TILAPIA AQUACULTURE IN THE NORTH CENTRAL REGION

Chairperson: Donald L. Garling, Michigan State University

Industry Advisory Council Liaison: Curt Stutzman, Kalona, Iowa

Extension Liaison: Donald L. Garling, Michigan State University

Funding Request: $120,000

Duration: 2 years (September 1, 1996 - August 31, 1998)

Objectives:

1a. Develop and/or identify cost-effective feeds for tilapia culture in recirculating systems that minimize waste generation.

1b. Compare and evaluate economically important traits of current commercial tilapia strains in the North Central Region with other strains cultured in recirculating systems.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Year 2	Total
Purdue University	Paul B. Brown	1a	$17,600		$17,600
Illinois State University	Kerry W. Tudor	1a	$16,400		$16,400
Michigan State University	Donald L. Garling	1a	$19,000		$19,000
Ohio State University	Konrad Dabrowski	1a	$8,000	$8,000	$16,000
	Paul A. Fuerst	1b	$9,500	$9,500	$19,000
Southern Illinois University-Carbondale	Christopher C. Kohler	1a & 1b	$16,000	$16,000	$32,000
		TOTALS	**$86,500**	**$33,500**	**$120,000**

Non-funded Collaborators:

Facility	Collaborator
USDA, NCAUR, Peoria, Illinois	Dr. Victor Wu

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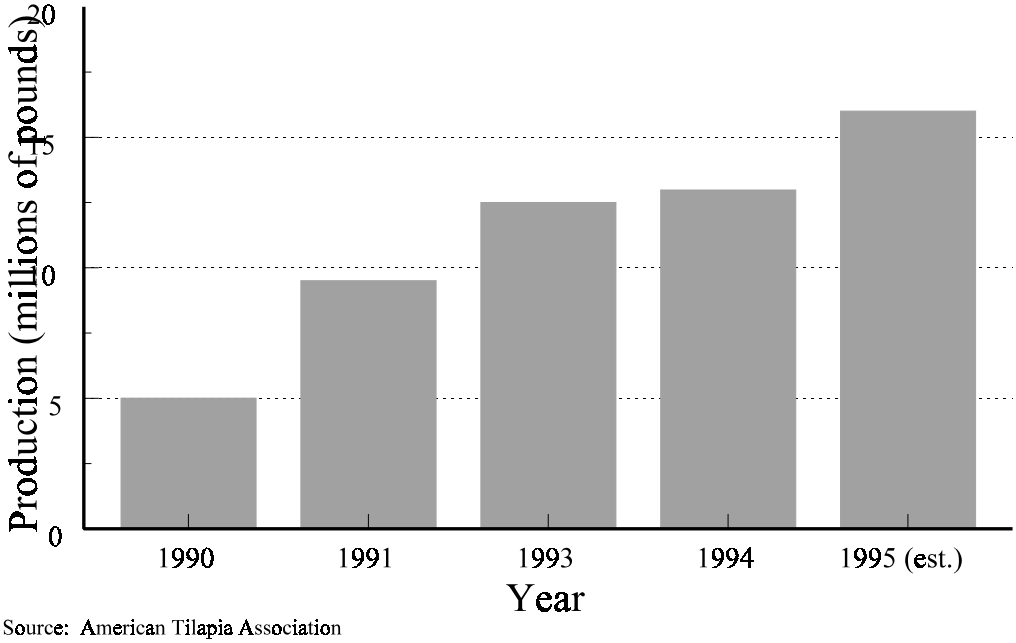
JUSTIFICATION

The American Tilapia Association (ATA) has compiled production and import statistics for a recent tilapia situation and outlook report (Anonymous 1995). Total U.S. sales of tilapia increased 38% from 1993 to 1994 from 20.3 to 28 million kg (44.8 to 61.8 million lbs). As a result, tilapia consumption surpassed trout consumption in the U.S. for the first time in 1994. Growth in sales increased primarily from imports of frozen fillets primarily originating in Taiwan, Thailand, and Indonesia. Estimated domestic tilapia production rose modestly in 1994 compared to earlier trends (Figure 1) increasing from 5.6 to 5.9 million kg (12.5 to 13 million lbs). Farm gate prices for live tilapia ranged from $2.60 to $3.30 per kg ($1.20 to $1.50 per lb) while fillet prices ranged from $7.27 to $11.00 per kg ($3.30 to $5.00 per lb) in 1994.

Figure 1. Farm Raised Tilapia Production in the United States



Source: American Tilapia Association

The North Central Region (NCR) produced an estimated 635,000 kg (1.4 million lbs) of tilapia, about 11% of total U.S. production, in 1994. With changes in management and renewed expansion activities at two of the major tilapia production facilities in the NCR, the ATA is projecting significant growth in tilapia production within the region (Anonymous 1995). While the opportunity for commercial tilapia culture in the U.S. and the NCR is real, there are several problem areas. In February of 1995, the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council (IAC) identified two areas of research needed to enhance the economic viability of the existing tilapia aquaculture operations and to facilitate industry expansion within our region. Each area was assigned equal importance as indicated by listing the research objectives as 1a and 1b. The two areas of research identified by the NCRAC IAC were feed development for tilapia emphasizing waste reduction culture in recirculating systems (Objective 1a) and evaluation of commercially available tilapia strains (Objective 1b).

Feed Development for Tilapia Culture in Recirculating Systems (Objective 1a)

This project objective will focus on development of commercial tilapia diets containing locally available plant protein sources (Purdue University and Illinois State University) or animal by-product meals (Ohio State University), enzymatic pretreatment of plant proteins to enhance utilization and minimize ammonia production (Michigan State University), and the use of an extract of the plant *Yucca shidigera* to reduce ammonia production (Southern Illinois University-Carbondale).

Feed costs are typically one of the most expensive annual variable costs in aquaculture; thus, reducing feed costs facilitates industrial development. More importantly, tilapia in the NCR are raised in recirculating systems. Excess feed or undigested portions of feeds in the system must be removed, often at great expense. Crude protein (CP) in excess of the animal's requirement leads to excretion of ammonia from the gills and multiple forms of nitrogen in the feces (Kaushik and Cowey 1991). Nitrogen in the feces can be converted to the typical ammonia-nitrite-nitrate cycle present in recirculating systems by the bacteria present (Liao and Mayo 1974). Thus, dietary formulation must consider maximizing dry matter and CP digestibility to minimize waste in the system and maximize profits.

To maximize efficiency, animals should be fed a diet that provides all required nutrients in adequate amounts. Ingredients used to formulate the diet should be low cost, yet high quality. About 10% of the world's fish meal is currently used for aquaculture diet formulation; demand for high quality fish meals is increasing causing increased prices. In diets fed to fish, one of the typical goals is to reduce the concentration of fish meal in diets for the target species by incorporating high-quality feedstuffs of plant origin or animal co-products. All-plant diets have been developed for tilapia (Wu et al. 1994; 1995). Diets containing no fish meal have been developed and are fed in commercial settings in this region. However, those diets are only preliminary attempts and the critical factors listed above have not been evaluated.

The minimal dietary CP concentration for tilapia varies with production system, age, and numerous other factors. Current production systems can be viewed as a continuum, starting with earthen culture ponds, then cages, then indoor recirculating systems. Feeds for tilapia reared in earthen culture ponds often contain lower levels of CP than feeds fed to fish reared in cages, with higher protein needs for fish reared in tank culture. Fish raised in outdoor settings can have significant sources of available food in the normal pond biota. The amount is usually greater in ponds than in cages because of the obvious limitation of space and access to natural pond biota. In tank culture, feeds must be more nutritionally complete due to the lack of other food items. As an example of this continuum, current estimates of the minimal level of CP that supports maximum weight gain in tilapia raised in ponds can be as low as 20-25%, while the minimum for cage culture is approximately 28%. The optimal CP concentration for tilapia raised in tanks is thought to be closer to 30-35% (Luquet 1991). Most of the estimates developed did not balance essential amino acid concentrations to meet the recently established requirements (Santiago and Lovell 1988). Dietary amino acid concentrations in excess of the dietary requirement lead to excretion of ammonia. The principal investigators on this project have funding in place to determine the minimal concentration of dietary CP in all-plant diets fed to tilapia (see **RELATED CURRENT AND PREVIOUS WORK**). The next logical step is determination of the optimal energy to protein ratio (E:P).

Nutrients ingested by animals provide the necessary substrates for biochemical reactions. One of the most important reactions is the synthesis of adenosine triphosphate (ATP). ATP serves as the primary source of cellular energy in vertebrates. Three different categories of macronutrients can supply the necessary substrate for synthesis of ATP; lipid, carbohydrate or protein. One of the fundamental goals in development of diets for target species is establishing optimal E:P, specifically the ratio of energy from lipid and carbohydrate to the concentration of protein. Essentially, nutritionists seek to provide adequate energy in the form of lipid and carbohydrate sparing the absorbed amino acids for protein synthesis. This has been termed the protein sparing effect. While the optimal E:P has been explored in tilapia, it has not been explored in fish fed all-plant diets and with diets containing the established essential amino acid requirements.

Utilization of plant protein sources by fishes may be enhanced by pre-treatment of plant feedstuffs high in phytin with the enzyme phytase. For example, soybeans have antinutritional factors (ANF) with the potential to reduce their biological value and result in pathological states in monogastric animals (Rackis 1974; Liener 1994). Phytic acid (1,2,3,5/4,6-hexakis dihydrogen phosphate), one such ANF, exists as a salt of mono- and divalent cations in legumes and cereals. Phytate, the salt of phytic acid, binds divalent cations making them unavailable, resulting in mineral deficiencies. Reduced mineral bioavailability has been shown in rainbow trout (Spinelli et al. 1983; Riche 1993; Cain and Garling 1995), channel catfish (Satoh et al. 1989), carp (Hossain and Jauncey 1993), and tilapia (McClain and Gatlin 1988). Treatment of soybean meal (SBM) with phytase prior to inclusion into fish feeds has improved phosphorous and protein utilization (Cain and Garling 1995).

