IMPROVING SALMONID AQUACULTURE IN THE NORTH CENTRAL REGION

Chairperson: Paul B. Brown, Purdue University

Industry Advisory Council Liaison: David A. Smith, Urbana, Ohio

Extension Liaison: Ronald E. Kinnunen, Michigan State University

Funding Request: $160,000

Duration: 2 Years (September 1, 1997 - August 31, 1999)

Objectives:

1. Develop and evaluate practical and economically viable diets that are fish-meal free or as fish-meal free as practical:
   - using soy, or other oil-seed products that are regionally available, and
   - using Shasta, Donaldson and Kamloop strains of rainbow trout and/or Arctic charr for the evaluation.

2. Evaluate the effects of water temperature on the growth/stress response in salmonid strains or species (as listed in Objective 1) under outdoor commercial culture conditions in the upper and lower portions of the North Central Region.

3. Investigate the effects of trace mineral supplementation on the growth and stress response of rainbow trout in high density culture, as evaluated by plasma cortisol levels and fin nipping behavior.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Year 2	Total
Purdue University	Paul B. Brown	1	$19,964	$20,036	$40,000
Michigan State University	Donald L. Garling	1 & 3	$20,439	$19,561	$40,000
Ohio State University	Konrad Dabrowski	1	$20,000	$20,000	$40,000
University of Wisconsin-Madison	Jeffrey A. Malison	2	$20,000	$20,000	$40,000
		TOTALS	**$80,403**	**$79,597**	**$160,000**

Non-funded Collaborators:

Facility	Collaborators
Rushing Waters Fisheries, Inc., Palmyra, Wisconsin	David Mueller
Animal Disease Diagnostic Laboratory, West Lafayette, Indiana	Randy White
Wenger, Inc., Sabetha, Kansas	Brad Strahm
USDA, NCAUR, Peoria, Illinois	Victor Wu, Kathy Warner
Freshwater Farms of Ohio, Urbana, Ohio	David Smith
Wisconsin Aquatic Veterinary Service, Madison, Wisconsin	Myron Kebus

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JUSTIFICATION

Aquaculture production in the U.S. increased from 140 to 302 thousand metric tons between 1983 and 1994 (National Marine Fisheries Service 1996) and is still the fastest growing sector of agriculture. By some estimates, U.S. aquacultural production should increase from approximately 10% at present to 25% of the total supply of fish by the year 2000. Consumption of fish in the U.S. has stabilized at approximately 6.4 kg/capita; that figure is 4-5 times lower than in the United Kingdom and 12-15 times lower than in Japan. Assuming that there are 225 million people in the U.S. consuming 6.4 kg of fish per year, then the increase from 10% of total supply to 25% represents an increase in domestic production of 33,750,000 kg. This figure represents substantial room for increased domestic production, but would still result in importation of the majority of fish consumed in the United States.

Channel catfish is the most popular cultured fish consumed in the U.S. and its deliveries to processing plants, based on inventories, are expected to expand by 7% in 1996 in comparison to the record volume of 203 million kg processed in 1995. Salmon and trout production are also on the rise and expected to continue as new sites become available (production of salmon in Maine increased by 50% between 1994 and 1995) and growers continue to become more efficient (ERS 1996).

Culture of salmonid fishes is the largest aquacultural industry in the North Central Region (NCR) and probably the oldest. Based on the most recent Situation and Outlook Report from the North Central Regional Aquaculture Center (NCRAC), sales from the 110 producers of salmonids were over $6.1 million or 33% of total aquacultural sales in the NCR (Hushak 1993). The number of producers and gross sales figures are underestimated as only 51% of the licensed producers in the region responded to the survey. For example, the most recent summary of global aquacultural production listed 99 producers of trout in just Michigan and Wisconsin (Anonymous 1996). Almost 50% of the trout production in the U.S. occurs in Idaho each year, but spread among fewer producers than in the NCR. Only 33 producers in Idaho generated over $33.0 million in sales in 1995. Thus, the salmonid industry in the NCR can be characterized as having more producers than in Idaho, but generating smaller annual sales of fish or lower production per operation. Lower production per operation requires efficiency of that production as producers cannot take advantage of economies of scale.

The Industry Advisory Council (IAC) of the NCRAC has consistently ranked salmonid research and extension activities among the highest priority items. Indeed, only yellow perch has been funded for a longer continuous period than salmonids. This situation is clearly due to the number of trout producers in the NCR, the smaller nature of individual operations, and the need for increased efficiency of production. At the 1996 NCRAC Annual Program Planning Meeting, salmonids were again designated among the highest priority species groups and several specific areas were targeted for research and extension funding.

Mature aquacultural industries often identify focused problem areas in need of research effort. This has been the case with producers of salmonids in the NCR. In this proposal, several key research areas were identified by the IAC that directly impact production efficiency. These include improved diets, the interaction between nutrition and genetics, interaction of temperature/stress/genetics, and agonistic behavior. While these topics do not make for a cohesive proposal, they are the primary factors impacting economic efficiency and growth of salmonid aquaculture in the NCR.

Practical Diet Development (Objective 1)

Diets for trout and salmon consist primarily of fish meal and oil as sources of crude protein and lipid. However, this situation, most likely, cannot continue into the foreseeable future. Use of fish meal and oil by the aquaculture industry is expected to more than double whereas predictions of fish meal production indicate a decline of 5% (Rumsey 1993). The 600,000 metric tons of fish feed projected in 2000 will require about 250,000 tons of fish meal or 25-33% of the available supply. Faced with increasing demand, diminishing supply, and higher feed prices, fish nutritionists must identify feedstuffs that could serve as substitutes for fish meal.

Numerous studies have examined the use of alternative feedstuffs in diets for trout and salmon (cf. Cho et al. 1974; Fowler and Banks 1976; Gropp et al. 1976; Spinelli et al. 1979; Tiews et al. 1979; Spinelli et al. 1983; Dabrowski et al. 1989; Olli and Krogdahl 1994; Rumsey et al. 1994; Bureau et al. 1996). None of the plant feedstuffs could completely replace fish meal when used as a sole source of crude protein. However, recent studies with rainbow trout (see **RELATED CURRENT AND PREVIOUS WORK**), have identified combinations of plant feedstuffs that promote weight gain and feed efficiency that is within 80-90% of fish fed standard fish meal-based diets. Individual plant feedstuffs have an inferior nutritional composition compared to fish meal, but selected ones can be combined to meet the nutritional requirements of the target species. However, replacement of fish meal with plant protein sources causes other problems other than simply meeting the nutritional requirements of the target species.

As commercial catches of seafoods decline, aquacultural development must increase to replace the diminishing supply. However, aquaculture development must proceed in an environmentally sound manner. Commercial aquaculture is developing at the time other animal production industries are experiencing increased regulation, a large portion of which is focused on waste management. Acceptance of a new animal production industry demands attention to waste management issues.

Phosphorus (P) and nitrogen (N) are the primary focal points when discussing waste management within aquaculture. These two nutrients are the most common limiting nutrients in both fresh and salt water (Wetzel 1983). Thus, addition from any source can cause environmental changes that are unacceptable within our society. The only organic input into aquaculture operations is feed for the target species; thus, the logical line of research should address dietary P and N dynamics (Brown 1992).

Both P and N are required nutrients in fish. Phosphorus is an essential mineral required for "almost every aspect of metabolism" (NRC 1980), including bone growth and development, high-energy phosphate bonds, nucleotide synthesis, and metabolic pathways involving amino acids, carbohydrates and lipid. The nitrogen component of diets is supplied as both essential and nonessential amino acids. Essential amino acids (EAA) cannot be synthesized by the fish, but must be supplied in the diet. A dietary deficiency of EAA causes growth retardation, decreased N utilization, and increased N excretion. The goal in dietary formulation is to provide the minimum amount of P and EAA to meet the requirements of the target species, while providing sufficient amounts for maximum growth and efficiency of feed utilization. Plant feedstuffs tend to exhibit inferior EAA patterns compared to fish meals. Thus, combinations of plant feedstuffs and supplementation with feed-grade forms of EAA are critical if there is to be a move away from fish meal.

The forms of both nutrients are important considerations as potential sources are not absorbed by the fish to the same degree. Thus, availability or absorption from the chosen ingredients is a critical piece of information that must be known. Plant feedstuffs contain a high percentage of P in the form of phytic acid, which is nutritionally unavailable to trout (Riche and Brown 1996), and simply exacerbates the effluent problem. Once the critical dietary components are known, then reduction in effluents should logically focus on movement of solid waste through the production system, solubilization of critical components from the solid waste, precise quantification of these factors, and methods of handling wastes.

Trout and salmon are the principal group of fishes raised in the NCR. Further, the NCR is the region where effluent concerns are acutely problematic. Production from a public hatchery operated by the Michigan Department of Natural Resources was restricted because of effluent concerns. If the salmonid industry is to maintain the current level of production, research studies aimed at improving effluents is needed. If the industry is to grow, improved effluents is mandatory.

