

HYBRID STRIPED BASS⁷

Project Component Termination Report for the Period
June 1, 1990 to August 31, 1996

NCRAC FUNDING LEVEL: \$269,000 (June 1, 1990 to February 29, 1996)

PARTICIPANTS:

Fred P. Binkowski	University of Wisconsin-Milwaukee	Wisconsin
George G. Brown	Iowa State University	Iowa
Paul B. Brown	Purdue University	Indiana
Konrad Dabrowski	Ohio State University	Ohio
James E. Ebeling	Ohio State University	Ohio
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
Jeffrey A. Malison	University of Wisconsin	Wisconsin
Robert J. Sheehan	Southern Illinois University-Carbondale	Illinois
Bruce L. Tetzlaff	Southern Illinois University-Carbondale	Illinois
M. Randall White	Purdue University	Indiana
<i>Extension Liaison:</i>		
Joseph E. Morris	Iowa State University	Iowa

REASON FOR TERMINATION

The objectives for this work on Hybrid Striped Bass were completed.

PROJECT OBJECTIVES

- (1) Develop larval diets and economically feasible techniques to convert hybrid striped bass young from zooplankton to prepared diets.
- (2) Develop intensive hatchery production techniques for white bass and to "domesticate" white bass by producing brood stock originating from induced spawns.
- (3) Improve methods for storage and transport of striped bass and white bass gametes.

PRINCIPAL ACCOMPLISHMENTS

In a comparative study conducted at Southern Illinois University-Carbondale (SIUC), hatching rates for embryos incubated in Heath trays (28.2%) were equivalent to tannic acid-treated (150 mg/L water) embryos incubated in Heath trays (22.9%) or McDonald jars (22.4%).

Facilities to intensively rear larval white bass were established at Ohio State University (OSU), SIUC, and the University of Wisconsin-Milwaukee (UW-Milwaukee). White bass larvae from three separate spawning trials were shipped by overnight freight to OSU and UW-Milwaukee. Attempts to rear larval white bass were minimally successful. Less than 1% survival rates were obtained by day 122 at UW-Milwaukee, day 45 at OSU, and day 24 at SIUC.

A group of white bass sac-fry shipped from SIUC to UW-Milwaukee was introduced evenly by volume into twelve 60-L (15.9-gal) flow-through aquaria. Each aquarium contained approximately 300 sac-fry. These fish were offered "green tank" water and the three experimental diets that were provided by Purdue University (Purdue). The length of the cylindrical food particles ranged from approximately 0.5 to 1.7 mm (0.02 to 0.07 in) and the diameter was 420-595 μm . White bass sac-fry are approximately 3.5 mm (0.14 in) in total length. The cross sectional diameter of the feed approximated the width of the entire head (550-630 μm) of white bass sac-fry, and was outside of the range of the width of the mouth. UW-Milwaukee researchers ground portions of the diets in a mortar and pestle and sieved it through a 150 μm mesh to obtain more suitable-size particles. From May 26-31, 1995, each of the three ground and sieved diets was offered to fry in triplicate aquaria along with "green tank" water. The controls received only "green tank" water.

No feeding activity or interest by the fry in the formulated diets was observed. Mortality of the sac fry was heavy in all the tanks and by May 31 (within 6 days), less than a dozen fry were observed in any of the aquaria and more than half of them had only one or no living fry. At this point the trial was terminated.

Researchers at SIUC found that both hybrid striped bass crosses at a 2-5 g (0.07-0.18 oz) size range readily convert from zooplankton to formulated feed. Over 90% of the fish converted to formulated feed within two days as compared to 70-85% after seven days for largemouth bass which were trained in a "side-by-side" study. Preliminary results indicate that white bass and reciprocal-cross hybrids are equivalent in this regard and can make the switch between day 21 and 28 after hatch. Original cross hybrids can generally be switched at day 7 after hatch.

A problem facing hybrid striped bass aquaculturists is that hybrid fry are not always available. Gametes must be obtained from two species that may not be spawning simultaneously or are located in different geographical areas. Therefore to facilitate hybrid production, viable *Morone* semina need to be readily available when ripe eggs are available.

To aid in the solution of this problem, procedures for reliable short-term (refrigerated) and long-term (cryopreservation) storage of striped bass (*Morone saxatilis*) semina were developed. Initially, the characteristics of high quality spermatozoa were examined to determine methods for assessing sperm quality and developing effective sperm handling techniques. This led to the formulation of extenders for short-term (less than 21 days) refrigerated (1°C; 33.8°F) storage. The quality of stored seminal samples was tested by determining sperm motility percentages and developing a sperm quality index (SQI). Refrigerated extended seminal samples were routinely stored for 14 days with 50% sperm motility.

