NUTRITION

Chairperson: Paul B. Brown, Purdue University

Industry Advisory Council Liaison: Curtis Harrison, Hurdland, Missouri

Extension Liaison: Donald L. Garling, Michigan State University

Funding Request: $200,000

Duration: 2 Years (September 1, 2004 - August 31, 2006)

Objectives:

1. Develop cost-effective fish meal-free diets for grow out of hybrid striped bass with an initial minimum weight of 100 g (3.5 oz).

2. Develop cost-effective fish meal-free diets for grow out of yellow perch with an initial weight of 10 g (0.35 oz).

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Year 2	Total
Purdue University	Paul B. Brown	1 & 2	$32,000	$33,500	$65,500
Southern Illinois University-Carbondale	Christopher C. Kohler	1	$23,750	$23,750	$47,500
Michigan State University	Donald L. Garling	2	$29,000	$28,500	$57,500
University of Wisconsin-Madison	Jeffrey A. Malison	2	$14,500	$15,000	$29,500
		Totals	**$99,250**	**$100,750**	**$200,000**

Non-funded Collaborator:

Facility	Collaborator
Land O'Lakes	M.E. Griffin

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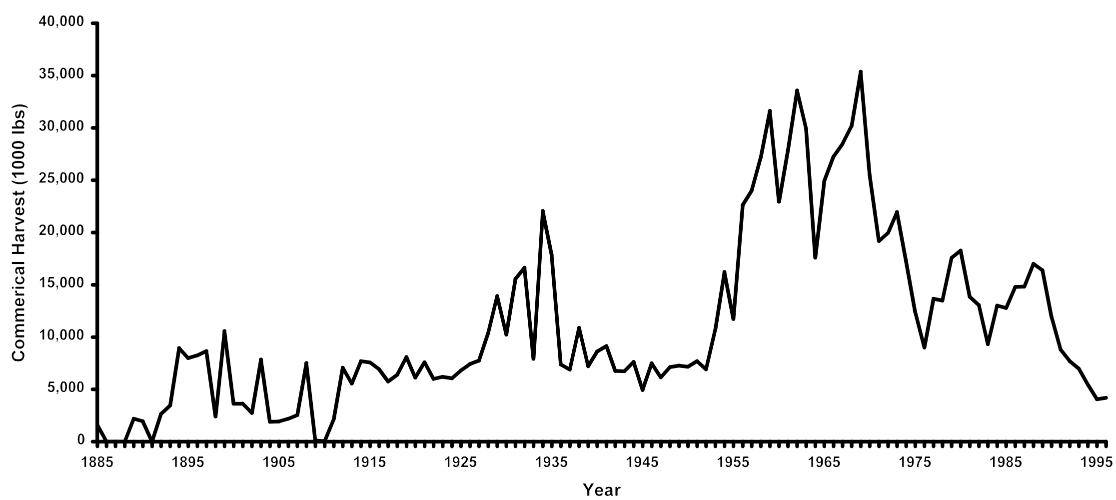
JUSTIFICATION

Yellow perch and hybrid striped bass are generally considered two of the most important aquaculture species in the North Central Region (NCR) of the United States. There have been eight funded research projects with yellow perch and seven with hybrid striped bass since inception of the North Central Regional Aquaculture Center (NCRAC) in 1988. Walleye (seven funded projects), sunfish (five funded projects), and salmonids (four funded projects) are three of the other species-oriented priority topics. Focal points of research with both yellow perch and hybrid striped bass have been on production systems, strains suitable for the NCR, feeds and feeding, and markets. In February 2003, the Industry Advisory Council of NCRAC convened at the Annual Program Planning Meeting and identified continued nutrition research with both species as one of the highest priority topics for the next funding cycle. The Board of Directors concurred with the ranking and allocated funds for a two-year project focused on development of fish meal-free diets for both species. A Project Review Committee was established by NCRAC and they considered nine Statements of Interest submitted by scientists in the NCR. Four scientists were selected to participate in the project. This proposal describes the proposed activities of the four selected participants.

The overarching justification for aquacultural research with both yellow perch and hybrid striped bass is based on market considerations. Commercial harvests of yellow perch from the Great Lakes and striped bass from the Mid-Atlantic region of the Atlantic Ocean were two of our larger commercial fisheries in U.S. waters. From 1970 through 1990 harvests declined, which significantly affected supply/demand economics. The classic decline in supply coupled with stable or increasing demand commonly leads to increase in price, which peaks the interest of potential or current fish producers. Such has been the situation with yellow perch and striped bass.

Figure 1 depicts total commercial harvest of yellow perch from all Great Lakes through 1996. There has been a distinct decline in harvest since 1970, which led to restrictions on both the recreational and commercial fisheries. The situation with striped bass from the Atlantic Ocean was similar in that commercial harvest declined, but population levels declined to the point that striped bass were declared rare and endangered by the U.S. Fish and Wildlife Service. Population levels have improved to the point where there is a limited recreational and commercial fishery, but the markets were presented with the hybrid striped bass during the harvest moratorium and the hybrid has developed name recognition and acceptance in the marketplace. Aquacultural production of both yellow perch and hybrid striped bass is increasing and experiencing some of the typical growing pains seen in other aquaculture industries. One of the initial focal points commonly identified as an area in need of research help is feeds. Feeds typically constitute a high percentage of annual variable costs in aquaculture production, occasionally as high as 60% (Riepe 1998).

Figure 1. Commercial harvest of yellow perch from the Great Lakes 1885-1996



Diets for carnivorous species often contain relatively high levels of fish meal which is a relatively expensive, yet high quality ingredient in diets. Fish meal contains all the nutrients needed by a fish for rapid growth. Diets for new aquaculture species are often trout/salmon diets or slightly modified versions of salmonid diets. Indeed, early evaluations of appropriate diets for both yellow perch and hybrid striped bass indicated that relatively high protein, high fat trout/salmon diets were best for both (Brown, P.B. et al. 1993; 1996). Fish meal is a high protein feed ingredient and is commonly incorporated into salmonid diets as the primary source of crude protein and essential amino acids. Thus, current diets for both species are some of the more expensive diets in the United States. Use of fish meal in diets fed to culture species has been the recent focus of significant criticism.

Aquaculture currently uses 34% of the global fish meal supply and 56% of the global fish oil supply (Pike and Barlow 2003). Projected use by 2010 is 48% and 79%, respectively. Given that fish meal and oil supplies have not increased significantly since 1985, this represents a significant movement of fish meal and oil away from other uses and into fish feeds. Growth in aquaculture and the increasing use of fish meal and oil associated with that growth has become a focal point of criticism. Naylor et al. (2000) argued that aquaculture is not alleviating the loss of global supply of fish and shellfish, but actually exacerbating the problem by demanding more fish meal and oil. Further, recent studies identified contaminants in farm-raised fish (Hites et al. 2004), most likely originating from fish meal in the diets. There are ongoing public debates about the validity of many of the criticisms leveled at fish meal use in aquaculture feeds (Hardy 2003). Regardless of the outcome of those debates, fish meal supplies are limited and unlikely to supply the needs in the growing aquaculture arena. Global supply of fish meal has varied between 6 and 7 mmt since 1985 and total supply is unlikely to increase (Pike and Barlow 2003). There are also additional reasons to rethink use of fish meal in diets.

Fish meal is a relatively expensive feed ingredient. The August 25, 2003 edition of Feedstuffs lists menhaden fish meal at $480–$570/ton. In contrast, the price for high-protein soybean meal averaged $213/ton, cottonseed meal averaged $156/ton, canola meal averaged $136/ton, sunflower meal averaged $85/ton and linseed meal averaged $141/ton. If feedstuff prices are expressed as a function of crude protein, fish meal is $7.38– $8.77/unit protein, while the plant feedstuffs range from $1.89–$4.35/unit protein. Aquatic animals grow best when fed relatively high concentrations of crude protein and crude protein is typically the most expensive macronutrient in diets. Thus, diets that contain high concentrations of fish meal cost more than diets containing high concentrations of plant protein feedstuffs. Prices of fish meal are also highly variable.

Fish meal is traded in most major markets around the world and is subject to customary fluctuations in price, some real and some perceived. For example, in years when the first fishing boats off South America do not immediately catch fish, fish meal prices start upwards very quickly. In most years, fish enter the harvesting areas and prices decline somewhat after an initial scare. However, there have been environmental conditions that inhibited fish from entering harvest areas and commercial harvest declined for an entire season. Production of fish meal declined during a recent El Nino period (1998) due to lack of fish off the coast of South America (Pike and Barlow 2003). During these periods, prices increase. All major feedstuffs are traded on world markets and subject to price fluctuations, but the variability in fish meal prices can be much more dramatic than seen in plant protein feedstuffs. Global supplies of plant protein feedstuffs are much higher than global supplies of fish meal, which limits price fluctuation. Global supplies of soybeans in 1995 was 137 million tons, cottonseed 32 million tons, sunflower 23 million tons, and canola 29 million tons (Soyatech 1995).