The U.S. rendering industry processed about 16.3 million metric tons of inedible co-products in 1991 from the slaughter of domestic animals (F. Bisplinghoff, personal communication). Most animal by-products cannot be used as direct replacements for fish meals in fish diet formulation due to imbalances in essential amino acids (EAA) or low digestibility of certain proteins. Additives of synthetic EAA produced in the U.S. (methionine and lysine) have facilitated development of specific guidelines for the application of animal by-products in salmonid diets. The data required for least-cost and least-polluting diet formulation for tilapia cultured in recirculated systems are not presently available to enable replacement using mixtures of animal by-products with or without additional synthetic amino acids.

In addition to nutritional manipulations to reduce fecal volume, other strategies can be taken to reduce the major elemental components of the feces, namely nitrogen and phosphorus. An extract of the *Yucca shidigera* plant has shown promise in the control of ammonia with various terrestrial livestock animals. It is not known if the reduction is due to urease inhibition, increased use of ammonia (Jacques and Bastien 1989), or direct binding of ammonia (Headon and Dawson 1990). The utility of using this extract for fish needs further examination (see **RELATED CURRENT AND PREVIOUS WORK**). Previous studies (Tidwell et al. 1992; Kelly and Kohler, Southern Illinois University-Carbondale, personal communication) utilized channel catfish and two different commercial sources of *Y. shidigera* extract. These extracts can contain at least three steroid saponins (Kaneda et al. 1987), but the exact extraction procedures utilized by different companies conceivably can result in significantly different levels of active compounds in the end products. Specifically, it appears that the saponin component of the extract can be removed without eliminating its ammonia reduction capabilities (D.R. Headon, University College, Galway, Ireland, personal communication to Tidwell et al. 1992). Accordingly, the extract used in the Kelly and Kohler study may prove highly useful in reducing nitrogen content in tilapia feces. Moreover, the long intestinal tract of tilapia compared to channel catfish may be more conducive for nitrogen reduction.

Evaluation of Commercially Available Tilapia Strains (Objective 1b)

Tilapia of the genus *Oreochromis* are native to the rivers and lakes of Eastern Africa. Several species including *O. niloticus* (the Nile tilapia) and *O. aureus*, are used widely in aquaculture. Genetic studies using molecular markers to characterize the various commercial strains of these species, and their hybrids, have only recently been started (Macaranas et al. 1986; Harris et al. 1991; Seyoum and Kornfield 1992). It is not clear how "strains" currently being used in U.S. aquaculture are related to one another, nor how homogeneous are different lines of the same "strain." The purpose of this portion of the proposal is to evaluate the genetic differentiation which has occurred among different strains in the U.S. aquacultural industry, to determine the levels of genetic variability which exist within the aquaculture strains in this country, and to evaluate the relative hybrid contributions of the two major pure species (*O. niloticus* and *O. aureus*) which contribute to U.S. stocks.

It is unclear which, if any, of the current strains/hybrids cultured in the NCR are most suitable for rearing in recirculating systems. Comparative performance evaluations in recirculating systems under controlled conditions are needed as an initial assessment. These strains/hybrids need to be compared to "pure" *O. niloticus* strains because many in the tilapia culture industry believe this to be the superior species (Curtis Stutzman, ATA, Kolona, Iowa, personal communication); also, *O. niloticus* are receiving considerable strain evaluation in the Philippines (Bolivar et al. 1993; Eknath et al. 1993). Alternatively, a hybrid between *O. niloticus* and *O. aureus* might prove more suitable as has been found in Israel (Hulata et al. 1993). However, strain evaluations taking place in the Philippines and Israel are not specifically aimed at performance in recirculating systems.

RELATED CURRENT AND PREVIOUS WORK

Feed Development for Tilapia Culture in Recirculating Systems (Objective 1a)

A major limiting constraint associated with rearing tilapia in reuse systems is the accumulation of nutrients, particularly nitrogenous products, and solids. The nitrogenous wastes associated with feeds and feeding are divided into a solid fecal fraction and a soluble fraction associated with gill and urinary excretions. A number of mechanical methods for the separation and removal of the solid fraction exist and this area continues to be a rich topic for aquaculture engineers. While numerous biofiltration devices exist for handling the soluble

fraction, they all utilize nitrifying bacteria for the oxidation of ammonia to the end product nitrate. However, the efficacy of nitrifying bacteria is variable and contingent upon relatively unpredictable and often hard to manage parameters such as dissolved oxygen (DO), pH, temperature, CO_2, BOD, and competitive heterotrophic bacteria, as well as circulating ammonia, nitrite, and nitrate levels (Wheaton 1993).

The difficulty and expense associated with nitrogen and solids removal makes reducing wastes entering the system the most viable and desirable alternative. Cho (1993) has estimated that 10% of ingested nitrogen is excreted in the feces with values as high as 66% excreted as ammonia. These values are based on feeding high quality fish meal proteins and are higher for lower quality plant protein substituted diets. Despite these statistics the industry is moving towards replacing expensive and sometimes unavailable fish meal products with less expensive plant protein feedstuffs. This makes solids reduction and increased nitrogen retention an obvious starting point for reducing nitrogen inputs into the system.

Nutritional research with various species and hybrids of tilapia has been conducted for many years (Luquet 1991; Lim 1989; National Research Council 1993). Indeed, tilapia nutrition should be considered similar to catfish and trout in that we know a great deal about their nutritional needs and ability to utilize various feedstuffs. However, the majority of those studies have been conducted with juvenile fish, usually with an initial weight of 1-10 g. There are limited evaluations of how the optimal E:P changes as fish increase in size, although this topic is being explored in diets fed to catfish (Dr. E. Robinson, Mississippi State University, personal communication). Winfree and Stickney (1981) identified that the E:P ratio changes as fish size increases, but the comparisons were with fish of initial weight of either 2.5 or 7.5 g. We suggest that if E:P ratios change at these sizes, then the ratio most likely changes as fish attain larger sizes. Nutritional studies with tilapia are numerous and the pertinent aspects related to this proposal are found in the reviews listed above.

The most promising plant protein alternatives identified are soybean products. However, partial or complete replacement of fish meal with soy products has met with mixed success in tilapia species (Davis and Stickney 1978; Jackson et al. 1982; Viola and Arieli 1983; Shiau et al.1987; 1989; 1990; Davies et al. 1989; De Silva and Gunasekera 1989; El-Dahhar and El-Shazly 1993). Generally poor performance was in direct relationship to the level of fish meal replacement (Shiau et al. 1990). Reasons cited for decreased performance (reduced growth, protein efficiency ratio [PER], net protein utilization [NPU], and increased feed conversion ratio [FCR]) were residual trypsin inhibitors, unbalanced amino acids, methionine deficiency or undigestible polysaccharide ANF. However, evidence to support these conclusions was lacking and fish failed to respond to attempts correcting for these factors.

SBM based diets supplemented with methionine (Shiau et al. 1987; Shiau et al. 1989) or methionine and lysine (El-Dahhar and El-Shazly 1993) did not increase performance to the level of control diets. Methionine was not limiting in diets with 24% CP but was limiting at 32% CP, and methionine supplementation only had a significant effect on performance parameters when diets contained SBM as the sole source of protein (Shiau et al. 1989). However, methionine levels in these studies were above requirements determined for tilapia (Jackson and Capper 1982). Substitution of SBM for at 30% of the protein had no significant effect on total protein or dry matter digestibility, but led to a significant reduction at greater than 33% substitution (Shiau et al. 1987; Shiau et al. 1989). These findings would indicate bioavailability of methionine is less than expected, or the available amino acids are unbalanced. The currently suggested maximum replacement of fish meal with SBM in tilapia diets is 30% of the protein (Shiau et al. 1990).

Phytic acid may be a primary ANF (see **JUSTIFICATION** section above) reducing the usefulness of soybean products. Phytin complexes with proteins via direct bonding with phytic acid and through mineral-phytate interactions (Cheryan 1980; Reddy et al. 1989). In acidic environments, such as Tilapine stomachs (pH 2.0), half of the phosphorus moieties of phytic acid are negatively charged creating an environment favorable for binding proteins with ϵ-amino groups on lysine, imidazole groups on histidine, and guanidyl groups on arginine (Cain and Garling 1995). In alkaline environments, such as Tilapine intestine (pH 8.5-8.8) protein-cation-phytate complexes are favored. These protein-phytate and protein-mineral-phytate complexes are more resistant to proteolytic digestion *in vitro* and *in vivo* (Sigh and Krikorian 1982; Satterlee and Abdul-Kadir 1983; Grabner and Hofer 1985; Knuckles et al. 1985; Carnovale et al. 1988; Vaintraub and Bulmager 1991; Caldwell 1992). Decreased protein digestibility of diets supplemented with salts of phytic acid led to depressed growth

and poor performance in rainbow trout (Spinelli et al. 1983), chinook salmon (Richardson et al. 1985), and carp (Hossain and Jauncey 1993).

Fish, like other monogastric animals, are unable to hydrolyze the complexing phosphorus due to a lack of intestinal secretions of the hydrolytic enzyme phytase. However, diets prepared with phytase either as an enzymatic pretreatment or as an additive result in better performance. Cain and Garling (1995) found rainbow trout fed diets pretreated with phytase exhibited superior growth compared to fish fed the same diets without pretreatment and suggested the increased performance may be attributed to improved protein quality.

The addition of microbial phytase to swine diets significantly increased total tract digestibility of CP and all amino acids except cystine and proline, and ileal digestibility of methionine and arginine were also significantly increased (Mroz et al. 1994). Additionally, nitrogen retention was increased and daily nitrogen excretion was reduced 20-25% indicating improved amino acid balance. Similarly, nitrogen balance studies in rats indicate significantly higher fecal and urinary nitrogen losses when fed a high phytate bran flour diet (Satterlee and Abdul-Kadir 1983). These investigators also demonstrated increased protein digestibility, PERs, and biological value with diets containing lower phytate levels using both *in vitro* and *in vivo* methods.

