CULTURE TECHNOLOGY OF SALMONIDS

Chairperson: Ronald R. Rosati, Illinois State University

Extension Liaison: Ronald E. Kinnunen, Michigan State University

Funding Request: $200,000

Duration: 2 Years (September 1, 1994 - August 31, 1996)

Objectives:

1. Develop practical rainbow trout diets using regionally available feed ingredients, including fish meal analogs.
2. Use stress response as a selection tool for developing strains of trout having improved performance under conditions found in the North Central Region.
3. Use stress and performance responses in trout to evaluate culture system design and operation under practical conditions.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Year 2	Total
Illinois State University	Ronald R. Rosati	1	$6,500	$6,500	$13,000
Purdue University	Paul B. Brown	1	$17,500	$17,500	$35,000
Michigan State University	Donald L. Garling	1	$18,000	$19,000	$37,000
Ohio State University	Konrad Dabrowski	1	$15,000	$15,000	$30,000
Univ. of Nebraska-Lincoln	Terrence B. Kayes	1-3	$22,662	$17,338	$40,000
University of Wisconsin-Madison	Jeffrey A. Malison Terence P. Barry	2	$22,380	$22,620	$45,000
		TOTALS	**$102,042**	**$97,958**	**$200,000**

Non-funded Collaborators:

Facility	Collaborator(s)
Archer Daniels Midland, Peoria, Illinois	Forrest Sawlaw
National Center for Agricultural Utilization, Agricultural Research Service, USDA, Peoria, Illinois	Y. Victor Wu
Seven Pines Trout Hatchery, Lewis, Wisconsin	Hugo Kettula
Lake Mills State Fish Hatchery, Lake Mills, Wisconsin	Wisconsin Dept. of Natural Resources
Sandhills Aquafarm, Keystone, Nebraska	Michael Wyatt
Calamus State Fish Hatchery, Burwell, Nebraska	Nebraska Game and Parks Commission

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JUSTIFICATION

Members of the salmonid family, including various species of trout and salmon, are among the principal commercially cultured fishes in the North Central Region (NCA-23 1987; Brown and Hushak 1991). At the annual planning meeting of the North Central Regional Aquaculture Center (NCRAC) in 1989, the advancement of the salmonid aquaculture industry in the region was identified as high priority by the NCRAC Industry Advisory Council (IAC), the Research and Extension Subcommittees of the NCRAC Technical Committee, and the NCRAC Board of Directors. Gross sales of regionally produced salmonids have been estimated at over $6 million, or 44% of the region's aquaculture revenues.

At the 1989 through 1992 annual planning meetings of NCRAC, high-priority research topics identified and subsequently funded on salmonids included: (1) evaluation of the effects of selected genetic manipulations, particularly the induction of chromosomal ploidy changes; (2) development of less-polluting diets, with an emphasis on reducing phosphorus and nitrogenous wastage into rearing waters and facility effluents; and (3) examination of the effects of high rearing densities on physiological and organismic stress responses, survival and growth, by comparing responses in experimental tanks and production raceways. Both the rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) have been identified as the primary salmonid species of importance regionally, though this does not preclude future efforts on other species.

The salmonid aquaculture industry in the North Central Region relies on raceways, net-pens, and ponds as culture units, and is relatively small and diffuse compared to the trout raceway industry in Idaho or the net-pen culture industries in northern Europe, Canada, and Chile. However, salmonid aquaculture adds significantly to the agricultural diversity of the North Central Region and to the overall national production of salmonids (NCA-23 1987; WASC 1988). Markets for regionally produced salmonids include food-size fish sold to wholesalers or retailers in the food industry, and catchable-size fish sold to fee-fishing operations, private fish clubs, municipalities or government agencies (WASC 1988).

Salmonid aquaculture, both in the region and worldwide, is a relatively mature industry. Past and ongoing research on the principal cultured species has provided and is continuing to add important new information that has resulted in significant advances in genetics, feeds and nutrition, stress and disease management, facility design, the understanding of rearing density and water-flow requirements, and other biological and environmental subject areas of importance to salmonid aquaculture. However, problems remain in all these areas, particularly when considered in relation to the growth of regulatory restrictions on agriculture in general and aquaculture in particular. In a world environment of ever-increasing costs and growing regulation, addressing these problems now through targeted well-conceived research, coupled with timely and effective extension education, will facilitate continued profitability by existing operations, and potentially offer attractive opportunities for new ventures in commercial salmonid culture.

At both the 1992 and 1993 annual NCRAC planning meetings, the IAC expressed a strong desire - across NCRAC project in general - for a greater linkage between laboratory and applied research and for more emphasis on field testing under practical production conditions. At the 1993 meeting, the priority topics identified by the IAC for 1994-96 salmonid research were: (1) the development of practical rainbow trout diets employing regionally available feed ingredients, (2) using the stress response to develop improved trout strains for the region, and (3) using stress and performance responses in trout to evaluate culture system design and operation. However, on top of these identified research topics, the IAC continued to express its concerns over existing and potential regulatory constraints, the need for less-polluting diets, and the need to field test laboratory findings under practical production conditions.

Aquaculture effluents and the need for less-polluting diets have recently received a great deal of attention in several nations (Cowey and Cho 1991), and recent legal precedents have limited production at certain aquaculture production facilities. Additional regulatory constraints on aquaculture effluents are under consideration at the federal level as well as in some states in the North Central Region. To meet these regulatory challenges and increase cost effectiveness, research is needed to develop lower-cost, less-polluting diets; identify genetic strains that perform better, yet are more resilient and less sensitive to stress under intensive rearing conditions; and develop culture systems and operational strategies that allow the production of fish at higher densities using less water. Addressing these multifaceted yet related needs is the goal of the proposed project.

Developing Practical Trout Diets Using Regionally Available Feed Ingredients (Objective 1)

Because of feed costs, the continued movement away from the use of fish meals to alternative high-quality regionally available protein sources is vital for the continued growth of salmonid aquaculture in the North Central Region. Presently, salmonid aquaculturists in the region are obliged to import feeds from other parts of the U.S. and experience an economic disadvantage because of this. The NCRAC IAC, recognizing that the region annually produces more high-quality feedstuffs than most other parts of the country, identified the

development of salmonid diets using regionally available ingredients as a top priority. This line of research will build on previous salmonid diet development studies in the region, as well as around the world.

Recently, concern has been growing over the level of phosphorus (P) and solids released from aquaculture production facilities due to the role of these nutrients in the aging process (eutrophication) of receiving waters. Liao (1970) reported water quality degradation in receiving streams from the effluents of salmonid hatcheries. Phosphorus from fecal wastes and uneaten food stimulates increased benthic and planktonic algal blooms and macrophyte growth in freshwater systems (Lall 1991). Decreased water quality resulting from increased P discharge from fish culture facilities has been well documented (Hinshaw 1973; Bergheim and Selmer-Olsen 1978; Korzeniewski et al. 1982; Ketola 1985; Folke and Kautsky 1989; Kendra 1991). Increased eutrophication has also been observed in salt water systems from increased nitrogen levels (Persson 1991).

Kendra (1991) showed significant increases in temperature, pH, suspended solids, ammonia, organic nitrogen, total phosphorus, and chemical oxygen demand in hatchery effluent waters compared to incoming waters at 11 state and 9 commercial salmonid production facilities in Washington. Wiesmann et. al. (1988) analyzed water entering and leaving nine commercial trout farms in northern West Germany and showed that concentrations of ortho-phosphate in the water increased substantially during passage through the farms. He suggested that in order to limit phosphate pollution of receiving streams, feed manufacturers should aim to avoid dietary P concentrations that exceed the nutritional requirements of the fish being fed.

National attention was focused on Michigan when the Department of Natural Resources (DNR) was ordered by a court to substantially reduce P discharge from the Platte River Salmon Hatchery (Honor, Michigan) to cut back on the eutrophication of Platte Lake. To meet the target P levels, costly modifications were made in the hatchery settling basin and raceways to improve solids removal. Feeds were also modified by reducing P levels and changing the form of inorganic P in the mineral mix. If similar costly requirements are imposed on commercial fish farmers, continued development of aquaculture in Michigan will be jeopardized. The National Aquaculture Association and the Michigan Fish Growers' Association list waste management among their top three constraints on aquaculture development.

Most of the fish meal in the U.S. is produced from whole fish (80% menhaden), and about 10% comes from the scraps remaining after the processing of mackerel and tuna. Total fish meal supplies for U.S. demand, as determined by the difference between domestic production and net imports, has been stable for the past decade; but net imports since 1984 have ranged from 57.4 to 200.2 thousand metric tons. About 80% of the fish meal consumed in the U.S. is used as an ingredient in poultry feed. Poultry consumption more than doubled in the U.S. from 15.5 kg per person in 1960 to 35.3 kg in 1987 (Thomson 1990), whereas the U.S. annual per capita consumption of commercial fish and shellfish (live weight equivalent) increased from 10.2 to 18.5 kg over the same period (Current Fishery Statistics 1990).

Aquaculture production in the U.S. increased from 92.1 to 243.6 thousand metric tons between 1980 and 1985 and is still the fastest growing sector of agriculture. About 10% of the world's fish meal is currently used for aquaculture diet formulation, and demand is increasing. By some estimates, U.S. aquaculture production should increase from about 10% at present, up to about 25% of the domestic aquatic harvest by the year 2000. Alternatives to fish meal must be found to meet the demands of the U.S. fish feed manufacturing industry. For domestic aquaculture to expand, finding solutions through the use of agricultural by-products would benefit both the livestock rendering and aquaculture industries.

The U.S. rendering industry processes about 16.3 million metric tons of inedible by-products each year from the slaughter of cattle, swine, and poultry (F.D. Bisplinghoff, personal communications). There is a problem in using most animal by-product meals in fish diet formulations due to an imbalance in essential amino acids or low digestibility of certain proteins (e.g., keratins). It is likely that with additives of essential amino acids, we can come up with needed guidelines for the application of animal by-products and *a priori* evaluation criteria. The novelty of this approach to the proposed research is the utilization of mixtures of animal by-products. The data essential for least-cost and least-polluting diet formulations for salmonids are not presently available.

The proposed project is also designed to develop less-polluting diets that provide optimum growth in trout, and to evaluate the effects of diet composition on the characteristics of solid wastes. This research will build on experiments conducted at Michigan State University (MSU) and Purdue University (Purdue) that demonstrated that phytin P from soy flour can be chemically modified to increase its bioavailability to fish (Cain 1993; Riche and Brown 1993). The conversion of the unavailable P to an available form dramatically reduced or eliminated the need for supplemental P in the diet. Available P concentrations in the feed were closer to the requirements of the fish, a higher proportion of P was retained in the body, and a lower percentage was released as waste. However, another limitation to the use of plant products in trout diets is that high carbohydrate diets alter energy metabolism and endocrine function. To determine the maximum amount of carbohydrate allowable in a diet, we must understand how dietary carbohydrate alters metabolism and the major hormones that control growth and nutrient partitioning and turnover. Fish feeds that are low in P but

otherwise nutritionally complete must be developed if aquaculture is to continue to advance in an environmentally sound manner.

A remarkable feature of the scientific literature on fish feeds and nutrition is that while research has been done and frequent reference is made relative to the effects of stress and the relationship between nutrition and disease (e.g., see reviews by Smith 1989a, 1989b; Ghittino 1989), few if any systematic studies have been published on the effects of different diets, overall nutritional status, or specific nutrient levels, on the biochemical and endocrine mechanisms regulating the stress response in fish. This is all the more remarkable considering the fact that the stress response is a major determining factor in fish health and greatly influences disease resistance through stress-mediated effects on the immune system and other metabolic functions (e.g., see Pickering 1981; Adams 1990; Barton and Iwama 1991).

Research on fish feeds and nutrition is typically done under laboratory conditions in which dietary factors or specific nutrients are systematically evaluated; but all tested fish are of the same species and genetic strain, and environmental variables are carefully controlled, usually at or near optimum rearing conditions. In such laboratory studies, the effects of potential stressors typical of aquaculture production systems - such as system design and operation, water flow rates and hydraulic patterns, high rearing densities, marginal dissolved oxygen levels and water turnover rates, and elevated ammonia concentrations - are rarely a consideration.

Since aquaculture is inherently a complex "multifactorial" enterprise, diets and nutritional concepts developed in the laboratory must be field tested under production conditions to verify their practical value. The proposed project will initiate such field testing of laboratory-developed trout diets, as they become available.

Using the Stress Response to Develop Improved Trout Strains for the Region (Objective 2)

Midwest trout farmers, through their representation on the NCRAC IAC, have identified the development of regional trout strains as a top research priority. Presently, the industry is highly dependent on trout eggs purchased from outside the North Central Region (primarily sources on the West Coast). Increasingly, the fisheries management agencies of Midwestern states are imposing restrictions on the importation of trout eggs from other parts of the country, primarily because of concerns related to disease transmission. Accordingly, the availability of trout eggs within the North Central Region is becoming an important concern to many commercial trout growers. An important consideration related to this is that little information is available that specifically compares the suitability of existing commercially available trout strains to the highly variable rearing conditions that exist in the region.

One of the most important considerations in developing regional sources of trout eggs is that the genetic strains selected should be well suited to the distinctive conditions that are found in the region. Trout farms in the North Central Region, although numerous, are diffuse, small in size and have relatively low water flows (e.g., compared to the large operations in Idaho). To maximize production at each location, trout are often reared at high densities and/or loadings. Water temperatures on many of these farms are seasonally sub-optimal for trout, often being lower than 10°C in the winter and reaching 18-20°C or higher in the summer. In addition, some farms suffer from seasonal reductions in water flow, which can result in transient problems of low dissolved oxygen and elevated ammonia levels. Successfully coping with such environmental factors requires trout that are highly resistant to stress.

