North American Journal of Aquaculture 63:238–241, 2001

The Effects of Oxygen on the Short-term Storage of Striped Bass Semen

KAREN JENKINS-KEERAN

Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20741, USA

PAUL SCHREUDERS AND KIMBERLY EDWARDS

Biological Resources Engineering, University of Maryland, College Park, Maryland 20741, USA

L. CURRY WOODS III*

Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20741, USA

Abstract.—The effect of different gaseous environments on the motility of spermatozoa from striped bass *Morone saxatilis* after 2 and 48 h of storage at 3 ± 1°C was examined. Storage in a 1.5-mL polypropylene snap-cap vial versus a 50-mL polystyrene tissue culture flask was also examined for any influence on motility. Motility declined with time within each treatment group and in comparison with the motility of freshly collected undiluted semen. After 2 h, there was no significant difference in the motility of sperm stored under ambient air or oxygen. After 48 h, however, semen stored under an oxygen atmosphere had a significantly ($P < 0.0444$) greater percentage of motile sperm (13 ± 2.4%) than did semen stored under ambient air (9 ± 1.9%) or nitrogen (4 ± 1.7%). These results suggest the importance of oxygen to the motility of striped bass sperm stored for short periods. After both 2 and 48 h of storage, the percentage of motile sperm obtained from semen stored in the 1.5-mL snap-cap vials was higher than that of sperm obtained from semen stored in the 50-mL tissue culture flasks in the same gaseous environment.

The demand for striped bass *Morone saxatilis* and its hybrids has made it one of the fastest-growing segments of finfish aquaculture in the United States (Striped Bass Growers Association 1998). One of the main problems faced by producers of hybrid striped bass is the different spawning times and geographical locations of the *Morone* species used to create the hybrids. To alleviate this problem, researchers have examined procedures such as cryopreservation (Kerby 1983, 1984), domestication of broodstock (Leffler 1999; Woods et al. 1999), and control of the reproductive cycle (Woods and Sullivan 1993; Sullivan et al. 1997). Another potential solution is short-term refrigerated storage of striped bass semen. Although no articles have been published on the short-term storage of striped bass semen, there is evidence of this technique being used by striped bass culturists (Harrell 1997; Brown and Brown 2000). Techniques for the short-term refrigerated storage of semen have been developed for several teleosts, such as walleye *Stizostedion vitreum* (Moore 1987), red drum *Sciaenops ocellatus* (Wayman et al. 1998), Atlantic sturgeon *Acipenser oxyrinchus* (DiLauro et al. 1994), milkfish *Chanos chanos* (Hara et al. 1982), tilapia *Tilapia mossambicus* (Harvey and Kelley 1984), paddlefish *Polyodon spathula* (Brown and Mims 1995), and several salmonids (Scott and Baynes 1980; Billard 1981; Erdahl et al. 1984; Jensen and Alderdice 1984; Erdahl and Graham 1987; McNiven et al. 1993; Christensen and Tiersch 1996). Investigators found that to obtain optimal sperm motility and viability, salmonid semen must have adequate exposure to air or oxygen (Scott and Baynes 1980; Billard 1981; Stoss et al. 1987; Bencic et al. 2000). However, exposure to oxygen has been shown to be detrimental to human spermatozoa (de Lamirande and Gagnon 1992a, 1992b; Aitken et al. 1998). This study investigates the effect of oxygen on the motility of striped bass spermatozoa after 2 and 48 h of storage at 3 ± 1°C.

Methods

Semen was collected from six male striped bass early in the spawning season. The fish were randomly selected from a population of captive and domesticated striped bass produced and maintained at the University of Maryland's Crane Aquaculture Facility in Baltimore. Each fish was anesthetized with quinaldine at a concentration of

* Corresponding author: LW60@umail.umd.edu

Received August 23, 2000; accepted January 9, 2001

70 mg/L (Woods et al. 1992). Urine in the bladder was expressed and the urogenital vent was wiped clean and dry to prevent contamination by water, urine, and feces. Semen was expressed directly into sterile 50-mL conical tubes by applying gentle abdominal pressure. All samples were kept at approximately 2 ± 1°C in an ice water bath until analysis could be performed. All fish appeared healthy before and after semen collection.

