ATTACHMENT C

PROJECT NAME: Culture of Bluegill and Crappie for Food Fish

FUNDING REQUEST: Year 1 - $62,883
Year 2 - $67,875

DURATION: 2 Years

ADMINIINISTRATIVE ADVISOR: Dr. Charles F. Cole, School of Natural Resources, Ohio State University, Columbus, OH 43210

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JUSTIFICATION

The sunfish (Centrarchidae) of the genus *Lepomis* and *Pomoxis* have significant economic impact in the North Central Region. In Illinois, 50.9 percent of the sportfish harvest is composed of these two genera (Baur 1988). Because of this popularity, the potential for marketing these fish for food is considerable. The Chicago Fish House, one of the major seafood suppliers in the North Central Region has expressed interest in sunfish aquaculture because "...at this time there is a ready market...". In attempts to profit by the popularity of sunfish, tilapia are currently being marketed in parts of the midwest as "bluegill".

Development of sunfish culture for food has been restricted in the past because of legal constraints limiting the sale of sportfish. However, because of the growing interest in aquaculture, these restrictions are being removed. But, because of these past regulations, little effort had been directed to the development of the culture technologies for production of sunfish as a food fish.

Several species of sunfish (particularly crappie, *Pomoxis annularis* and *P. nigromaculatus*, bluegill, *Lepomis macrochirus*, and various hybrids) are currently being raised for food fish in the North Central Region, yet little information is available to aquaculturists. Several impediments to successful production of food-sized sunfish currently exist, including; control of maturation, determination of optimal temperature for growth, and comparisons of performance of the various hybrids.

The life histories of the bluegill and crappie are generally similar. Both are sedentary, littoral fishes whose primary foods are insects and small fish (Pflieger 1975). Spawning behavior is also similar. The male excavates a shallow depression into which he entices a female. After spawning, the male guards the nest until the eggs hatch and the larvae are capable of swimming. This spawning behavior results in high survival of young bluegill and crappie, with overpopulation often the end result.

In food fish production, overpopulation reduces the growth rate 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 these species attain sexual maturity before attaining marketable size. In addition, 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 (F_1) of this cross is a fish which, although sexually fertile, has a sex ratio skewed to males. In some populations, males comprise greater than 95% of the population (Childers 1967; Lewis and Heidinger 1978). Recently, a hybrid between the white and black crappies has also been developed (Buck and Hooe 1986).

However, despite skewed sex ratios, production of F_2 progeny can occur. 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.

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 fish are also available.

Much has yet to be determined about the biology of the hybrid sunfish and hybrid crappie as it relates to aquaculture. For example, 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/hectare) 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 of nominal species has been examined to a limited extent. However, little, if any, attention has been paid to the relative performance of individuals in production or the high proportion of male progeny desired for hybrid aquaculture stocks. Demonstration of differential, heritable ability to produce male-skewed progenies would establish the basis for breeding schemes aimed at genetic improvement of hybrid sunfish aquaculture stocks. In addition, the feasibility of using the technology of chromosome manipulation should be examined. The use of sterile, triploid sunfish would eliminate the problem of overpopulation and stunting.

This project has been developed using an interdisciplinary approach, requiring the expertise and facilities of two institutions: Southern Illinois University (SIU) and Michigan State University (MSU). In addition, the facilities of the Evert Fish Farm, located in Missouri will also be utilized.

The overall goals of this project are to: 1) promote the genetic improvement of production characteristics for fingerling and food fish production; and 2) develop optimal production techniques for sunfish.

Upon successful completion of these goals, the potential of sunfish for food production will be determined at various temperatures and densities. In addition, the potential improvement in growth and control of reproduction by genetic manipulation will be evaluated.

RELATED CURRENT AND PREVIOUS WORK

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 it amenable to fish culture.

Ellison and Heidinger (1978) demonstrated that the hybrid sunfish grows 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) suggests that this characteristic make 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 made. In addition, optimum stocking rates in cages and ponds has yet to be determined. None of this data are 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.

The Industry Avisory Council of the North Central Regional Aquaculture Center has identified three major problems needing resolution for the development of sunfish aquaculture in the North Central Region: (1) the control of reproduction, either by the production of monosex fish or through the production of sterile polyploids, (2) the determination of the range of temperatures needed for growth, and (3) the requirements for intensively rearing sunfish larvae.