The limiting factor in plant protein substitution for fish meal in tilapia diets is the reduced digestibility and availability of nutrients from plant proteins. Pretreatment of plant proteins with phytase should render phytate incapable of sequestering proteins and minerals making these nutrients more available to the animal. Additionally, the hydrolysis of phytate should decrease the inhibitory effect observed on gastric and intestinal proteolytic enzymes and thereby increase CP digestibility. Assuming energy is not a limiting factor, increased amino acid availability would increase protein accretion and could decrease amino acid catabolism and ammonia excretion. Both solid and soluble nitrogen would be reduced minimizing nitrogen inputs into the system.

An extract of the *Yucca shidigera* plant has also been successfully used to control ammonia accumulation with various livestock animals (Berg 1977; Rowland et al. 1979; Jacques and Bastien 1989). It also appears to improve growth when incorporated into feeds for poultry (Johnston et al. 1981; 1982), swine (Foster 1983; Cromwell et al. 1985; Mader and Brumm 1987), and cattle (Goodall and Matsushima 1980). However, Tidwell et al. (1992) found that although *Yucca shidigera* may have value as a preconditioning agent for water recirculating systems, it decreased growth rates of juvenile channel catfish when used as a feed additive. This study utilized a powdered extract commercially sold under the trade name De-Oderase (Alltech Biotechnology Center, Nicholasville, Kentucky). Studies conducted at Southern Illinois University-Carbondale (SIUC) with a *Y. shidigera* extract sold under the commercial name Micro-Aid (Distributors Processing Inc., Porterville, California) were more positive (Kelly and Kohler, personal communication). While juvenile channel catfish (9.0 ± 1.0 g mean initial weight) fed 0.5 and 1.0 g Micro-Aid/kg feed did not grow significantly different than controls, channel catfish fry (0.2 ± 0.1 g initial mean weight) fed 1.0 g Micro-Aid/kg feed grew significantly faster than controls and the 0.5 g Micro-Aid/kg feed treatment. Moreover, nitrogen content of feces from channel catfish fed 1.0 g Micro-Aid/kg feed was significantly lower, possibly indicating improved protein digestion/utilization. No adverse effects in feeding or growth were noted when catfish were fed up to 5 g Micro-Aid/kg feed. It appears that Micro-Aid is a *Y. shidigera* product that offers considerably more potential for use in aquaculture than De-Oderase.

Diets used in intensive fish culture are formulated on the basis of measured values for metabolizable or digestible energy (DE). However, for tilapia only limited data of feedstuff energy and protein digestibility values are available (Hanley 1987; Anderson et al. 1991). DE values for plant-derived feedstuffs were higher for tilapia than those quoted in the literature for rainbow trout and channel catfish. DE values for animal-derived feedstuffs were lower for tilapia than those for trout, pigs or poultry. However, the level of tested compounds, poultry by-product meal or meat-and-bone meal were at 60% of the diet (Anderson et al. 1991). The experimental design of the research proposed for this work group project, where a fish meal analog composed of four animal co-products will be used, should overcome the shortcomings of using a single ingredient replacement.

Dabrowski (1982) has argued that despite the herbivorous or omnivorous feeding habits of tilapia, growth of fish fed diets with animal protein sources is superior to those fed exclusively on algal material or detrital aggregates. Furthermore, the optimum protein and energy requirements for maximum growth of tilapia are

not considerably different than in other teleost fish when fed animal protein based feeds. Numerous papers on optimum protein requirement in several tilapia species and their hybrids in freshwater and saltwater conditions have confirmed that the maximum growth and best protein utilization is obtained at the dietary protein level of 30-35% and fat level of 5-7% dry matter (Winfree and Stickney 1981; Wang et al. 1985; De Silva et al. 1989; Shiau and Huang 1989; El Dahhar and Lovell 1995). In most of these studies, semipurified, casein-gelatin based diets were used. De Silva and Perera (1985) used diets where fish meal was the only source of protein. Fish grew best on diets containing 30% fish meal protein and 10 ppt salinity seemed to be more advantageous than a freshwater environment.

Several authors have obtained better performance of tilapia fed diets composed of a mixture of fish meal and plant protein (soybean or yellow corn meal) or co-dried fish silage and plant meal than with a single protein source (Siddiqui et al. 1988; Clark et al. 1990; Fagberno and Jauncey 1994). However, no studies have been performed where fish meal protein was partially or entirely replaced with a mixture of animal co-products. The determination of essential amino acid requirements for tilapia (Santiago and Lovell 1988) allows balancing formulated practical diets for limiting amino acids by addition of synthetic amino acids.

Evaluate Commercial Tilapia Strains (Objective 1b)

Although numerous tilapia strains have been the subject of investigation for aquacultural purposes (see Tave 1988), only in the Philippines has a concerted effort been undertaken. A program of systematic documentation, evaluation and utilization of tilapia genetic resource to build a national breeding program commenced in 1988 with the Genetic Improvement of Farmed Tilapias (GIFT) project (Pullin et al. 1991). The chosen strategy was to combine new germplasm from Africa with the farmed strains available in the Philippines (Bolivar et al. 1993; Eknath et al. 1993). *Oreochromis niloticus* strains collected from Egypt, Ghana, Kenya, and Senegal were compared to four established Asian farmed strains (Eknath et al. 1993). There were no significant differences in growth among strains with the exception of the Ghanan strain which showed a significantly lower body weight at 210 days (Bolivar et al. 1993). The primary objective of the GIFT project is to build a base population with a broader genetic base and initiate a genetic improvement program to develop a more productive tilapia (Eknath et al. 1993). Ultimately, the various strains may be crossbred to form a mixed population unless specific crosses show heterosis.

In Israel, the hybrid *O. niloticus* × *O. aureus* has proved most suitable for the conditions under which tilapia are raised there. Hulata et al. (1993) evaluated six stocks of *O. niloticus* and four of *O. aureus* as parental stocks for hybridization. Ten different crosses were evaluated in polyculture with common carp, silver carp, and grass carp. Similar to findings in the Philippines, the Ghanan strain of *O. niloticus* appears less suitable for aquaculture. The Nile River strains of both *O. niloticus* and *O. aureus* were deemed as potential candidates for commercial hybridization (Hulata et al. 1993).

ANTICIPATED BENEFITS

Producers throughout the NCR are raising tilapia. However, the combination of species and culture systems are not operating at peak efficiency. Diets fed to tilapia are most often modified catfish diets. Those same diets are thought to cause increased muscle lipid concentrations in catfish. If the same problem exists in tilapia during the growout phase of production, then the same problems will occur as in catfish. Fish containing relatively high concentrations of lipid in the muscle are subject to more rapid uptake of off-flavor compounds from the water. Further, shelf life of the product can be impaired because of the higher degree of lipid oxidation that can occur. Higher lipid concentrations in fillets is often the result of imbalanced E:P. Thus, the benefits of this line of research are continued improvement of diets fed to tilapia in recirculating systems, continued development of all-plant diets, enzymatic feedstuff enhancement, and use of animal agriculture co-products that can be easily manufactured in this region, and continued improvement in product quality for the consumer.

The ability to evaluate genetic differences within and between strains, and to determine the degree of hybrid mixture within some strains will assist the design of future work to select strains which are better adapted to culture conditions which will be utilized in the northern United States, and to assist in the evaluation of genetic schemes such as the production of YY male lines, which can be used to improve aquacultural production.

Gene markers for hypervariable neutral polymorphisms have been shown to be able to discriminate among populations and species with better resolution than morphometric traits. These gene markers also have the potential for application in aquaculture, including identification of individuals, families and species and labeling of brood stocks (Harris et al. 1991). They can also be of importance in the identification of hybridization between stocks and species and in the monitoring of inbreeding rates in managed stocks for proper fisheries management.

PROGRESS TO DATE

New project.

OBJECTIVES

1a. Develop and/or identify cost-effective feeds for tilapia culture in recirculating systems that minimize waste generation.

1b. Compare and evaluate economically important traits of current commercial tilapia strains in the North Central Region with other strains cultured in recirculating systems.

PROCEDURES

Feed Development for Tilapia Culture in Recirculating Systems (Objective 1a)

Purdue University/Illinois State University

Researchers at Purdue University (Purdue) have a “pure” strain of *Orechromis niloticus*. These fish were initially acquired by Simplot Aquaculture, then transferred to several research groups around the U.S. A former employee of Simplot acquired fish for the group at Purdue. Descendants of the original strain fish will be used by all collaborators in this project.

The project at Purdue will focus on optimal E:P of fish with an initial starting weight of 20-50 g. Diets will be formulated at Purdue from all-plant ingredients. Those diets will be similar in ingredient composition to diets already proven to promote the same weight gain and feed conversion as commercial diets (Wu et al. 1994; 1995). Other studies are underway now and results from those will also be incorporated into diet formulation. All diets will be isonitrogenous, containing the minimum concentration of CP that supports maximum weight gain. That study will be completed in 1996 using diets that are isoenergetic, but with varying levels of CP. The approach in this proposed study will use isonitrogenous diets containing varying concentrations of energy. Thus, the combined approach should result in useful information.

All diets will be extruded at Illinois State University (ISU) under the direction of Kerry Tudor. All ingredients will be acquired as a collaborative effort between Purdue and ISU. All extrusion equipment is on site and available. Feeds have been made for other NCRAC projects including trout feeds that produced a near 1:1 FCR. Diets will be stored frozen (-20°C) prior to feeding.

