**ATTACHMENT C**

**PROJECT NAME**: Advancing Hybrid Striped Bass Culture in the North Central Region

**FUNDING LEVEL**: Year 1 (91-92) - $46,548
Year 2 (92-93) - $50,002

**DURATION**: 2 years

**CHAIRPERSON**: Dr. Christopher C. Kohler, Fisheries Research Laboratory, Southern Illinois University, Carbondale, IL 62901-6511, Telephone (618) 536-7761

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## JUSTIFICATION

Per capita consumption of seafood products has grown steadily in the United States, a demand largely being met by increased imports. For example, the U.S. purchased $4.8 billion in seafood from abroad in 1986 while only selling $1.3 billion worth overseas (U.S. Department of Commerce Statistics).

Thus, seafood imports represent a major cause of the U.S. balance of trade deficit. States within the North Central Region are a major source of demand for imported seafood. The North Central Region is rich in terrestrial resources, and not surprisingly, agriculture is a dominant industry. However, the movement of agriculture in the region toward production of commodities, such as corn and soybeans, with ultra-high efficiency has been accomplished at great cost; now surpluses in all major crops, combined with land devaluations and financial strains, have forced both the agriculture system and its participants to investigate other farm technologies (Illinois Dept. Agriculture 1986). Aquaculture is perceived within the region as a logical vehicle for diversification. Indeed, it is this perception and desire for aquaculture development that provided the impetus to establish a USDA regional aquaculture center in the Midwest.

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

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

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

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

According to the National Aquaculture Development Plan (JSA 1983), the principal constraint on commercial striped bass aquaculture in the U.S. is the "nonavailability of seed stock". Recent declines in the striped bass fisheries along the Atlantic coast, as well as legal constraints, have increasingly limited the availability of wild broodstock (especially females) as a source of gametes (Harrell 1984). In part, the problem of limited availability of striped bass gametes in the North Central Region could be greatly reduced by utilizing female white bass crossed with male striped bass to produce reciprocal cross hybrids. White bass are native and fairly common through-out 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 resolve the dilemma of gamete availability, strategies for domesticating broodstocks and associated methods of controlling reproduction need to be developed (Donaldson and Hunter 1983; Idler et al. 1987). The development of female white bass and male striped bass broodstocks for production purposes would obviate the need to use wild fish as gamete sources. Two additional benefits of using female white bass as broodstock are that they normally mature at an earlier age (age 3) and are much smaller (i.e., 250-580 g body weight) than female striped bass, which typically mature at age 4 or older and weigh 2-7 kg or more (Scott and Crossman 1973; Smith 1988). Earlier age-at-maturity and smaller size are characteristics that should greatly reduce the cost and effort of broodstock rearing and maintenance, and facilitate the development of methods to manipulate sexual maturation and out-of-season spawning.

The development of efficacious procedures to manipulate sexual maturation and induce out-of-season spawning is an important component of optimal broodstock management. The potential benefits of such procedures include: (1) greater predictability of gamete production; (2) reduced incidence of failed spawnings, gamete resorption and subsequent broodfish losses (e.g., due to toxemia); and (3) the production of fertilized eggs and fry at predetermined times throughout the year. The availability of fertilized eggs outside the normal spawning season would greatly facilitate research on the intensive culture of both hybrid and purebred striped bass fry. On a larger scale, the production of fertilized eggs out-of-season could facilitate a fuller, more efficient use of culture facilities and equipment, and might allow such innovative techniques as the double- or triple-cropping of fry in rearing ponds.

This proposal describes an ongoing cooperative regional research project that is interdisciplinary in scope and involves investigators from three institutions in three states: Southern Illinois University, University of Wisconsin-Madison, and University of Nebraska-Lincoln. The principal goal of the project is to address key problems that pertain to the development of commercial hybrid striped bass culture in the North Central Region. Problem areas that are addressed include: (1) broodstock development, (2) mechanisms regulating the natural reproductive cycle, and (3) manipulation of gonadal maturation and out-of-season spawning. Significant progress on these problem areas has been achieved during the first year of the two-year project. However, a third and fourth year of funding is needed for the three lines of research to reach full fruition and thus yield maximum benefits.

