CULTURAL TECHNOLOGY OF WALLEYE

| Chairperson: | Robert C. Summerfelt, Iowa State University |
|---------------------|--|
| Extension Liaisons: | Ronald E. Kinnunen, Michigan State University; Joseph E. Morris, Iowa State University |
| Funding Request: | \$150,000 |
| Duration: | 2 Years (September 1, 1993 - August 31, 1995) |

Objectives:

- 1. Measure genetic parameters required for efficient combined selection on sub-adult and adult traits, using a pedigreed population of walleye.
- 2. Compare performance (survival, growth, feed conversion) of walleye hybrids produced from different parental stocks reared under intensive and the tandem extensive-intensive culture systems.
- 3. Conduct field trials that compare effectiveness and costs of different pond and tank culture strategies for producing advanced fingerlings.

Proposed Budgets:

| Institution | Principal Investigator(s) | Objec- tive(s) | Year 1 | Year 2 | Total |
|---|--|-------------------|----------|----------|-----------|
| University of Minnesota | Anne R. Kapuscinski | 1 | \$19,354 | \$19,754 | \$39,108 |
| University of Wisconsin- Madison | Jeffrey A. Malison Terence P. Barry | 1-3 | \$15,000 | \$16,000 | \$31,000 |
| lowa State University and the McGraw Foundation | Robert C. Summerfelt Tom Harder | 2&3 | \$21,371 | \$24,521 | \$45,892 |
| University of Nebraska- Lincoln | Terrence B. Kayes | 3 | \$17,000 | \$17,000 | \$34,000 |
| | | TOTALS | \$72,725 | \$77,275 | \$150,000 |

Non-funded Collaborators:

| Facility | Collaborator(s) |
|--|-------------------------------------|
| Aurora-Aqua, Inc., Kandiyohi, Minnesota | Gene P. Hanson |
| North Platte State Fish Hatchery, North Platte, Nebraska | Nebraska Game & Parks Commission |
| Calamus State Fish Hatchery, Burwell, Nebraska | Nebraska Game & Parks Commission |

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JUSTIFICATION

The coolwater percid fishes (Family Percidae), the yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*), rank among the most heavily exploited species in North American commercial and recreational fisheries (Kendall 1978). Because of their popularity as food and game fishes in the Upper Midwest, these percids have been recognized by the North Central Regional Aquaculture Center (NCRAC) as species with unique aquacultural potential for the region. At the first joint meeting of the Industry Advisory Council and the Technical Committee of NCRAC in May 1988, and at every joint planning meeting since, the yellow perch and walleye have been assigned the highest ranking of any species for financial support from the Center. Research planning for the percids, under the auspices of NCRAC, has been divided into one work group for yellow perch and a second for walleye. This proposal is for research on walleye and walleye hybrids.

It should be noted that the walleye also was recognized in the National Aquaculture Development Plan (Joint Subcommittee on Aquaculture 1983) as a fish with substantial aquaculture potential, because it has a high market value and the supply from traditional commercial sources is limited. At present, food-size walleye production in the U.S. is limited to few tribal fisheries in Minnesota and Wisconsin. Elsewhere in the U.S., commercial fishing for walleye has been prohibited to protect the resource for exclusive exploitation by sport fishing. To supply the retail market, walleye, primarily as frozen fillets, are imported from Canada. In Canada, the total wild harvest of walleye has been declining. Between 1973 and 1979, the commercial harvest of walleye in the Province of Manitoba was only 65% of the average annual production between 1945 and 1954 (Sifa and Ayles 1981). The limited supply has produced exceptionally high retail prices for walleye fillets (\$16.09-25.35/kg). Such prices have stimulated strong private-sector interest in the production of food-size fish by aquaculture, for example, a fairly large-scale effort to raise walleye to food-size was started by Aquaculture Inc., Rolla, Missouri (NCRAC Journal 1990).

State, federal and provincial fisheries management agencies in North America have stocked more than one billion walleye fry and fingerlings (Conover 1986). Given the numbers of walleye fry and fingerlings needed for stocking, many fisheries management agencies in the U.S. and Canada have conducted applied research on various phases of walleye aquaculture for many years (Coolwater Culture Workshop 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992). The fish culture activities traditionally associated with producing walleye include spawning of wild broodstock, hatching fry, and rearing fingerlings in ponds. Numerically, fry comprise 98% of walleye stockings in the U.S. and Canada (Conover 1986). Large numbers of fingerlings can be produced to a size of 32-50 mm total length (TL) by traditional pond-culture methods (Beyerle 1979; Fox 1989; Fox and Flowers 1990; Harding 1991; Fox et al. 1992). However, survival and production are often highly variable (Li and Ayles 1981), and it is difficult in ponds to rear large numbers of walleye to sizes above 100 mm TL without the addition of forage fish. To meet the demands of fisheries managers for fingerlings larger than 100 mm TL, government agencies have focused their research efforts on developing procedures to train and further raise pond reared fingerlings on formulated feeds by intensive culture in tanks (Cheshire and Steele 1972; Nagel 1974, 1976, 1985, 1991; Beyerle 1975; Nickum 1986). A variety of factors have been studied, including stocking density, water temperature, light intensity, diet, and feeding frequency (Nickum 1986; Summerfeit 1988, 1990).

At present, commercial walleye aquaculture is geared almost entirely to the production of eggs, fry, and pond-reared fingerlings. Commercial walleye producers are selling fry and fingerlings to lake associations, sportsman's clubs, and individual lake and pond owners for stocking. Excellent market prices for walleye fry and fingerlings have bolstered commercial walleye production. Newly hatched fry sell for 1 to 1.5 cents each, and fish of 32 to 100 mm TL are sold by producers for \$0.25 to \$0.75, or more. The growth of private-sector pond production has been particularly marked in Minnesota, Nebraska, Wisconsin, Iowa, and Michigan. Experience with rearing walleye to food-size has been limited largely to a few researchers in the region, who have the expertise to train pond-reared fingerlings to formulated feeds. However, as previously noted, private-sector interest in the production of food-sized walleye is very high.

For commercial walleye aquaculture to be profitable, many aspects of the production process require further investigation, and field trials are needed to evaluate the effectiveness and costs of different production strategies. The focus of the proposed project is on: (1) selective breeding based on family selection; (2) performance comparisons of purebred and hybrid walleye; and (3) field trials comparing the effectiveness and costs of different strategies for producing advanced fingerling walleye (defined here as young-of-the-year walleye having a TL of 100 mm or longer). Throughout this project document, the term **"hybrid walleye" refers to walleye female x sauger male crosses.** The project, which is interdisciplinary in scope and could not be accomplished by a single institution, will involve the state fisheries management agencies of Iowa, Minnesota, Nebraska, and Wisconsin, the non-profit Max McGraw Wildlife Foundation of Illinois, Aurora Aqua, Inc., a private producer in Minnesota, and investigators from four of the 12 land grant universities in the North Central Region: Iowa State University

(ISU), the University of Minnesota at St. Paul (UMN), the University of Nebraska-Lincoln (UN-L), and the University of Wisconsin-Madison (UW-Madison). Cost data collected by the NCRAC Walleye Work Group will be given to the NCRAC Economics and Marketing Work Group for its analyses of the production economics of walleye aquaculture. This integrated collaboration among four land grant universities, two private sector groups, and four state agencies allows the project to access the required facilities and the best available expertise in the North Central Region. This, in turn, will greatly enhance success in meeting project objectives.

RELATED CURRENT AND PREVIOUS WORK

Review of Current NCRAC Walleye Projects

The present proposal describes a new two-year, cooperative regional research project by the NCRAC Walleye Work Group. The proposed research includes an extension of a current objective (Work Group 2, Year 3, Objective 2), on evaluating differences in performance of family lines, and two new objectives. One of the new objectives involves comparison of walleyes and hybrid walleyes reared intensively and in tandem pond-tank culture systems, and the other, field trials in Illinois and Nebraska to obtain comparative data on production costs to rear fingerlings to 100 mm or longer by intensive and extensive culture systems. Although NCRAC has not funded research on walleye-sauger (*S. v. vitreum x S. canadense*) hybrids, proposals for study of the hybrid were among the first walleye work group objectives; funding constraints prevented approval at that time.

The proposed research builds on prior NCRAC Walleye Work Group projects as explained below.

<u>Work Group 1</u>: This Work Group started in 1989 as a three year project, initially with funding for only the first two years (1989-90, 1990-91), however, a third year of funding was approved for all three objectives for FY 1992 (1991-92). Objectives of this work group were: (1) characterization of the natural reproductive cycle of walleye; (2) evaluation of various zooplankton seeding and clam shrimp control strategies for their effects on the pond production of walleye fingerlings; and (3) examination of the etiology of gas-bladder inflation problems in walleye fry reared in intensive culture. This work group involved cooperation from the U.S. Fish and Wildlife Service, several natural resources agencies (Kansas, Illinois, Iowa, Minnesota, Ohio, and Wisconsin) and investigators from six institutions in the North Central Region (Southern Illinois University at Carbondale (SIUC), ISU, Michigan State University (MSU), UMN, UN-L, UW-Madison). In the third year, the project was made inter-regional with the addition of University of California-Davis to draw upon the expertise of Dr. D. E. Hinton to conduct a histological study of walleye gas bladder development, a component to determining the cause for non-inflation of the gas bladder of larval walleye (Objective 3). Findings from the entire project will be reported in the next year as the various components reach termination. While completion reports for the project are due October 1, 1992, some components of the completion report will not be submitted until 1993 in order to provide time for data analysis and writing by investigators who carried out research in the 1992 culture season.

<u>Work Group 2, Years 1 and 2</u>: In the first two years (September 1, 1990 to August 31, 1992), this Work Group had two objectives: (1) to develop baseline information on genetic composition of walleye populations for potential use as broodstock; and (2) to conduct comparisons of phenotypic characteristics of progeny from selected walleye broodstock.

Objective 1 was proposed by Lisa W. and James E. Seeb, SIUC, however, in August, 1990, before the start of the project, the Seebs relocated to Alaska. The decision was made to retain the project and give SIUC an opportunity to find a competent replacement for the PI. SIUC completed an extensive search and hired Neil Billington, a population geneticist with prior research experience on walleye population genetics. Dr. Billington has initiated work for Objective 1 as planned, using allozyme and mitochondrial DNA (mtDNA) analysis for discriminating walleye populations in the North Central Region.

If populations possessing genetic marks are used in selective breeding programs, the marks are passed from generation to generation, and their use may facilitate the perpetual marking of aquaculturally important strains (reviewed in Utter and Seeb 1990). Additionally, work with other species suggests that occasional linkages observed between allozyme markers and genes controlling quantitative traits may provide information important for selective improvement (Liebowitz et al. 1987; Seeb 1987). Seeb et al. (1981), in a pilot study, resolved 40 allozyme loci in 52 walleye from Lake Erie. Ten loci were polymorphic; the average heterozygosity observed was 0.057, showing that walleye possess an abundance of genetic variation suitable for population structure and gene diversity analyses. Murphy and Lee (1986) only resolved four of ten polymorphic loci they detected in Minnesota populations; yet they observed substantial population differentiation between major watersheds. Billington and Herbert (1988) used 22 endonucleases to study mtDNA variation in ten populations of walleye from the Great Lakes basin. Their data clearly differentiated eastern and western Great Lakes populations and demonstrated

the utility of mtDNA analysis in walleye population studies. Population genetic data from allozymes and mitochondrial DNA will provide the baseline data needed to recognize and maintain pure strains for aquaculture; they will also provide genetic diversity estimates useful for identifying suitable strains for selective breeding programs.

Objective 2 was a comparative study of performance traits of different walleye stocks in the region. The performance of several candidate stocks was compared to identify stocks exhibiting the best overall performance for subsequent use in initiation of a selective breeding program. Identification of the stocks exhibiting the best overall performance is an important prerequisite to starting a selection program because it is significantly easier and less expensive to improve a population with an initially good genetic makeup than one with a poor genetic makeup (Kinghorn 1983; Shultz 1986; Tave 1987).

Work for Objective 2 was initiated in 1991 by an interdisciplinary group of researchers, involving faculty from three institutions: SIUC, ISU, and UMN. In addition to the principal investigators, there has been active collaboration from state agencies (Iowa, Minnesota, Ohio) and the U.S. Fish and Wildlife Service's Genoa National Fish Hatchery and the Garrison Dam National Fish Hatchery, Riverdale, North Dakota. Anne Kapuscinski, UMN provides assistance to collaborators at SIUC (Bob Sheehan and Bruce Tetzlaff) and ISU (Bob Summerfelt) with analysis of performance comparisons among different stocks.

The project termination date is August 31, 1992, however, given that the start date in 1990 was after the 1990 culture season, the termination date was extended to February 1993 to allow incorporation of both the 1991 and 1992 culture seasons and provide additional time for analysis and preparation of the completion report.

Stock evaluations have included both intensive (ISU) and tandem pond-tank culture (SIUC) systems. In the tandem culture component carried out at SIUC, 2-day-old fry are stocked in pond, reared to a 38-50 mm fingerling size, harvested from the ponds and transferred to tanks where they are trained to formulated feed. In the SIUC study, stocks are evaluated in terms of pond survival and success at habituating pond reared fingerlings to formulated feed.

ISU has evaluated offspring from wild broodstocks (obtained from natural waters) in 1991 and 1992 from: (1) Iowa (IA), (2) Kansas (KS), (3) the Mississippi River near Genoa, Wisconsin (MR), (4) Minnesota (MN-1, MN-2, two populations), and (5) North Dakota (ND). In addition, a semi-domesticated, captive brood stock maintained in ponds at the London Fish Hatchery, Ohio (OH) was evaluated each year. The OH broodstock was identified as a high priority for study because it is the only known stock to have been inbred for three generations and should therefore exhibit some domestication. Significant stock differences were found in egg size, size of newly hatched fry, incidence of cannibalism, congenital defects and adaptability to rearing in an intensive culture environment. In the 1991 season, four strains (IA, OH, MN-1, and MR) were evaluated at ISU for a number of performance criteria; so far, data on survival, gas bladder inflation, viability, length at harvest and growth rate have been analyzed. Survival differed among all stocks. The OH and MR stocks had significantly higher gas bladder inflation and viability than the other two stocks, but growth rate was highest for the MR and MN-1 stocks. A ranking system for a composite evaluation of the stocks is under development.

