CULTURE TECHNOLOGY OF SUNFISH

Chairperson: Robert S. Hayward, University of Missouri-Columbia

Industry Advisory Council Liaison: Curtis Harrison, Hurdsville, Missouri

Extension Liaison: Joseph E. Morris, Iowa State University

Funding Request: $200,000

Duration: 2 Years (September 1, 1999 - August 31, 2001)

Objectives:

1. Conduct field trials of bluegill and F_1 hybrid sunfish (female green sunfish × male bluegill) in commercial size production facilities defined as ponds >0.04 ha and indoor recycle systems in the upper and lower portions of the North Central Region. A minimum of three replicates will be used in all pond and recycle system studies; commercial feeds to be use will be those identified in previous studies.

2. Evaluate grading strategies to enhance grow out in commercial systems to market size ($\geq$227 g), including the culture potential of discards.

Proposed Budgets:

Institution	Principal Investigator(s)	Objective(s)	Year 1	Year 2	Total
Southern Illinois Univ.-Carbondale	Robert J. Sheehan	1	$20,500	$22,500	$43,000
Iowa State University	Joseph E. Morris	1	$17,250	$16,750	$34,000
Iowa State University	Robert A. Summerfelt	1	$12,600	$7,400	$20,000
University of Minnesota	Ira R. Adelman	1	$29,000		$29,000
North Dakota State University	Mark A. Sheridan	1	$21,500	$20,500	$42,000
Univ. of Missouri-Columbia	Robert S. Hayward	2	$16,000	$16,000	$32,000
		TOTALS	**$116,850**	**$83,150**	**$200,000**

Non-funded Collaborators:

Facility	Collaborator(s)
Kloubec Fish Farms, Amana, Iowa	Myron Kloubec
Harrison Fish Farm, Hurdsville, Missouri	Curtis Harrison

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JUSTIFICATION

Sunfish of the genera *Lepomis* and *Pomoxis* already have significant economic impact in the North Central Region (NCR). For example, 51% of the sportfish harvest in Illinois consists of fishes of these two genera (Baur 1988). The potential for marketing sunfish as food fish is considerable because of this popularity. As an example, Michigan consumers want locally produced, farm raised, fresh fish products (Chopak 1992a). Michigan brokers, wholesalers, retailers, and restaurants listed the bluegill as one of the top three species that they would like to purchase for their customers (Chopak 1992b). Representatives of the Chicago Fish House, until recently a major seafood supplier in the Midwest, 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 (R. Rubin, personal communication) of $4.41—5.51/kg to producers for 0.23 kg crappie indicates that sunfish are about double the value of the two major aquaculture species rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*). The significance of the market potential for sunfish has been exploited by producers of other fish species; in some cases tilapia have been marketed in parts of the Midwest through major retail outlets as "bluegill" (*Lepomis macrochirus*). However, a need for updated and more broad scale information on market potential for sunfish throughout the NCR and beyond is recognized.

A number of factors make sunfish strong candidates for commercial food fish production in the Midwest. According to Webber and Riordan (1976), the criteria for commercially marketable aquaculture species include both marketing and biological aspects of the species. The marketing criteria include taste, appearance, texture, and consumer recognition. Bluegill and hybrid sunfish both have good to excellent flavor with a slightly soft texture, which makes them acceptable to a large number of consumers (McLarney 1987). Bluegill and hybrid sunfish also possess biological characteristics that make them favorable for aquaculture including their acceptance of prepared diets (Ehlinger 1989), capacity to tolerate and grow over a wide temperature range (Heidinger 1975), potential to grow rapidly (Krumholz 1949; Breck 1993; Hayward et al. 1997), and the ability to spawn repeatedly (Stickney 1985). The hybrid sunfish produces mostly males (Childers and Bennett 1961; Laarman 1973; Brunson and Robinette 1986), and exhibits reduced reproductive ability (Ricker 1948; Childers 1967).

The suitability of crappies as an alternative aquaculture species has been substantially investigated by the NCRAC Sunfish Work Group. In general, crappie have proven to be much less tolerant to handling, more difficult to train to prepared diets, and more susceptible to disease-related mortalities than have all the lepomids evaluated in a similar manner. Black crappie (*Pomoxis nigromaculatus*) have, in general, survived better and been more easily trained to accept prepared feeds than white crappie (P. *annularis*). Crappie may have advantages in the market as compared with *Lepomis*; crappie may command a higher price, and they may be better suited for the production of a larger fish, but neither possibility is a certainty at this time. A number of impediments to successful crappie food fish culture have already been identified. Crappie, more so than the lepomids, require basic research on their biology to gain insight into how these impediments might be overcome. For example, recent work indicates that crappie do not consume food and grow well at temperatures above 26°C (Hayward and Arnold 1996; J. Triplett, Pittsburg State University, personal communication). Rearing these fish at temperatures that are too high could conceivably account for much of the difficulty that has been experienced in attempts to rear crappie.

A “side-by-side” comparison of production performance of hybrid sunfish and black crappies in ponds was recently completed at Southern Illinois University-Carbondale (SIUC) (R. Sheehan, personal communication). This study found that black crappie survived less well in pond settings than hybrid sunfish (49% versus 75%). Most importantly, mean production of harvestable fish (110 g) was 416.1 kg/ha for hybrid sunfish, but was actually negative for black crappie (-143.8 kg/ha). This result for black crappie confirms that serious impediments presently exist for using crappie in aquaculture. In part as a result of this recent finding, it was decided to focus efforts on lepomids in the near-term sunfish research efforts.

Investigations of the male bluegill (*Lepomis macrochirus*) × female green sunfish (*L. cyanellus*) F_1 hybrid (hereafter, hybrid sunfish) have been carried out to evaluate the usefulness of this fish in traditional recreational fishing pond stocking programs. However, there is increasing interest in hybrid sunfish, as well as bluegill, as a food fish. Hybrid sunfish have shown higher growth rates under colder temperatures than bluegill (Brunson and Robinette 1986) and the hybrid has proven superior to its parent species under intensive culture conditions (see **PROGRESS TO DATE**). Despite these apparent advantages, there appears to be an insufficient information base at present to justify focusing efforts on the hybrid sunfish alone. Consequently, most of the studies proposed herein deal with both the bluegill and the hybrid sunfish. In general, studies that provide clear indication of the potential to raise bluegill and hybrid sunfish to market size as a food fish, including grow-out times required to do so, are warranted in both pond and indoor recycle systems. Clarification of whether the bluegill or hybrid sunfish holds an advantage due to marketability and grow-out characteristics is needed as well, as is the need to develop techniques that will reduce grow-out times below those which are presently suggested.

Among other studies conducted by NCRAC participants (see **RELATED AND CURRENT PREVIOUS WORK**), recent work (Hayward et al.1997) at the University of Missouri-Columbia (UMC) indicates that substantial potential exists to increase growth rates of lepomids beyond those that have been observed to date. Hybrid sunfish fed mealworms, *Tenebrio molitor*, on a repeating no-feed/re-feed schedule which elicited the compensatory growth response, achieved twice the body weight of control counterparts fed daily in a 115-day feeding and growth experiment. This result suggests that grow-out times for this fish in aquaculture might be cut by a factor of two. The UMC study with hybrid sunfish was carried out under highly controlled laboratory conditions which included holding fish individually in small plexiglass test chambers and feeding a natural prey so that daily food consumption by test fish could be accurately measured. Follow-up experiments evaluated whether the growth benefits observed with the hybrid sunfish from compensatory growth feeding could be readily extrapolated to the commercial production setting. Effects of compensatory growth on flesh weight and composition (e.g., protein:fat ratio) were also assessed. Results of the follow-up work indicated that use of commercial feeds was not a problem in that hyperphagia (a component of compensatory growth) was observed when a pelleted commercial feed was used. In addition, proximate analysis of fish that had grown rapidly due to compensatory growth, versus those fed in more standard fashion, revealed no differences in flesh composition. However, the follow-up study did reveal that compensatory growth was slowed when fish were held in groups (as in aquaculture), due to declines in consumption, growth, and growth efficiency that result from social interactions (Hayward et al. In revision). This result, and findings of a related study at UMC (Wang et al. In review), demonstrate that strong social interactions that occur among hybrid sunfish impede growth and promote inter-individual size variation. McComish (1971) had previously demonstrated similar growth-impeding effects due to social interactions in bluegill. These findings indicate that efforts to reduce the negative effects of social hierarchy on growth in both bluegill and hybrid sunfish are warranted as developments in this area may substantially reduce grow-out times. Preliminary work towards ameliorating negative effects of strong social interaction in lepomids is proposed herein (see **PROCEDURES**).

In the NCR, recirculating systems are becoming increasingly important as commercial production units. Increased demand for quality water supplies, a substantially reduced growing season, and widely varying environmental conditions have made the controllability of recirculating systems far more attractive. New technological developments in monitoring, logging, decision processing, and adjusting rearing environments using computerized process control systems have permitted year-round production under optimal conditions. Although recirculating systems are inherently more expensive to operate than open systems, the ability to tightly control the rearing environment, eliminate growing season limitations, reduce water consumption requirements, and increase the number of locations possible for fish production have also opened the door for the commercial production of higher valued species previously considered not suitable for open production. Consequently, it is believed that studies of bluegill and hybrid sunfish production in indoor recycle systems as well as in outdoor ponds are warranted to fully explore the potential to produce these fish for the food fish market.

Commercial-Scale Field Trials (Objective 1)

In both the culture and research settings, most sunfish rearing has been done in ponds, with the objective of producing fish for stocking purposes (Engelhardt 1981; Dupree and Huner 1984; McLarney 1987; Stickney 1985). Consequently, relatively little is known of the potential and time periods that would be required to grow sunfish to the larger sizes necessary to market them as a food fish (≥227g). Tidwell and Webster (1994) found that hybrid sunfish stocked in experimental ponds in Kentucky at relatively large sizes (38 and 66 g) and at two densities (6,175 and 12,359 fish/ha), gained between 70—85 g in one year (April to April); these fish were fed a 36% protein diet. Little information was provided on fish size variation so it was not possible to determine weight gains of the faster growing individuals. Tidwell and Webster (1994) noted that similar rates of weight gain were observed for hybrid sunfish in ponds by Brunson and Robinette (1986), even though lower stocking densities were employed. However, recent work at SIUC (R. Sheehan, personal communication) indicates that at least a significant portion of hybrid sunfish reared in their experimental ponds grew from 1 g to 108 g (upper value approximately 113 g) in a single growing season. This higher weight gain by hybrid sunfish over a shorter time period than in the Tidwell and Webster (1994) study, provides a more promising indication about growth rate potential in ponds.

Concerning diets for sunfish, Tidwell et al. (1992) showed that the use of higher protein feeds (up to 37%) may improve growth and production potential of hybrid sunfish. Webster et al. (1997) found that the same hybrid will grow well on a practical diet containing 35—36% protein (with fish meal comprising 32% of the protein). However, recent studies conducted at Purdue University (Purdue) found significantly higher weight gains for bluegill and hybrid sunfish fed a 40% protein trout diet than for fish fed lower protein levels. Clearly, information concerning the pond rearing of sunfish to market size as food fish is sparse, and in some apparent disagreement. Approaches specifically geared towards increasing growth rates to reduce grow-out times of lepomids have, to date, been underexplored.

In addition to pond rearing, indoor recirculating systems offer controlled environments that can be optimized for the growth of sunfish. The potential to profitably rear sunfish to market size as a food fish would appear particularly promising here, given the potential to substantially extend periods of optimal growth conditions relative to those that are possible in outdoor ponds. Evaluation of the production factors involved in bringing bluegill and hybrid sunfish from fingerling to market size in indoor recycle systems has been identified as a priority by the aquaculture industry in the NCR.

Information concerning the culture of sunfish in indoor recycle systems is very sparse. Of the 169 references considered by Williamson et al. (1993) in their review of centrarchid culture, none dealt with the intensive culture of sunfish. At the program planning meeting for NCRAC in 1996 and 1998, the Industry Advisory Council specifically requested that some of the sunfish projects involve recycle systems to determine their potential for commercial production within the region. Recycle aquaculture systems hold the potential for substantial year-round growth of sunfish, yet, presently there is no information on appropriate rearing densities (kg/m^3) or loading rates(kg/Lpm) or growth and production potential.

Among fishes that develop nests as part of their reproductive strategy, as do bluegills and green sunfish, selective pressure for rapid growing males is apparent in relation to their role as nest guarders. Accordingly, there may be growth advantages associated with rearing male sunfish. Potentially, more rapid growth of males may underlie indicated growth advantages for the hybrid sunfish (versus the bluegill), because the latter are predominantly male. Accordingly, research is also needed to determine whether mixed-sex or single-gender stocks of bluegill show higher growth rates under otherwise identical conditions, and to determine how these growth rates compare to hybrid sunfish.