An overwhelming number of studies have shown that environmental stressors, including conditions such as those described above, can have a marked impact on the performance of cultured fish (e.g., see Pickering 1981; Adams 1990; and Barton and Iwama 1991 for reviews). In fact, the performance gains that have resulted from classical selective breeding programs can be completely offset by environmental stressors typically associated with aquaculture (Pickering 1992). Performance changes known to be induced by stress include reduced growth and feed conversion, reduced immunocompetence, and increased incidences of disease and mortality (see above cited reviews). To a large extent, environmental stressors exert these effects through their activation of the physiological stress response mechanism (see **RELATED CURRENT AND PREVIOUS WORK**).

The stress response mechanism evolved to protect fish from both acute and chronic stressors encountered in the natural environment, and there is little doubt that such a response mechanism is necessary for fish to survive actual life-threatening emergencies such as predation, lack of food, and rapidly changing environmental conditions (see Chester Jones et al. 1969; Barton and Iwama 1991). In well-managed aquaculture operations, however, cultured fish are often exposed to environmental conditions and procedures that are not harmful or life threatening in and of themselves, but nevertheless evoke a physiological stress response. This response, in turn, has detrimental effects on the growth and overall performance of cultured fish. In other words, under typical aquaculture conditions, the physiological stress response can be more detrimental to the performance than are the environmental stressors that trigger the response.

One approach to resolving this problem is to identify and directly select fish having a high resistance to stress (i.e., those having an attenuated stress response). Such a selection scheme is intimately linked to the domestication process itself, and the benefits of this approach have been well documented in other areas of agriculture, such as the poultry industry (e.g., Brown and Nestor 1973). As part of NCRAC's effort to help develop regional trout stocks, we propose to evaluate and begin using the physiological stress response (1) as a tool to identify those existing commercially available trout strains in the North Central Region that are presently most resistant to stress, and (2) as a genetic selection tool to improve the performance characteristics of trout strains being raised in the region.

Using Stress and Performance Responses to Evaluate System Design and Operation (Objective 3)

At the 1993 NCRAC planning meeting, the NCRAC IAC identified the use of "stress and performance responses in trout to evaluate culture system design and operation under practical conditions" as a top priority. In reality, this constitutes an expansion of a priority objective set forth by the IAC in 1991, to "determine the practical limits on rearing density of juvenile rainbow trout by examining the effects of selected high rearing densities on trout stress responses, survival and growth in both experimental tanks and production raceways." Research on the latter objective was begun in September 1992 and is still in progress. The primary focus of both of these objectives has been on one of NCRAC's main goals - to "optimize culture technologies of salmonids for the climactic and economic conditions existing in the North Central Region" (NCRAC 1992).

To be relevant to the production aquaculture situations, practical research on culture system design and operation requires a clear understanding of the fact that such research is intrinsically complex and "multifactorial" in nature. In overview, most culture manuals, literature reviews, and monographs on production system design and operation (e.g., Piper et al. 1982; Westers 1984; Soderberg 1986; Visscher and Godby 1987; Visscher and Dwyer 1990; Colt and White 1991) reveal that conclusions and decisions made on these topics are typically drawn from calculations, extrapolations, past experiences, and "try-it-and-see" evaluations - rather than from controlled experiments in which selected physical or biological parameters are systematically varied and all other variables possible are accounted for or regulated as part of an experimental design.

Tightly controlled experiments are rarely possible under practical aquaculture conditions - often due to facility, operational, and work-force limitations - but also because a total-systems approach to experimentation is generally considered more relevant. In reality, optimum culture system design and operation is influenced by a host of physical and biological variables, including siting, water availability, water temperature and chemistry, culture system geometry and hydraulic flow patterns, water turnover rate, fish species and genetic strain, rearing density and loading, diet composition and handling characteristics, and feeding procedures. Other potentially important considerations include: day length and the daily light/dark cycle, light intensity, water depth and background coloration, and disturbance due to background noise and vibration. Such complexity calls for a holistic approach to research on culture system design and operation.

For many years, irrespective of most physical and biological considerations and production objectives, raceway culture has been the preferred method of intensively rearing trout in the U.S. But both regionally and nationally, the high cost of raceway construction is a major constraint on the growth of trout aquaculture (JSA 1983; Senn et al. 1984). In 1992, the construction cost of new raceways was estimated at approximately \$125-320/m^3 of rearing water, depending on site, construction materials, numbers of raceways being built, and other factors (NCRAC 1992). (As a comparison, a 1993 survey and analysis of the sales literature on cylindrical-shaped tanks, conducted by University of Nebraska-Lincoln [UN-L] researchers, revealed costs ranging from \$33-235/m^3 of rearing water, depending primarily on size, construction materials, and if a sloped or flat bottom configuration is used - with most production-size tanks of 3 m diameter and larger costing \$50-165/m^3.) In conventional raceway culture, the production capacity of a given facility is usually determined by the specific water flow or "loading" requirements of the cultured species or strain. The term "loading rate" is defined as the weight of fish being raised per unit of water flow (e.g., kilograms of fish per liter per minute). Recommended loading rates are based largely on estimates of oxygen consumption, feeding rates, and production of metabolites (Westers and Pratt 1977; Piper et al. 1982; Westers 1984; Soderberg 1986; Brannon 1991; Klontz 1991).

In most conventional raceway systems, dissolved oxygen is the first factor that limits loading rate. This is true because the atmosphere is only 21% oxygen and the solubility of dissolved gases in water at normal atmospheric pressure is limited. Accordingly, even with the best aeration procedures, conventional raceway culture requires comparatively large volumes of water flow to provide sufficient oxygen for intensive fish production. This makes the trout industry heavily dependent on high-volume spring or artesian water sources, which are scarce and growing scarcer. Because of market price limitations, the cost of pumping water from wells in most commercial situations is prohibitive for trout raceway culture, even when sufficient volumes of water are available (Senn et al. 1984). Partly as a consequence of this, most if not all trout producers in the North Central Region rely on specialty niche markets and the local or regional sale of live trout for recreational

purposes, and have great difficulty competing in the general food-fish market with their Idaho counterparts, who typically obtain water ideal for trout production from high-volume springs.

In recent years, various new technologies that provide pure oxygen supplementation have received widespread attention as a potential means of increasing the carrying capacity or "loading" of fish, including salmonids, in intensive culture systems. Thus, if supplemental oxygen is used to sustain adequate dissolved oxygen levels, loading rates can theoretically be increased until the concentration of metabolites (primarily un-ionized ammonia) reach maximum permissible levels. The Michigan DNR and others (Visscher and Godby 1987; Colt and Watten 1988) have reported that pure oxygen systems can be used to greatly increase salmonid production (e.g., 1.7 to 2.8 times) in a given facility and at comparatively low cost (e.g., 2.5 to 5.5 times cheaper than increased water pumping).

Pure oxygen systems are available commercially and are being increasingly utilized for salmonid production in both private sector and government aquaculture operations (Visscher and Godby 1987; Colt and Watten 1988; Visscher and Dwyer 1990; W.J. Daley, KCM, Inc., Seattle, Washington, and H. Westers, Aquaculture Bioengineering Corporation, Rives Junction, Michigan, personal communications). However, the full potential benefits of pure oxygen supplementation remain uncertain, because of a general lack of substantive experimental evidence collected under practical conditions (as compared to engineering calculations and short-term trial-and-error equipment testing), regarding the best culture system design and operational criteria to employ to optimize the effects of oxygen supplementation, and due to the relative scarcity of definitive information on the density-related spacial requirements of salmonids independent of water quality and flow.

As a practical aid to fish culturists, Piper et al. (1982) developed a density index that is calculated by dividing kilograms of fish/m^3 (pounds of fish/ft^3) of rearing water by fish length in inches. This index is based on the premise that density can be safely increased with increasing fish size. Piper et al. (1982) recommended that a density index of 0.5 not be exceeded for salmonids, "to avoid undue crowding." However, many practical fish culturists have demonstrated and recent experimental evidence (including recent and ongoing research by the NCRAC Salmonid Work Group) support the proposition that some salmonid species, including the rainbow trout, can be safely reared at density indices considerably higher than 0.5 if good water quality is maintained (see **RELATED CURRENT AND PREVIOUS WORK**).

System design and operation have been the subjects of much discussion, calculation, and trial-and-error testing for many years (e.g., Burrows and Chenoweth 1970; Westers and Pratt 1977, Piper et al. 1982; Westers 1984; Soderberg 1986; Colt and Watten 1988; Watten and Johnson 1990; Brannon 1991; Klontz 1991; Youngs and Timmons 1991). Recently, Westers (Submitted) reviewed and analyzed a growing body of biological information, empirical evidence, and engineering design criteria which suggest that when pure oxygen supplementation is being used to intensively culture salmonids, properly configured circulating cylindrical-shaped tanks may be more operationally efficient and cost-effective than even the best designed traditional plug-flow raceways (discussed further under **RELATED CURRENT AND PREVIOUS WORK**). However, to our knowledge, the performance of rainbow trout in cylindrical tanks versus conventional raceways for intensive culture, using pure oxygen supplementation, has not been systematically compared under conditions when both types of systems are being operated at the same site using known optimum design and operational criteria and the same water source (same pH, alkalinity, etc.).

Regardless of technology, essentially all of the known environmental factors that affect the performance of trout under intensive culture conditions are also known potential stressors that can trigger a physiological stress response (e.g., see Pickering 1981; Adams 1990; Barton and Iwama 1991). Physiological stress presents one of the most insidious problems in aquaculture. From a practical perspective, procedures for identifying stress could be extremely useful in evaluating system design and operation. Potential procedures for examining stress responses and assessing the general health of trout under intensive culture conditions have been developed but have not been fully correlated and/or evaluated (see **RELATED AND CURRENT RESEARCH**). Two complicating factors in any comparison of production system design and operation or in any assessment of stress responses or general fish health are genetic strain and diet.

Our study will utilize stress and performance responses to begin comparing the operational efficiency and other merits of intensively culturing rainbow trout using oxygen supplementation in cylindrical tanks versus conventional raceways. The responses of two commercially available rainbow trout strains commonly produced in the North Central Region and to two diets developed to minimize the impact of effluent nutrient levels and fecal wastage in intensive trout culture will be examined in both cylindrical tanks and raceways under practical rearing conditions.

RELATED CURRENT AND PREVIOUS WORK

Developing Practical Trout Diets Using Regionally Available Feed Ingredients (Objective 1)

Westers (1991) has reported extensive reductions of the phosphorus (P) concentrations in the effluents from the Platte River fish hatchery in Michigan due to a reduction in feed wastage, removal of fines from the feed fed, lowering of the P levels in the fish diets, and use of dietary P sources that are more available to the fish's digestive system. Butz and Vens-Cappell (1982) found that the biochemical oxygen demand (BOD) in fish feces can decline by as much as 50% in 12 h. Sumari (1984) reported that 24-h old sludge, from a variety of diets, underwent reductions in BOD ranging from 35 to 46% in 15 d. The changes that occurred during the first 24 h were unknown.

The European Inland Fisheries Advisory Commission (EIFAC) work group on fish farm effluents recommended in 1987 that waste solids should be removed from fish rearing units as rapidly as possible, ideally without unnecessary disturbance to the structure of the solids. According to Westers (1991), the removal of intact fecal pellets is the key to effective solids and associated waste nutrient management. He stated that undivided excreta particles have a rapid settling velocity, but when fragmented, various fractions may become suspended, thus significantly reducing settling velocities. In addition, leaching of nutrients and decomposition will be accelerated on smaller suspended particles.

Fish activity facilitates the movement of fecal materials down raceways when existing water velocities are inadequate to produce such movement. The use of baffles in raceways to effect local acceleration of water flow to move fecal materials has been quite effective (Boersen and Westers 1986; Westers 1991). However, questions remain about the level of disturbance that can be tolerated as a function of diet ingredients, age of the fecal material, etc. Additional quantification can provide better direction for culture system design and determining allowable solids residence time in raceways before solids must be removed to minimize P solubilization and release, ultimately as an effluent waste.

The high costs and reduced availability of fish meal and other animal proteins have caused a shift in use to plant protein sources in the formulation of fish diets. Up to three-fourths of the total P in plant feedstuff sources is phytin P that is largely unavailable to fish (Riche and Brown 1993). Fish meal or inorganic P is added to fish diets that are high in plant proteins, to meet the minimum P nutritional requirement. The excess P from the plant proteins is voided primarily in semi-solid fish feces.

Feed composition may alter the physical properties of fecal wastes, which in turn, will affect their removal from fish culture systems. Stechey and Trudell (1990), from a study of methods of separating waste solids from three types of fish culture systems, concluded that: (1) feed related factors such as feed brand, feed type, and feeding method appear to be subordinate to facility design and culture practices with respect to pollution impact; and (2) the required level of effluent treatment (in Ontario) will be governed by total dissolved P concentration because solids can be removed to desired levels even at relatively high flow rates. Binding agents are used to stabilize fish feeds (Hardy 1989), and can have significant effects on pellet stability (Heinen 1981; Reinitz 1983), the stability of fecal materials, and on trout physiology (Storebakken 1985; Storebakken and Austreng 1987). High levels of polycellulose binders have been associated with hepato-renal syndrome in cultured turbot (Anderson et al. 1976; Roberts and Bullock, 1989).

Fish meal is traditionally used in the production of trout feeds. This commodity is expensive and often in short supply. There is increasing evidence that the nutritional value of several alternative protein sources is not inferior to that of fish meal, and their value can be increased significantly by an appropriate blend of ingredients or addition of limiting amino acids (Dabrowski and Dabrowska 1981; Dabrowski and Wojno 1984). For rainbow trout, poultry by-product meal combined with feather meal appeared to be an excellent replacement for part of the fish meal in the diet formulation. Higgs et al. (1979) found that replacing 75% of the herring meal protein in a coho salmon diet with poultry by-products meal produced the same growth rate as the control diet. Fowler (1990) found that in a chinook salmon diet, 38% of the fish meal could be successfully replaced by feather meal; and fish growth and condition did not differ at the end of a 20-week experiment.