Mechanisms of Sex Control and Polyploidy

The control of overpopulation in sunfish can be controlled in two ways; through the refinement of the techniques for producing interspecific hybrids to assure highly skewed sex ratios, and through the production of polyploids.

Relatively rapid progress in improving the production characteristics of sunfishes could be made through more efficient utilization of interspecific hybrids. Such hybrid crosses frequently show accelerated growth relative to the parental species, and male-skewed sex ratios (Hubbs and Hubbs 1933; Childers and Bennett 1961; Childers 1967). Skewed sex ratio is of interest because the growth of the males is more rapid than that of the females. Wide variation of sex ratios among hybrid sunfishes has been reported in the literature, even among particular crosses between the same parental species (Table 1). This phenomenon can also be noted in other crosses (e.g., 69% males among green sunfish female x redear sunfish male hybrids produced by Childers (1967), and 99% males from this cross produced by Heidinger and Lewis (1972). The unpredictability of sex ratios has limited the commercial utility of hybrid sunfishes.

The reasons for variability in sex ratios in *Lepomis* hybrid crosses are unknown, except that it is not a simple matter of selective mortality of the ova of one sex. Presumably the skewed sex ratio is the consequence of the genetic mechanism of sex determination. The mechanism of sex determination in this genus has not been studied. Systematic study of the variability of sex ratios among particular interspecific pairings of *Tilapia* species (Hulata et al 1983; Majumdar and McAndrew 1983) demonstrated intrapopulation variation for sex determination in these species, leading to the preposal of models of sex determination through the action of several autosomal loci (e.g., Hammerman and Avtalion 1979; Wohlfarth and Hulata 1981). Polygenic sex determination has also been proposed for poeciliids (Kallman 1984).

Studies of *Lepomis* hybrids have always examined populations composed of mixed progeny groups, thus overlooking the possibility of variability in the sex ratios among progenies of individual pairings. Examination of particular, replicated interspecific pairings would provide otherwise unavailable data useful for explaining the reported variability of sex ratios among *Lepomis* hybrids, and for determining whether the sex ratio of such pairings is repeatable, and thus heritable and exploitable as the basis for family selection for skewed sex ratio. Such knowledge would be useful in evaluating the likelihood of developing breeding programs aimed at reliable production of all-male hybrid aquaculture stocks, and in the development of a model of sex determination in the genus *Lepomis*.

Table 1. Sex ratios of green sunfish (GS) x bluegill (BG) hybrids.

Cross (Female x Male)	Brood Stock Source	% Males	Reference
GS x BG	Michigan	81	Hubbs and Hubbs (1933)
	Michigan	87	Laarman (1973)
	Central Illinois	97	Childers and Bennett (1961)
	Southern Illinois	80	Ellison and Heidinger (1978)
	Texas	66-78	Crandall and Durocher (1980)
	Mississippi	95	Brunson and Robinette (1987)
BG x GS	Central Illinois	64-70	Childers (1967)
	Southern Illinois	71	Lewis and Heidinger (1971a)

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. 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 fail 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 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. The resulting egg has two identical set 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. 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. 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). Both Michigan State University and Southern Illinois University have demonstrated the ability to induce high percentages of triploidy with high survival rates in a variety of salmonids and their hybrids (Seeb et al. 1988; Seeb and Miller 1989).

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 reguardless 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) speculate 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 puncatus*) 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 noblis*) 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; Blanc et al. 1987) 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. 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.

Comparisons of Performance of Various Hybrids

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. Among these comparisons in optimum temperature, growth dynamics, and food conversion, and determination of optimum stocking density for growth and survival.

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 thermal tolerance of 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, a mathmatical model can be constructed in conjunction with extension biologists to predict growth rates and feed costs for different geographical locations or different thermal regimes in water re-use systems. 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 production-scale studies outlined in this proposal.

The thermal requirements of the bluegill are well documented (see Reynolds and Casterlin 1979, 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 the 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 24.0ºC for bluegill, green sunfish, white crappie, and black crappie, respectively. We are unaware of any data on the thermal requirements for either hybrid. 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 10.4ºC.

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) recommend stocking hybrid sunfish at rates of 741 to 7,400 fish per hectare 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 per hectare to examine the effects of feeding on growth and condition during winter. These fish were harvested at mean weights of 34 to 45 grams, far below production weights of 200-225 grams. Lewis and Heidinger (1971b) suggest that hybrid sunfish could be grown at densities of 1,500 to 2,000 kg per hectare in ponds, although the maximum which they attained was 1,000 kg.