The principal goal of this objective is to replace high quality (high priced) fish meal in the diets for salmonid species with alternative ingredients of comparable crude protein concentrations supplemented with synthetic amino acids, lipids and minerals when needed and to understand the changes that occur in effluent management as these changes are made. Feed costs are a high proportion of total fish production cost (32-50% in salmon; Anderson and Bettencourt 1992); thus, research on the use of low cost ingredients in aquaculture diets has a long history, but seems to evolve from exclusively animal by-product replacers (meat and bone meal, feather meal, poultry by-product meal) towards plant proteins (soybean, rapeseed and cottonseed). It was realized that considerable benefit can be gained by designing the replacer which will combine the attributes of fish meal protein (available amino acids), remove antinutrients, and be supplemented with other essential nutrients.

Evaluate Water Temperature on the Growth/Stress Response (Objective 2)

Midwest trout farmers, through their representation on the IAC of the NCRAC, indicated that a top research priority was the identification of trout and salmon strains suited to the water temperatures characteristic of the region. Groundwater temperatures of 8-11°C are prevalent at many of the salmonid farms in Minnesota, Michigan, and Wisconsin. In addition, the water temperatures of many trout production ponds and raceways in the region vary significantly with season, dropping to 5-8°C in the winter and reaching 18-20°C in the summer. These conditions are not optimal for the production of Kamloops rainbow trout (*Oncorhynchus mykiss*), the strain most commonly reared in the U.S. Kamloops trout are ideally suited to stenothermal, 15°C, waters such as those found in the Hagerman Valley of Idaho. Under the thermal conditions found in the NCR, however, Kamloops trout often do not realize their maximum growth potential. If Midwest trout farmers reared other salmonid species or strains, they could possibly improve their profitability.

Water temperature has profound impacts on fish growth and performance. The growth rate of most fish species decreases at temperatures both above and below the optimum for the species (Stickney 1994). Water temperatures below the optimum range lower metabolism and thereby reduce feed consumption and growth. Temperatures above optimum elevate metabolic rate and thus increase energy demand.

In addition to its direct effects on growth, water temperature also has major effects on physiological stress in fish. Elliot (1981) defined thermal stress as any temperature change or condition that produces a significant disturbance in the normal behavior and function of fish, and further defined “critical temperature ranges” as temperatures either above or below optimum at which signs of thermal stress first become apparent.

Fish exposed to critical temperatures for even a short time may show signs of acute stress. Some of the most important and commonly measured parameters of acute stress include serum concentrations of cortisol, glucose and chloride (Schreck 1990). Fish exposed to temperatures in the critical range over a longer period of time may show signs of chronic stress. Chronic stress has been quantified by using an assessment method which measures manifestations of biochemical and physiological alterations at the organism level (e.g., see Goede and Barton 1990). Ultimately, exposure of fish to temperatures that are either in critical ranges or simply sub-optimal will result in reduced growth rates, increased incidence of disease, and/or higher mortalities, all factors that reduce the productivity and profitability of fish farms.

Different coldwater salmonid species and strains have optimal growth temperatures that range from 10-20°C. Additionally, some species and strains may tolerate fluctuating temperatures better than others. The Arctic charr (*Salvelinus alpinus*) has been identified by some regional aquaculturists as a species which may grow and perform well at facilities with relatively cool water temperatures. In addition to this potential advantage, Arctic charr commands a producer (farm gate) price of up to $7.50/kg in the round, compared to $5.50/kg for rainbow trout (R. Milton, Hidden Valley Charr Ltd., Prince Edward Island, Canada, personal communication). The Alvdal strain of Donaldson rainbow trout has been identified as a strain which may perform better than Kamloops under relatively warm or highly variable temperature conditions (T. Kayes, University of Nebraska-Lincoln, personal communication). Although Donaldson rainbow trout are the second most commonly reared trout strain in the NCR, little has been documented about their production characteristics under regional culture conditions. We propose to compare the effects of water temperature on the growth and stress responses of Arctic charr and Kamloop and Donaldson rainbow trout under outdoor commercial culture conditions found in the NCR.

Diets/Fin Nipping Behavior (Objective 3)

Several of the most experienced trout culturists in the NCR have been concerned that diets for trout reared in high density culture are not sufficient to meet the needs of the fish. The fundamental hypothesis is that increased fin nipping behavior occurs in these situations and could be caused by inadequate mineral supplementation in diets for fish under chronic stress conditions (high density culture). This topic has been discussed and ranked several times by the IAC and deserves attention. Clearly, inadequate diets can result in agonistic behavior in animals as evidenced by several early studies with red drum, as well as other species of fish, in which inadequate diets resulted in high degrees of cannibalism among cohorts (Brown et al. 1988).

Once adequate diets were identified, cannibalism was eliminated. Adequacy of diets for stressful rearing conditions has not been explored in fishes.

RELATED CURRENT AND PREVIOUS WORK

Practical Diet Development (Objective 1)

There have been several attempts to develop diets for trout that are free of fish meal. Those met with varying degrees of success. However, recent attempts have been more fruitful. Adelizi et al. (1996) identified two formulations that when fed to rainbow trout resulted in weight gains that were 82-85% of fish fed a positive control diet and feed conversion efficiencies that were 86-87% of fish fed the same control diet. Fish in those studies were fed to satiation and had a mean initial weight of 35 g. In the second study, additional modifications were made to one of the diets. Those included verification that astaxanthin masks the yellow pigmentation that develops when trout are fed processed corn products, identification that lysine is the first-limiting essential amino acid in all-plant diets, identification of a reduction in use of fish oil, and a second indication that trout fed all-plant diets are preferred by a trained taste panel. However, the all-plant diets developed at Purdue have only been fed to one strain of trout.

Smith et al. (1988) evaluated two dietary formulations fed to 10 strains of rainbow trout. No significant differences were detected between the various strains. However, the "primarily plant" diet used in that study contained 7.5% fish meal, 5.0% blood flour and a total of 10.7% crude protein supplied by animal sources. Thus, there has not been an evaluation of all-plant diets fed to various strains of rainbow trout. Further, only one strain evaluated in that study overlaps with this proposed evaluation; the Donaldson strain. A small degree of overlap with previous research efforts should be considered the best method in a continuing line of research.

Waste from aquaculture facilities is the focal point of several research groups around the world. In 1990, a Special Symposium, sponsored by the University of Guelph under the auspices of the International Union of Nutritional Sciences, was held in Canada. The focus of the Symposium was aquaculture wastes; specifically on improvements in diets that would diminish waste in effluents (Cho and Cowey 1991). A second symposium was held in 1994 (proceedings not published yet). At the World Aquaculture Society Annual Meeting in 1996, a three-day symposium on Sustainable Aquaculture Development was conducted. Many of the talks and much of the discussion centered on the interaction of nutrition and wastes.

The only organic input into aquaculture operations is feed. Uneaten food and metabolic wastes from ingested food are, therefore, the predominant source of P and N from facilities. This concept has been demonstrated with salmonids (Ketola 1985; Ketola and Harland 1993; Ketola and Richmond 1994) and led to a series of studies to improve waste effluents from trout production facilities in the NCR. The initial focus of those studies was on dietary P dynamics, specifically absorption or availability of P from dietary ingredients.

Supplementary enzymes have been used to increase digestibility of feeds and improve growth (Carter et al. 1994). Researchers at Michigan State University (MSU) demonstrated that pretreatment of soybean meal with the enzyme phytase increased the availability of phosphorus in salmonid diets (Cain and Garling 1995). Phytase also significantly increased availability of P when incorporated into diets as an ingredient (Riche and Brown 1996). Protein utilization of trout fed diets containing phytase-treated soybean meal was also enhanced. Phytates act as chelators to form protein-phytic acid complexes that can reduce the bioavailability of protein and minerals (NRC 1993). Work is currently underway at MSU to determine if growth differences between trout fed phytate treated and non-treated feeds resulted from increased protein or mineral availability.

The results of studies with cottonseed meal in catfish, salmonid and tilapia diets (Robinson and Brent 1989; Fowler 1980; Robinson and Li 1994) indicated that between 10 and 30% of solvent extracted, 40% crude protein cottonseed meal can be used in aquaculture diets without growth depression of fish. Cottonseed meals from the oilseed mill typically contain about 400 to 800 mg/kg free gossypol. In channel catfish raised at high densities in earthen ponds and fed a feed containing 51.25% cottonseed meal with supplemented lysine (0.65%), results have shown that growth rate, dressout percentage and chemical composition of fillet did not differ significantly from fish fed diet containing soybean meal (42%) (Robinson and Li 1994). Fowler

(1980) demonstrated that up to 34% cottonseed meal can be used in the diet for two Pacific salmon species to replace soybean meal without growth depression. Digestibility of solvent extracted and extruded cottonseed meal protein was not significantly different from similarly processed soybean meal in rainbow trout diets (Smith et al. 1995). Furthermore, when cottonseed meal was incorporated in rainbow trout diets at 15%, organoleptic tests showed no differences in flesh acceptability associated with diets, and all trout fillets were equally acceptable to taste panels (Smith et al. 1988).

Herman (1970) indicated that in rainbow trout, growth depression did not occur at gossypol concentrations lower than 290 mg/kg of diet, whereas histopathological changes in liver and kidney were noted at 95 mg/kg. Gossypol accumulated in trout liver when fish were fed 1000 mg of gossypol acetate/kg of diet for several months. However, free gossypol concentration was only 10.7 mg/kg of trout muscle after 18 months of feeding, and the level was undetectable after only five weeks on the gossypol devoid diet (Roehm et al. 1967).