Tacon and Jackson (1985) found that a mixture of meat and bone meal with blood meal (4:1) successfully replaced up to 50% of the fish meal in a rainbow trout diet formulation. However, meat and bone meal alone was a poor replacement for herring meal in a chinook salmon diet (Fowler and Banks 1976). A complete replacement of fish meal by animal by-products in salmon diets was not accomplished without a decrease in fish performance.

The results of a preliminary diet-testing experiment with rainbow trout fry (initial weight 1.6 g) by Ohio State University (OSU) investigators indicate that success in the ability to replace fish meal is related to total "target protein" level in the diet. At 36% protein, complete fish meal replacement with selected alternative protein sources resulted in a growth of trout not significantly different from that observed in trout fed a fish-meal based

diet. As part of the proposed project, we will test the performance of rainbow trout up to market size on diets made using similar alternative protein sources. Another important consideration in decreasing or replacing fish meal in production diets with certain alternative protein ingredients is a resulting major decrease in dietary P. However, many such ingredients are also low in certain other minerals - Mg, Mn, Cu, and particularly Zn, compared to fish meal. This complicating factor needs to be further investigated.

The plant feedstuffs evaluated in salmonid diets have been tested by "standard" nutritional methods, typically evaluating only one plant feedstuff at a time as a replacement for fish meal. Combinations of plant feedstuffs have only rarely been evaluated because of the confounding effects of the multi-variate approach. For example, soybean products, most often solvent-extracted soybean meal, have been evaluated in diets fed to rainbow trout (Cho et al. 1974; Rumsey and Ketola 1975; Gropp et al. 1976; Sanholm et al. 1976; Spinelli et al. 1978; Reinitz and Hitzel 1980; Lemm et al. 1988; Olli et al. 1989; Dabrowski et al. 1989) and Atlantic salmon (Arnesen et al. 1989; Olli et al. 1989; Hendricks et al. 1990; van den Ingh et al. 1991).

Soybean products have also been evaluated in diets fed to other salmonids (Higgs et al. 1979), but the majority of the published research has been on rainbow trout and Atlantic salmon because of the international focus on these two species. Another example of the replacement of fish meal with a plant protein source is a line of research using canola meal in diets fed to chinook salmon (Higgs et al. 1982; Higgs et al. 1983). While the list of published papers above is not complete, it should be clear that significant research has been done in the area of evaluating the replacement of fish meal with single plant protein feedstuffs. This line of research is continuing at Purdue, MSU, and other research laboratories around the world.

Most of the studies reported above found that high-quality plant protein sources could replace a portion, but not all of the fish meal in salmonid diets. A generalization that can be made from these studies is that plant protein sources can be added to salmonid diets at 15-25% of the dry matter, but will replace only a portion of the crude protein (typically, 35-45%, depending on species and life history stage). Tiews et al. (1979) summarized the results of research in which they evaluated several all-plant protein-source diets. They found that fish fed 23 out of 43 fish meal-free diets grew and converted feed as well as those fed a positive control diet. However, those 23 diets contained various animal feedstuffs (e.g., poultry by-product meal and feather meal), which can vary in nutritional content from lot to lot, or bacterial protein, krill meal, soy protein concentrates, etc., which were not commercially available at the time or were cost prohibitive.

The core problem with evaluating fish meal-free diets has been a lack of basic information on fish nutrient requirements. A major ingredient of plant feedstuffs is amino acids, but the indispensable amino acid requirements of salmonids have not all been quantified. Those that have been were published primarily in the 1980s (see Cho and Cowey 1991). With these data, all-plant feedstuff diet development using regionally available ingredients can be aggressively pursued. The research proposed by collaborators at Purdue and Illinois State University (ISU) is a continuation of work in progress on tilapia. These studies have identified several fish meal-free diets that are readily accepted by tilapia and do not compromise weight gain or feed conversion. The dietary formulation approach employed was to use readily available plant feed ingredients such as distillers grains, corn gluten products, as well as the more traditional soybean meal, corn grain, and wheat products. That formulation approach will be continued as part of the proposed project.

The proposed nutritional studies will continue and expand on previous salmonid research funded by NCRAC. Portions of the proposed research will continue evaluation of the use of phytase as a means of enhancing P absorption from plant feedstuffs (MSU) and will evaluate mechanisms of maintaining fecal pellet integrity to facilitate the management of solid wastes and diminish the solubilization of key nutrients. Another part of the proposed research has been designed to use the P and crude protein absorption estimates developed through previous salmonid studies at Purdue to develop and evaluate fish meal-free diets fed to rainbow trout that are nutritionally complete and less polluting. As an added component of the project, the two laboratory-developed diets that best facilitate the management of fecal wastes and minimize the release of effluent nutrients will be field tested under practical rearing conditions.

Using the Stress Response to Develop Improved Trout Strains for the Region (Objective 2)

The physiological stress response of fish is mediated by the nervous and endocrine systems (for reviews see Pickering 1981; Adams 1990; Wedemeyer et al 1990; Barton and Iwama 1991). Wedemeyer and McLeay (1981) classified the stress responses of fish as follows: (1) "primary alterations," reflected by increased activity of the endocrine system, including release of adrenocorticotropic hormone from the pituitary, and increased levels of circulating catecholamines and corticosteroid hormones (primarily cortisol); (2) "secondary alterations," characterized primarily by physiological responses such as increased plasma glucose and potassium titers, decreased plasma sodium and chloride, diuresis and impaired osmoregulation, reduced blood clotting time, leucopenia and decreased immunocompetence; and (3) "tertiary effects," referring to whole-animal and population responses such as reduced growth, and increased incidence of disease and death.

Numerous investigations have been done on the physiological stress responses of salmonids (for recent studies see, e.g., Patino et al. 1986; Barton and Schreck 1987; Pickering and Pottinger 1987a,b; Thomas and Rice 1987; Kebus et al. 1992; Barry et al. 1993a). Stressors frequently examined for their effects on fish include handling, crowding, forced swimming, hypoxia, changes or extremes in temperature or water chemistry, and exposure to various pollutants or toxicants (Pickering 1981, 1992; Adams 1990; Wedemeyer et al. 1990; Barton and Iwama 1991; and above cited references). Wedemeyer and McLeay (1981, see also Wedemeyer et al. 1989, 1990) proposed that acute stressors applied in challenge tests (e.g., acute bioassays) could be used to assess chronic "stress load" on fish, owing to prior exposure to other (known or unknown) stressors. Investigators at the University of Wisconsin-Madison (UW-Madison) have used such procedures to evaluate various factors, including gas supersaturation, rearing density and loading, as chronic stressors in trout (Kebus et al. 1992; Barry et al. 1993a; Barry et al. in press).

A wide range of studies have demonstrated that both chronic and acute stressors can have profound negative effects on the growth and survival of fish, both in the natural environment and under aquaculture conditions (Pickering 1992). Cultured fish are often unavoidably exposed to handling, crowding, tank cleaning, and variable and/or transiently sub-optimal water quality (e.g., in temperature, pH, and dissolved oxygen and ammonia concentrations). Such procedures and conditions are often comparatively harmless in and of themselves, but are nevertheless perceived by the fish as harmful, and thereby exert a physiological stress response (Donaldson, 1981; Pickering 1981; Adams 1990; Barton and Iwama 1991). Schreck (1981) theorized that the intensity of a fish's stress response has a major "psychological" component and is governed by the extent to which stressors cause fright, discomfort or pain. We propose that many aquaculture procedures have detrimental effects on the growth and performance of cultured fish primarily because they elicit a generalized stress response. Accordingly, fish having an intrinsically "low," "reduced," or "attenuated" stress response should show improved performance when reared under aquaculture conditions.

Considerable evidence supports this hypothesis. For example, numerous studies have shown that fish whose physiological stress response is reduced by the use of anesthetics during certain routine hatchery procedures ultimately perform better than untreated fish (e.g., Strange and Schreck 1978; Limsuwan et al. 1983a,b; Robertson et al. 1987, 1988; Laidely and Leatherland 1988; Thomas and Robertson 1991). In addition, there is a strong association, and probably a direct causal link, between elevated cortisol levels, immunosuppression, and the susceptibility of cultured fish to diseases (Ellis 1981; Pickering and Duston 1983; Pickering 1984; Thomas and Lewis 1987; Peters et al. 1988; Maule et al. 1989; Pickering and Pottinger 1989; Wiik et al. 1989; Peters et al. 1991).

Studies conducted by Refstie (1982, 1986) and Woodward and Strange (1987) demonstrated that certain components of the physiological stress response of fish are heritable traits. European strains of Atlantic salmon and rainbow trout having a specific "low" or "high" stress response (fish having low or high cortisol levels 0.5 to 2.0 h following an acute stressor) have been developed (Fevolden et al. 1991). Atlantic salmon having a low stress response showed improved growth and disease resistance, but the results for rainbow trout were equivocal (Fevolden et al. 1992, 1993). In a separate investigation, Pottinger et al. (1992) found a seven-fold difference in cortisol levels (1 h after an acute stressor) of individual trout within the same strain. To our knowledge, no additional studies on these topics have been published. As part of an ongoing project funded by the University of Wisconsin Sea Grant Institute, UW-Madison scientists are presently correlating a wide range of specific stress-response parameters of individual trout with their performance characteristics. The results of these investigations will provide the basis of the "within-strain" evaluations of rainbow trout performed as part of the proposed project.

Based on the above cited European studies and suggestive field observations such as the example given in the following paragraph, significant variations in intensity of the generalized stress response probably exist both between and within strains of rainbow trout that are commercially available to fish farmers in the North Central Region. To our knowledge, no specific information on this subject has been published. According to a survey done by Kinnunen (1990), the Kamloop strain is regionally predominant, leading all other strains in the number of producers raising it, as well as numbers of eggs and fingerlings purchased, produced, and sold. The Donaldson strain is second in importance. The commercial production and sale of other strains in the region is comparatively insignificant.

Both the Kamloop and Donaldson varieties of rainbow trout that are primarily raised in the North Central Region are domesticated strains, with the majority of eggs coming from the Pacific Northwest (Kinnunen 1990). One possible example of a potential stress-response variation between strains is the fact that the Alvdal (Swedish) strain of Donaldson trout are being produced in Nebraska under conditions of daily temperature variations and summer temperature highs that would usually be considered lethal for other strains of rainbow trout (T.B. Kayes, UN-L, personal field observations).

Our studies will begin with rainbow trout strains commonly raised in the North Central Region, and will be conducted under environmental conditions encountered in the region. Research is needed to compare the thermal tolerance limits and potential variations in the stress-response and performance patterns of trout

strains that are presently available to fish farmers in the region. The proposed research will launch a systematic effort to examine "between-strain" and "within-strain" variations in stress and performance responses in rainbow trout stocks presently being raised in the region, and will evaluate and develop methods for producing new strains of trout having improved performance traits under the aquaculture conditions found in the region.

Using Stress and Performance Responses to Evaluate System Design and Operation (Objective 3)

Optimum culture system design and operation is influenced by a broad array of physical and biological variables, ultimately depends on site considerations and water availability and chemistry, and from the management perspective, should be controlled by production objectives. Accordingly, in "real-world" terms, there is no one "best" culture system design or mode of operation that will fit every situation. Implicit to the concept of optimum system design and operation - particularly considering the information provided throughout the **JUSTIFICATION** and **RELATED CURRENT AND PREVIOUS WORK** sections of this proposal - is the strong likelihood that both diet type and genetic strain are extremely important factors, as are culture system geometry and hydraulic flow patterns, water flow and exchange rates, rearing density and loading, and feeding procedures.

Attesting to the complexity of culture system design, Westers (Submitted) recently compared, through calculations and an analysis of pertinent physical and biological information taken from the scientific literature, the relative merits of raceways and cylindrical tanks for salmonid production. According to Westers (Submitted), the principal advantages of raceways are: (1) they can support fish at higher rearing densities, owing to their hydraulic capacity to operate at higher water exchange rates; and (2) their narrow rectangular shape makes the crowding, size-grading, collection, and harvesting of fish easier than with cylindrical tanks. Properly designed raceways operate on the basis of plug hydraulic flow, and accordingly exhibit a progressive decline in water quality (i.e., declining oxygen concentration and increasing ammonia, carbon dioxide, and solid wastes levels) from their head to their tail end.

The principal disadvantages of raceways, according to Westers (Submitted), are: (1) the water velocity down them is dependent on the rate of water inflow and, applying normal design and operational criteria, is very low - too low to provide for solid wastes removal; and (2) they require thicker walls and 1.5 to 3.0 times as much wall area to enclose a given volume of water than do cylindrical-shaped tanks, which thus require far less material to construct. Conventional raceways, therefore, are not self-cleaning and are typically more expensive than cylindrical tanks. Baffles can be added to raceways to increase the water velocity along their bottoms, thereby facilitating solid wastes removal (Boersen and Westers 1986); but baffles add to the cost of raceways, greatly complicate their hydraulic flow patterns, and have uncertain effects on the physiology and behavior of the fish being produced (Westers Submitted).

The principal advantages of cylindrical tanks, according to Westers (Submitted), are: (1) their shape, combined with the far higher water velocities that can be achieved in them, make them potentially self-cleaning - particularly when an inwardly sloped floor, central drain, and external standpipe are used; (2) the water velocity and general flow pattern in them can potentially be controlled by the geometry of the inlet and outlet structures and are not totally dependent on the rate of water inflow; and (3) the near-homogeneous mixing that occurs in them greatly facilitates pure oxygen supplementation, with less chance of high-oxygen total gas supersaturation than is potentially possible in raceways. Other advantages include the facts that cylindrical tanks are comparatively easier to equip with and require fewer feeders than raceways, and provide better mixing when therapeutants or other chemicals are added to the rearing water.

