CULTURE TECHNOLOGY OF SALMONIDS

Chairperson: Paul B. Brown, Purdue University

Extension Liaison: Ronald E. Kinnunen, Michigan State University

Funding Request: $149,997

Duration: 2 Years (September 1, 1992 to August 31, 1994)

Objectives:

1. To continue evaluations of all-female diploid, all-female triploids, and mixed-sex diploids through sexual maturity and to use broodstock developed in the region to produce all-female diploid and all-female triploid trout populations.

2. To continue development of less-polluting diets by:
   a. quantifying absorption of crude protein in rainbow trout fed commonly-available feedstuffs substituted at varying levels in the diet (evaluation of associative effects);
   b. develop baseline effluent values from several types of salmonid aquaculture facilities located in the region;
   c. to develop and field test a mass balance method to estimate phosphorus levels from feed sources in hatchery effluents; and
   d. quantify phosphorus absorption from common feedstuffs fed to Atlantic salmon.

3. 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.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive	Year 1	Year 2	Total
Southern Illinois University-Carbondale	Robert J. Sheehan	1	$20,000	$0	$20,000
Purdue University	Paul B. Brown	2	$17,500	$17,500	$35,000
Michigan State University	Donald L. Garling	2	$16,125	$17,862	$33,987
University of Nebraska-Lincoln	Terrence B. Kayes	3	$12,480	$13,445	$25,925
University of Wisconsin-Madison	Jeffrey A. Malison Terence P. Barry	3	$17,169	$17,916	$35,085
		TOTALS	**$83,274**	**$66,723**	**$149,997**

TABLE OF CONTENTS

JUSTIFICATION

Members of the family salmonidae, including various species of trout and salmon, are among the principal fishes cultured in the North Central Region (NCA-23 1987). Advancement of the salmonid aquaculture industry in the region was identified as a high-priority item by the Industry Advisory Council, both Technical Committees, and the Board of Directors at a joint meeting held in May 1989, and in subsequent meetings. Specific research projects identified at the joint meetings included: 1) effects of genetic manipulations; 2) development of less-polluting diets; and, 3) evaluation of high rearing densities. Both rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) were identified as primary species of importance.

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 when compared to trout production in Idaho or net-pen culture in Europe, Canada or Chile. However, the industry adds significantly to agricultural diversity in the region and to 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 fishing clubs, municipalities or governmental agencies (WASC 1988).

Salmonid aquaculture, whether in this region or other parts of the world, is a relatively mature industry. Past research efforts have provided information on the major aspects of genetics, nutrition, and environmental physiology, as well as other biological characteristics, of the primary species cultured. However, problems remain in the areas listed above, particularly when we consider the regulatory restrictions on agriculture in general and aquaculture specifically. Addressing these problems with thorough, well-conceived research projects will allow continued profitability of existing operations and opportunities for new ventures. Because of the mature state of the salmonid industry and the diverse nature of the identified priorities, a fully-integrated research plan is not possible. However, this does not reduce the importance of the proposed topics.

Genetic manipulations of fishes have been receiving a great deal of attention in recent years and offer promise as a means of gaining some additional weight gain in fish that reach marketable size after sexual maturation. Less-polluting diets have also been receiving a great deal of attention (Cowey and Cho 1991), and recent legal actions have limited production at certain hatcheries. Further regulatory constraints are under consideration at the federal level as well as in many states in the North Central Region. Increasing rearing densities, or raising more fish in the same or even reduced amounts of water, will help producers maintain profitability in a era in which variable costs are rapidly increasing. To achieve the latter goal, cost-efficient, less-polluting diets are required, and genetic manipulations may result in a fish that is more tolerant of crowding. Thus, while the identified priority areas appear unrelated, they can be easily tied together, and all are vital for increased, as well as continued, production efficiency in the North Central Region.