## RELATED CURRENT AND PREVIOUS WORK

The conventional method of propagating striped bass involves capturing wild broodfish immediately before or during their normal spawning season and transporting them to a holding facility (Bonn et al. 1976; Kerby 1986). There, the females are usually injected with human chorionic gonadotropin (HCG) to ensure final ovarian maturation and ovulation. The males may also be injected with HCG to increase semen volume. Subsequently, either the ripe fish are artificially propagated (sperm and eggs are manually stripped and mixed), or males and females are allowed to spawn naturally together in tanks.

Similar propagation techniques have been utilized with white bass (W.E. Jenkins of the South Carolina Department of Wildlife and Marine Resources, personal communication; also Smith 1988; Smith and Jenkins 1986). Genetically and taxonomically, the striped bass and white bass are closely related; both spawn naturally in spring and early summer (Scott and Crossman 1973). However, the opposite sexes of the two species will not normally spawn when placed together. Because of this, artificial propagation techniques are used to produce both the SB x WB and WB x SB (female x male, respectively) hybrids (Kerby 1986).

In recent years, captive striped bass broodstock programs have been initiated and maintained by a few private producers and government agency fish hatcheries and laboratories, primarily along the Atlantic coast and in the South. Examples include: the Baltimore Gas and Electric Company's Crane Aquaculture Facility, the Marine Resources Research Institute of the South Carolina Department of Wildlife and Marine Resources, and the U.S. Fish and Wildlife Service's Southeastern Fish Cultural Laboratory in Marion, Alabama.

The Marine Resources Research Institute in South Carolina and Osage Catfisheries, Inc. in Missouri are two of the very few entities that maintain white bass broodstocks. For both species, the broodstocks developed to date consist almost entirely of "parental" (P) generation fish (white bass and striped bass) captured from the wild and acclimitized to pond or tank culture facilities, or "first filial" (F1) generation fish (primarily striped bass) raised from eggs. Typically, these broodstocks are maintained under ambient seasonal photoperiod/temperature conditions and become sexually mature during the normal spawning season.

To develop maximally effective methods of managing broodstock, baseline information on the physiological mechanisms regulating reproduction is needed (Donaldson and Hunter 1983; Idler et al. 1987). A starting point for obtaining such information is to characterize changes in specific circulating hormone titers and gonadal development during the annual reproductive cycle. Thereafter, with appropriate experimentation, precise practical methods of controlling reproduction and inducing out-of-season spawning can be developed.

The production of hybrid striped bass was first made practicable when researchers discovered that HCG could be used to (pharmacologically) induce final maturation and ovulation in female striped bass (reviewed by Kerby 1986). Numerous life history studies have been reported on the reproductive habits and fecundity of striped bass and white bass (Scott and Crossman 1973; Becker 1983). However, to our knowledge, no definitive investigations characterizing hormonal events and gonadal development during the annual reproductive cycle of either species has been published.

To help rectify this deficiency, Drs. Cheryl A. Goudi and Nick C. Parker of the U.S. Fish and Wildlife Service initiated a study to examine changes in circulating levels of the steroid hormones estradiol-17 Beta ($E_2$) and testosterone (T), gonadal development and pituitary histology during the annual reproductive cycle of broodstock striped bass maintained under ambient photoperiod/temperature conditions (C.A. Goudi and N.C. Parker, personal communications). As of this writing, the data from this study have not yet been analyzed.

In a similar investigation, Dr. Roger C. Cochran, lately of the John Hopkins University, collected blood and gonad samples at regular intervals during the annual reproductive cycle of striped bass in Chesapeake Bay, (R.C. Cochran, personal communication). The findings of his study have not yet been published. However, preliminary examination of the data indicate that $E_2$ levels in the females and T and 11-ketatestosterone levels in the males were elevated during autumn and winter, then peaked prior to spawning in May (R.C. Cochran, personal communication). Gonadal recrudescence was well under way in autumn in this species.

Considerable scientific evidence indicates that reproductive function and the initiation and maintenance of most, if not all, phases of the reproductive cycles of most fishes are controlled by specific "predictive" envi-ronmental cues, the exact nature of which may vary depending on species, and on entraining and modifying factors in the environment (Billard and Breton 1978; Lam 1983; Lam and Munro 1987). Photoperiod and temperature are thought to be the primary entraining factors for most temperate-water species, though other cues, such as the presence of reproductively active conspecifics or spawning substrate may also be involved in synchronizing reproductive events.