Cryopreservation procedures were developed and sperm quality of cryopreserved seminal samples of striped bass were assessed. Fertility tests with these samples were performed with white bass (*M. chrysops*) eggs and results were compared to those results when using (fresh) white bass semen.

Ten media containing dimethylsulfoxide (DMSO) were used to cryopreserve striped bass spermatozoa. Although all media successfully cryopreserved spermatozoa, the best motility (SQI 2.3: about 50%) was obtained with samples cryopreserved in the five media containing 4% DMSO. Using the criteria for high quality semen, the samples cryopreserved in media containing 4% DMSO with or without trehalose and bovine serum albumin gave the best motility results and were used in fertility tests with white bass eggs. Straws of the cryopreserved samples were transported from Florida to SIUC packed in dry ice. These were then stored in liquid nitrogen until used in fertility tests. Striped bass spermatozoa were cryopreserved with relatively simple methods. This may partially be because of the small size of the sperm, causing damage by the freezing process to be minimal since the cryogenic medium penetrates the whole cell very rapidly and the actual freezing may be rapid enough to prevent damaging ice crystal formation.

In the hybrid cross, the study was pursued until the hatch of normal larvae. Although success with cryopreserved spermatozoa has previously been reported for striped bass results were determined on the basis of cleavage, which does not necessarily indicate the normal development of diploid embryos. Fertility was tested using striped bass semen cryopreserved in cryogenic media and white bass eggs. The percent fertilization based on the number of hatched, normal larvae was 6.2 % for the cryopreserved semen and 2.5% for the eggs fertilized with fresh control white bass semen (dead and abnormal larvae were excluded). This represented a 251.2% hatch from cryopreserved semen related to control semen. No development was found in control eggs (unfertilized eggs) tested for parthenogenesis.

The motility intensity of thawed and activated cryopreserved spermatozoa was roughly equivalent to that of seminal samples activated after 14-21 days of refrigerated storage, indicating that

cryopreservation of striped bass semen may be the best option when storage time exceeds 21 days.

Emphasis was also focused on developing refrigerated and frozen storage methods for white bass spermatozoa. Evaluations of sperm motility and nuclear magnetic resonance (NMR) were used as measures of success in developing methodologies. NMR was used to measure the availability of high energy phosphorus compounds to power flagellar movements in spermatozoa.

Sperm quality was best when seminal samples were extended prior to shipping and when they were transported in tissue-culture flasks which provided a larger air space than the microcentrifuge tubes which were also tested as shipping containers. Extenders with simple formulations, including one that was essentially only a saline solution, were as good or better than a more complex extender solution for maintaining sperm quality during refrigerated storage at 1°C (33.8°F). The simple saline solution extender maintained good sperm quality for up to one month of refrigerated storage.

Declines in high-energy phosphorus compounds and increases in their breakdown product, as measured via NMR, corresponded with declines in sperm motility over time during refrigerated storage of semen. However, NMR detected differences in stored energy in spermatozoa among seminal samples when no such differences in sperm motility were detected, indicating that NMR may be a more sensitive measure of sperm quality.

It was found that a cryogenic solution consisting of a simple extender and DMSO as the cryoprotectant performed as well as more complex cryogenic media in sperm motility tests. Fertility was somewhat reduced using cryopreserved semen, as compared to semen which had been extended and stored at 1°C (33.8°F) for about one week. Cryopreservation reduced white bass sperm motility to 5 to 25% of motility in fresh semen samples, a reduction similar to that found in seminal samples which are extended and stored under refrigeration for about four weeks. It is recommended that refrigerated storage be used for white bass semen if storage times of one month or less are anticipated. Cryopreservation is the better option, if sperm storage is to exceed one month.

IMPACTS

Studies by the Hybrid Striped Bass Work Group demonstrate that:

- Improvements in hatching rates allows for increased hatchery production or reduction in brood stock needs.
- Improvements in larval rearing techniques of white bass will allow "true" domestication.
- Improvements in switching hybrid striped bass fingerlings from zooplankton to formulated feeds will increase production efficiency.
- *Morone* semen which is to be stored should be kept cold at all times subsequent to stripping.
- White bass injected with hCG once per month and held at 15°C (59.0°F) produced 2 to 3 times as many spermatozoa as compared to those either given hCG once per week or no hCG but otherwise treated similarly--using this approach allowed semen to be obtained from each fish once per week for several months.
- Semen should be diluted with an extender prior to shipping and transported on ice.
- Relatively simple extender solutions (saline solutions) are effective for refrigerated storage of *Morone* semen.
- Tissue culture flasks proved to be better than microcentrifuge tubes for shipping white bass semen--this difference was attributed to the oxygen in the larger air space of the former.
- *Morone* semen can be extended and stored at 1°C (33.8°F) and good motility can be retained for 3 to 4 weeks.
- Initial evaluations indicated that changes in NMR spectra of seminal samples are consistent with changes in sperm motility; however, NMR may provide a more sensitive measure of semen quality.
- Cryopreservation reduced sperm motility by about 50%, as compared to extended semen.