This project will be divided into two distinct phases. In the first study conducted at Purdue, juvenile tilapia (average initial weight of 20-50 g) will be stocked into 120-L glass aquaria. Triplicate groups of fish will be offered one of seven diets containing graded ratios of E:P. An eighth diet will be a positive control, commercially available diet routinely used in this region for tilapia. Every attempt will be made to maintain equal ratios of carbohydrate to lipid in the experimental diets (El-Sayed and Garling 1988). Differences in energy concentration between diets will be by changes in total amounts of lipid and carbohydrate. Standard methodology for determination of nutrient requirements in fish will be used (Gropp and Tacon 1994). Fish will be fed to satiation twice daily for a minimum of 10 weeks. At the end of the study, fish will be counted and weighed for determination of survival, weight gain and FCR. Further, dressout percentages will be determined. A sample of fish from each replicate will be filleted by hand.

In the second phase of the project, the same positive control diet and two of the experimental formulae will be fed to duplicate groups of fish on a larger scale at ISU. Each tank at ISU contains 31,000 L. Fish will be stocked at a density of approximately 30 g/L (or 0.25 lb/gal). The two experimental diets fed to tilapia in this phase of the project will be the same formulation identified in the first phase that contains the lowest E:P that promoted maximum response and the one containing the next lowest ratio of E:P. Feeding graded levels of nutrients to fish results in a mathematical relationship of responses usually described by a fitted broken line or a curvilinear relationship. We suspect that optimal nutritional values identified in small scale, highly controlled studies may be in excess of those that would be optimal in production situations. This has not been verified in recirculating systems, but is a common situation in fish reared in ponds.

The second phase of the study will be conducted for a minimum of 14 weeks. At the end of the study, final weights of fish and numbers will be determined for calculation of weight gain, survival and feed conversion. Further, samples of fish from each replicate will be used for determination of dressout percentages. This will result in direct comparisons of results from small and large scale studies.

Pertinent water quality variables will be monitored regularly at both sites. DO and temperature will be monitored daily. Ammonia and nitrate will be monitored at least weekly. Hardness, pH and alkalinity will be monitored monthly. Standard equipment (YSI DO meter, benchtop pH meter and a Hach DREL 1C Water Quality Test Kit) is available and in working order.

Michigan State University

Researchers at Michigan State University (MSU) will: 1) evaluate the effect of phytate on protein digestion and availability in tilapia feeds, 2) evaluate the efficacy of phytase pretreatment for increasing growth, feed conversion, and nitrogen retention in tilapia fed plant protein based diets, and 3) determine EAA digestibility values from soy protein substituted diets receiving enzymatic pretreatment and no pretreatment. Standard methodology for determination of nutrient requirements in fish will be used (Gropp and Tacon 1994).

An initial experiment will focus on the effects of phytic acid on CP digestibility in juvenile tilapia. Diets will be formulated to contain 32% CP and optimal E:P for growth in tilapia. All diets will contain complete mineral and vitamin premixes as well as an indigestible marker to evaluate CP digestibility. Dietary treatments will incorporate a 0% and increasing graded levels of either the calcium or sodium salt of phytic acid. Dietary treatments will be compared to a commercial diet serving as a control. Diets will be prepared, pelleted, and stored at -20°C at MSU.

“Pure” strain *Orechromis niloticus* originally obtained from Purdue will be used in all experiments. Triplicate groups of juvenile Nile tilapia will be randomly assigned the control diet or an experimental diet. Fish will be fed to satiation twice daily for 10 weeks. Fish will be weighed every two weeks. At the end of the trial fish will be anesthetized, weighed, and fecal samples collected by dissection. Feed and feces will be ashed and nitrogen determined by micro-Kjeldahl and converted to protein (N × 6.25). The marker will be determined by AOAC (1990) accepted protocols. CP digestibility will be determined by CP to marker ratios in feed and feces. Dietary treatments will also be evaluated for growth, survival, FCR, PER, NPU, and energy retention.

A second experiment will focus on the effects of enzymatic pretreatment of soy products with phytase before incorporation into the diet. Phytase of microbiological origin (BASF) will be used. Enzymatic pretreatment of solvent extracted SBM; raw, full-fat SBM; heat treated, full-fat SBM; and a soy protein concentrate, obtained from commercial sources, will be carried out by the manufacturer's recommendations. Protein sources will be incorporated into diets in graded levels of substitution into a fish meal diet on a percent protein basis. Dietary treatments will consist of 0%, 33%, 67% and 100% substitution of pretreated and untreated soy products. A commercial diet will serve as a control. Diets will be prepared and stored as above.

Triplicate groups of juvenile tilapia will be randomly assigned the control diet or an experimental diet. Fish will be fed to satiation twice daily for 10 weeks. Fish will be weighed every two weeks. At the end of the trial fish will be anesthetized, weighed, and fecal samples collected by dissection. Dietary treatments will be evaluated for growth, survival, FCR, PER, NPU, and energy retention. Feed and feces will be freeze-dried, pulverized, and then hydrolyzed for 18 h with 6N HCl. Free amino acids will be derivatized with phenylisothiocyanate

(PCT) before analysis with a reversed phase C18 HPLC column utilizing a Waters liquid chromatography system (Waters 1986). Digestibility of individual EAA will be determined as above.

If additional funding can be secured from the Michigan Soybean Promotion Committee or other sources, MSU researchers will:

1. Assess qualitative losses of amino acids excreted as phytate complexes.

2. Evaluate the effect of phytate and partially hydrolyzed phytate on *in vitro* and *in vivo* digestibility.

3. Examine digesta from different segments of the gastrointestinal tract to determine relative sites of amino acid uptake, develop a pH profile, and look for potential liganded phytate complexes.

4. Determine rate of ammonia excretion in fish fed the above soy protein diets to determine if phytase pretreatment enhances amino acid balance and improves amino acid utilization.

Ohio State University

Experiments to evaluate the animal by-products as substitutes for fish meal in tilapia diets will be carried out in the Piketon Research and Extension Center, Ohio State University (OSU). Nile tilapia obtained from SIUC maintained in our facility will be used in the first year of experimentation. After hatching, fish will be divided into two groups and one of them exposed to high temperatures of 32-34°C for 30 days. This procedure should increase the percentage of males up to 91% (Baroiller and Geraz 1995). The second group will be kept at 22-24°C. Tilapia of the approximate weight 5 g will be allocated to 36 experimental 40-L fiberglass tanks. Each tank will contain 20-25 fish of two different groups.

Five experimental diets and one reference diet (Table 1) will be fed to three tanks per diet over a 2-4 month period. Fish meal and animal by-products will be purchased from commercial sources and diets processed at the OSU feed meal, Wooster, Ohio.

The utilization of dietary protein for fish growth will be assessed by comparing the mean weight gain, growth rates (% per day), gross FCR and apparent protein utilization ratio (Dabrowski et al. 1989). For amino acid analysis of feeds, dried and pulverized samples will be hydrolyzed in 6N HCl at 110°C for 24 h under vacuum. After acid removal by rotary evaporation, coupling of amino acids with PCT will be carried out as described in the "Waters" Pico-tag procedures for HPLC amino acid analysis.

Studies to evaluate "between-species" or "between-hybrids" differences in the diet utilization will be carried out in the second year of this project. During both years, a collaborative arrangements with Paul Fuerst, OSU, will be made to determine "the purity" of species and/or hybrids to be used in feeding experiments.

SIUC

A 32% protein commercial feed will be used as the base diet to determine the efficacy of *Yucca shidigera* extract (Micro-Aid, Distributors Processing Inc., Porterville, California) to reduce N in feces and enhance overall growth performance of Nile tilapia. Treatments of 0, 0.5, 1.0, 1.5, and 2.0 g Micro-Aid/Kg feed will be prepared by mixing the prescribed amounts of dried extract in agar and then coating the feed. Production trials will be conducted in quadruplicate. Five fish weighing approximately 25 g each will be stocked in each of 20 75-L aquaria, all connected to a common biofilter. Water flow will be regulated to achieve approximately 12 turnovers per day. Water temperature will be maintained at 28°C. Photoperiod (14-h light/10-h dark) will be held constant. Fish will be stocked two weeks prior to trials and fed daily the control diet at a rate of 3% wet body weight, equally split into two meals (early morning/late afternoon). Excess feed and feces will be removed daily by siphon. Four aquaria will randomly be assigned to each of five treatments. The same rationing and feeding schedule will be maintained. All fish will be marked using fin clips or passive integrator transponder (PIT) tags. Fish will individually be weighed every two weeks and feed rations adjusted accordingly using treatment means. Major water quality parameters (temperature, DO, pH, total ammonia nitrogen [TAN], CO_2, alkalinity, and hardness) will regularly be monitored using standard aquaculture

procedures (Hach kits, DO meter, etc.). Specific growth rate (SGR), FCR, PER, and condition (K) will be determined bi-weekly as follows:

$$SGR = 100 [\ln \text{final liveweight (g)} - \ln \text{initial liveweight (g)}]/\text{time (d)}$$

Table 1. Diet formulation for fish meal analog study with tilapia. All numbers as percent.

