CULTURE TECHNOLOGY OF BLUEGILL AND CRAPPIE FOR FOOD FISH

Chairperson: Robert J. Sheehan, Southern Illinois University

Extension Liaison: Donald L. Garling, Michigan State University

Funding Request: $149,867

Duration: 2 Years (September 1, 1992 to August 31, 1994)

Objectives:

1. Production and evaluation of polyploid sunfish.
2. Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish.
3. Development of low cost, high performance sunfish diets.

Proposed Budgets:

Institution	Principal Investigator(s)	Objective	Year 1	Year 2	Total
Michigan State University	Donald L. Garling	1	$16,375	$17,612	$33,987
Illinois Natural History Survey	David H. Wahl Michael L. Hooe	2	$5,500	$5,500	$11,000
Pittsburg State University	James R. Triplett	2	$13,657	$15,343	$29,000
Southern Illinois University-Carbondale	Robert J. Sheehan Bruce L. Tetzlaff	2	$35,380	$20,000	$55,380
Purdue University	Paul B. Brown	3	$20,500	$0	$20,500
		TOTALS	**$91,412**	**$58,455**	**$149,867**

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JUSTIFICATION

Sunfish of the genera *Lepomis* and *Pomoxis* already have significant economic impact in the North Central Region. For example, 50.9% of the sportfish harvest in Illinois consists of these two genera (Baur 1988). The potential for marketing sunfish as food fish is considerable because of this popularity. Representatives of the Chicago Fish House, one of the major seafood suppliers in the Midwest, have indicated during North Central Regional Aquaculture Center (NCRAC) meetings a strong interest in obtaining an aquaculturally produced supply of sunfish because of their marketability. A June 1992 price quote from the Chicago Fish House (Bob Rubin, personal communication) of $4.41-5.51/kg ($2.00-2.50/lb) to producers for crappie indicates that sunfish are about double the value of the two major aquaculture species: rainbow trout and channel catfish. The significance of the market potential for sunfish has not been lost on others; tilapia are currently being marketed in parts of the Midwest through major retail outlets as "bluegill."

A number of factors make sunfish strong candidates for commercial food fish production in the Midwest. Sunfish broodstocks are easy for culturists to acquire in high numbers in contrast to species such as the walleye and striped bass. Even inexperienced culturists can readily spawn sunfish in ponds. *Lepomis* and *Pomoxis* sunfish are not inclined to be cannibalistic, unlike striped bass and walleye. A number of sunfish species and hybrids are known to readily accept pelleted feeds. Sunfish exhibit fast initial growth (see **PROGRESS TO DATE**) and grow at lower temperatures than channel catfish; an important characteristic for production in our region.

Despite these important attributes, several factors have slowed progress towards widespread commercialization of sunfish food fish production. The development of a sunfish food fish production industry has been impeded, because sexual maturation in sunfish species occurs prior to the attainment of market size. This leads to slow and inefficient growth during grow out and uncontrolled reproduction in culture ponds. Also, the development of sunfish food fish culture has historically been impeded by legal restrictions imposed on the sale of sportfish. This is one reason why little effort had been directed at developing sunfish grow-out technologies in the past.

Today, legal constraints on the sale of aquaculturally produced sunfish are diminishing in the Midwest. For example, passage of the Illinois Aquaculture Act has enabled aquaculturists in that state for the first time to legally produce and market sunfish. This has resulted in 35 licensed sunfish producers as of 1990 in Illinois alone. Thus, it is an ideal time to identify culture technologies and sunfish stocks uniquely suitable for our region. A report on the "Development Strategy for Kansas Aquaculture" by the Kansas Aquaculture Task Force indicated that crappie, bluegill, and hybrid bluegill production show promise for recreational fishing and food fish markets. Similarly, Hahn (1989) concluded that bluegill production in Kansas will likely become commercially viable within the next few years. It is clear that the development and optimizing sunfish production technologies will benefit sunfish fingerling producers already existing in the region by creating an additional market for their products, that being the sale of fingerlings to sunfish growout operations. Entrepreneurs interested in producing sunfish food fish will also benefit from the research proposed herein, because the proposed studies will provide needed information for developing economic forecasts.

Several species of sunfish, particularly crappie *Pomoxis annularis* and *P. nigromaculatus* and bluegill *Lepomis macrochirus* (and its hybrids), are already being raised as food fish on a limited basis in the North Central Region. The combination of this fledgling sunfish food fish industry and recent research successes (see **PROGRESS TO DATE**), suggests that sunfish food fish production is approaching widespread commercialization, yet production technologies have not been optimized, and little specific information is available to culturists regarding successful techniques. Even basic information, such as how many sunfish to stock in a given culture unit, is lacking, making it difficult for aquacultural extension specialists in our region to guide the development of this industry.

The development of suitable prepared diet formulations and the availability of inexpensive commercial feeds have been perhaps the most significant factors responsible for the success of the commercial rainbow trout and channel catfish industries, yet the qualitative and quantitative requirements for basic feed components, such as protein, are unknown for sunfishes. History tells us that the development of low cost, high growth diets for sunfish is essential to speed commercialization of this industry.

History also indicates that the choice of suitable species and stocks for production has been paramount to commercial aquaculture success in given geographic locations. The rainbow trout has been ideal for the cold waters of Idaho, whereas a warmwater fish, the channel catfish, has dominated pond production in the Lower Mississippi River Valley. The selection of these species was not by chance. For example, considerable research effort was expended on evaluations of the aquacultural and marketing characteristics of several ictalurid catfish species, including channel catfish, blue catfish, white catfish, and flathead catfish, as well as their hybrids. Genetic manipulation and refinement of stocks for production continue today; e.g., the largest rainbow trout producer in Idaho used gynogenesis and sex reversal to develop the broodstock necessary to convert all its facilities to all-female production (Jim Parsons, Clear Springs Trout Farm, Buhl, Idaho, personal communication). Similar stock identification approaches are needed for sunfish in our region.

Thus, it is essential that a number of research needs be addressed to expedite the development of a sunfish food fish production industry in our region. Studies are needed to determine: 1) how to reduce the detrimental effects of sexual maturation such as uncontrolled reproduction, diminished growth and reduced feed conversion; 2) relationships between temperature and growth and optimal temperatures for growth for various sunfish; 3) best performing sunfish species, hybrids, or genetically manipulated sunfish; 4) formulations for low cost, high performance sunfish feeds, and 5) stocking densities and yields under commercial aquaculture conditions. This proposal addresses all of these critical problems; its funding will be highly responsive to the needs of existing and future culturists in our region.

This is a proposal for a cooperative project that combines the talents and facilities of researchers from five institutions (Illinois Natural History Survey, Michigan State University, Pittsburg State University, Purdue University, and Southern Illinois University) in four states (Illinois, Kansas, Michigan, and Indiana). It is divided into three somewhat interrelated objectives, namely: 1) production and evaluation of polyploid sunfish, 2) determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish, and 3) development of low cost, high performance sunfish diets.

Production and evaluation of polyploid sunfish

Induced triploidy in sunfish could potentially overcome many impediments to the development of the sunfish foodish production industry in the Midwest. Induced triploidy in other species has resulted in individuals with reduced gonadal development, higher vitality, and delayed sexual maturation. If triploid sunfish show similar characteristics, then the problems associated with the onset of sexual maturation before attaining market size, such as the loss of dietary energy to gonadal development and spawning behaviors, will be diminished. Efficient growth through sexual maturation is especially important for sunfish, because market value will be greatly diminished for fish less than 0.51 kg (0.33 lb) (Bob Rubin, Chicago Fish House, personal communication). Induced triploidy could promote good growth and feed conversion through market size. Induced triploidy could also overcome problems regarding uncontrolled reproduction, since triploid individuals produce aneuploid gametes.

Michigan State University (MSU) and Southern Illinois University-Carbondale (SIUC) have worked jointly to develop methods for inducing triploidy in and evaluating polyploid sunfish. Donald L. Garling and colleagues at MSU were the first to produce, via induced polar body retention through cold shocks, triploid and tetraploid bluegills.

It currently is not possible to rear any of the *Lepomis* species from hatch to juvenile size under laboratory conditions. The only method available for producing *Lepomis* juveniles is by stocking in ponds, where natural forage organisms can satisfy nutritional requirements of larvae at the onset of exogenous feeding. For research on methods for inducing polar-body retention, the inability to efficiently rear *Lepomis* to the juvenile stage means that less expensive methods routinely used for confirming triploidy in larger specimens, such as via the Coulter Counter, are not suitable because of the high number of ponds that would be required to isolate each replicate from each experimental condition tested to induce polar body retention. However, MSU has refined methods which facilitate ploidy determinations in larval sunfish; this will greatly facilitate studies directed at optimizing techniques for inducing polar body retention.

Robert J. Sheehan and colleagues at SIUC were the first to produce triploid hybrid sunfish. They evaluated several shock types, magnitudes, and durations and found that hydrostatic pressure shocks were superior to temperature shocks, because high survival (90+ %), 100% triploidy, and no deformed individuals were produced using the former method. MSU subsequently produced triploid bluegill using hydrostatic pressure shocks.

The achievements to date clearly demonstrate that Garling at MSU and Sheehan at SIUC are the leaders in *Lepomis* ploidy manipulation. Further, the collaborative effort between these two institutions has been extremely successful. MSU has determined ploidy through flow cytometry in samples of hybrid sunfish from SIUC's experiments. MSU has agreed to cover SIUC's costs for ploidy determinations related to work proposed herein. SIUC has provided MSU specifications for constructing hydrostatic pressure chambers, and information regarding pressure-shock techniques. SIUC will evaluate the growth performance of triploid bluegill through grow-out (see Objective 2) that will be produced by MSU as part of Objective 1. MSU proposes herein to continue its evaluations of polyploids, polyploid induction, and the use of tetraploid broodstock to guarantee production of 100% triploid stocks. This collaboration will continue through funding of MSU in Objective 1 and through funding for Sheehan's work at SIUC in Objective 2.

Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish

Overpopulation reduces growth rate through competition for food. In addition, energy which could go into the production of marketable flesh is utilized in spawning activities and the production of gonadal products. Therefore, control of reproduction and population density is a major requirement for the efficient production of sunfish food fish. Sunfish, like the yellow perch, *Perca flavescens*, present a unique problem to aquaculturists, since they attain sexual maturity before attaining marketable size. Another problem is that spawning will occur in grow-out ponds.

To limit reproduction in bluegill sunfish, fish culturists have developed a hybrid of the bluegill male and green sunfish (*L. cyanellus*) female. The first filial (F_1) generation of this cross, although sexually fertile, is predominantly male. In some populations, males comprise greater than 95% of the population. Recently, a hybrid between the white and black crappies has also been developed.

However, production of F_2 progeny can occur despite the skewed sex ratios. In addition, a considerable amount of energy which could go into the production of usable flesh is expended in pre-spawning behaviors, such as nest building and aggressive territoriality. These behaviors could affect the density at which sunfish can be reared.