In summary, both the yellow perch and hybrid striped bass were historically important species commercially harvested by U.S. fishermen. Declines in populations led to restrictions on harvest and decreased supply of fish. Aquacultural production began as supplies decreased. Current diets for both are high-protein, high fish meal diets formulated around the general requirements for trout and salmon. Fish meal supplies are problematic on several fronts and can affect feed prices in the short term. Thus, alternative protein sources are needed. The fundamental challenge in making this change is that plant protein feedstuffs contain lower crude protein concentrations, lower quality balance of essential amino acids, and antinutritional factors that limit nutrient availability.

Numerous studies have examined the use of alternative protein feedstuffs in diets for carnivorous species such as 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). Probably none of the plant feedstuffs can completely replace fish meal when used as a sole source of crude protein. However, recent studies with rainbow trout 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 (Adelizi et al. 1998). 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, plant protein sources contain chemicals that inhibit their use by animals. There have been few formal evaluations of antinutritional factors in diets fed to fish.

Similarly, several alternative lipid sources have been evaluated in diets fed to carnivorous species of fish (Cho et al. 1974; Green and Selivonchick 1990; Nematipour and Gatlin 1993; Guillou et al. 1995; Dosanjh et al. 1998; Torstensen et al. 2000; Bell et al. 2001). Of these candidates, canola oil possesses minimal anti-nutritional factors (Tacon 1992), possesses a longer shelf life compared to fish oils due to natural antioxidant properties (Shahidi 2000), and is naturally rich in monounsaturated fatty acids (Khalil et al. 2001) and in a-linolenic acid (18:3n-3), both of which are precursors to long-chain fatty acids in many animals.

RELATED CURRENT AND PREVIOUS WORK

Fish Meal-Free Diets for Hybrid Striped Bass (Objective 1)

Detailed nutritional research with the hybrid striped bass began in the late 1980s. To date, nutritional requirements for lysine (Griffin et al. 1992), arginine (Griffin et al. 1994a), methionine (Griffin et al. 1994b), threonine (Keembiyehetty and Gatlin 1997), choline (Griffin et al. 1994c), and phosphorus (Brown, M.L. et al. 1993) have been quantified. Further, estimates of the remaining essential amino acid requirements are available (Brown 1995; Twibell et al. 2003). Given several of the critical nutritional requirements for this hybrid, practical diets were developed and are currently available. To date, only soy products (Brown et al. 1997) and meat and bone meal (Bharadwaj et al. 2002) have been systematically evaluated as alternative sources of crude protein and essential amino acids. Both ingredients could be incorporated at relatively high levels in diets (around 40% of the dry matter), replacing an isonitrogenous amount of fish meal. A low level of fish meal is considered important in practical diets as a flavor component (Brown, P.B. et al. 1993; Webster et al. 1997), but there are alternative flavor additives for diets fed to fish (Gould et al. 2003). This level of understanding is sufficient for development of cost-effective, fish meal-free diets.

Fish can be classified into “lean” or “fatty,” according to how lipid in triacylglycerol form is stored in their bodies. Lean fish store triacylglycerol in the liver, while fatty fish store triacylglycerol in the fillet. Hybrid striped bass are classified as lean fish with 3–4% total lipid (Fair et al. 1993). In a study evaluating the effects of dietary menhaden oil on hybrid striped bass growth and muscle fatty acid composition, Fair et al. (1993) found that hybrid striped bass acquired the fatty acid profile of the dietary lipid source. In this study, a linear relationship existed between essential fatty acids in the feed and essential fatty acids in the flesh of the bass. Fatty acid compositions of the edible muscle tissue in hybrid striped bass in a study by Fowler et al. (1994) also reflected the composition of the semipurified isocaloric diets containing varying levels of long-chain polyunsaturated fatty acids (20:5n-3 and 22:6n-3).

Fair et al. (1993) found that hybrid striped bass fed diets supplemented with 4 and 8% menhaden oil exhibited better growth and survival than those without. Moreover, Nematipour and Gatlin (1993) found that hybrid striped bass fed diets not supplemented with menhaden oil suffered tail and skin erosion, which resulted in mortalities approaching 80%, whereas no mortality or body erosion was observed in the groups fed diets supplemented with menhaden oil.

Dosanjh et al. (1988) assessed canola oil as a supplemental source of dietary lipid for juvenile Chinook salmon. The type of lipid did not significantly influence fish growth, and when fed an equal mixture of herring oil and canola, overall food and protein utilization was the highest. Groups that received supplemental canola oil, lard, or a combination of the two had elevated percentages of n-3 fatty acids in their body lipids. Dosanjh et al. (1998) also examined the effects of various dietary blends of canola and menhaden oil on growth, proximate composition, and flesh quality in Atlantic salmon. The author concluded as much as 47% of the lipid in high-energy grower diets could be canola oil without comprising performance. Bell et al. (2001) also replaced fish oil with canola oil in diets of Atlantic salmon and found no differences in growth or feed conversion, and that diets can be supplemented up to 50%. Fair et al. (1993) found that hybrid striped bass fed a diet of 8–12% menhaden oil had twice the levels of n-3 fatty acids (1.0 g/100 g tissue) as those without the oil (0.5 g/100 g tissue). Accordingly, hybrid striped bass have the potential to be an excellent source of nutritional n-3 fatty acids, provided sufficient quantities are incorporated into their diet (Greenberg and Harrell 1994).

Fish Meal-Free Diets for Yellow Perch (Objective 2)

Brown et al. (1996) reported that yellow perch fed practical trout diets gained more weight and had better feed conversion rates than yellow perch fed a practical catfish diet. One of the trout diets in that study contained the same amount of crude protein (36%) as did the catfish diet. While the trout diet had a slightly higher lipid content (8% versus 6%), that difference should not have significantly impacted the results (Cartwright et al. 1998). Reduced performance of yellow perch appears to be related to the increased level of plant proteins in the catfish diet.

Research conducted at Michigan State University (MSU) has shown that the optimal crude protein level for yellow perch is between 21–27%, although a slightly higher weight gain was observed for fish fed 34% crude protein (Ramseyer and Garling 1998). Brown et al. (1996) found that perch with an initial weight of 51g gained more weight when fed a commercial trout diet containing 40% crude protein/10% lipid (40/10) than fish fed diets containing 33/8, 38/12, or 50/17.

Several of the key nutritional requirements for yellow perch have been quantified. Twibell et al. (1998) reported the dietary lysine requirement of juvenile yellow perch as 1.1% of the diet. A series of studies were conducted to determine the methionine requirement of yellow perch (Twibell et al. 2000). In the first study, the diets contained 0.03% cysteine and the methionine requirement was 1.0–1.1% of the diet. In the second study, supplemental cysteine was added to the diet and a sparring effect on the methionine requirement was observed; 51% of the methionine requirement was spared by the addition of cysteine. A ratio of 51:49 cysteine:methionine was used in the third study and the total sulfur amino acid requirement was 0.85% of the diet. The dietary arginine requirement for juvenile yellow perch was reported as 1.4% of the diet (Twibell and Brown 1997).

Twibell and Brown (2000) conducted a study to determine the choline requirement of yellow perch using a ratio of 51:49 cyst(e)ine:methionine and total sulfur amino acid concentration of 0.85%. The conversion of sulfur amino acids to choline was thus limited (Kasper et al. 2000). In the same study, phosphatidylcholine (PC) was evaluated as a choline supply. The choline requirement was approximately 600 mg/kg diet and dietary PC could meet the choline requirement. This study demonstrated that perch are capable of using PC as a source of choline, but when the choline requirement was met by choline-Cl, there was no beneficial effect of dietary PC at the dietary concentrations used. These data provide an important framework for evaluation of practical feed ingredients.

Unpublished data from Purdue University (Purdue) indicated that yellow perch juveniles could effectively utilize up to 40% solvent-extracted or expelled soybean meals in practical diets. At concentrations above 40%, feed consumption, weight gain, and feed conversion ratio decreased. The use of soybean meal as a partial or total replacement of fish meal has potential economic and environmental benefits for the aquaculture industry. Of the antinutritients in soybean products, proteinase enzyme inhibitors such as trypsin inhibitors (TI) have been considered the most important. Trypsin is a pancreatic enzyme used to help digest protein. The goal of the study at MSU is to determine the effects of TI on the growth and performance in formulated fish feeds for yellow perch. Other antinutrients in plant protein feedstuffs include saponins, lectins, insoluble carbohydrates, and several other chemicals that impair utilization of diets or health of the animal consuming those diets. Several of those chemicals are being systematically examined in diets fed to rainbow trout and Atlantic salmon as part of a separate project funded by another funding source. Several of the Principal Investigators on this project are also involved in the separate project. Those results should be available before this project begins and will provide guidance on the critical factors in these studies.

A great deal of research has been conducted on replacing fish meal with alternative sources of protein. The primary potential replacement sources for fish meal in fish diets are various animal protein sources including meat meals, blood meal, and egg, and plant protein sources such as soybean and corn meal. Research to date has generally shown that for most non-herbivorous fish, animal protein sources have a better amino acid balance than plant protein sources. On the other hand, plant protein sources are generally less expensive than animal protein sources. Depending on fish species, diets high in plant protein can be fortified with relatively expensive ingredients (such as free amino acids) but still remain cost-effective.

