AQUACULTURE TECHNOLOGY OF WALLEYE

Chairperson: Jeffrey A. Malison, University of Wisconsin-Madison

Extension Liaison: Anne R. Kapuscinski, University of Minnesota

Funding Request: $75,000

Duration: 1 Year (September 1, 1992 - August 31, 1993)

Objectives:

1. Develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning.

2. Measure genetic parameters required for efficient selection on fry and fingerling traits, using pedigreed families. (Although this objective will be completed in the time period of this proposal, it is important to appreciate its long-term value from the perspective of future objectives. Establishment of pedigreed families via completion of objective 2 will allow consideration of the following future objectives: 1) Objective for Years 2-3 - Measure genetic parameters required for efficient combined selection on sub-adult and adult traits, using a pedigreed population of walleye; 2) Objective in Years 4-6 - Following implementation 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.)

Proposed Budgets:

Institution	Principal Investigator	Objective	Year 1
University of Nebraska-Lincoln	Terrence B. Kayes	1	$15,222
University of Wisconsin-Madison	Jeffrey A. Malison	1	$19,778
Iowa State University	Robert C. Summerfelt	2	$20,000
University of Minnesota	Anne R. Kapuscinski	2	$20,000
		TOTALS	**$75,000**

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JUSTIFICATION

The coolwater percid fishes (Family Percidae) have been designated a priority candidate group for development of commercial aquaculture in the North Central Region (Joint meeting of the Industry Advisory Council and the Technical Committee for Extension and Research, May 1988, East Lansing, Michigan). The coolwater fishes that have significant potential for commercial aquaculture are the yellow perch (*Perca flavescens*), walleye (*Stizostedion vitreum vitreum*) and the walleye-sauger (*S. v. vitreum x S. canadense*) hybrid. Research planning for the percids, under the auspices of the North Central Regional Aquaculture Center (NCRAC), has been divided into one work group for yellow perch and a second for walleye. This proposal concerns the walleye.

The yellow perch and walleye are the most exploited percid species in North American commercial and recreational fisheries (Kendall 1978). Although a small commercial fishery for yellow perch still exists on the Great Lakes, commercial harvest of walleye in the U. S., except for a few tribal fisheries, has been eliminated in favor of sport fishing. The walleye has been recognized by the National Aquaculture Development Plan (Joint Subcommittee on Aquaculture 1983) as a fish with substantial aquaculture potential. In 1983 and 1984, state, federal and provincial fisheries management agencies in North America stocked more than one billion walleye fry and fingerlings (Conover 1986).

Given the numbers of walleye fry and fingerlings reared for maintenance stocking, many fisheries 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). The fish culture activities traditionally associated with producing walleye include spawning of wild broodstock, hatching fry, and rearing small fingerlings in ponds. Numerically, fry comprise 98% of walleye stockings in the U.S. and Canada (Conover 1986). However, the relative survival of fingerling walleye after stocking is 16 to 60 times greater than with fry (Heidinger et al. 1985). Large numbers of fingerlings can be produced to a size of 35-50 mm total length (TL) by traditional pond-culture methods (Beyerle 1979; Fox 1989). Increasingly, the focus of research by government agencies is being directed to training pond-reared fingerlings to formulated feeds in intensive culture in order to meet the demand from recreational fisheries management for larger (100-150 mm TL) fingerlings (Cheshire and Steele 1972; Nagel 1974, 1976; Beyerle 1975; Nickum 1986). A variety of factors have been studied, including stock density, temperature, light, diet, and feeding frequency (Nickum 1986, Summerfelt et al. 1990).

At this time, commercial walleye aquaculture is primarily geared to the production of eggs, fry and pond-reared fingerlings. Commercial walleye producers are selling fry and fingerlings to lake associations, sportsman clubs and individual lake and pond owners for maintenance stocking. Given the incentive of excellent market prices, commercial walleye production has expanded rapidly in the past 5 years. Newly hatched fry sell for 1 to 1.5 cents, and fish of 35 to 100 mm TL are sold by producers for $0.25 to $1.00, respectively. 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 largely limited to a few researchers in the region, including the principal participants of this proposal at Iowa State University (ISU), University of Minnesota (UM), University of Nebraska-Lincoln (UN-L) and the University of Wisconsin-Madison (UW-Madison), who have the expertise to train pond-reared fingerlings to formulated feed. Most food-size walleye currently from Canadian capture fisheries and the only U.S. supply is from a few Indian tribal lakes. The limited supply has produced exceptionally high prices, with fillets commanding retail prices of $16.09-25.35/kg. Such high prices are a strong stimulus to private sector interest in the production of food-size fish. Although the contribution of aquaculture to food-size walleye has been limited (NCA-23 1987), in 1989 a fairly large scale effort to raise walleye to food-size was started by Aquaculture Inc., Rolla, Missouri (NCRAC Journal 1990).

For commercial walleye aquaculture to expand, especially in the direction of food-fish production, a sustained, collaborative, interdisciplinary research effort needs to be focused on critical bottlenecks, including: (1) the lack of procedures for manipulating reproduction and inducing spawning in walleye broodstock; (2) the lack of captive, domesticated broodstock; (3) the unreliability of present pond management and harvesting strategies for fingerling production; (4) disease identification and FDA-approved therapeutics to control disease problems; (5) the relatively poor growth rates of large fingerlings and sub-adult fish reared in captivity, and (6) the lack of commercially-produced diets for rearing advanced fingerlings to food-sized fish. Many aspects of the production process need further study to facilitate the development of a commercial aquaculture industry based on sound scientific principles.

This proposal describes an extension of ongoing cooperative regional research efforts by the NCRAC Walleye Work Group. The principal goal of the Work Group is to address key problems that presently constrain development of commercial walleye culture in the North Central Region. The focus of this project is on: (1) manipulation of the annual reproductive cycle of walleye to induce out-of-season spawning and (2) development of selected strains of domesticated broodstock that are adapted to the

environment and feeding regimes found in commercial aquaculture. Significant progress on these problem areas has been made during the first several years of our efforts. This proposal constitutes a request for an additional year of funding which is needed for these two lines of research to reach fruition and ultimately yield maximum benefits.

This research effort is an integrated interdisciplinary project which could not be completed by a single institution. It involves cooperation from federal and state agencies, and investigators from four institutions in the North Central Region: Iowa State University, the University of Minnesota at St. Paul, the University of Nebraska-Lincoln, and the University of Wisconsin-Madison.

Manipulation of the Annual Reproductive Cycle

Strategies for manipulating reproduction are needed to insure the availability of "seed stock" for commercial aquaculture and for selective breeding and other types of genetic manipulations such as gene implantation (see Donaldson and Hunter 1983; Idler et al. 1987). In turn, the development of efficacious procedures to manipulate sexual maturation and induce out-of-season spawning is an important component of optimal broodstock management. The benefits of such procedures include: (1) greater predictability of gamete production; (2) reduced incidence of failed spawnings, gamete resorption and subsequent broodfish losses; and (3) the production of fertilized eggs and fry at multiple and predetermined times during the year.

The availability of fertilized eggs outside the normal spawning season would also greatly facilitate research on the intensive culture of walleye fry. On a larger scale, the production of fertilized eggs out-of-season could facilitate a fuller, more efficient use of culture facilities and equipment, and might allow such innovative techniques as the double- or triple-cropping of fry in rearing ponds.

Considering the walleye's importance as a food and game fish, remarkably little is known about its reproductive physiology. Baseline information on the physiological mechanisms regulating the annual reproductive cycle of walleye is essential to the development of efficacious procedures for managing captive broodstock and manipulating walleye reproduction (Donaldson and Hunter 1983; Idler et al. 1987). A starting point for obtaining such information is to characterize changes in specific circulating hormone titers and gonadal development during the annual reproductive cycle. Thereafter, with appropriate experimentation, precise practical methods of controlling reproduction and inducing out-of-season spawning can be developed.

Selective Breeding of Walleye Strains for Commercial Aquaculture

Genetic research is critically important for world-wide development of aquaculture (Wilkins and Gosling 1983; Gall and Busack 1986). Implementation of rationally designed and long-term selective breeding programs into aquaculture operations is an essential means of improving the performance of cultured organisms (Gjedrem 1983; Kinghorn 1983; Tave 1987). The major benefits of selective breeding to commercial aquaculture operations are improvements in product quality and in cost-effectiveness, and increases in harvestable yields and profits. Genetic research aimed at improving culture technology for walleye was ranked as a high priority at joint meetings of the Industry Advisory Council and the Technical Committee of NCRAC, held in May, 1988 in East Lansing, Michigan. Initiation of selective breeding studies at an early stage of commercial walleye culture development is necessary because the generation time of three to four years for cultured walleye represents an inherent lag in the improvement of production traits. Two or more generations of selective breeding may be necessary to obtain substantial gains in some performance traits.

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 incre asing 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. 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.