Evaluate Water Temperature on the Growth/Stress Response (Objective 2)

Water temperature exerts pronounced effects on fish growth, both directly and indirectly. Because fish are poikilothermic, temperature directly governs metabolic rate and the energy requirements for metabolism, food consumption, and the efficiency of food conversion (Fry 1971). Water temperature can also have an indirect effect on growth when it evokes physiological stress responses that in turn impact a wide range of physiological functions (see below).

In many fish species, growth rate typically increases with increasing temperature, peaks at an optimum temperature range, and declines as temperature exceeds optimum (Brett 1979). For most species this optimum temperature range is relatively narrow, but can vary significantly between even closely related species or strains. For example, among coldwater salmonids, the optimum growth temperature has been reported to be 12.8°C for brown trout (*Salmo trutta*), 14.0°C for Arctic charr, 15.0°C for sockeye salmon (*Oncorhynchus nerka*), 15.5°C for coho salmon (*O. kisutch*), and from 15.0-17.0°C for rainbow trout (Brannon 1991; Klontz 1991).

The extent to which water temperature fluctuates can also have a significant effect on fish growth. In one study, rainbow trout held at an average of 17°C and exposed to daily temperature fluctuations of 4°C had lower growth rates than fish held at a constant 17°C (Hokanson et al. 1977), implying that even brief daily exposures to temperatures above or below optimum can be detrimental to growth. Similar negative effects of oscillating temperatures on growth in rainbow trout were reported by Wurtsbaugh (1973).

Temperatures either above or below the optimum range have direct effects on growth and performance. Temperatures below optimum directly reduce metabolic rate, daily food consumption and growth rate (Brett 1979). Temperatures above optimum increase the bioenergetic costs of routine metabolism, and are often coupled with poor food conversion and decreased feeding activity, ultimately resulting in decreased weight gain or even weight loss (Brett 1979).

In addition to the direct effects outlined above, temperatures significantly outside of the optimum range can have indirect effects on growth and performance through activation of physiological stress mechanisms (e.g., see Pickering 1981; Adams 1990; and Barton and Iwama 1991, for reviews). Performance changes known to be induced by stress include reduced growth and food conversion, and increased incidence of disease and mortality (see reviews cited above). The specific temperatures which elicit a stress response vary by species or strain in a manner similar to that of optimum temperature (Elliot 1981), and can also be affected by the temperature to which fish were previously acclimated (Strange et al. 1977; Strange 1980; Barton and Schreck 1987).

The physiological stress response of fish is mediated by the nervous and endocrine systems (reviewed in Pickering 1981). Wedemeyer and McLeay (1981) classified the stress responses of fish as follows: (1) "primary alterations," reflected by increased activity of the endocrine system, including release of adrenocorticotropic hormone from the pituitary, increased levels of circulating catecholamines and corticosteroid hormones (primarily cortisol); (2) "secondary alterations," characterized primarily by physiological responses such as increased plasma glucose and potassium titers, decreased plasma sodium and chloride, diuresis and impaired osmoregulation, reduced blood clotting time, leucopenia and decreased immunocompetence; and (3) "tertiary effects," referring to whole-animal and population responses such as reduced growth, increased incidence of disease and death.

Numerous investigations have been done on the physiological stress responses of fish, especially salmonids (e.g., Patino et al. 1986; Barton and Schreck 1987; Pickering and Pottinger 1987a,b; Kebus et al. 1992; Barry et al. 1993a). Both an actual stressor and the stress responses of fish can be categorized as acute (short-term) or chronic (long-term, Adams 1990). The instantaneous or acute stress level that a fish is experiencing has often been assessed by measuring baseline serum concentrations of cortisol, glucose and chloride (Schreck 1990). Wedemeyer and McLeay (1981, see also Wedemeyer et al. 1989) proposed that the overall (acute and chronic) stress load of fish can be evaluated by measuring changes in serum levels of cortisol, glucose and chloride following an acute stress challenge test. Investigators at the University of Wisconsin-Madison (UW-Madison) have used such procedures to evaluate various factors, including gas supersaturation, rearing density, and loading, as chronic stressors in trout (Kebus et al. 1992; Barry et al. 1993a, b).

Goede and Barton (1990) described an autopsy-based organismic assessment to measure the overall level of stress to which a fish has been exposed, and to determine “how well a fish is coping with its environment.” Some of the measures used in this health assessment index (HAI) include various body condition parameters (such as mesenteric fat), organ conditions (such as kidney and liver), and blood constituents (such as hematocrit). As part of a project funded by the Wisconsin Department of Agriculture, Trade and Consumer Protection, Dr. Myron Kebus (Wisconsin Aquatic Veterinary Service, Madison, Wisconsin, personal communication) has been successfully using an HAI to evaluate the overall rearing conditions and health and stress levels of rainbow trout at a number of commercial trout farms in Wisconsin. It is proposed that measurements of baseline serum levels of cortisol, glucose and chloride, changes in the serum levels of these compounds following acute stress challenge tests, and an HAI will be used to assess the acute and chronic stress loads of selected salmonid strains and species reared under different water temperature regimes found in the NCR.

Groundwater temperatures at many of the salmonid farms in the upper part of the NCR range from about 8-11°C. In the outdoor trout production ponds and raceways which utilize these waters, however, temperatures can vary significantly with season, dropping to 5-8°C in the winter and reaching 18-20°C in the summer. Under these conditions, Kamloops rainbow trout often do not realize their maximum growth potential. Kamloops strain rainbow trout have an optimum growth temperature of 15°C, and for each degree above or below the optimum temperature there is an 8.5% decrease in weight gain or growth (Klontz 1991). Many rainbow trout strains can also exhibit measurable signs of thermal stress at temperatures outside their normal range (Wedemeyer 1973; Hokanson et al. 1977).

The Arctic charr has several biological traits that may make it particularly well suited for commercial culture under the relatively cool environmental conditions that can be found in the NCR. First, the growth rates of Arctic charr are maximum at 14.0°C (Jobling 1983), lower than the optimum temperature of Kamloops rainbow trout. Second, the growth rate of Arctic charr at its optimum temperature is among the highest of several salmonid species (Jobling et al. 1993). At the optimum temperature for each species, the specific growth rate of Arctic charr was measured at 7.5%/day, compared to 6.9%/day for rainbow trout and 5.53% for coho salmon (Jobling 1983; Jobling et al. 1993). Third, some studies have shown that at 11.0-13.0°C, growth rates of Arctic charr are significantly better than those of several salmonids (Gjedrem and Gunnes 1987; Wandsvik and Jobling 1982; Heggberget et al. 1994). To date, however, nothing has been documented regarding the growth and performance traits of Arctic charr raised under conditions found in the NCR. Additionally, nothing is known about the stress responses of Arctic charr reared under these conditions.

Donaldson rainbow trout are used by fish farmers throughout the United States, and some believe that the Donaldson strain may perform better than Kamloops at relatively warm temperatures. For example, a Swedish strain (Alvdal) of Donaldson rainbow trout are being successfully reared at trout farms in Nebraska under conditions of high summer temperatures that may be lethal to other strains of rainbow trout (NCRAC 1994), suggesting that Donaldson trout may either have a higher optimum growth temperature or tolerate thermal stress better than Kamloops. The Donaldson rainbow trout reared in Nebraska also tolerate significant daily temperature fluctuations that would be detrimental to Kamloops (T. Kayes, University of Nebraska-Lincoln, personal communication). To our knowledge, however, little or no documented information is available on the growth, performance or stress responses of Donaldson rainbow trout reared under conditions found in the NCR. Research is needed to compare the thermal tolerance characteristics and potential variations in the stress responses and performance patterns of salmonid strains or species that are presently available to fish farmers in the region.

Diets/Fin Nipping Behavior (Objective 3)

Erosion of fins is a common occurrence in trout production facilities (Kindschi et al. 1991) and has been correlated with high rearing density (Bullock and Snieszko 1981; Bosakowski and Wagner 1994). Further, high rearing densities leads to agonistic behavior and fin nipping (Abbott and Dill 1985). Open wounds, regardless of source, can become infected and result in loss of fish and impairment of economic return. However, other than the survey conducted in Utah (Bosakowski and Wagner 1994), there have apparently been no formal evaluations of the prevalence or the perceived impact of the problem. The IAC has consistently ranked this as a priority area for investigation. Because of limited funds, the initial step in solving this problem will be a survey of salmonid producers in the NCR designed to gauge the severity and prevalence of the problem.

ANTICIPATED BENEFITS

Continual evaluations of all-plant diets will occur in Objective 1 and will continue providing alternative dietary formulae for producers of trout in the NCR. Those formulae, depending on ingredient prices, will benefit producers as fish meal prices inevitably change. As an example, the proposed research on Objective 1 will explore new diet formulations in rainbow trout where fish meal ($515/ton) will be replaced with soybean meal ($230/ton), cottonseed meal ($210/ton), meat-and-bone meal ($260/ton), and poultry by-product meal ($350/ton). Further, use of those formulae by various strains of trout will provide important information regarding the applicability of new feeds for the species and strains of interest in the region.