No literature is available on the density of hybrid crappie that can be reared in ponds. There is also no data on optimum numbers of hybrid sunfish or hybrid crappie which can be held in floating cages or intensively in tanks. Heidinger (1975) achieved a 30% weight gain over 75 days when hybrid sunfish were stocked in cages at a density of 6.8 kg per m^3 (200 fish per m^3). Since there was no attempt to reach production levels in that study, the density was far below the 100 kg per m^3 expected when rearing species such as channel catfish.

OBJECTIVES

1. To determine the mechanisms of sex control in sunfish and to produce and evaluate polyploid sunfish and hybrids.

2. To compare the optimum temperature-food conversion relationship of selected hybrids with those of the parentals, and to determine optimum stocking densities of hybrid sunfish.

ATTACHMENT C

PROCEDURES

Objective 1

Two potential mechanisms for population control in the sunfishes will be examined. Dr. Lisa Seeb of SIU will investigate the heritability of sex contol in interspecific sunfish hybrids while Drs. Donald Garling of MSU and James Seeb of SIU will examine the production and evaluation of induced polyploidy.

To determine the mechanisms of sex control, progenies of both reciprocal crosses involving the green sunfish and the bluegill will be produced. Adults will be obtained from populations producing almost exclusively male hybrid progeny (Illinois), and from populations producing a lesser proportion of males (e.g., Arkansas or Texas). Breeding sized males and females will be individually marked with PIT tags and held in net enclosures in ponds, and monitored regularly for reproductive readiness. Most females should be reproductively ripe in early summer with many yielding additional batches of eggs intermittently throughout the summer.

The basic design involves stripping eggs from females as they become ripe, dividing the egg mass into several groups, and fertilizing each group with semen of one of several different males of the other species. Accurate spawning records will be kept, and efforts will be made to produce all pairings needed to complete four female x four male diallele designs, ultimately involving on the order of 10 female and 5 males in 2 to 4 such diallele series. Each pairing-specific group of eggs will be reared separately in small vessels through yolk sac absorbtion, later in aquaria or in enclosures in raceways, tanks, or ponds. The sex ratio of all pairing-specific progeny groups will be determined as soon as the young fish become large enough for reliable sexing at 50 to 75 mm in total length.

Replicate pairing-specific progenies will be produced whenever possible. Limited numbers of such replicates should become available as females produce additional groups of eggs late in the summer of the first year of the project, and larger numbers would become available the following spring.

To examine population control in sunfish through induced polyploidy MSU will focus on determining the mechanisms of polyploid induction in the bluegill while SIU will examine the performance of triploid hybrid sunfish and triploids of the parentals.

It will be relatively easy to modify existing thermal shock protocols (e.g. Busack and Baldwin 1988) to produce some triploid individuals. Success rate of induced triploidy may be quite low, but the early production of even a few triploids and their subsequent grow-out will permit gross evaluation ofgonadal development by year two of this study. SIU will initiate such study in year one, using bracketing heat shocks 12ºC above and 12ºC below the ambient water temperature to guarantee the production of some triploids. SIU and MSU will collaborate in both years one and two to both develop polyploidy techniques for *Lepomis* species and evaluate performance characters including growth and food conversion.

To determine the mechanism of induced polyploidy in sunfish, MSU will first determine the time/temperature relationships of the second meiotic and first mitotic events of a normally developing bluegill zygote. Adult bluegill will be captured from lakes near MSU before they begin to spawn naturally, and throughout the spring of the year. The adult bluegill will be transported to the MSU Fisheries Research Lab and held in large holding tanks. This procedure will allow researchers access to ripening fish in the laboratory four to eight weeks prior to the bluegill spawning in nature. The additional time will provide sufficient opportunity to complete the first phase of the study.

Several days prior to the initial egg development experiments, selected bluegill will be injected with human chorionic gonadotropin (HCG) to assure conditioning of the fish for spawning. Eggs and milt will be mixed thoroughly in a watch glass and 50 mL of water will be added. The watch glass will be placed into an equilibrated electric water bath and maintained at 20ºC. A subsample of fertilized eggs will be observed under a light microscope at 20X to 50X to observe the extrusion of the polar body in the second meiotic division, and the first mitotic cleavage. The development of the bluegill zygote beyond the first cleavage has been documented by Morgan (1951).