A regional approach to selection studies is needed for three major reasons. First, it will permit access to the quantity of laboratory rearing tanks at Iowa State University (ISU) and University of Minnesota (UM) required to conduct the first phase of selection studies. Second, by conducting the later phases of

selection studies in production ponds in Iowa and Minnesota, it will allow evaluation of the selected families in a variety of culture conditions found in the region. Third, it will bring together the required expertise including: intensive walleye fry and fingerling culture (Robert Summerfelt) and quantitative genetic analyses (Anne Kapuscinski). The following preliminary study has also been done with a regional approach: under a current grant (June 1990 - August 1992), we are comparing the performance of several candidate stocks from different states in order to identify the most suitable stock(s) for use in a breeding program involving selection (see Current Work on Walleye Strain Comparisons below).

RELATED CURRENT AND PREVIOUS WORK

Manipulation of the Annual Reproductive Cycle

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

The reproductive cycles of many annually spawning fishes, including walleye, are regulated by several environmental factors, of which temperature and photoperiod are of primary importance (Lam 1983). Environmental cues (as well as other factors) are perceived by the brain, which in turn stimulates the endocrine system to control reproductive cycles. The hypothalamus produces gonadotropin-releasing and (at least in some species) release-inhibiting hormones that regulate the secretion of one or more gonadotropins from the pituitary glands (Peter 1983; Peter et al. 1986; Sherwood 1987). Gonadotropin (GTH), in turn, stimulates the production of sex steroid hormones from the gonads and possibly the interrenal (Idler and Ng 1983; Fostier et al. 1983, 1987; Fontaine and Dufour 1987; Kunimasa et al. 1988). Estradiol-17β (E_2) is the primary steroid hormone responsible for ovarian growth and development in female fishes (Fostier et al. 1983; Lazier et al. 1987). Testosterone (T) and 11-ketotestosterone (11-KT) are the primary steroid hormones responsible for testicular growth and development in males (Fostier et al. 1983; 1987).

Reproductive cycles in fish can be divided sequentially into periods of spawning, gonadal involution and quiescence, gonadal growth and recrudescence, final gonadal maturation and gamete release (ovulation or spermiation), which again leads to spawning (Billard and Breton 1978, Lam 1983; Lam and Munro 1987). During the period of gonadal quiescence, circulating levels of sex steroids are usually quite low (Idler and Ng 1983; Fostier et al. 1983, 1987; Fontaine and Dufour 1987). During the period of gonadal growth and recrudescence in females, increasing concentrations of E_2 stimulate vitellogensis (yolk protein formation and deposition) and oocyte growth (Fostier et al. 1983; Nagahama 1983; Idler and Ng 1983; Lazier et al. 1987; Wallace et al. 1987). In males, increasing levels of T and/or 11-KT stimulate spermatogenesis (Idler and Ng 1983; Fostier et al. 1983, 1987).

Sometime after completion of the gonadal growth phase in most species examined to date, a GTH rise (or surge) triggers final maturation and subsequent gamete release (Billard and Breton 1978; Idler and Ng 1983; Goetz 1983; Fostier et al. 1987). In females, final maturation typically involves migration of the oocyte nucleus (termed the germinal vesicle) to the cell periphery, followed by dissolution of the nuclear membrane and dispersal of the chromosomes (collectively termed germinal vesicle breakdown or GVBD) and by a resumption of meiosis. Concurrently, during final maturation, yolk globules and oil droplets in the cytoplasm of the oocytes coalesce, the degree of coalescence depending on species (Goetz 1983). The stimulatory effects of the GTH surge on final maturation and ovulation is at least partially mediated by C-21 steroids produced in the ovaries (Goetz 1983). In the species examined to date, the principal steroids identified as functioning in this role are 17α,20β-dihydroxy-4-pregnen-3-one(17,20-DHP)and 17α,20β-dihydroxy-4-pregnen-3-one (17,20DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (17,20,21-THP, see Goetz 1983; Trant et al. 1986; Goetz et al. 1987; Scott and Canario 1987; Patino and Thomas 1990). In some species, both 17,20-DHP and 11-KT have been implicated as playing major roles in spermiation in males (Fostier et al. 1983, 1987).

To date, endocrine studies on walleye reproduction have focused almost entirely on the pharmacological induction of final oocyte maturation and ovulation, generally during the normal spawning season. Agents, such as carp pituitary extracts, mammalian luteinizing hormones and human chorionic gonadotropin (hCG), have been used to induce final maturation and ovulation, both *in vivo* (Nelson et al. 1965; Lessman 1978; Hearn 1980) and *in vitro* (Goetz and Bergman 1978). In one study, Pankhurst et al. (1986) observed that hCG, LHRHa (a synthetic luteinizing hormone-releasing hormone analogue) and pimozide (a dopamine antagonist and presumptive blocker of endogenous GTH release-inhibiting hormone) all stimulated final oocyte maturation and ovulation in female walleye during the spawning season. An

examination of plasma steroid dynamics in relation to changes in oocyte development in fish treated with LHRHa or pimozide (either alone or in combination) revealed that E_2 and T declined prior to final maturation, but that 17,20-DHP levels increased coincident with GVBD. The latter finding suggested that 17,20-DHP may be a maturation-inducing steroid in walleye.

The primary goal of the first three years of our presently funded project is to document the endocrine and gonadal changes which occur during the annual reproductive cycle of walleye. Significant progress was made during the first two years of our study, and in general, our research is continuing to progress according to the time frame outlined in the original proposals (Attachment E Program Plan #1 for Grant #89-38500-4319 and Attachment A to Program Plan #1 for Grant #91-38500-5900). In the early months of the project we developed a detailed set of protocols designed to insure the proper and uniform sampling of adult-sized walleye in the field, and distributed these protocols to collaborating investigators,. We also developed and validated accurate and precise methods for directly measuring levels of E_2 and T in walleye serum. For E_2, a commercially available solid-phase-antibody radioimmunoassay (RIA) with an iodinated tracer ligand (Coat-a-Count Estradiol, Diagnostic Products Corporation [DPC], Los Angeles, CA) was adapted and validated for use in walleye. To measure T, both a solid-phase-antibody RIA (Coat-A-Count, DPC) and a double-antibody RIA (DPC) have been similarly validated. Histological procedures that produce acceptable results in evaluating adult walleye gonads at all (seasonal) stages of development were also developed and standardized.

Using these procedures, we are presently evaluating reproductive development in wild-caught walleye and walleye held in ponds. Blood and tissue samples are being provided by collaborating investigators from the University of Minnesota and Southern Illinois University, respectively. Beginning in April, 1990, walleye were sampled at regular intervals over the course of 13 months and two spawning seasons. The data in Figure 1 show some of our results from walleye captured from Mille Lac, MN.

Figure 1. Gonadosomatic indices (GSI = [gonad weight/body weight] x 100) and serum levels of estradiol-17β and testosterone in male and female walleye captured from Mille Lac, MN, at different times during the year.

From these results, as well as our histological examinations, we have concluded that gonadal growth in male walleye begins in August or September, and testes are in an advanced stage of development as early as January. In fact, mature spermatozoa can be expressed from males collected from January through the spawning season. In female walleyes, gonadal growth also begins in late summer. By early January vitellogenesis is nearing completion, as evidenced by the high GSIs of females collected at this time. During the ongoing third and final year of the project, critical or insufficient data sets will be replicated as needed, and more frequent sampling will be done during key periods of the reproductive cycle. Of particular importance in this regard is the need for more frequent winter sampling in order to determine precisely when vitellogenesis is effectively completed.

Researchers at the UW-Madison have also started work to identify and characterize the steroids responsible for final oocyte maturation and ovulation in walleye. We conducted a series of experiments to evaluate the effectiveness of various steroids at inducing GVBD and ovulation in walleye oocytes cultured *in vitro*. Of the steroids tested, 17,20-DHP and 17,20,21-THP were the most potent in this regard (see Figure 2 for the results of one such study).

We have also cultured oocytes *in vitro* with radiolabelled precursors and hCG in order to isolate steroids produced by the ovarian tissues and released into the culture media during final oocyte maturation. Our results indicated that little or no 17,20,21-THP and only a moderate amount of 17,20-DHP were produced. However, we isolated a large amount of a steroid that we have not yet identified. Whether the latter compound is a maturational steroid or has some other function has not yet been determined. During the third (present) year of the ongoing project, we will continue to characterize the maturational steroids in walleye, develop and validate assays for these steroids, and measure these hormones in blood samples collected from wild-caught and pond-held adult fish. The information gained from this three-year project should provide us with the basic knowledge needed to develop practical methods of controlling reproduction and inducing out-of-season spawning.

Figure 2. Stimulation of GVBD and ovulation in walleye ooctyes cultured for 24 h with various steroids at 10 ng/mL. 17-P = 17α-hydroxy-4-pregnen-3-one, 17,20α-P = 17α,20β-dihydroxy-4-pregnen-3one, S = 17α,21-dihydroxy-4-pregnen-3-one, DOC = 11-deoxycorticosterone.