When pure oxygen supplementation is not employed, cylindrical tanks cannot be used to produce fish at as high a rearing density as is possible in raceways, because their cylindrical shape does not allow for equally high water exchange rates, and because their homogeneous mixing environment results in a reduction in available dissolved oxygen due to fish respiration (Westers Submitted). Also because of this mixing, dissolved metabolites such as ammonia and carbon dioxide are not so effectively flushed from cylindrical tanks as from raceways. Thus, fish raised in cylindrical tanks will be continuously exposed to low levels of metabolically produced ammonia and carbon dioxide. At sufficiently high rearing densities, one or the other of these soluble waste products (depending on pH) will become the principal limiting factor on fish performance and production, even if sufficient oxygen is made available through oxygen supplementation.

The main conclusions that can be drawn from Wester's (1992) analyses are that: (1) properly designed raceways are the best type of culture unit in which to produce most salmonid species at high rearing densities, when pure oxygen supplementation is not being used and sufficiently high water flows are available; and (2) when pure oxygen supplementation is employed, properly designed and operated cylindrical tanks are presently the best type of culture unit to use for high density salmonid production, because of their lower cost, higher (potentially controllable) water velocities, and capacity for self-cleaning. Here, however, it should be emphasized that Wester's (1992) analyses were based almost entirely on calculations and the subjective interpretation of information drawn from the scientific literature - and <u>not</u> on the systematic side-by-side

comparison of different salmonid species or strains being raised in raceways versus cylindrical tanks using the same water source.

For many years, a guiding premise of much of the technology development on the intensive flow-through culture of salmonids has been that loading rate (rearing density divided by rate of water flow) is the primary factor determining carrying capacity and production limits, and that rearing density and/or the space requirements of the fish being raised (independent of water quality and flow) are by comparison far less important (e.g., Piper et al. 1982; Westers 1984; Soderberg 1986; Brannon 1991). While there is little question of the critical need to maintain adequate levels of dissolved oxygen and remove potentially toxic metabolic wastes, it should be kept in mind that, in part, the basis of this emphasis on loading rate is historically rooted in the fact that engineers need quantitative criteria to design and build fish hatcheries, and that hydraulic considerations, feeding rates, and the biophysics and movement of dissolved oxygen and soluble waste products fit nicely into engineering calculations and design projections. Other factors of potentially equal importance, however, have not received similar attention because they are not so readily quantified.

Thus, if flowing water of the type coming from high-capacity springs is both abundant and cheap, and capital construction costs are high (as is the case with most state and federal fish hatcheries), then the heavy emphasis on maximizing loading rates and optimizing raceway design is understandable, but is perhaps a reflection more of site constraints and immediate human (agency) needs than a systematic evaluation of the most effective way to produce salmonids at least cost. (Soderberg [1986] has presented a particularly interesting historical perspective of the evolution of present concepts underlying the technology of intensive flow-through salmonid culture, and Brannon [1991] has recently provided a very good overview on the need for a balanced approach and focus on management objectives in rainbow trout culture.) Unlike most government agencies that produce salmonids, few fish farmers in the North Central Region have access to high-capacity springs or have the capital resources to build properly engineered raceway systems.

The tendency by aquaculture systems designers, particularly in the U.S., to discount the importance of the rearing density and spatial requirements of salmonids derives in part from numerous undeniable observations that some salmonids, particularly rainbow trout, can be reared at remarkably high densities. The classic example of this is the report by Buss et al. (1970), in which the authors described successfully raising rainbow trout in 6.3-L cylindrical hatching jars to densities greater than 540 kg/m^3, as well as in an upwelling 4,557-L silo to a density of 136 kg/m^3, without any major disease problems or observations of aggressive behavior. The difficulty with interpreting such reports is that they are typically anecdotal; do not present systematically collected information on critical environmental, design, and operational parameters that can be analyzed and used to develop improved culture system designs and operational criteria; and do not provide comparative information on the performance of control groups of fish of the same strain or genetic lot raised under "standard" conditions.

Furthermore, most such examples of producing rainbow trout at ultra-high rearing densities are accomplished by using extremely high water exchange rates (thus very low loading rates) that would not normally be considered practical in commercial aquaculture. According to commercial trout producers (e.g., J.D. Erickson, Clear Springs Trout Company, Buhl, Idaho, and H.W. Kettula, Seven Pines Trout Hatchery, Lewis, Wisconsin, personal communications) aggressive behavior and subsequent fin damage and other injuries is a persistent problem in commercial rainbow trout culture at conventional raceway rearing densities (16 to 80 kg/m^3). Like many fishes, trout and salmon often exhibit intraspecific aggression and hierarchical behavior which can be stressful and result in physical injury and lowered disease resistance (Keenleyside and Yamamoto 1962; Abbot and Dill 1985; Abbott et al. 1985; Peters et al. 1988, 1991).

High population density typically increases aggressive behavior among trout and salmon as a result of territorial defense and/or competition for available food (Keenleyside and Yamamoto 1962; Cole 1976; Fenderson and Carpenter 1971; Abbott and Dill 1985; Abbott et al. 1985; Ferguson et al. 1983). Crowding often produces social hierarchies that have subordinate individuals which live in a state of chronic stress (e.g., Ejike and Schreck 1980; Noakes and Leatherland 1977; Leatherland and Cho 1985). Such fish generally have depressed growth rates (Refstie and Kittelsen 1976; Symons 1970; Fenderson and Carpenter 1971; Refstie 1977) due to reduced feeding rates and/or feed conversion. Relating behavior directly to stress, Noakes and Leatherland (1977) and Ejike and Schreck (1980) found that stress levels in rainbow trout and coho salmon, respectively, were inversely related to hierarchical status.

A remarkable feature of the salmonid literature is that few studies have been done on the effects of rearing density, independent of water quality and flow. Many investigators have simply considered altered water quality to be an intrinsic characteristic of different rearing densities, while others have attempted to compensate for differing water quality at various rearing densities by adjusting flushing or flow rates (for a detailed discussion of the problems inherent to both these experimental approaches, see NCRAC 1992). Using an experimental design developed to resolve the problem of differential water quality and flow in evaluating the effects of rearing density, Soderberg and Krise (1986) observed no significant differences in

the growth or condition of lake trout stocked at density indices of 0.25, 0.5, 1.0, and 2.0; though at 2.0 mortality was higher due to a disease outbreak.

Kebus et al. (1991), using an experimental design similar to that of Soderberg and Krise (1986), found that rainbow trout raised at low loading rates and a density index of 1.77 grew as well as fish raised at an index of 0.5, and showed no indications of being diseased or chronically stressed. Soderberg and Krise (1986) concluded that lake trout could be successfully raised at a density index of at least 1.0 (twice that recommended by Piper et al. 1982), provided that "the water requirements for respiration and waste dilution are met." Taken together, these studies clearly demonstrate that it is possible to raise salmonids under laboratory conditions at higher densities than was recommended by Piper et al. (1982) for fish hatcheries. However, neither study specifically determined the upper limits on rearing density for commercial-scale intensive trout culture.

Prior to the investigation by Kebus et al. (1991), no definitive studies examining the effects of high rearing densities on the physiological stress response in rainbow trout, independent of water quality and flow, had been reported. In September 1992, UW-Madison and UN-L researchers of the NCRAC Salmonid Work Group initiated an ongoing investigation to "determine the practical limits on rearing density of juvenile rainbow trout by examining the effects of selected high rearing densities on trout stress responses, survival and growth in both experimental tanks and production raceways." Preliminary findings, using cylindrical-shaped laboratory tanks, indicate that rearing trout at density indices up to 1.35 probably does not adversely affect survival or disease incidence, but suggest that density indices above 0.9-1.0 (independent of loading or water exchange rate) may reduce growth and feed conversion in a linear fashion with increasing density. The responses of trout to various rearing densities in production raceways is being examined in Year 2 of the project.

The stress-response patterns of rainbow trout to various rearing densities and loading rates are still being evaluated, and additional experiments by UW-Madison and UN-L investigators are underway or planned. Stress and its many deleterious effects have been identified as a high priority area for aquaculture research. However, established procedures for scientifically evaluating stress responses typically require highly controlled experimental designs, expensive laboratory equipment, and sophisticated measurements that are rarely practical under production conditions. Goede and Barton (1990) have reviewed the literature on possible organismic indicators of stress in fish and described an autopsy-based indexing system, based on various morphological and hematological determinants, to evaluate stress level and overall health.

Recently, Goede (1993) proposed the use of this autopsy-based indexing system to evaluate the relative health and condition of fish (applied to date primarily to trout) under either culture or free-ranging field situations. As part of the ongoing NCRAC salmonid project, UN-L researchers have begun contrasting potential organismic indicators with established physiological measures of stress under practical culture conditions. Such research is needed both to help validate and to potentially broaden the scope of the Goede indexing system - by examining the relationship between the generalized stress response and overall health and condition status.

The proposed study on culture system design and operation will expand on the ongoing NCRAC salmonid research project. Most of the proposed research will focus on utilizing stress and performance responses to compare the merits of intensively culturing rainbow trout using oxygen supplementation in cylindrical tanks versus raceways under practical rearing conditions. As part of a holistic approach to this line of research, both genetic strain and diet will be treated as an integral part of system design and operation, and two different strains and two different diets will be evaluated in both types of production systems. In addition, work will be continued on evaluating potential organismic indicators of stress and on validating the Goede indexing system of assessing general fish health and condition.

ANTICIPATED BENEFITS

The collaborative project proposed will address one of NCRAC's main goals - to "optimize culture technologies of salmonids for the climatic and economic conditions existing in the North Central Region" (NCRAC 1992). At the direction of the NCRAC IAC, the NCRAC Salmonid Work Group will focus its efforts entirely on rainbow trout on three priority research needs: the development of less-polluting lower-cost diets from regionally available feed ingredients; the evaluation and development of genetic strains having improved performance under regional conditions; and the evaluation of culture system design and operation under practical conditions.

The research proposed to develop less-polluting lower-cost diets from regionally available ingredients will benefit existing aquaculturists facing stricter regulatory pressures to reduce waste nutrients in effluents, as well as new aquaculturists facing increasingly complex permitting processes. Using regionally available plant protein and animal by-product protein sources as substitutes for fish meal in trout diets should reduce the cost of feed manufacture and help produce diets that are less polluting. The research proposed to evaluate and

develop improved trout strains will delineate practical methods for producing improved regional strains with enhanced growth rates, feed conversion, and disease resistance that will improve overall production efficiency and help decrease effluent wastes.

Evaluation of the best available "less-polluting" diets made from regionally available ingredients and of trout strains presently available to regional fish farmers will be initiated as part of the research proposed to evaluate culture system design and operation under practical conditions. Using a holistic approach and appropriate field testing, both diet and genetic strain will be treated as an integral part of system design and operation, and the performance of the different diets and different strains will be compared in cylindrical tank versus raceway production systems, using pure oxygen supplementation.

This overall approach will provide a strong element of integration between the proposed project's three objectives and should help facilitate the transfer of the new products and technologies developed to practicing fish farmers, potential fish farmers, feed manufacturers, aquaculture facility designers, and other user groups which will be done through research publications, extension fact sheets and bulletins, and other appropriate outreach mechanisms.

OBJECTIVES

1. Develop practical rainbow trout diets using regionally available feed ingredients, including fish meal analogs.

    a. Evaluate the effects of feed binders in diets formulated from locally available plant ingredients on trout performance and on the stability of trout feces to enhance the removal of solids from hatchery effluents.

    b. Evaluate the effectiveness of phytase treatment of plant feed ingredients on phosphorus and protein availability to trout.

    c. Develop and evaluate fish meal-free diets using regionally available feed ingredients.

2. Use the stress response as a selection tool for developing strains of trout having improved performance under conditions found in the North Central Region.

3. Use stress and performance responses in trout to evaluate culture system design and operation under practical conditions.

PROCEDURES

Developing Practical Trout Diets Using Regionally Available Feed Ingredients (Objective 1)

Experiments to evaluate the effects of different feed binders in all-plant protein-source diets on rainbow trout performance and the stability of trout feces will be conducted at MSU, under the supervision of Don Garling. Stabilizing trout feces should enhance solids removal of solids from hatchery effluents. Paul Brown of Purdue and Ron Rosati Of ISU will formulate experimental feeds using regionally available plant protein sources. Experimental feeds will be balanced for the supply of essential amino acids, total protein, and metabolizable energy.

At MSU, experimental diets and a control (open formula trout diet used by the Michigan DNR) will be fed to triplicate groups of rainbow trout for 10-week preliminary trials. Trout will be reared in 300-L tanks that have been modified to facilitate solid waste collection. Growth, feed conversion, body composition, protein efficiency ratio, and waste production will be determined using standard methods. In addition to whole body analysis, liver fat levels will be determined. Promising diets from preliminary trials will be fed to triplicate groups of 50-mm until they reach market size.

Solid wastes will be removed from experimental tanks daily. Samples for mass balance estimates will be collected during each feeding period, throughout randomly selected 2-d periods to calculate the total P recovered. Total P collected per day will be calculated and multiplied by the number of days in the feeding period.

P in effluents can be described using the following mass balance equations (Cain 1993) as:

$$P_{ef} = P_{in} + P_{ho}$$

where:

P_{ef} = P in hatchery effluent
P_{in} = P in incoming water
P_{ho} = P of hatchery origin (from feeding, solids, . . .)

P_{ef} and P_{in} will not be measured since P from water is not appreciably used by fish (Lall 1991) and P_{ef} should only change as a result of P_{ho}. P effluents of hatchery origin will be measured using a mass balance equation:

$$P_{ho} = P_{fed} - [P_f + (P_{tm} - P_{ti})]$$

where:

P_{ho} = P in effluent from hatchery origin
P_{fed} = P in feed
P_f = P in solid wastes (feces and uneaten feed)
P_{tm} = P in fish at the time measured during grow out cycle
P_{ti} = P in fish at the beginning of the grow-out cycle.