	Test Diets				
	#1	#2	#3	#4	#5
Fish meal (FM)	20	15	10	5	0
Fish meal Analog	0	5	10	15	20

	Test Diets					Reference Diet[1]	
Ingredient	#1	#2	#3	#4	#5	Ingredient	#6
FM menhaden	10.00	7.50	5.00	2.50	0.00	Casein	27.0
FM herring	10.00	7.50	5.00	2.50	0.00	Gelatin	6.7
FM analog	0.00	4.75	9.50	14.26	19.00	Corn starch	38.3
Yeast	9.00	9.00	9.00	9.00	9.00	Cod liver oil	3.0
Corn gluten meal	15.00	15.00	15.00	15.00	15.00	Corn oil	2.0
Soybean	12.00	12.00	12.00	12.00	12.00	Cellulose	14.3
Wheat middlings	26.00	26.00	26.00	26.00	26.00	CMC[2]	3.0
Whey	7.00	7.00	7.00	7.00	7.00	Vitamin mix	1.4
Methionine	0.00	0.00	0.05	0.10	0.15	Mineral mix	4.0
Lysine	0.00	0.00	0.06	0.12	0.18	Ascorbic acid	0.3
Cr_2O_3	0.50	0.50	0.50	0.50	0.50		
Vit. & min. premix	2.00	2.00	2.00	2.00	2.00		
Alphacel	1.60	1.38	1.04	0.69	0.36		
Tender J	1.00	1.00	1.00	1.00	1.00		
Vitamin C-MP	0.05	0.05	0.05	0.05	0.05		
Cod Oil	5.85	6.32	6.80	7.28	7.76		
TOTAL	100.00	100.00	100.00	100.00	100.00		100.0

	Diets					
Composition	#1	#2	#3	#4	#5	#6
Protein	35.9	36.0	36.1	36.3	36.4	30.1
Fat	9.1	9.4	9.8	10.2	10.5	5.0
Methionine	0.85	0.85	0.85	0.85	0.85	
Lysine	2.05	2.05	2.05	2.05	2.05	

[1]Source: El Dahhar and Lovell 1995
[2]CMC=Carboxymethylcellulose

FCR = feed offered (g)/liveweight gain (g)

PER = liveweight gain (g)/protein offered (g)

K = liveweight (g)/length $(cm)^3$

At termination of each study (10 weeks), 10 fish from each treatment will randomly be selected, taken off feed for a 24-h period, and then frozen pending whole body proximate analysis. Percent moisture, fat and ash will be determined using standard methods (AOAC 1990). CP will be determined using Hach's modification of the AOAC (1990) standards (Watkins et al. 1987).

Fecal samples will be collected from fish receiving each feed treatment to determine relative amounts of N. Each treatment will be tested independently in triplicate. Three 110-L glass aquaria will be used, each with a water flow rate of 1.2-L/min in a recirculating-water system with charcoal-filtered municipal water. Water quality variables, particularly temperature, DO, pH, and ammonia will be closely monitored and maintained at appropriate and comparable levels for each treatment and during holding periods. Each aquarium will be stocked with 10 juvenile fish (about 25 g mean weight), and will contain a plexiglass feces collector of sloping walls (see Ayala et al. 1993). With the feces collector in place, aquaria will be divided into two chambers: a feeding compartment (about one-third of the aquarium volume) and a fecal collector compartment. A removable screen will separate the two chambers.

For each treatment, fish will be fed the test diet for one week before fecal collections begin. Fish will be fed 3% of their wet body weights once each morning. Prior to feeding, the screen separating the two chambers will be removed and fish will manually be coaxed to swim to the feeding chamber, whereupon the screen will be reset. Feed residue will be siphoned out after each feeding session. Subsequently, the screen will be removed, the fish will be forced to return to the chamber containing the feces collector, after which the screen will be reset. Feces will be collected once each afternoon from a removable plexiglass box at the bottom of the collector. Feces from each replicate will be placed into a labeled petri dish and left uncovered in a refrigerator at 1-3°C for a 24-h drying period. Samples of feces will subsequently be dried in a vacuum oven at 95°C for 5 h and cooled in a desiccator. Feces will be collected for each treatment until sufficient quantity, about 1.0 g, has accumulated. Feces will be analyzed for nitrogen content utilizing Hach's modification of the AOAC (1990) standards (Watkins et al. 1987).

Evaluate Commercial Tilapia Strains (Objective 1b)

OSU

To accomplish this the research will focus on two levels of genetic markers: 1) RAPD markers (randomly amplified polymorphic DNA), and 2) microsatellite (STR or simple tandem repeat) markers. Both methods utilize the polymerase chain reaction (PCR) to obtain DNA for analysis. The RAPD method provides data which can be used to estimate the average genetic variability of a sample. They can also be used to compare the levels of variation in different populations and species. Statistical methods have been developed which allow RAPD data to be used to estimate the genetic variability within populations, and to estimate differentiation between populations and between species, and hybridization.

This information can be used to identify stock or species-specific gene markers and use these to assess the level of hybridization among the aquacultural strains. Of special interest will be the question of introgression of genes from *O. aureus* into strains which are considered to be *O. niloticus.* This has a bearing on the ability of such mixed strains to be used for manipulation of sex-ratios, since the two species appear to possess different genetic systems for sex-determination.

Sample Collection

Fin tissue or muscle tissue will be taken from at least 15 fish per population per line per strain to be examined. Samples will be taken from fish maintained at Purdue and OSU. Fin samples allow nondestructive sampling to be made wherever appropriate. It is estimated that this will involve at least eight strains with a minimum

of four different line samples from each strain. Tissue samples will be preserved in 95% ethanol and sealed in labeled vials for shipment to the laboratory for DNA extraction.

DNA Extraction

DNA will be extracted from the muscle tissue by the standard phenol/chloroform extraction method (Williams et al. 1990). The tissue will be homogenized in a lysis buffer and proteins degraded using a proteinase enzyme (Proteinase K) at 55°C for 24 hours. Phenol/chloroform extraction will be performed, followed by 95% ethanol precipitation of the DNA. Excess ethanol will be removed by a 70% ethanol wash, following which the sample is dried and resuspended in tris - EDTA buffer overnight at 65°C. DNA concentration will be calculated using spectrometric readings taken at a wavelength of 260 nm.

DNA Analysis

Several methods have been suggested for the study of genetic diversity (Williams et al. 1990; Hoelzel 1992; Russell et al. 1993; Hadrys et al. 1992).

RAPD Primers

DNA from 15 individuals per sample for the different lines will be used to examine a set of at least 15 oligonucleotide primers. Primers which give a manageable and yet high number of polymorphic bands will be selected for the population analysis. This has already been ongoing in our laboratory in work on natural population of tilapiine and haplochromine cichlids from East Africa (Fuerst et al. 1995; Booton 1995; Mwanja et al. 1995; Black et al. 1995; Booton et al. In preparation).

Microsatellite Analysis

Microsatellites are a form of repeated genetic marker. They share some similarities with traditional DNA fingerprinting (Harris and Wright 1995). However, microsatellite (or STR sequences) are more abundant in the genome, and are likely to be more useful for strain analysis and ultimately genetic mapping. We have been developing a set of microsatellite markers for the analysis of natural populations of tilapiine and haplochromine cichlids. At present a set of 25 microsatellite loci have been identified and sequenced, and five of these loci have been used to examine the structure of natural cichlid populations (Wu, L. et al. 1995 and In Preparation).

An additional set of microsatellite loci are being developed by Thomas Kocher of the University of New Hampshire and Irv Kornfield of the University of Maine, as part of the cooperative project headed by Kocher of the development of a genetic map for tilapia. This project (of which Paul Fuerst is a cooperating member) is facilitated by a tilapia mapping group coordinated by Kocher.

Genetic Variability

Strains can be compared by presence or absence of RAPD bands as a measure of genomic variability and differentiation. In this project comparison will made based on presence/absence data of polymorphic RAPD bands, within individuals of the same line, between lines of the same strain and among the different strains and species. Each line will be represented by a minimum of 15 individuals.

For microsatellites, standard population genetic approaches can be used for the analysis of strain variability and interstrain differentiation. Microsatellites are especially useful in assessing the effects of potential inbreeding, because of their highly variable nature.

Hybridization

Strain and species specific markers can be assessed. We will identify presumably pure lines of *O. niloticus* to identify *O. niloticus* specific bands. If necessary, we will use strains from a natural population, for which we currently have a number of population samples from East Africa (Uganda and Kenya). RAPD banding patterns will be used to estimate the degree of hybridization between strains.

Mendelian Analysis of RAPD Variants

A series of controlled matings using pairs of *O. niloticus* will be set up to evaluate the inheritance of RAPD variants, following the suggestion of Clark and Lanigan (1993). Genetic material from known crosses is available from Thomas Kocher at the University of New Hampshire, with whom we are interacting on the development of a genetic map in tilapia, as a member of the tilapia mapping group. We will also evaluate the inheritance patterns of our microsatellite sequences.

Statistical Analysis

For each population and species the frequency of each polymorphic locus, mean proportion of shared bands, and mean number of polymorphic loci per primer will be calculated. The primary methods used to analyze RAPD diversity will be adapted from those used for the analysis of DNA fingerprints, following the cautions documented in the paper of Clark and Lanigan (1993). Genetic diversity of populations will be estimated using the mean heterogeneity per polymorphic locus (Dawson et al. 1993). To measure the overall distribution of variability between and within populations, gene diversity statistics will be calculated for each RAPD locus. Using the mean proportion of shared bands per polymorphic locus for each population and species, a similarity matrix will be made to compare the different species. Hybridization will be estimated based on the frequency of occurrence of species specific gene markers among amplification products of alternate species.

SIUC

In Year 1, two sources for each of white tilapia, red tilapia, and "pure" Nile tilapia will be compared for performance in trials conducted in a recirculating system. Sources of fish will be identified in consultation with NCRAC IAC tilapia producers and the ATA. Samples of Nile tilapia will be sent to OSU for genetic verification. Additional strains/hybrids will be evaluated in Year 2 based on first-year results. The precise strains/hybrids will be selected in consultation with the aforementioned groups.

A water recycle system containing 18, 115-L fiberglass tanks, a 500-L submerged biofilter, a 250-L settling tank, and a 250-L head tank will be employed. Water will be maintained at 28˚C using electric heaters. Air stones will be supplied to each tank, biofilter, and head tank. Black plastic sheeting will be canopied over the tanks and artificial lighting will be maintained at 14-h light/10-h dark.

Three tanks will randomly be assigned for each of the six strains/hybrids. Ten fish each weighing approximately 25 g will be stocked into their assigned tanks. Each fish will individually be marked with a PIT tag. Fish will be fed a 32% protein commercial feed at a rate of 3% wet body weight, equally split into two meals (early morning/late afternoon). Excess feed and feces will be removed daily by siphon. Fish will individually be weighed every two weeks and feed rations adjusted accordingly using treatment means. Major water quality parameters (temperature, DO, pH, TAN, CO_2, alkalinity, and hardness) will regularly be monitored using standard aquaculture procedures (Hach kits, DO meter, etc.). SGR, FCR, PER, and K will be determined bi-weekly.