Selective Breeding of Walleye Strains for Commercial Aquaculture

Identification of the strain(s) 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). A NCRAC sponsored study (Summerfelt et al. 1990) on walleye strain comparisons was initiated in the spring 1991 by an interdisciplinary group of researchers, involving faculty from three institutions: Southern Illinois University-Carbondale (SIUC), Iowa State University (ISU), and the University of Minnesota at St. Paul (UM). In addition to the principal investigators, there is active collaboration from

state agencies (Iowa, Minnesota, Ohio) and the U.S. Fish and Wildlife Service's Genoa National Fish Hatchery. The role of Anne Kapuscinski, UM was one of assistance to collaborators at SIUC (Robert Sheehan and Bruce Tetzlaff) and ISU (Robert Summerfelt) with experimental design and analysis of performance comparisons among different stocks of walleye reared in captivity.

Comparisons of Population Genetic Characteristics

Allozyme and mtDNA analysis will be used by Neil Billington (SIUC) under a population genetics objective for the current Walleye Work Group project (Summerfelt et al. 1990). The fish examined by Billington will include but not be limited to four stocks currently under examination in performance trait comparisons (Tables 1-3), and two other stocks of potential value for selective breeding (discussed under Procedures for Objective 2, Choice of Walleye Strain for Initiation of Selection Program). Biochemical genetics is a powerful tool for discriminating walleye populations. Genetic marks may have important applications in the development of walleye culture. Fish possessing genetic marks may be selected during the domestication process. The marks are passed from generation to generation, and their use may facilitate the perpetual marking of aquaculturally important strains (Seeb et al. 1989; Gharrett and Seeb 1989, reviewed in Utter and Seeb 1989). Additionally, work with other species suggests that the 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. Quantitative genetics research on walleye will provide the baseline information needed to evaluate strains for culture and to improve their performance via selective breeding.

Comparisons of Performance Traits

The objectives of this project are to conduct comparisons of phenotypic characteristics of progeny from selected walleye broodstock, three from wild sources and one (Ohio) captive broodstock. The strains evaluated in 1991 included: (1) an Iowa stock (IA) from the Spirit Lake Hatchery; (2) an Ohio stock (OH) from the London State Fish Hatchery; a Mississippi River stock (MR) from the Genoa National Fish Hatchery, Wisconsin; and (4) a Minnesota (MN) strain from Lake Saganaga, a boundary water lake between Minnesota and Ontario. The MN and MR stocks should be the least domesticated, and the MR stock potentially the most heterozygous of the three strains because of the greater opportunity for mixture of gene pools in the river and its tributaries. The OH broodstock is in the third generation of hatchery rearing derived from parents from Pymatuning and Mosquito reservoirs. The OH broodstock was identified as a high priority source for development of domesticated and selected broodstocks for aquaculturists in the North Central Region because it is a third generation captive broodstock.

Performance of selected stocks of walleye are evaluated in intensive and extensive culture environments. Strain evaluations in 1991 include both extensive (pond) culture followed by training the pond-reared fish to formulated feed (SIUC), and complete tank rearing entirely on formulated feed (ISU).

Funding for this project began June 1, 1990, after the walleye spawning season, therefore, the work accomplished to date was in the 1991 season. At Iowa State, facilities for intensive culture were renovated in the fall of 1990; the old cylindrical rearing tanks were removed, and replaced with square tanks of the Loadman et al. (1989) design, but modified to improve surface drainage and with a surface spray to remove surface films. The facilities have pH control, a recycle system, and UV disinfection of the recycled water.

In the 1991 season, strains were evaluated using numerous performance traits; to date, data on survival, gas bladder inflation, viability, length at harvest and growth rate have been analyzed (Table 1). Survival differed among stocks: 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 stocks.

TABLE 1. Comparisons (analysis of variance, ANOVA) of performance of four stocks of walleye reared in the modified Loadman et al. (1989) square tanks from 1-3 days to 20-22 days posthatch at ambient pH (pH>8.3). All fish were fed the Kyowa fry feed (H+Kyowa Hakko Kogyo Company, Ltd., Japan)

Source (stock)	Age[1] (days posthatch)	Survival (%)	GBI[2] (%)	Viability[3] (%)	Length[4] (mm)	Growth[5] (mm/day)
OH-London Hatchery	22	19.9[a]	72.8[a]	15.2[a]	13.9	0.32
IA-Spirit Lake	20	5.0[b]	35.0[b]	1.7[b]	13.9	0.34
MR-Mississippi River	20	13.9[a]	69.0[a]	9.4[a]	15.6	0.41
MN-Lake Saganaga	21[b]	1.8[b]	33.6[b]	0.7[b]	15.5	0.38
ANOVA, significance of F[6]		p<0.01	p<0.05	p<0.01		

[1]Age at harvest = days posthatch for the experiment.
[2]GBI = percent of 100 fish at the end of the experiment; the fish were examined individually with dissecting microscope.
[3]Viability = percent of total number of fish stocked that had gas bladders when examined at the end of the experiment.
[4]Length at harvest (20-22 days posthatch).
[5]Growth = (length at harvest - length at stocking)/number of days in rearing interval.
[6]The analysis was done with proportions and arcsine transformation, both results were the same. Duncan's new multiple-range test was used to determine the difference between stocks, stocks with the same letter were not significantly different.

Egg diameter ranged from 1.72 to 1.99 mm and larval length at hatching from 6.05 to 7.60 mm (Table 2). There was a strong correlation (r = 0.915) between egg size and larval length at hatching. Egg diameter is a heritable trait, although it is also influenced by the maternal environment (e.g., dam's food supply, food quality, body size, and age). Generally, there is a positive correlation between egg size and growth rate (Tave 1987).

TABLE 2. Egg diameter and fry size (length) at hatching.

Source (stock)	Egg diameter (mm)	Larval length (mm) at hatching
OH-London Hatchery	1.72	6.05
IA-Spirit Lake	1.99	7.46
MR-Mississippi River	1.90	7.60
MN-Lake Saganaga	a	a

[a]Fish were hatching on arrival, therefore, due to urgency of the situation, neither egg diameter nor length at hatching were obtained.

Indicators of prior inbreeding of the stocks were examined by recording incidence of deformities (Table 3) and developmental instability (phenotypic similarity of the right and left sides of individuals). The latter data have not been analyzed yet. Incidence of deformities was low, only 24 of 5,988 fry examined (0.4%), but incidence did differ significantly among the four groups (Chi-square, p=0.028).

TABLE 3. Incidence of deformities in four stocks of walleye reared in intensive culture facility on formulated feed (Fry feed Kyowa).

Source (stock)	Type of Deformity				Number[a]	Incidence[b] (n/1000)
	Upturned tail	Curled tail	Loridosis	Arched spine		
OH-London Hatchery	5	1	4	2	12 (1521)	0.79
IA-Spirit Lake	0	0	2	0	2 (1435)	0.14
MR-Mississippi River	1	0	2	0	3 (1312)	0.23
MN-Lake Saganaga	1	0	5	1	7 (1720)	0.41

[a]Total number of deformities (total number of fry examined)
[b]Total number of deformities/total number of fry examined x 100 = %; Chi-square analysis of observed incidence compared to hypothesis of equal incidence among all groups (0.4%), Chi-square=9.12, p=0.028.

Selective Breeding

Nagel (1985) reported development of a domesticated walleye broodstock at the London (Ohio) State Fish Hatchery beginning in 1975 with progeny of parents from Pymatuning and Mosquito reservoirs (T. Nagel, Ohio Department of Natural Resources, personal com munication). The first generation of F_1 offspring of the captive broodstock were produced in 1980. After pond-rearing to 31-38 mm on natural food, Nagel obtained higher survival (80 to 90%) of the F_1 from the captive broodstock than the offspring of wild broodstock (40 to 50% survival to 12.5 cm) when trained to formulated feed in tanks (Nagel 1985). Subsequently, Nagel reared four F_1 generations (1980 through 1983) to spawning size, produced the first F_2 generation in 1986 (also in 1987 through 1989), and an F_3 generation in 1990 and 1991. An F_4 generation is expected in 1994.

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. Yet, results from breeding programs with other fish species strongly suggests that efficie nt selection of walleye will successfully yield fish that reach marketable size in a shorter time and with reduced operating costs.

To date, quantitative genetic research on aquacultural fish species has focused primarily on rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), tilapias (*Oreochromis* sp.), channel catfish (*Ictalurus punctatus*), and common carp (*Cyprinus carpio*). Studies have been conducted on strain evaluations, genetic parameter estimation, selection, crossbreeding, and inbreeding. Most of the discussion below refers to reviews by Tave (1987), Kapuscinski and Jacobson (1987), review papers in the proceedings of a World Symposium on Selection, Hybridization and Genetic Engineering (Tiews 1987), and papers from the Third International Symposium on Genetics in Aquaculture (Gjedrem 1990).

Previous research has shown that there is great potential for selective breeding in fish. Two major reasons for this are (Gjedrem 1983): considerable genetic variance for growth rate and age at maturation has been found in various species; and high selection intensities can be practiced because of the high fecundities in most species. The opportunity for selective breeding of walleye also should be high due to high fecundities and the great likelihood that levels of genetic variation for production traits are similar to those that have been found in other species. Additionally, realized responses to selection have been higher in fish than those reported in farm animals (Gjerde 1986). Gains in farm animals have been approximately 1% per year, where as, reported gains in salmonids for example, have been 3-7% gains per year, or 10-30% per generation from individual and family selection schemes (e.g., Kinghorn 1983; Gjedrem 1979; Gjerde 1986; Hershberger et al. 1990).

