ATTACHMENT D

PROJECT NAME: Advancing Hybrid Striped Bass Culture

FUNDING LEVEL: Year 1 - $50,500
Year 2 - $50,500

DURATION: 2 years

ADMINISTRATIVE ADVISOR: Dr. James E. Seeb, Fisheries Research Laboratory and Department of Zoology, Southern Illinois University, Carbondale, IL 62901

TABLE OF CONTENTS

ATTACHMENT D

PROJECT NAME: Advancing Hybrid Striped Bass Culture

FUNDING LEVEL: Year 1 - $50,500
Year 2 - $50,500

DURATION: 2 years

ADMINISTRATIVE ADVISOR: Dr. James E. Seeb, Fisheries Research Laboratory and Department of Zoology, Southern Illinois University, Carbondale, IL 62901

JUSTIFICATION

Per capita consumption of seafood products has grown steadily in the United States, a demand largely being met by increased imports. For example, the U.S. purchased $4.8 billion in seafood from abroad in 1986 while only selling $1.3 billion worth overseas (U.S. Department of Commerce Statistics). Thus, seafood imports represent a major cause of the U.S. balance of trade deficit. States within the North Central Region are a major source of demand for imported seafood. The North Central Region is rich in terrestrial resources, and not surprisingly, agriculture is a dominant industry. However, the movement of agriculture in the region toward production of commodities, such as corn and soybeans, with ultra-high efficiency has been accomplished at great cost; now surpluses in all major crops, combined with land devaluations and financial strains, have forced both the agriculture system and its participants to investigate other farm technologies (Illinois Dept. Agriculture 1986). Aquaculture is perceived within the region as a logical vehicle for diversification. Indeed, it is this perception and desire for aquaculture development that provided the impetus to establish a USDA regional aquaculture center in the Midwest.

The immediate concerns for aquaculture development in the North Central Region are the identification of suitable species/hybrids for culture, development of broodstocks, and modification of existing technologies for rapid deployment within the emerging industry. Striped bass (*Morone saxatilis*) x white bass (*M. chrysops*) hybrids offer considerable commercial potential within much of the region. As a clear indication of its regional market potentital, seafood processors in Chicago are willing to pay a minimum of $3.50/lb in the round for fresh hybrid striped bass (personal communication from Andy Roberts, Illinois Dept. Agriculture).

The striped bass is a temperate-water anadromous fish that is native to the Atlantic coast and is widely stocked in large lakes and reservoirs in many parts of the U.S., including the North Central Region. The striped bass is prized as a game fish throughout most of its range and commands high market prices as a food fish (Norton et al. 1983). In 1983, the striped bass was identified at the national level (JSA 1983) as having significant potential for commercial aquaculture development.

Since 1983, research related to the development of commercial striped bass aquaculture has focused increasingly on the culture of striped bass x white bass hybrids. Numerous studies have demonstrated that both the female striped bass x male white bass (SB x WB, original cross) and the female white bass x male striped bass (WB x SB, reciprocal cross) hybrids are faster growing (at least during the first 2 years of life), and more robust and more resistant to disease and environmental extremes than purebred striped bass (Kerby 1986).

The identification of the hybrid striped bass as a candidate for commercial aquaculture development in the North Central Region is appropriate because: (1) a number of fish farmers are producing this fish; and (2) much of the southern half of the region is at approximately the same latitude and has about the same seasonal water temperature conditions as the mid-and southern Atlantic states where hybrid striped bass culture is being pursued. Indeed, the potential for future collaboration between the North Central, Northeastern and Southern Regions in the development of a national hybrid striped bass industry seems clear, particularly in light of the fact that the white bass is a native species and fairly common in the North Central Region.

According to the National Aquaculture Development Plan (JSA 1983), the principal constraint on commercial striped bass aquaculture in the U.S. is the "nonavailability of seed stock". Recent declines in the striped bass fisheries along the Atlantic coast, as well as legal constraints, have increasingly limited the availability of wild broodstock (especially females) as a source of gametes (Harrell 1984). In part, the problem of limited availability of striped bass gametes in the North Central Region could be greatly reduced by utilizing female white bass crossed with male striped bass to produce reciprocal cross hybrids. White bass are native and fairly common throughout much of the region (Scott and Crossman 1973; Becker 1983). However, legal constraints also limit access to wild white bass stocks (though not so much as for striped bass on the Atlantic coast). To that end, a NCRAC-sponsored cooperative regional

hybrid striped bass research project that is interdisciplinary in scope and involves investigators from two institutions in two states: Southern Illinois University, and the University of Wisconsin-Madison, is currently underway. The principal goal of that project is to address key problems that pertain to the development of commercial hybrid striped bass culture in the North Central Region. Problem areas being addressed include: (1) broodstock development, (2) mechanisms regulating the natural reproductive cycle, and (3) manipulation of gonadal maturation and out-of-season spawning.

To effect additional cost savings in the production of "seed stock", improved methods of cryopreserving semen could be employed to minimize the number of male striped bass needed as broodfish (see Stoss 1983; Kerby 1983; Kerby et al. 1985). Broodstocks of female striped bass and male white bass would have to be maintained only for genetic selection and production of male striped bass and female white bass broodfish. Efficient methods of storing and transporting gametes, if made available, could greatly facilitate efforts to cross stocks that spawn at different times or are located at different stations. Although such methods need to be perfected for both semen and eggs, it is more likely that studies on semen will result in rapid development of technology for use in the aquaculture industry. In addition to development of methods for cryopreservation of semen (long-term storage), there is a need for improvement of procedures for short-term storage and transportation. The use of nuclear magetic resonance spectroscopy (NMR) provides a powerful tool by which the metabolic state of seminal samples can be monitored before and after storage and transportation, as well as during the pre-freezing and freezing steps of cryopreservation. The development of reliable techniques to store, cryopreserve, and transport gametes would improve breeding and production capabilities for culture technology of hybrid striped bass. Specifically, the development of these techniques would allow: (1) a continuous supply of gametes, (2) year around production, (3) facilitation of selective breeding, and (4) more efficient use of available gametes.

Larval hybrid striped bass are traditionally stocked in earthen ponds that have been fertilized to ensure an abundant source of live foods (Kerby 1986). Fingerlings are subsequently harvested and brought indoors to be trained to formulated feeds. The fingerlings readily undergo training to prepared diets. However, the above procedure would not always be an option where the hybrid is produced out-of-season (a specific objective of the on-going project) or when ponds are simply not available such as would be the case in proposed commercial systems designed to rear fish solely in indoor, recycle systems. Also, the survival of hybrid striped bass larvae in ponds is extremely variable. Accordingly, there is a need to develop intensive larval hybrid striped bass feeding strategies similar to those developed for striped bass (Lewis et al. 1981) in tanks from hatching to advanced fingerlings.

This proposal describes a cooperative regional research project that will be interdisciplinary in scope and involve investigators from two institutions in two states: Iowa State University (ISU) and Southern Illinois University (SIU). The principal goal of the project is to address key problems that pertain to the development of commercial hybrid striped bass culture in the North Central Region. Problem areas to be addressed include: (1) storage and transport of gametes, and (2) feeding strategies for intensive culture of larvae.