In this objective, two approaches to control reproduction and reproductive behaviors in sunfish will be addressed. Also, optimum stocking densities and growth performance under cage culture will be examined using the two approaches.

Sunfish spawn in shallow depressions in the substratum. One way to prevent sunfish reproduction in ponds is to utilize cage culture to isolate them from the substratum. Bruce L. Tetzlaff of SIUC has received funding through the NCRAC to determine optimum stocking densities and evaluate growth and yield of hybrid *Lepomis* sunfish under cage culture. This work will be continued with hybrid crappie (*Pomoxis*) by James R. Triplett, Pittsburg State University (PSU), through the funding of this proposal.

Unlike the bluegill, crappie are more specific in their requirements for spawning. They usually spawn only once a year and seldom reach sexual maturity during their first year. This difference permits the exploration of a slightly different production scheme. Crappie can be spawned in open ponds and numbers of progeny controlled by the number of broodstock introduced during the first year. The offspring can then be removed at the beginning of the next growing season and stocked into cages where spawning is prevented.

The application of cage culture for the second growing season has other advantages besides curtailing reproduction and associated activities. In cages, fish densities can be maintained at levels that will break down territoriality, and eliminate wasteful aggressive behaviors without posing a threat to water quality in the system. It also postpones the effort to train fish to commercial feeds until fish are larger and more developed. Other advantages include easy harvest and closer monitoring.

This production scheme is potentially close to implementation, at least as a first effort, with answers to a few basic questions. Once caged, will fish accept and grow on a commercial diet? What stocking density produces optimal trainability, feed conversion, growth and survival? Are white, black or hybrid crappie best suited for this approach?

Induced triploidy is another potential approach for overcoming many of the negative aspects of early sexual maturation in sunfish. There has already been a research focus, funded through the NCRAC on the development of technologies to produce and evaluate triploid sunfish (see above).

Reduced gonad production has been reported in a number of triploid fishes (see Ihssen et al. 1990). Since gonad production is inhibited, it follows that gonadal products, such as steroid hormones, may also be reduced in triploids. This could be significant, since hybrid *Lepomis* sunfish are often primarily males, and males are likely to be more aggressive than females. The gonadal steroid, testosterone, is known to increase aggressiveness throughout the vertebrates (see Hadley 1984), and its production and elaboration could be reduced in triploid sunfish. Reduced gonadal steroid production has been reported for some triploids (see Thorgaard 1992). Triploids have been reported to be less aggressive in some species (Cassani and Caton 1986; Lincoln and Bye 1987), so optimal stocking densities for triploids could differ from diploids. Bruce Tetzlaff of SIUC will progress from his cage-culture studies of diploid hybrid *Lepomis* sunfish to studies with triploid hybrid *Lepomis* sunfish through funding of this objective.

Full funding of this objective will permit Bob Sheehan, SIUC, to continue his work on triploid induction in hybrid sunfish (*Lepomis* and *Pomoxis*) and allow him to provide the triploid hybrid *Lepomis* sunfish necessary for Bruce Tetzlaff's cage-culture studies. Sheehan will have completed his grow-out evaluations of the effects of water temperature on the growth performance of bluegill, green sunfish, hybrid bluegill x green sunfish, and triploid bluegill x green sunfish hybrids prior to the initiation of the work proposed herein. Funding of this objective will also permit him to evaluate triploid bluegill, white crappie, black crappie, hybrid crappie, and triploid crappie in a similar manner. He will then compare the growth performance of the three best performers of these *Lepomis* and *Pomoxis* species, hybrids, or triploids across a range of temperatures in side-by-side comparisons. This overall approach, which includes tests of parental species, hybrids, and triploids, will permit a rational choice, based on empirical findings, of sunfish stocks to be developed for food fish production at water temperatures found in our region.

We are fortunate to have the experts on crappie hybridization, Michael L. Hooe and David H. Wahl of the Illinois Natural History Survey, in our region (Buck and Hooe 1986; Hooe and Buck In Press). Full funding of this objective will permit them to provide the white crappie, black crappie, and hybrid crappie for the studies by Triplett and Sheehan.

Development of low cost, high performance sunfish diets

The purpose of this objective is to identify diets which will promote maximal weight gain and health in sunfish. No commercial feeds specifically formulated for sunfish are available, and although commercial hybrid sunfish production is ongoing in our region, there are no published reports of the nutritional requirements of hybrid sunfish.

In terms of both the qualitative and quantitative aspects of prepared fish diets protein is perhaps the most important component. Proteins serve as the structural framework for protoplasm in cells, form many cell components, and control the rates of metabolic reactions. Further, proteins are generally considered the most expensive components in animal diets. This is indicated by the extensive published research concerning the manipulation of carbohydrates and fats in fish diet formulations in efforts to reduce protein requirements (see Cowey and Sargent 1979).

Paul B. Brown, Purdue University, has had great success applying the Ideal Protein Concept (Agricultural Research Council 1981) to the formulation of feeds for striped bass. This approach greatly diminishes the time normally necessary for developing species-specific feeds. Funding of this objective will permit Brown to use the Ideal Protein Concept to respond to an important existing need in our region; namely, the development of low cost, high performance sunfish feeds.

RELATED CURRENT AND PREVIOUS WORK

Overview

The life history of the bluegill provides insight into the problems of early sexual maturation and unwanted reproduction in culture units. The bluegill is a sedentary, littoral fish whose primary foods are insects and small fish (Pflieger 1975). The male excavates a shallow depression in the substratum into which he entices a female. After spawning, the male guards the nest until the eggs hatch and the larvae are capable of swimming. Thus, the spawning requirements for bluegill can be satisfied in any pond, and the high level of parental care results in high survival of young bluegill, with overpopulation and the loss of control over densities often the end results.

Overpopulation reduces the growth of the initial crop through competition for food. In addition, energy which could go into the production of marketable flesh is utilized in spawning activities and the production of gonadal products. Therefore, control of reproduction and population density is a major requirement for the development of sunfish as an aquacultural product. Sunfish, like the yellow perch, *Perca flavescens*, present a unique problem to aquaculturists, since they attain sexual maturity before attaining marketable size, and spawning will occur in grow-out ponds.

To limit reproduction in bluegill sunfish, fish culturists have developed a hybrid of the bluegill male and green sunfish (*L. cyanellus*) female (Childers 1967; Lewis and Heidinger 1971a). The first filial generation (F_1) of this cross, although sexually fertile, has a sex ratio skewed to males. Males comprise more than 95% of some populations (Childers 1967; Lewis and Heidinger 1978). Recently, a hybrid between the white and black crappies has also been developed (Buck and Hooe 1986).

Hybrid sunfish are currently an important aquacultural product in the North Central Region. Fingerlings are sold to pond owners in lieu of bluegill or redear sunfish, *L. microlophus*, in an attempt to reduce population fecundity (Lewis and Heidinger 1978; Heidinger 1983). The 1988 Aquaculture Buyers Guide

(Aquaculture Magazine, Asheville, NC) lists 19 suppliers of hybrid sunfish in this region, encompassing the states of Illinois, Indiana, Kansas, Michigan, Missouri, Ohio, and Michigan. Food-sized sunfish are also available.

Much has yet to be determined about the biology of hybrid sunfish as it relates to aquaculture. The growth rate and food conversion efficiencies of hybrids as related to parentals at various temperature regimes must be determined before they can be recommended to aquaculturists throughout the region. Also, production rates (kg/ha) must be determined for various stocking densities to estimate the production potential for a given facility.

Genetic improvement of production characteristics of crappie, bluegill, and hybrid sunfish would be a major contribution toward the advancement of aquaculture in the North Central Region. Genetic improvement of current commercial stocks is largely limited to the production of interspecific hybrids. Performance of the various hybrids under intensive fish culture conditions has been examined only to a limited extent. The feasibility of using the technology of chromosome manipulation should be examined. The use of sterile, triploid sunfish would in theory eliminate the problem of overpopulation and stunting.

The hybrid sunfish (bluegill male X green sunfish female) has been shown to be an attractive alternative to bluegill for stocking farm ponds and small impoundments (Brunson and Robinette 1986; Childers 1967; Heidinger 1975; Lewis and Heidinger 1971a, 1978). Hybrid sunfish possess several characteristics which make them amenable to fish culture. Ellison and Heidinger (1978) demonstrated that hybrid sunfish grow at a more rapid rate than the parental species. This hybrid will also readily accept commercially prepared feeds (Lewis and Heidinger 1971a,b). In addition, there is evidence that hybrid sunfish continue to grow at lower temperatures than the bluegill or channel catfish (Brunson and Robinette 1982, 1983; Heidinger 1975). Heidinger (1975) suggested that this characteristic makes hybrid sunfish an attractive candidate for food fish production in areas with a short growing season. However, the studies of Heidinger (1975) were limited to Illinois ponds while those of Brunson and Robinette (1982, 1983) were conducted in Mississippi. Studies examining growth over the full range of temperatures found in the North Central Region need to be conducted. In addition, optimum stocking rates in cages and ponds have yet to be determined. None of this type of data is available for hybrid crappie or polyploid sunfish.

Although use of the hybrid sunfish has substantially reduced the problem of overpopulation caused by reproduction, Ellison and Heidinger (1978) found that F_2 production can occur sufficiently to cause stunting. Buck and Hooe (1986) have also found that hybrid crappie have a normal sex ratio and that fertile gametes can be produced. Therefore, control of reproduction is still a factor limiting the development of sunfish aquaculture.

Production and evaluation of polyploid sunfish

In addition to controlling reproduction, production of sterile, triploid sunfish may result in accelerated growth and increased size because of reduced gonad function and size. Ihssen et al. (1990) recently reviewed research results on ploidy manipulations of fishes. Triploidy often results in the early development of the gametes and retarded gonadal development in most species. Gonads of triploid Atlantic salmon, *Salmo salar*, are reduced in size in males by 48% and in females by 92.3% (Benfey and Sutterlin 1984a). Triploid rainbow trout, *Oncorhynchus mykiss*, also show reduced gonad size and function, but not to the same degree as other species (Thorgaard and Gall 1979). Male triploid rainbow trout testes developed normally except for reduced size, and produced nominal amounts of milt (Lincoln and Scott 1984). Circulating androgen levels did not differ significantly from diploids and the fish exhibited normal spawning activities (Lincoln and Scott 1984). Male triploid rainbow trout produce aneuploid sperm (DNA content intermediate between haploid and diploid values) which failed to produce viable progeny when used to fertilize haploid eggs (Benfey et al. 1986). Triploid male grass carp, *Ctenopharyngodon idella*, can be induced to produce aneuploid sperm by hormone injection (Allen et al. 1986). Coho salmon, *O. kisutch*, males have greatly reduced gonads and fail to undergo the normal development of secondary sexual characteristics and postspawning mortality (Johnson et al. 1984). Female fishes have markedly reduced gonad size with low levels of gonadal steroids as compared to diploids in all species studied. If male bluegill triploids are similar to rainbow trout, they may compete with diploid males for spawning partners, but produce no viable offspring.