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 par ameters 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 since 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 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. Expected response of walleye strains to selection on dressing percentage is uncertain because heritabilities in other species tend to be low (e.g., Gjerde and Gjedrem 1984), although Gjerde and Schaeffer (1989) reported an adequately high value of 0.36 in rainbow trout. Very little is known about genetic parameters for disease resistance in fish because it is a very complex trait, meaningful measurement is difficult in genetic experiments, and few appropriate studies have been conducted (Tiews 1987; Chevassus and Dorson 1990).

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.

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. Two primary constraints in this regard are (1) the lack of procedures for manipulating reproduction and controlling spawning in walleye, and (2) the lack of captive, domesticated broodstock. Our proposed studies under Objective 1 should greatly improve the predictability of walleye egg production, and functionally extend the annual walleye spawning season by several weeks or months. Increased availability of walleye eggs will, in turn, lead to more efficient use of culture facilities, especially fingerling production ponds, and facilitate research and development of intensive walleye fry culture techniques. Studies under Objective 2 will serve as a starting point of a long-term project whose goal is the development of a domesticated selected line of walleye suitable for commercial aquaculture. In the 1993 culture season, performance traits will be measured in pedigreed families derived from the best stocks, based on comparative evaluations in an intensive culture environment.

OBJECTIVES

1. Develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning.

2. Measure genetic parameters required for efficient selection on fry and fingerling traits, using pedigreed families. Although objective 2 will be completed in the time period for this proposal, it is important to appreciate its long-term value from the perspective of future objectives. Establishment of pedigreed families via completion of objective 2 will allow consideration of the following future objectives: <u>Objective for Years 2-3</u>

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

Objective in Years 4-6

Following implementation 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.

PROCEDURES

Manipulation of the Annual Reproductive Cycle (Objective 1)

Research to manipulate the annual reproductive cycle of walleye will be done collaboratively by investigators from the University of Nebraska-Lincoln (UN-L) and the University of Wisconsin-Madison (UW-Madison). In general, the UN-L will be responsible for coordinating the capture and collection of adult walleye from the wild, and maintaining walleye in ponds and tanks during experiments. Researchers from both institutions will be responsible for conducting spawning-induction experiments in Nebraska, and UW-Madison scientists will analyze blood and tissue samples.

The long-term goal of this line of research is to develop methods of inducing spawning in walleye throughout the year. To achieve this goal, a well coordinated research effort spanning several years will be required. The current project is limited to a duration of only one year. Accordingly, we propose to focus our efforts on a hypothesis that can be tested within a one-year time frame, and a set of experiments that will produce results yielding immediate practical benefits, independent of (any) subsequent studies.

Our working hypothesis for Objective 1 is that appropriate hormone treatments, when applied after the completion of the gonadal growth period and ovarian vitellogenesis, can be used to advance spawning in walleye by 2 to 12 weeks. We propose to treat wild-caught walleye at regular intervals with one of four hormonal regimes during the period of time between the end of vitellogenesis (possibly in January) and when spawning would normally occur (in late March or April in most locales). The precise timing and duration of this period is not yet known, but will be clearly delineated by the completion of our presently funded studies in 1991 and 1992. These studies have already shown that vitellogenesis is completed at least two weeks prior to spawning, and may be functionally complete as early as January (see **RELATED AND CURRENT PREVIOUS WORK**). We are specifically not proposing to attempt hormonal induction of spawning before the completion of vitellogenesis, because such attempts have generally failed due to: (1) a lack of ovarian response to maturational hormones, (2) improper synchrony between GVBD and ovulation, or (3) low egg survival after spawning (Goetz 1983; Donaldson and Hunter 1983).

Obtaining the large number of wild broodfish (64-80 females and 16-32 males) at times of the year needed for the study (January to April) will require a major effort. This effort will be coordinated by the UN-L working in concert with the Nebraska Game and Parks Commission, the Iowa Department of Natural Resources, and Region 6 of the U.S. Fish and Wildlife Service. All three agencies have made a firm commitment of resources and manpower to the successful conduct of the project. As an operational objective, every effort will be made to use wild-caught walleye captured from the same body of water. If this proves impractical, a strategy of planned backups involving the capture of broodfish from more than one source will be employed.

Present plans are to capture 90-120 adult female and 40-50 adult male walleye in the autumn (October-November) of 1992 from Sherman Reservoir in central Nebraska, or from Merrit Reservoir in northwestern Nebraska. These fish will be transported and stocked with forage species into overwintering ponds at the Calamus State Fish Hatchery in Nebraska and/or the Gavins Point National Fish Hatchery near the Nebraska border in South Dakota, and will be re-captured as needed for spawning-induction experiments at the Calamus hatchery. The ponds employed will be provided with sufficient water flow (and aeration if needed) to keep them relatively ice free during the winter. To the extent possible, minimum stress procedures will be used in capturing, transporting, and handling fish. Walleye will be recovered from the overwintering ponds with trap-nets or by seining.

As a backup to this plan, advance arrangements will also be made by state agency field crews to capture wild walleye broodfish in January, February, March and April from the Niobrara, Missouri and Mississippi Rivers and/or selected lakes or reservoirs in Nebraska and Iowa. (Detailed planning for this will be initiated in the winter of 1991-92). Captured broodfish will be transported directly to the Calamus hatchery for use in experiments. In anticipation of circumstances under which this may not be possible, advance arrangements will also be made to conduct spawning-induction experiments, as needed, at the Gavins Point hatchery in South Dakota and at the Spirit Lake and Rathbun state fish hatcheries in Iowa.

As an added backup, walleye broodfish captured from the Mississippi River in the autumn of 1992 will be overwintered in flow-through ponds at the Lake Mills State Fish Hatchery in Wisconsin, and spawning

experiments can also be conducted there. Collectively, this strategy of planned backups is designed to ensure that despite the inherent difficulties of obtaining significant numbers of adult walleye in a scheduled manner during the winter and the uncertainties of overwintering adult walleye in ponds, we will be able to complete the proposed project in one year.

Irrespective of place or source of fish, four spawning-induction experiments will be conducted sequentially, and will be interspaced equally during the period between the end of vitellogenesis (in winter) and the normal spawning season (usually in early April). For each experiment, 16-20 female and 4-8 male walleyes will be captured in the field or re-captured from overwintering ponds , and immediately transported and placed (on day 0) into four 750-L flow-through tanks at the receiving hatchery. One or two males will be kept in each tank, because we observed in a preliminary experiment (unpublished) that the presence of males promoted GVBD and ovulation in walleyes, possibly due to a pheromonal effect (Liley and Stacy 1983). Environmental conditions will be maintained that should minimize stress and facilitate spawning (e.g., 9-11 ^{o}C water temperature, 14-h light/8-h dark photoperiod using low intensity lighting, and sufficient water flow and aeration to provide low loading rates and good water quality).

The females in each tank will be subjected to one of four injection regimes: (1) intraperitoneal saline as a control on days 0 and 2; (2) hCG at 150 IU/kg on day 0 and 500 IU/kg on day 2; (3) des-Gly^{10}-[D-Ala^{6}]-LHRH-ethylamide (LHRHa) at 35 µm/kg on day 0 and 100 µm/kg on day 2; or (4) hCG at 150 IU/kg on day 0 and 17,20-DHP at 2 mg/kg on day 2. Similar doses of these hormones have been used successfully in our laboratory and by others (e.g., Pankhurst et al. 1986) to induce ovulation in walleyes captured during the spawning season. Our proposed strategy of using a "priming" dose of maturational hormone followed by a larger "triggering" dose has proved more effective than a single injection at inducing ovulation in other fish species examined to date, particularly when used with relatively immature fish (Donaldson and Hunter 1983).

On handling during experiments, each female walleye will be anesthetized (MS-222), and blood and egg samples will be taken on day 0 and subsequently on alternate days through day 12, or until the fish spawn. (Although such procedures may be rather stressful to fish, in the past we have sampled walleye for blood and eggs daily for 7 days, and observed few mortalities and no negative impact on spawning). Blood samples will be immediately processed, and the serum frozen and stored for subsequent measurement of E_2, T and/or 11-KT, and 17,20-DHP and any other maturational steroids identified during our ongoing studies. All steroid measurements will be made using previously validated RIAs or enzyme-linked immunosorbent assays (ELISAs). Eggs will be cleared in a clearing solution to determine their maturational stage based on the position of the germinal vessicle. Spawning induction will be measured on days 2, 4 and 6 through 12 by attempting to strip eggs from each individual fish, as done routinely during the spawning season.

All of the eggs obtained will be fertilized using fresh semen collected and pooled from several males held in tanks. The motility of semen collected from each male will be assessed prior to its use. Fertilized eggs will be incubated in flow-through hatching jars, and the viability of eggs from each female will be measured two 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. Present plans are to conduct the egg quality and hatchability studies at the Calamus State Fish Hatchery in Nebraska. If spawning-induction experiments are done at other hatcheries (i.e., Gavins Point, Spirit Lake or Rathbun), then the resulting fertilized eggs will be transported to the Calamus hatchery for incubation and study. In addition, some of these eggs will be retained at the hatchery of origin for incubation and egg quality and hatchability assessments there. This arrangement provides a means of accounting for the effects of egg transport and differences between hatcheries.