RELATED CURRENT AND PREVIOUS WORK

Storage and Transport of Gametes

Cryopreservation of fish spermatozoa, in general, has met with variable success. Development of suitable cryogenic media and freezing procedures have permitted successful freezing of semen from a number of species (Stoss 1983). Existing methods facilitate experimental and hybridization programs within fish culture, however, the methods are often inadequate for use in production facilities (Kerby 1983).

A major barrier preventing use of cryopreservation on a large scale basis is the lack of reproducible and reliable results between researchers and techniques. Multiple steps during the freezing process such as stripping and handling of gametes, compatibility of semen with various freezing solutions, and the physical stress of applied freezing and thawing programs contribute degrees of variability. These factors fluctuate from one freezing program to the next making it impossible for researchers to account for quality variability. Hence, any one factor in this sequence of freezing steps may influence the results.

The quality of cryopreserved semen is traditionally assessed on the basis of motility and fertility studies. Unfortunately, both motility and fertility are all or nothing in that samples are completely spent following analysis. NMR provides a means to analyze samples prior to, during, and after frozen storage without activating (and hence exhausting the samples). NMR can be used to isolate the parameters within the freezing procedure that contribute degrees of variability. Pre-freezing factors such as extender quality and equilibration time, cryoprotectant toxicity, and

semen quality between males, as well as freezing and post-freezing stress can be analyzed separately. Information gained will provide a means to upgrade the quality of freezing programs and improve the reliability of the results. Following NMR analysis subsequent motility and fertility studies can be conducted to correlate metabolic changes, sample quality, and fertilizing capacity.

The present study intends to improve on the methods for cryopreservation of gametes by using information obtained from studies on the preservation of biological membranes (Crowe and Crowe 1984; Rudolph and Crowe 1985). Studies by Rudolph and Crowe (1985) have demonstrated that the most effective cryoprotectants for biological membranes were the dissaccharides, trehalose and sucrose, and the amino acid, proline. All of these are naturally occurring cryoprotectants. These investigators have shown that trehalose prevents damage to membranes by forming hydrogen bonds with the polar head groups of phospholipids, the predominant macromolecule in the membrane.

To date, Brown and colleagues (Iowa State University) have successfully cryopreserved striped bass and white bass semina and have observed a very high percent (75-100) of motility in the thawed samples. However, they have not performed fertility studies. The cryogenic media consisted of the Trout Extender #7 (Table 1) mixed with 3-4% dimethylsulfoxide and one or more of the following additives: bovine serum albumin, soybean flour, proline, and trehalose. In addition, a post-freezing schedule was used whereby the sample was thawed in ice water.

Researchers (Sheehan and Kohler) at SIU have recently initiated studies focusing on extender and cryopreservation solution formulations for short- and long-term gamete storage, respectively, utilizing several fish species. Refrigerated white bass, bluegill, and tilapia sperm, diluted with extender solutions (modified Trout Extender #7), have been successfully stored 8 to 10 times longer than controls (refrigerated neat semen) based on motility studies. Cryopreserved (Trout Extender #7 plus 7% DMSO) tilapia sperm retained 75-100% viability after thawing, based on both dye exclusion and sperm activation tests. Cryopreserved white bass and bluegill sperm suffered little loss in viability after thawing on the basis of sperm motility tests. Similar to Brown and colleagues at ISU, fertility tests with cryopreserved gametes have yet to be successfully conducted at SIU.

SIU has established a laboratory population of about 400 adult white bass for the purpose of studying reproductive hormone profiles and gonadal development in captive fish (a collaborative study with the University of Wisconsin, funded by the North Central Regional Aquaculture Center). Portions of this laboratory population will be placed on photoperiod and temperature regimes that artificially compress the annual cycle to 10 and 8 months, while the remainder will remain on a 12-month cycle. SIU will also maintain a similar number of adult white bass in aquaculture ponds. This ongoing research could potentially provide opportuities for researchers at both ISU and SIU to obtain white bass gametes for experimentation a number of times over the course of the year from manipulated laboratory populations, pond populations, and wild-caught fish.

Since the shipment of gametes is critical to this proposal, methods must be developed for the shipping not only of undiluted, extended, and cryopreserved semen but also for eggs and embryos. To date, Brown and colleagues have shipped several samples of striped bass semina from Virginia to Iowa. These have been either stored in extenders or shipped as undiluted semen. These semina do not seem to be sensitive to low oxygen levels, so stoppered flasks can be used. Better success with white bass semen was obtained with respect to maintaining motility after shipping.

The problems of storage and cryopreservation of gametes can be approached by using an unique procedure. Fourrier transform nuclear magnetic resonance spectroscopy (NMR) has become an important technique for studying metabolism in living tissue (Iles et al. 1982). The technique uses a powerful magnet and radiowaves to obtain information about chemical compounds. The presence, and in some cases the concentration, of certain atoms which are sensitive to these radio waves can be measured. As a result, important chemicals (metabolites) in the living cells can be monitored. Thus, the principal advantage of NMR is that information can be obtained non-invasively (i.e. no damage to the cell). The ability to measure metabolite concentrations without biochemical extraction of chemical manipulation has contributed to and reshaped important concepts regarding metabolic functions.

The use of ^{31}P-NMR has grown immensely in its application in living systems since the first landmark studies of erythrocytes by Moon and Richards (1973) and Henderson et al. (1974). Numerous researchers including Hoult et al. (1974), Burt et al. (1976), Coleman and Gadian (1976), Navon et al. 1977) have subsequently utilized ^{31}P-NMR to study living cells.

As with erythrocytes and other cells types, gametes can be readily studied with ^{31}P-NMR. Spermatozoa are particularly suited for such studies because: (1) they are plentiful and can be easily obtained, (2) their functions are highly specialized (motility, sperm-egg attachment) and can be studied separately, and (3) their environment can be controlled without disrupting the cells.

ATTACHMENT D

During the last four years, George Brown and colleagues at Iowa State University have performed extensive ^{31}P-NMR studies on semen and spermatozoa of several species of fish, including rainbow and brown trout, walleye, and muskellunge. The results of some of these studies on semen have been published (Robitaille et al. 1987a) and others are in preparation. In these studies, resonances for nucleotide triphosphates (NTP, primarily ATP), nucleotide diphosphates (NDP), inorganic phosphate (Pi), phosphoesters, phosphodiesters, and phosphocreatine (PCr) were observed. They examined spermatozoa immediately after spawning, prior to and following motility initiation and during refrigerated short-term storage. They examined motility initiation under anaeroboic conditions and determined as predicted by ^{31}P-NMR results that these fish spermatozoa are not capable of motility under such conditions. It would appear that high levels of free-NTP and/or PCr are a requirement for motility in fish spermatozoa. Also, they found a burst of motility reduces thethe level of phosphocreatine which is used in the energy shuttle between mitochondria and the dynein arms of the flagellar apparatus. They further demonstrated that this peak rapidly decreases during anaerobic conditions, but can be restored during aeration. They also found that these fish spermatozoa possess a bound NTP pool which is thought to correspond to NTP on the dynein arms of the flagellum much like the situation proposed in mammalian spermatozoa (Robitaille et al. 1987b).