An important consideration in developing fish-meal-free diets is palatability. Diets without fish meal are often not readily accepted by non-herbivorous fish. The palatability and acceptance of such diets can be greatly improved by the addition of highly flavorful ingredients such as krill, shrimp meal, and certain free amino acids (Gould et al. 2003).

ANTICIPATED BENEFITS

Concern has been raised whether aquaculture can sustain its rapid growth worldwide if the industry continues to rely on fish meal and oil as the major dietary protein and lipid constituents. Issues have been raised concerning cost, fluctuating availability, and even if aquaculture is growing at the expense of wild fisheries dependent upon the same forage fish being harvested for fish meal. The implications revolving around fish meal and oil are particularly critical in the NCR because both products must be imported. The studies proposed here will provide feed manufacturers with the information they will need to produce cost-effective feeds free of fish meal. This line of research is similar to the series of projects funded by NCRAC on Salmonids. Those projects were designed to develop fish meal-free diets for rainbow trout. Benefits derived from those studies included a new feed meal specializing in fish meal-free diets for the NCR. That new business is located in Ohio.

OBJECTIVES

1. Develop cost-effective fish meal-free diets for grow out of hybrid striped bass with an initial minimum weight of 100 g (3.5 oz).

2. Develop cost-effective fish meal-free diets for grow out of yellow perch with an initial weight of 10 g (0.35 oz).

PROCEDURES

Fish Meal-Free Diets for Hybrid Striped Bass (Objective 1)

Purdue

Studies conducted at Purdue will be continuations of previous studies designed to develop and evaluate all-plant diets for hybrid striped bass. In the first year of the study, practical diets will be fed to triplicate groups of fish (initial weight 100 g (3.5 oz) or greater, depending on availability). Six to ten practical diets will be developed based on results from recently completed studies. It is anticipated that diets will be developed along the lines of those evaluated in previous salmonid studies. That is, diets will contain mixtures of plant protein feedstuffs that meet the essential amino acid requirements for hybrid striped bass (Twibell et al. 2003). The study will start with soy/corn mixtures using solvent-extracted soybean meal (Brown et al. 1997) and incorporate both yellow and white corn gluten meals with and without flavor additives. If possible, canola/wheat mixtures and soy protein concentrates will also be evaluated. All diets will be formulated to contain 36% crude protein and 14% lipid. All diets will meet the established nutrient requirements for hybrid striped bass and all diets will be extruded by Wenger Manufacturing, Inc. (Sabetha, Kansas). Diets will be fed to quadruplicate groups of fish all housed in the same experimental system.

It is difficult to propose diets for the second year until results from the first evaluation are available. However, the approach taken with previous salmonid projects will be applied to this project, building on results from the first year, focusing efforts on expansion of ingredient combinations, or focusing on refinements in a diet that was accepted by hybrids in the first year and resulted in maximal or near-maximal weight gains. All methods will be the same in both years of the project.

The experimental system that will be used in both years is a series of 120-L (30 gal) 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 (0.8 and 1.3 gal/min), depending on initial fish size, and temperature will be maintained at 28°C (84°F) 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.

At the end of each study, final numbers and weights of fish will be determined. Fillet samples will be collected for determination of dress out percentage and proximate analysis using standard procedures (AOAC 1984). Liver samples will be collected for determination of hepatosomatic index and liver lipid concentrations (Folch et al. 1957). Intraperitoneal fat will be collected, weighed, and expressed as a function of wet weight of the fish.

Both studies will be statistically analyzed as completely randomized designs using diet as the main effect. If analysis of variance indicates significant differences among treatments, then Student Neuman Keuls will be used to separate mean values of weight gain, feed conversion, survival, hepatosomatic index, dress out percentage, and proximate composition of fillets.

Southern Illinois University-Carbondale (SIUC)

With NCRAC funding, SIUC researchers recently determined that phase-III grow out of hybrid striped bass can be accomplished with diets containing 36% crude protein with an E:P (kcal/100 g protein) of 9.3. This diet, which will serve as a control, contains ~20% fish meal and 8% menhaden fish oil. Three to five isocaloric (8% fish oil)/isonitrogenous experimental diets will be formulated using alternative protein sources (e.g., but not necessarily limited to: blood, bone, feather, meat, and poultry byproduct meals) in combination with traditional protein sources (e.g., corn, soybean, and wheat products). Properly replicated tank studies will be conducted using standard aquaculture practices and evaluated based on standard performance criteria. Once one or more acceptable fish meal-free diets are found, a second study will be conducted in which menhaden fish oil will be replaced with canola oil at 0, 25, 50, 75, and 100%. All feeds will be extruded by Wenger Manufacturing, Inc. (Sabetha, Kansas). Each of the diets fed to the fish will be housed in a refrigerated trailer (~0°C; 32°F). To account for oxidative losses in the feed, lipid profiles for each treatment will be compared between samples frozen (~-20°C; -4°F) at the onset and others taken at the end of the feeding trial.

A minimum of four fish from each replicate (minimum of three) will be subsampled at the end of the 10-week trials. Fish will be weighed to the nearest 0.1 g (0.0035 oz) to calculate feed conversion ratio (FCR), total length (TL) measured to the nearest millimeter and, to calculate hepatosomatic and gonadosomatic indices (HIS and GSI, respectively), the livers and gonads will be removed, and weighed to the nearest 0.1 g (0.0035 oz). A fillet sample will be taken from each fish (second study only) for determination of fatty acid profiles. Fillets and livers will be placed in appropriately labeled bags and stored in an ultra low freezer at -80°C (-112°F) (Revco Ultima II by Kendro Laboratory Products, Asheville, North Carolina).

Total lipids will be extracted by homogenizing the samples in chloroform/methanol using an Omni (Omni International, Warrenton, Virginia) mixer and measured gravimetrically using the method outlined in Folch et al. (1957). After extraction, lipids will be converted to fatty acid methyl esters (FAME) using an acid catalyzed transmethylation method from Christie (1982). Purification of FAME will be performed using the method of Ghioni et al. (1996). Samples will be diluted and injected into the gas chromatograph (GC 17A, Shimadzu Scientific Instruments, Columbia, Maryland), equipped with an Omegawax 250 column (Restek Corporation, Bellefonte, Pennsylvania). Hydrogen will be the carrier gas, and temperature programming will begin at an initial temperature of 50°C (122°F), held for 2 min, then increased to 220°C (428°F) at 4°C/min (39°F/min) increments. Once at 220°C (428°F), the temperature will be held for 20 min. Eluted fatty acids will be labeled using the program EZChrom (Scientific Software, Inc., Pleasanton, California) based on standards supplied by Supelco (Bellefonte, Pennsylvania).

Weight gain, FCR, HSI, and GSI will be quantified and compared among treatments. Calculated HSI and GSI values will be arcsine square-root transformed for statistical comparison. Liver texture and appearance will be quantified using an increasing scale, one for pink and healthy to five for yellow and fatty. The overall oxidation of the feed will be calculated by comparing lipid profiles in initial and final feed samples. One-way analysis of variance will be used to test for differences between treatments. Comparisons between treatment groups based on concentrations of essential fatty acids will be made using Tukey's HSD test, with an alpha of 0.05.

Fish Meal-Free Diets for Yellow Perch (Objective 2)

Purdue

Studies conducted at Purdue will be continuations of previous studies designed to develop and evaluate all-plant diets for yellow perch. In the first year, we will evaluate the effects of soybean lectins on yellow perch. In the second year, we will examine the effects of soybean saponins on yellow perch. Both antinutritional factors will be incorporated into diets at graded levels representing soybean meal incorporation through 63% of the diet. In past studies, decreased performance in perch fed soybean meal over 40% of the diet was identified and these studies will determine which of the antinutritional factors limit incorporation. All diets will meet the established nutrient requirements for hybrid striped bass and all diets will be extruded by Wenger Manufacturing, Inc. (Sabetha, Kansas). Diets will be fed to quadruplicate groups of fish all housed in the same experimental system.

The experimental system that will be used in both years is a series of 120-L (30 gal) 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 (0.8 and 1.3 gal/min), depending on initial fish size, and temperature will be maintained at 24°C (75°F) 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.

At the end of each study, final numbers and weights of fish will be determined. Blood will be collected for analysis of insulin in fish fed lectins and saponins will be measured in blood of fish fed that chemical. Liver samples will be collected for determination of hepatosomatic index and liver lipid concentrations (Folch et al. 1957).

Both studies will be statistically analyzed as completely randomized designs using diet as the main effect. If analysis of variance indicates significant differences among treatments, then Student Neuman Keuls will be used to separate mean values of weight gain, feed conversion, survival, hepatosomatic index, dress out percentage, and proximate composition of fillets.

