CULTURE TECHNOLOGY OF HYBRID STRIPED BASS

Chairperson: Christopher C. Kohler, Southern Illinois University-Carbondale

Extension Liaisons: Joseph E. Morris, Iowa State University; Daniel A. Selock, Southern Illinois University-Carbondale; LaDon Swann, Purdue University

Funding Request: $168,000

Duration: 2 Years (September 1, 1993 - August 31, 1995)

Objectives:

1. To develop intensive hatchery production techniques for white bass and to "domesticate" white bass by producing broodstock originating from induced spawns.

2. To perfect cryopreservation techniques for white bass/striped bass semen and to demonstrate feasibility of hybrid striped bass production using "stored" semen in industry-type settings.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Year 2	Total
Southern Illinois University-Carbondale	Christopher C. Kohler Robert J. Sheehan	1 & 2	$42,000	$48,000	90,000
University of Wisconsin-Milwaukee	Fred P. Binkowski	1	$15,000	$15,000	30,000
Ohio State University	Konrad Dabrowski James E. Ebeling	1	$8,000	$8,000	16,000
Purdue University	Paul B. Brown M. Randall White	1	$2,500	$2,500	5,000
University of Wisconsin-Madison	Jeffrey A. Malison	1	$1,500	$1,500	3,000
Iowa State University	George G. Brown	2	$12,000	$12,000	24,000
		TOTALS	**81,000**	**87,000**	**168,000**

TABLE OF CONTENTS

JUSTIFICATION

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 potential, seafood processors in Chicago are willing to pay a minimum of $1.59/kg ($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 North Central Region Aquaculture Center (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-Carbondale (SIUC), and the University of Wisconsin-Madison (UW-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. That project has been highly successful in meeting its objectives (see **RELATED CURRENT AND PREVIOUS WORK**). However, the project only requires rearing developing embryos to swim-up fry. Accordingly, larval rearing protocols need to be developed. There is also a need to produce "true" domesticated broodstock, i.e. F_1 hybrids reared in captivity.

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 magnetic 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. A second NCRAC-supported hybrid striped bass project is underway that aims to: (1) provide a continuous supply of gametes, (2) allow for year around production, (3) facilitate selective breeding, and (4) result in more efficient use of available gametes. That project has also been highly

successful (see **RELATED CURRENT AND PREVIOUS WORK**). However, additional research on developing this technology is needed before it can be adopted by commercial producers.

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. Although brine shrimp nauplii have successfully been used as a first food for striped bass larvae, the smaller size of the white bass and reciprocal-cross hybrid larvae necessitates the use of even smaller foods for first feeding.

Feeds are generally considered to represent a large portion of production costs in any animal production industry. This also applies to aquaculture. However, producers of hybrid striped bass have relatively few options in purchasing diets for any life history stage. Because of a lack of information on nutritional requirements of fish fry, diets for that life history stage have been formulated in a general manner regardless of feeding habits of the fish. Based on the available information for larger fish, a generic diet for fish is not feasible as there is significant variation in nutritional requirements, ability to use forms of nutrients, and acceptance of specific or combinations of ingredients (NAS 1981). Several of the available diets for fish fry have been evaluated in hybrid striped bass, but weight gain and survival have been relatively low and highly variable. Further, recent studies conducted at Purdue University (P. Brown, unpublished data) have identified several types of diets that juvenile hybrid striped bass accept.

Feed costs are usually a large portion of annual variable costs in fish culture. While cost of feed is usually considered less important in rearing fry, it is a vital consideration in terms of health and survival. Thus, savings in feed costs can improve economic viability, particularly in a new aquaculture industry in which losses of fish are inevitable. In addition to economic considerations, tissue development during early life history can be vital to future growth rates in animals (Hofer 1985). Few tissues are completely formed at hatching and adequate nutrition at first feeding can be critical to eventual development and function. Based on lines of research with other species of fish, we should be able to develop diets that, when fed to first feeding fish, result in weight gains greater than those observed in wild fish or those feeding strictly on zooplankton. Thus, research in diet development for fry of hybrid striped bass could significantly improve numbers of fish surviving to fingerling size, a time when survival of fish is greatly improved and acceptance of feed is not a problem. More fish of a larger size will effectively improve cash flow to the private aquaculturist.

Methods for short-term storage and transportation of striped bass and white bass semen have been developed and demonstrated over the last two years in a cooperative study between Iowa State University (ISU) and SIUC. In addition, methods for cryopreservation of semen (long-term storage) for these species have also been developed. Finally, successful fertility studies using these methods have been performed. However, these advances still represent initial approaches and improvement of procedures for use in the aquaculture industry is needed. Development of reliable techniques to store, cryopreserve, and transport gametes will contribute immensely to the culture technology of hybrid striped bass. Specifically, the enhanced development of these techniques will allow: (1) a continuous supply of gametes, (2) year-round production, (3) holding fertile gametes of either white or striped bass until the other is in spawning condition to facilitate hybrid crosses, (4) facilitation of selective breeding, and (5) more efficient use of available gametes.

RELATED CURRENT AND PREVIOUS WORK

This proposal is a continuation of and addition to two previously funded NCRAC projects on hybrid striped bass. Objective 1 (intensive hatchery production) will build on the project in which out-of-season spawning of white bass has been achieved while Objective 2 (use of "stored" semen) is aimed at demonstrating the feasibility of hybrid striped bass production using cryopreserved and/or extended semen in industry-type settings.

Domestication and Controlled Spawning of White Bass

Adult white bass *Morone chrysops* were collected via hook-and-line fishing from the Illinois River near LaSalle, Illinois in the fall (1989) and spring (1990). The fish were therapeutically treated for diseases/stress during hauling and for approximately 1 week after stocking into an indoor 10,000-L, water-recycle system at Southern Illinois University-Carbondale. White bass were trained to dry formulated feed by initially providing moist pellets consisting of commercial dry trout feed (40% crude protein, 11% crude fat), raw gizzard shad *Dorosoma cepedianum* and vitamins. The dry trout feed was concomitantly introduced after a few days, and

the relative proportions of each feed was altered (dry feed increased:moist feed decreased) until fish solely accepted the dry feed, usually about 2 weeks. In summer (1990) 300 white bass (300-600 g) of an approximate even sex ratio were spread over three separate 10,000-L water recycle systems, one maintained under an ambient photoperiod/temperature regime, one compressed to about 9 months and one held at a temperature at or above spawning temperature (20 ± 5 °C) and constant photoperiod (14 hour light/10 hours dark). Using human chorionic gonadotropin (hCG) injections (500 and 125 IU/kg of female and male fish, respectively), the compressed-cycle fish were induced to spawn in March, 1991; the ambient-cycle fish in May, 1991; and the constant-cycle fish in May, 1991. hCG-injected constant cycle fish failed to spawn in March, 1991. The spawning condition of wild and the three captive populations, as determined by UW-Madison researchers from serum levels of estradiol-17β and testosterone, as well as gonadal histology, followed patterns congruent with actual spawning events.

Historical Cryopreservation of Fish Semen

Cryopreservation of fish spermatozoa, in general, has historically 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, many of the methods are 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 to variability. These factors fluctuate from one freezing program to the next, making it impossible for researchers to account for variability in quality. Hence, any one factor in this sequence of freezing steps may influence the results.

Storage of White Bass and Striped Bass Semen

Efficient methods for storage of *Morone* gametes would provide an essentially continuous supply of gametes for use in: (1) year-round hybrid production including hybridization of stocks that spawn at different times, (2) experimental ploidy manipulations, and (3) as a tool in genetic conservation of *Morone* stocks. Protocols for the successful collection, short- and long-term storage, and transportation of *Morone* gametes have been developed collaboratively between ISU and SIUC.

White bass sperm were collected from three groups of fish given either monthly hCG injections, weekly hCG injections, or no hCG. Five extender solutions were evaluated. Extended and non-extended semen samples were shipped to ISU where motility was determined. Extended sperm had significantly greater post-shipment motility when compared to non-extended samples. Only two of the five extenders resulted in significant differences in motility; a simple sodium chloride solution performed as well or better than more complex solutions. Sperm from monthly injected fish had significantly better motilities than sperm from weekly injected fish. No significant differences in motility between control and monthly injected fish were found.

Sperm samples collected from captive white bass and wild Florida striped bass were cryopreserved in 0.5 mL freezing straws using three cryoprotectants. Cryopreserved samples were stored in liquid nitrogen vapors. A portion of the straws were shipped on dry ice to ISU thawed, and motility evaluated and compared to motilities of extended sperm. Extended samples from both species had a significantly higher motility than the cryopreserved samples; motility was reduced by about 50% with cryopreservation. Extended white bass sperm held in refrigerated storage for approximately thirty days exhibited a similar reduction in motility. There were no significant differences between motilities of cryopreserved samples from both species. The remainder of the straws were later used in fertility tests with white bass eggs stripped from captive females. No significant differences in percent hatch were detected between the three cryoprotectant treatments. Hatch from eggs was 26.1 to 52.8% and 40.2 to 85.0% of hatch from eggs fertilized with fresh extended semen for cryopreserved white bass and striped bass sperm, respectively. The results indicated that although motility was significantly reduced with cryopreservation, reasonably good health was still obtained. However, cryopreserved sperm can be stored indefinitely. Therefore, if *Morone* sperm must be stored prior to use for longer than thirty days, cryopreservation appears to be the best option.