Development of Triploid and Monosex Stocks

The production of triploid, triploid hybrid, and monosex stocks have potential benefits to salmonid aquaculture (see Thorgaard 1986). Sterile triploids and triploid hybrids, especially females, do not become sexually mature. Thus, they continue rapid growth through this phase of their life history. This can be beneficial for the production of larger fish (Purdom 1986; Donaldson 1986). The focus of this project will be on development and evaluation of all-female diploid and triploid rainbow trout and Atlantic salmon.

Midwest producers have developed a market for 2-3 kg rainbow trout and salmon, but losses due to sexual maturity continue to be problematic (H. Kettula, Seven Pines Trout Hatchery, Lewis, Wisconsin, personal communication). Thus, they may benefit from both the potential food conversion decrease of mono-sex culture as well as the sterility inherent in the culture of mono-sex triploids.

Chromosome set manipulation in fishes is commonly achieved by treating newly fertilized eggs either to induce retention of the second polar body or to interrupt completion of the first mitotic cleavage of the zygote (reviewed in Thorgaard 1986). Coupling these meiotic and mitotic blocks with interspecific fertilization produces polyploid hybrids; coupling these processes with radiation treatments to inactivate one of the parental chromosome complements produces androgens (all-paternal inheritance) or gynogens (all-maternal inheritance) (Thorgaard 1986; Seeb and Miller 1989).

Induced triploidy

Induced triploidy is one of the most frequently used applications of this genomic manipulation technology. No special equipment is necessary, and the procedures can be readily learned and used by hatchery

workers (Bye and Lincoln 1986). Easy-to-apply heat shocks can be employed to induce polar-body retention in normally fertilized eggs. The resulting individuals have three sets of chromosomes and are generally sterile; aquacultural advantages of triploidy such as extended growing season, increased weight gain, etc., are slowly being documented in a variety of organisms including oysters (Allen and Downing 1986), abalone (Fujino et al. 1987a,b), scallops (Komaru and Wada 1989), and salmonids (Scheerer et al. 1987).

Triploid males, though sterile, may develop secondary sexual characteristics and develop dysfunctional, presumably aneuploid gametes (Lincoln 1981; Lincoln and Scott 1984). Therefore, triploidization alone has not been a satisfactory treatment in salmonid culture situations where control of secondary effects of maturation is often desired (Purdom 1986). Such males drop out of production and are not marketable. An alternative for producing sterile fish for culture is to produce all-female triploids (Donaldson 1986; Purdom 1986, see below). All-female triploid Atlantic salmon do not produce secondary sexual characteristics and have improved meat production over diploids at sexual maturity (Benfey and Sutterlin 1984).

Triploidy avoids depressed growth rates observed during sexual maturation by inducing sterility. However, prior to maturity, growth rates of diploids and triploids are often similar when they are reared separately (Johnson et al. 1986). Interestingly, when triploids are grown in common tanks with diploids, the triploids can show a reduced growth rate (Cassani and Caton 1986; Lincoln and Bye 1987), indicating that they may have reduced agonistic or competitive behavior. Intuitively, changes in behavior seem possible as a result of endocrinological changes that come with sterility. Reduced agonistic behavior in sub-mature triploids may lead to reduced activity and improved food conversion efficiency.

Gynogenesis and the production of all females

It is critical to note that the aquacultural applications of polar-body gynogenesis are restricted to the breeding value of the F_1 progeny, rather than there being any immediate aquacultural value of the F_1 individuals themselves. These individuals are inbred equivalently to two or three generations of full-sib matings (Allendorf and Leary 1984). Thus, these F_1 progeny would have reduced fitness relative to related outbred individuals. However, such individuals would have aquacultural potential by accelerating first generation inbreeding for breeding programs involving line crossing (Thompson 1983).