A portion of the freshly collected semen from each fish was activated upon initial collection by using Fisher's deionized ultra-filtered water (DIUF). Each fish's semen was subjected to three activations, from which we determined the average percentage of motile sperm. The activations were recorded on videotape with a Hitachi model KP-140 video camera attached to a Zeiss model D-7082 compound microscope at a magnification of 400×. The percentage of motile sperm was determined later from the video tapes. The remaining semen from each fish was diluted to an extender: semen ratio of 2:1. The extender was a simple solution of 8.6 g of NaCl per liter of DIUF (Brown and Brown 2000). Only extended semen was used for this experiment because previous investigations (Jenkins 1999) had shown that striped bass semen stored undiluted exhibited no motility after 24 h of refrigeration, whereas semen diluted with the 8.6 g/L salt solution maintained as much as 25% of its original motility under the same conditions.

The extended semen from each fish was divided into six treatment groups. In the first four treatments, 0.5 mL of extended semen was placed in each of four 1.5-mL polypropylene snap-cap vials. The first vial was stored open, exposed to ambient air. The second vial was stored closed, exposed only to the limited quantity of ambient air enclosed within the vial. The third vial was purged with dry nitrogen gas and closed, and the fourth vial was purged with dry oxygen gas and closed. The snap-cap vials were stored at 3 ± 1°C in their normal vertical position in a vial rack until tested for motility. Although most aquaculture facilities would use larger containers such as tissue culture flasks to store extended semen, the total volume of semen collected for this experiment was limited, and therefore 1.5-mL snap-cap vials were used.

After the appropriate volume of extended semen from each fish was divided among the four previously described treatments, at least 6 mL of extended semen for each fish was left. This remaining semen was used to evaluate storage in 50-mL polystyrene tissue culture flasks instead of 1.5-mL snap-cap vials. For the fifth treatment, 3 mL of extended semen from each fish was placed in a tissue culture flask and closed, the semen being exposed only to the ambient air enclosed within the flask. For the sixth treatment, 3 mL of extended semen from each fish was placed in a tissue culture flask, purged with nitrogen as in the third treatment, and closed to examine the effects of the absence of oxygen on the motility of striped bass sperm. The tissue culture flasks were stored horizontally to expose the maximum surface area of semen to air or nitrogen. All flasks were stored at 3 ± 1°C until tested for motility.

Sperm from each treatment from each fish was activated after 2 and 48 h of storage in replicate samples of three, using the same procedures described for the activation of the undiluted semen. Each vial and tissue culture flask was purged again with the appropriate gas immediately after its 2-h activations were completed. The percentage of motile sperm was visually estimated for all samples by the same observer. Spermatozoa that simply vibrated or did not show progressive forward movement were not considered motile.

An SAS (SAS Institute 1996) mixed-model analysis of variance (ANOVA) was used to identify significant differences in the percentage of motile sperm between treatment groups. Because not all gaseous environments could be tested by using the tissue culture flasks, the closed tissue culture flasks with ambient air and the closed tissue culture flasks purged with nitrogen were compared only with the snap-cap vials of the same gaseous environments to determine container effects. Pairwise contrasts of means were performed with differences considered significant at $P \leq 0.05$. Results are reported as means ± SEs.

Results

The percentage of motile sperm in the freshly collected striped bass semen was 36.9 ± 16.9%, an unusually low value for fresh semen. All fish appeared healthy both before and after semen collection. The poor semen quality may be due to the collection date's being early in the spawning season. For the sperm that were active, movement was fast and vigorous.

Compared with the results for freshly collected semen, the percentage of motile sperm decreased significantly ($P < 0.0001$) in all treatments after both 2 and 48 h of storage. The percentage of motile sperm also decreased significantly ($P < 0.0001$) between 2 and 48 h of storage. As the storage time of extended semen samples increased,