The extent to which reproduction in broodfish can be controlled depends in large part on the degree to which management techniques reflect an understanding of the species' reproductive biology (Lam 1982; Donaldson and Hunter 1983; Idler et al. 1987). "First generation" procedures, such as those presently used with striped bass and white bass, typically involve treating fish in near-spawning condition with crude fish pituitary extracts or mammalian hormones, such as HCG, to induce or ensure final gonadal maturation and gamete release. Many fish species do not respond to treatment with mammalian gonadotropins, or require very high doses to elicit a response. In particular, high doses of HCG (e.g., 1,550 to 2,200 IU/kg) are used to induce gonadal maturation and ovulation in female white bass (Smith and Jenkins 1986).

The effectiveness of such first generation techniques, when they do work, is normally limited to the spawning season (Lam 1982; Donaldson and Hunter 1983). Depending on fish species, a frequently encountered problem with using mammalian gonadotropins to accelerate gonadal development is that they may disrupt the normal sequence of developmental events or stimulate some, but not all, stages of maturation. Thus, their use may induce the release of immature ova (i.e., ovulation without maturation) or final maturation without ovulation, which can result in egg resorption (see Lam 1982; Goetz 1983; also T.B. Kayes, personal observation). Repeated treatment with either crude pituitary extracts or mammalian gonadotropins can also potentially cause immune reactions in broodfish, resulting in hormone inactivation, or worse, anaphylactic shock. Both egg resorption and strong immune reactions can have lethal consequences for broodfish.

"Second generation" procedures to control broodstock reproduction typically involve the use of photoperiod/temperature manipulations to shift, compress or expand the annual cycle of gonadal development (Lam 1983; Lam and Munro 1987). Such techniques have been successfully applied to a number of commercially cultured species, including the rainbow trout, common carp and turbot (Bye 1987; Lam and Munro 1987). Subjecting female rainbow trout to constant long-day length starting before or during their first spawning season (i.e., puberty) can result in subsequent spawnings as frequently as every 26 weeks (Bromage et al. 1984; Duston and Bromage 1986). Compressed annual photoperiod/temperature cycles have been used to produce spawnings every 20 to 42 weeks in a variety of temperate-water species that normally reproduce only once a year (Lam 1983; Bye 1987).

Second generation methods of inducing spawning typically involve the use of partially purified fish GTHs, or more often, pharmacological agents that stimulate the production and/or release of endogenous GTH. Much research along this line has focused on the use of mammalian luteinizing-hormone releasing hormone (LHRH), salmon gonadotropin releasing hormone (sGnRH) and their analogues (LHRH-A and sGnRH-A), some of which are far more potent than the natural hormones (Donaldson and Hunter 1983; Peter et al. 1986; Idler et al. 1987). In some fishes dopamine receptor antagonists such as "pimozide" and "domperidone" potentiate the effects of LHRH-A or sGnRH-A on GTH release and subsequent ovulation in goldfish and several species of Chinese carp, some of which do not respond well to LHRH-A or sGnRH-A alone (Peter et al. 1986, 1987). Dopamine appears to act as a GTH release-inhibiting factor, at least in some species (Peter 1983; Peter et al. 1986). Thus, blocking the effects of dopamine may be another way of stimualting GTH release.

The advantage of employing techniques that stimulate endogenous GTH release is that they obviate many of the problems of species specificity and variable responsiveness inherent with the use of mammalian gonadotropins like HCG. Also, as small peptides, the gonadotropin releasing hormones and their analogues are comparatively easy to synthesize; several are available commercially (Donaldson and Hunter 1983). These releasing agents do not appear to trigger negative immune reactions in the fish species tested to date. Accordingly, second generation procedures applied to striped bass and white bass might ensure not only better, more predictable spawning, but also fewer problems with spawning-related mortality.