Larval Feeding Strategies

While the feeding of live brine shrimp nauplii to striped bass larvae for their first 25 days (Lewis et al. 1981) has been used successfully, the smaller size of the white bass and hybrid larvae may require smaller live foods for as long as 15-16 days before brine shrimp nauplii are readily accepted. Harrell (1984) used live rotifers to supplement the diet of brine shrimp nauplii when feeding the larvae of F_1 hybrid striped bass. Other combinations of appropriately sized live foods might also be used to bridge this gap. Some of these small sized live foods are longer lived in freshwater and may be less susceptible to settling than brine shrimp nauplii. These properties may be advantageous to enhancing the presentation of the food items to the larvae and

increase the availability of the food at high density in the water column. Also better survival of these food species, as compared to brine shrimp nauplii, may be less prone to foul water quality within the rearing units through decomposition of uneaten food. This would decrease the need for a rapid water turnover of the rearing units and assist in the maintenance of high food densities. Food density and presentation to the larvae are recognized as important factors (Al-Ahmad 1978; Lewis et al. 1981; Loadman et al. 1989) in the initiation of feeding and the prevention of cannibalism.

Attempts to rear early life history stages of striped bass on commercial feeds have not compared favorably to the growth and survival of larvae fed live brine shrimp nauplii as a control (Braid 1977; Carreon 1978; Bowman 1979; Webster and Lovell 1990b). Recently, new larval diets incorporating microencapsulation and freeze drying technologies are being developed which may permit earlier substitution or complete replacement of live foods with new formulated diets for larval rearing. Such diets have been used with some reported "success" on walleye (Loadman et al. 1989), whitefish, and muskellunge. However, the sizes of these fish at first feeding are considerably larger than the size of the white bass and hybrids. As yet, it has not been demonstrated that diets formulated using these technologies can be used as first feeding foods, or exactly how early they might be used as substitutes for live foods.

Differences which University of Wisconsin-Milwaukee (UW-Milwaukee) researchers have observed in comparisons of pond and intensively reared yellow perch suggest that nutritional factors affecting the growth and development of larval fish during intensive culture are poorly understood. In the intensive rearing of both marine and freshwater physoclistic fish larvae, abnormal inflation of the swim bladder can be a major problem (Doroshov et al. 1981; Hadley et al. 1987). A higher incidence of non-inflation of the swim bladder and skeletal deformities occurs during intensive rearing with some strains of fish, while pond reared fish from identical parentage show an extremely low incidence of these conditions. Physical properties of the intensive rearing environment, such as oil films which restrict access of the larvae to the surface where they might gulp air (Barrows et al. 1988) have been implicated as possible causes of this condition. This condition has also been attributed to developmental problems that are influenced by the maternal contribution (Hadley et al. 1987; Brown et al. 1988) to egg quality within a particular batch of larvae. At present whether these conditions result from physically stressful conditions during intensive rearing, from nutritional inadequacy of larval foods used during rearing or the past nutritional history of the mothers is unclear. Fatty acid boosters have been used to enrich brine shrimp nauplii to be fed to marine shrimp and fish larvae (Dhert et al. 1990; Eda et al. 1990; Koven et al. 1990; Webster and Lovell 1990a) with some reported degree of improvement in growth and survival. The benefit of similar enhancement of larval foods has yet to be demonstrated for white bass and hybrids.

Tuncer et al. (1990) fed one of the new feeds for fish fry and *Artemia* to both striped bass and hybrids and compared weight gain, feed efficiency, survival and proximate composition of whole fish. Several treatment groups were incorporated in the experimental design. Those were designed to evaluate time after first feeding that fish could be switched to a formulated diet. The best survival in that study was 4.8%.

Researchers at Mississippi State University evaluated several of the larval fish diets fed to hybrid striped bass. In those studies, diets manufactured by Provesta, Fripac, Zeigler, and Biokyowa, were fed to fry. Fish fed the diet manufactured by Provesta gained more weight and survived better than fish fed the other diets through 60 days (H.R. Robinette, Mississippi State University, personal communication). Survival was significantly lower in fish fed one of the more popular fry diets.

Based on the relatively low survival rates of fry, the general lack of dietary evaluations with fish fry, and the real need to develop better quality feeds for fry of coolwater species, nutritional comparisons and development offer the potential of significantly improving economics of coolwater aquaculture. Researchers at Purdue University have evaluated numerous different dietary formulations for hybrid striped bass and identified several that are accepted by fish initially weighing 3-5 g (Brown et al. In press). Similarly, SIUC researchers found that both hybrid striped bass crosses at that 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 7 days for largemouth bass which were trained in a "side-by-side" study. Several of the "new" larval diets will be evaluated on smaller hybrids in spring, 1993.

ANTICIPATED BENEFITS

The overall goal of this collaborative project is to enhance hybrid striped bass aquaculture in the North Central Region. Out-of-season spawning of white bass has been achieved in a ongoing NCRAC-sponsored project. The development of intensive larval culture techniques for this species will allow for its full domestication, and will preclude the initial need for outdoor ponds. Because reciprocal cross hybrid striped bass are the same size as white bass at the swim-up stage, the results of the study will be directly applicable to their culture. The perfection of techniques for semen storage (cryopreservation and extended) would preclude the need for maintaining large numbers of male striped bass broodstock. It would also allow for maintaining genetic

integrity among cultured hybrids since gametes could be readily obtained from various sources. The knowledge gained from this study should be of immediate use by the aquaculture industry.

OBJECTIVES

The overall goal of this project is to optimize culture technologies for the climatic and economic conditions existing in the North Central Region. Specific objectives to achieve this goal are:

1. To develop intensive hatchery production techniques for white bass and to "domesticate" white bass by producing broodstock originating from induced spawns.

2. To perfect cryopreservtion techniques for white bass/striped bass semen and to demonstrate feasibility of hybrid bass production using "stored" semen in industry-type settings.

PROCEDURES

Intensive Culture (Objective 1)

The studies conducted under this objective will involve researchers from SIUC, Ohio State University (OSU), UW-Madison, UW-Milwaukee, and Purdue University. SIUC researchers will conduct all spawning work and will provide white bass larvae to Wisconsin and Ohio researchers. With the exception of UW-Madison, researchers from all universities will be involved in larval feeding studies. SIUC and UW-Milwaukee researchers will attempt to rear white bass larvae to broodstock size.

SIUC
Approximately 300 "domesticated" adult white bass (approximately even sex ratio; 300-600 g) will be evenly spread over 3 separate water-recycle systems (10,000 L capacity each), one of which will be maintained under an ambient photoperiod/temperature regime, one will be compressed to about 6 months and one will be compressed to about 9 months. The compressed- and ambient-cycle fish will be maintained in water recycle systems situated in a greenhouse. Black plastic sheeting will be positioned over the compressed-cycle tanks, and photoperiod will be controlled by using artificial lighting (60-W incandescent light bulb/tank) controlled by automatic timers. Photoperiod will be adjusted weekly. Clear-plastic sheeting will be positioned over the ambient-cycle tanks so that fish will be exposed to ambient photoperiod. Water temperatures of all systems will be controlled by 1.0 HP chillers (Frigid Unit) or 1200-W heating elements, placed in each tank, depending upon need. Temperatures will be measured by continuous thermal recorders (Ryan TempMentor model RTM) placed in each system. Rock salt (NaCl) will be added to the systems to maintain salinity at about 2 ppt. Dissolved oxygen, temperature, salinity, pH, nitrites, total ammonia, and chlorine will be routinely measured.

hCG and possibly LHRHa (a synthetic luteinizing hormone-releasing hormone analogue) injections will be used to induce spawning. The compressed and ambient-cycle female fish may be administered 10 ug/kg of LHRHa followed approximately one-week later by hCG injections (100-200 IU/kg female body weight; 100 IU/kg male body weight) at the appropriate times and temperatures (approximately 15 ^{o}C). These dosages are based on previous work conducted at SIUC. hCG alone has proven effective for inducing spawning. At least two separate spawns of different groups of fish will be obtained from each temperature/photoperiod regime resulting in a minimum of 6 spawns each year of the study. Prior to injection, the fish will be anesthetized with 50-100 mg/L MS-222, weighed to the nearest gram, and corresponding doses will be administered intramuscularly just ventral to the first dorsal fin above the lateral line. Fish will be given unique marks (dorsal spine or caudal fish clips) for later identification.

Female white bass will be checked for ovulation every 2 hours starting 16-hour post-hCG injection by lightly exerting abdominal pressure to extrude a small amount of eggs. Eggs will be staged similar to procedures described for striped bass (Kerby 1986; Rees and Harrell 1990). In general, ovulation is indicated by the occurrence of clear, free-flowing, uniform-shaped eggs with fully intact inner chorion surfaces. Once a female has ovulated, she will be anesthetized with 50-100 mg/L MS-222, weighed, and dried with a paper towel. All planned spawning events will be obtained by manually stripping eggs (approximately 80% of egg mass) into weighed Teflon cups (15 mL volume). The egg-containing cups will be weighed to the nearest 0.1 g, and the number of eggs will estimated based on 3,800 eggs/g (Rees and Harrell 1990). Semen will be collected from males by inserting a Pasteur pipette in their urogenital openings while applying suction. Semen from two males will be placed with the eggs of each female in the Teflon cups, water will be added at twice the egg volume, and the contents of the cups mixed. For the compressed- and ambient-cycle spawnings, semen will be extracted from males of the same cycle.

Following fertilization (2 min), half of the eggs from each spawn will be placed in a modified Heath tray in labeled 6 x 6-cm individual compartments constructed from PVC plastic and 125 µm-mesh Nitex screen at

a rate of approximately 5,000 eggs per compartment. A continuous flow of 16-18 °C oxygenated water (5 L/min) will be circulated through the trays. Trays will be covered with black plastic sheeting to prevent egg/larvae damage from excessive light. The other half of the eggs will be treated with tannic acid to remove stickiness prior to placement in McDonald jars from hatching. The eggs generally begin to hatch after 36 to 48 hours; 24 hours is sometimes required for all to hatch. An additional 96 to 120 hours is required for the larvae to absorb their yolksacs.