<u>Work Group 2, Year 3:</u> A third year of research was approved by the NCRAC Board of Directors for two objectives: (1) to develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning and (2) to measure genetic parameters required for efficient combined selection on sub-adult and adult traits using a pedigreed population of walleye. This research is scheduled for one year, September 1, 1992 through August 31, 1993.

The first objective of this project is an outgrowth of Objective 1 of the first work group - characterization of the natural reproductive cycle of walleye. The focus of this research will be to use the baseline information on walleye reproductive cycles to artificially manipulate sexual maturation and induce out-of-season spawning in captive broodstocks. The potential benefits include: (1) greater predictability of gamete production; (2) reduced incidence of failed spawning, gamete resorption and subsequent broodfish losses; and (3) the production of fertilized eggs and fry at multiple and predetermined times during the year.

The second objective is the first stage in the implementation of a rationally designed and long-term selective breeding program. It involves creation of parent generation (G_0) pedigreed families and measures of early life stage performance traits on these families. The major benefits of selective breeding to be assigned commercial aquaculture operations are improvements in product quality and cost-effectiveness, and increases in harvestable yields and profits that are expected from genetic improvement of performance traits (Gjedrem 1983; Kinghorn 1983; Tave 1987). Recently in the North Central Region, the importance of walleye as a food-fish has risen tremendously and a number of private producers and researchers have initiated the development of culture methods for a food-fish product

(NCA-23 1987). Yet, virtually no systematic breeding of walleye has been attempted in North America. The overall goal of Objective 2, therefore, is to develop a North Central Regional breeding program for efficient selection to improve commercially important traits of walleye.

As the study will not begin until September 1, 1992, the first progress report will not be available until October 1993.

Review of Related Current and Previous Work for Proposed Objectives

Three topic areas related to the proposed objectives are:

- 1. Selective Breeding
- 2. Hybrids
- 3. Field trials: Costs for production of fingerlings 100 mm or longer

Selective Breeding (Objective 1)

Genetic research is critically important for world-wide development of aquaculture (Wilkins and Gosling 1983; Gall and Busack 1986). The success of the Norwegian Atlantic salmon (*Salmo salar*) farming industry provides an excellent example of the value of initiating genetic analyses at the early stages of an aquaculture industry. Selective breeding programs, focused primarily on increasing growth rates, were begun in an early phase of the industry (Gjerde 1984; Gjerde and Gjedrem 1984; Standal and Gjerde 1987; Refstie 1987) and were a major contributing factor to the current domination of the international salmon market by Norway (Rhodes 1987, 1988). Realized responses to selection for growth rate, a major production trait, have been higher in fish species than those reported for farm animals (Gjerde 1986). Responses in Atlantic salmon breeding, for example, have ranged from a 14% to 30% gain per generation (Gjerde 1986; Kinghorn 1983). These findings suggest that a regional selective breeding program for walleye will be of great economic benefit to private aquaculturists. However, to the best of our knowledge, no quantitative genetic analyses of walleye populations have been reported in the literature (Ebbers and Colby 1988). We are also unaware of any current research in the U.S. to measure heritabilities or to develop selection programs for specific walleye production traits.

The focus of this objective is to develop a captive domesticated broodstock from selected families derived from the wild stocks which exhibited the best performance traits under intensive, indoor culture (see **Review of Current NCRAC Walleye Projects** <u>Work Group 2, Years 1 and 2</u>). An efficient regional selective breeding program is needed to create a walleye broodstock that will produce progeny adapted to commercial food-fish culture environments.

Design of selective breeding program

A successful breeding program depends on prior knowledge that the targeted traits will respond well to selection. For a given trait in a given fish population, response to selection is primarily influenced by the degree of genetic control over the trait (measured by heritability), amount of total trait variation in the population (measured by phenotypic means and variances, coefficient of variation, and range), and relationship to other traits (measured by genetic and phenotypic correlations). Because trait heritabilities and other genetic parameters differ for different populations, they must be measured in the population of interest to ensure successful selective breeding (Tave 1987). Estimates of genetic and population parameters, combined with consideration of operational limitations (e.g., facility size, available financial resources), can be used to design an optimum selection program yielding the greatest response to selection possible under the given constraints. Maintenance of pedigreed fish populations is imperative for precise estimation of genetic parameters, and thus, for efficient design and success of selection schemes (Gall 1990).

Heritability values for a trait reflect the level of additive genetic variance, which is the component of genetic variance most readily exploited in selection programs (Falconer 1981). Although heritabilities greater than 0.1-0.2 are needed for efficient individual selection, family selection can be implemented when heritabilities are low (Falconer 1981; Tave 1987). Combined selection, in which only the best individuals from the best families are bred, is the selection scheme of choice for most aquaculture situations (Gjedrem 1983; Gall 1990). It yields greater genetic gain per generation than sole use of individual selection or family selection. It also can account for differences between families due to different experienced environments, which will occur in most fish breeding programs because incubation and early life rearing of pedigreed families usually occurs in separate containers until fish are large enough to assign unique individual marks.

Examples of traits that had relatively high heritabilities in other species and that may be important for selective breeding in walleye include growth rate, size at age, age at sexual maturity or spawning, and

belly wall thickness (e.g., Kinghorn 1983; Gjerde and Gjedrem 1984; Gall et al. 1988; Gjerde and Schaeffer 1989; Hershberger et al. 1990). Traits that had lower heritabilities, necessitating family or combined selection, and that would be important in walleye selection, include survival and perhaps food conversion.

Few determinations have been made of phenotypic and genetic correlations between concurrently or sequentially expressed traits in fish. Existing reports, however, suggest the value of estimating these correlations whenever possible. Such information provides a means of planning for potential counteracting natural selection on negatively correlated traits, and of ensuring that selection schemes achieve improvement of targeted traits without inadvertent damage of critical correlated traits. Finally, use of a selection index, i.e., an efficient method of improving two or more economically important traits, requires having prior data on genetic and phenotypic correlations for the traits and population targeted for selection.

Induced and accelerated spawning of captive broodstock

The expertise and techniques needed to successfully spawn walleye will be required to achieve this objective because a major fraction of captive walleye fail to undergo final oocyte maturation and ovulation without hormonal manipulations (Nelson et al. 1965; Lessman 1978; Hearn 1980; Pankhurst et al. 1986; Heidinger et al. 1989; Barry et al. 1992). The ability to artificially spawn captive walleye with hormone injections may also make it possible to accelerate the proposed selective breeding program, by allowing earlier application of combined selection on the matings in the parent generation (G_0), and, creation of the first progeny generation (G_1). Recent work by UW-Madison investigators indicates that it may be possible to accelerate the onset of sexual maturation in walleye and induce spawning in 2-year-old captive broodstock (Malison et al. unpublished).

The changes in gonadal morphology and reproductive steroids which occur during the annual reproductive cycle of wild-caught walleye have recently been characterized (Malison et al., 1992, In preparation). Techniques were developed to assess the stage of oocyte maturation in pre-spawning walleye, and to induce final oocyte maturation and spawning in wild-caught walleye at least two weeks prior to the normal spawning season (Barry et al. 1992, In preparation). In the spring of 1992, UW-Madison researchers used similar techniques to induce final oocyte maturation and spawning in both sauger and female walleye \mathbf{x} male sauger hybrids which had been reared exclusively in indoor tanks since the small fingerling stage (unpublished data). Studies this upcoming year are aimed at evaluating several hormonal treatment regimes with the goal of advancing spawning in wild walleye by as much as 12 weeks (Malison and Held 1992).

Walleye x Sauger Hybrids (Objective 2)

With certain fish species, interspecific crossbreeding has resulted in hybrids having behavioral and growth characteristics better suited for intensive culture than those of purebred species. For example, muskellunge **x** northern pike, striped bass **x** white bass and plaice **x** flounder hybrids accept formulated feeds more readily and are more easily habituated to intensive culture conditions than their respective parent species (see Bishop 1968; Purdom 1976; Graff 1978; Kerby and Joseph 1979). Muskellunge **x** northern pike, lake trout **x** brook trout and striped bass **x** white bass hybrids grow faster than either parental species, at least during the first few years of life (Hesser 1978; Fraser 1980; Kerby 1986). The improved performance resulting from hybridization is one of the factors responsible for the rapid growth of the hybrid striped bass aquaculture industry over the last several years. The NCRAC Walleye Work Group has consistently ranked the evaluation of walleye **x** sauger hybrids high on their research priority list, but to date has not funded any studies on hybrids.

Natural hybridization between walleye and sauger has been documented (Colby et al. 1979) and both reciprocal hybrids have been artificially propagated in the laboratory (Nelson et al. 1965). Several fisheries management agencies, including the Ohio Department of Natural Resources, have reared fingerling walleye female **x** sauger male (W **x** S) hybrids in ponds for stocking into reservoirs (Smith and Carline 1982, 1983).

Field studies of walleye and walleye hybrids have revealed more favorable survival and growth from the hybrids (Lynch et al. 1982, Humphreys et al. 1984; Leeds and Summers 1987; Leeds 1990; Johnson 1991). Because of the faster growth and higher survival rates, hybrid walleyes are fast becoming popular among natural resource agencies for stocking in reservoirs and small impoundments. In Ohio, since 1978, the "...numbers of stocked hybrids have risen to over 6.3 million fingerlings, while walleye stocking has declined from a high of about 2.25 million fingerlings in 1984 to 0.9 million in 1991" (Johnson 1991).

There is also evidence that hybrid walleyes are more adaptable to cultural conditions, they have faster growth and lower food conversions than walleye when training to formulated feed (Malison et al. 1990)

and in rearing 100 mm fingerlings to advanced sizes (Siegwarth and Summerfelt 1990, 1992). Evidence gathered to date indicates that walleye hybrids have significant potential for intensive culture for food fish. In fact, the information reported by Malison et al. (1990), and Siegwarth and Summerfelt (1990, 1992) suggests that walleye hybrids may be preferred over the pure strains because of superior performance and desirable behavioral characteristics of the hybrid for an intensive culture environment.

In spite of the favorable prospects for use of hybrid walleyes in aquaculture there is much to be learned before hybrid walleye can be said to be superior to walleye for commercial aquaculture. The findings of Malison et al. (1990), and Siegwarth and Summerfelt (1990, 1992) were all based on crosses made from progeny of Rock Lake, WI walleyes and Mississippi River saugers. Potential performance gains resulting from hybrid vigor will almost certainly be dependent upon the stocks of brood fish used to make hybrid crosses. Hybrids produced from stocks of walleye and sauger other than Rock Lake and Mississippi River, respectively, may have variable performance gains compared to purebred walleye. In one study conducted at the UW-Madison (unreported), walleye **x** sauger hybrids produced using Mississippi River walleye and Mississippi River sauger as brood fish did not show the performance improvements observed in hybrids of Rock Lake walleye and Mississippi River sauger. The comparative study of performance traits of different walleye stocks in the region by the NCRAC Walleye Work Group 2 (Years 1 and 2) showed that there are substantial differences in performance characteristics of different walleye stocks. This strongly suggests that the performance of the hybrids will also be affected by the specific walleye and sauger broodstocks crossed.

Although the studies by Malison et al. (1990), and Siegwarth and Summerfelt (1990, 1992), demonstrated that the walleye hybrids grew faster than walleye, their data were for fingerling fish, with the largest average size of the hybrid group at 222.3 mm TL. Because hybrid walleye have not been reared to food-size (i.e., 681 gm or 1.5 lb), it is not known whether the faster growth rate of the hybrids will continue until they reach marketable size or whether growth rates of pure walleye will eventually surpass that of the hybrids and allowing them to reach market size first.

Field Trials on the Production of Advanced Fingerlings (Objective 3)

The ability to predictably produce walleye fingerlings in large quantities is one of the most important problems in the culture of coolwater fishes today (Nickum 1978; Joint Subcommittee on Aquaculture 1983). In 1989, the NCRAC recognized the need to solve this problem and identified development of effective culture procedures for the walleye as one of its nine research priorities (Announcement, Culture Technology of Walleye and its Hybrids, NCRAC, 26 July 1988).

Aquaculture research is normally done on a small scale under controlled experimental conditions. To test the practicality of strategies and procedures developed by such research, their effectiveness and costs under production conditions need to be evaluated by field trials that answer critical questions and help fish farmers and investors make informed decisions. Large numbers of replicated experimental treatments are rarely possible under production conditions because of constraints in facilities, number of animals involved, and associated costs. Accordingly, field trials are best used to compare different production strategies, where each one is comprised of an assemblage of discrete techniques that in most instances have been evaluated by earlier research. Field trials are thus an important link between research and application.

Importance of field trials for commercialization

Irrespective of technical feasibility, walleye aquaculture will not be commercialized unless it can be proven cost-effective. Specific procedures for estimating production costs have been developed for catfish, trout, salmon, and a variety of other cultured fishes (Shang 1981; Keenum and Waldrop 1988; Meade 1989; Bjorndal 1990; Hinshaw et al. 1990; Pillay 1990). Fixed costs associated with aquaculture (e.g., for land, buildings, ponds, tanks, water supply and electrical systems, machinery and equipment, labor, insurance, property taxes, overhead, and management) are often highly variable, depending on region, site, management skills, and other factors. Variable costs of aquaculture (e.g., for seed stock or fingerlings, feed, pond fertilizers, herbicides, chemotherapeutants, electricity, and pumping) tend to be strongly influenced by species and production method (e.g., pond or intensive tank culture). To our knowledge, no systematic comparisons of different strategies for commercially producing advanced fingerling walleye have ever been made. Field trials, because of their focus on evaluating (small-scale) research findings under (larger-scale) practical conditions, provide a mechanism for making such comparisons.