Fecal characteristics will be compared between diets. Techniques used by Chen et al. (1993) to measure the characteristics of suspended solids from recirculating aquaculture systems will be modified to: (1) identify particle size distribution, (2) determine the specific gravity of fecal materials, and (3) examine stability by observing fecal settling characteristics leaching for various levels of disturbance.

A second set of experiments will build on a recent NCRAC Salmonid Work Group study designed to evaluate the use of phytase to reduce feed and effluent P levels and the development of mass balance methods to accurately measure P levels in hatchery effluents (Cain 1993). An experimental trout diet employed in earlier NCRAC funded research will be used as a positive control. The latter is based on the T2M diet developed by Ketola (Tables 1-3). An open formula trout feed used by the Michigan DNR will also be used as a positive control. Plant protein sources from at least two feeds formulated by Brown and Rosati will be treated with phytase to increase the bioavailability of P (Cain 1993). Non-phytase treated feeds will be fed as negative controls.

The diets will be fed to triplicate groups of 50-mm trout until they reach market size. Trout will be reared in 300-L tanks that have been modified to facilitate solid waste collection. Growth, feed conversion, body composition, and waste production will be determined using standard methods. In addition to whole body analysis, liver fat levels will be determined.

Solid wastes will be removed daily. Samples for mass balance estimates will be collected during each feeding period, throughout randomly selected 2-d periods to calculate the total P recovered. Total P collected per day will be calculated and multiplied by the number of days in the feeding period.

Data will be analyzed using one-way Analysis of Variance (ANOVA) on all growth and whole body composition data to determine if differences observed between fish fed different diets are statistically significant. Duncans/Newman-Keuls test ($p = 0.05$) will be conducted as a multiple comparison on all growth data for all experiments to identify the treatments that differed from one another (Gill 1978).

Table 1. Composition of T2M experimental diet (%). Diet formulation for experimental diets provided by George Ketola, U.S. Fish and Wildlife Service (USFWS), Tunison Laboratory of Fish Nutrition, Cortland, New York.

Ingredients	%	Notes
Soybean meal	31	
Corn gluten meal	30	
Blood flour	10	
Fish oil	11	
L-lysine HCl	0.4	
Vitamin pre-mix	1.0	Vitamin premix No. 30 as supplied by the USFWS (see Table 2)
Mineral mix	+	See Table 3
Deflourinated rock phosphate	+	Diet T2M was supplemented with enough finely ground deflourinated rock phosphate (DRP) to provide a level of dietary P equal to the difference between 0.66% of diet and the amount supplied by fish meal.
Herring meal	10	
Cellulose	+	

Table 2. USFWS vitamin premix No. 30 used in T2M experimental diet. Choline chloride and ascorbic acid were added at levels of 0.14 and 0.28 g/kg of diet respectively.

Vitamin	mg/g premix	Vitamin	IU/g
D Calcium pantothenate	26.5	Vitamin E	88.2
Pyridoxine	7.7	Vitamin D_3	110.25
Riboflavin	13.2	Vitamin A	1,653,750 USP
Niacinamide	55.1		
Folic acid	2.2		
Thiamin	8.8		
Biotin	0.0882		
Vitamin B_{12}	0.0055		
Menadione sodium bisulfite complex	2.76		

Table 3. Mineral mixture composition used in T2M experimental diet.

Minerals	Amount (mg/kg of diet)
Mn	100
Zn	100
Cu	10
Fe (ferrous)	100
I	5
Se (selenite	0.1
Mg	990

Experiments on the development and evaluation of fish meal-free diets made with plant-protein sources will be a collaborative effort between Paul Brown of Purdue and Ron Rosati of ISU. Diets will be formulated at Purdue using "Mixit-2+" (Agricultural Software Consultants, Kingsville, Texas), a least-cost feed formulation program, using the dietary restrictions suggested by Cho and Cowey (1991). Nine separate formulations will be developed in each year of this proposal. Those developed in the first year will be formulated using a similar approach to those developed for tilapia. For example, regionally available feed ingredients will be used, including distillers grains, corn gluten feed and meal, soybean meal, canola meal, wheat gluten, and wheat midds. Diets formulated in the second year of the study will depend on results from the first year, but will be a continuation of the same type of effort. For example, depending on price and availability of ingredients, it may be possible to incorporate and evaluate cottonseed products, sunflower seed meals, and flax. Dietary formulations will be transferred to ISU. Feed ingredients will be acquired, mixed, and extruded at the ISU Experimental Feed Mill, then transported to Purdue for diet testing. This collaborative approach should make the best use of regionally available expertise and equipment.

All diets will meet the known indispensable amino acid (IAA) requirements of rainbow trout. For those IAAs not yet quantified, we will use the A/E ratio and the published quantitative values to predict those levels (Wilson and Poe 1985; Brown In press). Purdue researchers have conducted whole-body amino acid analysis of rainbow trout; and those have been verified by a private laboratory (Multi-Foods AgriResearch Group, Courtland, Minnesota). All diets will be formulated to contain 35% crude protein, similar levels of lipid, the same energy:protein ratio, and nutritionally complete vitamin and mineral premixes. The Feed Mill at ISU has been established, and maximum levels of lipid incorporation are being evaluated. We anticipate using values in the range of 16-20% of the dry diet (Cho et al. 1991) as fish oil, canola oil, or combinations of both, making sure that all diets contain at least 10 g/kg 18:3n-3 (Cho and Cowey 1991). All nutritional levels will either meet or exceed those recommended for rainbow trout (Cho and Cowey 1991).

At Purdue, experimental diets will be randomly assigned to triplicate groups of juvenile rainbow trout. We anticipate using either Mount Shasta or London strain fish in these feeding trials. Broodstock of both strains are currently located at Purdue, and eggs have been collected and fertilized from the London strain this year. Feeding rates will vary as fish get older and will follow recommended levels (Piper et al. 1982). Fish will be grown to market or near-market size in an established rearing system. That recirculating system is a series of 48, 120-L tanks connected to a solids and biological filter. The system is currently in use with rainbow trout, and a second study with trout may be conducted prior to starting the proposed experiment. Fish will be randomly stocked into aquaria at below maximum loading rates (Cho and Cowey 1991). Through the course of the study, if loading exceeds the recommended levels, we will decrease biomass in all replicates by selective removal of "average" size fish. Therefore, loading or density should not impact our feeding trial. In addition to the experimental diets, triplicate groups of fish will be fed a commercially available control diet.

Water quality will be monitored on a regular basis. Ammonia-nitrogen, nitrite-nitrogen, temperature, and dissolved oxygen will be monitored daily and maintained within acceptable limits for salmonids.

At the end of the growth phase of the diet study, samples of fish will be collected for proximate composition of fillets. Fillets will be removed by hand from a subsample of fish from each replicate group, dress out percentages will be determined by expressing fillet weight as a function of total wet body weight, then frozen prior to analyses. Nutritional contents will be determined in the Purdue Fish Nutrition Laboratory using standard methods (AOAC 1989). Response parameters used to judge the adequacy of diets will be weight gain, feed conversion ratio, survival, dress out percentage, protein retention, and proximate composition of fillets. While few trout are marketed as fillets, this parameter will serve as an indication of gross muscle production and adverse impacts on muscle quality.

Data will be statistically analyzed by one-way analysis of variance using each tank of fish as a replicate. Accepted level of significance will be 0.05.

Studies conducted by OSU investigators will evaluate animal by-product as substitutes for fish meal in rainbow trout diets. Rainbow trout of approximate weight 20-30 g will be allocated to 15 experimental 250 l fiberglass tanks. Each tank will contain 25-50 fish of two strains, London, Ohio and Mount Shasta, California. Fish will be fin clipped (left or right) to distinguished strains during the weight controls and sampling for body proximate composition, feces collection etc. at the completion of the growth trial.

Five diets (Table 2) will be fed to three groups each over a 4-5 month period. Fisheries and animal by-products will be purchased from a 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 (% day^{-1}), gross feed conversion ratio, apparent net protein utilization ratio and digestibility (absorbability) of amino acids between dietary groups.

At the conclusion of the growth trial, fecal samples will be collected on alternate days by stripping all of the fish after anesthesia with a 1:10 000 dilution of MS222. The sampling will be divided into three periods of 3 days each, and three samples treated as replicates. Samples will be stored at -18°C and then freeze dried.

To determine Cr content, samples of food or feces of approximately 40 mg will be ashed at 500°C for 15 h in 50-mL digestion tubes. After cooling, 0.5 mL of an oxidation mixture containing 70% perchloric acid, concentrated sulfuric acid and 2% Na-molybdate will be added to each ashed sample and heated until the color changes from green to orange. After cooling, 0.2 mL of perchloric acid will be added and the sample heated again to boiling.

Apparent absorption of amino acids will be calculated according to the formula given by Dabrowski and Dabrowska (1981) using Cr in food and feces as the marker.

For amino acid analysis, freeze-dried and pulverized samples will be hydrolyzed in 6N hydrochloric acid, at 110°C for 24 h, under vacuum. After acid removal by rotary evaporation, coupling of amino acids with phenylisothiocyanate (PCT) will be carried out. Reverse-phase HPLC separation of PCT-derivatives of amino acid will be run according to the procedure described by Heinrickson and Meredith (1984).

Using the Stress Response to Develop Improved Trout Strains for the Region (Objective 2)

Studies to evaluate "between-strain" and "within-strain" differences in the intensity of the generalized stress response of rainbow trout that are commercially available to fish farmers in the North Central Region will be done by UN-L and UW-Madison investigators, respectively. Potential variations in the stress and performance responses of two trout strains that are presently available in the region will be compared by UN-L investigators and their collaborators, under the direction of Terry Kayes, as described under Objective 3. The principal hypothesis of the research proposed by UW-Madison investigators on Objective 2 regarding "within-strain" differences is that the offspring of broodfish selected for a consistent stress response correlated with superior performance will outperform the offspring of non-selected broodfish, in terms of endpoints such as growth, feed conversion, condition, disease resistance, and survival.

Research to evaluate the efficacy of using the generalized stress response as a selection tool for producing regional rainbow trout broodstocks having increased performance capacity will be conducted collaboratively by investigators from the UW-Madison and Seven Pines Trout Hatchery, Lewis, Wisconsin. Seven Pines Trout Hatchery has a large disease-free certified population of Kamloop-derived broodfish, and is a leading supplier of rainbow trout eggs in the North Central Region. These fish have been reared under conditions common to the North Central Region for several generations and therefore are particularly well suited for the proposed study. Seven Pines Trout Hatchery will supply the fish and some of the rearing facilities required for the project. Personnel from the UW-Madison, in collaboration with Seven Pines Trout Hatchery staff, will be responsible for implementing the experimental protocols and conducting the required analytical work.

Physiological stress responses will be evaluated by subjecting fish to a 1-min acute handling stressor and subsequently measuring the serum levels of various physiological stress indicators (including serum cortisol, glucose, chloride, and osmotic pressure; see Donaldson 1981; Schreck 1981; Wedemeyer and McLeay 1981; Adams 1990; Kebus et al. 1991; Barry et al. 1993 a,b). The specific physiological indicator(s) to be measured, and the time at which blood samples will be taken, will be determined by the results of an ongoing study at UW-Madison (funded by the University of Wisconsin Sea Grant Institute [See Grant]) designed to evaluate the extent to which the physiological stress responses of individual rainbow trout are correlated with subsequent growth and performance. In the Sea Grant study, various physiological stress indicators will be analyzed at different times (i.e., 1, 6, and 24 h) after the administration of an acute handling stressor. The subsequent growth and performance of each fish will then be correlated with combinations of physiological measures at selected times (e.g., cortisol at 1 h, glucose at 6 h, chloride at 3 h, etc.). The combination shown to have the greatest diagnostic value will be employed in the proposed investigation.

Techniques for measuring serum cortisol, glucose, and chloride in rainbow trout have been validated by UW-Madison investigators (Barry et al. 1993a). Cortisol will be measured using a microplate enzyme-linked immunosorbant assay (ELISA). Glucose and chloride will be measured using a standard enzymatic procedure (glucose oxidase diagnostic kit 510-DA, Sigma Chemical Company, St. Louis, Missouri) and a Corning model 925 chloride analyzer (Ciba-Corning, Medford, Massachusetts), respectively. Serum osmolality will be measured using a Wescor vapor pressure osmometer (model 5100 C, Logan, Utah). Preliminary experiments evaluating the effects of fish sampling techniques, anesthetization, time of day, and blood-collection methods on the stress responses of rainbow trout have already been conducted by UW-Madison investigators, and it is anticipated that procedural artifacts will be minimal in these experiments (Kebus et al. 1991; Barry et al. 1993 a,b).

The selection process will begin with 250 to 300 2-year-old fish randomly selected from the future broodfish population at Seven Pines Trout Hatchery. The fish will be individually tagged and held in 10 net-pens (25

to 30 fish per net-pen) set up in a rearing pond supplied with aerated well water. The fish will be acclimated to the net-pens for at least 3 weeks prior to the start of the experiment. Results from a previous investigation (Pottinger et al. 1992) indicate that 25 to 40% of the fish from a random population of rainbow trout will show an "attenuated" or "low" cortisol response 1 h following an acute stress. Assuming these values hold true for our study, we expect that 60 to 120 fish from the initial population will have stress responses correlated to superior performance characteristics (as determined by the Sea Grant-funded project described previously).

Trout showing a consistent stress response will be identified by subjecting all 60 to 120 of the initially selected fish to a standardized acute stressor and quantifying their physiological stress responses a minimum of four times over the subsequent year. Approximately 50% of these fish are expected to show a consistent stress response (see Pottinger et al. 1992). At the end of Year 1, therefore, 15 to 30 fish of each sex showing a consistent "superior" stress response will be identified. These fish will then be used as broodstock for the second phase of the study, which is designed to compare the performance of the offspring of the selected fish with that of the offspring of non-selected fish.