MSU

MSU researchers will conduct a 2-year nutritional study using high quality semi-purified diets on young-of-the-year (YOY) and one-year-old (one+) yellow perch (see experimental design, Box 1). The diets will contain approximately 40% crude protein and be formulated to meet known nutritional requirements of yellow perch. The semi-purified diets will be prepared and provided by collaborators at Purdue University. The test diets will contain graded levels of trypsin inhibitors equivalent to diets containing 0, 15, 30, 45, and 60% soybean meal. The control used in the study will be a commercial diet containing approximately 40% crude protein, 10% lipid.

TI Treatments
0% SBM
15% SBM
30% SBM
45% SBM
60% SBM
Commercial Control

Year 1:

Age	Size	Period	Analysis
YOY	5-10 g	8 weeks	Feed Conversion Growth Chemical Body Composition

Year 2:

Age	Size	Period	Analysis
One +	70-100 g	20 days	Trypsin Activity Levels Protein Digestion Chemical Body Composition Amino Acids (potential)

Experimental design summary for yellow perch study at MSU

Yellow perch used in the study will be obtained from commercial fish producers in the NCR, in accordance with regulations required by the Michigan Department of Agriculture. The study has been designed to examine short- and long-term effects of trypsin inhibitors on feed conversion, growth, and physiological effects on fingerling and grow-out sized yellow perch. YOY fish, 5–10 g (0.18–0.35 oz), will be fed for 8 weeks to evaluate feed conversion, growth, and body composition (year 1). One+ fish, 70–100 g (2.5–3.5 oz), will be fed over a 20 day feeding trial to analyze protein digestibility and pancreatic trypsin activities in the small and large intestine and feces (year 2). Amino acid composition will also be determined, provided sufficient intestinal and fecal sample sizes are collected. The study will be conducted in a recirculating aquaculture system at the MSU Aquaculture Research Laboratory, East Lansing, Michigan. The fish will be held in 125-L (33 gal) tanks. The flow rate will be approximately 4 L/min (1 gal/min) and the water temperature will be maintained at 18–20°C (64–68°F), which is optimal temperature for yellow perch.

University of Wisconsin-Madison (UW-Madison)

Presently, most yellow perch producers use trout feed (or diets similar in content to trout feed) containing a high percentage of fish meal. Many years of research will be required to develop an “ideal” fish-meal-free diet for yellow perch—one that performs as well as trout diets and is equally as cost-effective. Scientists do, however, currently have a reasonable understanding of the protein nutritional needs of yellow perch and believe that the time is here to test several practical fish meal-free diets in yellow perch—diets that perform well, are cost-effective, and can be commercially available in short order. Purina Mills, LLC, under the expertise of Dr. Mark Griffin, has manufactured and tested such diets in other non-herbivorous fish species, and has agreed to do so at no cost for this project.

Researchers at UW-Madison propose to conduct a grow-out trial on yellow perch comparing four diets. All diets will be formulated to be 41% crude protein and 10.5% crude fat and meet or exceed the nutritional requirements for rainbow trout. The control diet will be a commercial trout grower containing a high percentage of fish meal. The experimental diets will be similar to the control diet, except that the fish meal will be replaced with animal and plant meal mixes in the following approximate ratios: 75% animal meal mix/25% plant meal mix, 55% animal meal mix/45% plant meal mix, and 35% animal meal mix/65% plant meal mix (see Table 1). Each of the experimental diets will also contain 5% shrimp meal to enhance palatability. All diets will be made into extruded slow-sinking pellets approximately 1.5 and 2.5 mm (0.06 and 0.1 in) in diameter.

In year 1, yellow perch, initially age 0 and 10 g (0.35 oz) in weight, will be raised to a market size of 110–120 g, (3.9–4.2 oz) over a period of approximately 10 months. The diets will be tested using triplicate tanks per treatment, with 80 perch initially stocked into each tank. During the grow-out trial, the tanks will be provided with flow-through water at 21°C (70°F), and light on a 16-h light/8-h dark photoperiod. The water in each tank will be exchanged 1.5–2.0×/h, and the tanks will be supplied with aeration to keep dissolved oxygen levels >80% of saturation levels. The fish in each tank will be fed to satiation once or twice daily (depending on their size), and daily records of feed consumption will be kept. The fish will be weighed and measured every three weeks. Endpoints during the grow-out phase will be weight gain, length gain, food consumption, feed conversion, and survival.

At the end of the grow-out study, most of the fish will be killed to determine fillet weight. The livers of the killed fish will be analyzed histologically to compare the extent of hepatic fat accumulation. An organoleptic comparison of fillets from fish fed the four diets will be conducted in conjunction with the UW-Madison Department of Food Science Sensory Analysis Laboratory. The cost-effectiveness of each of the four diets will be determined with the economic analysis of each diet incorporating diet cost, fish growth rate, feed conversion, and fillet yield.

In year 2, because many yellow perch farmers use a portion of their production animals as brood fish, and because diet can have a significant impact on reproductive development, the impact of the four diets on reproductive development will be assessed. After the grow-out study is completed (October 2005), 30 fish from each treatment group will be held for an additional 6 months under a normal wintertime ambient temperature/photoperiod regime to induce reproductive development (i.e., the temperature will slowly decline to <5°C (41°F), and rise again with the approach of spring. Similarly, the photoperiod will decline to 8-h light/16-h dark, and then rise. During this time, the fish will continue to be fed using the four different diets. The following April these fish should be reproductively mature and will be spawned (using at least 4 single pair matings per treatment). Key reproductive endpoints will be compared including egg quantity, quality, and size, fertilization rate, sperm viability and motility, percent hatch, fry size, and early development.

<u>Extension Plan</u>

Outreach will be accomplished in a timely manner and under terms agreeable between research and extension scientists, and involve industry consultation to effectively fulfill the Regional Aquaculture Center program goal. The extension liaison will determine recommended mechanism(s) for information dissemination of research findings and/or outreach activities that facilitate information transference to feed manufacturers for the development of new fish diets for yellow perch and hybrid striped bass. Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins. Research results will also be disseminated through the NCRAC Annual Progress Reports. Annual Progress Reports are assembled and edited by the extension liaison with input by the Principal Investigators. These reports are available on the NCRAC Web site (http://aquanic.org/publicat/usda_rac/apr/ncracapr.htm).

FACILITIES

<u>Purdue</u>

Purdue has a 715 m^2 (7,700 ft^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>SIUC</u>

The SIUC proximate analyses laboratory is fully equipped to perform all standard AOAC methods to estimate crude protein, crude fat, moisture, crude fiber, and ash in fish/feeds. The lipid laboratory is fully equipped with glassware and modern instrumentation with analytical software to perform all of the lipid and fatty acid assays,

Table 1. A list of ingredients to be used in the diets of the study to be conducted at UW-Madison.

Control Diet

Ingredient	% of Diet
Fish Meal	40.0
Poultry Meal	0.0
Blood Meal	0.0
Egg	0.0
Porcine Meat Meal	14.3
Corn Gluten Meal	0.8
Soybean Meal	0.0
Soy Protein Concentrate	0.0
Corn	10.0
Wheat Flour	10.0
Wheat Midds	15.3
Shrimp Meal	5.0
Fish Oil	2.9
Vitamins and Minerals	1.7

Experimental Diet #2

Ingredient	% of Diet
Fish Meal	0.0
Poultry Meal	5.2
Blood Meal	6.9
Egg	2.8
Porcine Meat Meal	21.5
Corn Gluten Meal	1.0
Soybean Meal	27.3
Soy Protein Concentrate	0.0
Corn	10.0
Wheat Flour	10.0
Wheat Midds	4.7
Shrimp Meal	5.0
Fish Oil	3.7
Vitamins and Minerals	1.9

Experimental Diet #1

Ingredient	% of Diet
Fish Meal	0.0
Poultry Meal	7.1
Blood Meal	9.0
Egg	3.7
Porcine Meat Meal	24.1
Corn Gluten Meal	0.0
Soybean Meal	18.4
Soy Protein Concentrate	0.0
Corn	10.0
Wheat Flour	10.0
Wheat Midds	8.0
Shrimp Meal	5.0
Fish Oil	2.8
Vitamins and Minerals	1.9

Experimental Diet #3

Ingredient	% of Diet
Fish Meal	0.0
Poultry Meal	3.3
Blood Meal	4.4
Egg	1.7
Porcine Meat Meal	18.9
Corn Gluten Meal	1.0
Soybean Meal	38.0
Soy Protein Concentrate	1.1
Corn	10.0
Wheat Flour	10.0
Wheat Midds	0.0
Shrimp Meal	5.0
Fish Oil	4.7
Vitamins and Minerals	1.9

and includes a Shimadzu Gas Chromatograph (GC-FID; GC17-A) fitted with an auto-sampler (AOC-20i), a Iatroscan (TLC-FID; MK-6s), and developing chambers for thin layer chromatography. The lipid laboratory also houses analytical balances, spectrophotometers, a freeze dryer, and an -80°C (-112°F) freezer. A 771 m^2 (8,300 ft^2), temperature-controlled wet laboratory building houses approximately 150 tanks of varying sizes. At least 15 recirculating systems are employed allowing for numerous studies to be conducted simultaneously. In addition, the wet laboratory building houses feed storage and an equipped feed manufacturing room (pelleted feeds), as well as a new water chemistry laboratory.