It is generally recognized by investigators who have worked with bluegill and hybrid sunfish in laboratory environments that these fish develop strong social hierarchies when held in groups. This problem can lead to substantial inter-individual size variation within rearing groups, with substantially reduced mean growth rates of fish resulting (McComish 1971; Hayward et al. In revision; Wang et al. In review). No practical solution to this problem has yet been developed for sunfish, but size grading (to reduce size disparity) and holding fish at high densities have reduced the negative effects of social interaction on fish growth in some species.

Evaluate Grading Strategies (Objective 2)

As previously stated, a need exists to clarify grow-out times required for hybrid sunfish and bluegills to reach market size in ponds and in indoor recycle systems under the present state of technology. Early indications from recent NCRAC-funded work at SIUC (R. Sheehan, personal communication) are that two to three years are required in ponds in the NCR, underscores the need to explore approaches to increase growth rates to reduce grow-out times. A recent study supported by NCRAC at UMC (Wang et al. 1998a) has found that substantial inter-individual variation of inherent origin exists among hybrid sunfish in terms of their consumption and growth rates, as well as their growth efficiency. Juvenile hybrid sunfish (9.8—18.1 g initial weight) held individually (without social influence) and fed *ad libitum* for 50 days, showed marked differences in these three production-related variables. Moreover, magnitudes of consumption, growth, and growth efficiency by these fish were strongly correlated (positively) with initial body weight. This work indicates that hybrid sunfish vary substantially in their inherent (genetic) capacities to grow, but fortunately, slower growing individuals reveal themselves early on, by their slightly smaller sizes at the start of grow out. Hence, removal of smaller individuals at the start of grow out by size-grading, is expected to substantially increase consumption and growth of hybrid sunfish with the added bonus of improved feed conversion. This knowledge could lead to shorter grow-out times and more uniform sizes of sunfish reared in ponds and in indoor recycle systems. Objective 2 emanates largely from the findings of this study by Wang et al. (1998a).

RELATED CURRENT AND PREVIOUS WORK

Commercial-Scale Field Trials (Objective 1)

Sunfish versus Crappie Performance Comparisons

Several years of NCRAC-funded research at SIUC have focused on studies of the growth of diploid and triploid *Pomoxis* and *Lepomis* parental species and associated hybrids in tank-culture studies across a range of temperatures (NCRAC 1996). These studies indicate that diploid black crappie and hybrid sunfish performed as well or better than their respective congeneric taxa. Furthermore, data indicate that the hybrid sunfish performs better than the black crappie under tank-culture conditions. A study at SIUC showed that at temperatures ranging from 8—18°C, hybrid sunfish showed 56-day weight gains of 20—75% while black crappie gains were only 20—45%. Growth of hybrid crappie was much poorer (NCRAC 1997).

Preliminary results from recent work in ponds at SIUC support findings from comparisons of sunfish versus crappie in experimental tanks, and indicate that serious impediments presently exist for using crappie in aquaculture. "Side-by-side" comparisons of production performance of hybrid sunfish and black crappies, each stocked at two densities (8,000 and 14,000 fish/ha) in ponds found that black crappie survived less well in pond settings than hybrid sunfish (49% versus 75%). Mean production of fish ($\geq$110 g) was 416.1 kg/ha for hybrid sunfish, but was actually negative for black crappie (-143.8 kg/ha). There is indication that the dismal performance of black crappies in this study was related to difficulties in getting them to consume the commercial diet provided (R. Sheehan, SIUC, personal communication).

Sunfish Diets

The basis for the majority of commercial production of bluegill and hybrid sunfish has been the provision of relatively small fish for stocking farm ponds. Because of this historical setting where natural food supplies for reared sunfish were largely sufficient, relatively little has been known about the nutritional requirements of lepomids, until quite recently. Brunson and Robinette (1986) fed hybrid sunfish a supplemental diet of channel catfish feed and found that their growth was no better than when no supplemental feeding was given. Earlier, Lewis and Heidinger (1971) had fed hybrid sunfish a supplemantal diet of Purina Trout Chow® containing a higher protein percentage than the catfish diet, and found that these fish grew better than those not fed a supplemental diet. Taken together, these studies hinted that hybrid sunfish may require diets with higher protein levels. More recently, Tidwell et al. (1992) evaluated the protein requirements of 4.7 g hybrid sunfish using practical feeds. They found that fish fed the highest protein diet used in their study (37%) exhibited significantly higher weight gain than fish fed diets with less protein.

Michigan State University (MSU) and Purdue have recently completed NCRAC-funded diet studies for sunfish. In the 3rd NCRAC-funded Sunfish project, researchers at MSU determined the optimum energy level for growth and protein retention for 125 mm hybrid sunfish. In this same project, investigators at Purdue found that the dietary requirement of hybrid sunfish for available phosphorus is 0.5% of the dry diet, and that both bluegill and hybrid sunfish grow best when fed diets containing no less than 10% dietary lipid in the form of fish oil. In the current NCRAC-funded Sunfish project (4th Sunfish project), Purdue also determined that a crude protein level of 40% was required to support maximal growth and minimal feed conversion ratio of hybrid sunfish raised in intensive culture. However, a SIUC study found that growth and food conversion of hybrid sunfish raised in a recycle system and fed a commercial diet with 44% crude protein were significantly greater than fish fed diets with 32 and 36% crude protein. The SIUC investigators concluded "optimal crude protein levels are likely to be in excess of 40% for hybrid sunfish in recirculating culture systems." Wording for Objective 1 of the current proposal indicates that the diet used in the proposed work for sunfish should be selected on the basis of findings from the recent NCRAC-funded sunfish diet studies.

Techniques for Increasing Sunfish Growth

Available studies indicating growth of bluegills and hybrid sunfish reared under state-of-the-art culture technology (e.g., Brunson and Robinette 1986; Tidwell et al. 1992; R. Sheehan, SIUC, personal communication) suggest that technological developments to increase these fishes' growth rates will be necessary. Work in the following topic areas relate to this need.

Compensatory Growth

Compensatory growth is the capacity of animals to grow at higher-than-normal rates when food is replenished after a period (usually more than one day) of its low supply. Compensatory growth occurs in many animal classes including mammals, birds, and fishes (Jobling et al. 1993), with proximate mechanisms associated with the rapid growth state being hyperphagia and increased growth efficiency (Broekhuizen et al. 1994). Compensatory growth in mammals has received substantial attention in agricultural research (Ryan et al. 1993). Fewer, but a growing number, of such studies have been completed on fishes from the perspectives of fish ecology (e.g., Russell and Wootton 1992; Wieser et al. 1992) and aquaculture (Grayton and Beamish 1977; Dobson and Holmes 1984; Jobling et al. 1993). Among such studies aimed at aquaculture, efforts have focused on exploiting the compensatory growth response to improve fish growth rates and growth efficiency as well as to influence maturation rate and tissue composition (Jobling et al. 1993).

Studies have shown that elevated growth rates from compensatory growth do occur in various life stages of many fish families when food is resupplied *ad libitum* or to satiation after days or weeks of its total deprivation or restriction. In nearly all cases, however, the duration of this rapid compensatory growth elicited by prior food deprivation ceases, once these fish "catch up" to the size of control counterparts that have been fed without interruption (e.g., Miglavs and Jobling 1989; Russell and Wootton 1992). Consequently, there has been little success in fish growth rate improvement using compensatory growth.

Whereas the majority of compensatory growth studies in fishes have involved a single period of food restriction followed by unrestricted re-feeding (single feeding cycle), a very few studies have employed repeating restriction/re-feed cycles (repeated feeding cycles) (Kindschi 1988; Quinton and Blake 1990; Jobling et al. 1993). Repeated feeding cycles are potentially valuable because they may allow successive reactivation of the compensatory growth state (by successive food restriction bouts) each time the compensatory growth state decays. In this way, the total time fish spend in the compensatory growth state may be extended relative to single feeding cycles.

Studies which have used repeated feeding cycles to increase fish growth rates with compensatory growth have caught up to, but have not significantly surpassed, the growth achieved by daily-fed controls. However, the NCRAC-funded study (Hayward et al. 1997) which used repeated feeding cycles to elicit compensatory growth in hybrid sunfish at UMC, was successful in producing significant growth in excess of controls, primarily because re-feeding periods were linked to the duration of the compensatory growth response. In a 105-day study, hybrid sunfish fed according to a particular repeated feeding schedule gained twice the body weight as daily fed controls. Feed conversion for the rapidly-grown compensatory growth fish was no different than for the controls. Furthermore, proximate analysis of flesh composition revealed that the more rapidly grown fish were no different in their protein:lipid ratios than the controls.

The previously described study of compensatory growth in hybrid sunfish was carried out with individually held fish which were fed a natural (versus commercial) food item. A more recent NCRAC-funded study of compensatory growth sought to determine whether results from the previous study could be readily applied to the culture setting. Findings indicate that use of a commercial feed does not impede compensatory growth in hybrid sunfish based on the occurrence of strong hyperphagia when such a feed is used. However, an impediment to the immediate use of compensatory growth in the culture of hybrid sunfish was revealed and is described in a recent paper (Hayward et al. In revision). This study demonstrates that group holding of hybrid sunfish reduces the strength of the compensatory growth response to the extent that growth benefits are negated. A parallel study at Iowa State University (ISU) where hybrid sunfish were also held in groups showed a similar result; fish fed on the compensatory growth eliciting schedule did not outgrow control fish that were fed every day. Strong social interactions among grouped hybrid sunfish appeared to underlie this impeding effect (see next paragraph), and efforts are warranted to reduce these effects so that the otherwise excellent potential that exists in compensatory growth feeding strategies can be realized in the culture of sunfish.

Social Interactions

It is well known in aquaculture that social interactions among grouped fish hold the potential to reduce food consumption, growth rates, and growth efficiency (Jobling 1985; McCarthy et al. 1992), and to promote inter-individual size variation (Jobling and Wandsvik 1983). Social interactions among grouped fishes include activities associated with dominant and subordinate behaviors, as well as the physiological stress of subordination (Koebele 1985). In addition, access to food may be reduced by interference competition. Work in this area has focused mainly on salmonid fishes, however, one study has considered the effects of social interaction on growth of bluegills (McComish 1971), and a recent study at UMC has also considered hybrid sunfish (Wang et al. In review). McComish (1971) found that holding bluegills in groups of three with *ad libitum* feeding caused a 75% decline in mean growth rate relative to individually held bluegill. Wang et al. (In review) found that consumption, growth, and growth efficiency declined up to 24, 27, and 34%, respectively with increasing density of hybrid sunfish relative to individually held counterparts. Among other approaches, holding fish at relatively high densities (e.g., Fleming and Johansen 1984) and separating disparately sized fish via grading (Jobling and Reinsnes 1986), have shown some success in ameliorating negative effects of social interactions in fish production. Clearly, studies aimed at reducing the effects of social interaction on the consumption, growth, growth efficiency, and size variation among bluegill and hybrid sunfish hold the potential to vastly improve growth and production of lepomid fishes.

Feeding Frequency and Rhythms

Few studies have examined the effect of feeding frequency on food consumption and growth rate of lepomids. In a recent study, Wang et al. (1998b) compared consumption, growth, and feed conversion ratio (FCR) among hybrid sunfish (7.4 g mean starting weight) fed one, two, three, and four times daily between the hours of 0800 and 1800. Fish were held in groups and fed to satiation at each feeding time with a commercial diet with a 45% protein level, for 30 days. Consumption and growth increased with feeding frequency up to three feedings per day, and increased no further with four daily feedings. FCR did not differ with feeding frequency and averaged 0.87. Daily feeding rhythm differed with feeding frequency; at three feedings per day, hybrid sunfish consumed about 0.25, 1.0, and 0.5% body weight at 0800, 1300, and 1800 h, respectively. At four feedings per day, fish consumed more uniform amounts (about 0.75% body weight) at each feeding time. Inter-individual size variation was observed to decline with increasing feeding frequency with no increase in size variation being detected when fish were fed four times per day.

Extended Growing Seasons

Previous research at ISU has made it possible to produce both bluegills and hybrid sunfish in the laboratory out-of-season by manipulating temperature and photoperiod; the use of hormones has not been necessary. Mischke and Morris (1997) used these newly developed procedures at ISU to produce approximately 750,000 bluegill and 70,000 hybrid sunfish out-of-season in 1995. This is an important development because sunfish can be stocked into ponds in April, for example, rather than substantially later in the year (e.g., August or September) as typically occurs when naturally-spawned young-of-year sunfish are used. This gain of four to five months in the first year of production will likely lead to significant reduction in total time required to grow sunfish to market size as a food fish.