The limited amount of time (a few weeks) each year that walleye larvae are normally available has long been a major constraint to research on walleye larvae culture and feeding. If successful, one immediate benefit of our proposed project will be to provide the technology needed for making walleye larvae available for a much longer time period. To realize this benefit and to further foster regional and inter-regional cooperation on walleye culture research, some of the walleye sac-fry and prolarvae generated by our project (especially between January and mid-March) will be distributed to the Rathbun Hatchery in Iowa, the Valley City National Fish Hatchery in North Dakota, and the Bozeman Fish Technology Center of the U.S. Fish and Wildlife Service in Montana, for evaluation in feeding trials. The remaining larvae will be retained at the Calamus hatchery for state-funded projects.

Data collected on serum steroid levels, egg maturational stages, frequency of successful spawning induction and egg viability will be used to compare the relative effectiveness of the various hormone treatments. The data will be analyzed and the findings published in a timely manner in appropriate peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

The proposed selection program is designed to concurrently complete two tasks in the parent generation: (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.

Selective Breeding of Walleye Strains for Commercial Aquaculture (Objective 2)

Choice of Walleye Strain for Initiation of Selection Program

Given the current availability of culture facilities at ISU and UM, the selection program will be developed for one strain or one synthetic strain (created by interbreeding fish from two or more strains). Using results of strain comparisons from a current study initiated in June 1990 and ending August 31, 1992 before the start of this proposed project (Summerfelt et al. 1990), we will choose the strain(s) showing: (a) best expression of production traits under aquaculture conditions; and (b) greatest genetic variation detected by protein electrophoresis and DNA analyses. Using similar criteria, we may also consider use of (1) a strain from Minnesota, and a (2) Spirit Lake, Iowa stock. For both stocks, performance data are available from other studies and population genetic analysis can be included in the work of year 2 for the current NCRAC walleye project (Summerfelt et al. 1990).

The Minnesota stock has performed well for over three years of cage culture on artificial feed in Minnesota fish ponds (Anne Kapuscinski, unpublished results from research funded by the Legislative Commission on Minnesota Resources). It should contain numerous sexually mature individuals in year 1 of this proposed project. The Spirit Lake stock contains about 300 fish of the 1989 year class, including males from which sperm was easily stripped in spring 1991, and about 600 fish of the 1990 year class. Both year-classes were pond-reared to 43-51 mm total length, trained indoors in an intensive culture environment to formulated feed then reared indoors until stocked in a 5.0 ha pond at ISU Horticulture Experiment Station. Fish of the 1990 year-class have already visible implant (VI) tags on the ventral surface of the mandible. Reproductive adults from both the Iowa and Minnesota stocks will have experienced aquaculture environments from birth, including feeding of artificial diets since the fingerling stage.

Creation of Pedigreed Families

A balanced, nested mating design of full- and half-sib families will be used (Becker 1984), where each sire is mated to three dams and an equal number of progeny per full-sib family are individually marked. Three dams are an optimum number for precise estimation of genetic parameters (Robertson 1959). This mating design is desirable because: (a) data from half- and full-sibs allow precise estimation of necessary genetic parameters using data from only the parent generation of families (i.e., generation founded in the first year of this line of work in 1990-91); and (b) individual and family data allow application of combined selection on reproducing adults of this same parent generation. In the first year, 24 full-sib families nested within 8 half-sib families will be created by mating randomly chosen dams and sires. Some additional families will be created at the outset to replace any crosses lost due to poor fertilization or low survival up to hatching. Combined culture facilities at UM and ISU will be needed to rear and collect performance data on the resultant 24 families.

This balanced nested design will incur a 2% inbreeding rate on the parent generation, which should be acceptable for a fish breeding program. Selection on domestic livestock is known to offset a 2% inbreeding rate per generation (Pirchner 1979). Except for the captive broodstock maintained at the London (Ohio) State Fish Hatchery (Nagel 1991), any adults used to create the parent generation of pedigreed families will be no more than one generation removed from the wild, thus having accumulated much lower total inbreeding than most domesticated livestock populations.

Full-sib families will be incubated and reared in separate containers until individuals are large enough ($\geq$125 mm) to receive visible implant (VI) tags. At this point, approximately 222 randomly chosen individuals within each full-sib family will be uniquely marked with a VI tag (one tag on the lower surface of a mandible) and remaining sibs will be culled from the study. From hereon, it will be possible to pool different families into common rearing tanks, cages or outdoor ponds in Iowa and Minnesota. Based on conservative estimates of survival probabilities through different life stages up to reproduction at age four, tagging a minimum of 222 individuals per family should yield approximately 20 individually marked reproductive adults per family at age four (Table 4). This is the optimum size of dam (full-sib) families for precise estimation of genetic parameters, under the conservative assumption that the lowest heritability for a trait targeted for selection will be approximately 0.1 (Robertson 1959). Establishment of marked individuals and families is required for future application of efficient combined selection on a set of traits. Such tagging allows: (a) computation of genetic and phenotypic correlations for sequentially expressed

traits, as required for concurrent selection on two or more traits (use of a selection index); and (b) selection of the best individuals, as required for combined family and individual selection.

TABLE 4. Life table for pedigreed walleye families.

Life Stage	Percent Survival	x	Initial No./Family	=	No. Surviving/Family
Fertilization to hatch	60%		at least 3,703 eggs		2,220 newly hatched fry
Fry to fingerlings	10%		2,220 fry		222 fingerlings (≥127 mm)
Fingerlings to age 4	30%		222 fingerlings (≥127 mm)		67 adults
Spawners at age 4	30%[1]		67 adults		20 reproductively capable fish

[1]This figure assumes that a smaller proportion of females than males will be in spawning condition at age 4.

Creation of a Control Line

It is imperative to establish a control line 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, using the same strain of parents as for pedigreed families, a control line will be generated by random mating sufficient adults to keep the rate of inbreeding <2%. Sufficient control fry will be cultured to produce approximately 275 converted fingerlings at each facility, or 550 total. Assuming similar survival probabilities as for pedigreed families (Table 4), this should generate approximately 165 reproductive adults at age four (50 reproductively capable fish) which will be sufficient to keep the inbreeding rate in the progeny generation (approximately 1%) no greater than that experienced in the selected line. After fish in the pedigreed line are tagged and pooled into common rearing units, control fish will be reared in common with them. Control fish will not need individual tags, although it may be useful to apply a common fin clip in order to positively distinguish them from any pedigreed fish that might lose their VI tags.

Estimation of Genetic and Population Parameters

Performance data needed to estimate genetic and population parameters on juvenile traits of interest for selection (as discussed under related current and previous work on selective breeding) will be collected in Year 1. These will include all fry traits of interest and the fingerling traits that can be measured during the time period of the grant. Genetic parameters (e.g., heritabilities, genetic correlations), will be determined using the nested analysis of variance method for a fixed-effects model, using data collected from individuals according to half-sib and full-sib groupings (e.g., Becker 1984). This method allows unbiased estimation of heritabilities from the sire component of variation. All attempts will be made to maintain a balanced design because it prevents bias and greatly simplifies statistical analysis for estimation of genetic parameters. If mortalities, however, lead to unequal family sizes, genetic analysis techniques developed for unequal subclass numbers will be used (e.g., Grossman and Gall 1968).

Culture Methods

Matings needed for the nested design of families require simultaneous availability of gametes from sufficient males and females of the chosen stock or synthetic stock. To make the necessary crosses at each laboratory (ISU and UM), we will use techniques for effective short-term transportation, short-term refrigerated storage and, if necessary, cryopreservation of walleye semen (Moore 1987). Also , we will use walleye egg transport and short-term refrigerated storage techniques that have consistently yielded excellent results (>80% fertilization rate) in Anne Kapuscinski's lab (Hallerman et al. 1989). Unfertilized walleye eggs that have not contacted any water are placed in dry plastic zip-lock bags inflated with pure oxygen and kept cool in insulated containers or a refrigerator.

Different families will be reared at both UM and ISU, 13 families at UM and 11 at ISU plus each facility will concurrently rear approximately one-half of the control line. At each site (MN and IA), fish will be reared on formulated feeds in indoor tanks until fingerlings reach a size (~125 mm) when individuals within each pedigreed family can be individually marked with VI tags and pooled into common rearing units.

After gametes are crossed, each family of fertilized eggs will be incubated either in separate hatching jars according to standard procedures (Nickum 1978, 1986), or in separate cells of divided trays of Heath-Tecna incubators. Anne Kapuscinski's lab (UM) has repeatedly used the latter method to successfully incubate small lots of walleye eggs. Thus, it should be effective for the separate incubation of families involving relatively small egg lots per family.