To our knowledge, the only published accounts of controlled out-of-season spawning of striped bass are those of Smith and Jenkins (1984, 1986) of the South Carolina Department of Wildlife and Marine Resources. These investigators found that striped bass and F1 generation SB x WB hybrid broodfish maintained on a compressed (9-month) annual photoperiod/temperature cycle could, after an HCG injection, be spawned 2 to 3 months earlier than normal. However, the exact significance of their findings was clouded somewhat by: (1) the small numbers of fish examined, (2) differences in age and maturity between individual fish, (3) a fairly high incidence of pre- and post-spawning mortality and spawning failures, and (4) great variability in egg fertilization or hatching.

No published reports on the control of the reproductive cycle of white bass appear to exist. In a field study on the ovarian cycle and fecundity of white bass, Ruelle (1977) observed that oocyte development began in late summer, proceeded rapidly in autumn, then went through a rapid final growth phase immediately prior to spawning in late spring. He also noted that the ovaries of freshly spent females contained large numbers of developing but unripe "recruitment" ova, which were subsequently resorbed. Collectively, this information suggests that properly managed white bass broodfish might be induced to spawn more than once a year.

**Progress Toward Objectives**

Significant progress was made on Objectives 1, 2 and 3 during Year 1, and in general, our research is being conducted according to the time frame described in the original proposal.

To meet all objectives, over 600 white bass were collected via hook-and-line by SIU researchers from either the Illinois River or Lake Shelbyville, IL. Fish were placed in aerated live-wells upon capture, moved to a truck-mounted hauling tank equipped with an oxygen transfusion device, and transported to the SIU aquaculture facilities at Carbondale, IL. However, an epizootic of "Ich" resulted in numerous mortalities. Most other losses have occurred among white bass which failed to habituate to the formulated feed/fish pellets that were offered. Fish were trained to the pelleted feed over an intensive two to three week period. Approximately 300 healthy white bass are currently on feed and stocked in three separate recycle systems.

SIU researchers determined that the sex of white bass not in spawning condition can be discerned with 80 to 90% accuracy by external examination of the genital regions. The genital region among female white bass is usually highly convoluted as opposed to the smoothe surface of most males.

An arrangement was made with Dr. Louis Helfrich of Virginia Tech to obtain captive striped bass brookstock as they may be required at some future date (note: striped bass are not needed during this phase of the research). In return, SIU researchers will provide Virginia Tech with captive white bass brookstock when such a need arises for them. SIU has also developed close ties with Dr. Curry Woods, Crane Aquaculture Facility, University of Maryland. Collaborative research is currently underway to take advantage of the separate white bass and striped bass broodstock.

To begin studies on Objective 2, University of Wisconsin-Madison researchers developed a detailed set of protocols for sampling adult-sized white bass in the field. These protocols, which outlined specific procedures on "least stress" fish capture methods, sampling frequency and intervals, methods for collecting, processing and shipping blood and tissue samples, and the implementation of a uniform sample labelling system, were forwarded to Drs. Christopher C. Kohler and Robert J. Sheehan of Southern Illinois University. Subsequently, all principal investigators agreed upon a first-year sampling schedule.

Also in the first year of the project, UW-Madison researchers developed and validated accurate and precise methods for directly measuring levels of estradiol-17β ($E_2$) and testosterone (T) in white bass serum obtained from SIU. For $E_2$, a commercially available solid-phase antibody radioimmunoassay (RIA) with an iodinated tracer ligand (Coat-a-Count Estradiol, Diagnostic Products Corporation [DPC], Los Angeles, CA) has been adapted for use on white bass, using standards prepared from charcoal-stripped white bass serum. This assay employs duplicate 25 μL serum aliquots, and has minimal cross-reactivity with other naturally occurring steroids. Quality control procedures are routinely being carried out, and quality control data generated for the $E_2$ RIA to date are as follows:

Coefficient of Variation (CV) of intraassay standards = 1.28%
CV of interassay standards = 11.50% (and has been <10%, except for one assay)
Sensitivity = 10 pg/mL
Recovery = 80-92%