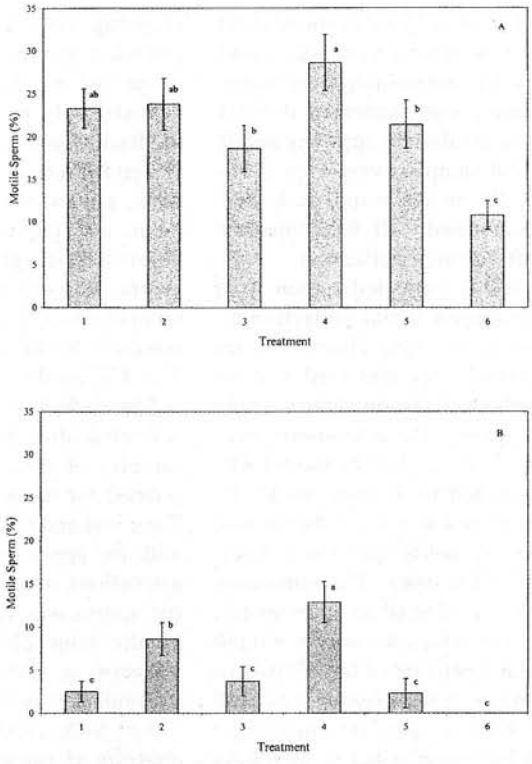

FIGURE 1.—Striped bass semen motility in an opened snap-cap vial (treatment 1), a closed snap-cap vial (treatment 2), a closed snap-cap vial purged with nitrogen (treatment 3), a closed snap-cap vial purged with oxygen (treatment 4), a closed tissue culture flask (treatment 5), and a closed tissue culture flask purged with nitrogen (treatment 6). Panel **A** shows motility after 2 h and panel **B** motility after 48 h. Data are means ± SE; treatments with different letters are significantly different from each other.

the vigorous activity associated with the motility of freshly collected spermatozoa decreased in all samples. Figure 1 shows the percentage of motile sperm obtained from the different treatments after 2 and 48 h of refrigerated storage. After 2 h of storage, no significant differences in the percentage of motile sperm were evident between semen samples stored under oxygen or in ambient air. However, the motility of the sperm treated with pure oxygen was statistically better than for those stored under a nitrogen atmosphere ($P < 0.0112$). After 48 h of storage, the percentage of motile sperm in semen samples stored in a snap-cap vial under an oxygen atmosphere was significantly higher than that of any other 48-h treatment group ($P < 0.0444$).

After 2 h of storage, there was no difference in the percentage of motile sperm obtained from semen stored in the snap-cap vials and that stored in the tissue culture flasks under ambient air. When the semen was stored under an atmosphere of pure nitrogen, however, the percentage of motile sperm in the snap-cap vials was significantly ($P < 0.0112$) greater than in the tissue culture flasks. After 48 h of storage under ambient air, the percentage of motile sperm obtained from the semen stored in the closed snap-cap vials was significantly ($P < 0.0033$) greater than that from the tissue culture flasks. Also, although the difference was not significant, the semen stored in the closed snap-cap vials purged with nitrogen had a higher average percentage of motile sperm than did the semen stored in the tissue culture flasks purged with nitrogen.

Discussion

The results of this experiment clearly demonstrate that the presence of oxygen is beneficial to striped bass sperm motility. Although human spermatozoa are highly susceptible to oxidative damage (de Lamirande and Gagnon 1992a, 1992b; Aitken et al. 1998), the results of this experiment support the results of previous investigations, in which the motility and viability of fish sperm were prolonged by the presence of oxygen (Scott and Baynes 1980; Billard 1981; Bencic et al. 2000). These data also support the theory that the sperm of animals that fertilize externally rely on an aerobic type of metabolism rather than a glycolytic type of metabolism (Mann 1964).

This study also suggests that the type of container used to store striped bass semen may affect semen quality. Under the conditions of our study, the 1.5-mL polypropylene snap-cap vial is the best storage container for small quantities of striped bass semen (3 mL or less), possibly because the vials have a lower ratio of surface area to volume for storing the semen than the tissue culture flasks provide. Given the greater volume of ambient air in the tissue culture flask than in the snap-cap vial, the sperm in the closed tissue culture flask are unlikely to have used up the oxygen reserve faster than the sperm in the closed snap-cap vial. Therefore, the smaller percentage of motile sperm observed in the tissue culture flask should be due to something other than a lack of oxygen. Although the extent of evaporation was not measured in any of the samples, an increase in semen viscosity was observed in both the opened snap-cap vials and the tissue culture flasks after 48 h of storage. Clearly, oxygen is necessary to maintain optimal striped bass sperm motility during short-term storage, but

further research is needed to determine whether the benefit of using pure oxygen rather than ambient air is commercially justified. In addition, further research is needed to determine the best ratio of semen surface area to volume for storage in both air and pure oxygen as well as the most appropriate storage container for larger volumes of striped bass semen.

References

Aitken, R. J., E. Gordon, D. Harkiss, J. P. Twigg, P. Milne, Z. Jennings, and D. S. Irvine. 1998. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biology of Reproduction 59:1037–1046.