At termination of the study (24 weeks), 10 fish from each strain/hybrid will randomly be selected, taken off feed for a 24-h period, and then frozen pending whole body proximate analysis. Percent moisture, fat and ash will be determined using standard methods (AOAC 1990). CP will be determined using Hach's modification of the AOAC (1990) standards (Watkins et al. 1987). Ten additional fish from each strain/hybrid will be randomly selected for determination of dressout and fillet percentages.

FACILITIES

Feeds Development for Tilapia Culture in Recirculating Systems (Objective 1a)

Purdue/ISU

Experimental systems are in place and functional at Purdue. These systems have been in routine use for the past two years. The experimental systems are a series of 24-48 glass aquaria (120-L). Each is operated

independently. The full system can be operated flow through or recirculating. A complete nutritional laboratory is available for this work.

Experimental tanks, fish and feed manufacturing equipment are available at ISU. That equipment has been used for the past five years for experimentation with tilapia in recirculating systems.

OSU

Konrad Dabrowski's laboratory in Kottman Hall includes a biofreezer (-85^{o}C), two refrigerated centrifuges, freeze-drier, drying ovens, two spectrophotometers DU-70, Beckman HPLC system, Varian 3400 gas chromatography system, electrophoresis system (Multiphor II, Pharmacia) and other accessories for biochemical research studies. A 167.2 m^2 (1,800 ft^2) wet laboratory in Kottman Hall is equipped with rearing tanks for fry, fingerling and brood stock; fish egg incubation apparatus; and acclimation chambers. This laboratory includes features for water temperature-control, supersaturation with oxygen and a sterilization system.

Konrad Dabrowski is in charge of the facilities at the Piketon Research and Extension Center, where brood stock fish of several species including rainbow trout, yellow perch, whitefish and channel catfish are maintained. This facility includes 14 ponds, an aquaculture building equipped with several hundred fish tanks, recirculation systems and temperature and light control rooms. The main building of the field station contains, among other things, an aquaculture laboratory for tropical fish and bioanalytical laboratory with Water Pico-Tag amino acid analyzer.

MSU

The MSU Aquaculture Laboratory has the necessary wet lab, tanks and water supply to conduct the proposed research. A water re-use system will be available to maintain fish in holding tanks and for spawning and early rearing. Nutritional experiments will be conducted in flow through tanks (40-L or 150-L) supplied with heated well water or water re-use system water. The tank system has been modified to facilitate collection of fecal materials. Plant protein feedstuffs will be treated with phytase and phosphorus determinations will be conducted in the analytical lab or with assistance from MSU's Department of Animal Sciences.

SIUC

Over 150 glass aquaria with volumes ranging from 38- to 209-L are maintained by the Fisheries Research Laboratory. Most of these are located in the University Vivarium, a National Institute of Health-approved animal housing facility under the direction of a veterinarian. Most aquaria are arranged in batteries with attachments to biofilters. The systems are highly flexible in terms of numbers that can be attached to a given biofilter. Several large indoor water recycle systems are also available at the University Wetlab. These systems will be used for housing fish prior to initiation of trials. All necessary equipment for measuring and maintaining water quality, measuring and weighing fish, preparation of feed, and conducting proximate analyses are available. A Lab-Line orbital shaker and all necessary glassware for conducting fecal studies are available.

Evaluate Commercial Tilapia Strains (Objective 1b)

OSU

Paul Fuerst's laboratory contains all required equipment for the molecular analysis of fish. The laboratory is a 111.5 m^2 (1,200 ft^2) wet lab, with separate hot space for radioisotope use in DNA sequencing. DNA sequencing apparatuses, PCR thermocyclers and RAPD electrophoretic equipment, centrifuges, spectrophotometer, etc. are available and in use by the laboratory. Computers with appropriate software for genetic and phylogenetic analysis are also available in the laboratory. A separate wet lab in the OSU Museum for Biological Diversity fishes section allows genetic crosses to be performed.

SIUC

See facilities description under Objective 1a.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Kerry W. Tudor Illinois State University	Economics/Aquaculture
	Christopher C. Kohler Southern Illinois University-Carbondale	Aquaculture
Indiana	Paul B. Brown Purdue University	Nutrition/Aquaculture
Michigan	Donald L. Garling Michigan State University	Nutrition/Aquaculture
Ohio	Konrad Dabrowski Ohio State University	Nutrition/Aquaculture
	Paul A. Fuerst Ohio State University	Fish Genetics

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Purdue University (Purdue)
Paul B. Brown

Illiinois State University (ISU)
Kerry W. Tudor

Michigan State University (MSU)
Donald L. Garling

Ohio State University (OSU)
Konrad Dabrowski
Paul A. Fuerst

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

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 University Department of Forestry and Natural Resources, 1159 Forestry Building West Lafayette, IN 47907-1159				**USDA AWARD NO.** Year 1 - Objective 1a	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul B. Brown				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. _1_ Graduate Students				$11,940	
d. _1_ Prebaccalaureate Students				$1,000	
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$12,940	
B. Fringe Benefits (If charged as Direct Costs)				$140	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$13,080	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,000	
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.) Telephone ($50), FAX ($50), Postage ($50), Photocopying ($75), Repairs ($295)				$520	
J. Total Direct Costs (C through I) . . . . . →				$17,600	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . . →				$17,600	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$17,600	$
O. Cost Sharing (If Required Provide Details)	$ 21,000				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR PURDUE UNIVERSITY

(Brown)

Objective 1a

A. Salaries and Wages. A graduate student (0.50 FTE) is required for acquisition of fish, coordination of diet manufacturing, feeding fish, water quality monitoring and harvesting. A prebaccalaureate student is required for supplementing these activities as fish will be fed 7 days per week.

B. Fringe Benefits. Standard fringe benefit rate is 1.13% for graduate students and 0.36% for prebaccalaureate students.

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

F. Travel. These funds will be used for acquisition of feedstuffs and dissemination of research results.

I. Other Direct Costs. Telephone ($50), FAX ($50), postage charges ($50) and photocopying charges ($75) associated with this project and unexpected repairs to critical equipment ($295).

UNITED STATES DEPARTMENT OF AGRICULTURE
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BUDGET

ORGANIZATION AND ADDRESS Illinois State University Department of Agriculture, Campus Box 5020 Normal, IL 61790-5020				**USDA AWARD NO.** Year 1 - Objective 1a	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Kerry W. Tudor				Duration Proposed Months: _____ **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. _1_ (Co)-PI(s)/PD(s)	1.2			$5,000	
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. _1_ Graduate Students				$8,400	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$13,400	
B. Fringe Benefits (If charged as Direct Costs)				$1,000	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$14,400	
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. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.)					
J. Total Direct Costs (C through I) . . . . . →				$16,400	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . . →				$16,400	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$16,400	$
O. Cost Sharing (If Required Provide Details)	$ 23,550				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR ILLINOIS STATE UNIVERSITY

(Tudor)

Objective 1a

A. Salaries and Wages. A graduate student (0.50 FTE) is required for acquisition of fish, coordination of diet manufacturing, feeding fish, water quality monitoring and harvesting. Salary for the Principal Investigator will be used for 1.2 months (0.10 FTE) during the critical phase of the study at ISU.

B. Fringe Benefits. Standard fringe benefit rate is 25% for PIs.

E. Materials and Supplies. These funds will be used for acquisition of feedstuffs and diet manufacturing at ISU ($1,000). Additionally, these funds will be used for routine maintenance of experimental and holding systems ($500), analytical chemicals ($300), and general laboratory supplies ($200).

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

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BUDGET

ORGANIZATION AND ADDRESS Michigan State University Department of Fisheries and Wildlife, 9 Natural Resources Building East Lansing, MI 48824-1222				**USDA AWARD NO.** Year 1 - Objective 1a	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Donald L. Garling				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. _1_ Graduate Students				$14,500	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$14,500	
B. Fringe Benefits (If charged as Direct Costs)				$650	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$15,150	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$2,250	
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.) Telephone ($100), FAX ($50), Postage ($100), Statistical consulting ($500), Freight/Shipping ($100)				$850	
J. Total Direct Costs (C through I) . . . . . →				$19,000	
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) . . . . . →				$19,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$19,000	$
O. Cost Sharing (If Required Provide Details)	$ 34,935				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR MICHIGAN STATE UNIVERSITY

(Garling)

Objective 1a

A. Salaries and Wages. A graduate student (0.50 FTE) is required for acquisition of fish, coordination of diet manufacturing, feeding fish, water quality monitoring and harvesting.

B. Fringe Benefits. Standard fringe benefit rate is 4.5% for graduate students.

E. Materials and Supplies. These funds will be used for analytical chemicals ($750), phytase treatment ($250), feedstuffs ($500), and for routine maintenance of experimental and holding systems ($250). Additionally, these funds will be used for office supplies associated with serving as Chairperson of the Work Group ($500).

F. Travel. These funds will be used for acquisition of fish ($350) and dissemination of research results ($400).

I. Other Direct Costs. Telephone ($100), FAX ($50), postage charges ($100), and statistical consulting ($500) associated with serving as Chairperson of the Work Group and for freight/shipping of treated feedstuffs to cooperator ($100).