<u>MSU</u>

MSU has a wet laboratory fully equipped to conduct grow-out studies. All studies will be conducted in experimental systems currently in place and operational. Systems contain between 12 and 50 tanks with varying capacities (20–340 L; 5–90 gal). Choice of tank size will be a function of initial size of fish. All experimental systems can be operated flow-through or recirculating.

UW-Madison

The UW-Madison Aquaculture Program has its main facility at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. For this project, two experimental wet-laboratory suites will be used. One suite contains 12, 220-L (60-gal) fiberglass tanks, and the other contains 12, 750-L (200-gal) tanks. The fish will initially be stocked into the 220-L (60-gal) tanks, but will be moved to the larger tanks as they grow. Photoperiod can be independently regulated in each suite, and all tanks will be provided with aerated, temperature-regulated (±1°C; 2°F) flow-through water.

REFERENCES

Adelizi, P.D., R.R. Rosati, K. Warner, Y.V. Wu, T.R. Muench, M.R. White, and P.B. Brown. 1998. Evaluation of fish meal-free diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture Nutrition 4:255-262.

Association of Official Analytical Chemists (AOAC). 1984. Official methods of analysis, 14th edition. Association of Official Analytical Chemists, Bethesda, Maryland.

Bell, J.G., J. McEvoy, D.R. Tocher, F. McGhee, P.J. Campbell, and J.R. Sargent. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. Journal of Nutrition 131:1535-1543.

Bharadwaj, A., W.R. Brignon, N.L. Gould, P.B. Brown, and Y.V. Wu. 2002. Evaluation of meat and bone meal in practical diets fed to juvenile hybrid striped bass *Morone chrysops* x *M. saxatilis*. Journal of the World Aquaculture Society 33:448-457.

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.

Brown, M.L., F. Jaramillo, and D.M. Gatlin. 1993. Dietary phosphorus requirement of juvenile sunshine bass, *Morone chrysops* x *M. saxatilis*. Aquaculture 113:355-363.

Brown, P.B., M.E. Griffin, and M.R. White. 1993. Experimental and practical diet evaluations with juvenile hybrid striped bass. Journal of the World Aquaculture Society 24:80-89.

Brown, P.B., K. Dabrowski, and D.L. Garling, Jr. 1996. Nutrition and feeding of yellow perch (*Perca flavescens*). Journal of Applied Ichthyology 12:171-174.

Brown, P.B., R. Twibell, Y. Jonker, and K.A. Wilson. 1997. Evaluation of three soybean products in diets fed to juvenile hybrid striped bass *Morone saxatilis* x *M. chrysops*. Journal of the World Aquaculture Society 28:215-223.

Bureau, D.P., A.M. Harris, and C.Y. Cho. 1996. The effects of a saponin extract from soybean meal on feed intake and growth of chinook salmon and rainbow trout. VII International Symposium on Nutrition and Feeding of Fish, College Station, Texas (abstract).

Cartwright, D.D., B. Watkins, J. Burgess, K. Warner, R. White, Y. Jonker, and P.B. Brown. 1998. Dietary lipid requirements of juvenile yellow perch. World Aquaculture ’98, Las Vegas, Nevada (abstract).

Cho, C.Y., H.S. Bayley, and S.J. Slinger. 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 31:1523-1528.

Christie, W.W. 1982. Lipid analysis, 2nd edition. Pergamon Press, Oxford.

Dabrowski, K., P. Poczyczynski, G. Kock, and B. Berger. 1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New in vivo test for exocrine pancreatic secretion. Aquaculture 77:29-49.

Dosanjh, B.S., D.A. Higgs, M.D. Plotnikoff, J.R. Markert, and J.T. Buckley. 1988. Preliminary evaluation of canola oil, pork lard and marine lipid singly and in combination as supplemental dietary lipid sources for juvenile fall Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 68:325-343.

Dosanjh, B.A., D.A. Higgs, D.J. McKenzie, D.J. Randall, J.G. Eales, N. Rowshandeli, M. Rowshandeli, and G. Deacon. 1998. Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition and thyroidal status of Atlantic salmon in sea water. Fish Physiology and Biochemistry 19:123-134.

Fair, P.H, W.P.Williams, and T.I. Smith. 1993. Effect of dietary menhaden oil on growth and muscle fatty acid composition of hybrid striped bass, *Morone chrysops x M. saxatilis*. Aquaculture 116:171-189.

Folch, J., M. Lees, and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226:497-509.

Fowler, K.P., C. Karahadian, N.J. Greenberg, and R.M. Harrell. 1994. Composition and quality of aquacultured hybrid striped bass fillets as affected by dietary fatty acids. Journal of Food Science 59:70-75.

Fowler, L.G., and J.L. Banks. 1976. Animal and vegetable substitutes for fish meal in the Abernathy diet. Progressive Fish-Culturist 38:123-130.

Ghioni, C., J.G. Bell, and J.R. Sargent. 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. Comparative Biological Physiology 114B:161-170.

Gould, N.L., M.M. Glover, L.D. Davidson, and P.B. Brown. 2003. Dietary flavor additives influence consumption of feeds by yellow perch, *Perca flavescens*. Journal of the World Aquaculture Society 34:412-417

Green, D.H.S., and D.P. Selivonchick. 1990. Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89:165-182.

Greenberg, N.J., and R.M. Harrell. 1994. Effects of dietary lipid supplements on subadult sunshine bass. Progressive Fish-Culturist 60:95-100.

Griffin, M.E., P.B. Brown, and A. Grant. 1992. Dietary lysine requirement of juvenile hybrid striped bass. Journal of Nutrition 122:1332-1337.

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

Griffin, M.E., M.R. White, and P.B. Brown. 1994b. Total sulfur amino acid requirement and cysteine replacement value for juvenile hybrid striped bass (*Morone saxatilis* x *M. chrysops*). Comparative Biochemistry and Physiology 108A:423-429.

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

Gropp, J., H. Koops, K. Tiews, and H. Beck. 1976. Replacement of fish meal in trout feeds by other feedstuffs. Pages 147-162 *in* T.V.R. Pillay and W.A. Dill, editors. Advances in aquaculture. Fishing News Books Limited, Surrey, England.

Guillou, A., P. Soucy, M. Khalil, and L. Adambounou. 1995. Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr (*Salvelinus fontinalis*). Aquaculture 136:351-362.

Hardy, R.W. 2003. Fish meal to farmed fish conversions. Aquaculture Magazine 29:36-40.

Hites, R.A., J.A. Foran, D.O. Carpenter, M.C. Hamilton, B.A. Knuth, and S.J. Schwager. 2004. Global assessment of organic contaminants in farmed salmon. Science 303:226-229.

Kasper, C.S., M.R. White, and P.B. Brown. 2000. Choline is required by tilapia when methionine is not in excess. Journal of Nutrition 130:238-242.

Keembiyehetty, C.N., and D.M. Gatlin, III. 1997. Dietary threonine requirement of juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilis*). Aquaculture Nutrition 3:217-221.

Khalil, I., A. Hassan, and S.Waqar. 2001. Fatty acid and sterol composition of vegetable oils. Scientific Khyber 14:11-18.

Naylor, R.L., R.J. Goldberg, J.H. Primavera, N. Kautsky, M.C.M. Beveridge, J. Clay, C. Folke, J. Lubchenco, H. Mooney, and M. Troell. 2000. Effect of aquaculture on world fish supplies. Nature 6790:1017-1024.

Nematipour, G. R., and D. M. Gatlin, III. 1993. Effects of different kinds of dietary lipid on growth and fatty acids composition of juvenile sunshine bass, *Morone chrysops* x *M. saxatilis*. Aquaculture 114:141-154.

Olli, J.J., and A. Krogdahl. 1994. Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in fresh water. Acta Agriculture Scandanavia, Section A. Animal Science 44:185-192.

Pike, I.H., and S.M. Barlow. 2003. Impact of fish farming on fish stocks. International Aquafeed Directory 2003:24-29.

Ramseyer, L.J., and D.L. Garling. 1998. Effects of dietary protein to metabolizable energy ratios and total protein concentrations on the performance of yellow perch, *Perca flavescens*. Aquaculture Nutrition 4:217-223.

Riepe, J.R. 1998. Yellow perch markets in the North Central Region: Results of a 1996/97 survey. Purdue University, Department of Agricultural Economics, Bulletin No. 756.

Rumsey, G.L., A.K. Siwicki, D.P. Anderson, and P.R. Bowser. 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Veterinary Immunology and Immunopathology 41:323-339.

Shahidi, F. 2000. Antioxidant factors in plant foods and selected oilseeds. Biofactors 13:179-185.

Soyatech. 1995. Soya Bluebook Plus. Soyatach, Inc., Bar Harbor, Maine. 292 pp.