Optimal Rearing Temperature

Some question remains as to what temperatures result in the most rapid growth rates of bluegill and hybrid sunfish. Researchers at UMC (Hayward et al. 1997; Wang et al. 1998a; Wang et al. 1998b) have conducted studies of hybrid sunfish at 24°C based on the study of Beitinger and Magnuson (1979) which indicated that this temperature falls within the range for maximal growth of bluegill. However, the study by Lemke (1977) indicated that age 0 bluegill (<8.0 g) grew well at 30°C. Growth studies of bluegill, green sunfish, and hybrid sunfish conducted at 8—18°C (5°C increments) at SIUC found that growth rates for all three fishes increased with temperature up to 23°C and increased no further at 28°C (Paret In preparation). FCR was found to be best at 28°C. Given the present lack of clarity concerning temperatures that produce the most rapid growth rates of bluegill and hybrid sunfish in their various life stages, more work on this topic is warranted.

Evaluate Grading Strategies (Objective 2)

Many studies aimed at reducing inter-individual size variation and increasing mean growth rates of fish in aquaculture have focused on minimizing the effects of social interactions (Jobling 1985; Carter et al. 1992; Jobling and Baardvik 1994; Wagner et al. 1996). In contrast, the effects of inherent inter-individual differences among fish, independent of social effects, on food consumption rates, growth rates, and growth efficiency, has received less attention (Jobling and Reinsnes 1986). This seemingly imbalanced approach to understanding inter-individual variation in growth rates persists, despite evidence that genetic-level differences among individuals may be involved (Thorpe 1977; Metcalfe 1986; Carter et al. 1992). A recent study at UMC (Wang et al. 1998a) has found that substantial inter-individual variation of inherent origin exists among hybrid sunfish in terms of their consumption and growth rates, as well as their growth efficiency. Juvenile hybrid sunfish (9.8—18.1 g initial weight) held individually (without social influence) and fed *ad libitum* for 50 days, showed marked differences in these three production-related variables. Moreover, magnitudes of consumption, growth, and growth efficiency by these fish were strongly correlated (positively) with initial body weight. The indication from this work is that hybrid sunfish vary substantially in their inherent, possibly genetically-based capacities to grow. Fortunately, individuals with poorer inherent growth capacity can be identified early on by their slightly smaller sizes at the start of grow out. Consequently, it is expected that grading off of smaller individuals at the start of grow out will result in higher growth rates, lower grow out times, and better FCRs. The extent to which these findings apply to bluegills are unknown, and investigation of this is warranted.

ANTICIPATED BENEFITS

Commercial-Scale Field Trials (Objective 1)

NCRAC has funded several years of research evaluating various centrarchid taxa in relation to their potential for production as a food fish. Taxa that have appeared most promising for this application from the lepomid and pomoxid groups, respectively, are the hybrid sunfish and the black crappie. The hybrid sunfish shows many of the characteristics sought in a commercial food fish, however, the main question is whether this fish can be grown to market size in short enough time periods to be economically feasible. The black crappie appears to perform better than the white or hybrid crappie under culture conditions and, due to its inherently larger size, has been thought to potentially be more suitable than the hybrid sunfish for production to the larger sizes required for marketing as a food fish. However, the black crappie has not performed as well as the hybrid sunfish. The black crappie has proven difficult to habituate to commercial feeds, and it has shown poorer survival in tank and cage culture than is typical of the hybrid sunfish. Moreover, a recent side-by-side study of hybrid sunfish versus black crappie in ponds (R. Sheehan, SIUC, personal communication), showed dismal performance by the latter, with production values that were actually negative. The problems with black crappie may relate to its habituation to commercial feeds, but could also relate to crappies' inability to consume food and grow well at temperatures ≥26°C (Hayward and Arnold 1996; J. Triplett, Pittsburg State University, personal communication). Given the problems that have persisted with crappie, it was decided to focus efforts on the more promising hybrid sunfish in this proposal. Despite evidence that the hybrid sunfish may be a better performer than the bluegill, the relative capacities of these two fishes to be reared economically to market size as a food fish has not been fully assessed. For this reason the present study will focus on both the hybrid sunfish and the bluegill.

The proposed study under Objective 1 is expected to provide sound indication of the economic feasibility of rearing hybrid sunfish versus bluegill to market size as a food fish. However, beyond this, a number of factors and procedures (some developed quite recently) will be evaluated for their potential to increase sunfish growth rates and thereby reduce rearing times required for grow out. These include: (1) an assessment of the influence of latitude (largely thermal regime) on growth rates of sunfish in ponds, (2) a preliminary effort to diminish the negative impacts that strong social interactions among sunfish have on size variation and growth rates, (3) application of recently acquired knowledge of optimal daily feeding frequencies for sunfish, including feeding rhythms, (4) use of fry that were spawned out-of-season, making them available for stocking earlier in the year and allowing substantially longer growth periods in year one, and (5) a rigorous evaluation of the capacity to grow sunfish to market size in indoor recycle systems where optimal growth environments can be maintained continuously.

Results from this objective are expected to provide a basis for assessing whether the rearing of sunfish for the food fish market can be economically feasible. Whether hybrid sunfish hold a distinct advantage over bluegill in this regard should also become more clear. The potential value of the previously listed approaches for reducing grow-out times of sunfish should be indicated from this work, allowing those that are less promising to be held back in favor of additional focusing on those approaches that show the most promise.

Evaluate Grading Strategies (Objective 2)

This objective will evaluate the potential to dramatically increase growth rates, improve feed conversion, and reduce size variation of commercially reared hybrid sunfish, through a relatively straightforward procedure. A recent study has shown that substantial inherent difference exists among individual hybrid sunfish in terms of their capacity to consume food and grow. Removal of those individuals that possess inherently low growth capacity would leave commercial producers with better performers in their rearing facilities. Because these poor growers are also poor feeders, producers are likely overfeeding these fish and adding to water quality problems. Fortunately, it has been found that the poor feeding and growing hybrid sunfish can be identified by their slightly smaller size at the start of grow out. The single study associated with this objective would compare the improvements in growth rates and the percentage of fish reaching market size at various times in pond-reared hybrid sunfish that were initially graded to remove smaller fish, versus those where no initial grading was done. Results of this work could bring about significant improvement in the economic feasibility of rearing hybrid sunfish as a food fish.

PROGRESS TO DATE

Overview

The initial centrarchid culture technology project funded through NCRAC sought to control sunfish reproduction through selective breeding and chromosome manipulation and to determine optimum rearing conditions for various sunfish and their hybrids. Modifications of techniques used to manipulate ploidy in other groups of fish were evaluated to develop optimal means of controlling sex determination and producing sterile sunfish.

The techniques used involved the application of temperature or pressure "shocks" to induce the retention in the developing eggs of additional sets of chromosomes that would normally be split off with the polar bodies during the process of oogenesis.

In 1990, MSU produced the first verified triploid bluegills through temperature shock induced polar body retention. The MSU workers also improved on the rearing time required before treated fish could be evaluated for ploidy level by devising a technique that uses 5—7-day-old larvae rather than 3-month-old fish. In 1992 a protocol for testing larval fish for ploidy was prepared. In addition, a Master's thesis describing the development of the fertilized bluegill egg was prepared. This information will assist researchers in determining optimum times for the application of shocks for production of polyploids. Pressure shock induction techniques have been extended to commercial sunfish producers.

In 1990, SIUC workers compared pressure and temperature shocking techniques for the production of triploid hybrid sunfish (bluegill female × green sunfish male). They found that hydrostatic pressure shocks were superior. One hundred percent triploids were produced at several pressure levels with good survival and no deformed individuals (Wills et al. 1994).

In 1992, researchers at SIUC refined the technique of using pressure shocks to produce triploid hybrid sunfish. They recommend subjecting the fertilized eggs to a pressure of 48,264 kPa for four minutes with the shock initiated two minutes after fertilization. Using this protocol they have produced 100% triploids with an actual survival of 30%. The survival rate of the shocked fish was higher than the survival of the unshocked control eggs produced from the same pairing of male and female fish (Wills et al. 1994). MSU researchers have also determined that pressure shocks will reliably produce triploid bluegill and have abandoned temperature shocks for the production of triploids. They were able to produce 100% triploidy in bluegill subjected to pressure treatments of 55,158 kPa for five minutes begun 1.5 minutes after fertilization.

Also at SIUC, irradiated spermatozoa (to destroy the DNA) have been used to fertilize bluegill eggs. Eggs from each female were divided into three lots. One lot (controls) was fertilized with normal spermatozoa. The other two lots were activated with irradiated spermatozoa. One lot of eggs activated with the irradiated spermatozoa was then subjected to pressure shock to induce polar body retention. These treatments were intended to produce gynogens, fish with exclusively the female genetic complement. The pressure-shocked individuals (putative diploid gynogens) showed poorer survival and abnormalities than the controls, but several hundred lived long enough to be stocked into a pond at SIUC. The controls developed normally, but the non-pressure shocked eggs all died, indicating that they were haploid, and that the irradiation treatment was successful. Seven sexually mature gynogens were recovered from the pond and all were female. The probability of obtaining seven fish which are all females from a fish population with a 50:50 sex ratio is 0.008. Thus, SIUC's results suggest that bluegill have an XY genetic sex-determination system, since all of the gynogens were female.

Investigators at MSU have also produced the first tetraploid bluegills using cold shock. Tetraploids mated with normal diploid bluegill should absolutely insure 100% sterile triploid offspring. Their results from 1992 suggest that pressure may not be a suitable shock for the production of tetraploids. They have so far achieved 10% tetraploid production using cold shocks.

The methodology developed for triploid sunfish production at SIUC will benefit those producers who wish to control reproduction in culture ponds. Triploid sunfish appear to be functionally sterile, and they do not complete gametogenesis, preventing much of the fish's energy from being diverted to the production of gonadal products (Wills et al. 1994). Histological and flow cytometric analyses of the gonads of male and female triploids by SIUC, in a collaborative study with Dr. Stan Allen at Rutgers University, showed that Meosis I is not completed during gametogenesis, and that the process stops when germ cells are in the hexaploid stage.

If tetraploid bluegill stocks can be produced and raised to maturity using improved cold or pressure techniques, as MSU is attempting to do, the tetraploid × diploid cross may be used to effectively and economically produce triploids in the future. Other researchers have shown that this cross will produce triploids with higher survival and better overall performance than triploids produced directly by shocks. If tetraploids could be raised to sexual maturity, brood stocks of tetraploids and diploids could be maintained in ponds or at hatcheries. Triploids could be produced from the tetraploid × diploid cross as needed with much less expenditures of time and money.

In 1991 and 1992 work began on evaluating the production characteristics of sunfish. SIUC researchers evaluated densities for cage culture of hybrid sunfish. They are now recommending that culturists stock cages at densities of less than 400 fish/m^3. In 1992, this same hybrid was evaluated in earthen ponds. The results suggested that densities of at least 370 fish/ha may be needed to stimulate feeding behavior on practical feeds in these fish.

Based on the results of work that has been completed, a recommendation to producers is to stock culture cages at densities less than 400 fish/m^3. Recommendations on stocking density, anticipated growth rate, and anticipated feed conversion over a range of temperatures for the hybrid sunfish and the triploid hybrid sunfish will be developed as part of this project.

Investigators at SIUC also determined the effects of temperature on the growth and feed conversion of bluegill, green sunfish, their hybrid, and their triploid hybrid. Their results show that the bluegill, green sunfish, and the hybrid have a linear increase in growth rate over the range of 8-23°C and that the best food conversion occurs at 18°C

ISU researchers have been able to both spawn and feed larval bluegills in an enclosed recirculating system. During a six-month period (December 1994 - May 1995), 40 spawns averaging 20,000 larvae each were obtained from 24 females. This NCRAC-sponsored research demonstrated that it is possible to induce bluegill to spawn in the laboratory out-of-season by manipulation of temperature and photoperiod without the use of hormones. A feeding protocol involving the use of brine shrimp and a commercial diet has also been established that allows for more than 40% survival and good growth rates of larvae through the first 30-days posthatch. This protocol allows for the production of bluegill larvae, regardless of season, for both laboratory studies and intensive culture of sunfish. A Master's thesis describing these protocols has been prepared (Mischke 1995). Research into production of hybrid bluegill is progressing using this same protocol at ISU.