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

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BUDGET

ORGANIZATION AND ADDRESS Ohio State University School of Natural Resources, 2021 Coffey Road Columbus, OH 43210				**USDA AWARD NO.** Year 1 - Objective 1a	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Konrad Dabrowski				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 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. _1_ Research Associates-Postdoctorates	3			$3,600	
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$3,600	
B. Fringe Benefits (If charged as Direct Costs)				$1,008	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$4,608	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,192	
F. Travel 1. Domestic (Including Canada)				$200	
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) . . . . . →				$8,000	
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) . . . . . →				$8,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$8,000	$
O. Cost Sharing (If Required Provide Details)	$ 8,500				

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

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

Form CSREES-55 (6/95)

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

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BUDGET

ORGANIZATION AND ADDRESS Ohio State University School of Natural Resources, 2021 Coffey Road Columbus, OH 43210				**USDA AWARD NO.** Year 2 - Objective 1a	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Konrad Dabrowski				Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 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. _1_ Research Associates-Postdoctorates	3			$3,600	
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$3,600	
B. Fringe Benefits (If charged as Direct Costs)				$1,008	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$4,608	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$3,192	
F. Travel 1. Domestic (Including Canada)				$200	
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) . . . . . →				$8,000	
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) . . . . . →				$8,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$8,000	$
O. Cost Sharing (If Required Provide Details)	$ 8,712				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR OHIO STATE UNIVERSITY

(Dabrowski)

Objective 1a

A. Salaries and Wages. Field and laboratory studies during 4-6 months each year will be conducted by a research associate. A research associate (0.25 FTE) will supervise chemical analysis of fish, feeds, and feces. Tasks will include diets preparation and analysis, preparation of daily, weekly, and monthly tables of laboratory experiments schedule, sampling at the Piketon Research and Extension Center, initial preparation of samples for analysis, transportation to the campus laboratory in Columbus and sample analysis. Approximately half of the labor in tank experiments will be supported by monies from the Piketon Center.

B. Fringe Benefits. Standard fringe benefit rate is 28% for research associates.

E. Materials and Supplies. First year will include only ingredients for diet production ($3,092). Second year will include general laboratory supplies ($1,000), reagents ($500), glassware ($592), and replacement parts for amino acid analyzer ($1,100).

F. Travel. These funds will support transportation for the collection of samples and feed processing.

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Ohio State University Department of Molecular Genetics, 481 W. 12th Avenue Columbus, OH 43210				**USDA AWARD NO.** Year 1 - Objective 1b	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul A. Fuerst				Duration Proposed Months: ___12___ **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →					
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →					
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$9,500	
F. Travel 1. Domestic (Including Canada)					
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) . . . . . →				$9,500	
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) . . . . . →				$9,500	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$9,500	$
O. Cost Sharing (If Required Provide Details)	$ 8,500				

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

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

Form CSREES-55 (6/95)

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Ohio State University Department of Molecular Genetics, 481 W. 12th Avenue Columbus, OH 43210				**USDA AWARD NO.** Year 2 - Objective 1b	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Paul A. Fuerst				Duration Proposed Months: ___12___ **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →					
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →					
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$9,500	
F. Travel 1. Domestic (Including Canada)					
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) . . . . . →				$9,500	
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) . . . . . →				$9,500	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$9,500	$
O. Cost Sharing (If Required Provide Details)	$ 8,713				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR OHIO STATE UNIVERSITY

(Fuerst)

Objective 1b

E. Materials and Supplies. Costs have been based on the need to analyze 200 fish/year. This includes PCR, primer synthesis, DNA sequencing, and electrophoresis for allele analysis. Supplies include thermostable DNA polymerase, nucleotides, at least one set each year of 60 PCR RAPD primers ($7/primer), 30 microsatellite primers ($30/primer), sequencing gels, radioisotopes, X-ray film, replacement glass plates for sequencing apparatuses, and miscellaneous chemicals for buffers, etc. These supplies have been calculated to cover molecular biology supplies neeed to analyze 100 RAPD loci and 10 microsatellite loci per year for 200 fish.

In addition, we expect to pursue an initial mtDNA analysis of tilapia haplotypes using heteroduplex analysis, which is already being done in our laboratory and which is very sensitive and cost effective. Estimates place the costs of microsatellite analysis at $2.00 per typing (2,000 tyings per year) and RAPD analysis at $0.75 per typing (3,000 typings per year), and heteroduplex analysis ($3.00 per typing; 400 typings per year) plus sequencing of 50 individuals for an 800 bp region of the mitochondria (@ $7.00 per individual). In addition, we request $380 per year for pipette recalibration and maintenance.

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

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS Southern Illinois University-Carbondale Fisheries Research Laboratory Carbondale, IL 62901-6511				**USDA AWARD NO.** Year 1 - Objectives 1a & 1b	
				Duration Proposed Months: ___12___	Duration Awarded Months: _____
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christoper C. Kohler				**FUNDS REQUESTED by PROPOSER**	**FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**				$
	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. _1_ Graduate Students				$11,500	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$11,500	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$11,500	
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)				$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.) Computer costs ($500), Report preparation ($100), Graphics ($250), Telephone ($100), FAX ($100), Equipment repairs ($350)				$1,500	
J. Total Direct Costs (C through I) . . . . . →				$16,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . . →				$16,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$16,000	$
O. Cost Sharing (If Required Provide Details)	$ 12,647				

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 Southern Illinois University-Carbondale Fisheries Research Laboratory Carbondale, IL 62901-6511				**USDA AWARD NO.** Year 2 - Objectives 1a & 1b	
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Christoper C. Kohler				Duration Proposed Months: ___12___ **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. _1_ Graduate Students				$12,000	
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$12,000	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$12,000	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$1,500	
F. Travel				$1,000	
1. Domestic (Including Canada)					
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.) Computer costs ($500), Report preparation ($100), Graphics ($250), Telephone ($100), FAX ($100), Equipment repairs ($350)				$1,500	
J. Total Direct Costs (C through I) . . . . . →				$16,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . . →				$16,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$16,000	$
O. Cost Sharing (If Required Provide Details)	$ 12,921				

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

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

Form CSREES-55 (6/95)

BUDGET JUSTIFICATION FOR SOUTHER ILLINOIS UNIVERSITY-CARBONDALE

(Kohler)

Objectives 1a & 1b

A. Salaries and Wages. A graduate student (0.50 FTE) is required to assist in culture studies.

E. Materials and Supplies. These funds will be used in Year 1 for acquisition of fish ($500), PIT tags ($500), glassware ($200), fish feed ($500), plumbing supplies ($100), and chemicals ($200) and Year 2 for acquisition of fish ($400), PIT tags ($400), glassware ($100), fish feed ($400), plumbing supplies ($50), and chemicals ($150).

F. Travel. These funds will be used for obtaining fish and travel associated with dissemination of research results at a professional meeting

I. Other Direct Costs. For each year of the project other direct cost include: computer costs ($500), report preparation ($100), graphics ($250), telephone ($100), FAX ($100), and equipment repair ($350).

TILAPIA CULTURE IN THE NORTH CENTRAL REGION

Budget Summary for Each Participating Institution for the First Year

	Purdue	ISU	MSU	OSU	SIUC	TOTALS
Salaries and Wages	$12,940	$13,400	$14,500	$6,900	$11,500	$59,240
Fringe Benefits	$140	$1,000	$650	$1,008	$0	$2,798
Total Salaries, Wages and Benefits	$13,080	$14,400	$15,150	$7,908	$11,500	$62,038
Nonexpendable Equipment	$0	$0	$0	$0	$0	$0
Materials and Supplies	$3,000	$2,000	$2,250	$7,892	$2,000	$17,142
Travel	$1,000	$0	$750	$200	$1,000	$2,950
Other Direct Costs	$520	$0	$850	$1,500	$1,500	$4,370
TOTAL PROJECT COSTS	**$17,600**	**$16,400**	**$19,000**	**$17,500**	**$16,000**	**$86,500**

Budget Summary for Each Participating Institution for the Second Year

	OSU	SIUC	TOTALS
Salaries and Wages	$7,300	$12,000	$19,300
Fringe Benefits	$1,008	$0	$1,008
Total Salaries, Wages and Benefits	$8,308	$12,000	$20,308
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$7,392	$1,500	$8,892
Travel	$200	$1,000	$1,200
Other Direct Costs	$1,600	$1,500	$3,100
TOTAL PROJECT COSTS	**$17,500**	**$16,000**	**$33,500**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

State/Institution		Year 1	Year 2
Purdue University			
Salaries and Benefits: SY @ 0.10		$6,000	$0
Supplies, Expenses, Equipment, and Waiver of Overhead		$15,000	$0
	Total	$21,000	$0
Illinois State University			
Salaries and Benefits: SY @ 0.20		$6,000	$0
Supplies, Expenses, Equipment, and Waiver of Overhead		$17,550	$0
	Total	$23,550	$0
Michigan State University			
Salaries and Benefits: SY @ 0.10 FTE		$8,715	$8,975
Supplies, Expenses, Equipment, and Waiver of Overhead		$12,025	$5,220
	Total	$20,740	$14,195
Ohio State University			
Salaries and Benefits: 2 SY @ 0.05 FTE		$8,500	$8,925
Supplies, Expenses, Equipment, and Waiver of Overhead		$8,500	$8,500
	Total	$17,000	$17,425
Southern Illinois University-Carbondale			
Salaries and Benefits: SY @ 0.05 FTE		$4,174	$4,367
Supplies, Expenses, Equipment, and Waiver of Overhead		$8,473	$8,554
	Total	$12,647	$12,921
	Total per Year	$94,937	$44,541
	GRAND TOTAL	$139,478	

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

LIST OF PRINCIPAL INVESTIGATORS

Paul B. Brown, Purdue University

Konrad Dabrowski, Ohio State University

Paul A. Fuerst, Ohio State University

Donald L. Garling, Michigan State University

Christopher C. Kohler, Southern Illinois University-Carbondale

Kerry W. Tudor, Illinois State University

VITA

Paul B. Brown
Department of Forestry and Natural Resources
Purdue University
1159 Forestry Building
West Lafayette, IN 47907-1159

Phone: (317) 494-4968
FAX: (318) 494-0409
E-mail: pb@forest1.fnr.purdue.edu

EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Institute of Nutrition
American Society of Zoologists
International Association of Astacology
World Aquaculture Society

SELECTED PUBLICATIONS

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

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

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

Swann, D.L., J.R. Riepe, J.D. Stanley, M.E. Griffin, and P.B. Brown. 1994. Cage culture of hybrid striped bass in Indiana and evaluation of diets containing three levels of dietary protein. Journal of the World Aquaculture Society 25:281-288.