Table 1. Extenders composition.

Extenders	E-G Ext[1]	Ext #5	Ext #7	Ext #9	Ext #13
$CaCl_3 \bullet 2H_2O$	0.250g	0.250g	0.250g	0.250g	____
$MgCl_2 \bullet 6H_2O$	0.440g	0.440g	0.440g	0.440g	____
$NaHCO_3$	0.470g	0.470g	0.470g	0.470g	____
KCl	5.115g	5.115g	5.115g	5.115g	____
NaCl	11.682g	11.682g	11.560g	11.158g	17.520g
glucose	20.0g	____	____	____	____
pyruvate	____	12.0g	12.0g	12.0g	____
citric acid	0.200g	0.200g	0.200g	0.200g	____
bicine (5.3g/100mL)	40mL	____	____	____	____
HEPES	____	4.76g	4.76g	9.52g	____
ddH_2O	2000mL	2000mL	2000mL	2000mL	2000mL
KOH (1.27g/100mL)	20mL	20mL	20mL	20mL	____
pen-strep[2]	____	20mL	20mL	20mL	20mL
osmolarity	310mOsm	310mOsm	310mOsm	319mOsm	310mOsm
pH	7.6	7.6	7.6	7.6	7.6

Extenders were mixed, pH adjusted, and then used or frozen.
[1] Modified Erdahl-Graham extender: Na_2HPO_4 has been replaced with $NaHCO_3$.
[2] Penicillin-streptomycin: Sigma, P 0906: 5,000 units of penicillin, 5mg streptomycin/mL of 0.9% NaCl.

More recently, Brown and colleagues examined the semina and spermatozoa of striped bass and white bass with ^{31}P-NMR procedures. Their initial results demonstrate that these semina are excellent material for the proposed study since high energy phosphorus compounds can be readily observed including phosphocreatine (PCr). It was found that the spermatozoa are not sensitive to low oxygen levels (contrary to results obtained with trout spermatozoa) or to mild centrifugations. They can be obtained in high concentrations (30-100 x 10^9 spermatozoa/mL) and in large volumes (2-20mL) two aspects necessary for NMR studies.

An excellent review by Scott and Baynes (1980) examined many of the methods and extenders commonly used for short-term storage of fish gametes. Interestingly, many of these studies have demonstrated that undiluted trout semen can be stored and remain fertile for one to two weeks (Ginsberg 1963). However, even with the development of various extenders, this time has not been significantly improved (Erdahl et al. 1984; Stoss and Donaldson 1982; Stoss 1983). Metabolism has received little emphasis (Benau and Terner 1980).

The Iowa State University investigators also performed extensive studies on short-term storage of spermatozoa of several fish species, including trout, walleye, and muskellunge. Semen volume, sperm concentration, dye exclusion, extracellular pH, and sperm motility were routinely determined for undiluted semen collected throughout the breeding period for all species. Although a great variation existed between individual samples in reference to sperm count and volume, the other factors seemed to be fairly consistent. With NMR studies, typical results included an initial decrease in ATP and ADP levels followed by the subsequent gradual decrease in PCr. The greatest decrease occurred during the first 24 hours of storage. Lower levels of motility were observed once the PCr levels began to decrease.

The trout undiluted semen was amenable to storage (1°C) and maintained motility for longer periods of time than the undiluted semina of walleye and muskellunge, which was short lived at 1°C and rapidly lost their motility function. NMR studies demonstrated that the energy levels of these species' semina decreased rapidly at 1°C, although the energy level of trout semen declined considerably slower.

In order to increase the viable period of these semina, extenders were used. In working with and testing the Erdahl-Graham's extender (Table 1), several problems were apparent: microbial infection, extracellular pH becoming alkaline, and substrate utilization. As these problems had to be solved individually, numerous experiments were performed. The trout spermatozoa were found not to utilize glucose, the pH could increase from 7.6 to 8.4 or higher within 24 hours, and microbial contamination, although variable, eventually occurred. Although spermatozoa were quite durable and were maintained routinely for 1-2 weeks, it was felt a better extender could be developed for routine work. The data for the extension of walleye and muskellunge semina is presently being examined by George Brown, Iowa State University.

Striped bass and white bass semen have been stored using Trout Extender #7, Extender #9, and Extender #13 which is only a saline solution (Table 1). Iowa State researchers have had considerable success with extenders #7 and #13 and have kept viable samples for 3-6 weeks. One difference from the semina of other fish species examined is the low requirement for oxygen during storage at 1°C. As a result, the lids of the culture flasks can be tightened, thus preventing dessication, a real problem with trout semen. Undiluted semina of both bass species were found to be so concentrated that storage without the use of extenders usually resulted in a great loss in the motility percentage within a 24 hour period.

Larval Feeding Strategies

Currently, the standard method of culturing hybrid striped bass involves the stocking of 1 to 4 day-old larvae into earthen ponds which were fertilized to promote production of zooplankton. The larvae are allowed to feed on zooplankton for a period of 30 to 40 days, and then harvested at a size of 40 to 60 mm. The intensive culture of hybrid striped bass necessitates the development of technologies for rearing the larvae of this fish in tanks. Combining a pond rearing facility with an intensive culture operation has several problems. First, construction and maintenance of ponds which are used only 30 days per year is not economical. Second, with the development of off-season spawning of hybrid striped bass, larvae could be produced during periods when seasonal weather conditions prevent pond use.

Early attempts to rear the larvae of striped bass and other coolwater fishes have met with limited success. Survival and/or growth of the larvae fed commercial feeds was lower than control fish fed live brine shrimp nauplii (Braid 1977; Carreon 1978; Bowman 1979). Therefore, the techniques for rearing striped bass larvae require the use of live brine shrimp nauplii as feed for the first 25 days (Lewis et al. 1981). However, due to the smaller size of hybrid striped bass larvae compared to pure striped bass, often the brine shrimp nauplii are too large for the fish to ingest. Harrell (1984) had to supplement a diet of brine shrimp with live rotifers when feeding the larvae of F_1 hybrid striped bass. However, the efficacy of constructing hatcheries for both brine shrimp and rotifers is questionable. Based on

the work of Al-Ahmad (1978), the space required to rear sufficient quantities of rotifers equals the space required to rear the fish.

Recently, several manufacturers using microencapsulation and freeze drying technologies have developed a new form of diets for larval fish. Loadman et al. (1989) used one of these feeds to achieve 30 percent survival when rearing walleye larvae from first feeding to 30 days. Millard (1986) also reported success with several of these diets for rearing whitefish and muskellunge.