Strategies for advanced fingerling production

There are three basic strategies for producing fingerling walleye: (1) intensively culture fry to advanced fingerlings entirely in tanks, feeding them brine shrimp nauplii and/or formulated feeds (Colesante et al. 1986; Krise and Meade 1986; Summerfelt et al. 1992); (2) produce walleye fingerlings by extensive

practices (e.g., fertilizer addition) entirely in ponds (Nickum 1986; Coolwater Culture Workshop 1989, 1990, 1991; Fox and Flowers 1990; Harding 1991; Fox et al. 1992); and (3) first culture the fish in ponds to an early fingerling size of 32-50 mm TL in 26-50 d (depending on food availability and temperature), and then intensively rear them to an advanced fingerling stage using formulated feeds (Beyerle 1979; Nickum 1978, 1986; Coolwater Culture Workshop 1989, 1990, 1991).

Intensive culture

Until recently, pond culture has been the only way to produce large numbers of 32-50 mm (TL) walleye. Based on these findings by the IDNR, it may now be possible to raise walleye on formulated feeds from first-feeding larvae to food-size fish. Successful tank culture of walleye from fry to advanced fingerlings was achieved by the Iowa Department of Natural Resources (IDNR) at the Rathbun Fish Hatchery (Summerfelt et al. 1991, 1992). However, average production in intensive systems is highly variable and further research is needed to improve consistency of results and reduce impediments to growth (due to low water temperatures in spring) and survival (due to high temperatures and disease in the summer).

Pond culture

There are two approaches for producing advanced walleye fingerlings in ponds: (1) release swim-up fry into ponds in the spring at low stocking rates (12,000-50,000 fry/hectare), and then harvest them as advanced fingerlings in the autumn and (2) stock hatchery ponds in the spring at relatively high rates (typically 175,000-250,000 fry/hectare), harvest early fingerlings at a size of 32-50 mm TL in 26-50 days, then restock them in ponds at a rate of 12,000-25,000 fish/hectare for continued rearing to advanced fingerlings. This latter strategy maximizes total numbers of fish produced per hectare in hatchery ponds. Early fingerlings not restocked into ponds for further growth can be released to public waters, or trained (habituated) to intensive tank culture and formulated feeds.

Research suggests that many fish species having small eggs and a larval feeding stage require similar procedures for optimizing pond production of juveniles. Both walleye and hybrid striped bass ponds, for example, are routinely treated with fertilizers to improve primary and secondary production, thus providing larger zooplankton forage base for the young fish (Richard and Hynes 1986; Buttner 1989; Harrell et al. 1990; Fox and Flowers 1990; Harding 1991; Fox et al. 1992). In addition, dense, stable plankton populations increase water turbidity and thereby inhibit the growth of aquatic macrophytes, which can compete with phytoplankton for available nutrients, restrict water circulation, interfere with fish harvest, and contribute to oxygen depletion and fish kills when the plants die (Chang 1986; Fast 1986). Optimal fertilization procedures vary from site to site, depending on differences in water chemistry, soil type, pond size, and other factors. A variety of organic and inorganic fertilizers have been used, including alfalfa pellets, various forms of phosphate, animal manures, seed meals, and hays.

A disadvantage of fertilization is that it increases the chemical and biochemical oxygen demand of a pond and can lead to dissolved oxygen depletion and massive fish kills. Oxygen depletion can be prevented by using emergency aerators when dissolved oxygen levels drop, or by the application of various supplemental aeration and water circulation techniques (reviewed by Boyd 1990 and Mével 1990). In addition to preventing dissolved oxygen depletion continuous or scheduled aeration and water circulation reduces chemical and thermal stratification, reduces diurnal fluctuations in dissolved oxygen, maintains aerobic conditions throughout a pond, allows a more constant decomposition of organic matter, and may help prevent phytoplankton die-offs (Fast 1986).

Aeration may also allow higher rates of fertilizer addition to ponds which could increase primary and secondary production. Parker (1979) demonstrated that a judiciously-managed program of stepped-up fertilization, combined with continuous aeration and water circulation through airlift pumps, could be used to increase the number of striped bass fingerlings produced per hectare of pond surface area by 2.5 times that achieved with conventional procedures. Preliminary studies at the UW-Madison, utilizing heavy carbon-nitrogen fertilization (see Jensen 1980) and a subsurface diffused-air aeration system, indicate that significantly enhanced production of walleye and yellow perch fingerlings in ponds should also be possible (J.A. Malison and T.B. Kayes, unpublished observations). Recently, Fox et al. (1992) found that continuous aeration with a subsurface diffused air distribution system helped maintain dissolved oxygen levels in heavily fertilized walleye production ponds.

Another very important facet of walleye pond culture is stocking rate. Research to date suggests that over a range of stocking rates (600,000-700,000 fry/hectare). Percent survival is unrelated to stocking rate or size at harvest, concluded that an increase in walleye size at harvest can be achieved at the expense of fish numbers and biomass produced, without affecting percent survival; or alternatively, that the numbers and biomass of fish harvested can be maximized by high stocking rates if (large) size is not critical (Harding 1991; Fox and Flowers 1990).

Pond+intensive culture

Successful implementation of the third strategy for producing advanced fingerling walleye, which involves first pond then intensive tank culture, depends on having procedures for habituating pond-reared early fingerlings to intensive culture conditions. Habituation is *normally* initiated in walleye 32-50 mm TL (Beyerle 1979; Nickum 1978, 1986; Coolwater Culture Workshop 1989, 1990, 1991), although recent work in perch indicates greater habituation success may be possible starting with much smaller fish (Malison and Held 1992). For example, it was found that: (1) the percent success of habituation of perch harvested from ponds at mean TLs of 16.9, 32.5, or 42.6 mm (53.3 \pm 5.5, 68.3 \pm 6.3, and 55.7 \pm 4.5[SE]%, respectively) were not significantly different, but habituation was faster in the smaller fish; (2) subsequent to habituation to intensive culture conditions, perch harvested at 16.9 mm TL were larger on days 131 and 215 post-hatch than fish harvested at 42.6 mm TL; (3) perch harvested at 16-20 mm TL and stocked into 750-L tanks at 13.7 fish/L exhibited higher percentages of habituation and less cannibalism than those stocked at 37.4 fish/L; and (4) perch reared using internal tank lighting had better rates of habituation than those reared under external overhead lights.

Collectively, the findings of Harding (1991), Fox and Flowers (1990), and Malison and Held (1992) suggest that walleye fingerling production might be greatly improved by a strategy of high pond stocking rates (e.g., 400,000-600,000 fry/hectare) and early pond harvest (e.g., when the fish reach 20-30 mm TL), in combination with appropriate tank stocking rates and internal tank lighting to facilitate habituation to intensive culture conditions. Implementation of this strategy will require procedures for harvesting large numbers of very small fingerlings from production ponds without undue stress. To perform their experiments, Malison and Held (1992) captured perch of 16-20 mm TL from a 0.4-hectare pond using an improved version of a system developed by Manci et al. (1983) for light-harvesting photopositive juvenile fish. Bulkowski and Meade (1983) reported that young walleye below 32 mm TL were attracted by light, while those over 32 mm TL became increasingly photonegative. Walleye between 9 and 30 mm TL were very photopositive. The potential of large-scale harvest of walleye from ponds using "light-response" and other low-stress procedures needs to be systematically evaluated.

ANTICIPATED BENEFITS

The overall goal of this project is to overcome the biological and technological constraints on the development and expansion of a commercial walleye food fish aquaculture industry. Three major constraints in this regard are: (1) lack of domesticated, selectively bred broodstock capable of spawning in captivity at early ages (2-3 yrs.); (2) uncertainty about the best choice of walleye and sauger stocks for producing hybrid walleye that will outperform purestrain walleye under captive culture conditions; and (3) uncertainty about the most cost-effective cultural technologies for producing advanced fingerlings.

Our proposed studies under Objective 1 will complete the *parent* generation of work in development of a domesticated line selected for high performance in different indoor systems for food-fish production. Benefits of improved performance of such a selected walleye strain will include reduced operating costs and harvest of a better quality product. If attempts under this objective to induce early reproduction are successful, the resulting shorter generation time will reduce: (1) costs of future selective breeding programs (in the private or public sector) and (2) costs of any production operations involving rearing of broodstock. Studies under Objective 2 will make it possible to identify one or more combinations of walleye and sauger stocks that yield hybrids exhibiting superior performance compared to purestrain walleye. Identification such superior crosses could substantially reduce the time, and therefore the costs, required to produce food-fish walleye. Field trials under Objective 3 will provide concrete comparisons of effectiveness and costs of alternative technologies for commercial-scale production of advanced fingerlings. Public availability of such comparative information will assist particular producers in making better choices about the best technology for their particular financial and facility constraints. Additionally, work under this objective should identify the best overall technology with respect to cost per viable advanced fingerling.

OBJECTIVES

1. Measure genetic parameters required for efficient combined selection on sub-adult and adult traits, using a pedigreed population of walleye.

[Completion of Objective 1 will allow consideration of the following future objective as a logical next step in a long-term selective breeding program: following application of combined selection on the parent generation, determine response to selection in the progeny generation and formulate practical guidelines for commercial-scale selection in future generations.]

- 2. Compare performance (survival, growth, feed conversion) of walleye hybrids produced from different parental stocks reared under intensive and the tandem extensive-intensive culture systems.
- 3. Conduct field trials that compare the effectiveness and costs of different pond and tank culture strategies for producing advanced fingerlings.

PROCEDURES

Selective Breeding for Commercial Aquaculture (Objective 1)

The proposed selection program is designed to concurrently complete two tasks in the parent generation or generation 0 (G_0): (a) estimate genetic parameters needed to design efficient selection on a set of traits and (b) apply combined selection that will yield optimum improvement of targeted traits. Such a selection program requires creation of pedigreed full- and half-sib families and statistically precise estimation of genetic and population parameters for the traits of interest.

Pedigreed Families at UMN

The NCRAC Walleye Work Group workplan for September 1, 1992 - August 31, 1993 (discussed **Review** of Current NCRAC Walleye Projects Work Group 2, Year 3), involves creation of 24 full-sib families nested within eight half-sib families. These families will constitute a balanced, nested mating design of full- and half-sib families (Becker 1984), where each sire is mated to three dams and an equal number of progeny per full-sib family are individually marked. Reasons for using this mating design were given in the NCRAC Walleye Work Group Workplan for September 1, 1992 - August 31, 1993.

Just prior to the start of Year 1 of this proposed project, approximately 222 randomly chosen fingerlings per full-sib family will have been marked with visible implant (VI) tags. Marked individuals from all 24 families will then be randomly pooled into common, indoor rearing tanks at the UMN culture facility. As fish grow larger and in order to meet associated budgetary and facilities constraints, it will be necessary to reduce the number of families in the design from 24 to approximately 12. This will be done by retaining the half-sib families (i.e., nested groups of three full-sib families) which exhibited the best overall survival and performance up to the life stage at culling. Family selection applied in this manner will maintain a balanced design, thus preventing bias and greatly simplifying statistical analysis for estimation of genetic parameters on traits expressed post-culling.

Using conservative estimates of survival probabilities up to age two and planning for random culling of surviving adults to 68 fish per family (in order to meet budgetary constraints), induced spawning should yield approximately 20 reproductive adults per family at age two (Table 1A, preferred option) or 14 per family at age three (Table 1B, fall-back option). For traits expressed at sexual maturity that may be of commercial importance (e.g., number of eggs per female, average egg size, sperm motility), the preferred option (spawning at age 2) will yield the optimum size of dam (full-sib) families for precise estimation of genetic parameters, given the *conservative* assumption that the lowest heritability will be approximately 0.1 (Robertson 1959). Nevertheless, the fall-back option (spawning at age 3) will yield sufficient numbers of individuals per family to obtain precise estimates of most commercially important adult traits. Both options will yield sufficient numbers of reproductively capable adults to allow application of combined selection at the time of mating and keep the rate of inbreeding $\leq 1\%$ in the progeny generation (G₁), thus well below rates that may cause inbreeding depression. Selection on domestic livestock is known to offset a 2% inbreeding rate per generation (Pirchner 1979).

 Table 1.
 Spawning schedule for pedigreed walleye families, using induced spawning.

| Generation 0 (G ₀) | Percent x | Number/Fam = | No. Surviving/Fam. |
|--------------------------------|--------------|------------------------------|---|
| Fingerlings to age 2 | 70% survival | 222 fingerlings (≥127 mm) | 155 adults (retain 68) |
| Spawners at age 2 ¹ | 30% mature | 68 adults retained | 20 reproductively capable fish ² |

| Α. | preferred | option: | spawning | at age 2 |
|----|-----------|---------|----------|----------|
| | p10101104 | optioni | opanning | at ago = |

B. fall-back option: spawning at age 3

| Generation 0 (G ₀) | Percent x | Number/Fam. = | No. Surviving/Fam. |
|--------------------------------|--------------|-----------------|---|
| Age 2 to age 3 | 70% survival | 68 age-2 adults | 48 adults |
| Spawners at age 3 | 30% mature | 48 adults | 14 reproductively capable fish ² |

¹If induced reproduction is successful on age 2 adults (in spring, 1995 or Year 2 of proposed project), it will be possible to accelerate by one year the first application of combined selection on G_0 and the creation of G_1 progeny.

²This figure assumes that a smaller proportion of females than males will be in spawning condition at the first time of reproduction.

Pedigreed Families at Aurora-Aqua, Inc.

An additional 200 tagged fingerlings per family, for the 12 or more selected families, will be raised in the indoor recirculating aquaculture system of Aurora-Aqua, Inc. (Gene Hanson, President), a private operation in Kandiyohi, MN (approximately 144 km west of UMN facilities). Performance of these fish will be compared to that of their relatives held at UMN. This will allow measurement of genotype-environment interactions, thus permitting us to select for good general performance across varied intensive culture environments.

This collaboration with Aurora-Aqua *is not necessary for completion of Objective 1*, but will add a valuable dimension to the proposed work without requiring any increase in this project's budget request. The collaboration involves the following arrangements. Aurora-Aqua will reimburse UMN for additional costs of feed and VI-tags needed to produce the additional fingerlings and cover all culture costs, in return for ownership of these fish once they leave the UMN facility. With technical assistance from UMN and UW-Madison investigators, Aurora-Aqua staff will follow procedures similar to those used at UMN for culture methods, data collection, induced spawning, and application of combined selection. All performance data will be shared with NCRAC Walleye Work Group members for use in various public domain media (e.g., NCRAC newsletters and reports, scientific journals, oral presentations, extension publications). Specific details of a collaborative agreement with Aurora-Aqua are pending review and approval by the NCRAC Board of Directors.