Spinelli, J., C. Mahnken, and M. Steinberg. 1979. Alternate sources of proteins for fish meal in salmonid diets. Pages 131-142 *in* J.E. Halver and K. Tiews, editors. Proceedings of the world symposium on finfish nutrition and fish feed technology, volume II. Berlin, Germany.

Spinelli, J., C.R. Houle, and J.C. Wekell. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30:71-83.

Tacon, A.G.J. 1992. Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fish Technical Paper, Rome: FAO 1-75.

Tiews, K., H. Koops, J. Gropp, and H. Beck. 1979. Compilation of fish meal-free diets obtained in rainbow trout (*Salmo gairdneri*) feeding experiments at Hamburg (1970-77/78). Pages 156-174 *in* J.E. Halver and K. Tiews, editors. Proceedings of the world symposium on finfish nutrition and fish feed technology, volume II. Berlin, Germany.

Torstensen, B.E., Ø. Lie, and L. Froyland. 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35:653-664.

Twibell, R.G., and P.B. Brown. 1997. Dietary arginine requirement of juvenile yellow perch. Journal of Nutrition 127:1838-1841.

Twibell, R.G., and P.B. Brown. 2000. Dietary choline requirement of juvenile yellow perch (*Perca flavescens*). Journal of Nutrition 130:95-99.

Twibell, R.G., D.D. Cartwright, K.A. Wilson, and P.B. Brown. 1998. Dietary lysine requirement of juvenile yellow perch. World Aquaculture '98. Las Vegas, Nevada.

Twibell, R.G., K.A. Wilson, and P.B. Brown. 2000. Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. Journal of Nutrition 130:612-616.

Twibell, R.G., M.E. Griffin, B. Martin, J. Price, and P.B. Brown. 2003. Predicting essential amino acid requirements for hybrid striped bass. Aquaculture Nutrition 9:373-382.

Webster, C.D., L.G. Tiu, and J.H. Tidwell. 1997. Effects of replacing fish meal in diets on growth and body composition of palmetto bass (*Morone saxatilis* x *M. chrysops*) raised in cages. Journal of Applied Aquaculture 7:53-68.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Indiana	Paul Brown Purdue University	Aquaculture/Nutrition
Illinois	Chris Kohler Southern Illinois University-Carbondale	Aquaculture
Michigan	Don Garling Michigan State University	Aquaculture/Nutrition
Wisconsin	Jeff Malison University of Wisconsin	Aquaculture/Endocrinology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Purdue University (Purdue)
Paul B. Brown

Southern Illinois University-Carbondale (SIUC)
Christopher C. Kohler

Michigan State University (MSU)
Donald L. Garling

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

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Purdue Research Foundation Office of Sponsored Programs West Lafayette, IN 47907-1021				**USDA AWARD NO.** Year 1: Objectives 1 & 2	
				Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul B. Brown				**FUNDS REQUESTED BY PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$17,606	
d. 1 Prebaccalaureate Students				$3,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$20,606	
B. Fringe Benefits (If charged as Direct Costs)				$1,310	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$21,916	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$8,084	
F. Travel 1. Domestic (Including Canada)				$2,000	
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) →				$32,000	
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) →				$32,000	
M. Other →					
N. Total Amount of This Request →				$32,000	$
O. Cost Sharing (If Required Provide Details)	$				

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 Purdue Research Foundation Office of Sponsored Programs West Lafayette, IN 47907-1021	**USDA AWARD NO.** Year 2: Objectives 1 & 2	
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)

	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				$18,134	
d. 1 Prebaccalaureate Students				$3,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$21,134	
B. Fringe Benefits (If charged as Direct Costs)				$1,396	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$22,530	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$8,970	
F. Travel 1. Domestic (Including Canada)				$2,000	
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) →				$33,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$33,500	
M. Other →					
N. Total Amount of This Request →				$33,500	$
O. Cost Sharing (If Required Provide Details)	$				

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 Purdue Research Foundation Office of Sponsored Programs West Lafayette, IN 47907-1021	**USDA AWARD NO.** Years 1 & 2: Objectives 1 & 2
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul B. Brown	Duration Proposed Months: 24 / Duration Awarded Months: ________

	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. 2 Graduate Students				$35,740	
d. 2 Prebaccalaureate Students				$6,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$41,740	
B. Fringe Benefits (If charged as Direct Costs)				$2,706	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$44,446	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$17,054	
F. Travel 1. Domestic (Including Canada)				$4,000	
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) →				$65,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$65,500	
M. Other →					
N. Total Amount of This Request →				$65,500	$
O. Cost Sharing (If Required Provide Details)	$				

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

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

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR PURDUE UNIVERSITY

(Brown)

Objectives 1 & 2

A. Salaries and Wages. Annual costs: one graduate student, and extra help (one summer hourly wage employee) to conduct the feeding trials and collect data.

B. Fringe Benefits. Benefit rates at Purdue are 0.4% for graduate students plus insurance ($937 in year 1 and $1,021 in year 2), and 10.1% for prebaccalaureate students.

E. Materials and Supplies. Year 1: fish ($1,000), fish food ($500), feed ingredients ($4,500), analytical reagents ($2,000), general wet-laboratory and record keeping supplies ($84). Year 2: fish ($1,000), fish food ($800), feed ingredients ($4,800), analytical reagents ($2,300), general wet-laboratory and record keeping supplies ($70).

F. Travel. Annual costs: partial support for PI to make presentation of project results at a multi-day conference in the United States. It is estimated that $1,500 per year will be needed to cover air and/or mileage and that $500 per year will be needed to help cover costs of food and lodging. U.S. Chapter meetings of the World Aquaculture Society, Fish Culture Section meetings of the American Fisheries Society, and World Aquaculture Society meetings when held in the U.S. are the likely meetings where presentations will be made.

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 Trustees Southern Illinois University-Carbondale Carbondale, IL 62901				**USDA AWARD NO.** Year 1: Objective 1	
				Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christopher C. Kohler				**FUNDS REQUESTED BY PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$14,000	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$3,750	
Total Salaries and Wages →				$17,750	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$17,750	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,000	
F. Travel 1. Domestic (Including Canada)				$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.)				$4,000	
J. Total Direct Costs (C through I) →				$23,750	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$23,750	
M. Other →					
N. Total Amount of This Request →				$23,750	$
O. Cost Sharing (If Required Provide Details)	$				

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	USDA AWARD NO. Year 2: Objective 1	
Board of Trustees Southern Illinois University-Carbondale Carbondale, IL 62901	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christopher C. Kohler	**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. ___ Other Professional					
c. 1 Graduate Students				$14,000	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 1 Technical, Shop and Other				$3,750	
Total Salaries and Wages →				$17,750	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$17,750	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,000	
F. Travel 1. Domestic (Including Canada)				$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.)				$4,000	
J. Total Direct Costs (C through I) →				$23,750	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$23,750	
M. Other →					
N. Total Amount of This Request →				$23,750	$
O. Cost Sharing (If Required Provide Details)	$				

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				USDA AWARD NO.	Years 1 & 2: Objective 1
Board of Trustees Southern Illinois University-Carbondale Carbondale, IL 62901				Duration Proposed Months: 24	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christopher C. Kohler				**FUNDS REQUESTED BY PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 2 Graduate Students				$28,000	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. 2 Technical, Shop and Other				$7,500	
Total Salaries and Wages →				$35,500	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$35,500	
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)				$2,000	
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.)				$8,000	
J. Total Direct Costs (C through I) →				$47,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$47,500	
M. Other →					
N. Total Amount of This Request →				$47,500	$
O. Cost Sharing (If Required Provide Details)	$				

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

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

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR SOUTHERN ILLINIOS UNIVERSITY-CARBONDALE

(Kohler)

Objective 1

A. Salaries and Wages. Salaries are needed for one 50% FTE graduate student per year, and extra help (one summer hourly wage employee) each year to conduct the feeding trials and collect and collate data.

E. Materials and Supplies. Annual costs: supplies needed include fish ($250), fish food ($500), general wet-laboratory, and office and record keeping supplies ($250) needed for the trials.

F. Travel. Annual costs: partial support for PI to make presentation of project results at a multi-day conference in the United States. It is estimated that $500 per year will be needed to cover air and/or mileage and that $500 per year will be needed to help cover costs of food and lodging. U.S. Chapter meetings of the World Aquaculture Society, Fish Culture Section meetings of the American Fisheries Society, and World Aquaculture Society meetings when held in the U.S. are the likely meetings where presentations will be made. All of these venues routinely hold sessions dealing with hybrid striped bass aquaculture and some include business meetings of the Hybrid Striped Bass Growers Association in which the PI holds Affiliate Membership.

I. All Other Direct Costs. Annual costs: telephone/fax ($250), photocopying ($250), equipment repair ($250), photos ($250), and feed manufacturing by Wenger Manufacturing, Inc. ($3,000).