The information gained from phase one will be used to complete phase two. Initially, triploidy or tetraploidy will be induced directly using heat shock, cold shock, or hydrostatic pressure. The experimental design for each treatment will be a randomized complete block, with three replicates. Analysis of variance will include partitioning for trend effects (e.g., linear or quadradic), for time with temperature held constant effects, and for temperature with time held constant effects, for heat, cold, or pressure treatments. Experiments will continue until an effective protocol can be found for polyploid induction.

Fertilized bluegill ova from a group of ripe adult females (8 to 12) will be collected as described above. The watch glass containing the eggs will then be immersed into a water bath of appropriate temperature.

The control group will be maintained at 20ºC while the treatment groups will be exposed to the appropriate shocks. Heat shocked eggs will be immersed in water baths at 30, 35, or 40ºC for periods of 2.5, 5.0, 7.5 or 10.0 minutes. Initially, these shocks will be induced 30 minutes after fertilization.

If heat shocks are not successful in producing tetraploid bluegill, cold shocks or pressure shocks will be examined in the second year of the study. Cold shocks will consist of immersion into water baths at 7ºC for 0 (control), 30, 60, or 90 minutes. A second series of experiments will hold time constant at 60 minutes, and vary temperature to 5, 7, 9, or 11ºC.

Following treatment, ova in each watch glass will be transferred, without acclimation, to individual 38-L aquaria, which are supplied with standard aeration and filtration. The eggs will be incubated at 26ºC to reduce hatching time and fungal growth.

Upon hatching, the bluegill larvae will initially be fed newly hatched brine shrimp naplii, and later, appropriately-sized commercial feeds. The fish will be reared to a size suitable for ploidy testing in 38-L aquaria. Bluegill should grow to a size of approximately 38 mm in three months. Blood samples will be collected and analyzed using flow cytometry to determine the presence and percentage of triploids and/or tetraploids. Experimental times and temperatures for shocking will be adjusted accordingly, based on egg viability and polyploid induction determined from the initial tests.

Flow cytometry has been found to be much more efficient than karyotyping for the determination of ploidy in fish. Flow cytometry has been used to determine the ploidy of salmonids (Thorgaard et al. 1982; Johnson et al. 1984; and others). Techniques used at the MSU Flow Cytometry Laboratory for identification of triploid chinook salmon should be applicable, with minor modification, for identification of diploid, triploid, and tetraploid sunfish.

Fifteen fingerlings from each experimental group will be used for ploidy determination. A sample of blood will be obtained from each fish by cardiac puncture and suspended in a sodium citrate buffer solution to prevent clotting. The samples will then be frozen in liquid nitrogen (-80ºC) for storage until analysis.

Procedures will be similar at SIU where diallele diploid and triploid replicate crosses will be made with bluegill and green sunfish. In collaboration with MSU, the effects of time, duration, and temperature of both heat and cold shocks on the induction of polar body retention will be measured. Comparative growth studies will also be made to evaluate the performance of the polyploids.

Both flow cytometry and allozyme electrophoresis will be used to determine ploidy of the crosses made at SIU. If electrophoresis of proteins provides a satisfactory means of detecting ploidy in sunfish and their hybrids (similarly, see Allen et al. 1982; Seeb et al. 1988; Seeb and Miller 1989), then it will provide a powerful tool which is more readily available to aquaculturists than flow cytometry.

Objective 2

To determine the extent to which the sunfish aquaculture industry can grow, several parameters pertaining to their culture must be determined. The goals of this objective are, thus, to determine the thermal requirements for adequate growth and food conversion, and to determine whether sunfish can be reared at economically high densities. Completion of this objective will allow the development of a model which could be used to determine the efficacy of either the extensive or intensive culture of sunfish in the North Central Region.

The growth performance in relation to temperature of hybrid sunfish (male bluegill x female green sunfish) or of white crappie, black crappie, and their hybrid will be determined in the first of two experimental trials. The second growth performance trial will be conducted with the hybrid (and its respective parentals) not examined in the first study or with a promising genetically manipulated sunfish identified in Objective 1. Two appropriate strains, species, or hybrids will be selected for study and examined "side-by-side" along with the genetically manipulated sunfish for comparison purposes, should the latter route be taken.

