

Gwo, J. 1994. Cryopreservation of yellowfin seabream (*Acanthopagrus latus*) spermatozoa (teleost, perciformes, sparidae). Theriogenology 41: 989–1004.

Gwo, J., K. Strawn, M. T. Longnecker, and C. R. Arnold. 1991. Cryopreservation of Atlantic croaker spermatozoa. Aquaculture 94:355–375.

Harrell, R. M., J. H. Kerby, T. I. J. Smith, and R. E. Stevens. 1990. Striped bass and striped bass hybrid culture: the next twenty-five years. Pages 253–261 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. American Fisheries Society, Bethesda, Maryland, USA.

Hodson, R. G. and C. V. Sullivan. 1993. Induced maturation and spawning of domestic and wild striped bass, Morone saxatilis (Walbaum), broodstock with implanted GnRH analogue and injected hCG. Aquaculture and Fisheries Management 24: 271–280.

Horvath, A. and B. Urbanyi. 2000. The effect of cryoprotectants on the motility and fertilizing capacity of cryopreserved African catfish *Clarias gariepinus* (Burchell 1822) sperm. Aquaculture Research 31:317–324.

Jenkins, K. 1999. The characterization and cryopreservation of striped bass (*Morone saxatilis*) semen. Master's thesis. University of Maryland, College Park, Maryland, USA.

Jenkins-Keeran, K. and L. C. Woods III. 2002a. The cryopreservation of striped bass *Morone saxatilis* semen. Journal of the world aquaculture society 33(1):70–77.

Jenkins-Keeran, K. and L. C. Woods III. 2002b. An evaluation of extenders for the short-term storage of striped bass milt. North American Journal of Aquaculture 64(4): 248–256.

Jenkins-Keeran, K., P. Schreuders, K. Edwards, and L. C. Woods III. 2001. The effects of oxygen on the short-term storage of striped bass semen. North American Journal of Aquaculture 63: 238–241.

Kerby, J. H. 1983. Cryogenic preservation of sperm from striped bass. Transactions of the American Fisheries Society 112:86–94.

Kerby, J. H. 1985. Growth, survival, and harvest of striped bass produced with cryopreserved spermatozoa. Transactions of the American Fisheries Society 114:761–765.

Lahnsteiner, F. 1996. Cryopreservation of semen of the grayling (*Thymallus thymallus*) and the Danube salmon (*Hucho hucho*). Aquaculture 144: 265–274.

Lahnsteiner, F., T. Weismann, and R. A. Patzner. 1992. Fine structural changes in spermatozoa of the grayling, *Thymallus thymallus* (Pisces: Teleostei), during routine cryopreservation. Aquaculture 103:73–84.

Leffler, M. 1999. Domesticated striped bass broodstock. Aquaculture Magazine 25(3):7–8.

Leung, L. K. P. 1991. Principles of biological cryopreservation. Pages 231–244 *in* B. G. M. Jamieson, editor. Fish evolution and systematics: evidence from spermatozoa. Cambridge University Press, New York, USA.

Linhart, O., R. Billard, and J. P. Proteau. 1993. Cryopreservation of European catfish (*Silurus glanis* L.) spermatozoa. Aquaculture 115:347–359.

Lubzens, E., N. Caube, I. Pekarsky, Y. Magnus, A. Cohen, F, Yusefovich, and P. Feigin. 1997. Carp (*Cyprinus carpio* L.) spermatozoa cryobanks—strategies in research and application. Aquaculture 155:13–30.

Morisawa, M. 1994. Cell signaling mechanism for sperm motility. Zoological Science 11:647–662.

Morisawa, M. and K. Suzuki. 1980. Osmolality and potassium ion—their roles in initiation of sperm motility in teleosts. Science 210:1145–1147.

Morisawa, M., K. Suzuki, and S. Morisawa. 1983a. Effects of potassium and osmolality on spermatozoan motility of salmonid fishes. Journal of Experimental Biology 107:105–113.

Morisawa, M., K. Suzuki, H. Shimizu, S. Morisawa, and K. Yasuda. 1983b. Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. Journal of Experimental Biology 107:95–103.