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BUDGET

ORGANIZATION AND ADDRESS
Department of Fisheries & Wildlife
Michigan State University
East Lansing, MI 48824

USDA AWARD NO. Year 1: Objective 2

Duration Proposed Months: 12 — Duration Awarded Months: ________

PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S)
Donald L. Garling

	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				$18,000	
d. 1 Prebaccalaureate Students				$5,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$23,000	
B. Fringe Benefits (If charged as Direct Costs)				$750	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$23,750	
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)				$750	
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.)				$500	
J. Total Direct Costs (C through I) →				$29,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$29,000	
M. Other →					
N. Total Amount of This Request →				$29,000	$
O. Cost Sharing (If Required Provide Details)	$				

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		

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COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 2: Objective 2	
Department of Fisheries & Wildlife Michigan State University East Lansing, MI 48824	Duration Proposed Months: 12	Duration Awarded Months: ________
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Donald L. Garling	**FUNDS REQUESTED BY PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel a. ___ (Co)-PI(s)/PD(s)					$
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. 1 Graduate Students				$18,500	
d. 1 Prebaccalaureate Students				$4,120	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$22,620	
B. Fringe Benefits (If charged as Direct Costs)				$800	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$23,420	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,330	
F. Travel 1. Domestic (Including Canada)				$1,250	
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.)				$500	
J. Total Direct Costs (C through I) →				$28,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$28,500	
M. Other →					
N. Total Amount of This Request →				$28,500	$
O. Cost Sharing (If Required Provide Details)	$				

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

Form CSREES-55 (6/95)

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BUDGET

ORGANIZATION AND ADDRESS Department of Fisheries & Wildlife Michigan State University East Lansing, MI 48824				**USDA AWARD NO.** Years 1 & 2: Objective 2	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Donald L. Garling				Duration Proposed Months: 24 **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. 2 Graduate Students				$36,500	
d. 2 Prebaccalaureate Students				$9,120	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages →				$45,620	
B. Fringe Benefits (If charged as Direct Costs)				$1,550	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$47,170	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$7,330	
F. Travel 1. Domestic (Including Canada)				$2,000	
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.)				$1,000	
J. Total Direct Costs (C through I) →				$57,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$57,500	
M. Other →					
N. Total Amount of This Request →				$57,500	$
O. Cost Sharing (If Required Provide Details)	$				

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

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

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR MICHIGAN STATE UNIVERSITY

(Garling)

Objective 1

A. Salaries and Wages. Laboratory studies will be conducted with the assistance of a graduate student and one undergraduate student. Responsibilities of the student will include diet analysis, general fish culture, and laboratory analyses.

B. Fringe Benefits. MSU requires medical coverage for graduate students estimated to be $750 for Year 1 and $800 for Year 2.

E. Materials and Supplies. Year 1: fish ($1,000), feed ingredients ($2,000), and chemicals ($1,000) will be required. Year 2: fish ($1,000), feed ingredients ($1,500), and chemicals ($800) will be required.

F. Travel. Domestic travel will be required to obtain fish. It is estimated that the PI and students will travel within the NCR to acquire fish, spending no more than 3 nights away from East Lansing in each year.

I. All Other Direct Costs. Annual costs: telephone ($300), fax ($100), and photocopying ($100).

UNITED STATES DEPARTMENT OF AGRICULTURE
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OMB Approved 0524-0022
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BUDGET

ORGANIZATION AND ADDRESS Board of Regents University of Wisconsin System 750 University Ave., Madison, WI 53706	**USDA AWARD NO.** Year 1: 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				
	Calendar	Academic	Summer	FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY CSREES (If Different)
A. Salaries and Wages					$
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. _1_ Technical, Shop and Other				$9,000	
Total Salaries and Wages . . . . →				$9,000	
B. Fringe Benefits (If charged as Direct Costs)				$2,970	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . →				$11,970	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,530	
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) . . . . →				$14,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . →				$14,500	
M. Other . . . . →					
N. Total Amount of This Request . . . . →				$14,500	$
O. Cost Sharing (If Required Provide Details)	$				

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
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Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Board of Regents University of Wisconsin System 750 University Ave., 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)
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. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. _1_ Technical, Shop and Other				$9,000	
Total Salaries and Wages →				$9,000	
B. Fringe Benefits (If charged as Direct Costs)				$2,970	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$11,970	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,030	
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) →				$15,000	
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) →				$15,000	
M. Other →					
N. Total Amount of This Request →				$15,000	$
O. Cost Sharing (If Required Provide Details)	$				

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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
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BUDGET

ORGANIZATION AND ADDRESS Board of Regents University of Wisconsin System 750 University Ave., Madison, WI 53706	**USDA AWARD NO.** Years 1 & 2: Objective 2	
	Duration Proposed Months: 24	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. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. _2_ Technical, Shop and Other				$18,000	
Total Salaries and Wages →				$18,000	
B. Fringe Benefits (If charged as Direct Costs)				$5,940	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$23,940	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,560	
F. Travel 1. Domestic (Including Canada)				$2,000	
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) →				$29,500	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) →				$29,500	
M. Other →					
N. Total Amount of This Request →				$29,500	$
O. Cost Sharing (If Required Provide Details)	$				

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

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

Form CSREES-55 (6/95)

BUDGET EXPLANATION FOR THE UNIVERSITY OF WISCONSIN-MADISON

(Malison)

Objective 2

A. Salaries and Wages. The salary for 3 months of a technician's time for each year is requested for the PI to perform the work indicated.

B. Fringe Benefits. The UW-Madison fringe benefit rate for faculty and academic staff is 33%.

E. Materials and Supplies. Year 1: chemical reagents ($1,000), and replacement air lines and air stones ($530) are needed for the project. Year 2: chemical reagents ($1,500), and replacement air stones ($530) are needed.

F. Travel. $1,000 is requested each year for travel between the UW-Madison campus and the Lake Mills State Fish Hatchery (50 trips per year, 70 miles per trip @ $0.286 per mile).

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

Year 1

	Purdue	SIUC	MSU	UW-Madison	TOTALS
Salaries and Wages	$20,606	$17,750	$23,000	$9,000	$70,356
Fringe Benefits	$1,310	$0	$750	$2,970	$5,030
Total Salaries, Wages, and Fringe Benefits	$21,916	$17,750	$23,750	$11,970	$75,386
Nonexpendable Equipment	$0	$0	$0	$0	$0
Materials and Supplies	$8,084	$1,000	$4,000	$1,530	$14,614
Travel	$2,000	$1,000	$750	$1,000	$4,750
All Other Direct Costs	$0	$4,000	$500	$0	$4,500
TOTAL PROJECT COSTS	$32,000	$23,750	$29,000	$14,500	$99,250

Year 2

	Purdue	SIUC	MSU	UW-Madison	TOTALS
Salaries and Wages	$21,134	$17,750	$22,620	$9,000	$70,504
Fringe Benefits	$1,396	$0	$800	$2,970	$5,166
Total Salaries, Wages, and Fringe Benefits	$22,530	$17,750	$23,420	$11,970	$75,670
Nonexpendable Equipment	$0	$0	$0	$0	$0
Materials and Supplies	$8,970	$1,000	$3,330	$2,030	$15,330
Travel	$2,000	$1,000	$1,250	$1,000	$5,250
All Other Direct Costs	$0	$4,000	$500	$0	$4,500
TOTAL PROJECT COSTS	$33,500	$23,750	$28,500	$15,000	$100,750

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

LIST OF PRINCIPAL INVESTIGATORS

Paul B. Brown, Purdue University

Christopher C. Kohler, Southern Illinois University-Carbondale

Donald L. Garling, Michigan State University

Jeffrey A. Malison, University of Wisconsin-Madison

VITA

Paul B. Brown
Department of Forestry and Natural Resources
Purdue University
715 West State Street
West Lafayette, IN 49707-2061

Phone: (765) 494-4968
Fax: (765) 496-2422
E-mail: pb@purdue.edu

EDUCATION

B.S. University of Tennessee, 1980, Wildlife and Fisheries Sciences
M.S. University of Tennessee, 1983, Fish Nutrition
Ph.D. Texas A&M University, 1987, Aquatic Animal Nutrition

POSITIONS

Professor (1997-present), Associate Professor (1993-1997), and Assistant Professor (1989-1993) of Aquaculture, Department of Forestry and Natural Resources, Purdue University
Assistant Professional Scientist (1987-1989), Illinois Natural History Survey
Adjunct Assistant Professor (1988-1989), Department of Animal Sciences, University of Illinois

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Institute of Nutrition
Society for Comparative Nutrition
Society for Integrative and Comparative Biology (formerly, American Society of Zoologists)
World Aquaculture Society

SELECTED PUBLICATIONS

Gould, N.L., M.M. Glover, L.D. Davidson, and P.B. Brown. 2003. Dietary flavor additives influence consumption of feeds by yellow perch, *Perca flavescens*. Journal of the World Aquaculture Society 34:412-417.

Twibell, R.G., M.E. Griffin, B. Martin, J. Price, and P.B. Brown. 2003. Predicting essential amino acid requirements for hybrid striped bass. Aquaculture Nutrition 9:373-382.

Brown, P.B., K.A. Wilson, Y. Jonker, and T.E. Nickson. 2003. Glyphosate-tolerant canola meal is equivalent to the parental line in diets fed to rainbow trout. Journal of Agricultural and Food Chemistry 51:4268-4272.