The selection procedure will be completed sometime before the autumn of 1995, at which time the fish will be 3-years-old and sexually mature. The selected broodfish will be allowed to ovulate naturally, and the eggs from at least six individual females will be fertilized with the pooled milt from at least six selected males. Eggs and milt from broodstock chosen randomly from the original Seven Pines population will be fertilized in an identical manner, and these will serve as the non-selected controls. The offspring from both the selected and control fish will be reared using standard procedures and identical environmental conditions.

The rearing environment will be kept suboptimal in order to evaluate the growth and performance of the trout subjected to the types of chronic stressors commonly associated with intensive fish farming. Suboptimal rearing conditions may include high rearing density (see Soderberg and Krise 1986; Pickering and Pottinger 1987; Kebus et al. 1991; Barry et al. 1994) and suboptimal water quality parameters, such as changes in temperature mimicking that often found on trout farms in the North Central Region (see **JUSTIFICATION**). Throughout the grow-out period, performance parameters including growth, feed conversion, incidence of disease, and mortalities will be monitored. The physiological stress responses of the offspring will be characterized at the termination of the study, and perhaps at other times, by applying acute stress challenge tests as described above.

Our data will be analyzed by analysis of variance using split-plot or multifactorial designs, as appropriate. The UW-Madison will be responsible for all data collection and analysis. Research findings will be published in a timely manner in appropriate peer-reviewed scientific journals. Extension information outlining the practical implications and benefits of the research will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

Using Stress and Performance Responses to Evaluate System Design and Operation (Objective 3)

Research to evaluate culture system design and operation under practical conditions using both stress and performance responses of rainbow trout as experimental criteria will be done by direction of Terry Kayes, and in cooperation with the Nebraska Game and Parks Commission and with Sandhills Aquafarm, a commercial trout production operation located near Keystone, Nebraska. All of the research conducted on this part of the proposed project will be performed at commercial-scale fish production facilities, and the results generated should be of near-term value to existing or potential trout producers.

The main focus of our investigation will be to utilize stress and performance responses to compare the operational efficiency and other merits of intensively culturing trout using pure oxygen supplementation in cylindrical tanks versus conventional raceways. Because type of diet and the stress-response and performance characteristics of different genetic strains of trout are factors that probably have a major influence on optimum culture system design and operation, two newly-developed "less-polluting" diets and two trout strains presently available to fish farmers in the North Central Region will be compared in both types of production systems. The two trout strains tested will be a domesticated Kamloop and the Alvdal (Swedish) variety of Donaldson trout.

Principal hypotheses for Objective 3 are: (1) when pure oxygen supplementation is used, overall trout performance and production will be better in cylindrical tanks than in raceways; (2) the average growth of trout will be about the same in both cylindrical tanks and raceways, but size variation and mortality, partly due to increased stress levels, will be greater in the latter; (3) performance and production of the Donaldson strain trout will be comparatively better than that of the Kamloop in cylindrical tanks than in raceways - i.e., there will be less difference in the Kamloop strain between culture systems; (4) the Donaldson trout will have a more "attenuated" generalized stress response than the Kamloop, and therefore will be less sensitive to stressors such as daily temperature fluctuations, temperature extremes, and elevated un-ionized ammonia levels; and (5) The fecal wastes generated from the "less-polluting" diets will be more readily and effectively removed from properly designed cylindrical tanks than from raceways, even ones equipped with baffles.

Comparisons of trout stress and performance responses in the two types of production systems will be performed primarily at the Calamus State Fish Hatchery near Burwell, Nebraska, though some may also be done at Sandhills Aquafarm. Unless otherwise indicated, most experiments comparing the merits of cylindrcal tanks versus raceways will have a duration of 6 to 12 weeks, and will be conducted using fish that are initially 65 to 130 mm in total length (TL) that will be further graded for uniform size before assignment in an experiment. All experiments will be run under ambient photoperiod and temperature conditions. The primary source of well water supplied to the raceways and other production tanks at the Calamus fish hatchery is a constant 13°C, though some 11°C well water is also available. The spring water supplying Sandhills Aquafarm can vary between 13 and 22°C in the summer, though is normally about 17-20°C, and can vary between 2 and 8°C in the winter, but is normally about 6-7°C.

Emphasis during the first 6 to 10 months of the proposed research at the Calamus hatchery will be placed on analyzing and improving operational protocols to optimize the performance and production of trout in cylindrical tanks and raceways, independent of any comparison between the two types of systems. In large part, the exact nature and direction of these future optimization studies will be determined by ongoing investigations and research that will be done at the Calamus hatchery over the next year, as part of a present NCRAC-funded effort to "determine the practical limits on rearing density of juvenile rainbow trout by examining the effects of selected high rearing densities on trout stress responses, survival and growth in both experimental tanks and production raceways" (NCRAC 1992). By the start of the proposed project in the autumn of 1994, we expect to have fairly close estimates of the maximum practical rearing densities and loading rates for raising Kamloop trout at the Calamus hatchery, given the groundwater chemistry on site.

Since pure oxygen supplementation through sealed packed columns is available at the Calamus hatchery and will be utilized as part of the proposed project, present plans for the first 6 to 10 months of research will be to determine maximum practical trout carrying capacities in both cylindrical tanks and raceways based on allowable un-ionized ammonia level. This will be done via a series of short-term (6-8 week) single- and multifactorial experiments, and using calculation procedures outlined by Soderberg (1986) and Westers (1984, Submitted). Ammonia levels will be manipulated through appropriate adjustments in rearing density and/or water exchange rate. Considerable uncertainty exists in the literature over the maximum allowable un-ionized ammonia level for salmonids. Westers (Submitted) recently suggested 0.025 mg/L as the maximum, in accordance with the recommendations of the European Inland Fishery Advisory Commission (1987) provided that the partial pressure of dissolved oxygen in the water does not go below 90 mm Hg, the water temperature is above 5°C, and the pH does not exceed 8.0. This recommended maximum is significantly higher than the traditionally reported maximum of 0.0125-0.016 mg/L (see discussions by Piper et al. 1982; Soderberg 1986).

In reality, the un-ionized allowable ammonia maximum probably varies with species, genetic strain, and a number of other physical and biological factors that are not well understood. Studies recently completed at the UW-Madison, as part of the ongoing NCRAC Salmonid Work Group project, suggest that un-ionized ammonia levels above 0.010-0.019 mg/L are detrimental to Kamloop rainbow trout, though part of this observation may have been related to the amount of solid wastes present in the tanks (J.A. Malison, UW-Madison, personal communications). Experiments at the Calamus hatchery will examine the practical un-ionized ammonia limits of both the Kamloop and Donaldson strains of rainbow trout. In all our performance and production experiments at the Calamus hatchery, dissolved oxygen and total dissolved gas pressure will be maintained at or slightly below saturation levels using pure oxygen supplementation through sealed packed columns. Most if not all of these experiments will be done using "single-pass" water delivery to the tanks and raceways, rather than by providing water to tanks and/or raceways arranged in series.

Also during the first 6 to 10 months of the proposed project, the design of various types of inlet structures for providing water to cylindrical tanks will be evaluated for their effects on water velocity and chemistry in the tanks and the "self-cleaning" characteristics of the tanks. As revealed by Tvinnereim and Skybakmoen (1989) and Westers (Submitted), most studies of inlet structure design to such tanks have been done without fish in the tanks, which to some extent compromises the practical relevance of these studies. Our experiments will employ near-optimal rearing densities and loading rates of fish in the tanks, and will use tanks with sloped bottoms, a central drain, and an external standpipe, as recommended by Westers (Submitted).

In all our performance and production experiments at the Calamus hatchery, total dissolved gas pressure, totaloxygen, pH and total ammonia-nitrogen, nitrite-nitrogen, total alkalinity, total phosphorus, orthophosphate, total suspended solids, turbidity, and BOD of the water in tanks and/or raceways and their effluents will be monitored at appropriate intervals, using a Sweeney "saturometer," a calibrated dissolved-oxygen probe, and standard procedures (AOAC 1980; APHA et al. 1986), as applicable. To facilitate stress assessments in short-term (6-8 week) experiments, fish will be assigned to tanks or raceways 2 to 3 weeks before the initiation of experimental treatments. Fish will be fed a set ration based on percent body weight, two times a day, either by hand or using timer-controlled feeders. Unless otherwise indicated, the feed used will be the open-formula diet routinely fed to trout by the Nebraska Game and Parks Commission. The amount fed will be calculated to produce near-optimum growth (Piper et al. 1982; Westers 1984; Soderberg 1986; and using standard

feeding tables), and will be adjusted every 2 weeks based on projected growth and monthly subsample determinations of weight gain.

The exact design and/or timing of each experiment, size and number of trout assigned to each treatment group, size of cylindrical tanks or raceways used, etc. at the Calamus hatchery will be determined by the objective(s) of the experiment, work-force availability, and the availability of fish and rearing units. Sufficient numbers of cylindrical tanks and raceways of the same type and in a variety of sizes are available at the Calamus hatchery (see **FACILITIES**), or can be provided by the UN-L, to conduct multivariate experiments using three or four replicate groups per treatment. When appropriate, basic or multifactorial variations of the experimental design of Soderberg and Krise (1988), Kebus (1992), and/or Barry et al. (1993b) will be used to evaluate the effects of various rearing densities, loading rates, or water exchange rates in tanks. Present plans are that most experiments will probably be run using either 1.2- or 1.8-m diameter cylindrical fiberglass tanks, and/or 0.61-m wide × 6.1-m long × 0.61-m deep or 0.91-m wide × 6.1-m long × 0.61-m deep fiberglass rceways. Both Kamloop and Donaldson strain trout will be procured from certified disease-free hatcheries.

The trout from each treatment group at the beginning and end of most experiments will be counted, and their total body weights and lengths measured. When appropriate, randomly selected samples of fish at the start of experiments or from individual treatment groups will be sacrificed for blood and tissue collection, or to assess relative health and condition (as per Goede 1993). For most experiments, fish randomly sampled from each treatment group will be weighted and measured at biweekly or monthly intervals. Except for acute stress challenge tests, all fish will be anesthetized with tricaine methanesulfonate (MS-222) prior to sacrifice, routine handling, or measurement. Measures of trout performance that will be recorded or calculated include: percent survival, morbidity, incidence of disease, length and weight growth, condition factor, feed ration, feed conversion, and carcass protein and fat contents - all determined by standard methods. The general health of fish throughout our study will be monitored with the assistance of the State Hatchery Biologist, who is based at the Calamus hatchery and is responsible for salmonid disease inspections statewide.

Standard physiological measures of the generalized stress response that will be routinely employed in the Nebraska experiments include assessments of serum glucose and chloride titers, serum osmolarity, hematocrit, and leucocrit (see Wedemeyer and Yasutake 1977; Wedemeyer and McLeay 1981; Barry et al. 1993a). For certain critical experiments, collected serum subsamples may also be sent to the UW-Madison for the determination of serum cortisol titers using a recently developed and validated ELISA (Barry et al. 1993a). As noted previously (under **PROCEDURES**, Objective 2), past studies, which have evaluated the effects of fish sampling techniques, anesthetization, time of day, and blood collection methods on the stress responses of rainbow trout, have shown that procedural artifacts should be minimal in our experiments, given the procedures that will be employed.

To assess chronic stress in trout subjected to different experimental treatments or production conditions, samples of fish from each treatment group or production lot will be subjected to a 1-min acute handling stressor about a week before the end of the experiment. The physiological responses (e.g., serum osmolarity and cortisol, glucose, and chloride titers) of these fish over time (e.g., 0, 3, 6, and 24-h post-stress) will be compared to fish not subjected to the acute handling stressor. In lengthy (over 10-week) experiments, acute stress challenge tests may also be administered midway through the experiment. Throughout the Nebraska study, potential organismic indicators of stress, of the type described by Goede and Barton (1990) and Goede (1993), will be systematically evaluated and contrasted with physiological measures of stress. Validation of the Geode (1993) indexing system as a means of evaluating stress would provide practicing fish culturists with an extremely useful management tool.

On completion of the experiments to optimize the performance and production of rainbow trout in cylindrical tanks and raceways independent of one another, an investigation will be initiated to contrast trout performance and production in the two types of systems under conditions in which both are operated in an optimal manner by the same personnel, using the same water source. As part of this investigation, the stress responses, performance, and production of Kamloop and Donaldson strain trout will be compared in cylindrical tanks versus raceways. This will be done using a 2 × 2 factorial design (genetic strain × type of culture system), with a minimum of three replicate groups per treatment. In this experiment, since pure oxygen supplementation will be employed and dissolved oxygen will not be limiting, effluent un-ionized ammonia concentration will be used as the standard for "setting" the carrying capacity of both the cylindrical tanks and raceways - specifically, either water flow rates or rearing densities will be adjusted to maintain effluent un-ionized ammonia levels at about 0.012 mg/L (as Piper et al. 1982; Soderberg 1986).

Depending on time and available resources, a second experiment of identical design (comparing the two genetic strains and culture systems) will be conducted, except that an effluent un-ionized ammonia concentration of 0.025 mg/L will be used as the standard for "setting" carrying capacity (as per Westers Submitted; and the recommendations of the European Inland Fishery Advisory Commission). This experimental approach should test the practical performance and production limits of both the Kamloop and Donaldson strains, as well as cylindrical tanks and raceways, and should help resolve the present uncertainty

that exists regarding the maximum allowable un-ionized ammonia level for trout. Two 2 × 2 factorial experiments that definitely will be done towards the end of the proposed project will separately contrast (in only one trout strain) the merits of the two best "less-polluting" diets developed by MSU, Purdue, and ISU investigators, with the fish production and waste-solids management characteristics of the open-formula diet routinely fed to trout by the Nebraska Game and Parks Commission. As part of each experiment, one of the "less-polluting" diets will be contrasted with the control open-formula diet in cylindrical tanks versus raceways, using standard optimum production procedures.