The University of Wisconsin-Milwaukee (UW-Milwaukee) studied feeding strategies for the culture of larval sunfish through a cooperative aquaculture development project with the Red Lake Band of Chippewa Indians, Red Lake, Minnesota and the Aquaculture Institute at the University of Wisconsin Great Lakes Research Facility. This group obtained pond reared black crappie brood fish and young-of-the-year black crappie. The smaller fish accepted adult frozen brine shrimp as a transitional food and were habituated to commercial starter feed within 14 days. The adult fish accepted adult frozen brine shrimp and other transitional foods after about 14 days and eventually habituated to Biodiet® within approximately three months. These stocks of crappies were then overwintered at the Aquaculture Institute. UW-Milwaukee attempted to spawn the adult fish by manipulating temperature and photoperiod this spring, but were unsuccessful in bringing the group of approximately 40 adult fish into spawning condition. They then attempted to bring in spawning fish from the wild and hired an experienced individual full-time for a month to locate spawning adults or nests with eggs in order to obtain larvae for feeding trials. This effort focused on an area from the Lake Winnebago region northward into the vicinity of Hayward in northwestern Wisconsin; however, all these efforts failed to reveal spawning fish, eggs or sac fry. Unusual temperature conditions contributed to the unpredictable spawning activity. UW-Milwaukee has attempted since 1996 to laboratory spawn their captive brood fish again; while they have yet to be successful, they continue to work on refining the process. Upon successful spawning of their captive brood fish, UW-Milwaukee will attempt to intensively rear black crappie using "green tank" organisms as first feeding foods.

This "green tank" procedure involves the use of batch cultures ("green tank") of live infusoria, brine shrimp nauplii, and ground beef heart and liver for first feeds. Over the last five to seven years, UW-Milwaukee has used and refined these techniques with yellow perch (*Perca flavescens*) to habituate intensively reared fingerlings to formulated starter diets. Perch can be habituated from live rearing foods to formulated starter diets in as little as 30 days post first feeding, and starter diet habituated fry can be produced at levels between 2,000—6,000 fry/m^3 of rearing volume. Techniques for intensive rearing of crappie larvae are not available. UW-Milwaukee hopes to bridge this information gap using the "green tank" procedure. Although it is debatable whether the "green tank" method will be feasible in commercial crappie food fish production, UW-Milwaukee will use it to facilitate its evaluations of procedures for habituating crappie to formulated starter diets. Should the "green tank" method fail with crappie, UW-Milwaukee will use the smallest size of pond reared fish that are available from commercial producers and habituate them to commercial feeds at the smallest feasible size.

Work at Pittsburg State University (PSU), has shown that white crappie can be held in recirculating systems, trained to feed on commercial pellets, and grown under those conditions. Early work indicated that trainability for smaller crappie, less than 150 mm total length (TL) was similar to largemouth bass in terms of the approach required and overall success of around 40—50%. Larger fish, however, appeared to require less conditioning with most, and in some cases nearly all, of the fish switching to pellets. Later work has focused on the persistent presence of fish that are classified as nonfeeders based on their emaciated appearance and a condition-factor of less than 0.9 K TL. Based on tank observations, aggressive interactions appeared to play a role in the continual development of nonfeeders.

The presence of feeding hierarchies in crappie was demonstrated in competition experiments in the laboratory at PSU. Although crappie are centrarchids, their relative timidity when compared to other members of the family made the levels of intraspecific aggressiveness surprising. Feeding strategies that made food appear scarce, or permitted dominant fish to prevent feeding, promoted inter-individual differences in feeding activity and thereby increased growth differences. Saturation feeding when all fish were hungry produced the best feeding response. The number of food particles available had to exceed the number of fish by two to three times before all fish would feed. This observation was reinforced in the results of the recirculating system trials. Feeding strategies to promote compensatory growth should greatly reduce the impact of the feeding hierarchy.

Feeding trials at PSU in cages and in the recirculating system using the Biodiet® semimoist pellets produced fatty livers and much surplus fat in the gut cavity. While this may primarily result from a feed not well balanced for this species, it could also represent overfeeding by dominant individuals in the hierarchy. Again, feeding strategies that promote compensatory growth may greatly reduce, if not eliminate, this development of excess fat. In all trials, growth was highest when temperatures were maintained around 20°C. Growth was poorest when temperatures reached or exceeded 26°C. This result is consistent with the findings of Hayward and Arnold (1996) who observed that food consumption rates of adult white crappie under *ad libitum* feeding conditions declined to low levels above 26°C.

An investigation of black crappie physiological responses to handling stress was initiated in 1997 by University of South Dakota (USD) and University of Wisconsin-Madison (UWM) researchers. Background data relating to initial pre-stress measures of blood characteristics were collected in 1997. Plasma samples were analyzed by USD and UWM for cortisol in 1998. Additional experiments were planned to evaluate the effects of water temperature and salt concentration in mitigating stress and enhancing survivorship.

A manual of sunfish culture practices was completed and is under review; it is scheduled for completion in 1999. In addition, a NCRAC video titled "Sunfish (*Lepomis* spp.) Culture" has been produced and is available for distribution.

In 1994-1995 researchers at UMC conducted a laboratory study on compensatory growth of the hybrid sunfish. Major objectives were to test various feeding schedules that elicited the compensatory growth response in these fish, for their capacity to increase growth rates and improve growth efficiency relative to fish fed every day. The hybrids were subjected to one of five treatment feeding regimes (N = 10 fish per treatment) involving repeated cycles of food deprivation followed by *ad libitum* re-feeding. Each treatment group's food deprivation periods were fixed at either 2, 4, 6, 10 or 14 days (designated as the D2, D4, D6, D10, and D14 treatment groups, respectively); re-feeding periods in all cases were continued until hyperphagia ceased (hyperphagia indicated active compensatory growth). Hyperphagia was considered to be present whenever a treatment group's daily consumption rate was significantly greater than that of the control group which was fed *ad libitum* every day. Fish in D2 and D14 significantly outgrew (wet weight) control fish (ANCOVA; $P < 0.0001$); D2 outgrew control fish two-fold (2.01), while D14 outgrew controls by a factor of 1.45 over the 115-day experiment. To our knowledge this is the first documentation of statistically significant outgrowing of daily-fed control fish of any species using compensatory growth. Although growth efficiency significantly exceeded controls during re-feeding periods for treatment groups, growth efficiency was no different than controls over the full experiment (which included both no-feeding and re-feeding periods). Details of this work can be found in Hayward et al. (1997).

In 1997-1998, a follow-up study of compensatory growth in hybrid sunfish was completed at UMC. This study's main objective was to evaluate the potential to use compensatory growth for rapid growth of hybrids under conditions similar to those in aquaculture. Results indicated that compensatory growth benefits can be achieved when commercial pellet feed is used rather than mealworms; hyperphagia associated with compensatory growth was regularly observed following periods of food deprivation. Most significantly, it was found that the magnitude of compensatory growth observed in the first study was substantially diminished when hybrid sunfish were held in groups (Hayward et al. In revision). This same result was found in a parallel study of compensatory growth conducted by Summerfelt at ISU. This appears to have been caused by intense social interactions among grouped hybrids; a parallel study at UMC demonstrated that consumption, growth, and growth efficiency of hybrid sunfish declines significantly (up to 24, 34, and 15%, respectively) when these fish are held in groups versus individually (Wang et al. In review). Studies are being considered to identify ways to diminish the effects of social interaction on growth performance in hybrid sunfish so that the benefits from compensatory growth as observed in the first study can be applied to the aquaculture setting.

Work at UMC concerning optimal feeding frequency and daily feeding rhythms of juvenile hybrid sunfish (initial weight of 7 g) has been completed (Wang et al. 1998). Consumption, growth, FCR, and the development of size variation (based on coefficients of variation [CVs]) was evaluated in a 50-day study where treatment groups were fed either one, two, three, or four times daily (satiation feeding at each time). Results showed that consumption and growth increased with feeding frequency up to three daily feedings, with no further increase occurring at four daily feedings. No change in FCR was observed with feeding frequency. Feeding rhythms (amounts consumed at each feeding time within a day) differed significantly (up to four times) across daylight periods, and daily patterns changed with feeding frequency. At three feedings per day, hybrid sunfish consumed about 0.25, 1.0, and 0.50% body weight at 0800, 1300, and 1800 h. The extent to which size variation among grouped hybrid sunfish developed as the 50-day experiment progressed was highest (CV increased 42%) when fish were fed once daily, and declined significantly with increasing feeding frequency, until CV did not increase at all with four daily feedings. Overall, results suggested that maximum growth can be achieved by feeding juvenile hybrid sunfish three times per day, morning, mid-day, and evening, but that four daily feedings may largely negate the development of size variation among individuals (which are of similar size at the start).

Research progress specifically addressing the objectives of the current Sunfish Work Group proposal is as follows:

Commercial-Scale Field Trials (Objective 1)

Crappie have not grown or survived well in previous studies in the region at PSU and SIUC under highly artificial conditions, such as in cages or tanks. Recently completed pond studies at SIUC which made side-by-side comparisons of survival, growth, and production of black crappie and hybrid sunfish showed far more promising results for the latter versus the former which actually showed negative production rates due to poor growth and high mortality. Consequently, it was decided that work in the near term should focus on lepomids for which the greatest promise has been shown to date. This SIUC study indicates it is likely that 113 g hybrid sunfish will reach the 227—453 g weight range in the NCR in a single growing season. However, fry are typically produced from spawns that occur primarily in May and June, leaving insufficient time for young-of-the-year to reach 113 g in their first growing season at high densities. Consequently, a third growing season must be entered to produce 227—453 g fish.

In the NCR it is common practice to stock sunfish brood stock into ponds in April—May and then harvest their offspring either in the fall or following spring. If culturists induced these fish to spawn out-of-season so that fingerlings were available earlier in the spring, 113 g fish could be produced by the end of the first year, with market size fish developing during the second year. Previous NCRAC-funded research at ISU has resulted in the capability to produce fingerlings in the spring through out-of-season spawning.

Two approaches will be evaluated to reduce costs for producing market size sunfish. The first approach entails out-of-season production of both bluegill and hybrid sunfish fry for stocking into ponds in April. (We note that bluegill have been included in addition to hybrid sunfish in the proposed work because it has not been fully demonstrated that they will perform less well then hybrid sunfish in all culture settings.) If good fry production and survival in ponds is obtained, then it should be possible to produce 113 g or larger sunfish during their first growing season. Grow out during a second growing season should produce market size sunfish, thus, compressing the production cycle from three to two years.

A second production approach, which involves reducing costs as opposed to production time, will be assessed by SIUC researchers. The approach will be to produce seed stock for a three-year production cycle at minimal cost during the first year. Small fingerlings (about 2.54 cm) of both taxa (bluegill and hybrid sunfish) will be produced at high densities from natural spawns of brood stock in ponds during 1999. Feeding will be kept to a minimum and only employed to ensure that progeny remain in good condition. It is hoped that by minimizing feeding costs, pond space requirements, and, therefore, related management costs, a three-year production cycle may be economically feasible. The 2.54-cm fish should achieve 113 g or more during year two of the cycle, and reach market size during year three.

Conduct Grading Strategies (Objective 2)

Recent research at UMC has shown that juvenile hybrid sunfish can grow at relatively slow and disparate rates in aquaculture-like settings, not only due to strong social interaction (Wang et al. In review), but also because of inherent, inter-individual differences in appetite, growth capacity, as well as growth efficiency (Wang et al. 1998a). This research has also revealed that hybrid sunfish “telegraph” their individual growth capacities early on by their relative lengths and weights; faster growers show sizes slightly larger than their counterparts at least as soon as they reach 9—18 g (and likely even earlier), and conversely for slower growing individuals. This objective seeks to take advantage of this new knowledge and determine the extent that growth rates, grow-out times, and FCRs can be improved by removing the smaller fish (which have been retained in the past) at the beginning of the grow-out period.

OBJECTIVES

1. Conduct field trials of bluegill and F_1 hybrid sunfish (female green sunfish × male bluegill) in commercial-size production facilities defined as ponds >0.04 ha and indoor recycle systems in the upper and lower portions of the North Central Region. A minimum of three replicates will be used in all pond and recycle system studies; commercial feeds to be use will be those identified in previous studies.

2. Evaluate grading strategies to enhance grow out in commercial systems to market size ($\geq$227 g), including the culture potential of discards.

PROCEDURES

Commercial-Scale Field Trials (Objective 1)

Pond Studies

SIUC and ISU researchers will investigate the pond culture of bluegill and hybrid sunfish in Illinois and Iowa, respectively. The emphasis will be to compare growth performance of these two fishes at two distinct latitudes. Accordingly, a number of variables will be held constant across the two study locations. These include stocking fish within a size range of 5.1—7.6 cm, stocking at densities of 12,000 fish/ha, using Silver Cup Trout Diet-sinking pellet (or a diet of identical composition from another feed manufacturer), and feeding three times daily (morning, mid-day, and evening) to apparent satiation levels at each feeding time. In addition, grading procedures will be identical across the two study locations. Also, temperature regimes will be monitored in ponds at both sites.