Larvae will be shipped via UPS overnight freight to UW-Milwaukee and OSU researchers. The critical periods for handling will be assumed to be the same as for striped bass (Lewis et al. 1981). Experience will dictate the best handling and shipping procedures for white bass. Success in working out the handling/shipping protocol and also carrying out the larval feeding studies will be greatly facilitated by having access to at least six spawning events each year.

During the first year of this study, three commercially available feeds will be examined as potential feeds for larval white bass. These include Zeigler Bioplancton^{R}, Zeigler AP-100^{R}, and Kyowa Fry Feed. Another feed will be obtained from Purdue University (see later section).

The study will be conducted at the SIUC Aquaculture Research Facility utilizing one 12,000-L water reuse 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 white striped bass at a density of 100 fish per liter. To eliminate 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.

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 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.

<u>UW-Milwaukee</u>
Studies of feasibility of intensive hatchery production of white bass will be conducted at UW-Milwaukee utilizing several larval feeding strategies applicable to rearing of fingerlings. The strategies to be tested would include those used at UW-Milwaukee to rear yellow perch and other species too small to accept formulated diet as a first feed, modifications of these techniques, and tests of new formulated diets. One or more batches of fertilized eggs/larvae per year of white bass will be obtained from SIUC. The following intensive larval rearing strategies will be tested:

1. The green tank water (GTW) - brine shrimp nauplii (BSN) - ground beef heart/liver mixture (GLM) - formulated commercial (Bioproducts Inc.) starter diet (FSD). This feeding scheme represents our basic strategy for rearing yellow perch from the on-set of first-feeding. However, the duration of the successive feeding phases will be adjusted appropriately for white bass by observing the feeding response of these fish.

2. Modification of the above scheme incorporating "improvements" in which the GTW organisms and the BSN would be enriched with commercially available fatty acid booster and/or vitamins.

3. First feeding trials of diets formulated commercially for Paul Brown (Purdue University) which would be of appropriate size for first feeding will be conducted.

For each of these sets of trials, duplicate insulated fiberglass rearing units would be stocked with equal numbers by volumetric measure of either fertilized eggs or recently hatched fry. These six rearing units will be capable of being operated either statically during early feeding phases or on a controllable flow-through basis to maintain water quality during later stages of the feeding regime. Each rearing unit would be equipped

with aeration and photoperiod controlled lighting. The standpipe would be equipped with a surrounding screen and a screened plug of appropriately sized mesh to prevent escape of the larvae. The water supply rearing temperature (20-25 °C) would be controlled through blending hot and cold dechlorinated water and running the inflow through a packed column to adjust gas saturation to near equilibrium.

Overall survival will be assessed as percentage of the initial estimated numbers of fish eggs or fry stocked in the rearing units and counts of those remaining after the first 8-10 week rearing period. Once daily siphoning is initiated (following the GTW phase), the timing of significant periods of mortality will also be determined through examination of the screened siphoned material taken from the rearing unit for numbers of dead larvae. During this 8 week period, weekly subsamples of approximately 30 fish will be drawn from each rearing unit for measurement of individual length, weight, degree of swim bladder inflation and gut fullness.

Growth, survival, and swim bladder inflation through the initial 8-10 week period will be described and compared in each of the rearing units using appropriate parametric or non-parametric statistical procedures. These trials will be repeated as necessary in the second year of the proposal until enough replicate rearing units have been examined. Our base feeding regime (GTW-BSN-BLM-FSD) will be used as a control to make comparisons of the success of the alternative rearing strategies.

These trials will determine which of the three intensive larval rearing strategies holds the most promise as a tool for the production of fingerlings and the subsequent development of domesticated broodstock of white bass.

OSU

The study will be conducted in a multiple tank system with a well water supply at the OSU-Piketon Research and Extension Center. Water in the system will be a well water filtered through sand filters. The system will be maintained at 22 °C. White bass larvae will be obtained from SIUC at the age of 1-2 days (or older if required) and the fish will be stocked in 36 40-L circular tanks at the rate of 2,000 per tank.

The experimental diets to be used will be: live freshwater rotifers *Brachionus calyciflorus* (LFR); the same strain of LFR enriched with the polyunsaturated fatty acid (PUFA) preparation (Dr. Fair, Southeast Fisheries Science Center, NOAA, Charleston, South Carolina) according to the method described by Bengston et al. (1991). The prepared diet to be used in this study will be similar to a diet formulated earlier in this laboratory for other fish larvae (see Dabrowski et al. 1984; Dabrowski and Poczycynski 1988). The freeze-dried diet will be ground and particles of less than 54, 54-105 and 105-220 µm will be separated. Several experimental diets will be tested. For instance, the prepared diets will be supplemental with 1, 2 or 3% of PUFA. Another set of diets will test the effect of the proteolytic enzyme preparation (Finnish Sugar, Helsinki, Finland) or betaine and other attractants.

Each diet will be assigned at random to three tanks of fish. Larvae will be fed at 1-hour intervals, 24 hours/day from day 5 until day 15. Then frequency of feeding will be re-adjusted and experiments continued until day 30. Growth of fish will be determined by measuring total length and weight of 10 fish from each tank every 10 days.

Enzyme assays will be used to evaluate the development of digestive tract functions in response to the dietary treatments. The entire digestive tract will be dissected from 10-20 fish and pooled for extraction of enzymes. The digestive tracts will be homogenized in 0.1 M Tris - HCl buffer pH 8.0 and centrifuged at 30,000 x g for 30 minutes at 4 °C. The filtrate will be concentrated with a 0.45 µm filter and frozen at -85 °C before further use. Protein determinations of the extracts will be made with Bio-Rad protein assay kits (BioRad Laboratories, Richmond, California). Trypsin and chymotrypsin activities will be measured with the synthetic substrates for amidase and esterase activities (Hummel 1959; see also Dabrowski et al. 1989; 1992) including electrophoretic separation of isoenzymes.

Purdue University

Research studies on development of a high-quality diet for hybrid striped bass fry will be a collaboration between the US Fish and Wildlife Service, Purdue University, UW-Milwaukee and SIUC. Diets will be formulated and mixed at Purdue University by Paul Brown, shipped to the US Fish and Wildlife Service Fish Technology Center in Bozeman, Montana and pelleted by Ric Barrows, then shipped to Fred Binkowski (UW-Milwaukee) and Chris Kohler/Bob Sheehan (SIUC) for the feeding studies. This collaboration will result in maximum use of available expertise. Brown has had good success formulating diets for new aquaculture species, Barrows has a new piece of feed manufacturing equipment that is capable of pelleting complete diets in small particles (<150 µm), and Binkowski and Kohler/Sheehan have experience with larval fish.

Feed ingredients will be purchased and mixed at Purdue using available equipment. Three dietary formulations will be evaluated in each year of this initial study. The first evaluation will include experimental diet 3 from our previous studies (Brown et al. In press), a standard fishmeal-based diet using the feeding stimulant betaine substituted into the diet as a partial replacement for choline, and a positive control diet

(Provesta larval fish diet). Experimental diet 3 from our previous studies has been readily accepted by juvenile hybrid striped bass and weight gain of fish fed that diet was 65-91% of weight gain in fish fed a variety of positive control (i.e., commercially-available) diets. Using the feeding stimulant betaine as a partial replacement for choline is an accepted method of incorporating this compound into diets for fish (J. Machado, US Food and Drug Administration, personal communication). Thus, the results could be applied in a practical setting. Purdue University is currently evaluating the dietary choline requirement of juvenile hybrid striped bass and those data will be available by the time this study begins. While the positive control diet is a closed formula, it is logical to assume that diet contains a relatively high level of fishmeal.

In the second year, we will narrow our focus. Clearly, dietary formulation approaches incorporated during the second year will be dependent on results observed during the first year, but a general project outline can be proposed. Hybrid striped bass are known to readily consume purified diets containing a combination of both essential and nonessential crystalline L-amino acids, but several of those L-amino acids incorporated alone in a purified diet did not elicit a feeding response (Brown et al. In press). Using combinations of the most inexpensive amino acids in conjunction with fish meal as a source of intact protein, two experimental diets will be formulated based on the results of the first year study and compared to fish fed Provesta.

In both years, triplicate groups of fish will be fed in excess for a minimum of 60 days. Primary indicators of dietary adequacy will be weight gain and survival. Additionally, whole body proximate analysis and routine histopathological examination will be conducted. Nutritional composition of fish can be an indicator of energy stores (fat) in fish fed various diets and a further indicator of dietary acceptance. Histopathological examination is a more thorough evaluation of tissues than simple gross weight gain and proximate composition and will provide additional information regarding tissue development. Proximate composition and histophathological examinations will be conducted on surviving fish from each dietary treatment in both years.

Four separate groups of fish will be shipped to Purdue from UW-Milwaukee and SIUC. The first two groups will be initial samples of fish collected prior to initiation of the study and the second two groups will be final samples collected at the end of the study. Within each shipment, one group will be frozen (-20 ^{o}C) and the second will be preserved in 10% neutral-buffered formalin. Frozen fish will be analyzed for moisture, crude protein, fat, and ash using routine nutritional methods (AOAC 1984) and fish preserved in formalin will be examined histologically using routine histotechniques (Sheehan and Hrapchak 1980). Histophathological examination will be conducted by Randy White, Purdue Animal Disease Diagnostic Laboratory.