Because of time, facilities, and funding constraints, experiments to directly compare the merits of a number of newly-developed "less-polluting" diets simultaneously in both cylindrical tanks and conventional raceways under practical production conditions will not be attempted as part of the proposed project. However, such experiments should be done in the future as part of a sustained effort to develop minimally polluting diets for various types of aquaculture production systems operated under different conditions. As indicated earlier in this section, critical water-quality parameters in the fish-rearing tanks and raceways, and their effluents, will be routinely monitored in all experiments conducted at the Calamus hatchery. Particular attention will be given to water-quality monitoring - especially of total ammonia nitrogen, nitrite-nitrogen, total phosphorus, orthophosphate, total suspended solids, turbidity, and BOD - during experiments done to evaluate the "less-polluting" diets. In addition, the time spent by fish culturists in cleaning and removing solid wastes from the tanks and raceways during these diet evaluation experiments will be recorded and compared between treatment groups.

Finally, in the summer of either 1995 or 1996, a field trial will be conducted at Sandhills Aquafarm to compare the thermal tolerance limits and stress-response patterns of Kamloop versus Donaldson strain rainbow trout. As stated earlier, the water temperature in the raceways at Sandhills Aquafarm can vary between 13 and 22°C in the summer, sometimes within 24 h. The basic experimental design will be to place 500 65- to 90-mm fish of each strain into each of four equivalent bar-screened sections at both the head and tail ends of four parallel raceways. In essence, this is a 2 × 2 factorial design, with genetic strain and location within each raceway being the experimental variables. The rearing density, feed, and production procedures used will be those routinely employed at Sandhills Aquafarm. Sandhills Aquafarm personnel will be responsible for all fish culture activities. Research personnel from the UN-L will help set up the experiment, and will collect the same types of stress-response and performance data as described for the Calamus hatchery. Acute stress challenge tests on the two trout strains at the two raceway locations will be conducted at approximately 6-week intervals throughout the summer.

Whenever possible, parametric statistical methods will be used to analyze numerical data. Nonparametric statistics will be employed when the application of parametric methods is found to be inappropriate or unfeasible. Research findings will be published in a timely manner in appropriate peer-reviewed scientific journals. Extension information outlining the practical implications and benefits of the completed research, and detailing practical new technologies, will be published through the regional and station bulletins in collaboration with the NCRAC Aquaculture Extension Work Group.

FACILITIES

Developing Practical Trout Diets Using Regionally Available Feed Ingredients (Objective 1)

Field testing, by the UN-L and its Nebraska cooperators, of the best available experimental trout diets developed by the project from regionally available feed ingredients will be coordinated by Terry Kayes. This effort will depend on informational input and the necessary diet formulations from the ISU, Purdue, MSU, and OSU investigators participating in Objective 1. The Nebraska facilities that will be used for this field diet testing are described under Objective 3.

MSU has the necessary wet lab, tanks and water supply to conduct the proposed research. Feeds will be manufactured and phosphorus determinations conducted with the help of MSU's Department of Animal Sciences.

Purdue recently completed a new 687.5 m^2 (7,400 ft^2) aquaculture research facility that will be used for this research. That laboratory currently houses two strains of rainbow trout, as well as chinook, Atlantic, and coho salmon. It is complete with back-up generators on both the dedicated well and the building. The Fish Nutrition Laboratory has all necessary equipment for completing the proximate analysis.

ISU provides specific expertise in aquacultural engineering and economics with an emphasis on the commercialization on intensive fish culture systems. Facilities at the university include complete farm-scale feed manufacturing equipment such as holding bins, grinders, mixers, scales, an extruder, dryers and the associated conveying equipment. Culture facilities include over 264,971 L (70,000 gallons) of intensive, indoor production devoted to commercial fish production. A variety of studies are in progress on the comparative

technical and economical feasibility of a variety of biological filters, particle filters, culture system management scenarios, and other engineering and economic aspects of commercial food fish culture.

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 broodstock 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 Piketon Research and Extension Center, where brookstock 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 aquaculture laboratory for tropical fish and bioanalytical laboratory with Water Pico-Tag amino acid analyzer.

The experiments conducted at the UW Aquaculture Program of the UW-Madison, under the direction of Jeff Malison and Terry Barry will be done at the UW Aquaculture Program's research facility located on the main University of Wisconsin campus and/or its research facility at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. The UW campus facility has a wet laboratory with ample supplies of carbon-filtered city water, nine 225-L, 13 750-L, and two 3020-L cylindrical fiberglass tanks with diameters of 0.76-m, 1.2-m, and 1.8-m, respectively. A three-probe computer-linked dissolved-oxygen and total gas pressure monitoring system will be operational by the summer of 1992. The UW Aquaculture Program also has a liquid oxygen injection system, which will be operational by early 1992. The campus analytical laboratory is equipped with a high-speed centrifuge, micro-ELISA plate reader, and other equipment required for our proposed research on stress physiology. The Lake Mills facility has 14 750-L tanks, over 100 110-L and 225-L tanks, and three 3020-L tanks, as well as the laboratory equipment and personnel support required to meet the needs of the proposal. Many of the procedures required to complete the proposed analyses (e.g., cortisol, glucose and chloride) have recently been developed and validated under an ongoing UW Sea Grant-funded project studying other aspects of stress physiology in salmonids (primarily lake trout) (Kayes 1988; Barry et al. 1991).

Using the Stress Response to Develop Improved Trout Strains for the Region (Objective 2)

Studies will be conducted jointly at the Seven Pines Trout Hatchery, Lewis, Wisconsin, and the University of Wisconsin Aquaculture Program's (UWAP) research facilities on the UW-Madison campus and the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. Seven Pines is one of Wisconsin's largest trout farms and trout egg producers, and has the necessary facilities and brood fish to conduct the proposed studies. The UWAP campus facility has a wet laboratory with numerous circular fiberglass tanks (110-L to 3,000-L) and ample supplies of carbon-filtered water which can be maintained at constant temperature by water heaters or chillers. The Lake Mills facility has over 100 tanks (110-L to 3,000-L), three water sources (dechlorinated city water, high capacity well and lake water), and laboratory equipment required to meet the objectives of the proposal. The UWAP analytical laboratory is equipped with a high speed centrifuge, micro-ELISA plate reader, chloride analyzer, microbalance, several compound and dissecting microscopes, and other equipment required for research on fish stress physiology and endocrinology, including regular access to a liquid scintillation counter, and a computer-controlled high pressure liquid chromatography (HPLC) system. We also have access to gamma counters and other analytical equipment through the UW-Madison Endocrinology-Reproductive Physiology Program, and an electron microscope through the UW-Madison Department of Anatomy.

Field testing, by the UN-L and its Nebraska cooperators, of two or more trout strains evaluated by the project will be done under the direction of Terry Kayes, working in close communication with UW-Madison investigators conducting research on Objective 2. The Nebraska facilities that will be used for this field testing between strains are described under Objective 3.

Using Stress and Performance Responses to Evaluate System Design and Operation (Objective 3)

The Nebraska studies on the proposed project will be done by UN-L investigators, under the direction of Terry Kayes, and in cooperation with the Nebraska Game and Parks Commission and with Sandhills Aquafarm, a commercial trout production operation. Nearly all the research done to evaluate the physical aspects of system design and operation, as wellas most of the field testing of experimental diets, will be done at the Calamus State Fish Hatchery, near Burwell, Nebraska. The field testung of trout strains will be done primarily at Sandhills Aquafarm, near Keystone, Nebraska. Some field testing of diets may also be done at Sandhills Aquafarm.

The Calamus State Fish Hatchery is a 24-hectare facility located immediately downstream of the 2,023-hectare Calamus Reservoir in northcentral Nebraska. Physical resources available at the Calamus hatchery

include: 11, 0.20-hectare and 40, 0.40-hectare fish production ponds; 8, 1.8-m wide × 1.2-m deep and 16, 2.4-m wide × 27-m long × 1.2 -m deep outdoor raceways; an 886-m^2 indoor fish production and research facility (which is equipped for water-temperature and light control and includes an analytical and fish pathology laboratory); 10, 0.61-m wide × 6.1-m long × 0.61-m deep indoor raceways; and numerous hatchery troughs, egg incubators and 1.2-, 1.5- and 1.8-m diameter cylindrical rearing tanks. Water resources at Calamus include: reservoir water (with a seasonal temperature variation from about 4°C in winter to about 22°C in summer) supplied to all the indoor and outdoor facilities via a 91-cm diameter main; and about 11-m^3/min and 1.1-m^3/min water flow from eight wells supplying 13 and 11°C water, respectively from two separate aquafers, to all the raceways and indoor facilities. A very large pure-oxygen supply system is in place at Calamus, and oxygen supplementation in individual tanks and raceways can be achieved through the use of sealed packed-columns.

Sandhills Aquafarm is a modern, well-designed trout production facility that was built in 1989. Michael Wyatt, the owner/operator of Sandhills and the current President of Nebraska Fish Farmers Association, is an experienced trout grower who is interested in evaluating the production potential of different trout strains under variable water-temperature conditions. The primary rearing facility at Sandhills consists of 12 concrete raceways (four side-by-side and three in a series). Each raceway is 2.4-m wide × 33.5-m long × 1.2-m wall height (with a 0.9 m- water depth) and is equipped with baffles and flow through partitions. The water flow through each raceway is 5.78 L/min for a total flow of 23,500 L/min through the facility. In 1990, the raceways were equipped with a low-head pure oxygen supplementation system. Production presently varies between 28,000 and 40,000 kg/year of Donaldson-strain rainbow trout, with a projected maximum capability of about 77,000 kg/year. The water supply at Sandhills comes from Whitetail Creek, which originates from springs about 1.6 km upstream of the raceways. Water temperatures in the raceways during the summer can vary between 13 and 22°C but are normally about 17-20°C. During winter, the water temperatures can vary between 2 and 8°C but are normally about 6-7°C.

The UN-L, Calamus State Fish Hatchery, and Sandhills Aquafarm all have fish transport trucks and equipment. Centrifuges, a micro-ELISA plate reader, spectrophotometer, chloridometer, osmometer, and other analytical equipment required to measure the physiological stress responses of trout are available in the Aquatic Biology Laboratory of the Department of Forestry, Fisheries and Wildlife on the UN-L East Campus. Depending on need, blood and tissue samples can be readily transported from the Calamus hatchery or Sandhills Aquafarm to the campus laboratory, or essential equipment can be moved to either of the two production facilities.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Ronald R. Rosati Illinois State University	Aquacultural Engineering
Indiana	Paul B. Brown Purdue University	Finfish and Crustacean Nutrition
Michigan	Donald L. Garling Michigan State University	Salmonid Culture/Finfish Nutrition
Ohio	Konrad Dabrowski Ohio State University	Finfish Nutrition
Nebraska	Terrence B. Kayes University of Nebraska-Lincoln	Fish Culture/Fish Endocrinology/ Extension
Wisconsin	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture/Physiology/Endocrinology
	Terence B. Barry University of Wisconsin-Madison	Aquaculture/Physiology/Endocrinology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Illinois State University (ISU)
Ronald R. Rosati

Purdue University (Purdue)
Paul B. Brown

Michigan State University (MSU)
Donald L. Garling

Ohio State University (OSU)
Konrad Dabrowski

University of Nebraska-Lincoln (UN-L)
Terrence B. Kayes

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

PROPOSED SALMONID BUDGET FOR
ILLINOIS STATE UNIVERSITY

(Rosati)

Objective 1

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)						
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students						
d. Prebaccalaureate Students					$1,500	$1,500
e. Secretarial-Clerical					$1,500	$1,500
f. Technical, Shop, and Other						
Total Salaries and Wages					$3,000	$3,000
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$3,000	$3,000
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$2,500	$2,500
F. Travel - Domestic (*Including Canada*)					$1,000	$1,000
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$6,500	$6,500
TOTAL PROJECT COSTS					$13,000	

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

BUDGET JUSTIFICATION FOR ILLINOIS STATE UNIVERSITY

(Rosati)

A. Salaries and Wages. One student worker is needed for this project for acquisition of diet materials and diet preparation (weighing, grinding, mixing, extrusion, drying). Clerical assistance in needed for coordinating and reporting the activities of the salmonid work group

E. Materials and Supplies. This budget is for acquisition of feed ingredients.

F. Travel. Funds will be used to disseminate experimental results, deliver materials to other workgroup participants and participate in workgroup meetings.

G. Other Direct Costs. These include photocopying, phone, FAX, postage and computer time for statistical analyses.

PROPOSED SALMONID BUDGET FOR
PURDUE UNIVERSITY

(Brown)

Objective 1

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.10				
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals	1	0.50			$10,400	$11,000
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$10,400	$11,000
B. Fringe Benefits (29% of 2b)					$3,016	$3,190
C. **Total Salaries, Wages and Fringe Benefits**					$13,416	$14,190
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$2,500	$2,000
F. Travel - Domestic (*Including Canada*)					$1,000	$1,000
G. Other Direct Costs					$584	$310
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$17,500	$17,500
				TOTAL PROJECT COSTS	$35,000	

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

BUDGET JUSTIFICATION FOR PURDUE UNIVERSITY

(Brown)

A. Salaries and Wages. One technician is needed for this project (0.50 FTE). The technician will be responsible for acquisition of fish, diet preparation, scientific feeding, and chemical analysis.

E. Materials and Supplies. This budget is for acquisition of feeds for broodstock and juveniles prior to starting the experiments, miscellaneous supplies for the experimental system (airline and airstones) and maintenance of chillers for temperature control.