Bencic, D. C., M. Krisfalusi, J. G. Cloud, and R. L. Ingermann. 2000. Short-term storage of salmonid sperm in air versus oxygen. North American Journal of Aquaculture 62:19–25.

Billard, R. 1981. Short-term preservation of sperm under oxygen atmosphere in rainbow trout (*Salmo gairdneri*). Aquaculture 23:287–293.

Brown, G. G., and S. D. Mims. 1995. Storage, transportation, and fertility of undiluted and diluted paddlefish milt. Progressive Fish-Culturist 57:64–69.

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.

Christensen, J. M., and T. R. Tiersch. 1996. Refrigerated storage of channel catfish sperm. Journal of the World Aquaculture Society 27(3):340–346.

de Lamirande, E., and C. Gagnon. 1992a. Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa on sperm axonemes. Journal of Andrology 13:368–378.

de Lamirande, E., and C. Gagnon. 1992b. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. Journal of Andrology 13:379–386.

DiLauro, M. N., W. F. Krise, M. A. Hendrix, and S. E. Baker. 1994. Short-term cold storage of Atlantic sturgeon sperm. Progressive Fish-Culturist 56:143–144.

Erdahl, A. W., D. A. Erdahl, and E. F. Graham. 1984. Some factors affecting the preservation of salmonid spermatozoa. Aquaculture 43:341–350.

Erdahl, A. W., and E. F. Graham. 1987. Fertility of teleost semen as affected by dilution and storage in a seminal plasma-mimicking medium. Aquaculture 60:311–321.

Hara, S., J. T. Canto, Jr. and J. M. E. Almendras. 1982. A comparative study of various extenders for milkfish, *Chanos chanos* (Forsskal), sperm preservation. Aquaculture 28:339–346.

Harrell, R. M. 1997. Hybridization and genetics. Pages 217–234 *in* R. M. Harrell, editor. Striped bass and other *Morone* culture. Elsevier, Amsterdam, The Netherlands.

Harvey, B., and R. N. Kelley. 1984. Chilled storage of *Sarotherodon mossambicus* milt. Aquaculture 36: 85–95.

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

Jensen, J. O. T., and D. F. Alderdice. 1984. Effect of temperature on short-term storage of eggs and sperm of chum salmon (*Oncorhynchus keta*). Aquaculture 37:251–265.

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

Kerby, J. H. 1984. Cryopreservation of striped bass spermatozoa: problems and progress. Pages 205–213 *in* J. P. McCraren, editor. The aquaculture of striped bass: a proceedings. Maryland Sea Grant Publication UM-SG-MAP-84–01. University of Maryland, College Park.

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

Mann, T. 1964. The biochemistry of semen and of the male reproductive tract. Methuen and Co., London.

McNiven, M. A., R. K. Gallant, and G. F. Richardson. 1993. Fresh storage of rainbow trout (*Oncorhynchus mykiss*) semen using a non-aqueous medium. Aquaculture 109:71–82.

Moore, A. A. 1987. Short-term storage and cryopreservation of walleye semen. Progressive Fish-Culturist 49:40–43.

SAS Institute. 1996. The SAS system for Windows. Cary, North Carolina.

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.

Stoss, J., L. Geries, and W. Holtz. 1987. The role of sample depth in storing chilled rainbow trout (*Salmo gairdneri*) semen under oxygen. Aquaculture 61: 275–279.

Striped Bass Growers Association. 1998. Results from the 1997 striped bass growers association producers survey. Aquaculture Magazine 24(5):51–56.

Sullivan, C. V., D. L. Berlinsky, and R. G. Hodson. 1997. Reproduction. Pages 11–73 *in* R. M. Harrell, editor. Striped bass and other *Morone* culture. Elsevier, Amsterdam, The Netherlands.

Wayman, W. R., T. R. Tiersch, and R. G. Thomas. 1998. Refrigerated storage and cryopreservation of sperm of red drum, *Sciaenops ocellatus* L. Aquaculture Research 29:267–273.

Woods, L. C., III, R. Bennett, and C. V. Sullivan. 1992. Reproduction of a domestic striped bass broodstock. Progressive Fish-Culturist 54(3):184–188.

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

Woods, L. C., III, E. M. Hallerman, L. Douglass, R. M. Harrell. 1999. Variation in growth rate within and among stocks and families of striped bass. North American Journal of Aquaculture 61:8–12.