UW-Madison

Researchers at the UW-Madison will continue to assess the reproductive status in maturing white bass as needed for the project. These assessments will include measurements of serum levels of estradiol-17β, testosterone, 11-ketotestosterone, and/or 17α,20β-dihydroxy-4-pregnen-3-one (17,20-DHP). At Madison, radioimmunoassays and/or enzyme-linked immunoassays for these hormones have already been developed and validated for use in white bass. The knowledge of these specific hormone levels will be critical in determining when out-of-season white bass are approaching reproductive maturity, and when the fish reach the optimum stage for induction of final maturation and spawning by injection of gonadotropins. The budget as submitted allows for assaying approximately 100-150 samples per year.

Stored Semen (Objective 2)

SIUC and ISU

Based on fertility tests with freeze-stored semen, cryopreservation procedures we developed have been fairly successful. Further, we have attempted to develop methodologies that will have potential under practical fish-culture conditions. However, additional work is needed to (1) optimize the use of available gametes, (2) reduce the variability in fertilization rates, and (3) adapt our laboratory procedures to commercial-scale aquaculture. The large volumes of semen that will be required for commercial-scale aquaculture will necessitate the use of larger freezing containers, causing difficulties in achieving uniform freezing and thawing rates. This will probably result in even greater variability in fertility. We are currently working with 0.5 mL freezing straws, and because of dilution with the cryogenic medium, this results in a relatively small amount of semen preserved in each straw.

Our findings to date suggest directions for improving techniques and addressing the needs of commercial-scale aquaculture. We have noted that frozen spermatozoa become motile during thawing, prior to activation. This suggests that thawing rate may be critical, and that achieving uniform thawing rates throughout large cryopreservation containers will be important. Also, duration and intensity of swimming upon activation of the spermatozoa are decreased after thawing, as compared to that of activated fresh semen. This suggests that improvements in the freezing medium, freeze-temperature regime, or both are needed.

To improve our laboratory-scale cryopreservation techniques, we propose the following. We will evaluate several concentrations of dimethyl sulfoxide (DMSO), the cryoprotectant that has given us the best success

thus far. We will also evaluate the use of ice water for thawing, in an attempt to reduce premature activation. Finally, we will evaluate vitrification procedures for long-term storage of semen.

Currently, vitrification (Armitage and Rich 1990; Fahy et al. 1990; Kono et al. 1988; Rall 1987) is being implemented for the cryopreservation of tissues. It is a physical process by which cryoprotectants (e.g., dimethylsulfoxide, acetamide, propylene glycol, 2,3-butanediol polyethylene glycol) solidify, forming a highly viscous, transparent, glass like substance when cooled. As vitrification occurs, all normal molecular and ionic distribution remain the same as found in the liquid state. The major advantage of vitrification is that rapid freezing is possible, and, theoretically, the spontaneous formation of ice nuclei within the membranes and cytoplasm of cells is prevented. Although methods have drawbacks in osmotic stress and toxicity, it has been used successfully to cryopreserve and recover mouse (Rall 1987) and rat (Kono et al. 1988) blastocysts.

To adapt our procedures to commercial-scale aquaculture, we will evaluate: (1) the use of larger, 4.5-mL straws, such as those employed by Wheeler and Thorgaard (1991) to freeze rainbow trout semen; (2) reducing the amount of cryogenic medium used to dilute the semen; and (3) the potential for storing samples on dry ice rather than liquid nitrogen. Storage on dry ice would be much more practical in an industry setting.

Because ripe eggs for fertility tests are available only infrequently, we will initially use motility tests and NMR to determine how modifications of our procedures affect sperm quality. 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. However, NMR requires large sample volumes. Our shift in emphasis from small freeze-storage containers and small volumes of sample to larger containers and larger sample volumes will be highly conducive to NMR analyses. Ultimately, fertility tests will be used to assess the most promising protocols.

For the following procedures, all sample collection, preparation, and motility and fertility tests will be conducted at SIUC. NMR analyses will be conducted at ISU.

Initially, NMR and motility tests will be used to assess a number of DMSO concentrations used in the cryogenic media. Once promising DMSO concentrations have been identified, fertility tests will be conducted with the three most promising DMSO concentrations. Eggs from a given female will be divided into four lots. One of the lots will be fertilized with fresh semen as a control, and the other three lots will be used to test the three DMSO concentrations. These procedure will be replicated with at least two other females.

To evaluate thawing regimes, semen will be taken from at least 6 males. The semen from each male will be divided into two lots and frozen in as many 0.5 mL straws as required, using the best cryogenic medium thus far identified; i.e., with the most promising DMSO concentration. Freezing straws obtained from the semen representing one lot from each male will then be thawed under tap water, the technique we are currently using for thawing. Once straws become thawed to the point of slush, they will be tested for premature activation and for intensity and duration of swimming following activation with fresh water. Straws from the other semen lot from each male will be thawed and held in ice water. Straws will be removed from the ice water once they are thawed to the point of slush, and at two, 15 minute intervals thereafter. Tests for premature activation and motility following activation will be performed as above at each time interval. Thus, we will compare the two thawing regimes and determine if holding thawed semen in ice water affects sperm quality.

Vitrification of semen will be attempted with semen from at least six males. NMR spectra and motility test results will be compared to those from fresh semen.

NMR and motility tests will be used to assess at least three different semen to cryogenic medium dilution solutions. Semen from each of six males will be divided into three lots. Each lot will be frozen with one of the three dilutions. However, DMSO concentration will be held the same in all three dilutions. The appropriate DMSO concentrations will be selected from the results of work described above. Half of the straws from each lot will be frozen on dry ice, the other half in liquid nitrogen. Thus, dilution rate and freezing regime will be examined in this experiment.

The best procedures identified to this point will then be used to freeze semen in large, 4.5-mL freezing straws (Wheeler and Thorgaard 1991). Semen from as many males as required for each of six of the larger volume straws will be combined. A subsample from each combined lot will be frozen in a 0.5-mL straw for comparison. Samples in the larger straws will be evaluated via NMR and spectra will be compared to those

obtained from previously evaluated frozen semen samples. Semen frozen in both the large and small straws will then be thawed and evaluated for premature activation and motility following activation.

Based on results of work in the first year, we will choose and refine a protocol(s) that appear(s) to be most suitable for practical applications. We will then test the protocol(s) under practical fish culture conditions by enlisting the cooperation of at least one commercial producer in the region. The goal of this second year of work will be to produce an entire crop of hybrid striped bass using cryopreserved spermatozoa.

FACILITIES

Intensive Culture (Objective 1)

SIUC
All activity involving obtaining and/or capture, acclimatization and long-term maintenance of broodfish will be done at the SIU Fisheries Research Laboratory in Carbondale, Illinois. The laboratory on the SIUC Campus has several 4.3 m-plus foot electrofishing and net boats available for collecting broodfish. The Laboratory also has a number of gill nets, trap nets, and other collection gears suitable for capturing white bass. Three pick-up truck hauling tanks are available for transport. These are equipped with surface agitator/aerators and compressed oxygen diffusers. Two of the hauling tanks are insulated. Four pick-up trucks are operated by the Laboratory which can be used to transport boats, collection gear, and hauling tanks. Equipment, boats, vehicles, and other facilities at the Laboratory's two satellite research stations in northern and central Illinois parallel those of the SIUC Campus laboratory. The two field stations, the Campus facilities, and a good working relationship with the Illinois Department of Conservation permit ready access to white bass broodfish populations anywhere in the state.

An indoor recirculated-water culture system will be used for photoperiod/temperature manipulations. Currently, six 9463-liter systems, each containing six 1325-liter circular tanks, are in operation at the SIUC wet lab facility. Two of these systems will be devoted to the proposed study for the full duration. One system is housed in a greenhouse allowing for a natural photoperiod. Black plastic tents are placed over three tanks to block natural light. These tanks are equipped with artificial lights set on timers to achieve the desired photoperiod for the compressed cycle. The natural- and compressed-cycle systems are maintained on separate biofilters and have individual temperature controls. Outdoor ponds are available to hold excess fish. Several hundred "domesticated" white bass broodstock should be available at completion of the on-going study on out-of-season spawning.

UW-Milwaukee
The aquaculture laboratories at the Center for Great Lakes Studies have over 740 m^2 of floor space for rearing units. They are supplied with municipal tapwater from a Lake Michigan source. This water supply is dechlorinated by chemical reduction with sodium sulfite. The minimum capacity of the dechlorination system is approximately 1900 Lpm (500 gpm). A portion of this water is heated by natural gas boilers and electrical immersion heaters. Hot and cold water supply lines and compressed air lines supply the rearing facilities. Rearing temperatures are typically controlled by blending the hot and cold supplies and passing the inflow to the rearing unit through packed columns to adjust gas saturation to near atmospheric equilibrium. Refrigeration units are available to maintain cold temperatures. The rearing units have photoperiod controlled lighting fixtures. A variety of large circular fiberglass tanks (2.4 m (8 ft), 1.2 m (4 ft), and 0.76 m (30 in) diameter) are available as rearing units. There are also rectangular and oval fiberglass tanks and a variety of small aquaria. Total tank capacity of the fisheries laboratory is approximately 77 m^3 volume (>20,000 gal). Rearing units are typically operated on a flow through basis, but we also have a limited ability to operate on a recirculating basis. We also have fish transporting units, the largest of which holds 1400 L (370 gal).

In addition there are a wide variety of instrument shop, analytical laboratory, research vessel, limnological equipment, library and computing facilities available at the Great Lakes Research Facility.

OSU
Konrad Dabrowski shares a 46.5 m^2 wet laboratory in Kottman Hall which is equipped with fish rearing tanks, fish egg incubation apparatus and acclimation chambers. This laboratory includes features for water temperature-control and sterilization systems.