To measure T, both a solid-phase antibody RIA (Coat-A-Count, DPC) and a double antibody RIA (DPC) have been adapted for use on white bass in a manner similar to that described for $E_2$ above. Like the RIA for $E_2$, these assays have low cross-reactivity with other steroids (except that the double antibody RIA exhibits 34% cross-reactivity with 5α-dihydrotestosterone). Quality control data generated to date for the T RIAs are as follows:

| | Solid-phase antibody | Double antibody |
|---|---|---|
| CV of intraassay standards | 2.06% | 2.25% |
| CV of interassay standards | 4.80% | 6.30% |
| Sensitivity | 0.15 ng/mL | 0.02 ng/mL |
| Recovery | 80-100% | 80-100% |

University of Wisconsin-Madison researchers have also examined various histological procedures and developed protocols that produce acceptable results in evaluating adult white bass gonads at all (seasonal) stages of development. One common problem with ovarian histotechnique in animals having relatively large eggs (e.g., many fishes, reptiles and birds) is that many routine procedures result in tissue hardening, or incomplete fixation, clearing and/or infiltration of large, maturing oocytes. This problem has been resolved by using Bouin's fluid as a fixative, xylene as a clearing agent and a two-part infiltration and embedding system available from Surgipath Medical Industries,Inc., Grayslake, IL.

To date, samples received from SIU by University of Wisconsin-Madison investigators and currently being analyzed cover two natural spawning periods of wild fish, and one cycle of the three captive populations (see below). Studies focusing on wild white bass will be completed in the initially funded project.

The approximately 300 white bass at SIU are currently spread over three separate recycle systems (see procedures and facilities sections for descriptions) to meet Objective 3. One system is being maintained under a normal photoperiod/temperature regime, one is being compressed to 9 months, and one is being held at a fairly constant temperature (24o + 2oC) and photoperiod (14h L:10h D) regime. Fish were sampled for hormone titers, etc., as specified in the procedures section. The compressed-cycle fish were spawned in late March, 1991; the normal cycle fish in May 1991 and limited spawning was achieved with the constant cycle fish in May, 1991. These data are being prepared for publications.

During the course of the project, Dr. Terrence B. Kayes, a principal investigator and author of much of the research proposed under Objectives 2 and 3, moved from the UW-Madison to the UNL. By mutual agreement, Dr. Jeffrey A. Malison is assuming the lead for the research being done at the UW-Madison. Dr. Kayes, from the UNL, will continue to assist UW-Madison investigators and the group with project coordination, the analysis and interpretation of research findings, and the preparation of reports and any subsequent publications.

## OBJECTIVES

1. Obtain and maintain populations of spawning size white bass and striped bass.

2. Evaluate the effects of selected photoperiod/temperature and hormonal manipulations on gonadal development and spawning in white bass broodstock. (Note: This was objective 3 in the initial project.)

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## PROCEDURES

### Objective 1

Research to characterize the natural reproductive cycle of white bass is being done collaboratively by investigators from Southern Illinois University (SIU) the University of Nebraska-Lincoln (UNL), and the University of Wisconsin-Madison (UW-Madison). Specific methods of capturing and/or maintaining fish, taking and processing blood and tissue samples, and collecting and analyzing data were agreed upon and developed jointly. In general, SIU is responsible for field collection and sampling, maintaining experimental broodfish in the laboratory and ponds, and the fish cultural aspects of the project. The UW-Madison is responsible for the analysis of blood and tissue samples and interpretation of endocrinological data. White bass were collected from the Illinois River and Lake Shelbyville, IL. Striped bass adults are being maintained at Virginia Tech in Blacksburg, VA., through a cooperative agreement with Dr. Louis Helfrich.

Approximately 150 white bass broodfish of each sex are being held in three 2500-gallon recirculated-water culture systems each containing four to six 350-gal circular tanks. These fish have been habituated to a high-protein, high oil commercial salmon diet ground with raw fish (gizzard shad) to produce a moist pellet.

### Objective 2

Studies on the effects of selected photoperiod/temperature and hormonal manipulations on gonadal development, maturation, and spawning in white bass are also being done collaboratively by investigators from SIU, UNL and the UW-Madison. In general, institutional responsibilities and methods of capturing and maintaining fish, taking and analyzing blood and tissue samples, and evaluating data will be as previously de-scribed. Here also, $E_2$, T, 17,20-DHP and 17,20,21-THP are the hormones being measured. All long-term experiments, such as those evaluating the effects of different photoperiod/temperature regimes, are being done at SIU. Some short-term experiments on the effects of various endocrine manipulations on final gonadal maturation and gamete release may be done at the UW-Madison using Lake Mendota fish.