Age 0 fish used at both study locations will be from the same source population. Brood stock (24 male bluegill, 12 female bluegill, and 12 female green sunfish) will be obtained from southern Illinois by SIUC researchers and transported to a holding facility at Kloubec Fish Farms, Amana, Iowa during fall 1998 or spring 1999. Out-of-season spawns will be obtained from these fish during spring 2000, and will be used by both universities. Prior to the onset of exogenous feeding, fry from both taxa will be shipped to SIUC for stocking into their fertilized production ponds in April 2000.

Analyses of genetic introgression will be conducted for fish used at both study sites. A total of 80 brood fish (20 bluegill and 20 green sunfish × 2 [ISU and SIUC stocks]) will be evaluated using mtDNA and allozyme electrophoresis.

SIUC

Fry survival will be assessed at SIUC at 2.5 cm TL. Five (or fewer replicates if necessitated by fry survival) production ponds each for bluegills and hybrid sunfish will then be stocked with 2.5 cm TL fish. In fall of the year 2000, all ponds will be harvested, survival and growth will be assessed, and the fish will then be stocked into five ponds per taxon (if warranted by survival) for grow out. Stocking of fry in April will have increased the length of the first year growing season by about two months. It is hoped that this will be sufficient to permit a production cycle where market-size fish can be produced within two years. Study procedures at SIUC will be coordinated with those at ISU to isolate the effects of latitude on hybrid sunfish growth.

In addition, a three-year production cycle which minimizes production costs during year one will be investigated at SIUC. Blosz (1948) found that stocking 247 brood bluegills/ha produced 929,083 2.5-cm TL age 0 bluegill/ha. Four ponds will be stocked with bluegill brood fish and four other ponds will be stocked with male bluegill and female green sunfish brood fish (to produce hybrid sunfish) at the above density in fall 1998 or early spring 1999. This portion of the work will be conducted without charge by SIUC prior to the funding period for the project, so that the entire three-year production cycle can be evaluated during the contract period. Ponds will be treated with organic fertilizers prior to the spawning period . Stocking rates, feeding rates, and other management practices in years two and three in the SIUC study will also conform to those used in pond studies at ISU to facilitate comparisons between survival and yield in the northern and southern portions of the region.

All work at SIUC will be conducted in 0.04 ha ponds. At least four (and sometimes five as stated above) replicates per taxon (bluegill and hybrid sunfish) will be used during all production phases of the project.

ISU

Pond studies to be conducted at Kloubec Fish Farms will evaluate both bluegill and hybrid sunfish. Young-of-the-year bluegill and hybrid sunfish used in the study will be provided by Kloubec Fish Farms, Amana, Iowa by mid-September 1999 at no cost to the project. All fish will be pond-reared to 5.1—7.6 cm at Kloubec Fish Farms; the trials will be initiated in October. Prior to stocking fish into production ponds at Kloubec Fish Farms, they will be sorted with the largest individuals being retained for the study (top 70% of the population). Fish will then be stocked into six production ponds at Kloubec Fish Farms (three per taxon) and grown until October 2000. At this time the market-size fish will be removed and populations reconstituted and cultured until June 2001. Fish will be fed by hand or by electronic feeders. Monthly samples will be obtained to establish monthly feeding rates unless precluded by elevated water temperatures that would make fish more susceptible to handling stress. Standard production information (yield, FCR, size-frequency distribution, and production costs) will be collected during the project. Kloubec Fish Farms will retain pond-reared fish for sale.

<u>Indoor Recycle Systems</u>

The broad goal of this work will be to determine production rates of bluegill and hybrid sunfish in indoor recycle systems, and to compare results to those achieved in the previously described pond studies (see above). A number of variables will be held identical to those in the pond studies. Starting sizes of bluegill and hybrid sunfish will be within the 5.1—7.6-cm length range. The feed will be identical to that used in the pond studies; feeding frequency and feeding rate will be identical to those used in the pond studies. Water temperature in all indoor recycle systems will be 25°C and photoperiod will be 16-h light/ 8-h dark. Grading schemes for the indoor recycle systems work will be matched, to the extent feasible, to those used in the pond studies. It is understood that fish densities in indoor recycle systems will vary across study locations, in part, because one study (ISU) will evaluate the effect of rearing density on sunfish growth performance (this portion of the study relates to ameliorating effects of social interaction). Fish in the ISU study will be from the same source as those used in the pond studies. Fish in the University of Minnesota (UMinn) and North Dakota State University (NDSU) studies will come from Spruce Creek Fish Farm, Miltona, Minnesota.

ISU

The goal of this research is to compare growth and production of bluegill and hybrid sunfish, and evaluate methods to increase growth rates and reduce inter-individual size variance by suppression of social hierarchy. The experimental design is to: (1) compare performance of bluegill and hybrid sunfish (female bluegill × male green sunfish) raised together in indoor recycle systems, and (2) evaluate fish holding density as a mediator of growth rates as well as inter-individual variance in growth rates.

Six, identical 1,135-L circular, fiberglass culture tanks with a center drain will be used for the research. The rearing system consists of the culture tanks, a microscreen (60 µm mesh) drum filter (Hydrotech AB, Vellinge, Sweden) for clarification, a packed column for degassing (moving water through air down a forced ventilation packed column) and biofiltration, and UV lights for disinfection of the return flow. The system is operated at ambient oxygen conditions (i.e., without oxygen supersaturation).

Each year, fish will be mechanically graded (bar grader) twice. The first grading will be done at the pond site in mid-September 1999, and again after six months of intensive culture at ISU. In both cases, the goal of grading will be to remove the smallest 30% of the population. At the pond site, the smaller group of fish would be retained by Kloubec for either further growth or sold to evaluate market potential of the smaller fish. Smaller fish from the grading at ISU (after six months) also will be returned to Kloubec for market evaluation. The sex ratio of a random sample of the larger and smaller groups of fish will be examined at each grading time. The larger group from the six-month grading will be retained for culture to the end of the study.

At ISU, bluegill and hybrid bluegill will be marked by injection of fluorescent elastomer marking at the base of the anal fin, using separate colors for each stock. Tests on walleye *Stizostedion vitreum* indicated 100% retention of elastomer marks for at least seven months (Clayton and Summerfelt 1997), and evaluation of those fish beyond that date indicates excellent retention for more than one year. Bluegill and hybrid bluegill will be raised together in the same culture tanks but distinguished by the fluorescent elastomer marks.

Two densities will be evaluated: (1) low density will be the density that will achieve a density index (Piper et al. 1982) of 0.4, which is an initial density of 10 g/L of each stock, and (2) a high density at a density index of 0.8, which is 20 g/L of each stock. Flows will be adjusted to maintain a constant loading (g/Lpm) of 0.2 kg/Lpm throughout the trial. Each treatment will be replicated three times. Density will be readjusted after the second grading (six months).

At the end of six months, fish will be removed from all six tanks, sorted as bluegill or hybrid sunfish, each group counted to determine comparative survival, and the group weight recorded. Then, a sample of 100 fish will be weighed and measured individually to calculate absolute, relative, and specific growth rates. The gender of all of the measured fish will be determined by microscopic examination of gonadal tissue. A sample will be obtained of both bluegill and hybrid bluegill for proximate analysis at the beginning of the study, at six months, and at the end of the study. At the end of the study, 100 fish from each tank will be hand-filleted to determine dress-out yield for a scaled but skin-on fillet. Temperature and dissolved oxygen (DO) will be recorded daily in each culture. Total ammonia nitrogen (TAN) will be measured three times per week in each culture tank by the phenate method (APHA et al. 1995) using a DR/3000 spectrophotometer (HACH Company, Loveland, Colorado). Unionized ammonia will be calculated from TAN concentrations using tables given by Thurston et al. (1979) with temperature and pH data obtained from the same water samples used for the TAN determination. Total alkalinity (pH = 4.6) and free CO_2 will be measured by titration in each culture tank, once per week (APHA et al. 1995). Nitrite (diazotization method), and chloride will be measured once per week and determined by colorimetric methods (APHA et al. 1995).

UMinn

Field trials of bluegill and F_1 hybrid sunfish will be conducted in commercial-scale, indoor recycle systems on the University of Minnesota-St. Paul campus. UMinn has six, 11.3 m^3 culture tanks in place for this study, each with an independent biofilter and rotating drum microscreen for solids removal. Three different biofilters are to be used: (1) trickling filters, (2) fluidized bed sand filters, and (3) submerged thin film filters.

Fingerling bluegills and F_1 hybrid sunfish will be obtained from Spruce Creek Fish Farm, Miltona, Minnesota. Each fish type will be stocked in each system type to give a comparison with three replicates. Fish will be stocked in September 1999. Initially, all fish will be held in floating cages within each culture tank to aggregate them for feeding. Fish weights will be sampled monthly to determine growth rate. FCR, size distribution, survival, and all cost information will be tracked for production estimates. Cost information will include feed, utilities, and labor. In October 2000, market-size fish will be removed. Ammonia-nitrogen, nitrite-nitrogen, DO, pH, alkalinity, and temperature will be monitored daily. Nitrate-nitrogen, CO_2 , and solids information will be collected weekly. All on-site water quality tests will be performed according to standard methods (APHA et al. 1995).

Differences in growth performance of bluegill and hybrid sunfish will be statistically tested by using a paired t-test (or a nonparametric analog if appropriate), with biofilter type being the "pairing variable." The sample size will be three observations for each fish type. The effect of biofilter type will not be statistically tested.

NDSU

Juvenile bluegill and hybrid sunfish will be obtained from a regional producer (Spruce Creek Fish Farm, Miltona, Minnesota) and transported to the Northern Aquaculture Center at NDSU. Fish will be in the 5.1—7.6 cm range. Fish will be quarantined and trained onto feed for a period of two weeks. During the quarantine period fish will be fed to apparent satiation with crumbles, three times per day. Following the two-week quarantine period, fish will be stocked into the six, 18,925-L indoor recycle systems. The stocking rate will be 3,000 fish/system. Each fish taxon will be randomly assigned to three tanks.

Water quality parameters will be monitored regularly. DO and temperature will be monitored daily. Other water quality variables including toxic ammonia, nitrite, pH, alkalinity, and hardness will be monitored weekly. Water quality equipment (YSI DO meter, Orion pH meter, and Hach spectrophotometer) is available and in working order at the facility. To develop growth curves, 150 fish will be collected at random with a net from each tank every 30 days and weighed (nearest g).

NDSU will conduct a 16-month grow-out study. All fish will be weighed at 12 months to determine if a 12-month grow-out period is sufficient for production in the indoor recycle systems. Fish will be grown for 16 months to determine if there is any production advantage beyond 12 months.

Upon completion of the study period(s), all fish in each tank will be counted and weighed. Absolute and percentage weight gain, FCRs, and percent survival and percentage reaching market size will be determined. Five percent of the remaining fish will be sacrificed from each replicate to determine dressed yield (gutted and gilled, heads on), and fillet yield. These yields will be compared to the other groups involved in field trials in recycle systems and ponds: ISU, SIUC, UMC, UMinn. Data will be analyzed using t-tests or two-sample nonparametric procedures if approriate.

Proximate analysis will be done to determine protein, lipid, ash, and moisture levels in fillets and whole fish. Taste tests will be completed in conjunction with the marketing team from NDSU; a blind taste test will be done to quantify overall consumer satisfaction, flavor, texture, and appearance.

Evaluate Grading Strategies (Objective 2)

UMC

This work will evaluate the potential to improve hybrid sunfish growth and feed conversion efficiency, and to reduce inter-individual size variation in ponds, by heavily grading-off smaller fish at the time that they are stocked. Objective 2 emanated from a laboratory study at UMC which found that individually held juvenile (5.1—7.6 cm TL) hybrid sunfish's consumption, growth, and feed conversion rates were positively correlated with their initial weights (Wang et al. 1998a). This indicates that poor performing hybrid sunfish reveal themselves early on by their smaller size; consequently, removal of smaller fish at stocking is expected to result in better overall growth performance.

Seven, 0.2 ha production ponds at Harrison Fish Farm in Hurdsville, Missouri will be used in this study. These ponds have aeration and drainage capabilities. Hybrid sunfish (5.1—7.6 cm TL) will be secured either from Harrison Fish Farm or Country Fish Farm, Salem, Missouri in September 1999 and stocked into the seven study ponds. In three ponds, fish will be mechanically graded at stocking so that the smaller 50% of fish are excluded. In three other ponds, fish will be stocked with no grading. Target stocking densities will be in the range of 9,000—12,000 fish/ha. However, adjustments to stocking densities will be made so that biomass of fish in ponds are similar across the two treatment groups. Random selection procedures will be used to allocate treatment groups to ponds. Finally, the seventh pond will be stocked with the "discard" fish, those excluded by grading, to evaluate their growth performance.