The biochemical laboratory in Kottman Hall includes biofreezer (-85 ^oC), refrigerated centrifuge, freeze drier, drying ovens, spectrophotometer DU-70, Beckman HPLC system, Varian 3400 gas chromatography system, and other accessories for biochemical research studies. Facilities at Piketon Research and Extension Center include 14 ponds, aquaculture building equipped with several fish tanks and recirculation system, and temperature and light control rooms. The main building of the field station contains aquaculture, chemical, and biological laboratories. The principal investigators also share a videoanalysis laboratory containing a BCS

486/33 megahertz computer housing Jandel Scientific's JAVA video analysis software and supported by two 43.2 cm Samsung monitors and a COHU 4815 video camera.

Purdue University
Facilities and equipment for mixing dietary ingredients are in place and functional. Similarly, the Fish Nutrition Laboratory at Purdue routinely conducts proximate composition of fish and crustaceans and is completely equipped. The Animal Disease Diagnostic Laboratory at Purdue conducts histopathological examinations of animal tissues daily and has been involved in a number of studies with fish tissues. The Fish Technology Center in Bozeman is fully equipped and capable of manufacturing the mixed diets.

UW-Madison
All hormone analyses will be done by the UW-Madison Aquaculture Program, which has its main research facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. These facilities include an analytical laboratory that is well equipped for histological, cytological, and endocrinological research. The Aquaculture Program also has additional analytical laboratory facilities on the main UW-Madison campus, including a high-speed, large-capacity centrifuge, a microplate reader, and all peripheral equipment needed to conduct a variety of immunoassays. The Program also has access to much of the laboratory facilities, equipment and instrumentation of the UW-Madison Endocrinology-Reproductive Physiology Program, Department of Poultry Science and Center for Limnology.

Stored Semen (Objective 2)

ISU
At ISU NMR is being utilized in the scientific community principally by chemists, biochemists and physicists. 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 background to utilize NMR facilities. They have four strong field instruments: Nicolet NMC300, Varians VR300, Bruker WM200, and a solid state Bruker MSL300. The Bruker MSL300 and Bruker WM200 are temperature control and have probes which permit the examination of any nucleus of our interest. We have had excellent access to the NMR spectrometers with a standard block of time scheduled.

Ample research space is available for use at ISU. Available are extensive facilities for routine laboratory studies including holding tanks, high quality optical microscopes, incubators, and centrifuges. A cryogenic laboratory is housed in the Veterinary Clinic at the Iowa State University Veterinary College. This laboratory supports both commercial and research endeavors and is staffed with research personnel with broad interest in gamete cryopreservation. The expertise and facilities of this laboratory may be used for the freezing studies of this proposal.

SIUC
See Facilities for Objective 1.

REFERENCES

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

AOAC (Association of Official Analytical Chemists). 1984. Official methods of analysis, 14th edition. Association of Official Analytical Chemists, Washington, D.C.

Armitage, W.J., and S.J. Rich. 1990. Vitrification of organized tissues. Cryobiology 27:483-491.

Barrows, F.T., W.A. Lellis and J.G. Nickum. 1988. Intensive culture of larval walleyes with dry or formulated feed: note on swim bladder inflation. The Progressive Fish-Culturist 50:160-166.

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

Bengston, D.A., P. Leger, and P. Sorgeloos. 1991. Use of *Artemia* as a food source for aquaculture. Pages 244-285 *in* R.A. Browne, P. Sorgeloos, and C.N.A. Trotman editors. Artemia Biology, CRC Press, Boca Raton, Florida.

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

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

Brown, C.L., S.I. Doroshev, J.M. Nunez, C. Hadley, J. Vaneenennaam, R.S. Nishioka, and H. Bern. 1988. Maternal triiodothyronine injections cause increases in swim bladder inflation and survival rates in larval striped bass, *Morone saxatilis*. Journal of Experimental Zoology. 248:168-176.

Brown, P.B., M.E. Griffin, and M.R. White. In press. Experimental and practical diet evaluations with juvenile hybrid striped bass. Journal of the World Aquaculture Society.

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

Dabrowski, K., and P. Poczyczynski. 1988. Intensive rearing of common carp and grass carp exclusively on dry diets. Aquaculture 69:317-332.

Dabrowski, K., P. Bergot, and S. Kaushik. 1984. Rearing of coregonid larvae using dry and live food. Preliminary data. Aquaculture 41:11-20.

Dabrowski, K., P. Poczyczynski, G. Kock, and B. Berger. 1989. Effect of fish meal protein substitution by soybean protein in diet on growth, diet utilization and proteolytic enzymes activities in rainbow trout. In vivo test for exocrine pancreatic secretion. Aquaculture 77:29-49.

Dabrowski, K., G. Krumschnabel, M. Paukku, and J. Labanowski. 1992. Cyclic growth and activity of pancreatic enzymes of Arctic charr (*Salvelinus alpinus L.*) alevins. Journal of Fish Biology 40:511-521.

Dhert, P., P. Lavens, M. Duray, and P. Sorgeloos. 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using omega-3-HUFA enriched live food. Aquaculture 90:63-74.

Doroshov, S.I., J.W. Cornacchia, and K. Hogan. 1981. Initial swim bladder inflation in the larvae of physoclistous fishes and its importance for larval culture. International Council for the Exploration of the Sea 178:495-500.

Eda, H., R. Murashige, Y. Oozeki, A. Hagiwara, B. Eastham, P. Bass, C.S. Tamaru, and C.S. Lee. 1990. Factors affecting the intensive larval rearing of striped mullet, *Mugil cephalus*. Aquaculture 91:281-294.

Fahy, G.M., J. Saur, and R.J. Williams. 1990. Physical problems with the vitrification of large biological systems. Cryobiology 27:492-510.

Hadley, C.G., M.B. Rust, and S.I. Doroshev. 1987. Factors influencing the initial swim bladder inflation in striped bass, *Morone saxatilis*. American Fisheries Society Symposium 2:164-169.

Harrell, R.M. 1984. Review of striped bass broodstock acquisition, spawning methods, and fry production. Pages 45-47 *in* J.P. McCraren, editor. The aquaculture of striped bass, a proceedings. University of Maryland Sea Grant Publication No. 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 Research Project F-41-R. Southern Illinois University-Carbondale.

Hofer, R. 1985. Effects of artificial diets on the digestive processes of fish larvae. *In* C.B. Cowey, A.M. Mackie, and J.G. Bell, editors. Nutrition and feeding in fish. Academic Press, New York.

Hummel, B.C.W. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. Canadian Journal of Biochemical Pharmacology 34:1393-1399.

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 nonsalmonid 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.

Kono, T., O. Suzuki, and Y. Tusunoda. 1988. Cryopreservation of rat blastocyst by vitrification. Cryobiology 25:170-173.

Koven, W.M., A. Tandler, G.W. Kissel, D. Sklan, O. Friezlander, and M. Harel. 1990. The effect of dietary (N-3) polyunsaturated fatty acids on growth survival and swim bladder development in *Sparus aurata* larvae. Aquaculture 91:131-141.

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

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

NAS (National Academy of Sciences). 1981. Nutritional requirements of fish and shellfish, revised. National Academy of Sciences, Washington, D.C.

Norton, V., T. Smith, and I. Strand, editors. 1983. Stripers: the economic value of the Atlantic coast commercial and recreational striped bass. University of Maryland Sea Grant Program No. UM-SG-TAS-83-12, College Park.

Rall, W.F. 1987. Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology 24:387-402.

Rees, R.A., and R.M. Harrell. 1990. Artificial spawning and fry production of striped bass and hybrids. Pages 43-72 *in* R.M. Harrell, J.H. Kerby, and R.V. Minton, editors. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division, American Fisheries Society, Bethesda, Maryland.

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

Sheehan, D.C., and B.B. Hrapchak. 1980. Theory and practice of histotechnology, second edition, C.V. Mosby Company, St. Louis, MO.

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

Tuncer, H., R.M. Harrell, and E.D. Houde. 1990. Acceptance and consumption of food by striped bass and hybrid larvae. Journal of the World Aquaculture Society 21:225-234.

Webster, C.D., and R.T. Lovell. 1990a. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture 90:49-61.

Webster, D.C., and R.T. Lovell. 1990b. Comparison of live brine shrimp nauplii and non-living diets as first food for striped bass larvae. The Progressive Fish-Culturist 52(3):171-175.

Wheeler, P.A., and G.H. Thorgaard. 1991. Cryopreservation of rainbow trout semen in large straws. Aquaculture 93:95-100.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Christopher C. Kohler Southern Illinois University-Carbondale	Aquaculture
	Robert J. Sheehan Southern Illinois University-Carbondale	Aquaculture/Physiology
Indiana	Paul B. Brown Purdue University	Aquaculture/Nutrition
	M. Randall White Purdue University	Veterinarian Medicine
Iowa	George G. Brown Iowa State University	Physiology
Ohio	Konrad Dabrowski Ohio State University	Larval Fish Culture/Nutrition/Physiology
	James E. Ebeling Ohio State University	Aquaculture
Wisconsin	Fred P. Binkowski University of Wisconsin-Milwaukee	Finfish Aquaculture/Larval Fish Culture
	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture/Physiology/Endocrinology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Southern Illinois University-Carbondale (SIUC)
Christopher C. Kohler
Robert J. Sheehan

University of Wisconsin-Milwaukee (UW-Milwaukee)
Fred P. Binkowski

Ohio State University (OSU)
Konrad Dabrowski
James E. Ebeling

Purdue University
Paul B. Brown
M. Randall White

University of Wisconsin-Madison (UW-Madison)
Jeffrey A. Malison

Iowa State University (ISU)
George G. Brown

PROPOSED HYBRID STRIPED BASS BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

(Kohler and Sheehan)

Objectives 1 and 2

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	2	0.20	2	0.20	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	2	0.75	2	1.00	$17,500	$25,500
d. Prebaccalaureate Students	1	0.75	1	0.61	$11,000	$9,000
e. Secretarial-Clerical	1	0.08	1	0.08	$1,500	$1,500
f. Technical, Shop, and Other						
Total Salaries and Wages					30,000	36,000
B. Fringe Benefits					$500	$500
C. **Total Salaries, Wages and Fringe Benefits**					30,500	36,500
D. Nonexpendable Equipment					$1,500	$1,500
E. Materials and Supplies					$4,000	$4,000
F. Travel - Domestic (*Including Canada*)					$4,500	$4,000
G. Other Direct Costs					$1,500	$2,000
TOTAL PROJECT COSTS PER YEAR (*C through G*)					42,000	48,000
TOTAL PROJECT COSTS					90,000	

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

BUDGET JUSTIFICATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

(Kohler and Sheehan)

A. Salaries and Wages. Two graduate assistants (0.75 FTE in Year 1 and 1.0 FTE in Year 2) to assist in collection, maintenance, spawning, and feeding of fishes. One student worker (0.75 FTE in Year 1 and 0.61 FTE in Year 2) to assist graduate students in above tasks. SIUC will serve as the lead institution and thus some secretarial support (0.08 FTE) will be required for report preparation, etc.