Principal hypotheses for this aspect of the study are: (1) maintenance of white bass broodfish on a compressed annual photoperiod/temperature cycle of 9 months duration will shorten their cycle of gonadal development; (2) the cycle of gonadal development in broodfish maintained on a constant long-day photoperiod and summer water temperature (24 C) will be 11-13 months long; (3) specific LHRH-As, used in concert with pimozide or domperidone, will be more efficacious than HCG at inducing final gonadal maturation and gamete release, particularly in brood fish subjected to the indicated compressed photoperiod/temperature cycle and constant photoperiod/temperature regime. Hypothesis 2 is based on the proposition that adult white bass have an endogenous circannual rhythm that functions (normally in concert with zeitgebers) in regulating reproductive cycling. As an alternative hypothesis, we suggest that the gonads of fish maintained on the constant long-days/summer temperature regime will be arrested in an early autumnal (early recrudescent) stage of development.

To assess the effects of different photoperiod/temperature regimes, at least 25 fish of each sex (all previously acclimatized to laboratory conditions and with prior spawning experience) will be assigned at the summer solstice (about June 22) to each of the following: (1) a simulated natural 12-month photoperiod/temperature cycle, (2) a compressed 9-month cycle, and (3) a constant photoperiod/temperature regime of 14-hours light/10 hours dark and 24 C. These regimes will be maintained for 2 years, or two complete cycles. These studies, coupled with our current successes, will provide ample demonstration and fine-tuning of procedures.

For each of the four regimes, blood and tissue samples from four fish of each sex will be collected at the midpoint or winter solstice of each year or cycle. Thus, sampling that requires the sacrifice of fish will be conducted twice for each treatment regime. At other times, particularly during the final quarter of each year or cycle, maturational status will be assessed by taking blood and analyzing hormone titers, by attempting to express semen from males, and by the catherization of females and examination of oocytes, as described by Smith and Jenkins (1986) and Bonn et al. (1976). In making these assessments, minimum stress procedures, including anesthesia, will be used to ensure good health and normal reproductive function.

Experiments on the effects of hormonal manipulations will include a preliminary comparision of the efficacy of selected high-potency LHRH-As, pimozide and domperidone with that of HCG. Different dosages plus appropriate combinations of the LHRH-As, pimozide and domperidone will be tested. Initial experiments will utilize near-mature but unripe wild fish captured immediately before or during the natural spawning season. Primary end-points examined will include: the response patterns of endogenous GTH levels in circulation, final maturation and release of gametes, numbers of ova and volume of semen released, fertilization success, embryo development and hatching success.

Collectively, these experiments will yield specific methodologies to manipulate and optimize the reproductive performance of white bass broodfish, and provide a firm foundation of further improvements in this technology as future needs demand. Many of the methods developed will no doubt also prove useful in the management of striped bass broodstock, given the close genetic and taxonomic relatedness of these two species.

**Dissemination of results**

Data collected by SIU, UNL and UW-Madison investigators will be collated on an ongoing basis, and the findings jointly (as appropriate) published in a timely manner in peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group. The industry will be kept apprised of progress. Based on our early successes, we are confident in bringing out-of-season spawning technology for white bass to a level that can be put into commercial practice at the termination of this project.

## FACILITIES

**Southern Illinois University-Carbondale**

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 SIU Campus has several 14-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 SIU 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 2500-gallon systems, each containing six 350-gal circular tanks, are in operation at the SIU 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. An addition system is being maintained in our wet lab for the constant photoperiod temperature regime. Outdoor ponds are available to hold excess fish.

**University of Wisconsin-Madison**

Blood and tissue samples collected in the field or at SIU, after appropriate preliminary processing, will be shipped or transported to the UW-Madison. All hormone and tissue analyses will be done by (or through) the UW Aquaculture Program, which has its main research facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. These facilities include--in addition to ponds, raceway tanks and a "wet" laboratory--an analytical laboratory that is well equipped for histological, cytological and endocrinological research.