The important breeding aspect of F_1 gynogens relates to all progeny being females. Females are the homogametic sex in salmonids (Donaldson and Hunter 1982), and gynogenetic progeny would all be female. This attribute can be used to create and perpetuate outbred lots consisting only of females. Such lots are created by (1) sex reversal of gynogens to produce phenotypic males capable of producing X-only sperm, and (2) crossing these males with outbred females (reviewed in Donaldson and Hunter 1982; Donaldson 1986). The advantage of integrating gynogenesis with hormonal sex reversal to produce all-female lots of fish rather than using sex reversal directly is that no food fish have been treated with drugs. The fact that no fish destined for human consumption have been treated with hormones can have considerable marketing advantages (Bye and Lincoln 1986). All-female monosex culture of this type has been used in Europe for a number of years (Bye and Lincoln 1986) and also can be valuable for rapidly increasing a broodstock.

Development of Less-Polluting Diets

The Industry Advisory Council (IAC) of the North Central Regional Aquaculture Center stated that there is an immediate need to develop less-polluting diets. The atmosphere among some regulatory agencies in the region is not encouraging toward aquaculture development and new regulations on effluents are expected. Commercial and public culture operations are facing closure and production limitations because of effluents released and development of new facilities is under close scrutiny due to regulatory concerns. Thus, addressing this topic is imperative for further development of salmonid aquaculture in the North Central Region and it is important to address this problem before aquaculturists are told to do so. Three primary areas have to be considered in research of this nature: phosphorus (P), nitrogen (N), and solids.

Reduction of P and N from aquaculture facility effluents is a current problem in the North Central Region, as well as many other parts of the world. The European Inland Fisheries Advisory Commission (EIFAC) recently established a Working Party to address this, but there has been little attention to this problem in North America. Because P and N are generally considered to be the limiting nutrients in freshwater environments, addition of these nutrients typically results in eutrophication (Wetzel 1983). Therefore, regulatory agencies are imposing stricter requirements on private and public facilities, and threatening closure and production limitations. Further, this has hindered the development and expansion of salmonid aquaculture in the region (eg., Minnesota Aquafarms, cage culture in the Great Lakes).

In recent years there has been a growing concern over the level of phosphorus released from fish hatcheries due to its potential role in increasing eutrophication in receiving waters. National attention was focused on this subject when the Michigan Department of Natural Resources (MDNR) was ordered by the court to substantially reduce P discharge from the Platte River Salmon Hatchery in Honor, Michigan to limit eutrophication of Platte Lake (H. Westers, MDNR, personal communication). Restrictions were placed on the total annual amount of P that could be released from the hatchery even though effluent P levels never exceeded Federal guidelines. To meet the target P levels, costly modifications of the hatchery settling basin and raceways were made. Other ways used to minimize P discharge included reduction in annual production and the lowering of P levels in the feeds by changing the form of inorganic P in the mineral mix (Ketola 1985).

Phosphorous has been identified as the primary limiting factor in freshwater aquatic plant growth and high levels of this nutrient in freshwater environments can promote eutrophication. Receiving waters downstream from fish hatcheries receive increased amounts of P (Hinshaw 1973; Bergheim/ and Selmer-Olsen 1978; Korzeniewski et al. 1982; Kendra 1991). Liao (1970) was one of the first to report water quality degradation from effluent of salmonid hatcheries. In 1972 the first regulation of hatchery waste discharge was implemented with the creation of the National Pollutant Discharge Elimination System (NPDES) permit program (Harris 1981). Kendra (1991) showed significant increases in temperature, pH, suspended solids, ammonia, organic nitrogen, total phosphorus, and chemical oxygen demand caused by hatchery effluent water compared to influent waters. He looked at 11 state and 9 commercial facilities during the summer of 1988. Wiesmann et al. (1988) analyzed water entering and leaving nine commercial trout farms of North-West Germany and showed that concentrations of ortho-phosphate in the water may increase substantially during passage through the farm. It was suggested that to limit phosphate pollution of water, feed manufacturers should aim to avoid phosphorus concentrations in feeds that exceed the requirements of the target fish.

Since the primary source of this P is from the feeds, the most desirable way to limit P in the effluents without sacrificing production is through increased utilization of P levels in feeds and feed management. Ketola (1985) showed that reduction in dietary P levels can markedly reduce effluent P from hatcheries. He also showed that less soluble forms of P in the mineral mix could reduce effluent P since the majority of P is initially found in uneaten feed or feces.