D. Nonexpendable Equipment. Our experience has been that we will need to replace a 1.0 HP chiller unit each year. Several chillers are used to manipulate spawning temperatures.

E. Materials and Supplies. Expendable supplies such as glassware, extender chemicals, liquid nitrogen, dry ice, miscellaneous labware, chemicals, fish feed, and plumbing supplies.

F. Travel-Domestic. Several collection trips will be made to maintain adequate numbers of white bass. There will also be several trips made between SIUC and ISU. In addition, there will be travel to hatcheries within and outside of the region for the purpose of obtaining gametes. NCRAC meetings and professional meetings for paper presentations will also require travel support.

G. Other Direct Costs: Computer costs, report preparation, graphics, telecommunications (telephone, FAX, and e-mail), postage, photocopying, and equipment repair.

PROPOSED HYBRID STRIPED BASS BUDGET FOR UNIVERSITY OF WISCONSIN-MILWAUKEE

(Binkowski)

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	0.17	1	0.17	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	2	0.25	2	0.25	$10,000	$10,000
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					10,000	10,000
B. Fringe Benefits (30% of 2b)					$3,000	$3,000
C. **Total Salaries, Wages and Fringe Benefits**					13,000	13,000
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,500	$1,500
F. Travel - Domestic (*Including Canada*)					$500	$500
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					15,000	15,000
TOTAL PROJECT COSTS					30,000	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MILWAUKEE

(Binkowski)

A. Salaries and Wages. Laboratory studies will be conducted by two full-time research specialists on 25% appointments. The tasks included are: monitoring laboratory egg incubations, maintaining "green tank" larval fish-food cultures, transforming incubation apparatus to larval-fingerling culture units, initiating larval feeding techniques from the onset of first-feeding through habituation on commercial feeds, and over the 60-80 day period, maintaining records on mortality, temperatures, etc., and implementing a daily experimental maintenance program.

B. Materials and Supplies. General laboratory supplies will include aquariums, hardware, fish food, and general construction costs for experimental apparatus.

F. Travel. These funds will support transportation, meals, and lodging for one research planning meeting with NCRAC participants and attend the annual work group meeting.

PROPOSED HYBRID STRIPED BASS BUDGET FOR OHIO STATE UNIVERSITY

(Dabrowski and Ebeling)

Objective 1

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.05	1	0.05	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	1	0.25	1	0.25	$3,600	$3,780
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other					$750	$750
Total Salaries and Wages					4,350	4,530
B. Fringe Benefits					$250	$100
C. **Total Salaries, Wages and Fringe Benefits**					4,600	4,630
D. Nonexpendable Equipment					$1,500	$0
E. Materials and Supplies					$1,500	$2,870
F. Travel - Domestic (*Including Canada*)					$400	$500
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					8,000	8,000
TOTAL PROJECT COSTS					16,000	

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

BUDGET JUSTIFICATION FOR OSU - PIKETON

(Dabrowski and Ebeling)

A. Salaries and Wages. Field and laboratory studies will be conducted by a graduate student and a research assistant. Their tasks include sampling at Piketon, initial preparation of samples for analysis, transportation to Piketon, fish and zooplankton sample analysis. Approximately half of the labor in pond and tank experiments will be supported by monies from the Piketon Center.

Additional responsibilities of a graduate student will include: diet preparation and analysis, preparation of daily, weekly and monthly tables and graphs of field and laboratory experiments schedule.

D. Nonexpendable Equipment. We request in addition to our DU-70 Beckman Spectrophotometer, an electrophoreris densitometer. This would allow us to use more precise and sensitive methods for enzyme analysis using isoenzyme separation.

E. Materials and Supplies. First year general laboratory and field supplies will include: reagents, glassware, diet ingredients, commercial feeds and replacement parts for laboratory equipment (homogenizers, spectrophotometer).

F. Travel. These funds will support transportation, meals and if necessary lodging for the collection of samples. Travel funds will also be used to attend the annual work group meetings and the NCRAC conference to present initial results.

PROPOSED HYBRID STRIPED BASS BUDGET FOR PURDUE UNIVERSITY

(P. Brown and White)

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	0.01	1	0.01	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students						
d. Prebaccalaureate Students					$750	$750
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					750	750
B. Fringe Benefits (3% of 2b)					$24	$24
C. **Total Salaries, Wages and Fringe Benefits**					774	774
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,500	$1,500
F. Travel - Domestic (*Including Canada*)					$0	$0
G. Other Direct Costs					$226	$226
TOTAL PROJECT COSTS PER YEAR (*C through G*)					2,500	2,500
TOTAL PROJECT COSTS					5,000	

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

BUDGET JUSTIFICATION FOR PURDUE UNIVERSITY

(P. Brown and White)

The budget reflects costs of feed ingredients, labor to acquire those ingredients and mix the experimental diets, shipping costs, and costs of analytical reagents for proximate analysis and histopathological examination.

PROPOSED HYBRID STRIPED BASS BUDGET FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison)

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	0.025	1	0.02	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.03	1	0.03	$800	$800
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					800	800
B. Fringe Benefits (32% of 2f)					$252	$252
C. **Total Salaries, Wages and Fringe Benefits**					1,052	1,052
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$448	$448
F. Travel - Domestic (*Including Canada*)					$0	$0
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					1,500	1,500
TOTAL PROJECT COSTS					3,000	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison)

A. Salaries and Wages. Salaries of personnel are needed to conduct analyses for serum estradiol-17β, testosterone, 11-ketotestosterone, and 17α, 20β-dihydroxy-4-pregnen-3-one (17,20-DHP).

E. Materials and Supplies. Biochemicals, reagents, hormone-assay and general laboratory supplies are needed to conduct hormone assays on serum samples.

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	$6,000
c. Graduate Students						
d. Prebaccalaureate Students					$500	$500
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					6,500	6,500
B. Fringe Benefits (33% of 2b)					$1,956	$1,956
C. **Total Salaries, Wages and Fringe Benefits**					8,456	8,456
D. Nonexpendable Equipment					$800	$500
E. Materials and Supplies					$1,000	$1,000
F. Travel - Domestic (*Including Canada*)					$500	$800
G. Other Direct Costs					$1,244	$1,244
TOTAL PROJECT COSTS PER YEAR (*C through G*)					12,000	12,000
TOTAL PROJECT COSTS					24,000	

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

BUDGET JUSTIFICATION FOR IOWA STATE UNIVERSITY

(G. Brown)

A. Salaries and Wages. A part-time technician will be paid hourly wages ($7.00) varying 15-20 hours/week for at least 9/12 year. Technician will be responsible for procedures involving the maintenance, transportation, short-term storage and cryopreservation of gametes.

D. Nonexpendable Equipment. Updating of computer hardware and graphic capabilities.

E. Materials and Supplies. Expendable supplies such as tissue culture flasks, disposable pipettes, chemicals for extenders, cryogenic chemicals and supplies and miscellaneous labware. Cost of postage service for transportation of samples.

F. Travel. Travel to fish hatcheries to obtain gametes at various times of the year. Travel to professional meetings for paper presentations.

G. Other Direct Costs. The NMR facilities at ISU charge the user $10/hr. Our expected usage will average 5 hours/week for 20 weeks of the year.