Fish will be fed to apparent satiation three times daily, once in the morning, mid-day, and evening, in accordance with the findings of Wang et al. (1998b). The feed will be matched to the type and composition of that used in Objective 1; however, the feed manufacturer may differ from those used in the other studies.

Temperature, DO, and Secchi disk depth will be determined and recorded weekly for all ponds, although DO will be monitored daily so that aerators can be activated as needed. Ponds will be seined monthly and collected fish will be measured (nearest mm TL) and weighed (nearest g). The data from seining will be used to adjust food amounts provided according to body weights, and to determine patterns of fish growth and CVs of length and weight over time, in relation to temperature.

As hybrid sunfish reach market size, they may be removed and sold by Harrison Fish Farm. The remaining fish will continue to be grown and monitored until August 2001. Ponds will be drained at the end of the study and all remaining fish will be counted and weighed.

Hybrid sunfish growth rates will be compared statistically between the ponds that were and were not initially graded. Other variables that will be compared between treatments include CVs of length and weight, condition factor, percent reaching market size, and percent mortality. Conversion efficiency will be calculated in two ways and compared between treatments: (1) from observed weight gain and feed weight provided, and (2) from observed weight gain and predicted food consumption. Predicted food consumption will be estimated for each pond, from a bluegill bioenergetics model (Hewett and Johnson 1992) which has been shown to effectively predict food consumption of hybrid sunfish from paired growth and temperature data (Whitledge et al. 1998). Finally, these variables will also be determined for the single pond of discard fish and compared non-statistically to results from the two main treatment groups.

FACILITIES

Commercial-Scale Field Trials (Objective 1)

SIUC

All work at SIUC will be conducted in 0.04 ha ponds. At least four (sometimes five as stated above) replicates per taxon (bluegill and hybrid sunfish) will be used during all production phases of the project. The Illinois Aquaculture Research and Demonstration Center at SIUC's Touch of Nature Facility has 90 fillable and drainable 0.04 ha aquaculture ponds and other equipment used in pond aquaculture, such as tractors, paddle-wheel aerators, hauling tanks, seines, etc. The SIUC campus also has an aquaculture pond complex consisting of 18, 0.06 ha ponds. SIUC also has two buildings and several rooms in other buildings which house tank culture systems which provide more than 1,115 m^2 of space for holding fish. Other SIUC aquaculture resources include fully equipped fish genetics, nutrition, feed preparation, and water quality labs.

ISU

Field trials involving hybrid sunfish and bluegills in ponds will be carried out at Kloubec Fish Farms. Kloubec Fish Farms is one the NCR's largest fish producers with operations in hatchery, fingerling, and food fish facilities. Kloubec Fish Farms has 50 culture ponds (32.4 ha) in Iowa.

In addition to ponds, brood stock collected from Illinois will be housed from fall 1998 through spring 2000 at Kloubec Fish Farms. This facility consists of six 1,135-L tanks connected to a common recirculating biofilter. Photoperiod and temperature regimes will be controlled using overhead lights and electric heaters, respectively.

Field trials of hybrid sunfish and bluegills will also be done in indoor recycle systems at the ISU aquaculture facility (Summerfelt). Fish will be cultured in six, 1,135-L tanks. These are circular, fiberglass culture tanks from which the water flows to a microscreen (60 µm mesh) drum filter (Hydrotech AB, Vellinge, Sweden) for clarification, then through a packed column for degassing (moving water through a 3 m, forced-ventilation packed column) and biofiltration, and then in series through six UV lights for disinfection. The flow passes through another packed column for reaeration and then to a holding tank for pH adjustment with pH controllers. High capacity heaters and a chilling system is available for precise temperature control. Other wet lab space with 30, 120-L tanks and 6, 275-L tanks are available for use in the project. A low humidity, temperature controlled room is available for feed storage. A fully equipped water analysis laboratory is located on the same floor; it is equipped with spectrophotometers, pH meters, turbidity meters, glassware, hood, and reagent storage.

UMinn

Uminn operates a 998.7 m^2 Fisheries and Aquaculture Laboratory on the St. Paul campus. The laboratory houses tanks ranging in size from 0.75 to 3 m in diameter served by either well water or recycled water from a central water treatment area. The central recycling system features microscreen particulate removal, biofiltration, and ozone and UV disinfection. Several individual recycling systems are also available.

The sunfish project will be carried out in six, commercial-scale 11.3-m^3 recirculating aquaculture systems located in the Fisheries and Aquaculture Laboratory. The systems utilize three types of biofilters: fluidized bed sand filter, submerged thin film filter, and high-rate trickling tower. Each system is replicated twice. All tanks have individual drum microscreens for solids removal, airstones for supplemental aeration, and immersion heaters for temperature control. Temperature, water level, aeration, and DO are continuously monitored and connected to a central laboratory alarm.

NDSU

NDSU currently operates a fully functional 90,840-L commercial-scale indoor recirculation system. Twenty-four 945-L stand-alone recirculating systems are currently used for strain comparisons and diet evaluations.

For the proposed work six 18,925-L recirculating systems will be used. The systems are each composed of 4.6 m grain-bin rings attached to concrete flooring. The systems are lined with PVC sheets. Water will exit the tanks via a center drain pipe, and an external stand pipe (to control water levels). The exiting water flows through a drum screen filter. Following solids removal, secondary solids removal and biofiltration occurs as water is pumped through an upwelling propeller-washed bead filter (PBF-10). The exiting water is pumped along the edge of the tank to create circular flow, for solids scrubbing of tank.

Photoperiod is electronically controlled on a continuous 16-h light/8-h dark cycle. Indoor temperature is maintained at 24°C.

Evaluate Grading Strategies (Objective 2)

UMC

Seven 0.2 ha production ponds at Harrison Fish Farm in Hurdsville, Missouri will be used in the two-year study to determine whether the initial removal of smaller hybrid sunfish will result in significantly improved production of market size fish. Each of these ponds are equipped with mechanical aeration systems, and are drainable to facilitate harvesting. As the study will take place on an active production aquaculture facility, most equipment necessary to conduct this study, including mechanical graders, is available on site.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Robert J. Sheehan Southern Illinois University-Carbondale	Fish Culture/Physiology
Iowa	Joseph E. Morris Iowa State University	Larval Fish Culture/Water Quality/Extension
	Robert C. Summerfelt Iowa State University	Fish Culture/Larval Fish
Minnesota	Ira R. Adelman University of Minnesota	Fish Feeding and Growth
Missouri	Robert S. Hayward University of Missouri-Columbia	Fish Bioenergetics/Feeding Strategies
North Dakota	Mark A. Sheridan North Dakota State University	Fish Physiology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

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

Iowa State University (ISU)
Joseph E. Morris
Robert C. Summerfelt

University of Minnesota (UMinn)
Ira R. Adelman

North Dakota State University (NDSU)
Mark A. Sheridan

University of Missouri-Columbia (UMC)
Robert S. Hayward

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Fisheries Research Laboratory Southern Illinois University-Carbondale Carbondale, IL 62901-6511				**USDA AWARD NO.** Year 1: Objective 1	
				Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Robert J. Sheehan				**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$12,900	
d. 1 Prebaccalaureate Students				$3,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$15,900	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$15,900	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,500	
F. Travel 1. Domestic (Including Canada)				$500	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Truck lease ($1,350), Telephone ($30), Fax ($20), Equipment repair ($200)				$1,600	
J. Total Direct Costs (C through I) →				$20,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$20,500	
M. Other →					
N. Total Amount of This Request →				$20,500	$
O. Cost Sharing (If Required Provide Details)	$19,173				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Fisheries Research Laboratory Southern Illinois University-Carbondale Carbondale, IL 62901-6511				**USDA AWARD NO.** Year 2: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Robert J. Sheehan				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$13,545	
d. 1 Prebaccalaureate Students				$3,150	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$16,695	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$16,695	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,900	
F. Travel 1. Domestic (Including Canada)				$700	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Truck lease ($1,860), Telephone ($45), Fax ($30), Equipment repair ($270)				$2,205	
J. Total Direct Costs (C through I) →				$22,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$22,500	
M. Other →					
N. Total Amount of This Request →				$22,500	$
O. Cost Sharing (If Required Provide Details)	$20,487				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	SIGNATURE	DATE
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

(Sheehan)

Objective 1

A. Salaries and Wages. A full-time graduate student will be involved with data collection, record keeping, and assist with acquisition of fish, supervision of undergraduate technician, and assist with fish husbandry as needed; a prebaccalaureate student hourly employee will handle fish husbandry (feeding fish, weighing and measuring fish), water chemistry, and data entry.

E. Materials and Supplies. Year 1: fish feed ($1,200), herbicide ($1,000), chemicals for water quality analyses ($100), general office supplies including paper, toner, pens, pencils, and notebooks ($75), and miscellaneous supplies such as plastic sampling bags and bottles ($125). Year 2: fish feed ($1,400), herbicide ($1,060), chemicals for water quality analyses ($110), general office supplies including paper, toner, pens, pencils, and notebooks ($150), and miscellaneous supplies such as plastic sampling bags and bottles ($180).

F. Travel. Year 1: $340 for transportation, lodging, and meal expenses for the graduate student to make a 2-day round trip from the campus of SIUC at Carbondale, Illinois to the campus of ISU at Ames, Iowa to deliver brood fish and $160 for transportation, lodging, and meal expenses for the partial support for the PI to attend a 3-day national aquaculture conference, destination to be determined. Year 2: $700 for transportation, lodging, and meal expenses for the partial support for the PI and/or graduate student to make a presentation of project results at a 3-day national aquaculture conference, destination to be determined.

I. All Other Direct Costs. Year 1: costs of a truck lease to travel from campus to the research facility ($150/month for nine months = $1,350), telephone ($30), fax ($20), and equipment repair ($200). Year 2: costs of a truck lease to travel from campus to the research facility ($155/month for 12 months = $1,860), telephone ($45), fax ($30), and equipment repair ($270).

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 1: Objective 1	
Department of Animal Ecology 124 Science II, Iowa State University Ames, IA 50011-3221	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Joseph E. Morris	**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS			FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer		$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$15,500	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . →				$15,500	
B. Fringe Benefits (If charged as Direct Costs)				$800	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$16,300	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$550	
F. Travel 1. Domestic (Including Canada)				$300	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($75), Fax ($25)				$100	
J. Total Direct Costs (C through I) . . . . →				$17,250	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$17,250	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$17,250	$
O. Cost Sharing (If Required Provide Details)	$16,300				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Department of Animal Ecology 124 Science II, Iowa State University Ames, IA 50011-3221				**USDA AWARD NO.** Year 2: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Joseph E. Morris				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$12,150	
d. 1 Prebaccalaureate Students				$1,100	
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$1,500	
Total Salaries and Wages →				$14,750	
B. Fringe Benefits (If charged as Direct Costs)				$600	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$15,350	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,000	
F. Travel 1. Domestic (Including Canada)				$300	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($75), Fax ($25)				$100	
J. Total Direct Costs (C through I) →				$16,750	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$16,750	
M. Other →					
N. Total Amount of This Request →				$16,750	$
O. Cost Sharing (If Required Provide Details)	$17,858				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	SIGNATURE	DATE
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR IOWA STATE UNIVERSITY

(Morris)

Objective 1

A. **Salaries and Wages.** A graduate student will be involved with data collection, record keeping, and assist with acquisition of fish, supervision of undergraduate, and assist with fish husbandry as needed; a prebaccalaureate student hourly employee will handle fish husbandry (tank hygiene, feeding fish, weighing and measuring fish), water chemistry, and data entry. The graduate student will be funded full-time in Year 1 and for 9 months in Year 2. The $1,500 in Year 2 is to pay for a technician to do genetic analysis of brood fish that will produce the offspring used in the ISU study.

B. **Fringe Benefits.** There will only be fringe benefits for the graduate student and the fringe benefit loading rate is $66.67/month.

E. **Materials and Supplies.** Year 1: fish feed ($550); Year 2: fish feed ($1,000).

F. **Travel**. Annual costs: $300 for mileage costs for the PI and/or graduate student to travel from the campus of ISU at Ames to Kloubec Fish Farms in Amana, Iowa and return—total round-trip distance is 221 miles (4 trips @ $0.34/mile).