In order to produce triploidy in fishes the second meiotic division of the egg must be inhibited (Chourrout and Itskovich 1983; Don and Avtalion 1986, 1988). The resulting egg has two identical sets of maternal chromosomes. Subsequent fertilization of this egg creates an individual with a triploid (3N) set of chromosomes. In fishes, the meiotic division is completed shortly after fertilization (Ginzburg 1972). Since fertilization is external in the sunfish, application of a meiotic division inhibitory treatment is possible. The second meiotic division may be prevented by any process which causes depolymerization of microtubules that are essential to the formation of the spindle apparatus (Purdom 1983; Chourrout 1986). The spindle apparatus is responsible for the separation of the chromosomes during the second meiotic division. Various treatments have been used to inhibit the second meiotic division in fish eggs. Treatments have included cold shocks, chemical treatments, hydrostatic pressure, and heat shocks (Refstie et al. 1977; Allen and Stanley 1979; Benfey and Sutterlin 1984a; Smith and Lemoine 1979; Lemoine and Smith 1980; Lincoln et al. 1974).

Conflicting reports of triploid growth potential are found throughout the literature. Most growth studies of triploid fish are not well replicated or controlled. Rainbow trout triploids were shown to grow slower than diploids (Solar et al. 1984). A 10-week growth study conducted at the MSU Aquaculture Laboratory failed to demonstrate differences in growth and feed utilization efficiency between diploid and triploid chinook salmon. However, they predict that expected increases in longevity of the triploids should result in a larger maximum size regardless of prematuration growth responses between diploids and triploids.

There may also be differences in survival (Thorgaard et al. 1981) and various hematological parameters (Graham et al. 1985) between triploids and diploids. Thorgaard et al. (1981) speculated that differences in the percentage of triploids between embryos and 18-month-old rainbow trout may indicate a higher mortality in the triploids. Graham et al. (1985) have shown that triploid Atlantic salmon have a lower hemaglobin-oxygen loading ratio than diploids and speculate that under extreme exertion may have a reduced ability to obtain oxygen. However, Benfey and Sutterlin (1984b) observed no difference between triploid and diploid Atlantic salmon in oxygen uptake under varying environmental oxygen tensions.

There are fewer studies of nonsalmonid triploids. In warm water species, triploids grow faster and reach heavier weights than diploids. Channel catfish, *Ictalurus punctatus*, triploids were significantly heavier than diploids at eight months and older (Wolters et al. 1982). Triploid male hybrids of grass carp X bighead carp (*Hypothalmichthys nobilis*) grew faster than diploid hybrids (Cassani et al. 1984). Purebred triploid grass carp have been produced by thermal shock of eggs and have been available commercially since about 1983 (Cassani and Caton 1985). Finally, triploid *Tilapia aurea* were larger than diploids at 14 weeks of age (Valenti 1975). Well designed experiments involving comparisons of growth rates, body composition, and nutritional requirements among triploid and diploid sunfish and their hybrids are required to determine if growth differences occur prior to sexual maturation.

Few papers have been published on the production of tetraploid fishes. Tetraploidy has been induced in rainbow trout (Chourrout 1982, 1984) and two species of tilapia, *Oreochromis niloticus* and *O. mossambicus*, and their hybrid (Myers 1986). Tetraploids mated with diploids produced viable offspring but at lower survival levels than normal diploids (Myers 1985). Under ideal conditions for production, tetraploid bluegill might be used to produce triploids through tetraploid X diploid crosses, eliminating the need to treat eggs separately (Purdom 1983).

Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish

Until recently, the culture of sunfish has been limited to the production of fingerlings for recreational fishing and pond stocking. Therefore, several performance characteristics which are important for the production of food fish need to be examined. Comparisons of growth dynamics and food conversion over a broad temperature range and determinations of optimum stocking densities for growth and survival are needed.

Knowledge of optimum rearing temperatures as well as the dynamics of growth and food conversion efficiencies at all possible temperatures is essential for determining the suitability of a fish species for different aquaculture practices. In pond situations where temperatures cannot be controlled the relationship between temperature and growth for a species will be a major factor in determining its success. Temperature can be controlled in water re-use systems, permitting production aquaculture at ideal temperatures to optimize or maximize growth and/or feed conversion. However, pumping and heating (or cooling) of water significantly increases the costs of production. Knowledge of growth performance and feed conversion at various temperatures could prove to be the key factors in determining the economic feasibility of water re-use systems.

Once data on growth performance and feed conversion at various temperatures have been collected, mathematical models can be constructed to predict growth rates and feed costs for different geographical locations, different thermal regimes in water re-use systems, and for all the tested species, hybrids, and triploids. This model would be extremely responsive to the needs of potential aquaculture entrepreneurs interested in estimating the viability of commercial sunfish culture in a given geographic region or a given culture system. A model such as this, developed under carefully controlled laboratory conditions, could be conveniently tested and refined in the production-scale studies outlined in this proposal.

The thermal requirements of the bluegill are well documented (see Reynolds and Casterlin 1977 for a review), but less information is available on the requirements of the green sunfish, white and black crappies, and almost no information is available on sunfish hybrids. Coutant (1977) summarized data on the preferred temperatures for numerous species. McCauley and Casselman (1981) have shown that final preferred temperature is a good predictor of the optimum temperature for growth. Final preferred temperatures in laboratory studies were 30.2 to 32.3, 27.3 to 30.6, 18.3 to 19.8, and 20.5 to 24.0 °C for bluegill, green sunfish, white crappie, and black crappie, respectively. We are unaware of any data on the thermal requirements for any hybrid sunfish. It is not possible to predict if the hybrids will be intermediate to the parental species or will show thermal heterosis. Both situations have been found (Bettoli et al. 1985). However, Brunson and Robinette (1983) have shown that hybrid sunfish do grow better than bluegill at mean water temperatures of 10.4 °C.

Among the characteristics which make an organism adaptable to aquaculture is its ability to grow well at high densities (Lewis and Heidinger 1971b). Intolerance to high densities can reduce yields through the development of dominant-submissive behavior, which reduces feed conversion, and through fighting, which can result in loss of fish from secondary infections. Maximizing density, and thus production, is necessary for economically successful aquaculture.

Lewis and Heidinger (1978) recommended stocking hybrid sunfish at rates of 741 to 7,400 fish/ha for farm ponds, with the higher rates being applied to ponds which will be fed and containing predators. Brunson and Robinette (1982) stocked hybrid sunfish at 2,471 fish/ha to examine the effects of feeding on growth and condition during winter. These fish were harvested at mean weights of 34 to 45 g, far below production weights of 200-225 g. Lewis and Heidinger (1971b) suggested that hybrid sunfish could be grown at densities of 1500 to 2000 kg/ha in ponds, although the maximum which they attained was 1000 kg.

Very little exists in the literature pertaining to the production of white, black or hybrid crappie in ponds, and nothing is found on their culture in cages. Buck and Hooe (1986) provided the most extensive, current comparison in ponds. Through controlled production of hybrids, they evaluated survival, growth and condition for parental stocks and F_1 hybrids. They found that F_1 hybrids grew significantly faster than either parent, and that the hybrids and the black crappie had higher condition factors and survival rates than the white crappie.

Efforts to look at the application of more intensive techniques to crappie production are also scarce. Smeltzer (1981) was able to train black crappie fingerlings to commercial feed and reduce losses due to handling stress by harvesting at night when water temperatures were at or below 20 °C. Amspacker (1991) was also able to reduce handling mortalities in white crappie by keeping water temperatures low, using salt during transport and anesthetizing fish during subsampling. His study looked at trainability, growth and food conversion efficiency for various size groups of white crappie in a small, closed system. Through several feeding trials, he found that trainability to a commercial diet (Mesa crumbles, Biodiet) was virtually 100% for adult white crappie (>160 mm) but only 45-65% successful for sub-adults (<130 mm).

Based on Amspacker's results, the role of stocking densities in trainability appeared to be most important for sub-adults. Adults readily switched to a commercial feed at densities as low as 1.64 kg/m^3, while trainability was 0 for sub-adults in three separate feeding trials when densities were between 0.89 and 0.96 kg/m^3. It wasn't until densities were increased to 4.0 kg/m^3 as recommended by Simco et al. (1986) for fingerling largemouth bass that sub-adult crappie showed any trainability.

During Year 1 of the first NCRAC Sunfish project, hybrid sunfish were held in cages at densities of 100, 200 and 400 fish/m^3. The variability in weight (as expressed as coefficient of variation) among these ranged from 25 to 40%, and appeared to be related to stocking density. This variability may be the result of dominant-submissive behavior or pre-spawning aggression.

No literature is available on the effects of density on triploid sunfish reared in floating cages or intensively in tanks. Currently, several experiments are being funded through the NCRAC evaluating triploid sunfish. The goals of that research include: the development of protocols for the production of polyploid sunfish and comparisons of food conversion and growth over a broad range of temperatures for parental species, hybrids, and triploids.

Development of low cost, high performance sunfish diets

There are apparently no published reports of nutritional studies with hybrid bluegill. Diets fed to the hybrid have included floating trout (Lewis and Heidinger 1971a) and floating catfish diets (Brunson and Robinette 1982). Nutritional research with catfish and salmonids is ongoing and diets change as new information becomes available. Thus, to state that a catfish or trout diet is optimal for hybrid bluegill may be correct one day, but not correct later when diets change. A better approach is to develop diets specifically for the target species, not extrapolating among fish species exhibiting wide ranges of food preferences and habits. We have recently adopted an approach to dietary formulation for new species that is rapid and appears to work.

Paul B. Brown and colleagues at Purdue University are currently evaluating commercially available and experimental diets fed to hybrid bluegill in a controlled situation. Through the early phases of that study, fish fed the trout diets are gaining weight more rapidly than those fed catfish diets. However, they have observed the same situation with hybrid striped bass in their laboratory, and those fish exhibited microvessicular hepatopathy when fed any of the existing diets and increased visceral fat with increasing dietary fat (salmonid diets). The goal of nutritional research is to define diets that promote maximal weight gain and health of fish. The salmonid diets fed to hybrid striped bass to date promote rapid weight gain, but do not promote health of fish as seen in the histopathological evaluations. So, the studies to date indicate that hybrid striped bass grow better when fed diets containing crude protein levels and essential amino acid balances similar to those in salmonid diets, but the lipid levels in current salmonid diets appears to be problematic. Researchers at Purdue will examine hepatic samples of hybrid bluegill at the end of their current study for determination of hepatocyte integrity and signs of hepatopathy and use that information as an indication of the appropriate levels of crude protein and lipid.