These studies will be conducted at Southern Illinois University, and similar protocols will be utilized in both growth trials. Growth performance trials will begin when the experimental fish are approximately 5 cm in length. 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 8-28ºC at 5ºC 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 evaluation of groups of 10-15 hybrids and similarly sized groups of each of the two parentals in each tank. Water inputs to the tanks will enter a central reservoir in which three holes are 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 1ºC per day from the initial acclimation temperature (about 20ºC) 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 once the trials begin. The feed will be a commercially available, nutritionally complete, 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. We anticipate 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. Standard water quality parameters will be monitored throughout the study, including dissolved oxygen, ammonia, nitrite, pH, and alkalinity.

Analysis of variance will be used to compare growth and feed conversion at the various temperatures for each species, hybrid, or genetically manipulated fish. The fish will be sexed at the end of the 90-day trial to determine sex ratios and relationships between growth, food conversion, and sex. 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-drived 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 effects of density on the production of food-sized hybrid sunfish will be conducted in cages the first year and in ponds during the second year. Yearling hybrid sunfish will be stocked into 1-m^3 cages at the following densities: 3 cages at 100 fish per m^3, 3 cages at 200 fish per m^3, and 3 cages at 400 fish per m^3. The cages will be placed into three 0.04-hectare ponds with one cage from each treatment in each pond. The fish will be fed daily at 3% bodyweight per 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 which will be developed in the laboratory. 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.

The examination of density in ponds will follow similar methodology. Six 0.2-hectare ponds located in southern Missouri will be stocked with yearling hybrid sunfish at the following densities: 2 ponds at 2,445 fish per hectare, 2 at 4,895 fish per hectare, and 2 at 7,340 fish per hectare. These stocking rates allow for 10% mortality and are designed to produce production levels of 500, 1,000, and 1,500 kg per hectare at harvest, assuming a 227 gram fish.

Growth, food conversion, and temperature data collected in the pond study will also be used to test the laboratory model.

Dependent on the initial evaluation of polyploid sunfish which will be made in Objective 1 of this study, the effects of density on any genetically-manipulated strain of sunfish which demonstrates potential for aquaculture will also be evaluated the second year. Methodology for this evaluation will be identical to the cage study described above.

ATTACHMENT C

FACILITIES

Objective 1

Production of hybrid sunfish larvae will be conducted under the direction of Dr. Lisa Seeb at the SIU aquaculture facility. The facility has a wide range of ponds, tanks, aquaria, and associated equipment. Production and evaluation of polyploid sunfish will also be conducted at this facility under the direction of Dr. James Seeb.

All genetic procedures at SIU, except flow cytometry, will be performed in the Genetics Lab of the SIU Fisheries Research Laboratory. The laboratory is equiped to perform allowzyme analyses, mitochondrial DNA analyses, induced polyploidy, and chromosomal studies. Flow cytometry will be conducted by Dr. Stan Allen, Rutgers University, who has perfected techniques to analyze the ploidy of tiny larvae at about the 2,000-cell stage (e.g., Chaiton and Allen 1985).

Identification of critical timing of meiotic and mitotic events, polyploid induction in bluegill, and bluegill grow-out will be conducted under the direction of Dr. 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.

Objective 2

Determination of the optimum temperature/food conversion relationship for sunfish will be directed by Dr. Robert Sheehan at the SIU Fisheries Research Laboratory. The SIU 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 SIU pond research facility and the Everet Fish Farm in southeast Missouri. The SIU facility consists of eighteen 0.04-hectare drainable ponds. The Everet Fish Farm contains twelve 0.2-hectare drainable ponds.

REFERENCES

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.

Allen, S.K., P.S. Gagnon, and H. Hidu. 1982. Induced triploidy in the soft-shell clam. Journal of Heredity 73:421-428.

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

Benfey, T.J., 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.

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

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

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.

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 Wilflife 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 6:156-167.

Brunson, M.W., and H.R. Robinette. 1987. Reproductive isolation between a hybrid sunfish and its parental species. Progressive Fish-Culturist 49:296-298.

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

Cassani, J.R., W. Caton, and B. Clark. 1984. Morphological comparisons of diploid and triploid hybrid grass carp (*Ctenopharvaodon idella*) female x (*Hypothalmichthus nobilis*) male. Journal of Fish Biology 25(3):269-278.