Consequently, diligent control of hatchery solid wastes is also essential. Weekly removal of solids from salmonid raceways and settling basins will remove the majority of P in the solid wastes before P leaching can occur (C. Starr, Bay Port Aquaculture, Inc., Bay Port, MI, personal communication). Poor feed management can be a major source of hatchery solids. Even if highly available low P diets are used in salmonid farming, overfeeding will result in excess P in the effluent. The method and rates of feeding will affect the amount of nutrient absorption and feed passage through the gut. Rates at which proper uptake of nutrients and limited food is wasted are essential for the fish to incorporate the maximum amount of P into its body.

Direct measurements using water samples from the hatchery effluent may not accurately indicate the level of P released by the hatchery. Many factors can affect P levels in the effluent water samples. Many of these factors are related to the P cycling in the hatchery settling pond as well as factors like the number of algal cells contained in a sample. Consequently, a better method to estimate the actual contribution of P from hatchery operations to receiving waters is needed. Today, with the increasing concern by the general public over the potential pollution of a waterway by fish hatcheries there is an urgent need for methods to accurately measure and minimize P in effluents.

There are two sources of P and N in aquaculture effluents: a small amount naturally present in the water, and a much larger amount excreted by the fish. The contribution from fish feces represents both dietary P and N that were not absorbed in the gastrointestinal tract as well as endogenous turnover from the fish. Thus, if diets are formulated on an available nutrient basis (i.e., maximizing P and N absorption), then fecal and effluent levels will be reduced. Additionally, more precise dietary formulation, based on availability of nutrients, should reduce feed costs and provide for fewer fluctuations in those costs. However, very little of this information is available for salmonids (NAS 1983).

We cannot promote increased production stocking densities in the region with the current regulatory atmosphere and the fact that existing facilities are facing closure and production limitations because of P and N in their effluents. We need to have a collaborative approach. Increased stocking densities seems feasible and will be beneficial to salmonid aquaculturists in the region, but we have to have the additional information from this objective regarding less polluting diets or the information from objective 3 cannot be implemented.

Effects of High Rearing Density on Stress, Growth and Production

Irrespective of species or marketing objectives, one major constraint on the growth of salmonid aquaculture, both regionally and nationally, is the high cost of raceway construction (JSA 1983; Senn et al. 1984). Raceway culture has historically been the preferred method of intensively rearing salmonids, though recently the production of food-size salmonids in net pens has become a rapidly expanding, multimillion-dollar industry (Parker 1988). However, this industry depends on raceways for the culture of young fish to the smolt stage of development. According to Senn et al. (1984), W. J. Daley of Fish Pro, Inc., Port Orchard, Washington, a well-known designer of aquaculture production systems, and J. D. Erickson of the Clear Springs Trout Company, Buhl, Idaho (personal communications), the construction cost of new raceways is approximately $\$125\text{-}320/m^3$ of rearing water, depending on site, construction materials, number of raceways being built and other factors.

Considering these high costs, perhaps the most economical way for fish farmers in the North Central Region to increase salmonid production would be to increase rearing densities. 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 (Piper et al. 1982; also see Westers and Pratt 1977).

In most conventional raceway systems, dissolved oxygen is the first factor that limits loading rate. Recently, pure oxygen supplementation has received widespread attention as a potential means of increasing the carrying capacity or "loading" of fish in raceways. Thus, theoretically, if supplemental oxygen is used to sustain adequate dissolved oxygen levels, loading rates can be increased until the concentration of toxic metabolites (primarily un-ionized NH_3) reach maximum permissible levels. The Michigan Department of Natural Resources and others (Visscher and Godby 1987) 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, 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 in both private-sector and government aquaculture operations (Visscher and Godby 1987; Visscher and Dwyer 1990; G. R. Bouck, Bonneville Power Administration, Portland, Oregon, and W. J. Daley, Fish Pro, Inc., personal communications). However, the limitations of these systems remain largely unknown because little definitive information exists on the density-related spatial requirements of salmonids independent of water quality and flow. In other words, the question remains: "If the main limiting factor of conventional loading is eliminated by adding supplemental oxygen, at what point does rearing density alone (defined as fish weight per unit of volume) have deleterious effects on the survival, performance, and growth of cultured salmonids?"