As a continuation of their studies with hybrid striped bass, they evaluated the Ideal Protein Concept (Agricultural Research Council 1981). This concept is based on the recognized fact that essential amino acid patterns of animals is similar to the optimal dietary pattern. This correlation has been verified with fish (Wilson and Poe 1985). By analyzing carcass amino acid composition of hybrid striped bass, and assuming some level of amino acid absorption from feedstuffs, diets were formulated using readily available feedstuffs in the Midwest and extruded by an Indiana-based feed mill. Those diets contained varying levels of protein and the essential amino pattern of whole fish. Brown and colleagues fed those diets to fish in their laboratory (mean initial weight of 48.1 g). Weight gain of fish fed a diet containing 36% crude protein was identical to that of fish fed one of the better hybrid striped bass diets formulated on the requirements of salmonids. Cost of their extruded diet was minimal compared to the cost of other diets for hybrid striped bass particularly when they considered transportation costs. An Indiana-based feed mill is now manufacturing that diet for sale to aquaculturists in this region.

Brown proposes using the same experimental methodology with hybrid bluegill. The time required to develop a diet based on essential amino acids can be reduced from 3-5 years to 2 with this approach, and he is developing most of the necessary information now.

ANTICIPATED BENEFITS

1. The development of procedures for tetraploid induction would greatly decrease labor costs for triploid *Lepomis* production and ensure 100% triploidy.

2. The development of methods for eliminating uncontrolled reproduction, making sunfish more suitable for commercial food fish production and more valuable for sale in the recreational fisheries market.

3. The determination of whether induced triploidy confers more rapid growth, more efficient growth, or both, are key factors in determining the cost-benefit ratio for induced triploidy.

4. Determinations of the temperature range for growth and temperatures where good growth and feed conversion are obtained for sunfish species, hybrids, and triploid sunfish; this information is essential for determining the economics of regional sunfish production and for selecting sunfish suitable for production under the various temperature regimes found in the region.

5. The formulation of feeds that will minimize production costs and maximize performance, enabling sunfish producers in our region to share advantages that culturists of other species, such as rainbow trout and channel catfish, in other regions have enjoyed for years.

6. The determination of sunfish yields under practical fish culture conditions is essential information for evaluating the economics of sunfish food fish production in the North Central Region.

7. The identification of optimal stocking densities will provide basic information for regional aquaculturists to improve production methods or initiate production trials.

8. The development of procedures for grow out of food-sized sunfish will open a new and potentially significant market for the significant number of existing sunfish fingerling producers in the North Central Region, that market being the supply of seed stock to grow-out operations.

PROGRESS TO DATE

Significant progress was made on development of polyploid sunfish since the summer of 1990. Several breakthroughs were made.

The first major breakthrough was the development by MSU of a less time consuming technique to test the ploidy level of bluegill and sunfish hybrids. Previously, the fish had to be raised in the lab for up to 3 months after hatching so sufficient quantities of blood would be available for flow cytometry testing. This obviously slowed experimental progress and made it difficult to build on successes. Also, more facility space had to be devoted to growing out a large number of treatment groups. In the early summer of 1990, MSU developed a method to test 5 to 7-day-old fry for ploidy level. Now many more treatments can be tested since less space is needed for growing out fingerlings and less time is needed to test the ploidy level of fish in the various treatments.

The second major accomplishment in the summer of 1990 was production of the first verified triploid bluegills through temperature-shock induced polar body retention by MSU researchers. Triploids bluegills are sterile and can be used to carefully control the numbers of bluegills in a production pond or small recreational impoundment. Researchers at SIUC (Sheehan and colleagues) at about the same time were the first to produce triploid hybrid *Lepomis* sunfish. Although both temperature shocks and hydrostatic pressure shocks were tested at SIUC, the latter proved to be superior because several tested hydrostatic pressure shock treatments yielded 100% triploids, good survival (90+ %), and no deformed individuals. They have subsequently in 1991 identified optimum hydrostatic pressure shocks for inducing triploidy in hybrid sunfish.

In 1991, MSU researchers further refined the triploid bluegill production techniques. They were able to produce 100% triploidy in bluegill subjected to cold shock (t_0 = 1.5 min. at 5 °C for 10 min.) or pressure treatments (t_0 = 1.5 min. at 562 kg/cm^2 (8000 psi) for 5 min). They are currently evaluating survival from the various 100% triploid treatments before final recommendations can be made. A large number of triploids are being produced so limited testing of their growth and survival can be done in 1992 by SIUC. The production of 100% triploids is very important for food fish producers and recreational fisheries managers since even a few normal diploid fish would result in reproduction and a loss of numbers control. This technique will provide a tool to eliminate stunting.

MSU also produced the first tetraploid bluegills using cold shocks. Tetraploids mated with normal diploid bluegill should result in 100% sterile triploid offspring. Use of tetraploids to produce triploids will enhance the supply of triploids and absolutely ensure the production of 100% triploid bluegill populations. By 1992, MSU researchers hope to refine the techniques to produce tetraploid bluegill. They will rear these tetraploid bluegill to maturity to test tetraploid X tetraploid, tetraploid X diploid and diploid X tetraploid crosses. These techniques will be extended to the commercial sector so production can begin as early as 1994.

SIUC (Tetzlaff) is currently studying relationships between stocking density and growth and yield of hybrid *Lepomis* sunfish. Sheehan of the same institution is currently evaluating the growth performance of bluegills, green sunfish, and bluegill X green sunfish hybrids across the temperature range of 8 to 28 °C, beginning with 5 g fish. Preliminary analysis of data from this work indicates that all three are capable of growth in excess of 4% body weight/day.

OBJECTIVES

1. Production and evaluation of polyploid sunfish.

2. Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish.

3. Development of low cost, high performance sunfish diets.

PROCEDURES

Production and evaluation of polyploid sunfish (Objective 1)

To examine population control in sunfish through induced polyploidy, MSU will focus on refining methods for induction of tetraploidy in bluegill. They will modify thermal and pressure shock protocols to determine the effect of different treatments on induction rates and survival. MSU and SIUC will collaborate in both years of this study to refine polyploidy induction techniques for Lepomine sunfishes and to evaluate performance characteristics including growth and food conversion.

The information gained from the first NCRAC Sunfish project will be used to refine triploidy and tetraploidy induction in bluegill. Cold shock and pressure shock treatments tested during this project will be clustered around treatments that produced the maximum polyploid induction rate with minimal mortalities. The experimental design will be a randomized complete block, with three replicates. Analysis of variance (ANOVA) will include partitioning for trend effects and for time and duration of cold and pressure shocks. Experiments will continue until the optimum protocol can be found for polyploid induction.

Mature, ripe bluegill will be collected from local wild stocks during the normal spawning season. The bluegill will be transported to the MSU Aquaculture Research Lab. Based on previous experience, egg quality is extremely important in survival of eggs subjected to cold and pressure shocks. Ova will be stripped from ripe females only. Ova and sperm will be mixed in a watch glass. Pressure or cold shocks will be administered at 1.5 minutes (triploid induction) or 35 to 45 minutes (tetraploid induction) post-fertilization. Pressure shocks will be administered for 2.5 to 5 minutes at pressures of 422 to 562 kg/cm^2 (6000 to 8000 psi). Cold shocks will be done at 5 ^oC for 5 to 10 minutes.

Following treatment, shocked ova will be transferred to specially designed flow-through containers and placed in Heath Incubator Trays. The trays are supplies with heated (to 26 ^oC) well water. Upon hatching, fry will be maintained in the trays for 5 days. A sample of up to 15 fry will be tested for ploidy using techniques developed at MSU during the first NCRAC Sunfish project.

Triploid bluegill will be grown to 5 cm for subsequent comparisons with normal diploid bluegill and diploid and triploid hybrid sunfish groups at SIUC. Tetraploid fish will be grown to maturity at MSU. Treatments which have produced tetraploid bluegill have been repeated and are currently being grown to 7.6 cm for subsequent ploidy testing. Upon hatching, the bluegill are fed newly hatched brine shrimp nauplii, and later, appropriately-sized commercial feeds. After 6 months, blood will be drawn by cardiac puncture. Flow cytometry techniques developed at MSU for identification of ploidy level in sunfishes will be used to identify tetraploids. Tetraploids will be grown to maturity so crosses of 2N X 4N and 4N X 4N can be made to determine fertility and the survival of offspring.

Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish (Objective 2)

Several institutions and researchers will participate in this objective. Jmaes R. Triplett, Pittsburg State University (PSU), will evaluate black crappie, white crappie, and hybrid crappie under cage culture conditions. Bruce L. Tetzlaff will evaluate triploid *Lepomis* hybrid sunfish under cage culture conditions. Robert J. Sheehan of Southern Illinois University-Carbondale (SIUC), will continue testing the growth performance of diploid sunfish, hybrid sunfish, triploid sunfish and hybrid triploid sunfish at various temperatures, but he will switch his emphasis from *Lepomis* to *Pomoxis* during this proposed project. He will also evaluate hydrostatic pressure shocks for producing triploid *Pomoxis* and produce the fish for Tetzlaff's studies. Ultimately he will test the best performing species, hybrids, triploids, or triploid hybrids in side-by-side comparisons to identify stocks for development for sunfish food fish production. David H. Wahl and Michael L. Hooe of the Illinois Natural History Survey (INHS) will provide the crappie parental species and hybrids necessary for the studies of Triplett and Sheehan. They will also collaborate with Sheehan to identify optimal shocks for producing crappie triploids.

In order to make a first estimate of trainability, growth and conversion by crappie in cages, white crappie will be obtained from local populations by trapping and electrofishing. Culture cages, 1 m^3 in volume will be placed in a 5.5 ha lake at the PSU Research Reserve. Individuals in the 100-130 mm size range will be stocked into six cages to provide replicated starting densities of 2, 4 and 6 kg/m^3. These densities will enable PSU to look at levels below and within estimated required densities to promote trainability. Final densities after a 180-day growing season should reach 34, 68 and 101 kg/m^3, which will approach production level expectations for channel catfish. Fish will be subsampled biweekly to provide an ongoing estimate of % trainability, growth and feed conversion efficiency. During the second year, fish in the 100-130 mm range will be obtained from specially cultured stocks at the INHS Sam Parr Biological Station. A comparison of three genetic stocks of crappie: white, black and F_1 hybrid, will be made in 12 cages, 1 m^3 in volume, placed in the 5.5 ha lake at the Research Reserve. This will provide four replicates for each stock. Although starting densities may be altered based on the results of the first year, original estimates follow the highest stocking density (6 kg/m^3) investigated in Year 1. Fish will be subsampled biweekly to provide an ongoing estimate of % trainability, growth and feed conversion efficiency.

The effects of density on the production of food-sized hybrid bluegill male X green sunfish females in cages and in ponds will have been determined by the fall of 1992. In addition, the effects of density on triploid green sunfish male X bluegill female hybrids will also be completed. This component of Objective

2, conducted by Tetzlaff of SIUC will further examine the effects of density on triploids using the original bluegill male X green sunfish female cross.

Yearling triploids will be stocked into 1 m^3 cages at the following densities: 3 cages at 100 fish/m^3, 3 cages at 200 fish/m^3, and 3 cages at 400 fish/m^3. The cages will be placed into three 0.04-ha ponds with one cage from each treatment in each pond. The fish will be fed daily at 3% body weight/day with a 40% protein floating trout diet. Feeding rates will be adjusted for fish growth bi-weekly. The study will continue through October, or until the bi-weekly samples reveal little or no growth. At the completion of the study, all fish will be weighed and measured, and the effects of density on growth, survival, food conversion, and dress-out percentage will be compared.