Maintenance of a Control Line

It is imperative to maintain control lines concurrent with creation of the pedigreed line of families that are destined for selective breeding (e.g., Tave 1987). Future comparisons between performance of the control line and the selected pedigreed line is the only reliable way to measure progress of the selection program because it permits discriminating between selection and improved husbandry or unintentional selection (e.g., a disease outbreak) as the true causes of improved performance. Thus, the control line produced under the NCRAC Walleye Work Group workplan for September 1, 1992 - August 31, 1993 will be maintained throughout this project. At each facility, approximately 160 control line, tagged fingerlings will be reared in common with pedigreed families. Assuming a similar spawning schedule as for pedigreed families (Table 1), this should generate sufficient numbers of spawning adults to keep the inbreeding rate in the progeny generation (approximately 1%) no greater than that experienced in the selected line.

Estimation of Genetic Parameters

Performance data needed to estimate genetic parameters on adult traits of interest for selection (as discussed under **RELATED CURRENT AND PREVIOUS WORK** on selective breeding) will be collected. Genetic parameters (e.g., heritabilities, genetic correlations), will be determined using nested analysis of variance methods on data collected for individuals according to half-sib and full-sib groupings (e.g., Becker 1984).

Induced Spawning of G₀ Adults

Natural history observations suggest that the onset of vitellogenesis and spermatogenesis will only begin in walleye after the fish reach a certain critical size (Hokanson 1977 and Colby et al. 1979). This is also true for the closely related yellow perch, and data from this species indicates that it is possible to accelerate the onset of sexual maturation by increasing the somatic growth rate (Malison et al. 1985). We hypothesize that the same will be true in walleye. Increasing the growth rate of captive walleye can be accomplished by rearing the fish for 18 months under optimal temperature (21-24 °C) and photoperiod (16 hours light/8 hours dark) conditions, and providing them with sufficient quantities of high-quality food. At the end of this period the fish should be 350 to 600 g, and large enough to spawn. UW-Madison investigators will lead efforts to induce spawning of selected broodstock held at UMN and Aurora-Aqua.

Natural history data, as well as recent data on the histological and endocrine changes that occur during the annual spawning season of walleye, suggest that male and female walleye require an over winter period to mature sexually (Hokanson 1977; Colby et al. 1979; Malison et al., 1992, In preparation). Therefore, in November, 1994, after the fish have been reared 18 months under optimal growth conditions, they will be placed under normal ambient temperatures and photoperiods to experience an "environmentally" normal winter. These conditions should stimulate the onset of gonadal growth and development. These events will take place in Year 2 of this proposed project.

In the spring (late March) of 1995 (Year 2), the fish will be assessed for their readiness to spawn by measuring (1) oocyte diameter and (2) position of the germinal vesicle (GV). In the morning hrs (approximately 9 am), groups of potential spawners will be anesthetized (in 0.6 mg/L MS-222). Oocytes will be aspirated through the genital pore with a polyethylene cannula and placed into an oocyte clearing solution (ethanol:formalin:glacial acetic acid, 6:3:1, v/v). The diameter of the oocytes will be measured to determine if vitellogenesis is complete. Fully vitellogenic walleye oocytes are approximately 1000-1500 mm in diameter (Malison et al., 1992, In preparation). The germinal vesicle (GV) is centrally located in immature oocytes, and migrates to the oocyte periphery over a 5-8 day period during final maturation. The position of the GV can be used as a precise indicator of the maturational stage of the oocyte and to predict germinal vesicle breakdown (GVBD=final maturation) and ovulation.

Female walleye in which vitellogenesis is determined to be complete will be subject to hormone treatment to induce final oocyte maturation and ovulation. Use of hormones will shorten the generation time by one year, thus accelerating genetic progress during the *research phase* of selective breeding. Because we expect to apply these hormones to laboratory animals treated in laboratory facilities, this aspect of the research should be exempt from needing an INAD. If it becomes desirable to apply hormones to two-year old fish being raised at the facility of our private sector collaborator, Aurora-Aqua, then Jeff Malison of UW-Madison has agreed to apply for an INAD for this purpose. Once descendants of selectively bred parents are disseminated to the private sector, we anticipate that the private producers will not need to apply hormones to propagate these genetically improved fish.

The specific hormone treatment(s) used to induce spawning will depend upon the results of studies conducted in 1993. These studies will be evaluating the following treatments: (1) human chorionic gonadotropin (hCG) at 150 IU/kg on day 0 and 500 IU/kg on day 2; (2) des-gly¹⁰ (D-ala⁶) LHRH-ethylamide (LHRHa) at 35 mg/kg on day 0 and 100 mg/kg on day 2; and (3) hCG at 150 IU/kg on day 0 and 17 α ,20 β -dihydroxy-4-pregnen-3-one at 2 mg/kg on day 2. Hormones will be dissolved in physiological saline and administered by single injection into the dorsal musculature. Previous studies have demonstrated that the stresses of handling, anesthetization, oocyte sampling, and injection have little or no effect on subsequent spawning success (Barry et al. 1992, In preparation). Fish will be sampled regularly (e.g., every 1-2 days) to monitor progress of oocyte maturation and ovulation. After ovulation, eggs will be stripped and fertilized by the dry method, and incubated using established methods. Techniques of refrigerated storage and cryopreservation of walleye semen (Moore 1991) will be used if needed to synchronize availability of ripe ova and semen. Previous studies have demonstrated that egg fertilization and embryo survival rates are not different in fish spawned naturally and those induced to spawn by injections of hCG and LHRHa (Barry et al. 1992, In preparation).

If none of the fish have completed vitellogenesis by the end of Year 2 of this project, the fish will be held under ambient conditions for a third year, and we will delay the hormonal induction of spawning until the following spring.

Application of Combined Selection

Based on results obtained for trait heritabilities and genetic correlations, and consultations with walleye producers in the region (facilitated by liaisons of the NCRAC Extension and Economics/Marketing Work Groups), a selection index will be formulated in order to impose efficient selection on a few traits at a time. Individual performance records will be evaluated to select the best individuals within the best families; these will be mated to produce the progeny generation, G_1 . The rate of inbreeding imposed on G_1 will be kept $\leq 1\%$ by mating at least 24 males and 24 females from G_0 .

Culture Methods

At UMN, intensive culture of fingerlings to sexually mature adults will be done in semi-square or circular tanks (2180-L rearing volume), using one exchange per hour and keeping loading densities \leq 48 g/L (3 lb/ft³). Rearing water will be heated to 21-24 °C and photoperiod will be 16 hours light/8 hours dark. Maximum room light levels will be approximately 25 lux and the tanks will be screened which reduces light about 50% over the water (10-15 lux maximum over the water surface). The Bioproduct "Biodry" formulation will be used to rear the fingerlings fish to sexual maturity. Feed will be dispensed by mechanical feeders actuated by electronic time-clock. Feeding begins when the fry are 3-day posthatch. Feed size will be upgraded to accommodate energetics of food capture.

Comparative Performance of Walleye Hybrids (Objective 2)

This study will compare the performance of walleye female **x** sauger male hybrids produced from several selected parental stocks and reared under intensive culture systems. In Year 1, performance comparisons will be made between hybrids produced by using a single stock of male sauger (Mississippi River) and three stocks (populations) of female walleyes: (1) Rock Lake, Wisconsin; (2) London Fish Hatchery, London, Ohio; and (3) Spirit Lake, Iowa. If the London Fish Hatchery stock of captive brood stock is no longer available by the spring of 1993, a Kansas stock (Milford State Fish Hatchery, Junction City, Kansas) or a western Nebraska stock (North Platte State Fish Hatchery, North Platte, Nebraska) will be substituted. Alternate sources of walleye stocks, if needed, would be northern Minnesota (Vermilion Lake), northern Wisconsin, or North Dakota (Devil's Lake or Lake Sakakawea), with the cooperation of the resource management agencies of these three states. In Year 2, performance comparisons will be made between hybrids produced from a single source of female walleye (Rock Lake) and three stocks of male sauger (Mississippi River, Ohio River, and Upper Missouri River). Rock Lake walleye will also be included as a purebred control in both years of the study.

In general, responsibility for obtaining the needed gametes will be shared between ISU and UW-Madison investigators, with the latter being responsible primarily for gametes from Wisconsin sources. For each group of fish produced, every effort will be made to collect a sample of gametes from at least 15-20 individual brood fish of each sex. Techniques of refrigerated storage and cryopreservation of walleye semen outlined by Moore (1991) will be used if it is necessary to store semen until ripe females of a particular fish stock become available. Eggs from ripe female brood fish will be stripped, pooled and fertilized with a pool of the appropriate semen using standard methods. Eggs will then be incubated to the eyed stage at the facilities at which they are collected and fertilized. Performance comparisons will be done at two sites, ISU for rearing fry to 75 mm fingerlings in an intensive culture system, and UW-Madison for rearing fingerlings to sub-adult (i.e., food) size. When fingerlings reach 75 mm, they will be transported to UW-Madison for further grow-out. The following provides specific information about culture facilities used at the two sites.

ISU

An intensive culture production system will be used to rear fry to fingerlings. Four groups of eyed eggs will be transported to ISU in ice-packed, insulated (Styrofoam[™]) shipping containers. For shipping, the eggs are wrapped in water saturated cotton muslin, placed on a middle tray with trays of ice above and below. From any of the proposed sites, the eggs can be driven to lowa or shipped express within 18 hours.

On arrival at ISU, the eggs are tempered to the water temperature, and then transferred to standard hatching jars, using a separate jar for each of the four groups of fish: the walleye control, and three groups of walleye hybrids (described above). At hatching, swim-up fry will flow from the hatching jar of its group into a catch tank for that group. Fry will be distributed to three replicate rearing tanks, each of 150 L capacity. Fry will be fed Kyowa B-400 and B-700 for 20 days and then weaned over to the larger Kyowa C-1700 feed. After 30 days, they will be weaned over to BioProducts BioDry feed for rearing to 75 mm TL. Performance data collected on the first phase of intensive rearing will include survival, gas bladder inflation, viability, incidence of cannibalism, and growth rate. At a mean TL of 75 mm, fingerlings will be transported to UW-Madison for rearing to food-size.

UW-Madison

For each year of our proposed study, the four groups of 75 mm TL fingerlings will be distributed among three 110-L tanks at a density of 25-50 fish/tank, depending on fish availability. Within each tank, 8-16 fish will be individually marked (using fin clips or tags) to monitor individual growth. Tanks will be equipped with internal lighting (16 hours light/8 hours dark photoperiod) and airstone aeration, and provided with tempered water (21 ± 1 °C) at 4-6 L/min. Fish will be fed 2-3 times daily by hand to excess, using BioDry or other suitable food. As the fish grow, they will be moved to larger tanks (220-L and 750-L) as needed. All fish will be counted, weighed and measured regularly (e.g., every 4-8 weeks). Fish will be reared for approximately one year, at which time they should approach a food-fish marketable size. Survival and gain in weight and length will be the primary performance characteristics measured within

the two year time frame of this project. Current plans are to evaluate other important characteristics (e.g., fillet yield, proximate carcass composition, organoleptic evaluation, etc.) in a subsequent study.

Field Trials for Advanced Fingerling Production (Objective 3)

Comparisons of the effectiveness and costs of different pond and tank culture strategies for producing advanced fingerling walleye will be done by investigators from ISU, the Max McGraw Wildlife Foundation of Illinois, UN-L, and UW-Madison. Costs associated with the different culture strategies will be evaluated in collaboration with the NCRAC Economics and Marketing Work Group.

ISU and the Max McGraw Wildlife Foundation will conduct field trials to compare intensive tank culture procedures (using only formulated feeds), with a culture system that involves rearing fish in ponds, followed by intensive rearing in tanks. The UN-L, working in cooperation with the Nebraska Game and Parks Commission, will conduct field trials to compare various pond culture procedures with several pond/intensive tank culture strategies. These studies will evaluate effects of pond fertilization and aeration on size of fish at pond harvest, and in conversation with UW-Madison researchers, will determine the minimum size at which walleye can be harvested from ponds and successfully habituated to intensive tank culture and formulated feeds.

Studies by ISU and the Max McGraw Wildlife Foundation

Field trials by ISU and the McGraw Foundation will be conducted in facilities operated by the McGraw Foundation near Dundee, Illinois. Bob Summerfelt of ISU will design the intensive tank culture facilities and be responsible for data analysis and the preparation of annual and final reports. Tom Harder of the McGraw Foundation will be responsible for all fish culture activities, supervision of personnel, record keeping, and data collection.

Source of fry

Ovulated walleye will be collected each year from populations in one or both of the two 12.15 hectare (30 acre) gravel quarry lakes on the McGraw Foundation property. These brood fish will be used as a source of gametes to produce the fry needed for both culture systems.

Assuming a 50% hatch from fertilized eggs, approximately 620,000 eggs will be needed to supply the 310,000 fry for the trials (200,000 for the extensive-intensive system, 110,000 for the intensive fry culture system, as explained below). Assuming average fecundity of 60,000 eggs per kilogram body weight (Nickum 1986), 12 to 20 females (1.5-2.0 kg each) will provide more than enough eggs and sufficient genetic diversity. Eggs from individual females will be fertilized with sperm from one or two males (dry pan method), then treated with Fuller's earth to remove adhesiveness, and incubated in standard hatching jars (Midland Plastics, Inc., Brookfield, Wisconsin) at the McGraw Foundation fish rearing facility.

Extensive-Intensive Rearing

After hatching, two 0.4 hectare (1 acre) ponds will be stocked with 100,000 2-day old fry (approximately 250,000 per hectare). Ponds will be fertilized with alfalfa pellets (2.4% total N) at 200 kg/hectare in six equal applications (total application of 1,200 kg/hectare). Fish will be harvested from ponds when the mean TL of fingerlings averages 38 mm (40-50 days). A portion (11,100) of the 38 mm pond-reared fingerlings will be reared intensively on formulated feed. Six tanks will be stocked with pond-reared fingerlings at a density of 2 fingerlings per L for continued rearing to a mean TL of 150 mm. This will require 2,400 fish per tank for the three 1,200-L tanks (7,200 total), and 1,300 fish per tank for the 650-L tanks (3,900 total). The total number of pond-reared fingerlings reared in the intensive phase of the tandem extensive-intensive system will be 11,100.