Chaiton, J.A., and S.K. Allen. 1985. Early detection of triploidy in larvae of Pacific oysters (*Crassostrea gigas*) by flow cytometry. Aquaculture 48:35-44.

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

Childers, W.F., and G.W. Bennett. 1961. Hybridization between three species of sunfish (*Lepomis*). Illinois Natural History Survey Biological Notes, No. 46, 15 pp.

Chourrout, D. 1982. Tetraploidy induced by heat shocks in the rainbow trout (*Salmo gairdneri* Richardson). 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.

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

Crandall, P.S., and P.P. Durocher. 1980. Comparison of growth rates, sex ratios, reproductive success and catchability of three sunfish hybrids. Annual Proceedings Texas Chapter of the American Fisheries Society (Texas Parks and Wild. Dept. Austin) 2:88-104.

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

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

Hammerman, I.S., and R.R. Avtalion. 1979. Sex determination in *Sarotherodon* (Tilapia). The sex ratio as a tool for the determination of genotype: a model of autosomal and gonosomal influence. Theoretical and Applied Genetics 55:177-187.

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. Proceedings of the Third British Freshwater Fish Conference, Liverpool, England.

Heidinger, R.C., and W.M. Lewis. 1972. Potential of the redear sunfish x green sunfish in pond management. Progressive Fish-Culturist 32:107-109.

Hubbs, C.L., and L.C. Hubbs. 1933. The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. Papers of the Michigan Academy of Science, Arts, and Letters 17:613-641.

Hulata, G., G. Wohlfarth, and S. Rothbard. 1983. Progeny-testing selection of tilapia brood stocks producing all-male progenies: preliminary results. Aquaculture 33:263-268.

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.

Kallman, K.D. 1984. A new look at sex determination in poeciliid fishes. Pages 95-11, *in* B.J. Turner, editor. Evolutionary Genetics of Fishes. Plenum Press, New York.

Laarman, P.W. 1973. Production from hybrid sunfish populations. Michigan Department of Natural Resources. Federal Aid in Fish Restoration, Project F-29-R-7, Final Report, Lansing.

Lemoine, H., Jr., and L. Smith. 1980. Polyploidy induced in brook trout by cold shock. Transactions of the 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(4):619-623.

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

Lewis, M.W., 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 approachs to the management of small impoundments. North-central 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., and A. Scott. 1984. Sexual maturation in triploid rainbow trout (*Salmo gairdneri* Richardson). Journal of Fish Biology 25:385-392.

Majumdar, K.C., and B.J. McAndrew. 1983. Sex ratios from interspecific crosses within the tilapias. Pages 261-269 *in* L. Fishelson and Z. Yaron, editors. Proceedings of the International Symposium on Tilapia in Aquaculture. Nazareth, Israel, 8-13 May, 1983.

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. Proceedings of the World Syposium on Aquaculture in Heated Effluents and Recirculation Systems, Vol. II. Heenemann, Berlin.

Morgan, G.D. 1951. The life history of the bluegill sunfish L.M. of Buckeye Lake, Ohio. Denison University Science Laboratory Journal 42:21-59.

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

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

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.

Seeb, J.E., and G.D. Miller. In press. The integration of allozyme analyses and genomic manipulations for fish culture and management. *In* D.H. Whitmore, editor. Application of electrophoresis and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, Florida.

Seeb, J.E., G.H. Thorgaard, and F.M. Utter. 1988. Survival and allozyme expression in diploid and triploid hybrids between chum, coho, and chinook salmon. Aquaculture 72:31-48.

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

Solar, I.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., 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.

Thorgaard, G.H., P. Rabinovitch, M. Shen, G. Gall, J. Prop, and F. Utter. 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture 29:305-309.

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

Wohlfarth, G.W., and G. Hulata. 1981. Applied genetics of tilapia. ICLARM Studies and Reviews 6:1-26.

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.