Intensive culture of fry on formulated feed will be standardized at the intensive culture sites using protocols developed by Robert Summerfelt in his current research at ISU (Summerfelt 1988, 1989). At ISU, fry are reared in tanks of the Loadman et al. (1989) design. They are 150-L capacity, cubical shaped tanks with a water flow pattern that facilitates keeping the food in suspension (100 food particles per L is considered critical). At initial fry densities of only 15 per L (2250 fry/tank), survival should be better than our conservative prediction of 10% survival (Table 4) because survival of postlarvae is affected by fish densities (Li and Mathias 1982) and 15 per L is less than 20/L used in the current walleye strain comparisons (e.g., Table 1). UM uses cylindrical-shaped tanks with a circular current but otherwise stocking densities, light and feeding schedules will be similar.

Larvae are counted gravimetrically after determining the mean weight of three samples of 500 fry . Water quality and current is maintained with a complete exchange twice per hour. Water temperature will be 16-17 °C up to 5 d posthatch, then gradually incremented to 20 °C by 15 d and 20-23 °C from 30-100 d. Water flow rates to the rearing tanks will be kept the same and will be adjusted to maintain calculated un-ionized ammonia below 0.001 mg/L. Dissolved oxygen, pH, alkalinity and total ammonia-nitrogen will be measured weekly. Dissolved-oxygen probe and standard analytical procedures will be used for measuring pH, alkalinity and ammonia-nitrogen (APHA et al. 1985).

Fish will be fed formulated feeds from fry to brood fish. The "Fry Feed Kyowa," Series B diets (Kyowa Hakko Kogyo Company, Ltd., Japan) will be used for rearing the larvae through 21 days, the Bioproducts "Biotrainer" diet will be used for 3 weeks and finally the Bioproduct "Biodry" formulation to rear the fingerlings to the reproductive adult stage. Feed will be dispensed by mechanical feeders actuated by an electronic time-clock. Fry feeding begins at 3-d posthatch. Feed size will be upgraded to accommodate changing requirements of the fish.

Fingerling culture practices for the tank reared fingerlings begin at 30 d. Maximum room light levels for fingerling culture are 25 lux but the tanks are screened which reduces light about 50% over the water (10-15 lux maximum over the water surface). The diurnal light regime (room lighting) is 18 h light and 6 h dark for the 70 d of fingerling rearing. Densities for intensive culture of fingerlings will start at 1.5 fish per liter when they are 15-20 mm total length in indoor tanks until the fish obtain a length of about 125 mm when they can be effectively tagged with the VI tag. After fish reach 125 mm total length and they are individually marked, they may be reared in common, either indoors as facilities permit, in cages in ponds, or at large in the pond(s). Biotrainer diet will be used for training fingerlings shifted to Biodry feed (both Biotrainer and Biodry are registered trademarks of Bioproducts Incorporated, Warrenton, Oregon).

FACILITIES

Manipulation of the Annual Reproductive Cycle (Objective 1)

The capture of walleye broodfish from the wild, maintenance of broodfish in holding ponds and experimental tanks, the conduct of spawning-induction experiments and egg quality and hatchability assessments of the UN-L portion of the proposed project will be coordinated by Terry Kayes, in cooperation with the Nebraska Game and Parks Commission, Iowa Department of Natural Resources, and Region 6 of the U.S. Fish and Wildlife Service. All three agencies have made a firm commitment to provide the capture gear, boats and motors, fish transport equipment, fish holding and hatching facilities, and manpower, assistance needed to perform the project. Broodfish captured from the wild will be held in ponds and/or tanks at the Calamus State Fish Hatchery in Nebraska and/or Gavins Point National Fish Hatchery in South Dakota. To the extent possible, spawning-induction experiments and egg quality and hatchability assessments will be done at the Calamus hatchery. This facility has 51 rearing ponds, indoor and outdoor raceways, 886 m^2 of floor space equipped for indoor hatching and rearing, numerous egg incubators and fiberglass cylindrical rearing tanks, three separate water sources (a river reservoir and two different aquifers), water-temperature control and pure oxygen aeration systems. Backup facilities for spawning experiments and egg hatching will also be available at the Gavins Point hatchery, and the Spirit Lake and Rathbun Fish Hatcheries in Iowa. Additional backup fish holding facilities in Wisconsin will be at the UW Aquaculture Program's main research laboratory at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. Facilities here include over 25 ponds from 0.2-0.7 ha, and a wet laboratory that has ample supplies of temperature-regulated (5 to 30 ± 0.5 °C) well or carbon-filtered city water. Equipment at Lake Mills includes a live-haul truck, over 100 circular fiberglass tanks ranging in size from 61-cm diameter (110-L capacity) to 1.8-m diameter (3,200-L capacity), and more than 20 hatching jars.

Determination of oocyte maturation and ovulation will be done using the four dissecting microscopes of the UW Aquaculture Program. Blood samples for hormone analysis will be collected, preliminarily processed, and shipped or transported to the UW-Madison. All hormone analyses will be done by the UW Aquaculture Program, which has its main research facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. These facilities include an analytical laboratory that is well equipped for histological, cytological and endocrinological research. The UW Aquaculture Program also has additional analytical facilities on the main UW-Madison campus, and has access to much of the laboratory facilities, equipment and instrumentation of the UW-Madison Endocrinology-Reproductive Physiology Program, Department of Poultry Science and Center for Limnology.

Selective Breeding oif Walleye Strains for Commercial Aquaculture (Objective 2)

Facilities for One Year of This Project

This research will be conducted in part at the UM fisheries wet lab which includes: a 284 m^2 fish culture lab supplied with filtered well water (7.6 L/sec., 10 ^oC, 6 on-line iron filters) and outfitted with on-line air compressor, on-line chiller unit, hot water heat exchangers, UV-sterilization filter, back-up electrical generator, tool shop, air compressor, and dechlorination filters (for use of city water in emergencies). Divided tray incubators and jar incubators, at least 17 circular fiberglass tanks (208.2 L rearing water/tank) supplied with heated water, and automatic feeders are available for separate culture of pedigreed walleye families. Adhering to a maximum walleye rearing density of 8 g/L, each circular tank is capable of rearing up to 97 walleye to the size (12.7 cm.) when they can be individually tagged, a value well above the 83 fish/family planned for this research. The water supply for all incubators and tanks is connected to a 24 hr. alarm system monitoring water level and temperature.

UM facilities for water chemistry analyses, and other analyses needed for incubating walleye eggs and rearing juveniles are located in four labs (203 m^2) of the Department of Fisheries and Wildlife. Major equipment includes: hoods, refrigerators, one -80 ^oC freezer (0.49 m^3), one walk-in freezer (5.3 m^3), electronic and triple-beam balances, one spectrophotometer, pH and oxygen meters, Winkler D.O. apparatus, on-line water de-ionizer, drying oven, muffle furnace, table-top centrifuges, microscopes, and a Beckman MDL J2-21 centrifuge. Access to the mainframe computer and numerous microcomputers, printers, software, and accessories are available for database storage, statistical analysis and reporting.

The aquaculture facilities at ISU include facilities in Science Hall on the main campus and a field site. On campus, the aquaculture facilities include two rooms for fish rearing and another room for analytical purposes. The latter is equipped with a hood, chemical sink, BOD incubator, and bench space for water chemistry, and microscopic analysis. One of the wet labs has 16,120 L tanks, and is supplied with both compressed air, pure oxygen and a pH controller. The other, larger wet lab, has 12,150 L tanks, a recycle system complete with biofilter, mechanical filter, and UV sterilizer and two pH controllers. Both wet labs are supplied with dechlorinated (activated charcoal) tap water, which is also treated with sodium sulfite from a chemical pump to eliminate residual free chlorine and chloramines. Excess nitrogen is reduced to safe levels by passing all incoming water through a packed column with plastic rings for degassing. Refrigerators/ freezers, analytical balance, pH meters, dissolved oxygen meters, microscopes (compound and dissecting), spectrophotometer, calorimeter, specific ion meter, gas saturometer, and glassware are available in the analytical lab for measuring water chemistry. The analytical laboratory has a PC and printer, the PC harddisk has software for word processing, graphics, spreadsheet, and statistics. It is also hardwired directly to the University mainframe (ISN port) for use of SAS (1986).