As changes away from fish meal are being made, effluent concerns must be addressed. The collaborative nature of this project and the past work conducted by the Work Group will ensure comprehensive evaluation of diets that can be used by producers in the region. The data generated in this portion of the proposal will provide producers with vital information regarding effluent concentrations that can be expected with various diets.

Studies under Objective 2 will provide detailed information on the growth and stress responses of Kamloops and Donaldson rainbow trout and Arctic charr reared under thermal conditions typically found in the NCR. Regional salmonid producers will be able to use this information to determine which of the three species/strains can be best utilized at their operation (i.e., under their specific thermal conditions) to maximize productivity and profitability.

The proposed survey under Objective 3 will determine the extent and severity of agonistic behavior in trout raised at high densities or loadings. This survey will help guide future objectives with salmonids and explore the causative factors in behavioral traits under conditions currently practiced in the NCR.

PROGRESS TO DATE

Two all-plant dietary formulae have been identified at Purdue University (Purdue) and modifications in the least expensive of those diets have been completed. That research built on over 50 years of previous nutritional research with trout and will continue in this project.

Researchers at Purdue established procedures for conducting P availability studies in diets fed to fish. Those studies focused on fecal collection methods (Brown 1993), evaluation of a new indicator of nutrient availability (Riche et al. 1995), quantification of P absorption (Riche and Brown 1996), and finally, evaluation of associative effects in diets (Brown and Riche 1996). This line of research began in 1990 and the results should facilitate rapid development of the data proposed in these studies.

Supplementary enzymes have been used to increase digestibility of feeds and improve growth (Carter et al. 1994). Researchers at MSU demonstrated that pretreatment of soybean meal with the enzyme phytase for salmonid diets increased the availability of phosphorus (Cain and Garling 1995). Protein utilization of trout fed diets containing phytase-treated soybean meal was also enhanced. Phytates act as chelators to form protein-phytic acid complexes that can reduce the bioavailability of protein and minerals (NRC 1993). Work is currently underway at MSU to determine if growth differences between trout fed phytate treated and non-treated feeds resulted from increased protein or mineral availability.

Objectives 2 and 3 of this proposal are new; thus, there is no progress to report.

OBJECTIVES

1. Develop and evaluate practical and economically viable diets that are fish-meal free or as fish-meal free as practical:
   - using soy, or other oil-seed products that are regionally available, and
   - using Shasta, Donaldson and Kamloop strains of rainbow trout and/or Arctic charr for the evaluation.

2. Evaluate the effects of water temperature on the growth/stress response in salmonid strains or species (as listed in Objective 1) under outdoor commercial culture conditions in the upper and lower portions of the NCR.

3. Investigate the effects of trace mineral supplementation on the growth and stress response of rainbow trout in high density culture, as evaluated by plasma cortisol levels and fin nipping behavior.

PROCEDURES

Practical Diet Development (Objective 1)

Purdue

Studies conducted at Purdue will be continuations of previous studies designed to develop and evaluate all-plant diets for rainbow trout. Specifically, the evaluation of the corn gluten meal/soybean meal-based diet identified in previous studies as promoting near maximal weight gain and feed conversion will continue, followed by evaluation of that revised diet in various strains of rainbow trout and, if possible, Arctic charr.

In the first year of the study, practical diets will be fed to triplicate groups of juvenile rainbow trout (initial weight 20-60 g, depending on availability). Ten separate diets will be developed based on results from recently completed studies. We anticipate the following diets. The lipid component has only partially been replaced with plant derived lipids; thus, we will explore flax seed as a source of n-3 fatty acids in place of fish meal, and evaluate the mineral supplementation necessary for complete use of plant protein feedstuffs for trout. There are numerous interactions between phytic acid and essential cations such as zinc, calcium, and magnesium (NRC 1980; Spinelli et al. 1983; Richardson et al. 1985). Lack of mineral availability might be inhibiting growth to the degree observed in previous studies. The remainder of the diets will systematically have additional zinc, magnesium, calcium and iron added to the diets as those are the most pressing concerns identified in animal nutrition (Erdman 1979). We anticipate two levels of each nutrient; one slightly below recommendations for nutritional adequacy and one slightly in excess (NRC 1993). Diets will be nutritionally complete, other than the nutrients in question, and meet the recommendations of Cho and Cowey (1991).

In the second year of the project, the new all-plant diets will be fed to Donaldson, Mt. Shasta and Kamloop strains of rainbow trout as well as Arctic charr, if available. Diets will be fed to quadruplicate groups of fish all housed in the same experimental system. All fish will be acquired from commercial suppliers in the NCR if available. It is likely that eggs or juveniles cannot be acquired at the same time. Thus, cold water temperatures will be used to restrict growth of those strains or species acquired early in the second year of the project, while those acquired later in the year will be raised at optimal temperatures. The goal is to start the study with similar size fish. Slow growth during selected periods should not adversely impact future growth in trout or charr. However, compensatory growth may lead to more rapid weight gain of those fish that experienced slowing of growth patterns. It is anticipated that all groups will be taken through a maximum feed intake and growth period at 14°C for three weeks prior to initiation of the study, which should diminish the impact of compensatory growth.

The experimental system that will be used in both years is a series of 120-L glass aquaria connected to a settling chamber and biological filter. The system can be operated either flow-through or recirculating. Water flow rate to each aquarium will be adjusted to between 3 and 5 L/min, depending on initial fish size, and temperature will be maintained at 14°C for both studies. Critical water quality parameters, such as dissolved oxygen, ammonia-N, nitrite-N and temperature will be monitored daily. Other water quality variables, such as pH, hardness and alkalinity, will be measured weekly.

All diets in both years of the project will be extruded by Wenger, Inc., Sabetha, Kansas. That group extruded diets in each of the last two years and provides complete information on extrusion conditions used.

At the end of each study, final numbers and weight of fish will be determined, then samples of fish collected for taste comparisons to be conducted at the USDA National Center for Agriculture Utilization Research in Peoria, Illinois. Additionally, fillet samples will be collected for proximate analysis using standard procedures (AOAC 1984). Liver samples will be collected for determination of hepatosomatic index, liver lipid concentrations (Folch et al. 1957) and samples submitted to the Animal Disease Diagnostic Laboratory for histological evaluation (Sheehan and Hrapchak 1980).

Both studies will be statistically analyzed as completely randomized designs using diet in the first year and strain/species in the second year as main effects. If analysis of variance (ANOVA) indicates significant differences among treatments, then Student Neuman Keuls will be used to separate mean values of weight gain, feed conversion, survival, hepatosomatic index, dressout percentage and proximate composition of fillets.

MSU

The studies conducted at MSU will overlap and expand on those conducted earlier with rainbow trout and concurrently with tilapia without direct duplication. Throughout all studies, the same genetic line of fish will be used. Initial fish size will be 50-150 g, representative of the grow out phase. Water quality will remain within acceptable tolerances and temperature will be 15°C. All studies will be conducted in experimental systems in place at MSU. The system contains 50 or 40 tanks of either 40 or 120 L capacity. Choice of tank size will be a function of initial size of fish. Density of fish used in all studies will be those found acceptable in previous studies. All experimental systems can be operated flow-through or recirculating. It is anticipated that operation of the system will be in the flow-through mode.

Tanks will be randomly assigned experimental or reference diets. Diet treatments will be: (1) negative reference (starvation), (2) positive reference (fish meal based), (3) reference diet (commercial feed provided by Zeigler Brothers, Inc.), (4) soybean meal substituted-untreated diet, or (5) soybean meal substituted-pretreated with phytase (Natuphos® provided by BASF). Soybean meal substitution will depend on EAA profile of the final treated product at a protein level at or slightly below the level for optimal growth. Feeds will be isocaloric with sufficient energy for protein synthesis. Each diet will be fed to three replicates per treatment for a grow out period of a minimum of 4-6 weeks. Fish will be weighed every two weeks and feeding rates will be adjusted accordingly.

Effluent P and N concentrations will be estimated using a modified mass balance equations as describe by Cain and Garling (1995). Growth, feed conversion (weight of feed fed/fish weight gain), and whole body analysis (APHA et al. 1989) will be determined and analyzed using appropriate statistical models.

At weeks 2, 4, 6 and at the conclusion of the feeding trial, fish will be randomly selected for blood and tissue samples. Blood will be collected via caudal puncture. Fish will be dissected to collect liver and muscle tissue samples. The hepatosomatic index will be calculated. All samples will be frozen in liquid nitrogen and stored at -80°C until subsequent analysis to determine the impact of phytase treatments on protein utilization. It is proposed that Insulin-like Growth Factors (IGF), IGFBP-3 (Insulin-like Growth Factor Binding Protein-3), and RNA/mRNA will be used as indicators of nitrogen balance and protein accretion.

There have been very few reports on the effects of an alteration in nutritional status on tissue protein metabolism in fish (McMillan and Houlihan 1992). Cellular growth response in fish is different than in mammals. Trout have been shown to exhibit indeterminate growth and exhibit both hypertrophic and hyperplastic growth throughout life (Matty and Lone 1985). Elevated growth hormone leads to better feed conversion ratios (Jobling 1994) and increases RNA:DNA ratio indicating enhanced efficiency of protein synthesis through its effects of IGFs (Jobling 1994).