UW Aquaculture Program facilities available for short-term holding of white bass include numerous 0.76- to 1.9-m diameter circular tanks equipped for flow-through of temperature-regulated (10 to 30 + 0.5 C) well or carbon--filtered city water. The Aquaculture Program has additional analytical and wet laboratory facilities on the main UW-Madison campus. The Program also has a live-haul truck, a work boat, plus 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.

Collectively these facilities and the support personnel operating them coupled with the personnel and equipment requested are more than adequate to meet the needs of the research proposed.

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**ATTACHMENT C**

## PROJECT LEADERS

| State | Name/Institution | Area of Specialization |
|---|---|---|
| **Illinois** | Christopher C. Kohler<br>Southern Illinois University | Aquaculture/Ecology |
| | Robert J. Sheehan<br>Southern Illinois University | Physiology/Aquaculture |
| **Wisconsin** | Jeffrey A. Malison<br>Univ. of Wisconsin-Madison | Physiology/Endocrinology |

**ATTACHMENT C**

INDIVIDUAL BUDGETS FOR PARTICIPATING INSTITUTIONS

**Illinois**

Southern Illinois University-Carbondale
Dr. Christopher C. Kohler
Dr. Robert J. Sheehan

**Wisconsin**

University of Wisconsin-Madison
Dr. Jeffrey A. Malison

**ATTACHMENT C**

**PROPOSED HYBRID STRIPED BASS BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY**

**(C. Kohler and R. Sheehan)**

**Objective 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 | 0.10 | 2 | 0.10 | $0 | $0 |
| b. Senior Associates | | | | | | |
| 2. No. of Other Personnel (Non-Faculty) & FTEs | | | | | | |
| a. Research Assoc./Postdoc | | | | | | |
| b. Other Professionals | | | | | | |
| c. Graduate Students | 2 | 1.00 | 2 | 1.00 | 19,200 | 20,400 |
| d. Prebaccalaureate Students | | | | | | |
| e. Secretarial-Clerical | | | 1 | 0.10 | | $ 1,500 |
| f. Technical, Shop, and Other | | | | | | |
| **Total Salaries and Wages** | | | | | $19,200 | $21,900 |
| B. Fringe Benefits (11.11% of 2e. + $267/mo Health Insurance) | | | | | $0 | $487 |
| C. **Total Salaries, Wages and Fringe Benefits** | | | | | $19,200 | $22,387 |
| D. Nonexpendable Equipment | | | | | $0 | $0 |
| E. Materials and Supplies | | | | | $4,300 | $3,917 |
| F. Travel - Domestic (*Including Canada*) | | | | | $4,000 | $4,000 |
| G. Other Direct Costs | | | | | $1,500 | $1,500 |
| **TOTAL PROJECT COSTS PER YEAR** (*C through G*) | | | | | $29,000 | $31,804 |
| **TOTAL PROJECT COSTS** | | | | | $60,804 | |

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

**ATTACHMENT C**

**PROPOSED HYBRID STRIPED BASS BUDGET FOR UNIVERSITY OF WISCONSIN-MADISON**

**(J. Malison)**

**Objective 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) | 1 | 0.07 | 1 | 0.07 | $0 | $0 |
| b. Senior Associates | 1 | 0.07 | 1 | 0.07 | $0 | $0 |
| 2. No. of Other Personnel (Non-Faculty) & FTEs | | | | | | |
| a. Research Assoc./Postdoc | | | | | | |
| b. Other Professionals | 1 | 0.50 | 1 | 0.50 | $12,075 | $12,115 |
| c. Graduate Students | | | | | | |
| d. Prebaccalaureate Students | | | | | | |
| e. Secretarial-Clerical | | | | | | |
| f. Technical, Shop, and Other | | | | | | |
| **Total Salaries and Wages** | | | | | $12,075 | $12,115 |
| B. Fringe Benefits (24% of 2b) | | | | | $2,898 | $2,908 |
| C. **Total Salaries, Wages and Fringe Benefits** | | | | | $14,973 | $15,023 |
| D. Nonexpendable Equipment | | | | | $0 | $0 |
| E. Materials and Supplies | | | | | $1,975 | $1,975 |
| F. Travel - Domestic (*Including Canada*) | | | | | $600 | $1,200 |
| G. Other Direct Costs | | | | | $0 | $0 |
| **TOTAL PROJECT COSTS PER YEAR** (*C through G*) | | | | | $17,548 | $18,198 |
| **TOTAL PROJECT COSTS** | | | | | $35,746 | |