Pond-reared fish will be trained to formulated feed using Kyowa C-1700, and fed BioProducts BioDry formula after they reach 50 mm (at approximately one month after the initiation of their training period). During training, fish will be fed Kyowa C-1700 every 5 minutes for 18 hours each day. After 30 days on the training diet, fish will be gradually switched to BioProducts BioDry feed for continued rearing to the 150 mm endpoint. At this point, all fish will be counted and weighed in mass, and 100 fish individually measured for length and weight.

Intensive Culture System

Three 1,200-L round tanks and three 650-L tanks will be used for intensive rearing of fry on formulated feed. In the first year, the stocking densities will be 20-40 fry/L, respectively. The first phase of the intensive rearing will be terminated when the fingerlings reach a TL of 38 mm, about 40 days at ambient water temperatures (filtered lake water). The fish will then be counted and the same six tanks (3 1,200 L and 3 650 L) restocked at 2 fingerlings per L for continued rearing to a TL of 150 mm.

The tank shape and inflow create a circular flow which reduces the rate of feed settling, facilitates feed distribution, and forces the fish to orient into the current, a factor which appears to reduce cannibalism

(Loadman et al. 1989). Sidewalls of the fry rearing tanks will be painted a flat black to deter the tendency of fry to cling to shiny surfaces. Water temperature of fry rearing tanks will follow those of ambient lake water. To keep tank temperatures below 21 °C, however, well water will be available to mix with lake water. Temperature, oxygen, pH, alkalinity, ammonia, and nitrite will be monitored weekly by APHA et al. (1989) or Hach (1992) methods and maintained within recommended limits for continuous exposure (Piper et al. 1983) by increasing flow rates to reduce metabolite concentrations.

Fry will be fed "Fry Feed Kyowa," Series B (Kyowa Hakko Kogyo Company, Ltd., Japan). This feed has been used by many investigators who have reported walleye fry survival equal to or better than that obtained using live feed (Loadman et al. 1989; Kindschi and MacConnell 1989). The Kyowa B-400, 250suggested by Merna (1977) and McElman and Balon (1979) for first-feeding walleye, and close to the recommendation of 0.2-0.25 μ m by Nickum (1978). After feeding begins, feed size will be increased. The B-700 (400-700 μ m) feed is started on the 9th day posthatch at 25% of the daily ration, then increased by 25% each day until the 12th day posthatch. Feed will be dispensed by a auger feeder every 3 minutes on a 22-hour basis until termination of the rearing interval. Feeding will begin when fry are 5-days posthatch. After 20 days, the Kyowa C-1700 feed will be started at 25% of the daily ration, then increased by 25% every 3 days until it is 100% of the diet. This phase I for rearing interval will be terminated when by 25% every 3 days until it is 100% of the diet. This phase I fry rearing interval will be terminated when the fish average 38 mm TL. These fingerlings will then be used to compare rearing success and costs of continued rearing of these tank reared fingerlings with training of pond-reared fish to formulated feeds.

During the rearing interval, daily counts of dead fish will be made to relate daily mortality to fish age and environmental conditions. Survival during the fry rearing phase (first 21 days) will be determined from a final count of fish in the tank. A sample of 100 fish from each tank will be used to determine the occurrence of an inflated gas bladder.

<u>Studies by the UN-L and UW-Madison</u> The overall goal of this research is to compare the effectiveness and costs of rearing advanced fry and early fingerling walleye initially in ponds, then producing the advanced fingerlings either extensively in ponds or by intensive tank culture using formulated feeds. Our hypothesis is that a strategy of intensive pond fertilization and aeration, combined with early (pond) harvest and habituation to intensive tank culture, will prove optimal as a cost-effective means of producing advanced walleye fingerlings.

Fertilized eggs for the Nebraska and Wisconsin components of the project will be obtained from broodstock walleye collected from Merritt Reservoir in Cherry County, Nebraska, and Rock Lake Wisconsin, respectively fertilized eggs will be incubated at the North Platte State Fish Hatcherv. Nebraska and the UW-Madison's research laboratory at the Lake Mills State Fish Hatchery, Wisconsin.

At both facilities, fertilized eggs will be incubated in flow-through McDonald jars, using standard procedures (e.g., Richard and Hynes 1986). The percentage viability of eggs in each hatching jar will be determined 2 days after fertilization and again just prior to hatch. Embryo development during incubation will be monitored, as will the timing and percent success of hatch (normally % hatching ranges between 50 and 70%). Sac fry will be stocked into ponds within 24-48 hours of hatch.

Throughout the project, principal end points examined will be percent survival, number, size, size variation, condition factor, and general health of the fish produced. Water temperature and dissolved oxygen concentrations will be measured routinely during both pond and tank culture trials. Cost inputs will be recorded or determined, as prescribed by the NCRAC Economics and Marketing Work Group. For the Nebraska pond studies, other water quality and biological parameters that will be routinely determined, using standard methods, include total ammonia-nitrogen, pH, Secchi disc reading, and possible chlorophyll-a and zooplankton population status (Wetzel and Likens 1979; APHA et al. 1989; Boyd 1990). Records will also be kept on climatic conditions relevant to pond production (e.g., cloud cover, rain events, temperature).

In Year 1 of the project, Nebraska investigators will focus their efforts on (1) evaluating effects of intensive pond fertilization and aeration on production of advanced fry and early fingerlings; (2) improving procedures for large-scale pond harvest of fish that are less than 38 mm TL; and (3) assessing effectiveness and costs of producing advanced fingerlings extensively in ponds. UW-Madison researchers will focus on determining the minimum size at which walleye can be harvested from ponds and habituated to intensive culture conditions.

Nebraska pond studies will be conducted at the North Platte hatchery in 16 0.4-hectare (1.0-acre) ponds. having an average depth of about 1.0 m. All ponds are rectangular (about 80-m-long x 50-m-wide) and have bottoms sloped to an outlet and external harvesting basin. At present, nine ponds are each equipped with about 1.5 kW of electrical service for aeration and other uses. By the start of the project, at least 12 of the ponds will be so equipped. By Year 2, aeration should be possible in all 16 ponds.

In Year 1 of the project, the 16 ponds will be used in a 2 x 2 factorial experiment to evaluate effects of two different levels of pond fertilization, combined with either no aeration or aeration supplied by a subsurface diffused-air distribution system. This experimental design provides four replicate ponds per treatment. After the spring thaw, ponds will be filled with water 7-14 days before stocking. Present plans are to stock all ponds with 500,000 fry/hectare, which is twice the traditional stocking rate employed at the North Platte hatchery and 33% higher than the highest rate examined by Harding (1991) at that facility.

Alfalfa pellets will be the only fertilizer employed. Present plans are to compare fertilizer application rates of 150 and 225 kg/hectare per week, with application initiated one week before stocking and continued on a weekly basis thereafter. This fertilization strategy may be modified, however, depending on the maintenance of dissolved oxygen concentrations in the ponds above critical levels. Samples of alfalfa pellets will be submitted to the UN-L Department of Agronomy's Soil and Plant Analytical Laboratory prior to fertilization for determination of total nitrogen, phosphorus, organic and mineral contents.

The eight aerated ponds will be equipped with subsurface diffused-air distribution systems because of concerns that mechanical aerators or airlift pumps might kill walleye fry. Depending on preliminary aeration trials conducted before the start of the production experiment, the specific type of subsurface diffused-air distributor used will be either the "FAT CATTM" system, sold by Aquatic Ecosystems, Inc. of Apopka, Florida, or the "QUAD-AIRTM" system, sold by AREA, Inc. of Homestead, Florida. Both systems essentially consist of a delivery pipe from a blower and floating plastic air lines with hanging air tubing or piping fitted with airstones, which are suspended above the pond bottom. Principal differences between the systems are in the relative size, number and arrangement of airstones. Both systems are widely used in the ornamental fish industry in Florida and the penaid shrimp industry in Central and South America, and have the advantages of simplicity of design and easy removal from ponds to facilitate repairs, fish harvest and other management activities.

The Nebraska pond fertilization and aeration study in Year 1 will be terminated in 40-50 day, when walleye typically reach a TL of 32-45 mm. Following harvest, fish will be placed in 244-cm-long x 61-cmwide x 61-cm-deep black fiberglass holding tanks located indoors at the North Platte hatchery. Immediately thereafter (or within 24 hours), randomly selected samples of walleye from the tanks will be stocked into eight refilled 0.4-hectare ponds at the North Platte hatchery at a rate of 25,000 fish/hectare for the extensive production of advanced fingerlings. Walleye returned to ponds at the North Platte hatchery will be reared to advanced fingerlings using the standard procedures employed by the Nebraska Game and Parks Commission. This procedure depends on the natural productivity of the ponds and does not involve any supplemental fertilization or aeration.

For the extensive pond culture field trials on the production of advanced fingerlings, no experimental treatments of ponds will be compared. However, detailed records of cost inputs will be maintained for comparison with intensive tank culture methods (examined by ISU and UN-L in Year 2), and emergency aeration procedures may be employed to ensure adequate dissolved oxygen levels. These extensive pond culture trials will be continued until fish reach about 100 mm TL, which normally occurs in mid-September in Nebraska. At that time, ponds will be harvested and the walleye stocked in public waters by the Nebraska Game and Parks Commission.

Concurrent with the Nebraska pond fertilization and aeration study, UW-Madison researchers will focus on determining the minimum size at which walleye can be harvested from ponds and habituated to intensive tank culture and formulated feeds. For this study, one or two 0.4 hectare ponds at the Lake Mills State Fish Hatchery will be stocked with walleye fry at rates of 150,000-250,000 fry/hectare. The ponds will be supplied with supplemental aeration and fertilized with soybean meal and inorganic phosphorus and nitrogen at rates which have proven effective for fingerling production at the Lake Mills hatchery. Growth of the fish in the ponds will be monitored by measuring subsamples of fish captured twice weekly. When the fish reach each targeted size, several thousand will be harvested from the ponds using either a light trap system similar to that described by Manci et al. (1983) for fish <38 mm TL, or trap nets set overnight for larger fish. Our current plans are to evaluate habituation to intensive culture conditions of three or four sizes of walleye fingerlings harvested between 17 and 45 mm TL. Specific sizes used will be determined by results of a preliminary study conducted in the spring/summer of 1993.

After each harvest, walleye will be immediately stocked into three 220-L flow-through fiberglass tanks provided with tempered water $(21 \pm 0.5 \,^{\circ}C$ at a flow rate of 8-10 L/min) and airstone aeration. Tanks will be stocked at 3 fish/L, and in all cases loading rates (kg of fish/L/min) will be low enough to maintain good water quality (e.g., dissolved oxygen concentrations not lower than 6.0 mg/L). During their first 2 day in the laboratory, fingerlings will be treated prophylactically to reduce stress and minimize bacterial disease outbreaks with a daily, 4-hour static bath of NaCl (7 g/L). For the duration of all studies, fish will be fed continuously throughout the day with automatic feeders, as well as several times daily by hand, using Ziegler Salmon Starter (Ziegler Bros. Inc., Gardner, Pennsylvania). Tanks will be cleaned and dead fish removed and counted on a daily basis. The tanks will be kept in a dark room with no overhead lighting,

and each tank will be continually illuminated with a submerged internal light. We have found that the use of internal lights increases the survival of walleye during the habituation period (unpublished results), as observed for perch (Malison and Held 1992). The end points measured will be: (1) habituation, defined as the percentage of fish that survive the transition to intensive culture conditions; (2) starvation, defined as the percentage of fish that die and are recovered; and (3) cannibalism, defined as the percentage of fish that die and are recovered; and (3) cannibalism, defined as the percentage of fish that can not be accounted for at the end of the habituation intervals. In related studies (e.g., Malison and Held 1992), our definition of starvation was substantiated by observations that virtually all dead fish recovered were extremely emaciated, and losses which could be attributed to disease or other causes (e.g., mechanical injury) were negligible. Our definition of cannibalism was substantiated by observations of cannibalistic behavior and the fact that fish could not escape from the tanks through the standpipe screens or by any other means. Obvious cannibals will be regularly removed during the experiment. We expect that the habituation intervals will last from 19 to 51 days, and the study will be terminated when the number of dead fish recovered daily drops to less than 0.05% of the total initial stocking number, and all remaining fish are actively feeding. Growth of the fish will be monitored by weighing and measuring subsamples of fish from each tank at regular intervals both during the study and for an additional eight weeks.

In 1983, UW-Madison investigators (including Terry Kayes, who is now at the UN-L) published a procedure for harvesting walleye and other photopositive juvenile fish from ponds using a lift net and light (Manci et al. 1983). Since then, an improved light-harvesting system has been developed which can capture 3,000-8,000 walleye of 20-30 mm TL per 15-min set (J.A. Malison and T.B. Kayes, unpublished observations). However, a possible limitation of this particular system is that it is probably too cumbersome and labor-intensive to be used for large-scale pond harvest.

In Year 1 of the project, UN-L investigators will develop and test one or more alternative light-harvesting systems for their utility in large-scale pond culture. In addition, one or more new types of passive capture gear, presently under development at the UN-L, may also be evaluated. All these evaluations will be performed at either the Calamus hatchery or the North Platte hatchery in 0.4-hectare ponds separate from those used for the pond fertilization and aeration study. The most promising procedures will be evaluated further in Year 2.

In Year 2 of the project, Nebraska researchers will focus their attention on examining the effects of pond stocking rates and harvesting strategies related to fish size at initiation of harvest on the production of advanced fry and early fingerlings, and on assessing the effectiveness and costs of producing advanced fingerlings intensively in tanks. Both endeavors will build on the findings made in Year 1 by UN-L and UW-Madison investigators, particularly with respect to pond stocking rates, the minimum size at which walleye can be harvested from ponds and habituated to intensive tank-culture conditions, and optimum procedures to achieve habituation.