Oda, S. and M. Morisawa. 1993. Rises of intracel-

lular Ca^{2+} and pH mediate the initiation of sperm motility by hyperosmolality in marine teleosts. Cell Motility and the Cytoskeleton 25:171–178.

Ohta, H. and T. Izawa. 1996. Diluent for cool storage of the Japanese eel (*Anguilla japonica*) spermatozoa. Aquaculture 142(1–2):107–118.

Ogier de baulny, B., C. Labbe, and G. Maisse. 1999. Membrane integrity, mitochondria activity, ATP content, and motility of the European catfish (*Silurus glanis*) testicular spermatozoa after freezing with different cryoprotectants. Cryobiology 39: 177–184.

Perchec, G., J. L. Gatti, J. Cosson, C. Jeulin, F. Fierville, and R. Billard. 1997. Effects of extracellular environment on the osmotic signal transduction involved in activation of motility of carp spermatozoa. Journal of Reproduction and Fertility 110:315–327.

Perchec, G., C. Jeulin, J. Cosson, F. Andre, and R. Billard. 1995. Relationship between sperm ATP content and motility of carp spermatozoa. Journal of Cell Science 108:747–753.

Piironen, J. 1993. Cryopreservation of sperm from brown trout (*Salmo truttam.lacustris* L.) and Arctic charr (*Salvelinus alpinus* L.). Aquaculture 116: 275–285.

Rana, K. J., R. M. Muiruri, B. J. McAndrew, and A. Gilmour. 1990. The influence of diluents, equilibration time and pre-freezing storage time on the viability of cryopreserved *Oreochromis niloticus* (L) spermatozoa. Aquaculture and Fish Management 21:25–30.

Scott, A. P. and S. M. Baynes. 1980. A review of the biology, handling and storage of salmonid spermatozoa. Journal of Fish Biology 17:707–739.

Sherwood, N. M., L. W. Crim, J. L. Carolsfeld, and S. M. Walters. 1988. Sustained release I: Characteristics of *in vitro* release of gonadotropin-releasing hormone analogue (GnRH-a) from pellets. Aquaculture 74:75–86.

Stoss, J. and T. Reftsie. 1983. Short term storage and cryopreservation of milt from Atlantic salmon and sea trout. Aquaculture 30:229–236.

Striped Bass Growers Association. 1998. Results from the 1997 Striped Bass Growers Association producers survey. Aquaculture Magazine 24(5): 25–27.

Strussmann, C.A., P. Renard, H. Ling, and F. Takashima. 1994. Motility of pejerrey *Odontesthes bonariensis* spermatozoa. Fisheries Science 60(1): 9–13.

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

Tiersch, T. R., C. A. Goudie, and G. J. Carmichael. 1994. Cryopreservation of channel catfish sperm: storage in cryoprotectants, fertilization trials and growth of channel catfish produced with cryopreserved sperm. Transactions of the American Fisheries Society 123:580–586.

Woods, L. C. III and C. V. Sullivan. 1993. Reproduction of striped bass, Morone saxatilis (Walbaum), broodstock: monitoring maturation and hormonal induction of spawning. Aquaculture and Fisheries Management 24:211–222.

Woods, L. C. III, R. O. Bennett, and C. V. Sullivan. 1992. Reproduction of a domestic striped bass broodstock. The Progressive Fish-Culturist 54: 184–188.

Woods, L. C. III, C. C. Kohler, R. J. Sheehan, and C. V. Sullivan. 1995. Volitional tank spawning of female striped bass with male white bass produces hybrid offspring. Transactions of the American Fisheries Society 124:628–632.

Woods, L. C. III, J. Woiwode, M. McCarthy, D. Theisen, and R. O. Bennett. 1990. Non-induced spawning of captive striped bass in tanks. The Progressive Fish-Culturist 52:201–202.

Yao, Z., L. W. Crim, G. F. Richardson, and C. J. Emerson. 2000. Motility, fertility and ultrastructural changes of ocean pout (*Macrozoarces americanus* L.) sperm after cryopreservation. Aquaculture 181:361–375.