F. Travel. Funds will be used to disseminate experimental results and participate in workgroup meetings.

G. Other Direct Costs. These include photocopying, phone, FAX, postage and computer time for statistical analyses.

PROPOSED SALMONID BUDGET FOR
MICHIGAN STATE UNIVERSITY

(Garling)

Objective 1

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.05				
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	1	0.50	1	0.50	$13,500	$14,000
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$13,500	$14,500
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$13,500	$14,500
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,500	$1,550
F. Travel - Domestic (*Including Canada*)					$750	$750
G. Other Direct Costs					$2,250	$2,200
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$18,000	$19,000
TOTAL PROJECT COSTS					$37,000	

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

BUDGET JUSTIFICATION FOR MICHIGAN STATE UNIVERSITY

(Garling)

A. Salaries and Wages. One level 2 Graduate Research Assistant to assist in the research project.

E. Materials and Supplies. Feeds during acclimation to lab conditions, chemicals for proximate analysis, phytase treament, etc., and general lab materials and supplies.

F. Travel. In-state travel to obtain fish and materials and to participate in Work Group meetings.

G. Other Direct Costs. Utilities for off-campus wet lab are paid by department through grants and contracts.

PROPOSED SALMONID BUDGET FOR
OHIO STATE UNIVERSITY

(Dabrowski)

Objective 1

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.05	1	0.05	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc	1	0.25	1	0.25	$6,000	$6,000
b. Other Professionals						
c. Graduate Students	1	0.25	1	0.25	$3,600	$3,780
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$9,600	$9,780
B. Fringe Benefits					$2,105	$2,105
C. **Total Salaries, Wages and Fringe Benefits**					$11,705	$11,885
D. Nonexpendable Equipment					$2,100	$0
E. Materials and Supplies					$995	$2,915
F. Travel - Domestic (*Including Canada*)					$200	$200
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$15,000	$15,000
TOTAL PROJECT COSTS					$30,000	

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

BUDGET JUSTIFICATION FOR OHIO STATE UNIVERSITY

(Dabrowski)

A. Salaries and Wages. Field and laboratory studies during one quarter will be conducted by a graduate student. A research associate will supervise chemical analysis of fish, feeds and faeces. Their tasks include diets preparation and analysis, preparation of daily, weekly and monthly tables of laboratory experiments schedule, sampling at Piketon, initial preparation of samples for analysis, transportation to Columbus and sample analysis. Approximately half of the labor in tank experiments will be supported by monies from the Piketon Center.

D. Non-expendable Equipment. We request $2,100 for purchasing 2 mm die for processing fish diets in feed mill in Wooster. This installation is only occasionally producing diets for fish and the size of the trout to be used.

E. Materials and Supplies. First year will include only ingredients for diet production. Second year will include general laboratory supplies, reagents, glassware and replacement parts for amino acid analyzer.

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

PROPOSED SALMONID BUDGET FOR
UNIVERSITY OF NEBRASKA-LINCOLN

(Kayes)

Objective 1-3

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	Year 1		Year 2			
	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.05	1	0.05	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other	1	0.50	1	0.50	$10,085	$10,590
Total Salaries and Wages					$10,085	$10,590
B. Fringe Benefits (25% of 2f)					$2,521	$2,648
C. **Total Salaries, Wages and Fringe Benefits**					$12,606	$13,238
D. Nonexpendable Equipment					$1,056	$0
E. Materials and Supplies					$2,400	$1,100
F. Travel - Domestic (*Including Canada*)					$3,800	$2,500
G. Other Direct Costs					$2,800	$500
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$22,662	$17,338
TOTAL PROJECT COSTS					$40,000	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF NEBRASKA-LINCOLN

(Kayes)

Objectives 1-3

A. Salaries and Wages. A Research Technician (0.50 FTE) is needed to assist: (1) the project cooperators (the Nebraska Game and Parks Commission and Sandhills Aquafarm) with the set up and operation of experiments and field trials, and (2) the principal investigator with the conduct of experiments, stress challenge tests, water sampling and analyses, performance data collection, and the analyses of physiological and organismic indicators of stress.

B. Fringe Benefits. The UN-L has a standard fringe benefit rate of 25% of faculty and staff salaries.

D. Nonexpendable Equipment. Funds are needed to help procure additional portable dissolved-oxygen and gas-supersaturation monitoring equipment to support the project. The UN-L presently owns some such equipment, but it is not adequate to meet project requirements. The funds requested represents about 40% of the cost of the additional equipment needed. The other 60% will come from other funding sources.

E. Materials and Supplies. Funds are needed in both Year 1 and 2 of the project to help procure ingredients for and manufacture the (laboratory-evaluated) experimental diets that will be field tested under practical rearing conditions by the UN-L and its cooperators. While most of this field testing will not be initiated until Year 2, much of the planning, experimental set up, and ingredients purchasing will be done in the second half of Year 1. In regard to Objective 3, in Year 1 of the project, about $1,100 is needed to procure hardware, nets, buckets and totes, and other materials required to set up the experimental systems and field trials. In both Year 1 and 2, about $900 is needed to purchase expendable supplies (e.g., reagents, biochemicals, buffers, alcohol, tissue fixatives, dry ice, microscope slides, glassware, and computer and office supplies) to conduct experiments, water analyses, blood and organismic indicator analyses; and to otherwise run the project. The Nebraska Game and Parks Commission and Sandhills Aquafarm will provide all the standard formulated trout feed that will be used in the project.

F. Travel. The UN-L component of the project will require considerable in-state travel during both funding years. Critical travel distances (one way) are as follows: (1) from the UN-L to the Calamus State Fish Hatchery, about 200 miles; (2) from the UN-L to Sandhills Aquafarm, about 300 miles; and (3) from the Calamus hatchery to Sandhills Aquafarm, about 215 miles. The cost of long-term stays at the two project sites by UN-L researchers will be covered by mechanisms separate from NCRAC. Total estimated in-state travel costs for short-term lodging, meals, and fleet vehicle rental for Year 1 and 2, respectively, are $5,500 and $3,300. About 40% of these costs can be covered by pooling appropriate travel expenses on various UN-L projects under the principal investigator's supervision, which brings the total in-state travel funds requested for Year 1 and 2 down to $3,300 and $2,000, respectively. An additional $500 per year is needed to help support travel to a scientific meeting and attend a NCRAC Salmonid Work Group meeting.

G. Other Direct Costs. Funds are needed in Year 1 to help procure the Kamloops rainbow trout eggs and fingerlings required for the project. While the Nebraska Game and Parks Commission and Sandhills Aquafarm may contribute some fish, neither cooperator can provide all the trout of the right strain, age, and size required on an "as-needed" basis. So, significant procurement of fish from outside sources will be necessary. While the UN-L comparisons of the two trout strains will be conducted primarily in Year 1, some experiments may be continued into Year 2. Additional funds are needed each year to meet postage, shipping, photographic processing, telecommunications, and photocopying expenses related to the project.

PROPOSED SALMONID BUDGET FOR
UNIVERSITY OF WISCONSIN-MADISON

(Malison and Barry)

Objective 2

	Year 1		Year 2		Year 1	Year 2
A. Salaries and Wages	Year 1		Year 2			
	No.	FTEs	No.	FTEs		
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	2	0.08	2	0.08	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students	1	0.50	1	0.50	$16,000	$16,630
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$16,000	$16,630
B. Fringe Benefits					$1,680	$1,790
C. **Total Salaries, Wages and Fringe Benefits**					$17,680	$18,420
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$2,000	$2,500
F. Travel - Domestic (*Including Canada*)					$2,500	$1,500
G. Other Direct Costs					$200	$200
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$22,380	$22,620
TOTAL PROJECT COSTS					$45,000	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

(Malison and Barry)

C. Wages and Salaries. A graduate student is needed to assist the PIs and staff with the conduct of experiments including the collection of biological samples, the conduct of stress and performance tests, analysis of physiological stress indicators, and analysis and publication of results.

E. Supplies. Biochemicals, reagents and laboratory supplies are needed to conduct analyses of plasma cortisol, glucose and/or chloride. Fish feed and wet laboratory supplies are required for fish husbandry and to conduct fish culture experiments.

F. Travel. Approximately 65% of the travel budget requested will be needed for 6-8 trips to Seven Pines Trout Hatchery, Lewis, Wisconsin. The remainder of the travel budget will be used to attend NCRAC salmonid work group meetings.

G. Other Direct Costs. Approximately $200 is needed for telephone, fax, postage and photocopying.

CULTURE TECHNOLOGY OF SALMONIDS

Budget Summary for Each Participating Institution at $102.0K for the First Year

	ISU	PURDUE	MSU	OSU	UN-L	UW-MADISON	TOTALS
Salaries and Wages	$3,000	$10,400	$13,500	$9,600	$10,085	$16,000	$62,585
Fringe Benefits		$3,016		$2,105	$2,521	$1,680	$9,322
Total Salaries, Wages and Benefits	**$3,000**	**$13,416**	**$13,500**	**$11,705**	**$12,606**	**$17,680**	**$71,907**
Nonexpendable Equipment				$2,100	$1,056		$3,156
Materials and Supplies	$2,500	$2,500	$1,500	$995	$2,400	$2,000	$11,895
Travel	$1,000	$1,000	$750	$220	$3,800	$2,500	$9,270
Other Direct Costs		$584	$2,250		$2,800	$200	$5,834
TOTAL PROJECT COSTS	**$6,500**	**$17,500**	**$18,000**	**$15,020**	**$22,662**	**$22,380**	**$102,062**

Budget Summary for Each Participating Institution at $98.0K for the Second Year

	ISU	PURDUE	MSU	OSU	UN-L	UW-MADISON	TOTALS
Salaries and Wages	$3,000	$11,000	$14,500	$9,780	$10,590	$16,630	$65,500
Fringe Benefits		$3,190		$2,105	$2,648	$1,790	$9,733
Total Salaries, Wages and Benefits	**$3,000**	**$14,190**	**$14,500**	**$11,885**	**$13,238**	**$18,420**	**$75,233**
Nonexpendable Equipment							$0
Materials and Supplies	$2,500	$2,000	$1,550	$2,915	$1,100	$2,500	$12,565
Travel	$1,000	$1,000	$750	$200	$2,500	$1,500	$6,950
Other Direct Costs		$310	$2,200		$500	$200	$3,210
TOTAL PROJECT COSTS	**$6,500**	**$17,500**	**$19,000**	**$15,000**	**$17,338**	**$22,620**	**$97,958**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

State/Institution		Year 1	Year 2
Illinois State University			
Salaries and Benefits: SY @ 0.05 FTE		$5,550	$6,050
Supplies, Expenses, and Equipment		$10,000	$10,000
	Total	$15,550	$16,050
Purdue University			
Salaries and Benefits: SY @ 0.05 FTE		$5,750	$6,250
Supplies, Expenses, and Equipment		$15,000	$15,000
	Total	$20,750	$21,250
Michigan State University			
Salaries and Benefits: SY @ 0.50 FTE		$5,740	$5,940
Supplies, Expenses, and Equipment		$8,100	$9,000
	Total	$13,840	$14,940
University of Nebraska-Lincoln			
Salaries and Benefits: SY @ 0.05 FTE		$10,713	$10,456
Supplies, Expenses, and Equipment		$18,784	$14,300
	Total	$29,497	$24,756
University of Wisconsin-Madison			
Salaries and Benefits SY @ 0.08 FTE		$3,600	$4,100
TY @ 0.08 FTE		$2,500	$2,800
Supplies, Expenses, and Equipment		$18,250	$20,200
	Total	$24,350	$27,100
	Total per Year	$103,987	$104,096
	GRAND TOTAL	$208,083	

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

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

LIST OF PRINCIPAL INVESTIGATORS

Terence P. Barry, University of Wisconsin-Madison

Paul B. Brown, Purdue University

Konrad Dabrowski, Ohio State University

Donald L. Garling, Michigan State University

Terrence B. Kayes, University of Nebraska

Jeffrey A. Malison, University of Wisconsin-Madison

Ronald R. Rosati, Illinois State University

VITA

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EDUCATION

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

POSITIONS

Assistant Researcher, University of Wisconsin Aquaculture Program, U.W.-Madison (1990-present)
Fulbright Graduate Research Fellow, The University of Tokyo, Japan (1988-89)
Research Associate, USAID, Iloilo, Philippines (1986-88)
U.S. Peace Corps Volunteer, High School Biology Teacher, Western Samoa (1977-80)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Society of Zoologists
World Aquaculture Society
Asian Fisheries Society
American Fisheries Society

SELECTED PUBLICATIONS

Barry, T.P., T.B. Kayes, J.A. Malison, and C.H. Amundson. In press. Validation of an ELISA for measuring cortisol in fish and comparison of stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture.

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SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

SELECTED PUBLICATIONS

Brown, P.B., W.H. Neill, and E.H. Robinson. 1990. Preliminary evaluation of whole body energy changes as a method of estimating maintenance energy needs of fish. Journal of Fish Biology 36:107-108.

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.

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POSITIONS

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SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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

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

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Assistant Professor of Fisheries Science, Department of Fisheries and Wildlife Sciences, Virginia Institute and State University (1976-1980)

SELECTED PUBLICATIONS

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Instructor, Department of Biological Sciences, Chico State College (1968-1970)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

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SELECTED PUBLICATIONS

Barry, T.P., A.F. Lapp, T.B. Kayes, and J.A. Malison. In press. Validation of an ELISA for measuring cortisol in fish and comparison of stress responses of rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Aquaculture.

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SELECTED PUBLICATIONS

Rosati, R., P.D. O'Rourke, K.T. Tudor, and R.D. Henry. 1993. Performance of a raceway and vertical screen filter while growing *Tilapia nilotica* under commercial conditions. Techniques for Modern Aquaculture. American Society for Agricultural Engineering. St. Joseph, Michigan.

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