- A relatively simple cryogenic medium (4% DMSO in a simple extender solution) was effective for storing *Morone* semen.
- Excellent fertility in white bass eggs was obtained using cryopreserved striped bass semen, and good fertility was obtained using cryopreserved white bass semen.
- Based on reductions in sperm motility, cryopreservation is the better option for *Morone* semen if it is to be stored for more than 3 to 4 weeks, whereas refrigerated storage is better for shorter storage times.

RECOMMENDED FOLLOW-UP ACTIVITIES

NCRAC is currently funding studies aimed at comparing different geographical strains of hybrid striped bass and white bass in ponds. These studies are incorporating spawning, sperm storage, and hatchery procedures developed in this project. The sperm storage protocols are also being tested in industry settings. Collectively, the results from past and current studies should pave the way to economically undertake hybrid striped bass culture in the NCR. Continued demonstration of the technologies developed need to be undertaken with industry partners.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the [Appendix](#) for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

SUPPORT

YEAR S	NCRAC- USDA FUNDIN G	OTHER SUPPORT					TOTAL SUPPOR T
		UNIVER- SITY	INDUSTR Y	OTHER FEDERA L	OTHE R	TOTAL	
1990- 93	\$101,000	\$94,000				\$94,000	\$195,000
1993- 96	\$168,000	\$119,44 0				\$119,44 0	\$287,440
TOTAL	\$269,000	\$213,44 0				\$213,44 0	\$482,440

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Publications in Print

Kelly, A.M., and C.C. Kohler. 1996. Sunshine bass performance in ponds, cages, and indoor tanks. *Progressive Fish-Culturist* 58:55-58.

Kohler, C.C., and R.J. Sheehan. 1991. Hybrid striped bass culture in the North Central Region. Pages 207-209 *in* Proceedings of North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.

Kohler, C.C., R.J. Sheehan, C. Habicht, J.A. Malison, and T.B. Kayes. 1994. Habituation to captivity and controlled spawning of white bass. *Transactions of the American Fisheries Society* 123:964-974.

Woods, L.C., 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.

Manuscripts

- Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. In preparation. Short-term and long-term storage of striped bass *Morone saxatilis* semen. Journal of the World Aquaculture Society.
- Brown, P.B., R. Twibell, Y. Hodgins, and K.A. Wilson. In review. Use of soybean products in diets fed to juvenile hybrid striped bass. Journal of the World Aquaculture Society.
- Kohler, C.C. In press. Chapter 6 in Harrell, R.M., editor. White bass production and broodstock development. Elsevier Press, Amsterdam.
- Morris, J.E., and C.C. Kohler. In press. Pond culture of hybrid striped bass fingerlings in the North Central Region. NCRAC Fact Sheet Series, NCRAC Publications Office, Iowa State University, Ames.
- Suresh, A.V., J.B. Rudacille, M.L. Allyn, V. Sheehan, R.J. Sheehan, and C.C. Kohler. In review. Induction of ovulation in white bass (*Morone chrysops*) using hCG and LHRHa. Aquaculture.