As a practical aid to fish culturists, Piper et al. (1982) developed a density index that is calculated by dividing the weight of fish per volume of rearing water by fish length. 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, "to avoid undue crowding." According to this criterion, for example, the maximum rearing density for 15.2 cm fish should not exceed 48 kg/m^3 and for 28.3 cm fish 64 kg/m^3. However, recommendations on the use of this index are based largely on experience and empirical considerations rather than on data derived from controlled testing.

In apparent contrast to Piper et al.'s (1982) recommendation, many practical fish culturists over the years have demonstrated, and recent experimental evidence supports the proposition, that some salmonids can be reared at a density index considerably higher than 0.5 if good water quality is maintained (Buss et al. 1970; Soderberg and Krise 1986; Kebus et al. In Press). However, the upper limits on rearing density for most species and strains remain unknown. Some fish culturists maintain that rearing density by itself is not a limiting factor in high intensity salmonid culture and cite isolated examples and anecdotal evidence to support this contention. However, anecdotal information and unpublished reports do not provide the specific numbers needed to design optimal production systems or make informed business decisions.

In fact, considerable scientific evidence suggests that spatial requirements and rearing density, independent of water quality, may be extremely important in salmonid culture (see **RELATED CURRENT AND PREVIOUS WORK**). A major factor that probably determines the minimum spatial requirements of salmonids is the extent to which various species, strains and different life history stages are tolerant of crowding. Crowding can act as a potent environmental stressor and elicit a physiological stress response in fish (see **RELATED CURRENT AND PREVIOUS WORK**). Physiological stress, in turn, can slow growth, impair disease resistance and directly cause death (for reviews see Pickering 1981; Adams 1990; Wedemeyer et al. 1990; Barton and Iwama 1991).

Physiological stress, including its effects on fish health, presents one of the most insidious problems in aquaculture. From a practical perspective, identifying and then minimizing sources of stress could have immediate beneficial impacts on salmonid culture. However, physiological stress is difficult to measure, and potential procedures for evaluating stress have rarely been evaluated under practical conditions. Our study will examine the functional relationship between rearing density and physiological stress as applied to high-intensity culture of rainbow trout under both experimental laboratory and production conditions. In addition, potential procedures for evaluating stress under practical rearing conditions will be examined and correlated with standard laboratory measures.

The information generated by our study will facilitate efforts to optimize the use of rearing space in raceways, provide valuable information on possible indicators of stress under practical rearing conditions, and help evaluate the potential (and limitations) of pure oxygen systems to increase production rates and loading in salmonid culture.

RELATED CURRENT AND PREVIOUS WORK

This proposal is a continuation of and addition to a previously funded NCRAC project on salmonids. Objectives 1 (development of triploid and monosex stocks) and 2 (develpoment of less-polluting diets) are continuations of the previous project while objective 3 on high-rearing densities is new.

Development of Triploid and Monosex Stocks

Researchers at the University of Minnesota (UM) and Southern Illinois University-Carbondale (SIUC) worked jointly in the first year of the previous project to fulfill the criteria established for this objective; namely, to produce all-female stocks of rainbow trout for diploid and sterile triploid production. The specified procedures for this objective also included an evaluation of the performance (survival, growth, and feed conversion) of mono-sex diploid and mono-sex triploid rainbow trout as compared to mixed-sex /diploids.