The Year 2 pond studies will be conducted in the same 16 0.4-hectare ponds as Year 1. The ponds will be used in a 2 x 2 factorial experiment to examine the effects of two different stocking rates in combination with two different harvesting strategies related to fish size at initiation of harvest. As in Year 1, this experimental design provides four replicate ponds per treatment. Except for experimental treatments, pond management and fish propagation and husbandry procedures will be similar to those employed in Year 1. Present plans are to uniformly aerate and apply fertilizer (alfalfa pellets) at a rate of 225 kg/hectare per week to all 16 ponds, though changes in the application rate may be made depending on Year-1 results and the need to maintain dissolved oxygen concentrations above critical levels.

To test the production limits of these aerated fertilized ponds, present plans are to stock eight of them with 400,000 fry/hectare, and eight with 600,000 fry/hectare. The latter value is about the same as the highest stocking rate examined by Fox and Flowers (1990), which, to our knowledge, is the highest pond stocking rate for walleye fry reported to date in the scientific literature. The two stocking rates tested, however, may be changed depending on Year-1 findings.

In addition to stocking rate, the Year-2 pond experiment will compare the traditional method of pond harvesting, which targets on walleye of 32-45 mm TL and involves pond drainage and the collection of fish from a harvesting basin about 6 weeks after pond stocking, with an alternative strategy involving intensive cropping of smaller-size fish from ponds using light-harvesting and other capture gear. This latter strategy will be initiated when the walleye in the ponds first reach the size at which they can be readily habituated to intensive tank culture and formulated feeds, as determined by the UW-Madison investigations in Year 1. Studies by Malison and Held (1992) on yellow perch suggest that 19-25 mm TL in walleye might be an appropriate size to initiate the "pond-cropping" strategy, but this hypothesis needs to be verified. At 6 weeks after stocking, any walleye remaining in the ponds subjected to cropping will be harvested by conventional pond-drainage procedures.

Throughout the Year-2 pond study, types of data collected and collection procedures will be similar to those of Year 1. In addition, effects of the pond-cropping strategy on the size, growth and condition of the walleye remaining in the ponds subjected to cropping will be evaluated, as will the effects of different harvesting techniques (i.e., pond drainage, light harvesting, capture by seining or a new type of small-meshed trap net presently being developed at the UN-L) on general fish health and post-harvest survival.

The disposition of the walleye harvested from ponds at the North Platte hatchery in Year 2 will be similar to that of Year 1. Specifically, some of the fish will be immediately restocked into ponds for the extensive production of advanced fingerlings, while most of the others will be transported to lakes and reservoirs for release. Field trials on the extensive pond production of advanced fingerling walleye will be repeated (as in Year 1) as part of the Nebraska Game and Parks Commission's normal operations. In Year 2, about 30,000 young walleye harvested by the pond cropping strategy (thus having a TL of less than 32 mm) will be transported to the Calamus hatchery, where they will be intensively reared to advanced fingerlings.

At the Calamus hatchery, the fish will be habituated to intensive tank culture and formulated feeds, then raised to advanced fingerlings using procedures developed under controlled laboratory conditions by UW-Madison researchers in Year 1. The Calamus study will thus be a scaled-up field test of these procedures under practical rearing conditions. The study will be done using either gray or blue 1,000-L cylindrical fiberglass tanks equipped with automatic feeders and using filtered water from the Calamus Reservoir at ambient temperature. Internal tank lighting will probably also be employed, as described by Malison and Held (1992). Present plans are to habituate the fish to a conventional salmon starter diet fed continuously throughout the day, then rear them on a pelleted salmon feed fed at regular intervals three to five times daily. Walleye stocking densities in the tanks for both habituation and rearing will be determined in Year 1 of the project.

The Calamus study will be terminated in September, concurrent with the completion of the extensive pond culture field trials at the North Platte hatchery, and all the fish will be stocked into public waters by the Nebraska Game and Parks Commission. Growth, production, and cost input data from the Calamus and North Platte investigations will be compared with each other and with data generated by the ISU/McGraw Foundation field trials.

FACILITIES

Selective Breeding for Commercial Aquaculture (Objective 1)

Culture of pedigreed families at UMN

Culture of pedigreed families and the control line will be conducted in a new 743.2 m², indoor facility at UMN, which will be operational in July, 1993 (i.e., two months prior to the start of this project). This facility will easily meet the culture requirements for this project because it will have the following features: filtered well water (10 °C) supplying the equivalent of 28.4 l/sec flow-through to one flow-through and one recirculating aquaculture system; heat exchanger and chiller units to modify ambient water temperatures (allowing us to rear walleye at optimum temperatures of 21-24 °C); ozonation, low pressure air system; alarm system; standby generator; and submerged trickling filter for the recirculating system. Commontank rearing of the various families for this objective will use a small portion of the new lab's water capacity; it will require a maximum of 4.6 L/sec water flow (based on one exchange per tank, for 10 rearing tanks with 1,639 L rearing volume per tank). UMN facilities for water chemistry analyses and other analyses needed for rearing walleye are located in four labs (203 m²) of the Department of Fisheries and Wildlife, and include the necessary scientific instruments and computers.

Culture of additional fish from pedigreed families at Aurora-Aqua

As explained in the procedures for this objective, this work will be enhanced by but is not dependent on plans to culture additional individuals from each family at facilities of Aurora-Aqua, Inc., in Kandiyohi, MN (approximately 144 km west of UMN facilities). In order to rear and selectively breed these additional fish, and then rear their selected progeny, Aurora-Aqua is prepared to devote required portions of their facility not only during the two year duration of this NCRAC project, but also for at least another four years beyond this project. Over the entire six year period, Aurora-Aqua proposes to devote \$50,000 of capital and operating costs to the culture of this selected line. Their facility consists of an insulated building (368.6 m²), currently containing five 5,677.5 L recirculating intensive culture systems (and with the capacity and plans to hold 20 systems in total). Each system is self-contained with its own biofilter, and a heater is available to raise water temperatures as needed. Sub-adult walleye (formerly converted to artificial feed at the fingerling stage and reared in outdoor cages for several years) have been successfully reared in one of these systems for a number of months. In order to further demonstrate the suitability of this facility for the proposed collaboration, Aurora-Aqua staff have recently begun using one of these recirculating systems to convert pond-reared fingerlings to artificial feed, a task much harder than

that proposed for this collaboration, i.e., culturing walleye which were raised from hatching on artificial rations in indoor tanks. One full time technician will be available to conduct culture activities and data collection.

Induced spawning

Mature brood fish will be induced to spawn at the new UMN facility, which has the required capability to chill and heat water beyond ambient temperatures. Depending on availability of water chilling capacity at the Aurora-Aqua, induction of spawning may also be attempted on mature brood fish held at that facility. Researchers from the UW-Madison will travel to Minnesota to evaluate the reproductive status and induce spawning in the walleye brood fish, bringing with them all the necessary supplies and equipment (e.g., dissecting microscopes). If needed, however, some brood fish can be transported to Wisconsin and the spawning induced at the University of Wisconsin Aquaculture Program's (UWAP) research facilities at the Lake Mills State Fish Hatchery, Lake Mills, WI. The Lake Mills facility has over 100 tanks (110-L to 3,000-L), three water sources (dechlorinated city water, high capacity well, and lake water), and all of the laboratory equipment required to meet the objectives of the proposal.

Comparative Performance of Walleye Hybrids (Objective 2)

ISU fry culture facilities consist of 12-150 L, and 6-250 L circular flow fiberglass tanks. Each tank is equipped with individual flowmeter to control exchange rate, an auger feeder, and rheostat to control light intensity. A timer is available to control on/off interval for feeders. Temperature and pH are controllable to provide options for experimental evaluations. A separate analytical laboratory is used for analysis of pH, dissolved oxygen, gas pressures, hardness, ammonia-N, nitrite-N, all forms of alkalinity, and residual chlorine. Other analyses can be conducted to meet special needs or sample analysis carried out in the laboratory of the Engineering Research Institute. A room adjacent to the Aquaculture Laboratory has research microscopes (dissection and compound) equipped for color and B&W photomicrography (135 mm and Polaroid).

<u>UW-Madison</u> The UW Aquaculture Program has its main wet and analytical laboratories at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. The facility has an ample supply of temperature-regulated (10 to 30 ± 0.5 °C) well or carbon-filtered city water, and over 100 tanks ranging in size from 110 to 3,000 L. The UW Aquaculture Program also has all of the equipment necessary to capture and transport brood fish and fingerlings, and collect and incubate eggs. This equipment includes a live-haul truck, two boats, 11 trap nets, and over 30 McDonald hatching jars. For the proposed growth studies we will initially use 12 110-L tanks, and as the fish grow they will be moved to larger tanks as needed.

Field Trials for Advanced Fingerling Production (Objective 3)

ISU - Max McGraw Wildlife Foundation

The field trials will be carried out at the Max McGraw Wildlife Foundation, Dundee, Illinois. The McGraw Foundation has 526.5 hectares of land about 50 km west of Chicago, in Kane County, Illinois. Adult walleye can be captured during the spawning season by night time electrofishing from two of the 30 acre gravel quarry lakes on the McGraw Foundation property. The McGraw Foundation has a new fish culture facility of about 150 m² containing eight fiberglass rectangular raceways and 12 circular fiberglass tanks. Six of the circular fiberglass tanks have a 1,200 L capacity (1.65 m mean diameter, 0.56 m standpipe height) and 6 tanks a 650 L capacity (1.22 m diameter, 0.56 m standpipe height).

UN-L

Field trials in Nebraska on the production of advanced fingerling walleye will be done by UN-L investigators, under the direction of Terry Kayes and in collaboration with the Nebraska Game and Parks Commission. All pond rearing of walleye for the Nebraska field trials will be done at the North Platte State Fish Hatchery, a 39-hectare facility located below the (647-hectare) Maloney Reservoir, near North Platte, Nebraska. Physical resources available at the North Platte hatchery include: 39 0.40-hectare fish production ponds supplied with reservoir water through a 41-cm-diameter main, four 1.2-m-wide x 18-mlong x 1.2-m-deep outdoor raceways, several 1.8-m-diameter cylindrical outdoor rearing tanks, and a 223-m² hatch house equipped with 80 McDonald hatching jars and eight 0.61-m-wide x 2.4-m-long x 0.61-mwide black fiberglass rearing tanks. All raceways, tanks and hatching jars are supplied with reservoir water, which varies seasonally in temperature from about 2 °C in winter to about 25 °C in summer. The North Platte hatchery also has a work shop and a garage and storage building. The UN-L and Nebraska Game and Parks Commission both have trucks equipped with live-haul tanks for the transport of fish.

All intensive tank rearing of walleye for the Nebraska field trials will be done at the Calamus State Fish Hatchery, a 59-hectare facility located immediately downstream of the (2,023-hectare) Calamus

Reservoir, near Burwell, Nebraska. Physical resources available include: 11 0.20-hectare and 40 0.40hectare fish production ponds; 8 1.8-m-wide x 20-m-long x 1.2-m-deep and 16 2.4-m-wide x 27-m-long x 1.2-m-deep outdoor raceways; an 886-m² indoor fish production and research facility (which is equipped for water-temperature and light control and includes an analytical and fish pathology laboratory); 10 0.61-m-wide x 6.1-m-long x 0.61-m-deep and 8 0.91-m-wide x 6.1-m-long x 0.61-m-deep indoor raceways; and numerous hatchery troughs, egg incubators (including McDonald jars) and 1.2-, 1.5- and 1.8-m-diameter cylindrical rearing tanks. Water resources include: reservoir water (with a seasonal temperature variation from about 4 °C in winter to about 22 °C in summer) supplied to all the indoor and outdoor facilities via a 91-cm-diameter main; and about 11-m³/minute and 1.1-m³/minute water flow from eight wells supplying 13 and 11 °C water, respectively, from two separate aquifers, to all the raceways and indoor facilities. A very large pure-oxygen supply system is in place, and oxygen supplementation in individual tanks and raceways can be achieved through the use of sealed packed-columns.

<u>UW-Madison</u> The study on the habituation of walleye fingerlings by the UW-Madison will be conducted at the UW Aquaculture Program's main wet and analytical laboratories at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. In addition to the facilities and equipment described under Objective 2, the hatchery has over 30 ponds ranging in size from 0.2 to 1.0 hectares. We will use one or two 0.2-0.4 hectare ponds to raise the walleye fingerlings needed for this study.

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PROJECT LEADERS

| <u>State</u> | Name/Institution | Area of Specialization |
|--------------|---|--|
| Illinois | Tom Harder Max McGraw Wildlife Foundation | Finfish Aquaculture and Fisheries Management |
| lowa | Robert C. Summerfelt Iowa State University | Finfish Aquaculture (general)/Larviculture/ Fish Health |
| Minnesota | Anne R. Kapuscinski University of Minnesota | Aquaculture/Quantitative Genetics |
| Nebraska | Terrence B. Kayes University of Nebraska-Lincoln | Finfish Aquaculture/Fish Physiology/Endo- crinology |
| Wisconsin | Jeffrey A. Malison University of Wisconsin-Madison | Aquaculture/Physiology/Endocrinology |
| | Terence P. Barry University of Wisconsin-Madison | Aquaculture/Physiology/Endocrinology |

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

University of Minnesota (UMN) Anne R. Kapuscinski

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

Iowa State University (ISU) (includes funds for field project at Max McGraw Wildlife Foundation) Robert C. Summerfelt

Max McGraw Wildlife Foundation Tom Harder

University of Nebraska-Lincoln (UN-L)

Terrence B. Kayes

PROPOSED WALLEYE BUDGET FOR UNIVERSITY OF MINNESOTA

(Kapuscinski)

Objective 1

| | | | | | | | | Year 1 | Year 2 |
|----|-----|-------|--|--------|-------|--------|---------|----------|----------|
| | • | | | Ye | ear 1 | Ye | ar 2 | | |
| Α. | Sa | larie | es and Wages | No. | FTEs | No. | FTEs | | |
| | 1. | No | o. of Senior Personnel & FTEs ¹ | | | | | | |
| | | a. | (Co)-PI(s) | 1 | 0.10 | 1 | 0.10 | \$0 | \$0 |
| | | b. | Senior Associates | | | | | | |
| | 2. | | o. of Other Personnel (Non- culty) & FTEs | | | | | | |
| | | a. | Research Assoc./Postdoc | | | | | | |
| | | b. | Other Professionals | | | | | | |
| | | C. | Graduate Students | | | | | | |
| | | d. | Prebaccalaureate Students | | | | | \$2,496 | \$2,496 |
| | | e. | Secretarial-Clerical | | | | | | |
| | | f. | Technical, Shop, and Other | 1 | 0.50 | 1 | 0.50 | \$11,150 | \$11,700 |
| | | | Total Salaries and Wages | | | | | \$13,646 | \$14,196 |
| В. | Fri | nge | Benefits (25% of 2f) | | | | | \$3,537 | \$3,704 |
| C. | То | tal S | Salaries, Wages and Fringe Benef | its | | | | \$17,183 | \$17,900 |
| D. | No | nex | pendable Equipment | | | | | \$0 | \$0 |
| E. | Ma | ateri | als and Supplies | | | | | \$1,371 | \$1,054 |
| F. | Tra | avel | - Domestic (Including Canada) | | | | | \$600 | \$600 |
| G. | Otl | her | Direct Costs | | | | | \$200 | \$200 |
| то | TAL | _ PR | ROJECT COSTS PER YEAR (C thro | ough G |) | | | \$19,354 | \$19,754 |
| | | | | | TOTAL | PROJEC | т соѕтѕ | \$39 | ,108 |

¹FTEs = Full Time Equivalents based on 12 months.