CULTURE TECHNOLOGY OF HYBRID STRIPED BASS

Budget Summary for Each Participating Institution at $81.0K for the First Year

	SIUC	UW-Milwaukee	OSU	Purdue	UW-Madison	ISU	TOTALS
Salaries and Wages	$30,000	$10,000	$4,350	$750	$800	$6,500	52,400
Fringe Benefits	$500	$3,000	$250	$24	$252	$1,956	5,982
Total Salaries, Wages and Benefits	**30,500**	**13,000**	**4,600**	**774**	**1,052**	**8,456**	**58,382**
Nonexpendable Equipment	$1,500	$0	$1,500	$0	$0	$800	3,800
Materials and Supplies	$4,000	$1,500	$1,500	$1,500	$448	$1,000	9,948
Travel	$4,500	$500	$400	$0	$0	$500	5,900
Other Direct Costs	$1,500	$0	$0	$226	$0	$1,244	2,970
TOTAL PROJECT COSTS	**42,000**	**15,000**	**8,000**	**2,500**	**1,500**	**12,000**	**81,000**

Budget Summary for Each Participating Institution at $87.0K for the Second Year

	SIUC	UW-Milwaukee	OSU	Purdue	UW-Madison	ISU	TOTALS
Salaries and Wages	$36,000	$10,000	$4,530	$750	$800	$6,500	58,580
Fringe Benefits	$500	$3,000	$100	$24	$252	$1,956	5,832
Total Salaries, Wages and Benefits	**36,500**	**13,000**	**4,630**	**774**	**1,052**	**8,456**	**64,412**
Nonexpendable Equipment	$1,500	$0	$0	$0	$0	$500	2,000
Materials and Supplies	$4,000	$1,500	$2,870	$1,500	$448	$1,000	11,318
Travel	$4,000	$500	$500	$0	$0	$800	5,800
Other Direct Costs	$2,000	$0	$0	$226	$0	$1,244	3,470
TOTAL PROJECT COSTS	**48,000**	**15,000**	**8,000**	**2,500**	**1,500**	**12,000**	**87,000**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

State/Institution		Year 1	Year 2
Southern Illinois University-Carbondale			
Salaries and Benefits: SY @ 0.20 FTE		$8,000	$9,000
Overhead Waived		$17,000	$17,000
	Total	$25,000	$26,000
University of Wisconsin-Milwaukee			
Salaries and Benefits: SY @ 0.17 FTE		$7,654	$8,036
Supplies, Expenses, and Equipment		$4,800	$5,200
	Total	$12,454	$13,236
Ohio State University			
Salaries and Benefits: SY @ 0.05 FTE		$2,500	$2,750
Supplies, Expenses, and Equipment		$3,000	$3,000
	Total	$5,500	$5,750
Purdue University			
Salaries and Benefits: SY @ 0.10		$500	$550
Supplies, Expenses, and Equipment		$1,500	$1,500
	Total	$2,000	$2,050
University of Wisconsin-Madison			
Salaries and Benefits: SY @ 0.02		$1,044	$1,044
TY @ 0.01		$556	$556
Supplies, Expenses, and Equipment		$125	$125
	Total	$1,725	$1,725
Iowa State University			
Salaries and Benefits: SY @ 0.15 FTE		$6,000	$6,000
Supplies, Expenses, and Equipment		$6,000	$6,000
	Total	$12,000	$12,000
	Total per Year	$58,679	$60,761
	GRAND TOTAL	$119,440	

[1]Since cost sharing is not a legal requirement some universities chose not to provide resource commitment from institutions

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

LIST OF PRINCIPAL INVESTIGATORS

Fred P. Binkowski, University of Wisconsin-Milwaukee

George G. Brown, Iowa State University

Paul B. Brown, Purdue University

Konrad Dabrowski, Ohio State University

James E. Ebeling, Ohio State University

Christopher C. Kohler, Southern Illinois University-Carbondale

Jeffrey A. Malison, University of Wisconsin-Madison

Robert J. Sheehan, Southern Illinois University-Carbondale

M. Randal White, Purdue University

VITA

Fred P. Binkowski
Senior Scientist
Center for Great Lakes Studies
600 E. Greenfield Avenue
University of Wisconsin-Milwaukee
Milwaukee, WI 53204

Phone: (414) 382-1700
FAX: (414) 382-1705

EDUCATION

B.S. University of Wisconsin-Milwaukee, 1971
M.S. University of Wisconsin-Milwaukee, 1974

POSITIONS

Senior Scientist (1991-present) and Associate Scientist (1987-1990), Center for Great Lakes Studies/University of Wisconsin Great Lakes Research Facility (GLRF)
Senior Fisheries Biologist (1984-1986), Associate Fisheries Biologist (1981-1983), and Assistant Fisheries Biologist (1978-1980), Center for Great Lakes Studies/University of Wisconsin GLRF
Research Specialist, Fisheries, Dept. of Zoology, University of Wisconsin-Milwaukee (1975-1978)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Early Life History and Fish Culture Sections
International Association for Great Lakes Research (Associate Editor)
World Aquaculture Society

SELECTED PUBLICATIONS

Miller, T., L. Crowder, J. Rice, and F.P. Binkowski. 1992. Body size and the ontogeny of the functional response in fishes. Canadian Journal of Fisheries and Aquatic Sciences 49:805-812.

Miller, T., L. Crowder, and F.P. Binkowski. 1990. Zooplankton size dynamics and recruitment success of bloater in Lake Michigan. Transactions of the American Fisheries Society 119:484-491.

Luecke, C, J.A. Rice, L.B. Crowder, S.E. Yeo, and F.P. Binkowski. 1990. Recruitment mechanisms of bloater in Lake Michigan: an analysis of the predatory gauntlet. Canadian Journal of fisheries and Aquatic Sciences 47:524-532.

Seale, D.B., and F.P. Binkowski. 1988. Vulnerability of early life intervals of *Coregonus hoyi* to predation by a freshwater mysid, *Mysis relicta*. Environmental Biology of Fishes 21:117-125.

Rice, J.A., L.B. Crowder, and F.P. Binkowski. 1987. Evaluating potential sources of mortality for larval bloater (*Coreogonus hoyi*): starvation and vulnerability to predation. Canadian Journal of Fisheries and Aquatic Sciences 44:467-472.

Sommer, C.V., F.P. Binkowski, M.A. Schalk, and J.M. Bartos. 1986. Stress factors that can affect studies of drug metabolism in fish. Veterinary and Human Toxicology 28 (Supplement 1):45-54.

Stewart, D.J., and F.P. Binkowski. 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: an energetics-modeling synthesis. Transactions of the American Fisheries Society 115:643-661.

VITA

George G. Brown
Professor
Department of Zoology and Genetics
Iowa State University
Ames, Iowa 50011-3223

Phone: (515) 294-3145

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 and Genetics, Iowa State University, Ames, Iowa (1976-present)
Associate Professor, Department of Zoology and Genetics, Iowa State University, Ames, Iowa (1972-1976)
Assistant Professor, Department of Zoology and Genetics, Iowa State University, Ames, Iowa (1967-1972)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

Brown, G.G., and C.D. Drewes. 1992. ^{31}P-NMR analysis of phospholombricine and other phosphorus-containing metabolites in selected freshwater and terrestrial oligochaetes. Comparative Biochemistry and Physiology. 102B:389-396.

Wilson, E.F., G.G. Brown, and C.D. Drewes. 1992. Characterization of phosphorus metabolites during six stages of development in the earthworm *Eisenia foetida*. Comparative Biochemistry and Physiology 102B:383-388.

Robitaille, P-M.L., P.A. Robitaille, G.G. Brown Jr., and G.G. Brown. 1991. An analysis of the pH-dependent chemical-shift behavior of phosphorus-containing metabolites. Journal of Magnetic Resonance 92:72-84.

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

VITA

Paul B. Brown
Associate Professor
Department of Forestry and Natural Resources
Forestry Building
Purdue University
West Lafayette, IN 47907

Phone: (317) 494-4968
FAX: (317) 494-0409

EDUCATION

B.S. University of Tennessee, 1981
M.S. University of Tennessee, 1983
Ph.D. Texas A&M University, 1987

POSITIONS

Associate Professor, Department of Forestry and Natural Resources, Purdue University (1993-present)
Assistant Professor, Department of Forestry and Natural Resources, Purdue University (1989-1993)
Assistant Professional Scientist/Field Station Director, Illinois Natural History Survey (1987-1989)
Research Associate, Texas A&M University (1986-1987)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Fisheries Society: Indiana Chapter; Membership Concerns Committee (National) 1985-present; Walleye Technical Committee and Walleye Technical Committee (North Central Division) 1988-present; Fish Culture Section
American Institute of Fishery Research Biologists
International Association of Astacology
World Aquaculture Society
Sigma Xi, Gamma Sigma Delta

SELECTED PUBLICATIONS

Brown, P.B., M.E. Griffin, and M.R. White. In press. Experimental and practical diet evaluations with juvenile hybrid striped bass. Journal of the World Aquaculture Society.

Brown, P.B., and E.H. Robinson. 1992. Vitamin D studies with juvenile channel catfish (*Ictalurus punctatus*) reared in calcium-free water. Comparative Biochemistry and Physiology 193A:213-219.

Griffin, M.E., P.B. Brown, and A. Grant. 1992. Dietary lysine requirements of juvenile hybrid striped bass. Journal of Nutrition 122:1332-1337.

Reigh, R.C., E.H. Robinson, and P.B. Brown. 1991. Effects of dietary magnesium on growth and tissue magnesium content of blue tilapia *Oreochromis aureus*. Journal of the World Aquaculture Society 22:192-200.

Brown, P.B., W.H. Neill, and E.H. Robinson. 1990. Preliminary evaluation of whole body energy changes as a method of estimating maintenance energy needs of fish. Journal of Fish Biology 36:107-108.

Brown, P.B., and E.H. Robinson. 1989. Comparison of 26 and 30 percent protein feeds for channel catfish. The Progressive Fish-Culturist 51:149-151.

VITA

Konrad Dabrowski
Professor
School of Natural Resources
2021 Coffey Road
Ohio State University
Columbus, OH 43210

Phone: (614) 292-4555
FAX: (614) 292-7162

EDUCATION

M.S. Agriculture and Technical University, Olsztyn, Poland, 1972
Ph.D. Agriculture and Technical University, Olsztyn, Poland, 1976
D.Sc. Agricultural University, Szczecin, Poland, 1984

POSITIONS

Visiting Professor of Aquaculture, Ohio State University (1989-present)
Visiting Professor, University of Innsbruck, Austria (1987-1989)
Visiting Professor, Tokyo University of Fisheries, Japan (1984-1985)
Associate Professor, Agriculture and Technical University, Olsztyn, Poland (1972-1985)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Editorial Board Member for Aquaculture and Aquatic Living Resources
Fisheries Society of British Isles
Japanese Fisheries Society
National Research Council, Washington, Subcommittee on Fish Nutrition (1990-1992)
World Aquaculture Society

SELECTED PUBLICATIONS

Dabrowski, K., G. Krumschnabel, M. Paukku, and J. Labanowski. 1992. Cyclic growth and activity of pancreatic enzymes of Arctic charr (*Salvelinus alpinus L.*). Journal of Fish Biology 40:511-521.