Researchers from UM and SIUC produced sex-reversed gynogens (XX phenotypic males) at Hugo Ketula's Seven Pines Trout Farm in Lewis, Wisconsin during the first year of work. Some gynogens were transported to SIUC for growout while the remainder are being grown at Seven Pines. Thus, the broodstock necessary for producing mono-sex (all-female) rainbow trout in the region have been produced, and further production of sex-reversed gynogens planned for Year 2. UM and SIUC researchers also successfully tested 29 ^{o}C heat-shock procedures for inducing triploidy in rainbow trout at Seven Pines Trout Farm during the first year of the project.

Crosses have been made to evaluate the production performance of three genetic treatments: 1) mixed-sex diploids, 2) all-female diploids, and 3) all-female triploids. Thirty female rainbow trout from Seven Pines Fish Farm, thirty normal males from the Isle of Man Hatchery in the United Kingdom, and thirty sex-reversed XX males were used for crosses. Eggs from each female were divided into three lots. One normal male from the Isle of Man Hatchery was then crossed with one of the lots from a female. The other two lots from each female were fertilized with all-female sperm from a single male from the Isle of Man Hatchery, producing all XX (all-female) individuals, and then one of the two all-female lots was subjected to a heat shock to induce triploidy. Thus, one lot of eggs from each female was crossed with a unique normal male, a second lot was crossed with a unique XX male, and triploidy was induced in the third lot from each female crossed with the same XX male. This experimental design permits an evaluation of the effects of individual females and individual males as well as of the three genetic treatments.

Egg lots from 5-7 of the females are being evaluated for survival and growth through 90 days at UM. Egg lots from the same number of females are being monitored at SIUC through 180 days, as part of the ongoing salmonid study.

None of the Atlantic salmon induced gynogens survived during initial experiments. UM will again attempt to produce sex-reversed Atlantic salmon in Year 2 of the previous project.

Development of Less-Polluting Diets

Objectives proposed in the previous proposal have been met. Researchers at Purdue University have defined fecal collection methods for quantifying phosphorus (P) absorption from commercially-available feedstuffs (Brown In Press). In that initial study, the concern regarding a leachable form of P was confirmed and an invasive fecal collection procedure has been selected for the remainder of the work. Those studies are in progress.

Researchers from Michigan State University (MSU) are in the final stages of studies comparing weight gain, survival, feed conversion and excreted P from fish fed phytase-supplemented and low-phosphorus diets.

Treatment of soy flour with the enzyme phytase converted the biologically unavailable organic phytin phosphorus to the available inorganic form. Feeding experiments are in progress to determine the potential of using converted phytin P from soy flour to meet the nutritional requirements of rainbow trout and to minimize the amount of P lost in feces. The reduction of P in the feces has the potential to significantly reduce the P concentration in the effluent from trout hatcheries.

In MSU's initial experiment, they fed triplicate groups of fingerling rainbow trout the following diets:

DIET	TYPE	SOURCE	PHYTASE TREATMENT	% SUPPLEMENTAL P
A	Commercial reference	Zeigler Salmon Starter	NA	NA
B	Semi-purified reference	Ketola[1] T2M experimental diet	-	100
C	Semi-purified experimental	MSU Modified T2M	+	100
D	Semi-purified experimental	MSU Modified T2M	-	25
E	Semi-purified experimental	MSU Modified T2M	+	25
F	Semi-purified experimental	MSU Modified T2M	+	0

[1]Diet formulation provided by George Ketola, USFWS, Tunison Laboratory of Fish Nutrition, Cortland, NY.

They will determine the effects of dietary phytase treatments on growth and P effluent values. P effluent values will be determined using a modified P mass balance study. P levels from samples of fish at the beginning and termination of the study and from feed samples are being measured. The concentrations of P added to the effluent can be determined by subtracting P concentrations assimilated by the fish from the P levels fed. After the first 7 weeks of the feeding experiment, there has been no signs of abnormalities or reduced growth between fish fed the various diets.