Feed presentation and density have also been shown to be a factor in the successful rearing of larval fish. Lewis et al. (1981) and Loadman et al. (1989) feel that upwelling currents maintain feed in the water column longer and thus make it more available to the fish. These authors, as well as Al-Ahmad (1978) also show that food density is critical, both for the initiation of feeding and to prevent cannibalism.

OBJECTIVES

1. To improve methods for storage and transport of striped bass and white bass gametes.

2. To develop larval diets and economically feasible techniques to convert hybrid striped bass young from zooplankton to practical diets.

PROCEDURES

Objective 1

Studies focusing on gametes of seasonal spawners have time limitations for accomplishing objectives. We intend to circumvent this constraint in two ways: (1) out-of-season spawning research with white bass is simultaneously being conducted at SIU; (2) we will take stored samples to hatcheries located at various latitudes for fertility studies, as well as to obtain additional gametes.

Cryopreservation of semen will be performed at SIU. Seminal samples are often extended using #7 in a 1:2 ratio then stored on ice for a time period varying from a few minutes to one or more days. After evaluation of sperm motility, semen will then be mixed with cryogenic medium containing Extender #7 and including one or more of the following additives: bovine serum albumin, soybean flour, promine D, trehalose, sucrose, proline, and dimethylsulfoxide. This prepared medium, glassware, and equipment are kept on ice to avoid temperature shock. Without delay this mixture is siphoned into 0.5 mL freezing straws and placed on dry ice for 15-20 minutes. These straws are then stored in the vapor medium of liquid nitrogen for 1 or more days. Frozen samples are then thawed and sperm suspensions are examined for motility immediately. Other frozen samples will be shipped to ISU for NMR examination.

Since we are attempting some innovative approaches to the freezing of semen, each step will be carefully examined for any effect on sperm motility and metabolism. Motility percentages and viability of spermatozoa will be determined. NMR techniques will be used to examine the energy levels of spermatozoa before and after freezing.

We have already had success freezing and thawing striped bass, white bass, and tilapia semina, although we have not used such samples in fertility studies. We need to improve our methods and develop ways to freeze large amounts of materials rapidly and efficiently. In the final analysis, fertility studies will be the sole test to determine efficacy of procedures developed.

Gametes, primarily semen, that have been treated for short-term storage (extenders) and cryopreserved materials will regularly be shipped between Ames, Iowa and Carbondale, Illinois. Shipments will also be made to other striped bass/white bass hatcheries in the United States. Semen will be stored in culture flasks with various extenders. Although samples will be analyzed before shipping, they will be analyzed again after transportation. Since preliminary trials have shown the bass semen to be sensitive to continuous agitation, methods will be used to reduce such actions. For example, studies have shown that striped bass samples are not overly sensitive to low oxygen, therefore storage in tubes with little or no air space might be workable. As with the previous studies, the "acid test" for determining efficacy of transporting gametes will be fertility tests.

The procedures for the determination of metabolic energy levels with NMR of striped bass and white bass spermatozoa will aid in the development of improved and reliable short-term and frozen storage techniques. Nuclear

Magnetic Resonance Spectroscopy (NMR) will be used to monitor the metabolic response of samples exposed to various temperatures, chemical environments, extenders, and freezing solutions. The presence, absence, and changes of metabolic products during these treatments will serve as an indicator of those conditions most suitable for semen storage.

From various sources striped and white bass gametes will be obtained during the spring months. Samples will be collected in tissue culture flasks and packed on ice for transportation to ISU or SIU. In the laboratory, volume, concentration, pH, motility, extender evaluation, and NMR observations are to be performed for each (when possible) seminal sample.

For NMR analysis, 3 mL of the prepared sample are pipetted into a 10 mm NMR tube. This tube is inserted into the super conducting magnet of a Bruker WM300 spectrometer operating at 121 MHz for ^{31}P-NMR observation. An external capillary containing 0.1 M methylene diphosphonic acid (Alfa Products), a chemical shift reference (delta = 18.69 ppm) in 99.8% D20 (Columbia) is added to the 10 mm NMR tube to provide a lock signal. All shifts are reported such that 85% phosphoric acid is assigned a chemical shift of 0.00 ppm. Spectra are collected at 1, 10, or 20^{o}C depending on the particular study. Since the concentration of spermatozoa in each sample is very high, relatively good spectra can be obtained in less than 2 min of acquisition. Spectra are observed using a 78 degree pulse (10.6 microsec), a 0.5 sec receiver delay, a 13,889 Hz sweep width and an acquisition time of 0.59 sec without proton decoupling. The number of scans will range from 50 to 3,500.

Use of ^{31}P-NMR, a method which identifies phosphorus metabolites in the mM range, will allow identification of metabolites and their concentrations in spermatozoa and in the medium which they are suspended. These metabolites are adenosine monophosphate (cAMP), phosphocreatine (PCr), inorganic phosphages (Pi), phosphosugars, and nicotinamide adenine dinucleotide (NAD+ and NADH). Identification and measurement of these compounds will be made with semen which has been freshly collected and shipped to ISU within 24 hours. Samples will be aerated and cooled to 1 C prior to analysis (to prevent anaerobic respiration during collection). Following NMR analysis, samples will be checked again for motility percentage. The intracellular pH will be determined from a calibration curve obtained by titrating the cells in the presence of a proton ionophore.

After these results are obtained, spectra for semen will be examined at temperatures of 1, 10, and 22^{o}C in an aerated or an anoxic environment during short-term storage to determine the rate that high energy phosphorus compounds are used during a resting (maintenance) stage. An anoxic environment can be created in tightly stoppered NMR tubes containing the sample and can be analyzed directly as metabolic compounds decrease. Most importantly, measurements of recovery by such anoxic spermatozoa (when aerated) can be obtained. In addition, seminal fluid (void of spermatozoa) will be examined for high energy compounds. These experiments should show the effects of anaerobic conditions in stored high energy compounds and should indicate possible irreversible conditions. To determine if the metabolic profile is affected seasonally, analysis of freshly shipped semen will occur periodically throughout the proposed time period.

One of the major purposes of the energy storage of spermatozoa is preparation for motility. Energy consumption prior to and during motility can be measured by the quantity of peaks heights of ATP/ADP and PCr. Motility can also be monitored in an aerobic environment when recovery is not possible. Results are expected to show a decrease in PCr and ATP/ADP following activation. Recovery of the original levels of these compounds should occur in the presence of oxygen.

These analysis are designed to develop metabolic profiles for the *Morone* spermatozoa. Percent of motility and level of activity will serve as the criteria for semen viability and sample quality. ^{31}P-NMR of semen of the two species showed peaks representing phosphomoesters, phosphodiesters, inorganic phosphates, phosphocreatine, and adenosine triphosphates. On a comparison, the relative values of these compounds are similar in both species.

Samples will be stored either as undiluted or extended semen in 25 cm^2 tissue culture flasks with a liquid depth of no greater than 0.3 mm to prevent anoxia. Flask will have lids tightened for a minimum gas exchange and will be incubated in a Hotpack refrigerated incubator at 1^{o}C. After varying periods of storage time, aliquots of semen will be removed and analyzed.