Concurrent with the examination of the effects of density, data will be collected which will be used to test the temperature/food conversion model developed from the diploid hybrids and the triploid reciprocal cross. Recording thermographs will measure water temperatures in the vicinity of the cages at hourly intervals for the duration of the study. Growth and food conversion data determined from the bi-weekly samples can then be correlated with the mean water temperature during that two-week period. These results will then be compared to those of the laboratory study.

Ongoing studies evaluating relationships between water temperature and growth performance will be continued at SIUC as part of Objective 2. The growth performance in relation to temperature of white crappie, black crappie, and their hybrid will be determined by Sheehan. He will then produce, with cooperation from the INHS, triploids of either black crappie, white crappie, or their hybrid, depending on which performs the best. Garling, MSU, has agreed to cover the costs (with funds from Objective 1 above) of the flow cytometry ploidy analyses necessary for fish produced by Sheehan in the work proposed herein.
Subsequent growth performance/temperature tests will be conducted three stocks at a time with triploid crappie, triploid bluegill (from MSU; see Objective 1) and the best performing stocks from studies previously funded through the NCRAC.

Growth performance trials will begin when the experimental fish are approximately 5 g in size. The trials will be conducted in ten circular fiberglass tanks (1.2 x 0.9 m), each equipped with a biofilter, heating and/or cooling capability, and an aeration/de-gasification head tank. Five temperatures, ranging from 8-28 °C at 5 °C intervals will be maintained in replicate tanks. Photoperiod will be maintained at an L:D 14:10. The tanks will be divided into three cells with plastic mesh screen, permitting the simultaneous evaluation of 10-15 individuals of each of three stocks (parentals, hybrids, triploids, or hybrid triploids) in side-by-side comparisons. Water inputs to the tanks will enter a central reservoir in which three holes were drilled such that the water is divided and equally distributed into each of the three cells per tank; a technique employed successfully in the past.

Temperatures will be manipulated no more than 1 °C per day from the initial acclimation temperature (about 20 °C) during the period used to attain the experimental temperatures. All fish will be held for at least 10 days at their respective experimental temperatures before growth trials begin. At the start of each trial, the fish will be measured, weighed, and marked with a cold brand, dorsal fin clip, or by some other method, allowing identification of individuals. Fish will be hand fed to satiation two times per day, and food consumption will be monitored. The feed will be a commercially available floating pellet of appropriate size consisting of at least 35% protein. Trials will last for a total of 90 days, but will be interrupted after 30 and 60 days to allow measurements of growth. It is anticipated that some of the experimental fish (especially in the higher temperature groups) will have reached a desirable marketable size (greater than 0.22 kg) by the end of the 90-day growth trial. Water quality parameters will be monitored throughout the study, including dissolved oxygen, ammonia, nitrite, pH, and alkalinity using Standard Methods (APHA et al. 1989).

ANOVA will be used to compare growth and feed conversion at the various temperatures for each species, hybrid, or genetically manipulated sunfish. The fish will be sexed at the end of the 90-day trial to determine sex ratios and whether sex influences growth, food conversion, and dress-out. Mathematical models will be constructed from the data describing instantaneous growth and feed conversion efficiency as functions of temperature. The derived models should be capable of accurately predicting growth and feed costs under constant temperature conditions, such as in water re-use systems. However, the models will also be tested using temperature, growth, and feed conversion data collected from production-scale pond studies described elsewhere in this objective. We anticipate some disparity between values for growth and feed conversion predicted by the model and the empirically derived pond production data. The laboratory studies will be conducted under constant photoperiod and temperature conditions, and excellent water quality will be maintained, whereas photoperiod, temperature, and water quality will fluctuate in the ponds. Comparison of the empirically-derived data to predicted values derived from the models will provide insight into how large a role these factors play under real-world conditions, and it will also permit refinement of the model to fit pond production conditions.

The INHS will provide the parental crappie species and hybrids necessary for this work. The white crappie, black crappie, and white crappie female x black crappie male F_1 hybrids used in the comparative growth rate and cage studies will be produced at the INHS Sam Parr Biological Station. This hybrid was chosen because of ease of production and because pond studies have shown growth rates for the reciprocal F_1 hybrids are similar (Buck and Hooe 1986; Hooe and Buck 1991).

Ripe white and black crappie brood fish will be collected from natural populations in central Illinois in April of 1993 and 1994, transported to the laboratory, and spawned in vitro (Buck and Hooe 1986). Each of the three genetic stocks will be reared in the laboratory to the free-swimming stage (approximately 5 days post-hatch) and then stocked into rearing ponds at the Sam Parr Biological Station and at SIUC. Each genetic stock will be reared in separate ponds to facilitate positive identification. To allow production of 50 mm fish in early July and 100-125 mm fish by late September, fry will be stocked at a rate of 7,500 to 12,500 per ha. The genetic integrity of the brood fish used to produce the three genetic stocks will be confirmed by using vertical starch-gel electrophoresis (Hooe and Buck 1991).

Development of low cost, high performance sunfish diets (Objective 3)

All work on this objective will be conducted by Paul B. Brown at Purdue University. The green sunfish female x bluegill male hybrids will be used in all evaluations and will be obtained from Clear Creek Hatchery.

Juvenile fish will be transported to the Purdue Aquacultural Research Facility and stocked into 2000 L holding tanks. Five fish will be sampled from the holding tank for analyses of whole-body essential amino acid profile using standard methods. Oven-dried samples will be hydrolyzed in 6N hydrochloric acid under constant vacuum. Hydrolyzates will be dried, then dervitized with phenylisothiocyanate (PITC) as described by Sarwar and Botting (1990). Aliquots will be injected into a gradient liquid chromatography system with constant column temperature of 47.5 °C (Waters Chromatography Division, Milford, MA). PITC-labelled amino acids will be identified and quantified by comparisons with known standards using a UV/VIS detector set at 254 nm.

Individual essential amino acids will be expressed as a ratio with total essential amino acids (A/E ratio). However, this provides only the optimal essential amino acid pattern, not absolute amounts (Wilson and Poe 1985) of amino acids necessary for dietary formulation. Thus, quantification of at least one dietary essential amino acid prior to formulating diets is requisite. We propose defining the dietary lysine requirement of juvenile fish.

In our current studies, juvenile hybrid bluegill are accepting a purified crystalline amino acid diet and growth of fish fed that diet has been good. Thus, we will use a version of our crystalline amino acid diet for determining the dietary lysine requirement. Diets will contain the optimal level of crude protein as determined in our current studies. Diets will be nutritionally-complete based on our current knowledge of fish nutritional requirements and formulated to contain graded levels of L-lysine in 0.2% gradations ranging from 0.8-2.2% of the dry diet, which encompasses the range of known dietary lysine requirements (Brown et al. 1988; Wilson 1989; Griffin and Brown 1991). The diets will be formulated to contain the essential amino acid pattern of whole fish, Dry ingredients will be mixed, adjusted to pH 7.0 (Wilson et al. 1977), and pelleted in our laboratory using standard procedures. All diets will be dried at 60 °C using a forced-air oven and stored frozen (-20 °C) prior to feeding.

Groups of 15 fish will be randomly stocked into 40-L aquaria and diets will be randomly assigned to triplicate groups of fish. All fish will be fed their respective diets for 2 weeks prior to initiation of the experimental period. After this time, all fish will be weighed and offered their respective diet at a rate of 4% body weight/day (on a dry matter basis) divided into two equal meals. We are using this feed rate now and it appears to be an acceptable level as all groups are consuming the daily allotment. All fish will be weighed every two weeks and feed allowances adjusted based on weight gain of each replicate.

At the end of the study, 5-6 fish from each replicate will be removed from the tank, anesthetized with tricainemethanosulfonate, and blood samples collected via the caudal vein. Blood samples will clot on ice, then mixed with acetonitrile, and centrifuged at 4 °C to precipitate intact proteins. Serum samples will be stored frozen prior to analyses of free-amino acids by the procedures listed above.

All data (weight gain, feed efficiency, survival, and serum lysine levels) will be subjected to ANOVA using SAS for determination of statistical differences among treatment means. All data will be examined for their appropriateness in defining the requirement. Those parameters that are responsive to graded levels of dietary lysine will be used to define the dietary requirement using standard quantitative models. These models include a fitted-broken line or quadratic curve using the dietary level that corresponds to 95% of the asymptote (Robbins et al. 1979).

This proposed research, together with our current studies, will provide the necessary information to use computer-aided least-cost dietary formulation based on the Ideal Protein Concept for formulating diets for hybrid bluegill.

FACILITIES

Production and evaluation of polyploid sunfish (Objective 1)

Identification of critical timing of meiotic and mitotic events, polyploid induction in bluegill, and bluegill performance tests will be conducted under the direction of Donald L. Garling at the MSU Aquaculture Laboratory.

Flow cytometry analysis of diploid, triploid, and tetraploid bluegill will be made at the MSU Flow Cytometry Laboratory.

Determinations of optimum stocking densities and relationships between temperature and growth for sunfish, sunfish hybrids, and triploid sunfish (Objective 2)

Determination of the optimum temperature/food conversion relationship for sunfish will be directed by Robert J. Sheehan at the SIUC Fisheries Research Laboratory. The SIUC facility contains a wet lab containing sufficient temperature and photoperiod-controlled tank facilities for the execution of this study. Ancillary facilities include refrigerated feed storage, a water quality analytical laboratory, a fish "work-up" room, and other support laboratories.

Pond studies will be conducted under the direction of Bruce Tetzlaff at the SIUC pond research facility. The SIUC facility consists of eighteen 0.04 ha drainable ponds.

The evaluation of different densities and genetic stocks on crappie culture in cages will be directed by James R. Triplett at the PSU Research Reserve. This facility contains several strip mine lakes of excellent water quality. The largest at 5.5 ha is where the cages will be placed. The laboratory at the Reserve has lake and treated water supplies with four, large cement tanks and numerous movable, smaller tanks.

The facilities at the Sam Parr Biological Station are available to the INHS researchers, David H. Wahl and Michael L. Hooe, to support their contributions to this objective. The Station has numerous ponds for producing fingerlings and sufficient fish tanks for in vitro spawning.

Development of low cost, high performance sunfish diets (Objective 3)

Research on application of the Ideal Protein Concept to dietary development for hybrid bluegill will be conducted at the Purdue University Aquacultural Research Facility. Currently, this facility contains 502 m^2 of space equipped with well water, 2 hp blowers for supplemental aeration, and temperature control. Experimental aquaria are in place and currently in use with hybrid bluegill nutritional studies. Purdue is in the process of developing a new research facility. If available, this research will be conducted in the new facility.