IGF is produced in the liver in response to growth hormone (Brier et al. 1986; Baxter 1988; Nam et al. 1990) and mediates the growth effects associated with growth hormone. In higher vertebrates, plasma/serum concentrations of IGF increase during periods of good nutrition. The form IGF-I enhances amino acid uptake into cells *in vitro*, increases anabolic activity on skeletal muscle, increases protein synthesis and decreases protein degradation *in vitro* (Magi et al. 1991), and decreases circulating levels of amino acids by reducing protein breakdown as opposed to stimulating protein synthesis (Jacob et al. 1989). Urinary urea-N excretion decreased after administration of IGF-I to humans (Hizuka et al. 1991) which implies better N retention. Increases in IGF-I in humans is highly correlated with increased N retention (Elasser et al. 1989).

Concentration of total and free IGF-I in serum will be measured by radioimmunoassay (RIA). Free IGF-I will be measured without removal of IGFBP and total IGF-I will be measured after removal of IGFBP by formic acid -ethanol extraction (Sharma et al. 1994). Rabbit anti-human IGF-I will be used as the antibody, and iodinated recombinant human IGF-I as the tracer (Bristow et al. 1990). Relative concentrations of IGFBP in serum will be examined using western blotting (Kelly et al. 1992; Sharma et al. 1994). Relative concentrations of various IGFBPs will be determined by densitometry after separation on SDS-gel (Sharma et al. 1994). Researchers at MSU have demonstrated that these methods work for determination of rainbow trout IGF-I. Other assays specifically developed for salmonids may be used to increase assay sensitivity such as protein binding assay specific for rainbow trout (Niu et al. 1993) or use of recombinant salmon or trout IGF-I.

Total RNA from muscle and liver tissues will be prepared by the method of VandeHaar et al. (1995). Specific mRNA will be determined by northern blot after hybridization to ^{32}P labeled human IGF-I cDNA (Bell et al. 1984). Concentrations of total and free IGF-I in serum and total RNA from muscle and liver tissues will be compared between fishes fed reference and phytase treated diets.

<u>Ohio State University</u>

At Ohio State University (OSU), practical diets will be formulated based on composition already tested with rainbow trout and channel catfish (Dabrowski et al. 1989; Robinson and Tiersch 1995). The diets will be mixed and steam pelleted at the Ohio State University Feed Meal, Wooster, Ohio. In order to determine the suitability of soybean meal, a cottonseed meal and animal by-product mixture will replace 0, 25, 50 and 75% of the total dietary protein supplied by fish meal. The diet with the highest replacement should contain no more than 450 mg gossypol/kg, a limit set by the U.S. Food and Drug Administration for both animal feed and human diet. In all diets containing the alternative protein source, the required profile of EAA (lysine, methionine and arginine found limiting) will be supplemented with crystalline forms.

Six hundred rainbow trout of the Mt. Shasta strain (spring spawners) will be used in the first experiment, all 1 year old fish. Fifteen experimental groups will be fed experimental diets for 6-8 months to obtain fish of approximately 0.5 kg. Each diet will be assigned to three tanks (250 L volume) of randomly distributed fish. Fish will be weighed at bi-monthly intervals and their feeding rate adjusted to current biomass. At the Piketon Research and Extension Center, well water is supplied and temperature increases during the growing season from 10 to 15°C. At the completion of growth study, fish will be weighed, 10 fish per dietary treatment will be killed and their fillet used for further analysis.

The second experiment (second year of study) will be conducted with the same diets (stored in a freezer at -20°C) using the Donaldson strain (fall spawners). The utilization of dietary protein for fish growth will be assessed by comparing the mean weight gain, growth rate (%/day) gross feed conversion ratio, protein utilization based on proximate analysis of feeds and fish bodies.

Evaluate Water Temperature on the Growth/Stress Response (Objective 2)

The principal hypothesis for Objective 2 is that Kamloops and Donaldson rainbow trout and Arctic charr will exhibit differential stress and performance responses (including growth, feed conversion, condition, and survival) under outdoor commercial culture conditions typically found in the NCR. The present plans are to conduct one "summer" and one "winter" experiment at Rushing Waters Fisheries, Inc., Palmyra, Wisconsin. This facility has water temperatures which are above optimum for Kamloops trout in summer and below their optimum in winter. Accordingly, these studies will provide information relevant to the broad range of environmental conditions found throughout the region. Rushing Waters is a leading supplier of market-sized rainbow trout in the NCR, and also has two years of experience with Arctic charr production, making this hatchery particularly well suited for the present study.

The specific studies outlined under this objective were prepared in collaboration with Mr. William Johnson, former vice-president and manager of Rushing Waters. Just prior to the submission of this proposal, however, a management change occurred at Rushing Waters, and Mr. Johnson is no longer with the company. The current production manager at Rushing Waters, Mr. David Meuller, has indicated that he would like to continue with the studies as scheduled.

In the event that Rushing Waters is not able to participate in these studies, the following back-up arrangements have been made. Freshwater Farms of Ohio, Inc., Urbana, Ohio, a trout producer that has summer water temperatures similar to those found at Rushing Waters, has agreed to conduct a summer study similar to the Rushing Waters study described below. Bullfrog Fish Farm, Menomonie, Wisconsin, a trout producer that has winter water temperatures similar to those found at Rushing Waters, has agreed to conduct a winter study similar to the Rushing Waters study. As an alternative back-up, both a summer and winter experiment can be conducted at the campus facilities of UW-Madison Aquaculture Program, where water of the appropriate temperatures is available year-round to simulate the summer and winter conditions of regional trout farms. If any of these back-up procedures are implemented, the environmental conditions and experimental designs described below for Rushing Waters will be matched as closely as possible, and all other parameters, such as fish densities, loadings, etc., will be within the normal ranges used by regional salmonid aquaculturists.

Rushing Waters will supply the fish and rearing facilities needed for the proposed study. Personnel from UW-Madison, in collaboration with Rushing Waters staff, will be responsible for implementing the experimental protocols and conducting the required analytical work.

Kamloops strain rainbow trout eggs will be obtained from Troutlodge Inc., Sumner, Washington, Donaldson eggs or fingerlings from Crystal Lake Fisheries, Ava, Missouri, and Arctic charr eggs from Hidden Valley Charr Ltd., Prince Edward Island, Canada (or other producers of these species, depending on availability). Eggs will be hatched at Rushing Waters, and fry or fingerlings will be raised in indoor raceways supplied with flow-through water until they reach a total length of 7.5-12.7 cm. At this time they will be stocked into outdoor grow-out raceways at a rate of 4,000-5,000 fingerlings/raceway. The fish will be fed a standard trout grow-out diet (Silver Cup, Nelson and Sons, Inc., Murray, Utah) using demand feeders.

Rushing Waters has a total of fifty 5×30 m earthen grow-out raceways connected in two linear series. Artesian wells provide the farm with constant 10°C groundwater, the quantity of which varies by season (see below). Two 8-9 month experiments will be conducted, one encompassing a summer production season (March through November) and the other a winter season (September through May). At the beginning of the summer experiment, fingerlings from the Donaldson and Kamloops strain will be stocked into two raceways per strain at the head of the raceway series, where water temperatures remain relatively close to 10°C, and two raceways per strain near the rear of the raceway series, where water temperatures fluctuate above 10°C during the summer, reaching as high as 18-20°C. At the beginning of the winter experiment, Arctic charr and Kamloops rainbow trout will be stocked into two raceways per strain at the head of the raceway series, where water temperatures remain relatively close to 10°C, and into two raceways per strain near the rear of the raceway series, where water temperatures fluctuate during the winter and reach as low as 5°C. After the fingerlings are stocked they will be raised to market size under standardized conditions.

Throughout the grow-out periods, performance parameters including growth, feed conversion, incidence of disease and mortality will be accurately monitored and recorded. Water quality parameters such as temperature, dissolved oxygen, and pH will be measured frequently at both the beginning and end of the raceway series. Other water quality parameters, such as ammonia, nitrite and water hardness will be measured periodically.

Physiological stress measures will be taken three times during each experiment, at approximately the beginning, middle, and end of each production season. This sampling schedule will allow an evaluation of stress levels under several water temperature/flow rate combinations. For example, during the course of the summer production season, water temperatures in the spring and autumn are normally moderate with high flow rates, whereas water temperatures during mid-summer become more extreme, in conjunction with lower flow rates.