Facilities for Future Selective Breeding Research

Although work during the one year of this proposed project will not involve growout of tagged fish to broodstock size, facilities are available in Minnesota and Iowa for doing so in subsequent years. At least one Minnesota fishfarmer, Don Winson, has expressed interest in being a co-investigator on future walleye selective breeding studies by rearing fish from this proposed project to the breeding stage. He would use covered cages outfitted with automatic feeders and suspended in ponds supplied with well water. The cage setups were installed during a prior collaborative project with Anne Kapuscinski, funded by the Legislative Commission on Minnesota Resources (LCMR), in which Don Winson used them to rear converted walleye fingerlings to food-fish size. Under the same LCMR project, Anne Kapuscinski also collaborated with other Minnesota walleye aquaculturists from the private sector (their ponds of various sizes were outfitted with covered cage/automatic feeder setups and aerators to keep ice open in the winter) and the Leech Lake Indian Reservation (their indoor heated water facility was outfitted with rectangular rearing tanks and automatic feeders). Some of these parties may also be interested in serving as co-investigators in future walleye selection work by rearing fish to the breeding stage. Growout options in Iowa include the facilities at the Iowa State University Horticulture Experiment Station: (a) three indoor 946 L tanks; and (b) cages outfitted with automatic feeders and aeration apparatus to prevent ice

cover over the cages. The cages will be suspended in a 4.9 ha pond alongside a small building with a power supply.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Iowa	Robert C. Summerfelt Iowa State University	Finfish Aquaculture: General larvi-culture, fish health
Minnesota	Anne R. Kapuscinski University of Minnesota	Aquaculture/Quantitative Genetics
Nebraska	Terrence B. Kayes University of Nebraska-Lincoln	Aquaculture Production/Fish Physiology and Nutrition Aquaculture Extension
Wisconsin	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture Physiology/Endocrinology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

University of Nebraska-Lincoln
Terrence B. Kayes

University of Wisconsin-Madison
Jeffrey A. Malison

Iowa State University
Robert C. Summerfelt

University of Minnesota
Anne R. Kapuscinski

PROPOSED WALLEYE PROJECT BUDGET FOR
UNIVERSITY OF NEBRASKA-LINCOLN (UN-L)

(Kayes)

Objective 1

			Year 1
A. Salaries and Wages	No.	FTEs	
1. No. of Senior Personnel & FTEs[1]			
a. (Co)-PI(s)	1	0.08	$0
b. Senior Associates			
2. 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	0.40	$7,858
Total Salaries and Wages			$7,858
B. Fringe Benefits (25% of 2f)			$1,964
C. **Total Salaries, Wages and Fringe Benefits**			$9,822
D. Nonexpendable Equipment			$0
E. Materials and Supplies			$1,500
F. Travel - Domestic (*Including Canada*)			$3,500
G. Other Direct Costs			$400
TOTAL PROJECT COSTS (*C through G*)			$15,222

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF NEBRASKA-LINCOLN

A. Salaries and Wages. The UN-L component of the proposed project will require a great deal of manpower in October-November and from early January through late April. Most of the field collection of walleye broodfish and much of the technical support at the participating fish hatcheries will be provided by personnel of the Nebraska Game and Parks Commission, the Iowa Department of Natural Resources, and the U.S. Fish and Wildlife Service. To implement the project, a Technicial (0.40 FTE) is needed to assist (1) state and federal personnel with the field collections, set up of experiments and general fish husbandry, and (2) the principal investigator with spawning induction experiments and assessments of egg quality and hatchability.

E. Materials and Supplies. Hand-nets, buckets, fiberglass tote containers, cold-weather protective gear, glassware, microscope slides, ice, dry ice, insulated containers, hypodermic needles and syringes, catheters, anesthetic, hormones (cost shared with UW-Madison), and miscellaneous reagents and other supplies are needed for the field collections, conduct of experiments, egg quality and hatchability assessments, and to ship blood samples to the UW-Madison.

F. Travel. The UN-L component of the proposed project will require extensive regional travel in October-November and from early January through late April. Field collections from the Mississippi River in the east to Merrit Reservoir in western Nebraska may be needed to do the proposed research. To the extent possible, spawning-induction experiments and egg quality and hatchability assessment will be done at the Calamus State Fish Hatchery in Nebraska, but holding ponds, holding tanks, hatching facilities and manpower of the Gavins Point National Fish Hatchery in South Dakota and the Spirit Lake and Rathbun fish hatcheries in Iowa may also be utilized. Estimated minimum travel costs for five or six visits of three- to six-days duration to field or hatchery work sites by two (possibly three) UN-L investigators are as follows: $900 for meals, $950 for lodging, and $1,250 for fleet truck rental and gasoline, for a total of $3,100. In addition, $400 is needed to help pay for travel to a NCRAC Walleye Work Group meeting and a national

G. Other Direct Costs. Extensive telecommunications (telephone and FAX) will be required to coordinate the proposed project. About $300 is needed for telephone and FAX, as well as photocopying and postage. An additional $100 is required for overnight express shipment of frozen blood samples to the UW-Madison.

PROPOSED WALLEYE PROJECT BUDGET FOR
UNIVERSITY OF WISCONSIN-MADISON (UW)

(Malison)

Objective 1

			Year 1
A. Salaries and Wages	No.	FTEs	
1. No. of Senior Personnel & FTEs[1]			
a. (Co)-PI(s)	1	0.04	$0
b. Senior Associates	1	0.04	$0
2. No. of Other Personnel (Non-Faculty) & FTEs			
a. Research Assoc./Postdoc			
b. Other Professionals	1	0.50	$12,500
c. Graduate Students			
d. Prebaccalaureate Students			
e. Secretarial-Clerical			
f. Technical, Shop, and Other			
Total Salaries and Wages			$12,500
B. Fringe Benefits (29.5% of 2b)			$3,688
C. **Total Salaries, Wages and Fringe Benefits**			$16,188
D. Nonexpendable Equipment			$0
E. Materials and Supplies			$1,500
F. Travel - Domestic (*Including Canada*)			$1,800
G. Other Direct Costs			$290
TOTAL PROJECT COSTS (*C through G*)			$19,778

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

A. Salaries and Wages. Salaries of personnel (Other Professional, 0.50 FTE) are needed to (1) assist UN-L personnel with the capture and maintenance of broodfish, (2) conduct experiments in conjunction with UN-L personnel, including the collection of blood and egg samples, and (3) conduct analyses for serum hormones and egg maturation and viability.

E. Materials and Supplies. Biochemicals, reagents, hormone-assay and general laboratory supplies are needed for hormone injections (the latter cost shared with UN-L) and to conduct assays on serum and eggs.

F. Travel. $900 will be needed for trips to Nebraska and/or Iowa hatcheries to conduct experiments, $400 will be used to attend NCRAC walleye group meetings, and $500 will be used to present findings and results at a scientific conference.

G. Other Direct Costs. $290 is needed for telephone, FAX, postage and photocopying.

PROPOSED WALLEYE PROJECT BUDGET FOR
IOWA STATE UNIVERSITY (ISU)

(Summerfelt)

Objective 2

			Year 1
A. Salaries and Wages	No.	FTEs	
1. No. of Senior Personnel & FTEs[1]			
a. (Co)-PI(s)	1	0.05	$0
b. Senior Associates			
2. No. of Other Personnel (Non-Faculty) & FTEs			
a. Research Assoc./Postdoc			
b. Other Professionals			
c. Graduate Students	1	0.50	$12,500
d. Prebaccalaureate Students	1	0.20	$2,500
e. Secretarial-Clerical			
f. Technical, Shop, and Other			
Total Salaries and Wages			$15,000
B. Fringe Benefits ($40/month for 2c)			$480
C. **Total Salaries, Wages and Fringe Benefits**			$15,480
D. Nonexpendable Equipment			$0
E. Materials and Supplies			$3,500
F. Travel - Domestic (*Including Canada*)			$800
G. Other Direct Costs			$220
TOTAL PROJECT COSTS (*C through G*)			$20,000

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

BUDGET JUSTIFICATION FOR IOWA STATE UNIVERSITY

A. Salaries and Wages. A Graduate Student (0.50 FTE), is needed to (1) conduct incubation, rearing, and tagging of fish; (2) supervise undergraduate lab aide; (3) collect, compile and assist with analysis of performance data for genetic parameter estimation. A Prebaccalaureate Student lab aide (0.20 FTE) is needed to assist with maintenance of the fish rearing facility (cleaning tanks, filling feeders, maintaining water system) and conducting routine water quality monitoring of the system (oxygen, gas pressures, ammonia, pH, temperature, alkalinity). The study plan includes continuous rearing from egg to broodstock, thus, once fish are acquired, the fish will require daily care, year-around.

B. Fringe Benefits. Health insurance for graduate research student.

E. Materials and Supplies.

Fish food:	
Biokyowa B-400 (k kg), Biokyowa B700 (5 kg)	$ 700
BioProducts Biotrainer and Biogrower diets	200
Reagents and glassware needed for water quality monitoring; and for removal of chlorine (activated carbon and sodium sulfite), and maintaining buffering (sodium bicarbonate)	340
Visible implant tags and injector:	
2,500 VI tags (for 11 families and extras to replace lost tags)	1,500
2 syringes for VI tag insertion	160
Utility (installation and monthly service) for fish rearing facility at Horticulture Experiment Station (6 mo @ $40/month)	240
Repairs (water pump) and maintenance	360
SUBTOTAL	$ 3,500

F. Travel. For PI to attend annual Work Group meeting, or for PI and/or graduate student to attend professional meeting (annual Coolwater Fish Culture Workshop), and travel between Ames and the University of Minnesota to collect eggs from chosen walleye strain.

G. Other Direct Costs. Communications with Work Group members and NCRAC administration (FAX, telephone, postage, electronic mail networks, photocopies).