Papers Presented

- Brown, G.G., L.D. Brown, K. Dunbar, C. Habicht, R.J. Sheehan, C.C. Kohler, and L. Koutnik. 1991. Evaluation of white bass semen with ³¹P-NMR for the improvement of transportation, storage, and fertility methods. 53rd Midwest Fish and Wildlife Conference, Des Moines, Iowa, November 30-December 4, 1991.
- Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. 1995. Use of cryopreservatives. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995.
- Brown, P.B., R. Twibell, Y. Hodgins, and K. Wilson. 1995. Soybeans in diets fed to hybrid striped bass. 24th Annual Fish Feed and Nutrition Workshop, October 19-21, 1995, Columbus, Ohio.
- Brown, P.B., Y. Hodgins, R. Twibell, and K.A. Wilson. 1996. Use of three soybean products in diets fed to hybrid striped bass. World Aquaculture '96, January 29-February 2, 1996, Bangkok, Thailand.
- Habicht, C., R.J. Sheehan, C.C. Kohler, G.G. Brown, and L. Koutnik. 1991. Routine collection, storage, and shipping of white bass sperm. 29th Annual Meeting Illinois Chapter of the American Fisheries Society, Champaign, Illinois, March 5-7, 1991.
- Kohler, C.C. 1993. The farm fish of the future: hybrid stripers. AQUA '93: 7th Annual Minnesota Aquaculture Conference, Alexandria, Minnesota, March 5-6, 1993. (Invited paper)
- Kohler, C.C. 1994. Hybrid striped bass aquaculture. Yellow Perch and Hybrid Striped Bass Production: From Fry to Frying Pan, Piketon, Ohio, July 3, 1994. (Invited speaker)
- Kohler, C.C. 1995. Broodstock management of white bass. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, November 2-4, 1995, Champaign, Illinois.
- Kohler, C.C. 1996. Induced out-of-season spawning of fishes. Missouri Aquaculture Industry Association Annual Meeting, February 3-4, 1996, Jefferson City, Missouri.
- Kohler, C.C. 1996. Advancing hybrid striped bass culture in the North Central Region and elsewhere. Aquaculture America '96, U.S. Chapter of the World Aquaculture Society, February 14-17, 1996, Arlington, Texas.

- Kohler, C.C., R.J. Sheehan, M.L. Allyn, J.B. Rudacille, and A. Suresh. 1996. Controlled spawning of white bass. Aquaculture America '96. U.S. Chapter of the World Aquaculture Society, February 14-17, 1996, Arlington, Texas.
- Kohler, C.C., R.J. Sheehan, C. Habicht, J.A. Malison, and T. B. Kayes. 1992. Acclimation to captivity and out-of-season spawning of white bass. Aquaculture '92, 23rd Annual Meeting of the World Aquaculture Society, Orlando, Florida, May 21-25, 1992.
- Kohler, C.C., R.J. Sheehan, C. Habicht, V. Sanchez, J. Finck, J.A. Malison, and T.B. Kayes. 1991. Domestication and out-of-season spawning of white bass. 53rd Midwest Fish and Wildlife Conference, Des Moines, Iowa, November 30-December 4, 1991.
- Kohler, C.C., R.J. Sheehan, C. Habicht, V. Sanchez, J.A. Malison, and T.B. Kayes. 1993. Development of white bass brood stock and spawning protocol. U.S. Chapter World Aquaculture Society Annual Meeting, Hilton Head Island, South Carolina, January 27-30, 1993. (Invited paper)
- Kohler, C.C., R.J. Sheehan, and T.B. Kayes. 1989. Advancing hybrid striped bass culture in the Midwestern United States. 51st Midwest Fish and Wildlife Conference, Springfield, Illinois, December 5-6, 1989.
- Kohler, C.C., R.J. Sheehan, C. Habicht, V. Sanchez, J.A. Malison, and T.B. Kayes. 1992. Collection, acclimation to captivity, and out-of-season spawning of white bass. American Fisheries Society Annual Meeting, Rapid City, South Dakota, September 14-17, 1992.
- Kohler, C.C., R.J. Sheehan, V. Sanchez, and A. Suresh. 1994. Evaluation of various dosages of hCG to induce final oocyte maturation and ovulation in white bass. 25th Annual Meeting of the World Aquaculture Society, New Orleans, Louisiana, January 12-18, 1994.
- Kohler, C.C., R.J. Sheehan, A. Suresh, L. Allyn, and J. Rudacille. 1996. Effect of hCG dosage on hatching success in white bass. International Congress on the Biology of Fishes, July 15-18, 1996, San Francisco, California.
- Kohler, S.T. 1995. Cost of production. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, November 2-4, 1995, Champaign, Illinois.
- Koutnik, L.A., R.J. Sheehan, C.C. Kohler, C. Habicht, and G.G. Brown. 1992. Motility and fertility of extended and cryopreserved *Morone* sperm: when is cryopreservation the best option? Annual Meeting, Illinois/Wisconsin Chapters of the American Fisheries Society, Waukegan, Illinois, February 10-13, 1992. (Awarded Best Student Paper)
- Morris, J. 1995. Pond preparation for larval fish. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, November 2-4, 1995, Champaign, Illinois.
- Rudacille, J.B., and C.C. Kohler. 1996. Relative performance of white bass, sunshine bass, and palmetto bass fed a commercial diet. Aquaculture America '96, U.S. Chapter of the World Aquaculture Society, February 14-17, 1996, Arlington, Texas. (Awarded Best Student Presentation)
- Sheehan, R.J. 1995. Use of sperm extenders. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, November 2-4, 1995, Champaign, Illinois.
- Swann, L. 1995. Cage culture. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, November 2-4, 1995, Champaign, Illinois.