Routine tests will be performed at both ISU and SIU and will include one or more of the following: sperm counts, dye exclusion, motility percentage, extracellular pH, oxygen uptake, and NMR observations. To determine a viable count for spermatozoa, an eosin solution (0.113 g of Esoin B/100 mL of extender) is used for a dye exclusion tests. The solution of mixed (5:1) with semen and counts are made using a cytohematometer. Viable spermatozoa do not absorb dye. Since percentage of motility is very difficult to estimate accurately, we are using the following numerical scale: 5 (75-100 motility), 4 (approximately 50%), 3 (5-25%), 2 (<5%), and 1 (0%). Motility is recognized

as active forward motion, not vibratory. The extracellular pH is determined on fresh samples of semen also after storage or various analyses. A Chemtrix pH meter is used in a cold room (4-5°C) to measure pH's of cold extenders and seminal samples. Because of the large amount of protein in seminal fluid, the pH reference is rechecked between each measurement.

After significant results are obtained from short-term storage of undiluted semen, other samples will be diluted with various extenders. These will usually be performed in a 1:2 ratio (semen:extender) although other ratios will also be used. The original extender used in our studies is described by Erdahl and Graham (1980) but several modifications have now been implemented. For clarity, the extenders and their ingredients are listed in Table 1. Extenders will be tested for ability to maintain motility, energy levels, and viability of spermatozoa. Particular interest will be given to extracellular pH, antibiotics, buffers, and substrates.

Morone semen is collected "dry" (undiluted), in which condition the spermatozoa are nonmotile. Upon dilution with 1%, 10%, or 25% artificial sea water, the spermatozoa undergo vigorous motility for 30-45 seconds. The effect of short-term storage (1 to 21 days) on the viability of fish spermatozoa will be determined to answer several questions. In particular, what are the effects on motility activation during storage at 1, 10, and 22°C, both under aerobic and anaerobic conditions? How rapidly is motility activation affected and can significant decreases in activation be recovered particularly in the cases of anoxia? What are the maximum time periods for sperm storage? Initially, we will increase our data bank on spermatozoa stored in seminal fluid and then examine spermatozoa mixed with extenders which have been successfully used for storage. Important observations at this point are which extenders are best maintaining the sperm viability, particularly over extended periods of time. We expect that the levels of motility activation will decrease during storage and hope to identify nutritional and chemical requirements that will maintain high levels. We are also interested in the recovery of "energy depleted" spermatozoa. Another concern is whether spermatozoa can be stored anaerobically at low temperatures and then be recovered sufficiently to perform motility and fertility studies.

We will be obtaining seminal samples from different males. This will allow us to determine variation in energy levels occurring between individuals, between males from different location and maybe between seasonal spawners. The mass of data collected for many males should be meaningful. Large sample size and frequency of collection will permit analysis of seminal quality between individuals and will continue to be an accurate data base that will apply to all individuals.

Objective 2

During the first year of this study, three commercially available feeds will be examined as potential feeds for larval hybrid striped bass. These include Zeigler Bioplancton^{R}, Zeigler AP-100^{R}, and Kyowa Fry Feed. The first feed is a freeze-dried natural plankton while the latter two are microencapsulated high nutrient feeds.

The study will be conducted at the SIU Aquaculture Research Facility utilizing one 12,000-L water re-use system. This system is comprised of six 1,800-L circular fiber glass tanks. Wastewater from these tanks is pumped through a sand filtration system to remove particulate matter, and then reconditioned by biofiltration and aeration. Three conical bottomed larval fish cages, each containing approximately 110 liters of water will be placed in each of three circular tanks. Each cage will be supplied with in-flowing water directed to the bottom of the cone via a shad-tube, thus providing a slight upwelling current. Each cage will be stocked with pre-feeding larval hybrid striped bass at a density of 100 fish per liter. To eliminmate the possibility of feed transfer among cages in a tank, all three cages of fish in a given tank will be offered the same experimental diet.

Hybrids striped bass larvae will be obtained either from the SIU Quad Cities Aquaculture Facility or from a commercial source. After acclimation to the 15°C rearing temperature, the fish will be stocked into the experimental units. Numbers of larvae stocked will be determined through standard volumetric techniques.

When the fish are 4.5 days old, feeding trials will commence. The experimental diets will be offered to the fish at 10 minute intervals, 24 hours per day through the use of automatic feeders. Feeding rates will be adjusted such that each group of fish is exposed to a density of feed of 100 particles per liter every 10 minutes. After several feedings, a relatively high density of feed will accumulate due to limited loss of food particles from the system. High feed density is necessary to initiate feeding and to control cannibalism in the larvae of predacious fish (Lewis et al. 1981; Heidinger and Tetzlaff 1985; Loadman et al. 1989).

Standard water quality parameters (D.O., NH_3-N, NO_2-N) will be monitored daily. Tank maintenance such as removal of organic matter, will be performed as required. The study will be terminated after 40 days. Performance criteria for the three diets tested will include percentage survival, growth and percent of fish with inflated swimbladders.

During the second year of the study, the best performing diet from the first year will be compared to two additional larval diets. Selection of these diets has yet to be made, but will probably include the diet currently being developed for red drum by Aquaculture Products Company and a graded flake feed.

Data collected at the four research stations will be collated on an ongoing basis, and the findings jointly (as appropriate) published in a timely manner in peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

FACILITIES

Objective 1

At Iowa State University, NMR is being utilized in the scientific community principally by chemists, biochemists, and physicists. By virtue of this fact, NMR time has often been difficult to obtain by members of the biological sciences. Poor access to NMR instruments is often the rule for the biologist. Indeed, this may be slowing down the infiltration of NMR as a practical technique outside of chemistry and biochemistry. At Iowa State University, however, NMR machines are under the supervision of a branch of the Graduate College known as Instrument Services. This service encourages all potential users regardless of their department to utilize NMR facilities. They have four strong field instruments: Nicolet NMC300, Bruker WM300, Bruker WM200, and a solid state MSL 300. The Bruker WM300 is equipped with temperature control and has probes which permit the examination of virtually every nucleus which is theoretically observable by NMR. This is a specialty instrument and routine work is discouraged on this machine by Instrument Services. We have had excellent access to the NMR spectrometers with a standard block of time scheduled.

Ample research space is available for use at Iowa State University. Available are hoods, work tables, autoclaves, air, gas, distilled water, refrigerators, freezers, and minor equipment (pH meters, balances, clinical centrifuges, mixers, etc.). In the laboratory of Dr. George Brown, the major equipment includes the following: trout holding tanks, various microscopes of high optical quality, Sorvall centrifuges, and an ultracold freezer (-70^{o}C). Other major equipment necessary for the proposed research is readily available in nearby laboratories.