PROPOSED WALLEYE PROJECT BUDGET FOR UNIVERSITY OF MINNESOTA (UM)

(Kapuscinski)

Objective 2

			Year 1
A. Salaries and Wages	No.	FTEs	
1. No. of Senior Personnel & FTEs[1]			
a. (Co)-PI(s)	1	0.10	$0
b. Senior Associates			
2. No. of Other Personnel (Non-Faculty) & FTEs			
a. Research Assoc./Postdoc			
b. Other Professionals			
c. Graduate Students			
d. Prebaccalaureate Students	1	0.10	$900
e. Secretarial-Clerical			
f. Technical, Shop, and Other	1	0.50	$10,443
Total Salaries and Wages			$11,343
B. Fringe Benefits (27.25% of 2f)			$2,846
C. **Total Salaries, Wages and Fringe Benefits**			$14,189
D. Nonexpendable Equipment			$1,350
E. Materials and Supplies			$3,840
F. Travel - Domestic (*Including Canada*)			$400
G. Other Direct Costs			$221
TOTAL PROJECT COSTS (*C through G*)			$20,000

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF MINNESOTA

A. Salaries and Wages. A Principal Lab Technician (Technical, Shop, and Other, 0.50 FTE) is needed to collect gametes from broodstock of chosen strain(s); conduct incubation, rearing, and VI tagging of fish constituting 13 pedigreed families and a control line (approx. 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. A Prebaccalaureate Student is needed (0.10 FTE or 118 hrs) to assist with maintenance of the fish rearing facility (cleaning tanks, filling feeders, maintaining water system) and conducting routine water quality monitoring of the system (oxygen, gas pressures, ammonia, pH, temperature, alkalinity).

D. Nonexpendable Equipment. 15 Boatcycle agitators (1/15 Hp.) are needed to provide emergency aeration to each circular rearing tank used in this study during periodic maintenance and breakdown/repair of central water pump and aeration system.

E. Materials and Supplies.

Fish food:	
Biokyowa B-400 (5 kg), Biokyowa B-700 (5 kg)	$ 700
BioProducts Biotrainer and Biogrower diets	200
Chemicals (MS-222, Formalin, disinfectant, water chem. reagents)	340
Alarm monitoring service and pager	440
3,000 VI tags (for 13 families and extras to replace lost tags)	1,800
2 syringes for VI tag insertion	160
Repairs and maintenance of fish culture equipment	200
SUBTOTAL	**$ 3,840**

F. Travel. Trips to collect gametes from chosen walleye strain (University car rental, lodging and meals for lab technician) and for local trips to pick up expendable culture supplies from local vendors (PI is currently the Extension Liaison for the Walleye Work Group and therefore hopes to receive some Extension Work Group funds to attend annual Work Group meeting during period of this project).

G. Other Direct Costs. Communications with Work Group members and NCRAC administration (FAX, telephone, postage, electronic mail networks, photocopies).

AQUACULTURE TECHNOLOGY OF WALLEYE

Budget Summary for Each Participating Institution at $75.0K for One Year

	UN-L	UW	ISU	UM	TOTAL
Total Salaries and Wages	$7,858	$12,500	$15,000	$11,343	$46,701
Fringe Benefits	$1,964	$3,688	$480	$2,846	$8,978
Total Salaries, Wages and Benefits	$9,822	$16,188	$15,480	$14,189	$55,679
Nonexpendable Equipment	$0	$0	$0	$1,350	$1,350
Materials and Supplies	$1,500	$1,500	$3,500	$3,840	$10,340
Travel	$3,500	$1,800	$800	$400	$6,500
Other Direct Costs	$400	$290	$220	$221	$1,131
TOTAL PROJECT COSTS	**$15,222**	**$19,778**	**$20,000**	**$20,000**	**$75,000**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

Institution/Item		Amount
University of Nebraska-Lincoln		
Salaries and Benefits		$5,184
Supplies, Expenses, and Equipment		$6,850
	Total	$12,034
Nebraska Game and Parks Commission, Iowa Department of Natural Resources, and U.S. Fish and Wildlife Service (combined)		
TY @ 1.00 FTE, Travel, Fish Hauling Equipment, Field Gear, Fish Facilities, Supplies, and Waiver of Overhead		$40,990
University of Wisconsin-Madison		
Salaries and Benefits: SY @ 0.08 FTE and		$4,194
TY @ 0.04 FTE		$2,396
Supplies, Expenses, and Equipment		$7,851
	Total	$14,441
Iowa State University		
Salaries and Benefits: SY @ 0.05 FTE (PI)		$4,986
Contribution of Indirect Costs (@42% of Total Direct Costs)		$8,400
	Total	$13,386
University of Minnesota		
Salaries and Benefits: SY @ 0.10 FTE and		$6,961
TY @ 0.05 FTE		$1,275
Supplies, Expenses, and Equipment		$8,206
	Total	$16,442
	GRAND TOTAL	$97,293

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: Completed in Year 1.

Objective 2: Completed in Year 1.

LIST OF PRINCIPAL INVESTIGATORS

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

VITA

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

Social Security No. 075-48-3375
Phone: 612-624-3019
FAX: (612) 625-5299

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 Section, Genetics Section, NCD Fish Genetics Tech. Comm.
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. In Press. Molecular analysis and growth evaluation of northern pike (*Esox lucius*) microinjected with growth hormone genes. Aquaculture.

Kapuscinski, A.R., and E.M. Hallerman. In Press. Implications of introduction of transgenic fish into natural ecosystems. Canadian Journal of Fisheries and Aquatic Sciences.

Kapuscinski, A.R. 1990. Integration of transgenic fish into aquaculture. Food Reviews International 6(3):373-388. (Invited Paper).

Kapuscinski, A.R., and L.D. Jacobson. 1987. Genetic guidelines for fisheries management. Minnesota Sea Grant, St. Paul.

Lannan, J.E., and A.R.D. Kapuscinski. 1986. Application of a genetic fitness model to extensive aquaculture. Aquaculture 57:81-87.

VITA

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

Social Security No. 573-62-7889
Phone: (402) 472-8183
FAX: (402) 472-2964

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, Department of Forestry, Fisheries and Wildlife, University of Nebraska-Lincoln (1990-present)
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)
Teaching Assistant, Department of Zoology, University of Wisconsin-Madison (1972-1974)
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 Society of Zoologists: Divisions of Comparative Endocrinology, Comparative Physiology and Biochemistry, Ecology and Comparative Immunology.
American Fisheries Society: Fish Culture, Bioengineering, Fish health, Water Quality and Early Life History Sections.
World Aquaculture Society

SELECTED PUBLICATIONS

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. In Press. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health, in press.

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. 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-17ß. Canadian Journal of Fisheries and Aquatic Sciences 45:1942-1948.

Kim, K.I., T.B. Kayes, and C.H. Amundson. 1987. Effects of dietary tryptophan levels on growth, feed/gain, carcass composition and liver glutamate dehydrogenase activity in rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology 88B:737-741.

VITA

Jeffery A. Malison
Assistant Director
University of Wisconsin Aquaculture Program
Dept. of Food Science, 103 Babcock Hall
University of Wisconsin-Madison
Madison, WI 53706

Social Security No. 395-50-7597
Phone: (608) 263-1242
FAX: (608) 262-6872

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 (1990-present)
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 Sciences
American Fisheries Society
American Society of Zoologists
World Aquaculture Society

SELECTED PUBLICATIONS

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. In Press. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health.

Malison, J.A. and J.A. Held. In Press. 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.

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

Malison, J.A., T.B. Kayes, B.D. 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.

Malison, J.A., C.D. Best, T.B. Kayes, C.H. Amundson, and B.C. Wentworth. 1986. Sexual differentiation and the use of hormones to control sex in yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 43:26-35.

VITA

Robert C. Summerfelt
Professor
Department of Animal Ecology
Iowa State University
Ames, IA 50011

Social Security No. 514-28-4947
Phone: (515) 294-6107
FAX: (515) 294-5468

EDUCATION

B.S. University of Wisconsin-Stevens Point, 1957
M.S. Southern Illinois University, 1959
Duke University Marine Laboratory, 1962
Ph.D. Southern Illinois University-Carbondale, 1964

POSITIONS

Professor, Department of Animal Ecology, Iowa State University (1976-present)
Associate Director of the North Central Regional Aquaculture Center, Iowa State University (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)
Visiting Professor: Utah State University (1983), Oregon Institute of Marine Biology (1975), and Southern 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; Walleye Technical Committee of the North Central Division; and Iowa Chapter.
American Institute of Fishery Research Biologists (Fellow)
Fisheries Society of the British Isles
Iowa Academy of Sciences
North American Lake Management Society
Societas Internationalis Limnologiae
World Aquaculture Society
Honorary: Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta

SELECTED PUBLICATIONS

Hussain, M., and R.C. Summerfelt. 1991. The role of mechanical injury in an experimental transmission of *Flexibacter columnaris* to fingerling walleye. Journal of the Iowa Academy of Science 98:93-98.

Siegwarth, G.L., and R.C. Summerfelt. 1990. Growth comparison between fingerling walleyes and walleye x sauger hybrids reared in intensive culture. Progressive Fish-Culturist 52:100-104.

Marty, G.D., and R.C. Summerfelt. 1990. Wound healing in channel catfish by epithelization and contraction of granulation tissue. Transactions of the American Fisheries Society 119:145-150.

Summerfelt, R.C., and L.S. Smith. 1990. Anesthesia and surgery. Pages 213-272 *in* C.B. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Summerfelt, R.C., and G.E. Hall, editors. 1987. Age and growth of fish. Iowa State University Press, Ames.