I. **All Other Direct Costs.** Annual costs: telephone ($75) and fax ($25).

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OMB Approved 0524-0022
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BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 1: Objective 1	
Department of Animal Ecology Iowa State University Ames, IA 50011-3221	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Robert C. Summerfelt	**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel					$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. 1 Prebaccalaureate Students				$8,200	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$8,200	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$8,200	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,700	
F. Travel 1. Domestic (Including Canada)				$500	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($50), Fax ($25), Shipping ($25), Photocopying ($100)				$200	
J. Total Direct Costs (C through I) →				$12,600	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$12,600	
M. Other →					
N. Total Amount of This Request →				$12,600	$
O. Cost Sharing (If Required Provide Details)	$16,300				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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BUDGET

ORGANIZATION AND ADDRESS
Department of Animal Ecology
Iowa State University
Ames, IA 50011-3221

PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S)
Robert C. Summerfelt

USDA AWARD NO. Year 2: Objective 1

Duration Proposed Months: 12

Duration Awarded Months: ________

Item	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages					$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. 1 Prebaccalaureate Students				$5,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$5,000	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$5,000	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,600	
F. Travel 1. Domestic (Including Canada)				$600	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($50), Fax ($25), Shipping ($25), Photocopying ($100)				$200	
J. Total Direct Costs (C through I) →				$7,400	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$7,400	
M. Other →					
N. Total Amount of This Request →				$7,400	$
O. Cost Sharing (If Required Provide Details)	$17,858				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR IOWA STATE UNIVERSITY

(Summerfelt)

Objective 1

A. Salaries and Wages. A prebaccalaureate student hourly employee will handle fish husbandry (tank hygiene, feeding fish, weighing and measuring fish), water chemistry, and data entry.

E. Materials and Supplies. Year 1: fish feed ($600); reagents and glassware for water quality analyses ($400); replacement of expendable analytical equipment, such as sensors, probe, and cables ($600); repair and maintenance of the recycle system which will include new tanks, PVC pipe, filter medium, and pumps ($1,200); proximate analysis of fish feed ($280); and proximate analysis of fish samples ($620). Year 2: fish feed ($300); reagents and glassware for water quality analyses ($200); replacement of expendable analytical equipment, such as sensors, probe, and cables ($300); repair and maintenance of the recycle system which will include filter medium ($100); proximate analysis of fish feed ($280); and proximate analysis of fish samples ($420).

F. Travel. Year 1: $150 for mileage costs for the PI to travel from the campus of ISU at Ames, Iowa to Kloubec Fish Farms in Amana, Iowa and return—total round-trip distance is 221 miles (2 trips @$0.34/mile) and $350 for transportation, lodging, and meal expenses for the partial support for the PI to attend a 3-day national aquaculture conference, destination to be determined. Year 2: $600 for transportation, lodging, and meal expenses for the partial support for the PI to make a presentation of project results at a 3-day national aquaculture conference, destination to be determined.

I. All Other Direct Costs. Annual costs: telephone ($50), fax ($25), shipping ($25), and photocopying ($100).

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OMB Approved 0524-0022
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BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 1: Objective 1	
Department of Fisheries & Wildlife University of Minnesota St. Paul, MN 55108-6124	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Ira R. Adelman	**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS				
	Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES
A. Salaries and Wages					$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. _1_ Technical, Shop and Other				$15,500	
Total Salaries and Wages →				$15,500	
B. Fringe Benefits (If charged as Direct Costs)				$4,278	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$19,778	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$9,000	
F. Travel					
1. Domestic (Including Canada)				$222	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$29,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$29,000	
M. Other →					
N. Total Amount of This Request →				$29,000	$
O. Cost Sharing (If Required Provide Details)	$16,946				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR UNIVERSITY OF MINNESOTA

(Adelman)

Objective 1

A. Salaries and Wages. A technician is needed for data collection and daily operation of the project.

B. Fringe Benefits. Fringe benefits are 27.6% for a technician.

E. Materials and supplies. Fish ($3,000), fish feed ($5,250), and general laboratory supplies and reagents including chemicals, glassware, nets, and batteries ($750).

F. Travel. The amount budgeted will be for mileage cost for the technician to travel from UMinn's campus at St. Paul to Miltona, Minnesota to acquire fish and return—total round-trip distance is 694 miles (1 trip @ $0.32/mile).

UNITED STATES DEPARTMENT OF AGRICULTURE
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OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS North Dakota State University Carrington Research Extension Center P.O. Box 219, Carrington, ND 58421	**USDA AWARD NO.** Year 1: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Mark A. Sheridan	Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel					$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. 1 Research Associates-Postdoctorates	4.0			$10,500	
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$10,500	
B. Fringe Benefits (If charged as Direct Costs)				$3,150	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$13,650	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$7,000	
F. Travel 1. Domestic (Including Canada)				$650	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($75), Fax ($25), Postage ($50), Photocopying ($50)				$200	
J. Total Direct Costs (C through I) →				$21,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$21,500	
M. Other →					
N. Total Amount of This Request →				$21,500	$
O. Cost Sharing (If Required Provide Details)	$44,770				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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BUDGET

ORGANIZATION AND ADDRESS North Dakota State University Carrington Research Extension Center P.O. Box 219, Carrington, ND 58421				**USDA AWARD NO.** Year 2: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Mark A. Sheridan				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. 1 Research Associates-Postdoctorates	4.0			$10,500	
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$10,500	
B. Fringe Benefits (If charged as Direct Costs)				$3,150	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$13,650	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$6,000	
F. Travel 1. Domestic (Including Canada)				$650	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($75), Fax ($25), Postage ($50), Photocopying ($50)				$200	
J. Total Direct Costs (C through I) →				$20,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$20,500	
M. Other →					
N. Total Amount of This Request →				$20,500	$
O. Cost Sharing (If Required Provide Details)	$20,170				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR NORTH DAKOTA STATE UNIVERSITY

(Sheridan)

Objective 1

A. Salaries and Wages. A research associate will be required to acquire fish, coordinate diet acquisition, feed fish, monitor water quality parameters, and collect data.

B. Fringe Benefits. Fringe benefits are 30% for a research associate.

E. Materials and Supplies. Year 1: fish ($2,500) and fish feed ($4,500); Year 2: fish feed ($4,500) and proximate analysis of fish samples ($1,500).

F. Travel. Year 1: $249 for mileage costs for the research associate to travel from NDSU's Carrington facility to Miltona, Minnesota to acquire fish and return—total round-trip distance is 638 miles (1 trip @ $0.39/mile) and $401 for mileage costs for the research associate to travel from the Carrington facility to Fargo, North Dakota to obtain fish feed—total round-trip distance is 343 miles (3 trips @$0.39/mile). Year 2: $401 for mileage costs for the research associate to travel from the Carrington facility to Fargo, North Dakota to obtain fish feed—total round-trip distance is 343 miles (3 trips @$0.39/mile) and $249 for transportation, lodging, and meal expenses for the partial support for the PI to make a presentation of project results at a 3-day national aquaculture conference, destination to be determined.

I. Other Direct Costs. Annual costs: telephone ($75), fax ($25), postage ($50), and photocopying ($50).

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OMB Approved 0524-0022
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BUDGET

ORGANIZATION AND ADDRESS University of Missouri-Columbia 302 Anheuser-Busch Natural Resources Bldg. Columbia, Missouri 65211-7240				**USDA AWARD NO.** Year 1: Objective 2	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Robert S. Hayward				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$11,000	
d. 1 Prebaccalaureate Students				$1,500	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$12,500	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$12,500	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,000	
F. Travel 1. Domestic (Including Canada)				$2,500	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$16,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$16,000	
M. Other →					
N. Total Amount of This Request →				$16,000	$
O. Cost Sharing (If Required Provide Details)	$5,340				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS University of Missouri-Columbia 302 Anheuser-Busch Natural Resources Bldg. Columbia, Missouri 65211-7240				**USDA AWARD NO.** Year 2: Objective 2	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Robert S. Hayward				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$11,000	
d. 1 Prebaccalaureate Students				$1,500	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$12,500	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$12,500	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,000	
F. Travel 1. Domestic (Including Canada)				$2,500	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$16,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$16,000	
M. Other →					
N. Total Amount of This Request →				$16,000	$
O. Cost Sharing (If Required Provide Details)	$5,415				

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR THE UNIVERSITY OF MISSOURI-COLUMBIA

(Hayward)

Objective 2

A. **Salaries and Wages.** A graduate student will be responsible for study site visits and data collection, and will be assisted by a prebaccalaureate student.

E. **Materials and Supplies.** Annual costs: Data paper, buckets, and small nets required for conducting the field work ($250) and fish feed ($750).

F. **Travel.** Year 1: $1,901 for mileage costs for the PI and/or graduate student to travel from the campus of UMC at Columbia, Missouri to Harrison Fish Farm in Hurdsville, Missouri and return—total round-trip distance is 233 miles (24 trips @$0.34/mile) and $599 for transportation, lodging, and meal expenses for the partial support for the PI to attend a 3-day national aquaculture conference, destination to be determined. Year 2: $1,901 for mileage costs for the PI and/or graduate student to travel from the campus of UMC at Columbia, Missouri to Harrison Fish Farm in Hurdsville, Missouri and return—total round-trip distance is 233 miles (24 trips @$0.34/mile) and $599 for transportation, lodging, and meal expenses for the partial support for the PI to make a presentation of project results at a 3-day national aquaculture conference, destination to be determined.

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

Year 1

	SIUC	ISU	Uminn	NDSU	UMC	TOTALS
Salaries and Wages	$15,900	$23,700	$15,500	$10,500	$12,500	$77,100
Fringe Benefits	$0	$800	$4,278	$3,150	$0	$9,228
Total Salaries, Wages and Fringe Benefits	$15,900	$24,500	$19,778	$13,650	$12,500	$86,328
Nonexpendable Equipment	$0	$0	$0	$0	$0	$0
Materials and Supplies	$2,500	$4,250	$9,000	$7,000	$1,000	$23,750
Travel	$500	$800	$222	$650	$2,500	$4,672
All Other Direct Costs	$1,600	$300	$0	$200	$0	$2,100
TOTAL PROJECT COSTS	**$20,500**	**$29,850**	**$29,000**	**$21,500**	**$16,000**	**$116,850**

Year 2

	SIUC	ISU	UMinn	NDSU	UMC	TOTALS
Salaries and Wages	$16,695	$19,750	$0	$10,500	$12,500	$58,445
Fringe Benefits	$0	$600	$0	$3,150	$0	$4,750
Total Salaries, Wages and Fringe Benefits	$16,695	$20,350	$0	$13,650	$12,500	$63,195
Nonexpendable Equipment	$0	$0	$0	$0	$0	$0
Materials and Supplies	$2,900	$2,600	$0	$6,000	$1,000	$12,500
Travel	$700	$900	$0	$650	$2,500	$4,750
All Other Direct Costs	$2,205	$300	$0	$200	$0	$2,705
TOTAL PROJECT COSTS	**$22,500**	**$24,150**	**$0**	**$20,500**	**$16,000**	**$83,150**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

Institution		Year 1	Year 2
Southern Illinois University-Carbondale			
Salaries and Benefits SY 0.10 FTE		$7,637	$7,987
Wavier of Overhead (41%)		$11,536	$12,500
	Total	$19,173	$20,487
Iowa State University			
Salaries and Benefits 2 SY 0.05 FTE		$16,500	$29,060
Wavier of Overhead (44%)		$16,100	$6,656
	Total	$32,600	$35,716
University of Minnesota			
Salaries and Benefits SY 0.20 FTE		$2,816	$0
Supplies, Expenses, Equipment, and Wavier of Overhead		$14,130	$0
	Total	$16,946	$0
North Dakota State University			
Salaries and Benefits SY 0.30 FTE		$10,450	$10,850
Supplies, Expenses, Equipment, and Wavier of Overhead (32%)		$34,320	$9,320
	Total	$44,770	$20,170
University of Missouri-Columbia			
Salaries and Benefits SY 0.05 FTE		$3,100	$3,175
Wavier of Overhead (14%)		$2,240	$2,240
	Total	$5,340	$5,415
	Total per Year	$118,829	$81,788
	GRAND TOTAL	**$200,617**	

[1]Because cost sharing is not a legal requirement universities are not required to provide or maintain documentation of such a commitment.