Physiological stress levels will be evaluated as follows. First, baseline serum concentrations of serum cortisol, glucose and chloride will be measured by gently crowding the fish in a seine, quickly removing a sample of at least six fish with a dip net, and immediately anesthetizing them in 50 mg/L unbuffered tricaine methanesulfonate (MS-222). The fish will be bled via the caudal vasculature within 8 min of being placed in the anesthetic, a time period that has been shown to be insufficient to allow for changes in levels of cortisol, glucose or chloride (unpublished data). Second, a group of fish will be quickly netted from the seine and given an acute stress challenge test by holding out them of the water for 1 min. These fish will then be randomly placed into separate holding pens for each sampling point, and then netted, anesthetized and bled at 1, 3, 6 or 24 hr following the stressor. It is understood that moving the fish to somewhat different conditions (into net-pens) after the challenge test may constitute an additional source of stress, it is the most practical experimental design available under the constraints of commercial culture conditions. The blood will be allowed to clot for at least 1 hr at 4°C, and centrifuged at 4000× g for 15 min. The serum will be stored at -35°C until assayed. Third, an autopsy-based HAI similar to that originally developed by Goede and Barton (1990) will be used. The HAI has proven to be a rapid, simple and inexpensive means of assessing fish health in field situations. In this study the HAI will be performed by Dr. Myron Kebus of the Wisconsin Aquatic Veterinary Service. Dr. Kebus, a licensed veterinarian specializing in aquatic animal health, recently received a development grant from the Wisconsin Department of Agriculture, Trade and Consumer Protection to use an HAI to evaluate the health and stress levels of fish at several commercial Wisconsin fish producers. In the proposed study, 20 fish of each strain will be obtained from each raceway at each of the three sampling times, and the fish will be necropsied at Rushing Waters. Parameters to be measured will include body condition (such as mesenteric fat, gonad maturity, condition factor and extremity damage), organ condition (such as eyes, gills, kidney and liver), and blood constituents (such as hematocrit, cortisol, glucose and plasma protein). These parameters are then ranked on a semi-quantitative scale from normal (healthy) to severely abnormal.

Techniques for measuring serum cortisol, glucose, and chloride in salmonids have been validated and are regularly used by UW-Madison investigators (Barry et al. 1993a). Cortisol will be measured using a microplate enzyme-linked immunosorbant assay (ELISA). Glucose will be measured using a standard enzymatic procedure (glucose oxidase, Sigma diagnostic kit 510-DA, St. Louis, Missouri), and chloride using a Corning model 925 chloride analyzer (Ciba-Corning, Medford, Massachusetts).

The UW-Madison will be responsible for all data collection and analysis. The data from each of the two experiments will be analyzed using a 2×2 factorial ANOVA with the factors being species (or strain) and temperature condition (position in raceway series). Research findings will be published in a timely manner in appropriate peer-reviewed scientific journals. Extension information outlining the practical implications and benefits of the research will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

Diets/Fin Nipping Behavior (Objective 3)

Fin nipping/erosion has been identified by the NCRAC IAC as a problem occurring on trout farms in the NCR. MSU will design and administer an outreach survey to determine the extent of the problem and identify factors which may be related to the causes of the problem. A survey will be developed that asks trout farmers to rank the severity of fin nipping (extent of fin nipping and impact on product value) at their farm for each of the trout species they rear and for three size classes. They will also be asked to characterize their water supply, rearing conditions (maximum rearing density (kg/m), and loading (kg/Lpm) and feeds management (feed source, type, regimes) for each of the size classes and species raised.

The questionnaire will be limited to one page, front and back. Replies will be facilitated by developing questions that ask the participant to check the appropriate boxes. A cover letter will describe the purpose of the survey and explain the return procedure to ensure the anonymity of the participants. Two envelopes will be included in the mailing; a slightly larger, numbered, return addressed envelope to identify respondents and a smaller blank envelope for the questionnaires. Upon receipt, the envelopes will be separated and the return envelop will only be used to remove the participant's name from follow up mailings and phone calls. Respondents who would like to receive copies of the survey analysis will be asked to enclose a business card in the outer return envelope.

The cover letter and questionnaire will be reviewed by extension specialists with experience in trout production or survey technique. The cover letter and questionnaire will be pretested by a preliminary mailing to Michigan public and private trout producers who will be asked to complete the form and make suggestions for improving the questions.

The questionnaires will be mailed during the winter of 1998 to up to 120 survey participants. Participants will be identified by asking state extension contacts to provide the names and addresses of the public sector and five private sector aquaculturists in their state that produce trout. Participants will be asked to respond within 30 days. After the initial mailing, non-respondents will be sent reminder postcard after 45 days and a phone call after 75 days.

Data will be tabulated and evaluated to determine the extent of the problem of fin nipping/erosion in the NCR and its relationship to limnological and cultural practices at affected farms. The results can be used to prioritize future research directions.

FACILITIES

<u>Purdue</u>

Purdue has a 715 m^2 wet laboratory fully equipped to conduct the proposed research. Five experimental aquaria systems are in place and functional. A dedicated well, equipped with emergency generator, supplies water to the building. There are 36 water outlets in the building each equipped with temperature control. Water quality test kits and dissolved oxygen meters are in place. Water quality in each experimental system and each holding tank are checked daily. The chemistry laboratory contains, among other things, an analytical microwave for wet digestion of samples and Shimadzu double-beam spectrophotometer, liquid chromatography system, and scintillation counter. Kjejdahl equipment is maintained on campus in departmental laboratories. A Perkin-Elmer atomic absorption spectrophotometer is also maintained by the Department of Forestry and Natural Resources.

<u>MSU</u>

MSU has the wet laboratory fully equipped to conduct the growout studies. Tissue preparation, RIA, and specific mRNA determinations by northern blot after hybridization to ^{32}P labeled human IGF-I cDNA will be carried out in the laboratory of and in consultation with Dr. VandeHaar, Department of Animal Science, MSU.

<u>OSU</u>

At OSU, Konrad Dabrowski's wet laboratory in Kottman Hall (167 m^2) is equipped with fish rearing tanks, fish egg incubation apparatus and acclimation chambers. This laboratory includes features for water temperature-control and sterilization systems. The biochemical laboratory in Kottman Hall includes a biofreezer (-85°C), refrigerated centrifuge, freeze-drier, drying ovens, spectrophotometer DU-70, Beckman HPLC system, Varian 3400 gas chromatography system, and other accessories for biochemical research studies. Facilities at Piketon Research and Extension Center include 14 ponds, and an aquaculture building equipped with several fish tanks and a recirculation system, and temperature and light control rooms. The main building of the field station contains aquaculture, chemical, and biological laboratories.

UW-Madison

Studies will be conducted jointly at Rushing Waters Fisheries, Inc., Palmyra, Wisconsin, and the UW-Madison Aquaculture Program's research facilities on the UW-Madison campus. Rushing Waters is one of Wisconsin's largest trout farms, and has committed 4, 5×30 m earthen raceways for the proposed project (see **RELATED AND CURRENT AND PREVIOUS WORK** for additional details). The UW-Madison campus facility has a wet laboratory with numerous circular fiberglass tanks (110-L to 3,000-L) and ample supplies of carbon-filtered water which can be maintained at 6-20±1°C by water heaters or chillers. The UW-Madison analytical laboratory is equipped with a high speed centrifuge, micro-ELISA plate reader, chloride analyzer, microbalance, several compound and dissecting microscopes, and other equipment required for research on fish stress physiology and endocrinology, including regular access to a liquid scintillation counter, and a computer-controlled HPLC system. Gamma counters and other analytical equipment are available through the UW Endocrinology-Reproductive Physiology Program. The Wisconsin Aquatic Veterinary Service owns or has access to all diagnostic equipment necessary to perform the HAI.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Indiana	Paul B. Brown Purdue University	Aquaculture/Nutrition
Michigan	Donald L. Garling Michigan State University	Aquaculture/Nutrition/Extension
Ohio	Konrad Dabrowski Ohio State University	Aquaculture/Nutrition
Wisconsin	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture/Endocrinology/Phys- iology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Purdue University (Purdue)
Paul B. Brown

Michigan State University (MSU)
Donald L. Garling

Ohio State University (OSU)
Konrad Dabrowski

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

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

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BUDGET

ORGANIZATION AND ADDRESS Purdue Research Foundation Office of Sponsored Programs West Lafayette, IN 47907-1021				**USDA AWARD NO.** Year 1: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul B. Brown				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	6			$10,067	
c. ___ Graduate Students					
d. 1 Prebaccalaureate Students				$1,500	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$11,567	
B. Fringe Benefits (If charged as Direct Costs)				$4,897	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$16,464	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,500	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$19,964	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$19,964	
M. Other →					
N. Total Amount of This Request →				$19,964	$
O. Cost Sharing (If Required Provide Details)	$26,500				

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

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

Form CSREES-55 (6/95)

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

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BUDGET

ORGANIZATION AND ADDRESS Purdue Research Foundation Office of Sponsored Programs West Lafayette, IN 47907-1021				**USDA AWARD NO.** Year 2: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul B. Brown				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	6			$10,469	
c. ___ Graduate Students					
d. 1 Prebaccalaureate Students				$1,500	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$11,969	
B. Fringe Benefits (If charged as Direct Costs)				$5,087	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$17,056	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,980	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) →				$20,036	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$20,036	
M. Other →					
N. Total Amount of This Request →				$20,036	$
O. Cost Sharing (If Required Provide Details)	$27,000				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR PURDUE UNIVERSITY

(Brown)

Objective 1

A. Salaries and Wages. A technician (0.50 FTE) is required for acquisition of fish, coordination of diet manufacturing, feeding fish, water quality monitoring and analytical work. A prebaccalaureate student is required for supplementing these activities as fish will be fed seven days per week.

B. Fringe Benefits. Standard fringe benefit rate is 47.41% for technicians and 8.3% for prebaccalaureate students.

E. Materials and Supplies. These funds will be used for acquisition of feedstuffs and diet manufacturing. Additionally, these funds will be used for routine maintenance of experimental and holding systems.