ATTACHMENT C

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	James E. Seeb Southern Illinois University	Fish Genetics
	Lisa W. Seeb Southern Illinois University	Fish Genetics
	Robert J. Sheehan Southern Illinois University	Aquaculture/Physiology
	Bruce L. Tetzlaff Southern Illinois University	Aquaculture/Water Re-use
Michigan	Donald L. Garling Michigan State University	Fish Culture/Polyploidy

ATTACHMENT C

INDIVIDUAL BUDGETS FOR PARTICIPATING INSTITUTIONS

Michigan

Michigan State University
Donald Garling

Illinois

Southern Illinois University
Lisa Seeb
James Seeb
Robert Sheehan
Bruce Tetzlaff

**PROPOSED SUNFISH BUDGET FOR
MICHIGAN STATE UNIVERSITY**

(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	0.05	1	0.05	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	1	0.25	1	0.25	$6,000	$7,200
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other ...						
Total Salaries and Wages					$6,000	$7,200
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$6,000	$7,200
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$500	$700
F. Travel - Domestic (*Including Canada*)					$700	$700
G. Other Direct Costs					$600	$600
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$7,800	$9,200
				TOTAL PROJECT COSTS	$17,000	

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

ATTACHMENT C

PROPOSED SUNFISH BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY

(J. Seeb and L. Seeb)

Objective 1

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.05	1	0.05	$0	$0
b. Senior Associates	1	0.085	1	0.085	$3,258	$3,421
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.25	1	0.25	$3,900	$4,173
c. Graduate Students	1	0.50	1	0.50	$9,312	$9,777
d. Prebaccalaureate Students	1	0.20	1	0.20	$1,500	$1,700
e. Secretarial-Clerical						
f. Technical, Shop, and Other ...						
Total Salaries and Wages					$17,970	$19,071
B. Fringe Benefits					$1,863	$1,911
C. **Total Salaries, Wages and Fringe Benefits**					$19,833	$20,982
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$4,157	$3,800
F. Travel - Domestic (*Including Canada*)					$2,400	$2,600
G. Other Direct Costs					$1,600	$1,800
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$27,990	$29,182
				TOTAL PROJECT COSTS	$57,172	

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

ATTACHMENT C

PROPOSED SUNFISH BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY

(Tetzlaff and Sheehan)

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	2	0.15	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	2	0.875	2	0.875	$17,437	$18,656
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$17,437	$18,656
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$17,437	$18,656
D. Nonexpendable Equipment					$1,335	$0
E. Materials and Supplies					$4,205	$7,300
F. Travel - Domestic (*Including Canada*)					$1,000	$1,300
G. Other Direct Costs					$3,116	$2,237
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$27,093	$29,493
TOTAL PROJECT COSTS					$56,586	

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

CULTURE OF BLUEGILL AND CRAPPIE FOR FOOD FISH

Budget Summary for Each Participating Institution at 62.9K for the First Year

	MSU	SIU	TOTALS
Salaries and Wages	$6,000	$35,407	$41,407
Fringe Benefits	$0	$1,863	$1,863
Total Salaries, Wages and Benefits	**$6,000**	**$37,270**	**$43,270**
Nonexpendable Equipment	$0	$1,335	$1,335
Materials and Supplies	$500	$8,362	$8,862
Travel	$700	$3,400	$4,100
Other Direct Costs	$600	$4,716	$5,316
TOTAL PROJECT COSTS	**$7,800**	**$55,083**	**$62,883**

Budget Summary for Each Participating Institution at 67.9K for the Second Year

	MSU	SIU	TOTALS
Salaries and Wages	$7,200	$37,727	$44,927
Fringe Benefits	$0	$1,911	$1,911
Total Salaries, Wages and Benefits	**$7,200**	**$39,638**	**$46,838**
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$700	$11,100	$11,800
Travel	$700	$3,900	$4,600
Other Direct Costs	$600	$4,037	$4,637
TOTAL PROJECT COSTS	**$9,200**	**$58,675**	**$67,875**

ATTACHMENT C

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

(Salaries, Supplies, Expenses and Equipment)

Institution/Item		Year 1	Year 2
Michigan State University			
Salaries and Benefits: SY @ 0.05 FTE		$2,940	$2,940
Supplies, Expenses and Equipment		$6,371	$6,371
	TOTAL PER YEAR	**$9,311**	**$9,311**
Southern Illinois University			
Salaries and Benefits: SY @ 0.25 FTE		$9,982	$9,982
Supplies, Expenses and Equipment:		$27,213	$30,412
	TOTAL PER YEAR	**$37,195**	**$40,893**
	GRAND TOTAL	**$46,506**	**$50,204**

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

ATTACHMENT C

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

ATTACHMENT C

LIST OF PRINCIPAL INVESTIGATORS

Donald L. Garling, Michigan State University

James E. Seeb, Southern Illinois University

Lisa W. Seeb, Southern Illinois University

Robert J. Sheehan, Southern Illinois University

Bruce L. Tetzlaff, Southern Illinois University

ATTACHMENT C

VITA

Donald L. Garling, Jr.
Associate Professor and
Fish Culture and Fisheries Extension Specialist
Department of Fisheries and Wildlife
Michigan State University
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EDUCATION

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

POSITIONS

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

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 gaidneri*). 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. Presented at the Annual Meeting of the World Aquaculture Society, January 4-9, Honolulu.