At ISU, the cryogenic laboratory, developed by the Veterinary Clinical Sciences is one of the few laboratories charged with research and commercial endeavors, supported by a university. The unique aspect is that both commercial production of cryopreserved gametes and research on gametes are actively supported. The staff for this facility have broad backgrounds in equine, bovine, ovine, canine, and porcine gamete cryopreservation. The cryogenics laboratory is presently producing male and female gametes for commercial distribution on a national and international basis for the bovine species. The equipment and facilities for cryopreservation are housed and maintained in Dr. Steven Hopkins' laboratory in the Veterinary Clinic at the Iowa State University Veterinary College. This equipment is routinely used for cryopreservation of bull semen. However, the expertise and the facilities of this laboratory may also be used for the vitrification studies outlined in this proposal.

At Southern Illinois University the cryogenic lab is housed by the Central Hybridoma Laboratory, SIU School of Medicine, which has extensive facilities to perform tissue culture procedures. Specifically, the laboratory contains a Programmed Automatic Cryo-Med Freezer and a Cryostar (-100^{o}C) Cryogenic Preservation System. The laboratory is operated by highly-trained specialists and operates as a central research shop available to all SIU researchers. The Central Hybridoma Laboratory is located contiguous to the SIU Fisheries Research Laboratory, and collaborative studies are currently in progress.

The Fisheries Research Laboratory (SIU) also has available extensive tank and pond facilities dedicated to aquacultural research. The facilities currently house adult breeding fish of a number of species that are routinely utilized for procurement of gametes for ongoing short- and long-term storage studies.

Gametes will be mailed by various carriers. Since this is only a shipment operation, special facilities are not needed. However, the type of containers and the manner of packing needs to be determined.

Objective 2

Facilities for the larval feeding experiments will be housed in the SIU Aquaculture Facility. For these experiments, one recirculating system consisting of six 1800-liter fiber glass tanks will be committed. This system has the capability for heating and limited cooling of the water, and is equipped with automatic control of photoperiod and feeding.

REFERENCES

Al-Ahmad, T.A. 1978. Evalution of rotifers as food for striped bass (*Morone saxatilis* Walbaum) fry under controlled conditions. M.S. thesis. Auburn University, Auburn, Alabama.

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.

Bowman, J.R. 1979. Survival and growth of striped bass (*Morone saxatilus* Walbaum) fry fed artificial diets in a closed recirculating system. M.S. thesis. Auburn University, Auburn, Alabama.

Braid, M.R. 1977. Factors affecting the survival and growth of striped bass (*Morone saxatilis*) fry in recirculating systems. Doctoral dissertation. Auburn University, Auburn, Alabama.

Burt, C.T., T. Glonek, and M. Barany. 1976. Analysis of phosphate metabolites, the intracellular pH, and the state of adenosine triphosphate in intact muscle of phosphate nuclear magnetic resonance. Journal of Biological Chemistry 251:2585-2591.

Carreon, J.A. 1978. Studies on the culture of the larval striped bass, *Morone saxatilis* (Walbaum), in closed recirculating systems. Doctoral dissertation. Auburn University, Auburn, Alabama.

Coleman, A., and D.G. Gadian. 1976. ^{31}P-NMR nuclear magnetic resonance studies of the developing embryos of *Xenopus laevis*. European Journal of Biochemistry 61:387-396.

Crowe, J.H., and L.M. Crowe. 1984. Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223:701-703.

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. 1980. Preservation of gametes of freshwater fish. Pages 317-326 *in* International Congress on Animal Reproduction and Artificial Insemination, Volume II, 16-20 June, Madrid, Spain.

Ginsberg, A.S. 1963. Sperm-egg association and its relationship to the activation of the egg in the salmonid fishes. Journal of Embryology and Experimental Morphology 11:13-33.

Harrell, R.H. 1984. Review of striped bass broodstock acquisition, spawning methods, and fry production. Pages 45-57 *in* J.P. McCraren, editor. The aquaculture of striped bass, a proceedings. University of Maryland Sea Grant Publication Number UM-SG-MAP-84-01, College Park.

Heidinger, R.C., and B.L. Tetzlaff. 1985. Illinois walleye research. Project completion report, Federal Aid to Fish Restoration Project F-41-R. Southern Illinois University-Carbondale.

Henderson, T.O., A.J.R. Costello, and A. Omachi. 1974. Phosphate metabolism in intact human erythrocytes: determination by phosphorus-31 nuclear magnetic resonance spectroscopy. Proceedings of the National Academy of Science, USA 71:2487-2490.

Hoult, D.I., S.J.W. Bushby, D.G. Gadian, R.K. Radda, R.E. Richards, and P.J. Seeley. 1974. Observation of tissue metabolites using ^{31}P nuclear magnetic resonace. Nature 252:285-287.

Iles, R.A., A.N. Stevens, and J.R. Griffiths. 1982. NMR studies of metabolites in living tissue. *In* J.W. Emsley, J. Feeney and L.H. Suteliffe, editors. Progress in Nuclear Magnetic Resonance Spectroscopy 15:49-200.

Illinois Department of Agriculture. 1986. The Illinois aquaculture industry: its status and potential. Springfield. JSA (Joint Subcommittee on Aquaculture of the Federal Coordinating Council on Science, Engineering and Technology). 1983. Striped bass species plan. Pages 137-145 *in* National aquaculture development plan, Volume 2. Washington, D.C.

JSA (Joint Subcommittee on Aquaculture of the Federal Coordinating Council on Science, Engineering and Technology). 1983. Striped bass species plan. Pages 137-145 *in* National aquaculture development plan, volume 2. Washington D.C.

Kerby, J.H. 1983. Cryogenic preservation of sperm from white bass. Transactions of the American Fisheries Society 113:86-94.

Kerby, J.H. 1986. Striped bass and striped bass hybrids. Pages 127-147 *in* R.R. Stickney, editor. Culture of non-salmonid freshwater fishes. CRC Press, Boca Raton, Florida.

Kerby, J.H., J.D. Bayless, and R.M. Harrell. 1985. Growth, survival, and harvest of striped bass produced with cryopreserved spermatozoa. Transactions of the American Fisheries Society 114:761-765.

Lewis, W.M., R.C. Heidinger, and B.L. Tetzlaff. 1981. Tank culture of striped bass: production manual. Fisheries Research Laboratory, Southern Illinios University-Carbondale.

Loadman, N.L., J.A. Mathias, and G.E.E. Moodie. 1989. Method for the intensive culture of walleye. Progressive Fish-Culturist 51:1-9.

Moon, R.B., and J.H. Richards. 1973. Determination of intracellular pH by ^{31}P nuclear magnetic resonance. Journal of Biological Chemistry 248:7276-7278.

Norton, V., T. Smith, and I. Strand, editors. 1983. Stripers: the economic value of the Atlantic coast commercial and recreational striped bass.

Robitaille, P.M., K.G. Mumford, and G.G. Brown. 1987a. Phosphorous-31 NMR study of trout spermatozoa at rest, after motility, and during short-term strorage. Biochemistry and Cell Biology 65:474-485.