F. Travel. These funds will be used for acquisition of feedstuffs and dissemination of research results at international aquaculture meetings. Costs will include transportation, lodging, and meals.

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COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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BUDGET

ORGANIZATION AND ADDRESS Department of Fisheries and Wildlife Michigan State University East Lansing, MI 48824-1222				**USDA AWARD NO.** Year 1: Objectives 1 and 3	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Donald L. Garling				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer		$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$12,650	
d. ___ Prebaccalaureate Students					
e. 1 Secretarial-Clerical				$1,337	
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$13,987	
B. Fringe Benefits (If charged as Direct Costs)				$1,055	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$15,042	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,897	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Survey costs ($500), IGF analyses ($2,000)				$2,500	
J. Total Direct Costs (C through I) →				$20,439	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$20,439	
M. Other →					
N. Total Amount of This Request →				$20,439	$
O. Cost Sharing (If Required Provide Details)	$20,740				
NOTE: Signatures required only for Revised Budget				This is Revision No. →	

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

Form CSREES-55 (6/95)

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

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BUDGET

ORGANIZATION AND ADDRESS Department of Fisheries and Wildlife Michigan State University East Lansing, MI 48824-1222				**USDA AWARD NO.** Year 2: Objectives 1 and 3	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Donald L. Garling				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				
1. No. of Senior Personnel	Calendar	Academic	Summer		$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$13,936	
d. ___ Prebaccalaureate Students					
e. 1 Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$13,936	
B. Fringe Benefits (If charged as Direct Costs)				$625	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$14,561	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,000	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) IGF analyses ($2,000)				$2,000	
J. Total Direct Costs (C through I) →				$19,561	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$19,561	
M. Other →					
N. Total Amount of This Request →				$19,561	$
O. Cost Sharing (If Required Provide Details)	$14,195				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR MICHIGAN STATE UNIVERSITY

(Garling)

Objectives 1 and 3

A. **Salaries and Wages.** Laboratory studies will be conducted with the assistance of a graduate student. Responsibilities of the student will include; diet preparation and analysis, general fish culture, and laboratory IGF-1 experiments. The graduate student will participate in Objective 3 (two months during Year 1). Secretarial/clerical assistance has been requested for preparation and mailing of the questionnaire for Objective 3.

B. **Fringe Benefits.** MSU requires medical coverage for graduate students estimated to be $600 for Year 1 and $625 for Year 2. Fringe benefits for secretarial assistance at MSU is 34% of salary.

E. **Materials and Supplies.** Fish ($1,000), feed ingredients ($2,000), and chemicals ($897) will be required to complete Objective 1.

F. **Travel.** Domestic travel will be required to obtain fish for Objective 1. Costs will include transportation, lodging, and meals.

I. **All Other Direct Costs.** Survey costs to administer the questionnaire for Objective 3 ($500) and laboratory assistance from the MSU Animal Science Department to complete the IGF (Insulin-like Growth Factor) analyses ($2,000/year).

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

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BUDGET

ORGANIZATION AND ADDRESS Ohio State University Research Foundation 1960 Kenny Road Columbus, OH 43210-1063				**USDA AWARD NO.** Year 1: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Konrad Dabrowski				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$11,332	
d. 1 Prebaccalaureate Students				$2,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . →				$13,332	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$13,332	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$5,233	
F. Travel 1. Domestic (Including Canada)				$785	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($450), Fax ($200)				$650	
J. Total Direct Costs (C through I) . . . . →				$20,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$20,000	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$20,000	$
O. Cost Sharing (If Required Provide Details)	$21,000				
NOTE: Signatures required only for Revised Budget				This is Revision No. →	

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

Form CSREES-55 (6/95)

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

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BUDGET

ORGANIZATION AND ADDRESS Ohio State University Research Foundation 1960 Kenny Road Columbus, OH 43210-1063	**USDA AWARD NO.** Year 2: Objective 1	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Konrad Dabrowski	Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)

Item	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED by PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages					$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$11,332	
d. 1 Prebaccalaureate Students				$2,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . →				$13,332	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$13,332	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$5,223	
F. Travel 1. Domestic (Including Canada)				$795	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Telephone ($450), Fax ($200)				$650	
J. Total Direct Costs (C through I) . . . . →				$20,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$20,000	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$20,000	$
O. Cost Sharing (If Required Provide Details)	$22,900				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR OHIO STATE UNIVERSITY

(Dabrowski)

Objective 1

A. **Salaries and Wages.** Field and laboratory studies will be conducted by a postdoctoral fellow. His tasks include sampling initial preparation of experimental diets and their analysis. Approximately half of the labor in tank experiments will be supported by monies from the Piketon Center.

   Additional responsibilities of a postdoctoral fellow will include: diet preparation and analysis, preparation of daily, weekly and monthly tables and graphs of field and laboratory experiments schedule.

E. **Materials and Supplies.** First year general laboratory and field supplies will include: reagents, glassware, diet ingredients, commercial feeds and replacement parts for laboratory equipment (homogenizers, spectrophotometer).

F. **Travel.** These funds will support transportation, meals and, if necessary, lodging for the collection of samples in Piketon (round trip distance 160 miles). Travel funds will also be used to attend the annual Work Group meetings and the NCRAC conference to present initial results. Costs will include transportation, lodging, and meals.

I. **All Other Direct Costs.** Annual costs: telephone ($450) and fax ($200).

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Board of Regents, University of Wisconsin System 750 University Avenue Madison, WI 53706	**USDA AWARD NO.** Year 1: Objective 2	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Jeffrey A. Malison	Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: ________ **FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS				
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer		$
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	2.6			$6,000	
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$5,000	
Total Salaries and Wages . . . . →				$11,000	
B. Fringe Benefits (If charged as Direct Costs)				$3,465	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$14,465	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$4,000	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Fax ($100), Postage ($200), Equipment repair ($235)				$535	
J. Total Direct Costs (C through I) . . . . →				$20,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$20,000	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$20,000	$
O. Cost Sharing (If Required Provide Details)	$24,400				

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

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

Form CSREES-55 (6/95)

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Board of Regents, University of Wisconsin System 750 University Avenue Madison, WI 53706	**USDA AWARD NO.** Year 2: Objective 2	
	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Jeffrey A. Malison	**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS				
A. Salaries and Wages	Calendar	Academic	Summer		$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. 1 Other Professional	2.6			$6,000	
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$5,000	
Total Salaries and Wages . . . . →				$11,000	
B. Fringe Benefits (If charged as Direct Costs)				$3,465	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$14,465	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$4,000	
F. Travel 1. Domestic (Including Canada)				$1,000	
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) Fax ($100), Postage ($200), Equipment repair ($235)				$535	
J. Total Direct Costs (C through I) . . . . →				$20,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$20,000	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$20,000	$
O. Cost Sharing (If Required Provide Details)	$25,050				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison)

Objective 2

A. **Salaries and Wages.** Technical help is needed to assist the PI with the conduct of experiments including the collection of biological samples, the conduct of stress tests, the analysis of physiological stress indicators, and analysis and publication of results.

B. **Fringe Benefits.** The fringe benefit rate for professional/technical positions is 31.5%.

E. **Materials and Supplies.** Biochemicals, reagents and laboratory supplies are needed to conduct analyses of plasma cortisol, glucose, chloride and other stress measures.

F. **Travel.** Approximately 35% of the travel budget requested will be needed for six trips to Rushing Waters Trout Fisheries, Inc., Palmyra, Wisconsin. The remainder of the travel budget will be used to attend NCRAC Salmonid Work Group meetings. Costs will include transportation, lodging, and meals.

I. **All Other Direct Costs.** Annual costs: fax ($100), postage ($200), and equipment repair ($235).

IMPROVING SALMONID AQUACULTURE IN THE NORTH CENTRAL REGION

Budget Summary for Each Participating Institution for the First Year

	Purdue	MSU	OSU	UW-Mad.	TOTALS
Salaries and Wages	$11,567	$13,987	$13,332	$11,000	$49,886
Fringe Benefits	$4,897	$1,055	$0	$3,465	$9,417
Total Salaries, Wages, and Fringe Benefits	$16,464	$15,042	$13,332	$14,465	$59,303
Nonexpendable Equipment	$0	$0	$0	$0	$0
Materials and Supplies	$2,500	$1,897	$5,233	$4,000	$13,630
Travel	$1,000	$1,000	$785	$1,000	$3,785
All Other Direct Costs	$0	$2,500	$650	$535	$3,685
TOTAL PROJECT COSTS	**$19,964**	**$20,439**	**$20,000**	**$20,000**	**$80,403**

Budget Summary for Each Participating Institution for the Second Year

	Purdue	MSU	OSU	UW-Mad.	TOTALS
Salaries and Wages	$11,969	$13,936	$13,332	$11,000	$50,237
Fringe Benefits	$5,087	$625	$0	$3,465	$9,177
Total Salaries, Wages and Fringe Benefits	$17,056	$14,561	$13,332	$14,465	$59,414
Nonexpendable Equipment	$0	$0	$0	$0	$0
Materials and Supplies	$1,980	$2,000	$5,223	$4,000	$13,203
Travel	$1,000	$1,000	$795	$1,000	$3,795
All Other Direct Costs	$0	$2,000	$650	$535	$3,185
TOTAL PROJECT COSTS	**$20,036**	**$19,561**	**$20,000**	**$20,000**	**$79,597**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