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

Wetzel, J.E., II, and P.B. Brown. 1993. Growth and survival of juvenile *Orconectes virilis* and *O. immunis* at different temperatures. Journal of the World Aquaculture Society 24:339-343.

Brown, P.B., and E.H. Robinson. 1992. Vitamin D studies with juvenile channel catfish (*Ictalurus punctatus*) reared in calcium-free water. Comparative Biochemistry and Physiology 103A:213-219.

VITA

Konrad Dabrowski
School of Natural Resources
Ohio State University
2021 Coffey Road
Columbus, Ohio 43210

Phone: (614) 292-4555
FAX: (614) 292-7432

EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Fisheries Society of British Isles
Japanese Fisheries Society
National Research Council, Washington, Subcommittee on Fish Nutrition (1990-1992)
World Aquaculture Society

SELECTED PUBLICATIONS

Blom, J.H., and K. Dabrowski. 1995. Reproductive success of females rainbow trout in response to graded dietary ascorbyl mono-phosphate levels. Biology of Reproduction 52:1073-1080.

Ciereszko, A., and K. Dabrowski. 1995. Spectrophotometric measurement of aspartate aminotransferase activity in mammalian and fish semen. Animal Reproductive Science 38:167-176.

Ciereszko, A., and K. Dabrowski. 1995. Sperm quality and ascorbic acid concentration in rainbow trout semen are affected by dietary vitamin C: an across season study. Biology of Reproduction 52:982-988.

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

Dabrowski, K., and J. Blom. 1994. Deposition of ascorbic acid in rainbow trout (*Oncorhynchus mykiss*) eggs and survival of embryos. Comparative Biochemistry and Physiology 1008A:129-135.

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

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

VITA

Paul A. Fuerst
Department of Molecular Genetics
Ohio State University
484 W. 12th Avenue
Columbus, OH 43210

Telephone: (614) 292-6403
FAX: (614) 292-4466
E-mail: pfuerst@magnus.acs.ohio-state.edu

EDUCATION

A.B. Manhattan College, 1970
M.S. Brown University, 1972
Ph.D. Brown University, 1975

POSITIONS

Associate Professor of Molecular Genetics (1986-present), Ohio State University
Associate Professor of Zoology (1987-present), Ohio State University
Associate Professor of Anthropology (1994-present), Ohio State University
Director (1988-1991), Molecular, Cellular, and Developmental Biology Program, Ohio State University
Assistant Professor (1980-1986) of Genetics, Ohio State University
Visiting Scientist (1983; 1985), Lab. Evolutionary Genetics, National Institute of Genetics, Mishima, Japan
Senior Research Associate (1975-1980), Univ.Texas Health Science Center at Houston, Center for Demographic and Population Genetics

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Genetics Association
American Society of Human Genetics
American Society for Microbiology
Genetics Society of America
Ohio Academy of Sciences Sigma Xi
Society for the Study of Evolution
Society for Molecular Biology and Evolution

SELECTED PUBLICATIONS

Fuerst, P. In press. G enetic considerations for the Lake Victoria cichlid Species Survivial Plan. J ournal of Aquaculture and Aquatic Sciences.

Parker, P., A. Snow, G. Booton, M. Schug, and P.A. Fuerst. In press. Molecular markers for population ecology. Ecology.

Schug, M., B. Porter, P. Parker, T. Cavender, and P. Fuerst. Submitted. Genetic variability in the endangered lake sturgeon (*Acipenser fulvescens*) revealed using VNTR markers and implications for population management. Canadian Journal of Fisheries and Aquatic Sciences.

Fuerst, P.A., W. Mwanja, G. Booton, M. Black, M. Chandler, and L. Kaufman. 1995. RAPD's as nuclear gene markers of population structure and hybridization in Lake Victoria cichlids. Journal of Cellular Biochemistry 19B:339.

Mbahinzireki, G., and P. Fuerst. 1995. Improvement of aquaculture in Uganda. Pages 7-14 *in* J.E. Haldeman, editor. Sustainable agriculture research in Uganda. Cornell University ARTP, Ithaca, N.Y.

Stothard, D.R., and P.A. Fuerst. 1995. Phylogenetic analysis of the Spotted Fever and Typhus Groups of *Rickettsia* using 16S rRNA gene sequences. Systematic and Applied Microbiology 18:52-61.

VITA

Donald L. Garling, Jr.
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI 48824

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

EDUCATION

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

POSITIONS

Professor (1990-present), Associate Professor (1985-1990), 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 Fisheries Science (1976-1980), Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture and Fisheries Educators
World Aquaculture Society,
Sigma Xi
Gamma Sigma Delta

SELECTED PUBLICATIONS

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

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

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

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

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

Garling, D.L. 1992. Making plans for commercial aquaculture in the North Central Region. Fact Sheet Series #101. North Central Regional Aquaculture Center.

Garling, D.L. 1991. NCRAC research programs to enhance the potential of yellow perch aquaculture in the region. Pages 253-255 *in* Proceedings of the North Central Aquaculture Conference. Michigan Department of Natural Resources, Wolf Lake Fish Hatchery, Mattawan, Michigan.

El-Sayed, A.F.M., and D.L. Garling. 1988. Carbohydrate-to-lipid ratios in diets for *Tilapia zilli* fingerlings. Aquaculture 73:157-163.

VITA

Christopher C. Kohler
Department of Zoology/Fisheries Research Laboratory
Southern Illinois University-Carbondale
Carbondale, IL 62901-6511

Phone: (618) 453-2890
FAX: (618) 536-7761
E-Mail: ckohler@siu.edu

EDUCATION

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

POSITIONS

Professor (1993-present), Associate Professor (1989-1993), Assistant Professor (1982-1988), and Research Associate, (1980-1981), Department of Zoology, Southern Illinois University-Carbondale
Associate Director (1991-present) and Assistant Director (1988-1991), Fisheries Research Laboratory, Southern Illinois University-Carbondale
Assistant Professor (1980), Department of Fisheries and Wildlife Science, Virginia Polytechnic Institute and State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Culture, Management, Introduced, Education and International Sections
World Aquaculture Society (USA Chapter)
Sigma Xi, Phi Kappa Phi

SELECTED PUBLICATIONS

Woods, III, L.C., C.C. Kohler, R.J. Sheehan, and C.V. Sullivan. 1995. Volitional tank spawning of female striped bass with male white bass produces hybrid offspring. Transactions of the American Fisheries Society 124:628-632.

Kelly, A.M., and C.C. Kohler. 1994. Human chorionic gonadotropin injected in fish degrades metabolically and by cooking. World Aquaculture 25(4):55-57.

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

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Killian, H.S., and C.C. Kohler. 1991. Influence of 17-α-methyltestosterone on red tilapia under two thermal regimes. Journal of the World Aquaculture Society 22:83-94.

Phillips, P.C., and C.C. Kohler. 1991. Establishment of tilapia spawning families providing a continuous supply of eggs for *in vitro* fertilization. Journal of the World Aquaculture Society 22:217-223.

Roem, A.J., C.C. Kohler, and R.R. Stickney. 1990. Vitamin E requirements of the blue tilapia, *Oreochromis aureus* (Steindachner) in relation to dietary lipid level. Aquaculture 87:155-164.

Stickney, R.R., and C.C. Kohler. 1990. Maintaining fishes for research and teaching. Pages 633-663 *in* C. Schreck and P. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

VITA

Kerry W. Tudor
Department of Agriculture
Illinois State University
Campus Box 5020
Normal, IL 61790-5020

Phone: (309) 438-2412
FAX: (309) 438-5037
E-mail: ktudor@ilstu.edu

EDUCATION

B.A. University of Northern Iowa, 1974
M.S. Iowa State University, 1979
Ph.D. Iowa State University, 1985

POSITIONS

Associate Professor (1994-present) and Assistant Professor (1987-1994) of Agricultural Economics, Illinois State University
Assistant Professor (1981-1987) of Agricultural Economics, West Texas A & M University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Agricultural Economics Association
American Economic Association
Aquacultural Engineering Society
Southern Agricultural Economics Association
World Aquaculture Society
Gamma Sigma Delta

SELECTED PUBLICATIONS

Tudor, K.W., R.R. Rosati, P.D. O'Rourke, Y.V. Wu, D.Sessa, and P. Brown. 1996. Technical and economical feasibility of on-farm fish feed production using fishmeal analogs. Journal of Aquacultural Engineering 15: 53-65.

Rosati, R., P.D. O'Rourke, K. Tudor, and P. Foley. 1994. Technical and economical considerations for the selection of oxygen incorporation devices in a recirculating aquaculture system. World Aquaculture '94, New Orleans, Louisiana. World Aquaculture Society Abstracts:34.

O'Rourke, P.D., K. Tudor., and R. Rosati. 1994. The selection and use of economic tools in the aquaculture engineering decision making process to determine the comparative costs of alternative technical solutions. World Aquaculture '94, New Orleans, Louisiana. World Aquaculture Society Abstracts: 41.

Rosati, R., P.D. O'Rourke, K. Tudor, and P. Foley. 1994. Production of *Oreochromis niloticus* in a modified Red Ewald-style recirculating system operated under commercial conditions. World Aquaculture '94, New Orleans, Louisiana. World Aquaculture Society Abstracts:46.

Foley, P., R. Rosati, P.D. O'Rourke, and K. Tudor. 1994. Combining equipment components into an efficient, reliable, and economical commercial recirculating aquaculture system. World Aquaculture '94, New Orleans, Louisiana. World Aquaculture Society Abstracts:50.

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