Bharadwaj, A.S., N.L. Gould, P.B. Brown, M.R. White, and A. Moore. 2002. Evaluation of experimental and practical diets for walleye *Stizostedion vitreum*. Journal fo the World Aquaculture Society 33:244-253.

Bharadwaj, A., W.R. Brignon, N.L. Gould, P.B. Brown, and Y.V. Wu. 2002. Evaluation of meat and bone meal in practical diets fed to juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis*. Journal of the World Aquaculture Society 33:448-457.

Brown, P.B., J.E. Wetzel, J. Mays, K.A. Wilson, C.S. Kasper, and J. Malison. 2002. Growth differences between stocks of yellow perch (*Perca flavescens*) are temperature dependent. Journal of Applied Aquaculture 12:43-56.

Twibell, R.G., B.A. Watkins, and P.B. Brown. 2001 Dietary conjugated linoleic acids and lipid source alter fatty acid composition of juvenile yellow perch, *Perca flavescens*. Journal of Nutrition 131:2322-2328.

Twibell, R.G., B.A. Watkins, L. Rogers, and P.B. Brown. 2000. Dietary conjugated linoleic acids alter hepatic and muscle lipids in hybrid striped bass. Lipids 35:155-161.

VITA

Donald L. Garling, Jr.
Department of Fisheries and Wildlife
Michigan State University
9A Natural Resources Building
East Lansing, MI 48824-1222

Phone: (517) 353-1989
Fax: (517) 432-1699
E-mail: garlingd@msu.edu

EDUCATION

B.S. University of Dayton, 1970, Biological Sciences
M.S. Eastern Kentucky University, 1972, Biological Sciences
Ph.D. Mississippi State University, 1975, Zoology–Fisheries Option

POSITIONS

Professor (1990-present), Associate Professor (1985-1990), and Assistant Professor (1980-1985), Department of Fisheries and Wildlife, Michigan State University

Aquaculture and Fisheries 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 Institute and State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Comparative Nutrition Society
World Aquaculture Society

SELECTED PUBLICATIONS

Nichols, S.J., and Garling, D.L. 2003. Evaluation of substitute diets for live algae in the captive maintenance of adult and subadult freshwater Unionidae. Journal of Shellfish Research 21(2):875-881.

Riche, M., N.L. Trottier, P.K. Ku, and D. Garling. 2001. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. Fish Physiology and Biochemistry 25(3):181-194. (Note: This article was actually published in 2003 with a 2001 publication date.)

Ramseyer, L.J., D.Garling, G. Hill, and J. Link. 1999. Effect of dietary zinc supplementation on phytase pre-treatment of soybean meal or corn gluten meal on growth, zinc status and zinc-related metabolism in rainbow trout, *Oncorhynchus mykiss*. Fish Physiology and Biochemistry 20:251-261.

Ramseyer, L.J., and D.L. Garling. 1998. Effects of dietary protein-to-metabolizable energy ratios and total protein concentrations on the performance of yellow perch, *Perca flavescens*. Journal of Aquaculture Nutrition 4:217-224.

Ostrowski, A.C.,and D.L. Garling. 1997. Test species and protocol guidelines: marine and freshwater finfish. Pages 288-302 *in* Proceedings of the Workshop on Expanding Agriculture Co-Product Uses in Aquaculture. Midwest Feeds Consortium, Des Moines, Iowa, December 5-7, 1994.

Brown, P., K. Dabrowski, and D. Garling. 1996. Nutrition and feeding yellow perch (*Perca flavescens*). Journal of Applied Ichthyology 12:171-174.

VITA

Christopher C. Kohler
Fisheries & Illinois Aquaculture Center
Southern Illinois University-Carbondale
Mail Code 6511
Carbondale, IL 62901-6511

Phone: (618) 453-2890
Fax: (618) 453-6095
E-mail: ckohler@siu.edu

EDUCATION

B.S. St. Mary's College of Maryland, 1973, Biology
M.S. University of Puerto Rico, 1975, Marine Biology/Aquaculture
Ph.D. Virginia Polytechnic Institute and State University, 1980, Fisheries Science

POSITIONS

Director (1993-present), Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale
Professor (1993-present), Associate Professor (1989-1993), and Assistant Professor (1986-1989), Department of Zoology, Southern Illinois University-Carbondale
Senior Scientist (1980-1986), Fisheries Research Laboratory, Southern Illinois University-Carbondale

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society (President, Fish Culture Section, 1999-2000; AFS 2nd Vice President-President, 2002-2006)
World Aquaculture Society

SELECTED PUBLICATIONS

Kelly, A.M., and C.C. Kohler. 2003. Utilization of Mcro-Aid (*Yucca* sp.) As a feed additive for channel catfish*Ictalurus punctatus* and the tilapia hybrid *Oreochromis mossambicus* × *O. niloticus*. Journal of the World Aquaculture Society 34:156-161.

Kelly, A.M., C.C. Kohler, and D.M. Hughes. 2002. Menhaden meal in practical diets for channel catfish fry and fingerlings reared in indoor recirculating systems. North American Journal of Aquaculture 64:290-293.

Kohler, C.C., R.J. Sheehan, J.J. Myers, J.B. Rudacille, M.L. Allyn, and A.V. Suresh. 2001. Performance comparison of geographic strains of white bass to produce sunshine bass. Aquaculture 202:351-357.

Kohler, C.C. 2000. Striped bass and hybrid striped bass culture. Pages 898-907 *in* R.R. Stickney, editor. Encyclopedia of aquaculture. John Wiley & Sons, Inc., New York.

Suresh, A.V., J.B. Rudacille, M.L. Allyn, V. Sheehan, R.J. Sheehan, and C.C. Kohler. 2000. Single injections of hCG or mGnRHa at low dosages induce ovulation in white bass. North American Journal of Aquaculture 62:87-94.

Rudacille, J.R., and C.C. Kohler. 2000. Aquaculture performance comparison of sunshine bass, palmetto bass, and white bass. North American Journal of Aquaculture 62:114-124.

Kelly, A.M., and C.C. Kohler. 1999. Cold tolerance and fatty acid composition in striped bass, white bass, and their hybrids. North American Journal of Aquaculture 61:278-285.

Kelly, A.M., and C.C. Kohler. 1996. Sunshine bass performance in ponds, cages, and indoor tanks. Progressive Fish-Culturist 58:55-58.

Brecka, B.J., C.C. Kohler, and D.H. Wahl. 1995. Effects of dietary protein concentration on growth, survival, and body composition of muskellunge and tiger muskellunge fingerlings. Journal of the World Aquaculture Society 26:416-425.

VITA

Jeffrey A. Malison
University of Wisconsin Aquaculture Program
Department of Animal Sciences
University of Wisconsin-Madison
1675 Observatory Dr.
Madison, WI 53706

Phone: (608) 263-1242
Fax: (608) 262-5157
E-mail: jmalison@wisc.edu

EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Sciences
American Fisheries Society
World Aquaculture Society

SELECTED PUBLICATIONS

Roberts, S., T. Barry, J. Malison, and F. Goetz. In press. Production of a recombinantly derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture.

Barry, T.P., and J.A. Malison, editors. 2004. Proceedings of Percie III: The Third International Percid Fish Symposium. University of Wisconsin Sea Grant Institute, Madison, Wisconsin. 136 pp.

Mandiki, R.S.M., J.A. Malison, J.A. Held, C. Rougeot, G. Blanchard, C. Mélard, and P. Kestemont. 2004. Hybridization of Eurasian and yellow perch increases growth rate of offspring. Pages 47-48 *in* Barry, T.P. and J.A. Malison, editors. Proceedings of Percis III: The Third International Percid Fish Symposium. University of Wisconsin Sea Grant Institute, Madison, Wisconsin.

Jentoft, S., J.A. Held, J.A. Malison, and T.P. Barry. 2002. Ontogeny of the cortisol stress response in yellow perch (*Perca flavescens*). Fish Physiology and Biochemistry 26:371-378.

Brown, P.B., J.E. Wetzel, J. Mays, K.A. Wilson, C.S. Kasper, and J. Malison. 2002. Growth differences between stocks of yellow perch (*Perca flavescens*) are temperature dependent. Journal of Applied Aquaculture 12:43-55.

Barry, T.P., M.J. Unwin, J.A. Malison, and T.P. Quinn. 2001. Free and total cortisol levels in semelparous and iteroparous chinook salmon. Journal of Fish Biology 59:1673-1676.

Malison, J.A., J.A. Held, L.S. Weil, T.B, Kayes, and G.H. Thorgaard. 2001. Manipulation of ploidy in walleye (*Stizostedion vitreum*) by heat shock and hydrostatic pressure shock. North American Journal of Aquaculture 63:17-24.

Weil, L.S., T.P. Barry, and J.A. Malison. 2001. Fast growth in rainbow trout is correlated with a rapid decrease in post-stress cortisol concentrations. Aquaculture. 193:373-380.