The Fish Nutrition Laboratory within the Department of Forestry and Natural Resources at Purdue has the necessary equipment for completing these studies. That equipment includes feed mixing and pelleting equipment, ovens for drying feed and feed samples, a liquid chromatography system, amino acid hydrolysis work station, analytical evaporator, refrigerated centrifuge, UV/VIS spectrophotometer, analytical microwave, hot and cold water baths, atomic absorption spectrophotometer, associated glassware and least-cost feed formulation computer program.

REFERENCES

Agricultural Research Council. 1981. The nutrient requirements of pigs. Commonwealth Agricultural Bureaux, Slough, UK.

Allen, S., Jr., and J. Stanley. 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon (*Salmo salar*). Transactions of the American Fisheries Society 108:462-466.

Allen, S., Jr., R.G. Thiery, and N.T. Hagstrom. 1986. Cytological evaluation of the likelihood that triploid grass carp will reproduce. Transaction American Fisheries Society 115:841-848.

Amspacker, Troy. 1991. Trainability, Growth, and Conversion Efficiency of White Crappie *Pomoxis annularis* on an Artifical Diet. M.S. thesis. Pittsburg State University, Pittsburg, KS.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, 17th edition. American Public Health Association, Washington, DC.

Baur, R.J. 1988. 1986 Illinois sport fishing survey. Illinois Department of Conservation. Special Fisheries Report Number 53.

Benfey, T., and A. Sutterlin. 1984a. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture 36:359-367.

Benfey, T., and A. Sutterlin. 1984b. Oxygen utilization by triploid land locked Atlantic Salmon *Salmo salar* L. Aquaculture 42:59-73.

Benfey, T., I.I. Solar, G. DeJong, and E.M. Donaldson. 1986. Flow-cytometric confirmation of aneuploidy in sperm from triploid rainbow trout. Transactions American Fisheries Society 115:838-840.

Bettoli, P.W., W.H. Neill, and S.W. Kelsch. 1985. Temperature preference and heat resistance of grass carp, *Ctenopharyngoden idella* (Valenciennes), bighead carp, *Hypophthalmichthys nobilis* (Gray), and their F_1 hybrid. Journal of Fish Biology 27:239-247.

Brown, P.B., D.A. Davis, and E.H. Robinson. 1988. An estimate of the dietary lysine requirement of juvenile red drum Sciaenops ocellatus. Journal of the World Aquaculture Society 19:109-112.

Brunson, M.W., and H.R. Robinette. 1982. Supplemental feeding of hybrid sunfish in Mississippi. Proceedings of the Annual Conference Southeast Association of Fish and Wildlife Agencies 36:157-161.

Brunson, M.W., and H.R. Robinette. 1983. Winter growth of bluegills and bluegill x green sunfish hybrids in Mississippi. Proceedings of the Annual Conference Southeast Association of Fish and Wildlife Agencies 37:343-347.

Brunson, M.W., and H.R. Robinette. 1986. Evaluation of male bluegill x female green sunfish hybrids for stocking Mississippi farm ponds. North American Journal of Fisheries Management 6(2):156-167.

Buck, H., and M. Hooe. 1986. The production and growth of F_1 hybrid crappie. Biological Notes No. 125. Illinois Natural History Survey, Champaign.

Cassani, J.R., and W.E. Caton. 1985. Induced triploidy in grass carp, *Ctenopharyngodon idella.* Val. Aquaculture 46:37-44.

Cassani, J.R., and W.E. Caton. 1986. Growth comparisons of diploid and triploid grass carp under varying conditions. Progressive Fish-Culturist 48:184-187.

Cassani, J.R., W. Caton, and B. Clark. 1984. Morphological comparisons of diploid and triploid hybrid grass carp., *Ctenopharyngodon idella* female X *Hypothalmichthys nobilis* male. Journal of Fish Biology 25:269-278.

Childers, W.F. 1967. Hybridization of four species of sunfishes (Centrarchidae). Illinois Natural History Survey Bulletin 29:159-214.

Chourrout, D. 1982. Tetraploidy induced by heat shocks in the rainbow trout (*Salmo gairdneri* R.). Reproduction and Nutritional Development 22:569-574.

Chourrout, D. 1984. Pressure-induced retention of the second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. Aquaculture 36:111-116.

Chourrout, D. 1986. Genetic manipulation in fish. *In* Klaus Tiews, editor. Selection, Hybridization, and Genetic Engineering in Aquaculture, Proceedings of a World Symposium sponsored by EIFAC and ICES, Bordeaux, June 27-30, 1986.

Chourrout, D., and J. Itskovich. 1983. Three manipulations permitted by artificial insemination in tilapia: induced diploid gynogenesis, production of all triploid population and intergeneric hybridization. Pages 246-255 *in* International Symposium on Tilapia in Aquaculture. Nazereth, Israel.

Coutant, C.C. 1977. Compilation of temperature preference data. Journal of the Fisheries Research Board of Canada 34:739-745.

Cowey, C.B., and J.R. Sargent. 1979. Nutrition. Pages 1-69 *in* W.S. Hoar, D.J. Randall, and J.R. Brett, editors. Fish physiology, volume III, energetics and growth. Academic Press, New york.

Don, J., and R.R. Avtalion. 1986. The induction of triploidy in *Oreochromis aureus* by heat shock. Theoretical and Applied Genetics 72:186-192.

Don, J., and R.R. Avtalion. 1988. Comparative study on the induction of triploidy in tilapia, using cold and heat shock techniques. Journal of Fish Biology 32:665-672.

Ellison, D.G., and R.C. Heidinger. 1978. Dynamics of hybrid sunfish in southern Illinois farm ponds. Proceedings of the 30th Annual Conference, Southeast Game and Fish Commissioners 30:82-87.

Ginzburg, A.S. 1972. Fertilization in fishes and the problem of polyspermy. *In* T.A. Detlaf, editor. Israel Program for Scientific Translations.

Graham, M, G. Fletcher, and T. Benfey. 1985. Effect of triploidy on blood oxygen content of Atlantic salmon. Aquaculture 50:133-139.

Griffin, M.E., and P.B. Brown. 1991. The dietary lysine requirement of juvenile hybrid striped bass. 22nd Annual Meeting of the World Aquaculture Society, San Juan, Puerto Rico.

Hadley, M.E. 1984. Endocrinology. Prentice Hall, Englewood Cliffs, New Jersy.

Heidinger, R.C. 1975. Growth of hybrid sunfishes and channel catfish at low temperatures. Transactions of the American Fisheries Society 104(2):333-334.

Heidinger, R.C. 1983. The use of hybrids in sportfish management and aquaculture in the United States. Pages 170-177 *in* Proceedings of the Third British Freshwater Fish Conference, Liverpool, England.

Hooe, M.L., and D.H. Buck. In Press. Evaluation of the interspecific F_1 hybrid crappies as sport fish in small impoundments. North American Journal of Fisheries Management.

Ihssen, P.E., L.R. McKay, I. McMillan, and R.B. Phillips. 1990. Ploidy manipulation and gynogenesis in fishes: cytogenetic and fisheries applications. Transactions of the American Fisheries Society 119:698-717.

Johnson, O.W., P.R. Rabinovich, and F.M. Utter. 1984. Comparison of the reliability of a coulter counter with a flow cytometer in determining ploidy levels in Pacific salmon. Aquaculture 43:99-103.

Lemoine, H., Jr., and L. Smith. 1980. Polyploidy induced in brook trout by cold shock. Transaction American Fisheries Society 109:626-631.

Lewis, W.M., and R.C. Heidinger. 1971a. Supplemental feeding of hybrid sunfish populations. Transactions of the American Fisheries Society 100:619-623.

Lewis, W.M., and R.C. Heidinger. 1971b. Aquaculture potential of hybrid sunfish. The American Fish Farmer 1971(Apr.):14-16.

Lewis, W.M. and R.C. Heidinger. 1978. Use of hybrid sunfishes in the management of small impoundments, Pages 104-108 *in* C. Novinger and J. Dillard, editors. New approaches to the management of small impoundments. Northcentral Division of the American Fisheries Society Special Publication No. 5.

Lincoln, R., D. Aulstad, and A. Grammeltvedt. 1974. Attempted triploid induction in Atlantic salmon (*Salmo salar*) using cold shocks. Aquaculture 4:287-297.

Lincoln, R.F., and V.J. Bye. 1987. Growth rates of diploid and triploid rainbow trout (*Salmo gairdneri* R.) over the spawning season. Page 134 *in* D.R. Idler, L.W. Cum and J.M. Walsh, editors. Proceedings of the Third International Symposium on the Reproductive Physiology of Fish. Memorial University of Newfoundland, St. John's, Newfoundland.

Lincoln, R., and A. Scott. 1984. Sexual maturation in triploid rainbow trout, (*Salmo gairdneri* Richardson). Journal of Fish Biology 25:385-392.

McCauley, R.W., and J. Casselman. 1981. The final preferendum as an index of the temperature for optimum growth in fish. Pages 81-92 *in* K. Tiews, editor. Proceeding of the World Symposium on Aquaculture in Heated Effluents and Recirculation Systems, volume II. Heenemann, Berlin.

Myers, J.M. 1986. Tetraploid induction in *Oreochromis* spp. Aquaculture 57:281-287.

Pflieger, W.L. 1975. The fishes of Missouri. Missouri Department of Conservation. Jefferson City.

Purdom, C.E. 1983. Genetic engineering by the manipulation of chromosomes. Aquaculture 33:287-300.

Refstie, T., V. Vassuik, and T. Gjedrem. 1977. Induction of polyploidy in salmonids by cytochalasin B. Aquaculture 10-65-74.

Reynolds, W.W., and M.E. Casterlin. 1979. Behavioral thermoregulation and the "final preferendum" paradigm. American Zoologist 19:221-224.

Robbins, K.R., D.H. Baker and H.W. Norton. 1979. Estimation of nutrient requirements from growth data. Journal of Nutrition 109:1710-1714.

Sarwar, G., and H.G. Botting. 1990. Rapid analysis of nutritionally important free amino acids in serum and organs (liver, brain and heart) by liquid chromatography of precolumn phenylisothiocyanate derivatives. Journal of the Association of Official Analytical Chemists 73:470-475.

Simco, B.A., J.H. Williamson, G.J. Carmichael, and J.R. Tomasso. 1986. Centrarchids. Pages 73-89 *in* R.R. Stickney, editor. Culture of nonsalmonid freshwater fishes. CRC Press, Inc., Boca Raton, Florida.

Smeltzer, J.F. 1981. Culture, handling, and feeding techniques for black crappie fingerlings. M.S. Thesis, Colorado State University, Fort Collins.

Smith, L., and H. Lemoine. 1979. Colchicine-induced polyploidy in brook trout. Progressive Fish Culturist 41:86-88.

Solar, I., E. Donaldson, and G. Hunter. 1984. Induction of triploidy in rainbow trout (*Salmo gairdneri* R.) by heat shock, and investigation of early growth. Aquaculture 42:57-67.