State/Institution			Year 1	Year 2
Purdue University				
Salaries and Benefits:	SY @ 0.10 FTE		$6,500	$7,000
Supplies, Expenses, Equipment, and Waiver of Overhead			$20,000	$20,000
		Total	$26,500	$27,000
Michigan State University				
Salaries and Benefits:	SY @ 0.10 FTE		$8,715	$8,975
Supplies, Expenses, Equipment, and Waiver of Overhead			$12,025	$5,220
		Total	$20,740	$14,195
Ohio State University				
Salaries and Benefits:	SY @ 0.05 FTE		$6,000	$6,400
Supplies, Expenses, Equipment, and Waiver of Overhead			$15,000	$16,500
		Total	$21,000	$22,900
University of Wisconsin-Madison				
Salaries and Benefits:	SY @ 0.08 FTE and TY @ 0.08 FTE		$6,400	$6,550
Supplies, Expenses, Equipment, and Waiver of Overhead			$18,000	$18,500
		Total	$24,400	$25,050
		Total per Year	$92,640	$89,145
		GRAND TOTAL	$181,785	

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

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

LIST OF PRINCIPAL INVESTIGATORS

Paul B. Brown, Purdue University

Donald L. Garling, Michigan State University

Konrad Dabrowski, Ohio State University

Jeffrey A. Malison, University of Wisconsin-Madison

VITA

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EDUCATION

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

POSITIONS

Professor (1997-present), Associate Professor (1993-1997), and Assistant Professor (1989-1993), Department of Forestry and Natural Resources, Purdue University
Assistant Professional Scientist/Field Station Director (1987-1989), Illinois Natural History Survey
Adjunct Assistant Professor (1988-1989), University of Illinois, Department of Animal Sciences
Research Associate (1986-1987), Texas A&M University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Institute of Nutrition
American Oil Chemists' Society
International Association of Astacology
Society for Comparative Nutrition
Society for Integrative and Comparative Biology (formally, American Society of Zoologists)
World Aquaculture Society
Gamma Sigma Delta, Sigma Xi

SELECTED PUBLICATIONS

Riche, M., and P.B. Brown. 1996. Absorption of phosphorus from feedstuffs fed to rainbow trout. Aquaculture 142:269-282.

Riche, M., M.R. White, and P.B. Brown. 1995. Barium carbonate as an alternative indicator to chromic oxide for use in digestibility experiments with rainbow trout. Nutrition Research 15:1323-1331.

Brown, P.B. 1995. A review of nutritional research with crayfish. Journal of Shellfish Research 14:20-28.

Brown, P.B. 1995. Using whole-body amino acid patterns and quantitative requirements to rapidly develop diets for new species such as striped bass (*Morone saxatilis*). Journal of Applied Ichthyology 11:342-346.

Griffin, M.E., K.A. Wilson, and P.B. Brown. 1994. Dietary arginine requirement of juvenile hybrid striped bass. Journal of Nutrition 124:888-893.

Griffin, M.E., K.A. Wilson, M.R. White, and P.B. Brown. 1994. Dietary choline requirement of juvenile hybrid striped bass. Journal of Nutrition 124:1685-1689.

Wu, Y.V., R. Rosati, D.J. Sessa, and P. Brown. 1994. Utilization of protein-rich ethanol co-products from corn in tilapia feed. Journal of the American Oil Chemists Society 71:1041-1043.

VITA

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EDUCATION

M.S. Agriculture and Technical University, Olsztyn, Poland, 1972
Ph.D. Agriculture and Technical University, Olsztyn, Poland, 1976
D.Sc. Agricultural University, Szczecin, Poland, 1984

POSITIONS

Professor (1989-present), School of Natural Resources, Ohio State University
Visiting Professor (1987-1989), University of Innsbruck, Innsbruck, Austria
Visiting Professor (1984-1985), Tokyo University of Fisheries, Tokyo, Japan
Associate Professor (1972-1987), Agriculture and Technical University, Olsztyn, Poland

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Editorial Board Member for Aquaculture and Aquatic Living Resources
Fisheries Society of British Isles
Japanese Fisheries Society
National Research Council, Washington, Subcommittee on Fish Nutrition (1990-1992)
World Aquaculture Society

SELECTED PUBLICATIONS

Matusiewicz, M., and K. Dabrowski. 1996. Utilization of the bone/liver alkaline phosphatase activity ratio in blood plasma as an indicator of ascorbate deficiency in salmonid fish. Proceedings of the Society for Experimental Biology and Medicine 212:44-51.

Matusiewicz, M., K. Dabrowski, L. Volker, and K. Matusiewicz. 1995. Ascorbate polyphosphate is a bioavailable vitamin C source in juvenile rainbow trout: tissue saturation and compartmentalization model. Journal of Nutrition 125:3055-3061.

Cierszko, A., and K. Dabrowski. 1994. Some biochemical constituents of fish semen: relationship between semen quality and fertility changes. Fish Physiology and Biochemistry 12:357-367.

Matusiewicz, M., K. Dabrowski, L. Volker. and K. Matusiewicz. 1994. Regulation of saturation and depletion of ascorbic acid in rainbow trout. Journal of Nutritional Biochemistry 5:204-212.

Dabrowski, K., A. Ciereszko, L. Ramseyer, D. Culver, and P. Kestemont. 1994. Effects of hormonal treatment on induced spermiation and ovulation of yellow perch (*Perca flavescens*). Aquaculture 120:171-180.

Dabrowski, K., G. Krumschnabel, M. Paukku, and J. Labanowski. 1992. Cyclic growth and activity of pancreatic enzymes of Arctic charr (*Salvelinus alpinus* L.). Journal of Fish Biology 40:511-521.

VITA

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EDUCATION

B.S. University of Dayton, 1970
M.S. Eastern Kentucky University, 1972
Ph.D. Mississippi State University, 1975

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Comparative Nutrition Society
World Aquaculture Society

SELECTED PUBLICATIONS

Brown, P., K. Dabrowski, and D. Garling. 1996. Nutritional requirements and commercial diets for yellow perch. Journal of Applied Ichthyology 12-171-174.

Nichols, S.J., T. Dietz, J. Lynn, H. Silverman, and D.L. Garling. 1995. Non-siphoning feeding behavior in zebra mussels, quagga mussels, and freshwater clams. 5th International Zebra Mussel and Other Aquatic Nuisance Species Conference. Toronto, Ontario, Canada.4, February 22-24 (abstract).

Brown, P., K. Dabrowski, and D. Garling. 1995. Nutritional requirements and commercial diets for yellow perch. Pages 42-43 *in* P. Kestemont and K. Dabrowski, editors. Aquaculture of percids. Presses Universitaires De Namur, Namur, Belgium.

Cain, K.D., and D.L. Garling. 1995. Pretreatment of soy bean meal for salmonid diets with phytase to reduce phosphorus concentration in hatchery effluents. Progressive Fish Culturist 57:114-119.

Ramseyer, L.J., and D.L. Garling. 1994. Amino acid composition of the ovaries, muscle, and whole body of yellow perch (*Perca flavescens*). Progressive Fish-Culturist 56:175-179.

Belal, I.E., D.L. Garling, and H. Assem. 1992. Evaluation of practical tilapia feed using a saturation kinetic model. Comparative Biochemistry and Physiology 102A:785-790.

Dean, J.C., L.A. Nielsen, L.A. Helfrich, and D.L. Garling, Jr. 1992. Replacing fish meal with seafood processing wastes in channel catfish diets. Progressive Fish-Culturist 54:7-13.

VITA

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EDUCATION

B.S. University of Wisconsin-Stevens Point, 1976
M.S. University of Wisconsin-Madison, 1980
Ph.D. University of Wisconsin-Madison, 1985

POSITIONS

Director (1995-present), Assistant Director (1990-1995), Associate Researcher (1987-1990), and Project Associate (1985-1987), University of Wisconsin Aquaculture Program, University of Wisconsin-Madison

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Sciences
American Fisheries Society
Wisconsin Aquaculture Association
Wisconsin Aquaculture Industry Advisory Council
World Aquaculture Society

SELECTED PUBLICATIONS

Barry, T.P., J.J. Parrish, and J.A. Malison. 1995. Ontogeny of the cortisol stress response in rainbow trout. General and Comparative Endocrinology 97:57-65.

Malison, J.A., L.S. Procarione, A.R. Kapuscinski, and T.B. Kayes. 1994. Endocrine and gonadal changes during the reproductive cycle of walleye. Fish Physiology and Biochemistry 13:473-484.

Barry, T.P., A.F. Lapp, T.B. Kayes, and J.A. Malison. 1993. Validation of a microtitre plate ELISA for measuring cortisol in fish and comparison of stress responses of rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture 117:351-363.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Measurement of resting and stress-elevated serum cortisol in rainbow trout *Oncorhynchus mykiss* in experimental net-pens. Journal of the World Aquaculture Society 23:83-88.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Malison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. Journal of Aquatic Animal Health 4:1-6.