Robitaille, P.M.L., P.A. Robitaille, P.A. Martin, and G.G. Brown. 1987b. Phosphorus-31 nuclear magnetic resonance studies of spermatozoa from the boar, ram, goat, and bull. Comparative Biochemistry and Physiology 87B:285-296.

Rudolph, A.S., and J.H. Crowe. 1985. Membrane stabilization during freezing: the role of two natural cryoprotectants, trehalose and proline. Cryobiology 22:367-377.

Scott. A.P., and J.H. Baynes. 1980. A review of the biology and handling and storage of salmonid spermatozoa. Journal of Fisheries Biology 17:707-739.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 1984, Ottawa.

Stoss, J. 1983. Fish gamete preservation and spermatozoa physiology. Pages 305-350 *in* Fish Physiology W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. Vol. IX Reproduction, Part B: Behavior and Fertility Control, Academic Press, New York.

Stoss, J., and E.M. Donaldson. 1982. Preservation of fish gametes. Pages 114-122 *in* C.J.J. Richter and H.J. Th. Goos, editors. Proceedings of the International Symposium on the Reproductive Physiology of Fish. Wageingen, The Netherlands.

ATTACHMENT D

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Christopher C. Kohler Southern Illinois University	Aquaculture
	Robert J. Sheehan Southern Illinois University	Physiology/Aquaculture
	Bruce L. Tetzlaff Southern Illinois University	Aquaculture
Iowa	George G. Brown Iowa State University	Cell Physiology

ATTACHMENT D

INDIVIDUAL BUDGETS FOR PARTICIPATING INSTITUTIONS

Illinois

Southern Illinois University
Christopher C. Kohler
Robert J. Sheehan
Bruce L. Tetzlaff

Iowa

Iowa State University
George G. Brown

ATTACHMENT D

PROPOSED HYBRID STRIPED BASS BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY

(Kohler, Sheehan and Tetzlaff)

Objectives 1 and 2

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	3	0.15	3	0.15	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	2	1.00	2	1.00	$19,200	$20,400
d. Prebaccalaureate Students						
e. Secretarial-Clerical			1	0.10	$0	$1,500
f. Technical, Shop, and Other						
Total Salaries and Wages					$19,200	$21,900
B. Fringe Benefits (11% of 2 + $267/month health insurance)					$0	$487
C. **Total Salaries, Wages and Fringe Benefits**					$19,200	$22,387
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$4,300	$3,113
F. Travel - Domestic (*Including Canada*)					$4,000	$4,000
G. Other Direct Costs					$1,500	$1,500
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$29,000	$31,000
TOTAL PROJECT COSTS					$60,000	

[1]FTEs = Full Time Equivalents based on 12 months.1

ATTACHMENT D

PROPOSED HYBRID STRIPED BASS BUDGET FOR IOWA STATE UNIVERSITY

(G. Brown)

Objective 1

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.15	1	0.15	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.5	1	0.5	$6,000	$6,500
c. Graduate Students						
d. Prebaccalaureate Students	2		2		$5,000	$5,000
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$11,000	$11,500
B. Fringe Benefits (32.6% of 2b)					$1,956	$2,119
C. **Total Salaries, Wages and Fringe Benefits**					$12,956	$13,619
D. Nonexpendable Equipment					$2,000	$0
E. Materials and Supplies					$2,000	$2,000
F. Travel - Domestic (*Including Canada*)					$1,500	$1,500
G. Other Direct Costs					$3,044	$2,381
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$21,500	$19,500
TOTAL PROJECT COSTS					$41,000	

[1]FTEs = Full Time Equivalents based on 12 months.

ATTACHMENT D

ADVANCING HYBRID STRIPED BASS CULTURE

Budget Summary for Each Participating Institution at 50.5K for the First Year.

	SIU	ISU	TOTALS
Salaries and Wages	$19,200	$11,000	$30,200
Fringe Benefits	$0	$1,956	$1,956
Total Salaries, Wages and Benefits	**$19,200**	**$12,956**	**$32,156**
Nonexpendable Equipment	$0	$2,000	$2,000
Materials and Supplies	$4,300	$2,000	$6,300
Travel	$4,000	$1,500	$5,500
Other Direct Costs	$1,500	$3,044	$4,544
TOTAL PROJECT COSTS	**$29,000**	**$21,500**	**$50,500**

Budget Summary for Each Participating Institution at 50.5K for the Second Year.

	SIU	ISU	TOTALS
Salaries and Wages	$21,900	$11,500	$33,400
Fringe Benefits	$487	$2,119	$2,606
Total Salaries, Wages and Benefits	**$22,387**	**$13,619**	**$36,006**
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$3,113	$2,000	$5,113
Travel	$4,000	$1,500	$5,500
Other Direct Costs	$1,500	$2,381	$3,881
TOTAL PROJECT COSTS	**$31,000**	**$19,500**	**$50,500**

ATTACHMENT D

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

(Salaries, Supplies, Expenses and Equipment)

Institution/Item		Year 1	Year 2
Southern Illinois University			
Salaries and Benefits: SY @ 0.15 FTE		$6,000	$7,000
Supplies, Expenses and Equipment		$20,000	$21,000
	TOTAL PER YEAR	**$26,000**	**$28,000**
Iowa State University			
Salaries and Benefits: SY @ 0.15 FTE		$9,000	$9,000
Supplies, Expenses and Equipment:		$11,000	$11,000
	TOTAL PER YEAR	**$20,000**	**$20,000**
	GRAND TOTAL	**$46,000**	**$48,000**

[1]Since cost sharing is not a legal requirement and due to the difficulty in accounting for small items, documentation will not be maintained.

ATTACHMENT D

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: Initiated Year 1 and completed in Year 2[1].

Objective 2: Initiated in Year 1 and completed in Year 2.

[1] Significant progress will be made on Objective 1 during Years 1 and 2. However, a third year of effort will probably be needed to fully complete the research proposed and thus yield maximum

ATTACHMENT D

LIST OF PRINCIPAL INVESTIGATORS

George G. Brown, Iowa State University

Christopher C. Kohler, Southern Illinois University

Robert J. Sheehan, Southern Illinois University

Bruce L. Tetzlaff, Southern Illinois University

ATTACHMENT D

VITA

George G. Brown
Professor
Department of Zoology
Iowa State University
Ames, IA 50011

Phone: (608) 263-1242

EDUCATION

B.S. Virginia Polytechnic Institute and State University 1959
M.S. Virginia Polytechnic Institute and State University 1961
Ph.D. University of Miami 1966

POSITIONS

Professor, Department of Zoology, Iowa State University, Ames (1977-present)
Associate Professor, Department of Zoology, Iowa State University, Ames (1971-1977)
Assistant Professor, Department of Zoology, Iowa State University, Ames (1967-1972)
Assistant Professor, Department of Biology, University of Miami, Coral Gables (1966-1967)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Iowa Academy of Science
American Society of Zoologists
American Association for the Advancement of Science
Sigma Xi

SELECTED PUBLICATIONS

Robitallie, P.A., P.A. Martin, and G.G. Brown. 1987. ^{31}P-NMR studies of semen and spermatozoa from boar, bull, ram, and goat. Comparative Biochemistry and Physiology 87B:285-296.