Thorgaard, G.H. 1992. Application of genetic technologies to rainbow trout. Aquaculture 100:85-97.

Thorgaard, G.H., and G. Gall. 1979. Adult triploids in a rainbow trout family. Genetics 93:961-973.

Thorgaard, G.H., M. Jazwin, and A. Stier. 1981. Polyploidy induced by heat shock in rainbow trout. Transactions of the American Fisheries Society 110:546-550.

Valenti, R. 1975. Induction of polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock. Journal of Fish Biology 7:519-529.

Wilson, R.P. 1989. Amino acids and proteins. *In* J.E. Halver, editor. Fish nutrition. Academic Press, New York.

Wilson, R.P., and W.E. Poe. 1985. Relationship of whole body and egg essential amino acid patterns to aminoacid requirement patterns in channel catfish, *Ictalurus punctatus*. Comparative Biochemistry and Physiology 80B:385-388.

Wilson, R.P., D.E. Harding, and D.L. Garling, Jr. 1977. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling channel catfish. Journal of Nutrition 107:166-170.

Wolters, W., G. Libey, and C. Chrisman. 1982. Effect of triploidy on growth and gonad development of channel catfish. Transactions of the American Fisheries Society 111:102-105.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Robert J. Sheehan Southern Illinois University-Carbondale	Aquaculture/Physiology
	Bruce L. Tetzlaff Southern Illinois University-Carbondale	Aquaculture
	David H. Wahl Illinois Natural History Survey (INHS)	Fish Population Dynamics
	Michael L. Hooe Illinois Natural History Survey	Aquaculture
Indiana	Paul B. Brown Purdue University	Aquaculture Nutrition
Kansas	James R. Triplett Pittsburg State University	Fisheries/Aquaculture
Michigan	Donald L. Garling Michigan State University	Aquaculture Production/ Nutrition and Physiology/ Aquaculture Extension

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Michigan State University
Donald L. Garling

Illinois Natural History Survey
David H. Wahl
Michael L. Hooe

Pittsburg State University
James R. Triplett

Southern Illinois University-Carbondale
Robert J. Sheehan
Bruce L. Tetzlaff

Purdue University
Paul B. Brown

PROPOSED SUNFISH PROJECT BUDGET FOR
MICHIGAN STATE UNIVERSITY (MSU)

(Garling)

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	0.10	1	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	1	0.50	1	0.50	$12,500	$13,750
d.	Prebaccalaureate Students						
e.	Secretarial-Clerical						
f.	Technical, Shop, and Other						
	Total Salaries and Wages					$12,500	$13,750
B. Fringe Benefits						$0	$0
C. **Total Salaries, Wages and Fringe Benefits**						$12,500	$13,750
D. Nonexpendable Equipment						$0	$0
E. Materials and Supplies						$250	$250
F. Travel - Domestic (*Including Canada*)						$500	$500
G. Other Direct Costs						$3,125	$3,112
TOTAL PROJECT COSTS PER YEAR (*C through G*)						$16,375	$17,612
					TOTAL PROJECT COSTS	$33,987	

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

BUDGET JUSTIFICATION FOR MICHIGAN STATE UNIVERSITY

A. **Salaries and Wages.** A Graduate Student (0.50 FTE) to care for fish, induce ploidy manipulations, check ploidy, and make diploid and triploid crosses. (Year 1 - $14,875; Year 2 - $16,363)

E. **Material and Supplies.** Feeds (pelleted for adults, *Artemia* for larvae) ($200); chemicals for ploidy analysis preparation ($250); and miscellaneous fish culture supplies ($50).

F. **Travel.** To obtain fish from local stocks for ploidy manipulation (Year 1) and partial travel to NCRAC Work Group Meetings (Year 1 and 2)

G. **Other Direct Costs.** Flow cytometer time to check ploidy of fish from MSU and SIUC (Sheehan) ($1,250) and utilities for the MSU Aquaculture Research Lab ($4,987).

PROPOSED SUNFISH PROJECT BUDGET FOR
ILLINOIS NATURAL HISTORY SURVEY (INHS)

(Hooe and Wahl)

Objective 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	0.05	1	0.05	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.10	1	0.10	$4,180	$4,180
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$4,180	$4,180
B. Fringe Benefits					$320	$320
C. **Total Salaries, Wages and Fringe Benefits**					$4,500	$4,500
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$500	$500
F. Travel - Domestic (*Including Canada*)					$500	$500
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$5,500	$5,500
TOTAL PROJECT COSTS					$11,000	

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

BUDGET JUSTIFICATION FOR ILLINOIS NATURAL HISTORY SURVEY

A. Salaries and Wages. PIs will need assistance in hand stripping and spawning brood fish, pond fertilization, routine water quality/disease monitoring, harvesting, and transporting fish.

E. Materials and Supplies. Fish feeds, water quality analysis glassware and reagents, fertilizers, and other chemicals.

F. Travel. Travel to Work Group meeting and/or to meet with other PIs.

PROPOSED SUNFISH PROJECT BUDGET FOR
PITTSBURG STATE UNIVERSITY (PSU)

(Triplett)

Objective 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	0.10	1	0.10		
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	1	0.50	1	0.50	$7,000	$7,000
d. Prebaccalaureate Students	1		1		$2,300	$2,300
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$9,300	$9,300
B. Fringe Benefits					$227	$227
C. **Total Salaries, Wages and Fringe Benefits**					$9,527	$9,527
D. Nonexpendable Equipment					$350	$0
E. Materials and Supplies					$2,780	$4,300
F. Travel - Domestic (*Including Canada*)					$700	$1,040
G. Other Direct Costs					$300	$476
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$13,657	$15,343
TOTAL PROJECT COSTS					$29,000	

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

BUDGET JUSTIFICATION FOR PITTSBURG STATE UNIVERSITY

A. Salaries and Wages. Graduate Student (0.50 FTE) to conduct cage culture experiments and a Prebaccalaureate Student to assist in cage culture experiments.

D. Nonexpendable Equipment. D.O. meter for monitoring water quality to determine feeding rates.

E. Materials and Supplies. Cage construction materials, fish feeds, analytical reagents for water quality parameter measurements, and miscellaneous laboratory glassware.

F. Travel. To collect experimental animals and attend Work Group meetings.

PROPOSED SUNFISH PROJECT BUDGET FOR
SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE (SIUC)

(Sheehan and Tetzlaff)

Objective 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	0.15	1	0.10	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.38			$8,500	
c. Graduate Students	1	0.50	1	0.50	$10,914	$11,569
d. Prebaccalaureate Students	1	0.20	1	0.20	$2,288	$2,288
e. Secretarial-Clerical	1	0.08	1	0.08	$450	$477
f. Technical, Shop, and Other						
Total Salaries and Wages					$22,152	$14,334
B. Fringe Benefits					$2,770	$0
C. **Total Salaries, Wages and Fringe Benefits**					$24,922	$14,334
D. Nonexpendable Equipment					$2,170	$1,400
E. Materials and Supplies					$2,280	$1,950
F. Travel - Domestic (*Including Canada*)					$1,250	$400
G. Other Direct Costs					$4,758	$1,916
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$35,380	$20,000
				TOTAL PROJECT COSTS	$55,380	

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

BUDGET JUSTIFICATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

A. Salaries and Wages. Bruce Tetzlaff's work on cage culture will only be conducted during the first year of the project. He will need a part-time (0.38 FTE) researcher (Other Professional) to assist. Bob Sheehan's involvement will be for both years and requires the other personnel listed. A Graduate Student (0.5 FTE) to assist in pond production of triploids and conduct laboratory temperature/growth experiments; a Prebaccalaureate Student (0.2 FTE) to help feed and monitor fish in laboratory studies; and a Secretary (0.08 FTE) to assist Sunfish chairperson with clerical/secretarial work.

D. Nonexpendable Equipment. Laboratory study requires up to eight cooling units in continuous duty operation. Experience indicates that about one unit will break down and be lost per year; $1,400 will cover the replacement cost of one unit per year. Also one Ryan Tempmentor ($770) will be needed during the cage culture work of Year 1 to record water temperature within the ponds in the vicinity of cages.

E. Materials and Supplies. $1000 will be needed for Tetzlaff during Year 1 for a 100-foot seine ($450), fish feed ($400), and cage repair materials, dip nets, and miscellaneous sampling supplies ($150). $3230 will be needed by Sheehan for the two years of the project for feed, water quality glassware and reagents, screeing, dip nets, and miscellaneous sampling supplies and chemicals.

F. Travel. Travel to Work Group Meetings and to other PI's facilities.

G. Other Direct Costs. Tetzlaff's work will require $2,700 for truck leasing and fuel for 6 months for field work with the remainder being needed for telecommunications (telephone, FAX), shipping charges, repair of equipment, mailing costs, graphics, library somputer searches, and costs associated with report and publication preparation.

PROPOSED SUNFISH PROJECT BUDGET FOR
PURDUE UNIVERSITY

(Brown)

Objective 3

		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	0.10			$0	
b.	Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs							
a.	Research Assoc./Postdoc						
b.	Other Professionals						
c.	Graduate Students	1	0.50			$12,200	$0
d.	Prebaccalaureate Students	1	0.20			$1,000	$0
e.	Secretarial-Clerical						
f.	Technical, Shop, and Other						
	Total Salaries and Wages					$13,200	$0
B. Fringe Benefits						$252	$0
C. **Total Salaries, Wages and Fringe Benefits**						$13,452	$0
D. Nonexpendable Equipment						$0	$0
E. Materials and Supplies						$4,000	$0
F. Travel - Domestic (*Including Canada*)						$1,428	$0
G. Other Direct Costs						$1,620	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)						$20,500	$0
					TOTAL PROJECT COSTS	$20,500	

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

BUDGET JUSTIFICATION FOR PURDUE UNIVERSITY

A. Salaries and Wages. A Graduate Student (0.50 FTE) is required for completion of these studies. The student's responsibilities will include acquisition of fish, amino acid analyses, diet manufacturing, scientific feeding of fish, blood collection and analyses, and data analyses. A Prebaccalaureate Student (0.20 FTE) is needed to assist the PI and Graduate Assistant.

E. Materials and Supplies. Funds for supplies include liquid chromatography-grade reagents for amino acid analyses, crystalline amino acids for incorporation in diets, vitamins and minerals.

F. Travel. Travel includes partial cost of one trip to the next International Symposium on Fish Feeds and Nutrition to present our application of Ideal Protein to fish feed development. The meeting will be held in either Hobart, New Zealand, Adelaide, Australia, or both sites. It is anticipated that Purdue University's XL International Travel Grants Program will supply the remainder of the necessary travel funds as they did this past year for attendance at the same symposium held in Biarritz, France.