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

LIST OF PRINCIPAL INVESTIGATORS

Ira R. Adelman, University of Minnesota

Robert S. Hayward, University of Missouri-Columbia

Joseph E. Morris, Iowa State University

Robert J. Sheehan, Southern Illinois University-Carbondale

Mark A. Sheridan, North Dakota State University

Robert C. Summerfelt, Iowa State University

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EDUCATION

B.A. University of Vermont, 1963
M.S. State University of New York at New Paltz, 1964
Ph.D. University of Minnesota, 1969

POSITIONS

Assistant, Associate, Full Professor (1974-present), University of Minnesota
Department Head (1982-present), University of Minnesota
Special Assistant to the Director (1990 sabbatical position), Division of Fish & Wildlife, Minnesota Department of Natural Resources

SCIENTIFIC AND PROFESSIONAL MEMBERSHIPS

American Institute of Fisheries Research Biologists
American Association for the Advancement of Science
American Fisheries Society
National Association of University Fisheries and Wildlife Programs

SELECTED PUBLICATIONS

Woiwode, J.G., and I.R. Adelman. 1992. Effects of starvation, oscillating temperatures, and photoperiod on the critical thermal maximum of hybrid striped by white bass. Journal of Thermal Biology 17:271-275.

Woiwode, J.G., and I.R. Adelman. 1991. Effects of temperature, photoperiod, and ration size on growth of hybrid striped × white bass. Transactions of the American Fisheries Society 120:217-229.

Woiwode, J.G., and I.R. Adelman. 1989. Influence of density and multipass water use on channel catfish performance in raceways. Progressive Fish-Culturist 54:183-188.

Ji, Y.Q., J.J. Warthesen, and I.R. Adelman. 1998. Thiamin nutrition, synthesis, and retention in relation to lake trout reproduction in the Great Lakes. American Fisheries Society Symposium 21: 99-111.

Cai, Y., J. Wermerskirchen, and I.R. Adelman. 1996. Ammonia excretion rate indicates dietary protein adequacy for fish. Progressive Fish-Culturtist 58:124-127.

Bushong, D.L., I.R. Adelman, and A.S. Csallany. 1996. Vitamin E, fatty acids, and other characteristics of eggs from the fish, walleye (*Stizostedion vitreum vitreum* Mitchill). Recent Research Developments in Nutrition 1:27-32.

Busacker, G.P., I.R. Adelman, and E.M. Goolish. 1990. Growth. Pages 363-387 *in* P. Moyle and C. Schreck, editors. Methods in fish biology, American Fisheries Society, Bethesda, Maryland.

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EDUCATION

B.S. Cornell University, 1977
M.S. Tennessee Technological University, 1980
Ph.D Ohio State University, 1988

POSITIONS

Associate Professor (1995-present) and Assistant Professor (1988-1995), Fisheries and Wildlife, University of Missouri-Columbia
Aquatic Ecologist (1985-1987), Battelle Memorial Institute
Research Associate I&II (1980-1984), Aquatic Ecology Program, Ohio State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society (Member of following technical committees: Aquaculture, Physiology, Reservoir Fisheries, Education)
Missouri Chapter of American Fisheries Society
American Institute of Fishery Research Biologists

SELECTED PUBLICATIONS

Wang, N., R.S. Hayward, and D.B. Noltie 1998. Variation in food consumption, growth, and growth efficiency among juvenile hybrid sunfish held individually. Aquaculture 167:43-52.

Wang, N., R.S. Hayward, and D.B. Noltie. 1998. Effect of feeding frequency on food consumption, growth, size variation, and feeding pattern of age-0 hybrid sunfish. Aquaculture 165:261-267.

Zweifel, R.D., R.S. Hayward, and C.F. Rabeni. In press. A bioenergetics evaluation of black bass distributions in Ozark Border region streams. North American Journal of Fisheries Management.

Whitledge, G.W., R.S. Hayward, D.B. Noltie, and N. Wang. 1998. Testing bioenergetics model predictions of fish growth and food consumption under feeding regimes that elicit compensatory growth. Transactions of the American Fisheries Society 127:740-746.

Hayward, R.S., and M.A. Weiland. 1998. Gastric evacuation rates and maximum daily rations of rainbow trout fed chironomid larvae at 7.8, 10 and 12.8°C. Environmental Biology of Fishes 51:321-330.

Whitledge, G.W., and R.S. Hayward. 1997. Laboratory evaluation of a bioenergetics model for largemouth bass at two temperatures and feeding levels. Transactions of the American Fisheries Society 126:1030-1035.

Weiland, M.A., and R.S. Hayward. 1997. Cause for the decline of large rainbow trout in a tailwater fishery: too much putting or too much taking? Transactions of the American Fisheries Society 126:758-773.

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EDUCATION

B.S. Iowa State University, 1979
M.S. Texas A&M University, 1982
Ph.D. Mississippi State University, 1988

POSITIONS

Fisheries and Aquaculture Specialist/Associate Professor (1995-present), Specialist/Assistant Professor (1988-1995), Department of Animal Ecology, Iowa State University and Associate Director (1990-present), North Central Regional Aquaculture Center
Graduate Research Assistant (1986-1988), Mississippi State University
Aquaculture Manager (1982-1986), Stiles Farm Foundation
Graduate Research Assistant (1981-1982), and Research Technician I (1980-1981), Texas A&M University
Fisheries Biologist Aide (1979), Indiana Department of Natural Resources

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Iowa Chapter; Education, Fish Culture, Early Life History, Computer Users, and Fish Management Sections
American Chapter - World Aquaculture Society
Epsilon Sigma Phi
Iowa Aquaculture Association
Phi Kappa Phi
Sigma Xi
World Aquaculture Society

SELECTED PUBLICATIONS

Morris, J.E., and C.C. Mischke. 1998. Sunfish (*Lepomis* spp.) culture. NCRAC Video Series #104, NCRAC Publications Office, Iowa State University, Ames.

Mischke, C.C., and J.E. Morris. 1998. Growth and survival of larval bluegills in the laboratory under different feeding regimes. Progressive Fish-Culturist 60:206-213.

Mischke, C.C., and J.E. Morris. 1997. Out-of-season spawning of sunfish (*Lepomis* spp.) in the laboratory. Progressive Fish-Culturist 59:297-302.

Latka, D.C., J.S. Ramsey. and J.E. Morris. 1995. Selection of tributary confluence habitat by shovelnose sturgeon in the channelized Missouri River. *In* A.D. Gershanovich and T.I.J. Smith, editors. Second International Symposium on Sturgeon, Moscow, Russia.

Bryan, M.D., J.E. Morris, and G.J. Atchison. 1994. Methods for culturing bluegill in the laboratory. Progressive Fish-Culturist 56:217-221.

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EDUCATION

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

POSITIONS

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

Associate Director (1995-present) and Assistant Director (1986-1995), Fisheries Research Laboratory, Southern Illinois University-Carbondale

Assistant Professor (1984-1986), Department of Fisheries and Wildlife Science, Virginia Polytechnic Institute and State University

SELECTED PUBLICATIONS

Sheehan, R.J., S.P. Shasteen, A.V. Suresh, A.R. Kapuscinski, and J.E. Seeb. In press. All-female triploids and diploids outgrow mixed-sex diploid rainbow trout. Transactions of the American Fisheries Society.

Suresh, A.V., and R.J. Sheehan. 1998. Muscle fibre growth dynamics in diploid and triploid rainbow trout. Journal of Fish Biology 52:570-587.

Suresh, A.V., and R.J. Sheehan. 1998. Biochemical and morphological correlates of growth in diploid and triploid rainbow trout. Journal of Fish Biology 52:588-599.

Sheehan, R.J. , and M. Konikoff. 1998. Wetlands and fisheries resources of the Mississippi River. Pages 628-647 *in* S.K. Majumdar, E.W. Miller, and F.P. Brenner, editors. Wetlands and associated systems. Pennsylvania Academy of Science, Harrisburg.

Wills, P.S., R.J. Sheehan, R.C. Heidinger, B.M. Burr, and M. Nuevo Alarcon. 1998. Range expansion for the golden topminnow, *Fundulus chrysotus* (Gunther), and its rediscovery in Missouri. Journal of Freshwater Ecology 13:253-254.

Fetzner, J., R.J. Sheehan, and L. Seeb. 1997. Population genetics of crayfish species of potential aquacultural importance in the Midwest. Aquaculture 154:39-55.

Conover, G.A., and R.J. Sheehan. 1996. Validation of black crappie OTC otolith marks. North American Journal of Fisheries Management 16:686-688.

Kohler, C.C., R.J. Sheehan, C. Habicht, J.A. Malison, and T.B. Kayes. 1994. Habituation to captivity and controlled spawning of white bass. Transactions of the American Fisheries Society 123:964-974.

Wills, P.S., R.J. Sheehan, and J. Paret. 1994. Induced polyploidy in sunfish. Journal of the World Aquaculture Society 25(4):507-511.

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EDUCATION

A.B. Humboldt State University, 1980
M.A. Humboldt State University, 1982
Ph.D. University of California-Berkeley, 1985

POSITIONS

James A. Meier Professor (1999-present), Director, Regulatory Biosciences Cluster (1991-present), Professor (1998-present), Associate Professor (1991-1998), Assistant Professor (1985-1991), and Graduate Program Coordinator (1986-1991), Department of Zoology North Dakota State University, Fargo
Visiting Professor (1998), University of Sao Paulo, Sao Paulo, Brazil
Visiting Professor (1996), University of Gotëborg, Goteborg, Sweden
Visiting Professor (1994), University of Tokyo, Ocean Research Institute, Japan
Visiting Scientist (1992), Institut Nationale Researches Agronomique, France
Visiting Scientist (1991), Humboldt State University, Arcata, California
Visiting Scientist (1986 and 1988), National Marine Fisheries Service, Seattle
Visiting Scientist (1985), Department of Zoology, University of Washington, Seattle

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Fisheries Society
Endocrine Society
North Dakota Academy of Sciences (President, 1999-2000)
Society for Integrative and Comparative Biology (Division of Comparative Endocrinology, Program Officer 1996-1999)
Sigma Xi (Chapter President, 1993)

SELECTED PUBLICATIONS

Holloway, A.C., M.A. Sheridan, G. Van Der Kraak, and J.F. Leatherland. In press. Correlations of plasma growth hormone, with somatostatin, gonadal steroid hormones and thyroid hormones in rainbow trout during sexual recrudescence. Comparative Biochemistry and Physiology.

Sheridan, M.A., and Y.-H. Kao. 1998. Regulation of metamorphosis-associated changes in lipid metabolism of selected vertebrates. American Zoology 38:350-368.

Sheridan, M.A., C.D. Eilertson, and T.H. Kerstetter. 1998. Changes in plasma somatostatin associated with seawater adaptation and stunting of coho salmon, *Oncorhynchus kisutch*. Aquaculture 168:195-203.

Pesek, M.J., N. Howe, and M.A. Sheridan. 1998. Insulin and glucagon modulate somatostatin binding to hepatocytes isolated from rainbow trout, *Oncorhynchus mykiss*. General Comparative Endocrinology 112:183-190.

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EDUCATION

B.S. University of Wisconsin-Stevens Point, 1957
M.S. Southern Illinois University, 1959
Ph.D. Southern Illinois University, 1964

POSITIONS

Professor (1976-present), Department of Animal Ecology, Iowa State University
Associate Director (1988-1990), North Central Regional Aquaculture Center
Chairman (1976-1985), Department of Animal Ecology, Iowa State University
Leader, (1966-1976) Oklahoma Cooperative Fishery Research Unit, Oklahoma State University
Assistant Professor (1964-1966), Department of Zoology, Kansas State University
Lecturer (1962-1964), Southern Illinois University-Carbondale

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
American Institute of Fishery Research Biologists (Fellow)
Aquacultural Engineering Society
Iowa Academy of Sciences
World Aquaculture Society
Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta

SELECTED PUBLICATIONS

Clayton, R.D., T.L. Stevenson, and R.C. Summerfelt. 1998. Fin erosion in intensively cultured walleye and hybrid walleye. Progressive Fish-Culturist 60:114-118.

Phillips, T.A., R.C. Summerfelt, and R.D. Clayton. 1998. Feeding frequency effects on water quality and growth of walleye fingerlings in intensive culture. Progressive Fish-Culturist 60:1-8.

Rieger, P.W., and R.C. Summerfelt. 1997. The influence of turbidity on larval walleye, *Stizostedion vitreum*, behavior and development in tank culture. Aquaculture 159:19-32.

Summerfelt, R.C., B.A. Barton, R.D. Clayton, and B. Pottebaum. 1997. Comparison of plasma cortisol and chloride measures of stress in fingerling walleye after handling and transporting from hatchery to stocking sites in vented and unvented transport tanks. Pages 38-58 *in* M. B. Timmons and T. Losordo, editors. Advances in aquacultural engineering. NRAES-105.

Summerfelt, R.C., editor. 1996. Walleye culture manual. NCRAC Culture Series #101, NCRAC Publications Office, Iowa State University, Ames.

Summerfelt, R.C. 1996. Intensive culture of walleye fry. Pages 161-185 *in* R.C. Summerfelt, editor. Walleye culture manual. NCRAC Culture Series #101, NCRAC Publications Office, Iowa State University, Ames.