Robitaile, P.M.L., K.G. Mumford, and G.G. Brown. 1987. ^{31}P-NMR study of trout spermatozoa at rest, after motility and during short-term shortage. Biochemistry and Cell Biology 65:474-485.

Robitaille, P.A., P.M.L. Robitallie, and G.G. Brown. 1986. ^{31}P-NMR study of motility initiation in the sperm of *Limulus polyphemus* L. Journal of Experimental Zoology 238-89-98.

Barnum, S.R., D.L. Clapper, and G.G. Brown. 1985. Modification of the fertilized egg surface following the cortical reaction in *Limulus polyphemus* L. as viewed with the scanning electron microscope. Journal of Morphology 183:185-198.

Behlmer, S.D., and G.G. Brown. 1984. Viability of cryopreserved spermatozoa of the horseshoe crab (*Limulus polyphemus* L.). International Journal of Invertebrate Reproduction and Development 7:193-199.

ATTACHMENT D

VITA

Christopher C. Kohler
Associate Professor, Department of Zoology and Phone: (618) 453-2890
Assistant Director, Fisheries Research Laboratory
Southern Illinois University
Carbondale, IL 62901

EDUCATION

B.S. St. Mary's College of Maryland 1973
M.S. University of Puerto Rico 1975
Ph.D. Virginia Polytechnic Institute and State University 1980

POSITIONS

Associate Professor, Department of Zoology, Southern Illinois University, Carbondale (1989-present)
Assistant Director of Fisheries Research Laboratory, Southern Illinois University, Carbondale (1988-present)
Assistant Professor, Department of Zoology, Southern Illinois University, Carbondale (1982-1988)
Research Associate, Department of Zoology, Southern Illinois University, Carbondale (1980-1981)
Assistant Professor, Virginia Polytechnic Institute and State University (1980)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Cultural, Management, Introduced, Education and International Sections
World Aquaculture Society
Sigma Xi, Phi Kappa Phi

SELECTED PUBLICATIONS

Kohler, C.C. In Press. Captive conservation of endangered fish. *In* E.F. Gibbons, J. Demarest, and B.S. Durrant, editors. Captive conservation of endangered species. State University of New York Press

Stickney, R.R., and C.C. Kohler. In Press. Chapter 20: Maintaining fishes for research and teaching. *In* C. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society

Roem, A.J., R.R. Stickney, and C.C. Kohler. In Press. Note on the vitamin requirements of blue tilpia (*Tilapia aurea*) in a recirculating system. Progressive Fish-Culturist

Kohler, C.C., and H.S. Killian. 1987. Evaluation of corn distiller's grains in practical diets for channel catfish. Second Symposium on Alternative Energy in the Midwest: Research and Application. Department of Energy and Natural Resources. Springfield, IL ILENR/AE-87/06:305-315.

Neto, J.R., C.C. Kohler, and W.M. Lewis. 1987. Water re-use system for production of fingerling fishes in Brazil, with emphasis on South American catfishes (*Rhamdia quelens* and *R. sapo*). Tropical Agriculture 64(1):2-6.

ATTACHMENT D

VITA

Robert J. Sheehan
Assistant Director, Cooperative Fisheries Research Lab and Phone: (618) 536-7761
Assistant Professor, Department of Zoology
Southern Illinois University
Carbondale, IL 62901

EDUCATION

B.S. Northeastern Illinois University 1973
M.A. Southern Illinois University 1976
Ph.D. Southern Illinois University 1984

POSITIONS

Assistant Professor, Department of Zoology, Southern Illinois University, Carbondale (1986-present)
Assistant Professor, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University (1983-1986)
Researcher, Fisheries Research Laboratory, Southern Illinois University, Carbondale (1981-1983)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture Section, Fisheries Educators Section, Water Quality Section, Exotic Fishes Section, Early Life History Section

SELECTED PUBLICATIONS

Sheehan, R.J., R.J. Neves, and H.E. Kitchel. 1989. Fate of freshwater mussels transplanted to formerly polluted reaches of the Clinch and North Fork Holston Rivers, Virginia. Journal of Freshwater Ecology 5:139-149.

Nielsen, L.A., R.J. Sheehan, and D.J. Orth. 1987. Impacts of navigation on riverine fish production in the United States. Proceedings of the International Symposium on Fish Production in Rivers. Polish Archives of Hydrobiology 33(3/4):277-294.

Helfrich, L.A., R.J. Sheehan, and J.S. Odenkirk. 1986. Fishing for sale: fee-fishing opportunities in Virginia. Virginia Cooperative Extensive Service Publication 420-898.

Sheehan, R.J., and W.M. Lewis. 1986. Relationships between the toxicity of aqueous ammonia solutions, pH, ammonia salt formulations, and water balance in channel catfish fingerlings. Transactions of the American Fisheries Society. 115:891-899.

Lewis, W.M., and R.J. Sheehan. 1977. Channel catfish culture: state of the art 1976. Proceedings Southeastern Conference of Game and Fish Commissioners, 31st Annual Conference, 1976:234-238.

ATTACHMENT D

VITA

Bruce L. Tetzlaff
Research Project Director
Fisheries Research Laboratory
Southern Illinois University
Carbondale, IL 62901-6511

Phone: (618) 536-7761

EDUCATION

B.S. Southern Illinois University at Carbondale 1974
M.A. Southern Illinois University at Carbondale 1979

POSITIONS

Research Project Director, Fisheries Research Laboratory, Southern Illinois University-Carbondale, Illinois (1980-present)
Researcher, Fisheries Research Laboratory, Southern Illinois University (1976-1980)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture Section, Bioengineering Section, Fish Management Section

SELECTED PUBLICATIONS

Heidinger, R.C., J.H. Waddell, and B.L. Tetzlaff. 1985. Relative survival of walleye fry versus fingerlings in two Illinois reservoirs. Proceedings of the Annual Conference, Southeast Association of Fisheries and Wildlife Agencies 39:306-311.

Miller, S.J., and B.L. Tetzlaff. 1985. Daily growth increments in otoliths of larval walleye (*Stizostedion vitreum*). Transactions of the Illinois Academy of Science 78:115-120.

Heidinger, R.C., B.L. Tetzlaff, and J. Stoeckel. 1985. Evidence of two feeding subpopulations of white crappie (*Pomoxis annularis*) in Rend Lake, Illinois. Journal of Freshwater Ecology 3:133-143.

Heidinger, R.C., L.J. Wawronwicz, and B.L. Tetzlaff. 1983. Applications of water re-use technology for overwintering threadfin shad (*Dorosoma petenense*) at northern latitudes. Aquaculture Engineering 2:153-162.

Lewis, W.M., R.C. Heidinger, and B.L. Tetzlaff. 1981. Tank culture of striped bass: Production manual. Fisheries Research Laboratory, Southern Illinois University, Carbondale, Illinois, 115pp.