G. Other Direct Costs. Needed to pay for repair and fabrication of equipment.

CULTURE OF BLUEGILL AND CRAPPIE FOR FOOD FISH

Budget Summary for Each Participating Institution at $91.4K for the First Year

	MSU	INHS	PSU	SIUC	Purdue	TOTAL
Total Salaries and Wages	$12,500	$4,180	$9,300	$22,152	$13,200	$61,332
Fringe Benefits	$0	$320	$227	$2,770	$252	$3,569
Total Salaries, Wages and Benefits	$12,500	$4,500	$9,527	$24,922	$13,452	$64,901
Nonexpendable Equipment	$0	$0	$350	$2,170	$0	$2,520
Materials and Supplies	$250	$500	$2,780	$2,280	$4,000	$9,810
Travel	$500	$500	$700	$1,250	$1,428	$4,378
Other Direct Costs	$3,125	$0	$300	$4,758	$1,620	$9,803
TOTAL PROJECT COSTS	**$16,375**	**$5,500**	**$13,657**	**$35,380**	**$20,500**	**$91,412**

Budget Summary for Each Participating Institution at $58.4K for the Second Year

	MSU	INHS	PSU	SIUC	Purdue	TOTAL
Total Salaries and Wages	$13,750	$4,180	$9,300	$14,334	$0	$41,564
Fringe Benefits	$0	$320	$227	$0	$0	$547
Total Salaries, Wages and Benefits	$13,750	$4,500	$9,527	$14,334	$0	$42,111
Nonexpendable Equipment	$0	$0	$0	$1,400	$0	$1,400
Materials and Supplies	$250	$500	$4,300	$1,950	$0	$7,000
Travel	$500	$500	$1,040	$400	$0	$2,440
Other Direct Costs	$3,112	$0	$476	$1,916	$0	$5,504
TOTAL PROJECT COSTS	**$17,612**	**$5,500**	**$15,343**	**$20,000**	**$0**	**$58,455**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

Institution/Item		Amount
Michigan State University		
Salaries and Benefits		$10,750
Supplies, Expenses, and Equipment		$20,000
	Total	$30,750
Illinois Natural History Survey		
Salaries and Benefits		$14,000
Supplies, Expenses, and Equipment		$10,000
Waiver of Overhead		$5,830
	Total	$29,830
Pittsburg State University		
Salaries and Benefits		$182,000
Supplies, Expenses, Equipment, and Waiver of Overhead		$4,000
	Total	$186,000
Southern Illinois University-Carbondale		
Salaries and Benefits: SY @ 0.10 FTE		$13,320
Supplies, Expenses, and Equipment		$24,000
Waiver of Overhead		$24,000
	Total	$61,320
Purdue University		
Salaries and Benefits: SY @ 0.05 FTE		$5,130
Supplies, Expenses, and Equipment		$30,130
	Total	$35,260
	GRAND TOTAL	$343,160

[1]Since cost sharing is not a legal requirement institutions do not need to maintain documentation.

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.

Objective 3: Completed in Year 1.

LIST OF PRINCIPAL INVESTIGATORS

Paul B. Brown, Purdue University

Donald L. Garling, Michigan State University

Michael L. Hooe, Illinois Natural History Survey

Robert J. Sheehan, Southern Illinois University-Carbondale

Bruce L. Tetzlaff, Southern Illinois University-Carbondale

James R. Triplett, Pittsburg State University

David H. Wahl, Illinois Natural History Survey

VITA

Paul B. Brown
Assistant Professor
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West Lafayette, IN 47907

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EDUCATION

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

POSITIONS

Assistant Professor, Department of Forestry and Natural Resources, Purdue University (1989-present)
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 Fisheries Society: Membership Concerns Committee (National) 1985-present; Walleye Technical Committee; Fish Culture Section 1985-present; Walleye Technical Committee (North Central Division) 1988-present, Fish Culture Section, Indiana Chapter
World Aquaculture Society, United States Chapter
International Association of Astacology
American Institute of Fishery Research Biologists
American Association for the Advancement of Science
Sigma Xi, Gamma Sigma Delta

SELECTED PUBLICATIONS

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. Progressive Fish-Culturist 51:149-151.

Brown, P. B., D. A. Davis, and E. H. Robinson. 1988. An estimate of the dietary lysine requirement of juvenile red drum. Journal of the World Aquaculture Society 19:109-112.

Robinson, E. H., D. LaBomascus, P. B. Brown, and T. L. Linton. 1987. Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. Aquaculture 64:267-276.

Robinson, E. H., S. D. Rawles, P. B. Brown, H. E. Yette, and L. W. Green. 1986. Dietary calcium requirement of channel catfish *Ictalurus punctatus*, reared in calcium-free water. Aquaculture 53:263-270.

Brown, P. B., R. J. Strange, and K. R. Robbins. 1985. Protein digestibility coefficients for yearling channel catfish fed various high-protein feedstuffs. Progressive Fish-Culturist 47:94-97.

VITA

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Professor and Fish Culture and
Fisheries Extension Specialist
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI 48824

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EDUCATION

B.S. University of Dayton, 1970
M.S. Eastern Kentucky University, 1972
Ph.D. Mississippi State University, 1976

POSITIONS

Professor, Department of Fisheries and Wildlife, Michigan State University (1990-present)
Aquaculture and Fisheries Extension Specialist, Department of Fisheries and Wildlife, Michigan State University (1980-present)
Associate Professor, Department of Fisheries and Wildlife, Michigan State University (1985-1990)
Assistant Professor, Department of Fisheries and Wildlife, Michigan State University (1980-1985)
Assistant Professor of Fisheries Science, Department of Fisheries and Wildlife Sciences, Virginia Institute and State University (1976-1980)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture and Fisheries Educators Sections
Beta Beta Beta
Sigma Xi
Gamma Sigma Delta

SELECTED PUBLICATIONS

Garling, D. L. 1991. NCRAC research programs to enhance the potential of yellow perch aquaculture in the region. Pages 253-255 *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.

Westmaas, A., W. Young, and D. Garling. 1991. Induction of polyploids in bluegill and chinook salmon. Pages 110-112 *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.

Machado, J. P., T. G. Bell, D. L. Garling, Jr., N. R. Kevern, and A. L. Trapp. 1989. Effect of carbon monoxide and exposure on gas-bubble trauma in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 46:74-80.

Westerhoff, R., D. L. Garling, and H. A. Tanner. 1988. Development of techniques to produce triploid chinook salmon for stocking the Great Lakes. Journal of the World Aquaculture Society 19:73A.

Garling, D. L., and L. A. Helfrich. 1984. Making plans for commercial fish culture in Michigan. Michigan Cooperative Extension Service Bulletin No. 3-1775. Michigan State University, East Lansing.

VITA

Michael L. Hooe
Associate Research Biologist — Phone: (618) 245-6348
Illinois Natural History Survey
Sam Parr Biological Station
RR 1 Box 174
Kinmundy, IL 62854

EDUCATION

B.S. Eastern Illinois University, 1978
M.S. Eastern Illinois University, 1980

POSITIONS

Associate Research Biologist, Illinois Natural History Survey, Sam Parr Biological Station, Kinmundy, IL (1990-present)
Assistant Research Biologist, Illinois Natural History Survey, Sam Parr Biological Station, Kinmundy, IL (1984-1990)
Research Technician, Illinois Natural History Survey, Sam Parr Biolgical Station, Kinmundy, IL (1981-1984)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Management Section
Organizer and Chairman of the symposium "Crappie Biology and Management" held in conjunction with the 120th Annual Meeting of the AFS 1990.

SELECTED PUBLICATIONS

Hooe, M.L., and D.H. Buck. In Press. Evaluation of the interspecific F_1 hybrid crappies as sport fish in small impoundments. North American Journal of Fisheries Management.

Buck, D.H., and M.L. Hooe. 1987. Evaluation of induced white crappie and black crappie hybridization. Final report for Federal Aid in Fish and Wildlife Restoration Project F-42-R. INHS Technical Report 87/7.

Buck, D.H., and M.L. Hooe. 1986. The production and growth of F_1 hybrid crappie. Illinois Natural History Survey Biological Notes No 125.

Buck, D.H., and M.L. Hooe. 1986. Comparative growth of northern largemouth bass and F_1 hybrid largemouth bass through three growing seasons. Transactions of the American Fisheries Society 115:296-304.

Buck, D.H., and M.L. Hooe. 1983. Evaluation of the hybrid bass (smallmouth male x largemouth female) as a sport fish. Final report for Federal Aid in Fish and Wildlife Restoration Project F-33-R.

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Ph.D. Southern Illinois University-Carbondale, 1984

POSITIONS

Associate Professor, Department of Zoology, Southern Illinois University-Carbondale (1992-present)
Assistant Professor, Department of Zoology, Southern Illinois University-Carbondale (1986-1992)
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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 Behaviour.

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.

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SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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SELECTED PUBLICATIONS

Heidinger, R.C., J.H. Waddell, and B.L. Tetzlaff. 1986. Relative survival of walleye fry versus fingerlings in two Illinois reservoirs. Proceedings of the Annual Conference, Southeast Association of Fisheries and Wildlife Agencies 39:306-311

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

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

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

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

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SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture, Fisheries Educators, Water Quality, and Fisheries Mangement Sections
Kansas Academy of Sciences
Kansas Commercial Fish Growers
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SELECTED PUBLICATIONS

Triplett, J.R., D.A. Culver, and G.B. Waterfield. 1981. An annotated bibliography on the effects of water-level manipulations on lakes and reservoirs. Ohio State University, Columbus.

Drenner, R.W., G.L. Vinyard, W.J. O'Brien, J.R. Triplett, and J. Wagner. 1980. The zooplankton community of LaCygne Lake: A cooling pond in Kansas. Southwestern Naturalist 26:243-249.

Gash, S.L., R. Gash, J.R. Triplett, R. Taffanelli, and J.C. Bass. 1972. Helminth parasites of Dry Wood Creek fishes in Bourbon and Crawford counties, Kansas. Transactions of the Kansas Academy of Science 75:245-250.

Bass, J.C., J.R. Triplett, and W.T. Waller. 1970. Fishes in the Kansas segment of the West Fork of Drywood Creek. Southwestern Naturalist 15:138-141.

Branson, B.A., J.R. Triplett, and R. Hartmann. 1969. A partial biological survey of the Spring River drainage in Kansas, Oklahoma and Missouri. Part II: the fishes. Transactions of the Kansas Academy of Science 72(4):429-472.

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American Fisheries Society
Ecological Society of America
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SELECTED PUBLICATIONS

Wahl, D.H., and R.A. Stein. 1990. Food consumption and growth among three esocids: field tests of a bioenergetics model. Transactions of the American Fisheries Society 120:230-246.

Wahl, D.H., and R.A. Stein. 1989. Comparative vulnerability of three esocids to largemouth bass (*Micropterus salmoides*) predation. Canadian Journal of Fisheries and Aquatic Sciences 46:2095-2103.

Mather, M.E., and D.H. Wahl. 1989. Comparative mortality of three escocids due to stocking stress. Canadian Journal of Fisheries and Aquatic Sciences 46:214-217.

Wahl, D.H., and R.A. Stein. 1988. Selective predation by three esocids: the role of prey behavior and morphology. Transactions of the American Fisheries Society 117:142-151.