BUDGET JUSTIFICATION FOR UNIVERSITY OF MINNESOTA

(Kapuscinski)

- A. Salaries and Wages. A Technician (0.5 FTE) working at the UMN culture facility is needed to: conduct rearing of fish constituting 13 pedigreed families and a control line (approximately 100 fish); collect, compile and assist with analysis of performance data for genetic parameter estimation; and respond to alarms triggered by rearing tank flow rates and temperature sensors. When appropriate, the technician will also assist UMN and UW-Madison investigators in collaborations with Aurora-Aqua, Inc. A prebaccalaureate student is needed for six hours per weekend (\$8/hr.) to conduct daily fish culture tasks and to respond to alarms as needed.
- E. Materials and Supplies. (Prices include shipping and handling)

| Fish food: BioProducts Biogrower diet | \$1000 |
|---|---------------|
| Chemicals (MS-222, Formalin, antibiotics, disinfectant, etc.) | 171 |
| Alarm monitoring service and pager | 200 |
| | TOTAL \$1,371 |

- **F. Travel.** Funds are for attendance (by PI) at annual workgroup meeting and for local trips to pick up culture supplies, and for 1-3 visits to Aurora-Aqua (144 km west of UMN facility).
- G. Other Direct Costs. \$200 for telephone, FAX, postage, electronic mail networks, and photocopies.

PROPOSED WALLEYE BUDGET FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison and Barry)

Objective 1-3

| | | | | | | Year 1 | Year 2 |
|----|---|-------------------|-------|--------|---------|----------|----------|
| • | | Ye | ear 1 | Ye | ear 2 | | |
| ۹. | Salaries and Wages | No. | FTEs | No. | FTEs | | |
| | 1. No. of Senior Personnel & | FTEs ¹ | | | | | |
| | a. (Co)-PI(s) | 1 | 0.06 | 1 | 0.06 | \$0 | \$0 |
| | b. Senior Associates | 1 | 0.04 | 1 | 0.04 | \$0 | \$0 |
| | 2. No. of Other Personnel (No Faculty) & FTEs | n- | | | | | |
| | a. Research Assoc./Postc | loc | | | | | |
| | b. Other Professionals . | 1 | 0.40 | 1 | 0.40 | \$10,000 | \$10,600 |
| | c. Graduate Students | | | | | | |
| | d. Prebaccalaureate Stud | ents | | | | | |
| | e. Secretarial-Clerical | | | | | | |
| | f. Technical, Shop, and C | other | | | | | |
| | Total Salaries and Wag | jes | | | | \$10,000 | \$10,600 |
| З. | Fringe Benefits (30% of 2b) . | | | | | \$3,000 | \$3,200 |
| С. | Total Salaries, Wages and Frir | ge Benefits | | | | \$13,000 | \$13,800 |
| D. | Nonexpendable Equipment | | | | | \$0 | \$0 |
| Ξ. | Materials and Supplies | | | | | \$1,400 | \$1,400 |
| F. | Travel - Domestic (Including Ca | nada) | | | | \$600 | \$800 |
| G. | Other Direct Costs | | | | | \$0 | \$0 |
| го | TAL PROJECT COSTS PER YE | AR (C through G) |) | | | \$15,000 | \$16,000 |
| | | , | TOTAL | PROJEC | т соѕтѕ | \$31 | ,000 |

¹FTEs = Full Time Equivalents based on 12 months.

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison and Barry)

- **A. Salaries and Wages.** Salaries of a Professional (0.4 FTE) are needed to monitor reproductive development and induce spawning of fish; assist PIs with conduct of growth and habituation experiments.
- E. Materials and Supplies. Hormones, general laboratory supplies, and fish food are needed for the activities proposed.
- **F. Travel.** Travel money will be used to travel to Minnesota to monitor reproductive development and induce spawning. In addition, money will be used to attend NCRAC Walleye Work Group meetings.

PROPOSED WALLEYE BUDGET FOR IOWA STATE UNIVERSITY

(Summerfelt and Harder)

Objectives 2 and 3

| | | | | | | | Year 1 | Year 2 |
|-----|---------|--|--------|-------|--------|---------|----------|----------------------|
| ~ | | | Ye | ear 1 | Ye | ear 2 | | |
| . S | Salarie | es and Wages | No. | FTEs | No. | FTEs | | |
| 1 | . No | o. of Senior Personnel & FTEs ¹ | | | | | | |
| | a. | (Co)-PI(s) | | | | | \$0 | \$0 |
| | b. | Senior Associates | | | | | \$0 | \$C |
| 2 | | o. of Other Personnel (Non- culty) & FTEs | | | | | | |
| | a. | Research Assoc./Postdoc | | | | | | |
| | b. | Other Professionals | | | | | \$0 | \$0 |
| | C. | Graduate Students | 1 | 0.25 | 1 | 0.50 | \$6,600 | \$13,892 |
| | d. | Prebaccalaureate Students | | | | | \$1,600 | \$1,800 |
| | e. | Secretarial-Clerical | | | | | \$446 | \$446 |
| | f. | Technical, Shop, and Other | | | | | | |
| | | Total Salaries and Wages | | | | | \$8,646 | \$16,13 |
| . F | ringe | Benefits (30% of 2b) | | | | | \$325 | \$683 |
| . Т | otal S | Salaries, Wages and Fringe Benef | its | | | | \$8,971 | \$16,82 ⁻ |
| . N | lonex | pendable Equipment | | | | | \$0 | \$ |
| . N | lateri | als and Supplies | | | | | \$8,200 | \$3,80 |
| . Т | ravel | - Domestic (Including Canada) | | | | | \$3,600 | \$3,10 |
| . C | Other | Direct Costs | | | | | \$600 | \$80 |
| ΟΤΑ | AL PR | ROJECT COSTS PER YEAR (C three | ough G |) | | | \$21,371 | \$24,52 |
| | | | | TOTAL | PROJEC | т соѕтѕ | \$45 | ,892 |

¹FTEs = Full Time Equivalents based on 12 months.

BUDGET JUSTIFICATION FOR IOWA STATE UNIVERSITY

(Summerfelt and Harder)

- A. Salaries and Wages. Graduate student: A research assistantship is requested to provide a stipend for a graduate student to carrying out the laboratory research. This includes making repairs and renovation of the culture facilities, analysis of water quality parameters needed to maintain the quality of the culture environment, acquisition of gamete (walleye eggs and sauger semen), hatching, then husbandry of walleye fry in an intensive culture environment to a TL of 75-100 mm. The stipend for the first year (\$6,660) for 0.25 FTE is prorated based on the \$12,600 0.5 FTU stipend for the current (1992-93) academic year plus 5%; the stipend for the second year, 1994-95, which is 0.5 FTE, includes another 5% increase. Prebaccalaureate students: prebaccalaureate students will be assigned to assist the graduate student with fish husbandry, cleaning tanks and water quality analysis. Secretarial support is for Bob Summerfelt as chair of the Work Group.
- **B.** Fringe benefits. The fringe benefit rate for graduate assistant is presently 4.92% of their salaries for health benefits; the budget did not anticipate an increase in the fringe benefit rate. Undergraduate student employees are not eligible for fringe benefits.
- E. Materials and supplies. For larviculture major items are activated carbon media for dechlorination of the water supply (\$1,800); larval feed (\$800); and plumbing supplies to update the facilities (flow meters, valves, PVC pipe). Other items include reagents and glassware for water chemistry, and miscellaneous supplies (\$800).

To avoid additional paperwork required to develop a subcontract between Iowa State University and the McGraw Foundation, or a separate agreement between NCRAC and McGraw Foundation, all supply items will be purchased by ISU and shipped directly to the McGraw Foundation. This includes the following:

The major portion of the supply budget will be needed in the first year (\$6,200) as set up costs to modify the McGraw facilities for fry culture: (1) Feeders, (2) Flowmeters, 12 large (227 Lpm) for the basic inflow supply, and 12 small (3 Lpm) for the surface sprays, (3) Valves, 12-2.54 cm, 12-1.27 cm, and (4) PVC pipe and fittings and Nalgene tubing to construct inflow supply lines for water exchange and sprays.

Fish feed (larval and transition diets) will be needed in both years, and additional funds will be needed to make minor modifications and repairs in the second year.

F. Travel.

- Research Functions: (a) Travel required to carry out research functions (\$600): to pickup eggs from UW-Madison, or the hatcheries which will be asked to supply them for the research, and/or travel to confer with collaborators at the UW-Madison, (b) for Bob Summerfelt (\$1,000/year) to travel (2 trips per year) to the McGraw Foundation to provide guidance on design and construction features of the culture facilities and to coordinate preparation of annual and final reports, and (c) for Tom Harder (\$500, Year 1) to visit the ISU and Rathbun Hatchery sites to observe details of the culture facilities.
- 2. Travel to attend Work Group meeting or meetings of professional societies (annual Coolwater Fish Culture Workshop) to present results of research: (a) Bob Summerfelt (\$500/year), (b) Tom Harder (\$500/year), and (c) Graduate Student (\$500/year).
- **G.** Other Direct Costs. Miscellaneous: shipment (overnight delivery) of fish eggs or fry from sources too far from campus for direct pickup; communication expenses: FAX, express mail, photocopies, and phone calls between collaborators and other Work Group members.

PROPOSED WALLEYE BUDGET FOR UNIVERSITY OF NEBRASKA-LINCOLN

(Kayes)

Objective 3

| | | | | | | Year 1 | Year 2 |
|----|--|---------|------|--------|------|----------|----------|
| | | Year 1 | | Year 2 | | | |
| Α. | Salaries and Wages | | FTEs | No. | FTEs | | |
| | 1. No. of Senior Personnel & FTEs ¹ | | | | | | |
| | a. (Co)-PI(s) | 1 | 0.05 | 1 | 0.05 | \$0 | \$0 |
| | b. Senior Associates | | | | | | |
| | No. of Other Personnel (Non- Faculty) & FTEs | | | | | | |
| | a. Research Assoc./Postdoc | | | | | | |
| | b. Other Professionals | | | | | | |
| | c. Graduate Students | | | | | | |
| | d. Prebaccalaureate Students | | | | | | |
| | e. Secretarial-Clerical | | | | | | |
| | f. Technical, Shop, and Other | 1 | 0.50 | 1 | 0.50 | \$8,600 | \$9,200 |
| | Total Salaries and Wages | | | | | \$8,600 | \$9,200 |
| З. | Fringe Benefits (25% OF 2F) | | | | | \$2,150 | \$2,300 |
| С. | Total Salaries, Wages and Fringe Benefi | ts | | | | \$10,750 | \$11,500 |
| D. | Nonexpendable Equipment | | | | | \$0 | \$0 |
| Ε. | Materials and Supplies | | | | | \$1,600 | \$1,000 |
| F. | Travel - Domestic (Including Canada) | | | | | \$3,900 | \$3,900 |
| G. | Other Direct Costs | | | | | \$750 | \$600 |
| то | TAL PROJECT COSTS PER YEAR (C thro | ough G) |) | | | \$17,000 | \$17,000 |
| | TOTAL PROJECT COSTS | | | | | \$34,000 | |

¹FTEs = Full Time Equivalents based on 12 months.