Masterson, M.F., and D.L. Garling. 1986. Effect of feed color on feed acceptance and growth of walleye (*Stizostedion vitreum* v.) fingerlings. Progressive Fish-Culturist 48:306-309.

Ostrowski, A.O., and D.L. Garling. 1986. Dietary androgen-estrogen combinations in growth promotion in fingerling rainbow trout. Progressive Fish-Culturist 48:268-272.

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

VITA

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Assistant Professor
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Southern Illinois University
Carbondale, Illinois 62901-6511

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EDUCATION

B.S. University of Puget Sound 1974
M.S. University of Washington 1982
Ph.D. University of Washington 1987

POSITIONS

Assistant Professor, Southern Illinois University-Carbondale (1988-present)
Research Assistant Professor, University of Idaho, Moscow (1987-1988)
Graduate Assistant, University of Washington, Seattle (1982-1986)
Fish Biologist, Washington Department of Fisheries, Olympia (1978-1980)
Fish Biologist, Pacific Fisheries Research, Seattle (1976-1978, 1980-1982)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
American Genetics Association
American Society of Ichthyologists and Herpetologists
Genetics Society of America
International Association for Genetics in Aquaculture
Sigma Xi

SELECTED PUBLICATIONS

Seeb, J.E., and G.D. Miller. In press. The integration of alloenzyme analyses and genomic manipulations for fish culture and management. *In* D.H. Whitmore, editor. Application of electrophoresis and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, Florida.

Seeb, J.E., F.M. Utter, and G.H. Thorgaard. 1988. Survival and alloenzyme expression in diploid and triploid hybrids between chum, chinook, and coho salmon. Aquaculture 72:31-48.

Seeb, J.E., L.W. Seeb, D.W. Oates, and F.M. Utter. 1987. Genetic variation and postglacial dispersal of populations of northern pike (*Esox lucius*) in North America. Canadian Journal of Fisheries and Aquatic Sciences 44:556-561.

Scheerer, P.D., G.H. Thorgaard, and J.E. Seeb. 1987. Performance and developmental stability of triploid tiger trout (brown trout x brook trout male). Transactions of the American Fisheries Society 116:92-97.

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EDUCATION

A.B. University of California-Berkeley 1973
M.A. University of Montana 1977
Ph.D. University of Washington 1986

POSITIONS

Research Assistant Professor, Southern Illinois University-Carbondale (1988-present)
Research Assistant Professor, University of Idaho, Moscow (1984-1988)
Graduate Assistant, University of Washington, Seattle (1982-1988)
Fish Biologist, Pacific Fisheries Research, Olympia, WA (1978-1981)
Fish Geneticist, National Marine Fisheries Service, Seattle (1977-1979)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
American Society of Ichthyologists and Herpetologists
Genetics Society of America
Society for the Study of Evolution
Sigma Xi.

SELECTED PUBLICATIONS

Seeb, L.W., J.E. Seeb, R.L. Allen, and W.K. Hershberger. 1990. Evaluation of adult returns of genetically marked chum salmon with suggested future applications. American Fisheries Society Symposium 7:418-425.

Seeb, L.W., and D.R. Gunderson. 1988. Genetic variation and population structure of Pacific ocean perch (*Sebastes alutus*). Canadian Journal of Fisheries and Aquatic Sciences 45:78-88. Copeia 1984(1):120-132.

Seeb, J.E., and L.W. Seeb. 1986. Gene mapping of isozyme loci in chum salmon (*Oncorhynchus keta*). Journal of Heredity 77:399-402.

Seeb, J.E., L.W. Seeb, and F.M. Utter. 1986. Use of genetic marks to assess stock dynamics and management programs for chum salmon. Transactions of the American Fisheries Society 115:448-454.

ATTACHMENT C

VITA

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EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

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

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

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

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

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

ATTACHMENT C

VITA

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EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

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

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

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

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

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