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

**ATTACHMENT C**

**HYBRID STRIPED BASS**

**Budget Summary for Each Participating Institution 46.5K for the First Year**

| | SIU | UW-M | TOTAL |
|---|---|---|---|
| Salaries and Wages | $19,200 | $12,075 | $31,275 |
| Fringe Benefits | $0 | $2,898 | $2,898 |
| **Total Salaries, Wages and Benefits** | $19,200 | $14,973 | $34,173 |
| Nonexpendable Equipment | $0 | $0 | $0 |
| Materials and Supplies | $4,300 | $1,975 | $6,275 |
| Travel | $4,000 | $600 | $4,600 |
| Other Direct Costs | $1,500 | $0 | $1,500 |
| **TOTAL PROJECT COSTS** | $29,000 | $17,548 | $46,548 |

**HYBRID STRIPED BASS**

**Budget Summary for Each Participating Institution 50.3K for the Second Year**

| | SIU | UW-M | TOTAL |
|---|---|---|---|
| Salaries and Wages | $21,900 | $12,115 | $34,015 |
| Fringe Benefits | $487 | $2,908 | $3,395 |
| **Total Salaries, Wages and Benefits** | $22,387 | $15,023 | $37,410 |
| Nonexpendable Equipment | $0 | $0 | $0 |
| Materials and Supplies | $3,917 | $1,975 | $5,892 |
| Travel | $4,000 | $1,200 | $5,200 |
| Other Direct Costs | $1,500 | $0 | $1,500 |
| **TOTAL PROJECT COSTS** | $31,804 | $18,198 | $50,002 |

**ATTACHMENT C**

## RESOURCE COMMITMENT FROM INSTITUTIONS[1]

(Salaries, Supplies, Expenses and Equipment)

| Institution/Item | | Year 1 | Year 2 |
|---|---|---|---|
| **Southern Illinois University** | | | |
| Salaries and Benefits: SY @ 0.10 FTE | | $5,000 | $6,000 |
| Supplies, Expenses and Equipment | | $10,000 | $9,000 |
| | TOTAL PER YEAR | 15,000 | 15,0000 |
| **University of Wisconsin-Madison** | | | |
| Salaries and Benefits: SY @ 0.14 FTE | | $3,850 | $4,410 |
| TY @ 0.01 FTE | | $1,190 | $1,250 |
| Supplies, Expenses and Equipment | | $6,098 | $7,519 |
| | TOTAL PER YEAR | 11,138 | 13,179 |
| | **GRAND TOTAL** | 26,138 | 28,1790 |

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

**ATTACHMENT C**

## 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.

**ATTACHMENT C**

## LIST OF PRINCIPAL INVESTIGATORS

**Dr. Christopher Kohler,** Southern Illinois University

**Dr. Jeffrey A. Malison,** University of Wisconsin-Madison

**Dr. Robert Sheehan,** Southern Illinois University

## VITA

Christopher C. Kohler
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### EDUCATION

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

### POSITIONS

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

### SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

### SELECTED PUBLICATIONS

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

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

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

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

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

**ATTACHMENT C**

**VITA**

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**EDUCATION**

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

**POSITIONS**

Associate Researcher, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1987-present)
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**

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.

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Malison, J.A. 1985. Growth promotion and the influence of sex-steroids on sexually related dimorphic growth and differentiation in yellow perch (*Perca flavescens*). Doctoral dissertation. University of Wisconsin-Madison.

Malison, J.A., C.D. Best, T.B. Kayes, C.H. Amundson, and B.C. Wentworth. 1985. Hormonal growth promotion and evidence for a size-related difference in response to estradiol-17'in yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 42:1627-1633.

Malison, J.A., C.D. Best, and T.B. Kayes. 1983. Hormonal control of growth and size dimorphism in yellow perch (*Perca flavescens*). American Zoologist 22:955.

**ATTACHMENT C**

# VITA

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## EDUCATION

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

## POSITIONS

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

## SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

## SELECTED PUBLICATIONS

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

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

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

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

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