BUDGET JUSTIFICATION FOR UNIVERSITY OF NEBRASKA-LINCOLN

(Kayes)

- A. Salaries and Wages. A Research Technician (0.5 FTE) is needed to assist (1) Nebraska Game and Parks Commission personnel with the set up and operation of both pond and tank culture field trials, and (2) the principal investigator and a (state-funded) graduate student research assistant with the conduct of experiments, equipment maintenance, data collection, and analyses of water and fish samples.
- E. Materials and Supplies. Biochemicals, reagents, and field and laboratory supplies (e.g., sample bottles, nets, insulated containers, glassware, microscope slides, dissecting equipment, etc.) are needed to collect and analyze water and fish samples. Hand nets, harvesting gear, miscellaneous hardware, pond fertilizers, and fish feeds are required to conduct the proposed pond and tank culture field trials. Computer supplies are needed for data entry, statistical analyses and graphics production. Part of the costs of some of these materials and supplies will be supported by funds from other sources.
- F. Travel. The UN-L component of the proposed project will require extensive in-state travel from March through September of both funding years, as well as lengthy stays by UN-L researchers at both the North Platte State Fish Hatchery and Calamus State Fish Hatchery. Round-trip driving distances from the UN-L main campus to the North Platte and Calamus hatcheries are about 500 and 400 miles, respectively. The distance between the two hatcheries is about 136 miles. The total estimated cost of lodging, meals, and fleet vehicle rental for the project is about \$9,400 per year. The cost of long-term stays by student researcher(s) and the UN-L staff technician at the two hatcheries will be covered by mechanisms separate from NCRAC. Of the funds requested, \$3,400 per year is needed to (partially) meet fleet vehicle rental costs and for short-term trips by the principal investigator and others to the project sites, and \$500 per year is needed to attend NCRAC Walleye Work Group meeting(s).
- **G.** Other Direct Costs. About \$200 per year is requested to meet telephone, FAX, postage and photocopying expenses. The remainder of the funds requested is needed to cover the costs of fertilizer and water sample analyses -- including total Kjeldahl nitrogen, total phosphorus, and orthophosphate. These analyses will be done by the UN-L Department of Agronomy's Soil and Plant Analytical Laboratory. Other routine analyses will be done by project personnel, under the supervision of the principal investigator.

| RESOURCE COMMITMENT | FROM INSTITUTIONS ¹ |
|----------------------------|--------------------------------|
|----------------------------|--------------------------------|

| State/Institution | | | Year 1 | Year 2 |
|--|---------------------------------|----------------|-----------|----------|
| University of Minnesota | | | | |
| Salaries and Benefits: | SY @ 0.10 FTE | | \$7,304 | \$7,669 |
| | TY @ 0.05 FTE | | \$1,455 | \$1,527 |
| Supplies, Expenses, a | nd Equipment | | \$8,300 | \$8,300 |
| | | Total | \$17,059 | \$17,496 |
| University of Wisconsin-N Salaries and Benefits: | ladison SY @ 0.10 FTE | | \$4,900 | \$5,200 |
| | TY @ 0.05 FTE | | \$1,700 | \$1,800 |
| Supplies, Expenses, a | nd Equipment | | \$2,400 | \$2,400 |
| | | Total | \$9,000 | \$9,400 |
| owa State University Salaries and Benefits: | SY @ 0.05 FTE | | \$5,200 | \$5,450 |
| | SY @ 0.05 FTE | | \$5,200 | \$5,450 |
| | | Total | \$10,400 | \$10,900 |
| Max McGraw Wildlife Foundation Salaries and Benefits: PI @ 0.20 FTE | | | \$7,400 | \$7,400 |
| Other Personnel | @ 0.30 FTE | | \$7,500 | \$7,500 |
| | | Total | \$14,900 | \$14,900 |
| Jniversity of Nebraska-Li | | | | |
| Salaries and Benefits | SY @ 0.05 FTE | | \$3,370 | \$3,540 |
| | TY @ 0.24 FTE | | \$3,000 | \$3,150 |
| Supplies, Expenses, and Equipment | | | \$68,200 | \$29,100 |
| | | Total | \$74,570 | \$35,790 |
| | | Total per Year | \$125,929 | \$88,486 |
| | | | \$214,415 | |

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

- Objective 1: Initiated in Year 1 and completed in Year 2.
- Objective 2: Initiated in Year 1 and completed in Year 2.
- Objective 3: Initiated in Year 1 and completed in Year 2.

LIST OF PRINCIPAL INVESTIGATORS

Terence P. Barry, University of Wisconsin-Madison
Tom Harder, Max McGraw Wildlife Foundation
Anne R. Kapuscinski, University of Minnesota
Terrence B. Kayes, University of Nebraska-Lincoln
Jeffrey A. Malison, University of Wisconsin-Madison
Robert C. Summerfelt, Iowa State University

Phone: (608) 263-1242

Terence P. Barry Assistant Researcher University of Wisconsin Aquaculture Program 103 Babcock Hall, 1605 Linden Drive University of Wisconsin-Madison Madison, WI 53706

EDUCATION

- B.S. Zoology, University of Wisconsin-Madison, 1977
- M.S. Zoology, University of Hawaii and Hawaii Institute of Marine Biology, 1989
- Ph.D. Endocrinology-Reproductive Physiology, University of Wisconsin-Madison

POSITIONS

Assistant Researcher, University of Wisconsin Aquaculture Program, UW-Madison (1990-present) Fulbright Graduate Research Fellow, The University of Tokyo, Japan (1988-1989) Research Associate, USAID, Iloilo, Philippines (1986-1988) U.S. Peace Corps Volunteer, High School Biology Teacher, Western Samoa (1977-1980)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Asian Fisheries Society American Society of Zoologists World Aquaculture Society

SELECTED PUBLICATIONS

- Barry, T.P., T.B. Kayes, J.A. Malison, and C.H. Amundson. In press. Validation of an ELISA for measuring cortisol in fish and comparison of stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture.
- Barry, T.P., P. Thomas, and G.V. Callard. In press. Identification and stage-related synthesis of 11deoxycorticosterone (DOC) by the dogfiah (*Squalus acnathias*) testis. Journal of Experimental Zoology.
- Barry, T.P., K. Aida, T. Okumura, I. Hanyu. 1990. The shift from C-19 to C-21 steroid synthesis in spawning male common carp, *Cyprinus carpio*, is regulated by the inhibition of androgen production by progestogens produced by spermatozoa. Biology of Reproduction 43:105-112.
- Barry, T.P., A.J.G. Santos, K. Furukawa, K. Aida, and I. Hanyu. 1990. Steroid profiles during spawning in male common carp. General and Comparative Endocrinology 80:223-231.
- Barry, T.P., K. Aida, and I. Hanyu. 1989. The effects of 17α,20β-dihydroxy-4-pregnen-3-one on the in vitro biosynthesis of 11-ketotestosterone by testicular fragments of the common carp, *Cyprinus carpio.* Journal of Experimental Zoology 251:117-120.
- Barry, T.P., and E.G. Grau. 1986. Estradiol-17β and thyrotropin-releasing hormone stimulate prolactin release from the pituitary gland of a teleost fish *in vitro*. General and Comparative Endocrinology 62:306-314.

Tom Harder Fishery Biologist Max McGraw Wildlife Foundation P.O. Box 9 Dundee, Illinois 60118

Phone: (708) 695-4610 FAX: (708) 741-8157

EDUCATION

B. S. University of Wisconsin-Stevens Point, Natural Resources, 1971

POSITIONS

Fisheries manager, Max McGraw Wildlife Foundation (1975-present) Fish culturist, Max McGraw Wildlife Foundation (1990-present) Commercial Aquaculture, Arkansas (1974) Commercial Aquaculture, Wisconsin (1974) Fish culturist, U. S. Peace Corps, Philippines (1973) Fish culturist, U. S. Peace Corps, India (1971-1973)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society North American Lake Management Society

SELECTED PUBLICATIONS

- Harder, T. 1992. Use of tanks and cages to train pond-reared walleye fingerlings to accept a commercial feed. Pages 23-30 *in* Coolwater Fish Culture Workshop Minutes, Carbondale, Illinois, January 6-8, 1992.
- Harder, T., and R. C. Summerfelt. 1991. Training pond-reared walleye fingerlings in cages to accept formulated feed. Pages 167-179 *in* Coolwater Fish Culture Workshop Minutes, Springfield, Missouri, January 7-9, 1991.

Anne R. Kapuscinski Associate Professor Department of Fisheries and Wildlife 130 Hodson Hall University of Minnesota St. Paul, MN 55108

EDUCATION

B.A. Swarthmore College, 1976 M.S. Oregon State University, 1980 Ph.D. Oregon State University, 1984

POSITIONS:

Associate Professor/Extension Specialist (Aquaculture), University of Minnesota (1989-present) Assistant Professor/Extension Specialist (Aquaculture), University of Minnesota (1984-1989) Instructor/Project Leader/Research Assistant Oregon State University (1980-1984) Research Assistant, Oregon State University (1977-1980) Aquaculture Research Technician, Weyerhaeuser Company (1976-77)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS:

American Fisheries Society: Fish Culture and Genetics Section; North Central Division Fish Genetics Technical Committee Genetics Society of America International Association of Genetics in Aquaculture (Charter Member) Society for the Study of Evolution World Aquaculture Society Sigma Xi, Phi Kappa Phi, Phi Sigma, Gamma Sigma Delta

SELECTED PUBLICATIONS:

- Nelson, D.M., and A.R. Kapuscinski. In press. Application of diallel crosses to lake trout (Salvelinbus namaycush) stock evaluation. Transactions of the American Fisheries Society.
- Gross, M., J. Schneider, N. Moav, C. Alvarez, S. Myster, Z. Liu, C. Hew, E. Hallerman, P.B. Hackett, K.S. Guise, A.J. Faras, and A.R. Kapuscinski. 1992. Molecular analysis and growth evaluation of northern pike (Esox lucius) microinjected with growth hormone genes. Aquaculture 103:253-273.
- Kapuscinski, A.R. 1990. Integration of transgenic fish into aquaculture. Food Reviews International 6(3):373-388. (Invited Paper).
- Hallerman, E.M., J.F. Schneider, M.L. Gross, A.J. Faras, P.B. Hackett, K.S. Guise, and A.R. Kapuscinski. 1989. Enzymatic dechorionation of goldfish, walleye and northern pike eggs. Transactions of the American Fisheries Society 117:456-460.
- Kapuscinski, A.R., and L.D. Jacobson. 1987. Genetic guidelines for fisheries management. Minnesota Sea Grant, St. Paul.
- Kapuscinski, A.R.D., and J.E. Lannan. 1986. A conceptual genetic fitness model for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 43:1606-1616.

Phone: 612-624-3019 FAX: (612) 625-5299

Terrence B. Kayes Associate Professor Department of Forestry, Fisheries and Wildlife 12 Plant Industry, East Campus University of Nebraska-Lincoln Lincoln, NE 68583-0814

EDUCATION

B.A. Chico State College, 1968

M.A. California State University at Chico, 1972

Ph.D. University of Wisconsin-Madison, 1978

POSITIONS

Associate Professor, Dept. of Forestry, Fisheries and Wildlife, University of Nebraska-Lincoln (1990present)

Assistant Director and Associate Scientist, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1979-1990)

Project Biologist, Aquaculture Research Laboratory, University of Wisconsin-Madison (1974-1979) EPA Trainee, Laboratory of Limnology, University of Wisconsin-Madison (1970-1972) Instructor, Department of Biological Sciences, Chico State College (1968-1970)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture, Bioengineering, Fish Health, Water Quality, and Early Life History Sections

American Society of Zoologists: Divisions of Comparative Endocrinology, Comparative Physiology and Biochemistry, Ecology, and Comparative Immunology

World Aquaculture Society

SELECTED PUBLICATIONS

- Malison, J.A., T.B. Kayes, J.A. Held, T.P. Barry, and C.H. Amundson. In press. Manipulation of ploidy in yellow perch (*perca flavescans*) by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture.
- Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health 4:1-6.

Kim, K.I., T.B. Kayes, and C.H. Amundson. 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Oncorhynchus mykiss*). Aquaculture 96:57-67.

Malison, J.A., T.B. Kayes, J.A. Held, and C.H. Amundson. 1990. Comparative survival, growth and reproductive development of juvenile walleye (*Stizostedion vitreum*), sauger (*S. canadense*) and their hybrids reared under intensive culture conditions. The Progressive Fish-Culturist 52:73-82.

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

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

EDUCATION

B.S. University of Wisconsin-Stevens Point, 1976

M.S. University of Wisconsin-Madison, 1980

Ph.D. University of Wisconsin-Madison, 1985

POSITIONS

Assistant Director, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1990present)

Associate Researcher, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1987-1990)

Project Associate, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1985-1987)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

- Barry, T.P., A.F. Lapp, T.B. Kayes, and J.A. Malison. Submitted. Validation of an (ELISA) for measuring cortisol in fish and comparison of stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture.
- Malison, J.A., T.B. Kayes, J.A. Held, T.P. Barry and C.H. Amundson. In press. Manipulation of ploidy in yellow perch (*Perca flavescens*) by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture.
- Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health 4:1-6.
- Malison, J.A., and J.A. Held. 1992. Effects of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (*Perca flavescens*) to intensive culture conditions. Aquaculture 104:67-88.
- Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Measurement of resting and stress-elevated serum cortisol in rainbow trout *Oncorhynchus mykiss* in experimental net-pens. Journal of the World Aquaculture Society 23:83-88.
- Malison, J.A., T.B. Kayes, B.C. Wentworth, and C.H. Amundson. 1988. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Canadian Journal of Fisheries and Aquatic Sciences 45:1942-1948.

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EDUCATION

B.S. University of Wisconsin-Stevens Point, Biology, 1957

- M.S. Southern Illinois University, Zoology, 1959
 Duke University Marine Laboratory, Summer 1962
 Ph.D. Southern Illinois University, Zoology, 1964

POSITIONS

Professor, Dept. of Animal Ecology, Iowa State University (1976-present) Associate Director of the North Central Regional Aquaculture Center (1988-1990) Associate Director of the North Central Regional Aquaculture Center (1988-1990)
Chairman, Department of Animal Ecology, Iowa State University (1976-1985)
Leader (Fishery Research Biologist, U.S. Fish and Wildlife Service, GS-13), Oklahoma Cooperative Fishery Research Unit, Oklahoma State University (1966-1976)
Assistant Professor, Department of Zoology, Kansas State University (1964-1966)
Lecturer, Department of Zoology, Southern Illinois University, Carbondale (1962-1964)
Visiting Professor: Utah State University (1983), Oregon Institute of Marine Biology (1975), and Southern Illinois University (1965)

Illinois University (1965)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture, Fish Health (Charter member), Education (Charter member), Bioengineering, Computer User, and Fisheries Management Sections; Iowa Chapter American Institute of Fishery Research Biologists (Fellow) Fisheries Society of the British Isles Iowa Academy of Sciences North American Lake Management Society Societies Internationalis Limnologiae World Aquaculture Society Honorary: Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta

SELECTED PUBLICATIONS

Cai, J., and R.C. Summerfelt. 1992. Effects of temperature and size on oxygen consumption and ammonia excretion in walleye. Aquaculture 104: 127-138.

Harding, L.M., C.P. Clouse, R.C. Summerfelt, and J.E. Morris. 1992. Pond culture of walleye fingerlings. Fact Sheet Series #102. North Central Regional Aguaculture Center.

Siegwarth, G.L., and R.C. Summerfelt. 1992. Light and temperature effects on performance of walleye and hybrid walleye fingerlings reared intensively. The Progressive Fish-Culturist 54: 49-53.

Forsberg, J. A., and R. C. Summerfelt. 1992. Ammonia excretion of fingerling walleye fed two formulated diets. The Progressive Fish-Culturist 54:45-48.

Forsberg, J. A., and R. C. Summerfelt. 1992. Effect of temperature on diel ammonia excretion of fingerling walleye. Aquaculture 102:115-126.

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