Dabrowski, K., and G. Kock. 1989. Absorption of ascorbic acid and ascorbic sulfate and their interaction with minerals in digestive tract of rainbow trout. Canadian Journal of Fisheries and Aquatic Science 46:1952-1957.

Dabrowski, K. 1989. Formulation of a bioenergetic model for coregonine early life history. Transactions of the American Fisheries Society 118:138-150.

Dabrowski, K., P. Boczyczynski, G. Kock, and B. Berger. 1989. Effect of fish meal protein substitution by soybean protein in diet on growth, diet utilization and proteolytic enzymes activities in rainbow trout. New in vivo test for exocrine pancreatic secretion. Aquaculture 77:29-49.

Dabrowski, K., F. Takashima, and Y.K. Law. 1988. Bioenergetical model of planktivorous fish feeding, growth and metabolism. Theoretical optimum swimming speed in fish larva. Journal of Fish Biology 32:443-458.

Georgopoulou, U., K. Dabrowski, M.F. Sire, and J.M. Vernier. 1988. Absorption of intact proteins by the intestinal epithelium of trout. Demonstration by luminescence enzyme immunoassay and cytochemistry. Cell and Tissue Research 251:145-152.

VITA

James M. Ebeling
Aquaculture Research and Extension Associate
P.O. Box 549
OSU-Piketon Research and Extension Center
Piketon, OH 45661-0549

Phone: (614) 289-2071
FAX: (614) 289-4591

EDUCATION

B.A. Albion College, 1971
M.S. Washington State University, 1974
M.S. Washington State University, 1977

POSITIONS

Research and Extension Associate, Piketon Research and Extension Center, Ohio State University (1991-present)
Project Manager, Recirculation Aquaculture Demonstration Project, North Carolina State University (1990-1991)
Research Coordinator, Mariculture Research & Training Center, University of Hawaii (1988-1990)
Research Assistant, Department of Agricultural Engineering, University of California-Davis (1983-1988)
Research Technologist II, Department of Agricultural Engineering, Washington State University (1981-1983)
Technical Specialist, Washington Energy Extension Service-Cooperative Extension Service (1979-1981)
Research Technologist II, Department of Agricultural Engineering, Washington State University (1977-1979)
American Peace Corps Volunteer, Secondary Education Program, Ghana (1971-1972)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Society of Agricultural Engineers
Sigma Xi
World Aquacultural Society

SELECTED PUBLICATIONS

Ebeling, J.M. 1991. A computer based water quality monitoring and management system for pond aquaculture. Pages 233-248 *in* Proceedings from the Aquaculture Symposium, Cornell University, Ithaca, NY, NRAES-49.

Ebeling, J.M., and T.M. Losordo. 1989. Continuous environmental monitoring systems for aquaculture. Pages 54-70 *in* J.A. Wyban and E. Antill, editors. Instrumentation in aquaculture. Proceedings of the World Aquaculture Society, January, Los Angeles, CA.

Losordo, T.M., R.H. Piedrahita, and J.M. Ebeling. 1988. An automated water quality data acquisition system for use in aquaculture ponds. Aquacultural Engineering 7:265-278.

VITA

Christopher C. Kohler
Associate Professor, Department of Zoology and
Associate Director, Fisheries Research Laboratory
Southern Illinois University-Carbondale
Carbondale, IL 62901

Phone: (618) 453-2890
FAX: (618) 536-7761

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 fo 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. 1990. Maintaining fishes for research and teaching. Pages 633-663 *in* C. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society, Maryland.

Roem, A.J., R.R. Stickney, and C.C. Kohler. In press. Note on the vitamin requirements of blue tilapia (*Tilapia aurea*) in a recirculating system. The 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.

Kohler, C.C., and S.P. Krueger. 1986. Use of pressed brewer's grain as feed for freshwater shrimp (*Macrobrachium rosenbergii*). Journal of the World Mariculture Society 16:181-182.

VITA

Jeffrey A. Malison
Assistant Director
University of Wisconsin Aquaculture Program
103 Babcock Hall, 1605 Linden Drive
University of Wisconsin-Madison
Madison, WI 53706

Phone: (608) 263-1242
FAX: (608) 262-6872

EDUCATION

B.S. University of Wisconsin-Stevens Point, 1976
M.S. University of Wisconsin-Madison, 1980
Ph.D. University of Wisconsin-Madison, 1985

POSITIONS

Assistant Director, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1990-present)
Associate Researcher, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1987-1990)
Project Associate, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1985-1987)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Fisheries Society
American Society of Zoologists
World Aquaculture Society

SELECTED PUBLICATIONS

Barry, T.P., A.F. Lapp, T.B. Kayes, and J.A. Malison. Submitted. Validation of an (ELISA) for measuring cortisol in fish and comparison of stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture.

Malison, J.A., T.B. Kayes, J.A. Held, T.P. Barry, and C.H. Amundson. In press. Manipulation of ploidy in yellow perch (*Perca flavescens*) by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health 4:1-6.

Malison, J.A., and J.A. Held. 1992. Effects of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (*Perca flavescens*) to intensive culture conditions. Aquaculture 104:67-88.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Measurement of resting and stress-elevated serum cortisol in rainbow trout *Oncorhynchus mykiss* in experimental net-pens. Journal of the World Aquaculture Society 23:83-88.

Malison, J.A., T.B. Kayes, B.C. Wentworth, and C.H. Amundson. 1988. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Canadian Journal of Fisheries and Aquatic Sciences 45:1942-1948.

VITA

Robert J. Sheehan
Associate Professor, Department of Zoology and
Assistant Director, Fisheries Research Laboratory
Southern Illinois University-Carbondale
Carbondale, IL 62901

Phone: (618) 453-6089
FAX: (618) 536-7761

POSITIONS

Associate Professor, Department of Zoology, Southern Illinois University-Carbondale (1992-present)
Assistant Director, Fisheries Research Laboratory, Southern Illinois University-Carbondale (1986-present)
Assistant Professor, Department of Zoology, Southern Illinois University-Carbondale (1986-1992)
Assistant Professor, Department of Fisheries and Wildlife Science, Virginia Polytechnic Institute & State University (1984-1986)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

Bodensteiner, L.R., R.J. Sheehan, W.M. Lewis, and P.S. Wills. In press. Effects of long-term repetitive formalin treatments during winter on channel catfish fingerlings. Journal of Aquatic Animal Health.

Krum, H.N., and R.J. Sheehan. In press. Development of a magnetic activity-detection system. Animal Behavior.

Sheehan, R.J., L.R. Bodensteiner, W.M. Lewis, P.S. Wills, and A.M. Brandenburg. In press. Flowing water: an effective treatment for ichthyophthiriasis. Transactions of the American Fisheries Society.

Sheehan, R.J., P.S. Wills, and W.T. Davin. 1991. Crayfish production: a promising enterprise for the Midwest. Pages 219-225 *in* Proceedings of the North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. Michigan Department of Natural Resources, Wolf Lake Fish Hatchery, Mattawan, Michigan.

Sheehan, R.J., L.R. Bodensteiner, W.M. Lewis, D.E. Logsdon, and S.D. Scherck. 1990. Long-term survival and swimming performance of young of the year fishes at low temperatures: links between physiological capacity and winter habitat requirements. Pages 98-108 *in* R. Sauer, editor. Proceedings of the Restoration of Midwestern Stream Habitat Symposium, Rivers and Streams Technical Committee, North-Central Division, American Fisheries Society, Minneapolis, Minnesota, December 4-5, 1990.

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.

VITA

Melvin Randall White
Veterinary Pathologist
Animal Disease Diagnostic Laboratory
Purdue University
1175-ADDL
West Lafayette, IN 47907-1175

Phone: (317) 494-7456

EDUCATION

D.V.M. Auburn University, 1982
Ph.D. University of Georgia, 1988

POSITION

Professional Staff, Purdue University (1988-present)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Diplomate, American College of Veterinary Pathologists, 1991
American Veterinary Medical Laboratory Diagnosticians, members, 1988-present
Xi Chapter of Phi Zeta, 1986-present
Recipient of C.L. Davis, D.B.M. Foundation's 1987 Veterinary Pathology Scholarship Award

SELECTED PUBLICATIONS

Brown, P.B., M.E. Griffin, and M.R. White. In press. Experimental and practical diet evaluations with juvenile hybrid striped bass. Journal of the World Aquaculture Society.

White, M.R. 1990. What's your diagnosis? Bilateral ovarian neoplasia in an aged gerbil. Laboratory Animal 19(6):20-23.

White, M.R., W.L. Chapman, Jr., and W.L. Hanson. 1989. Chemotherapy of experimental visceral leishmaniasis in the opossum. Journal of Parasitology 75(1):176-178.

White, M.R., W.L. Chapman, Jr., and W.L. Hanson. 1989. A comparison of experimental visceral leishmaniasis in the opossum, armadillo and ferret. Laboratory Animal Science 39(1):47-50.

White, M.R., W.L. Chapman, Jr., W.L. Hanson, K.S. Latimer, and C.E. Green. 1989. Experimental visceral leishmaniasis in the opossum. Veterinary Pathology 26:314-321.

White, M.R., W.A. Crowell, and B.L. Guy. 1988. Cecocolic intussusception in a foal with *Eimeria leuckarti* infection. Equine Practice 10(5):15-18.

White, M.R., W.A. Crowell, and J.L. Blue. 1986. A nepthrotic-like syndrome with and associated mesangio-proliferative glomerulopathy in a cow. Veterinary Pathology 23:439-442.