A second set of experiments will be run to compare the Ketola T2M reference diet with MSU modified T2M feeds with phytase treatment and graded levels of P supplements and a MSU modified T2M diet without phytase treatment or P supplements. The final experiment will be a long-term grow-out experiment rearing rainbow trout to market size on commercial and T2M reference diets and a MSU modified T2M diet with phytase and a % of P supplement based on the results of our earlier experiements.

This proposal addresses one of the other major nutritional concerns in diets fed to fish - nitrogen - as well as continuing work on P, and initiating studies with Atlantic salmon. Nitrogen, provided primarily by protein or amino acids, is generally considered the most expensive component in diets fed to fish and is considered one of the primary nutritional concerns in aquaculture effluents. There have been several protein digestiblity studies with fish and salmonids in particular (for a review, see Wilson 1989). However, the typical experimental approach involves feeding one protein source at a time and measuring digestion or absorption of the crude protein component in that feedstuff. Practical diets are, for the most part, not formulated to contain only one source of protein; they contain several ingredients that provide a portion of the protein. Studies with several animals have demonstrated the concept of associative effects, or a change in absorption of ingredients when several ingredients are used in pratical diets. Those effects can be positive (more than additive absorption with combinations of feedstuffs), negative (less than additive absorption), or nonassociative depending on the specific feedstuffs used and diet manufacturing methods. This was recently demonstrated for crayfish (Brown et al. 1989) and in the second year evaluation of the current P studies with rainbow trout. There have been no evaluations of associative effects in rainbow trout diets regarding their ability to absorb protein and has been listed as a primary area of interest by such renowned salmonid scientists as S. Kaushik (INRA, St. Pee, France).

This proposal will be a partial continuation of the P work underway, but represents a departure from those studies. P will be monitored in effluents from different types of aquaculture facilities. The Michigan Department of Natural Resources has agreed to cooperate in this proposal and allow monitoring of effluents at the Harietta Hatchery. That hatchery has a settling pond. Additionally, effluents from two Indiana Department of Natural Resources salmonid hatcheries have been chosen; one with a settling pond and one set up as a flow-through hatchery. Knowledge of their feeds, feed rates, water flow rates and loading densities will allow comparisons regarding P amd N discharges. Direct measurements of this nature will allow more precise hatchery design in the future and will probably identify advantageous concepts in use and areas that could use some modification. These data will be shared with the NCRAC Work Group on Characterization of Aquaculture Effluents.

Additionally, Purdue researchers have located and received a committment for Atlantic salmon from the US Fish and Wildlife Service. Those fish have been in raceway culture situations for 8-10 years and are certified free of diseases. Ancillary studies conducted by the Service have involved raising offspring from their broodstock at 22 °C in freshwater closed, recirculating systems (G. Ketola, USFWS, Tunison Laboratory of Fish Nutrition, Cortland, NY, personal communication). Based on their observations, they feel the growth of Atlantic salmon raised at 22 °C in freshwater is as good as that observed at colder temperatures. Thus, there appears to be a promising potential salmonid that might fit into the specific climatic conditions in the North Central Region. As an initial step, we will compare P absorption in Atlantic salmon fed several of the feedstuffs we are currently using (including blood meal, the various fish meals, and soybean meal) by methods identical to those in use in the current studies with rainbow trout.

Effects of High Rearing Densities on Stress, Growth and Production

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, impaired reproduction, and increased incidence of disease and death.

Numerous investigations have been done on the physiological stress responses of rainbow trout, brown trout and various species of salmon (for recent studies see, e.g., Barton et al. 1986; Patino et al. 1986; Barton and Schreck 1987; Pickering and Pottinger 1987a,b; Thomas and Rice 1987; Kjartansson et al. 1988; Maule et al. 1989; Peters et al. 1988, 1991). In operational terms, a stressor could be any biotic or abiotic factor that requires a physiological response to maintain homeostasis (Wedemeyer and McLeay 1981). Stressors frequently examined for their effects on fish include crowding, handling, forced swimming, hypoxia, changes or extremes in temperature or water chemistry, and exposure to various pollutants or toxicants (Pickering 1981; Adams 1990; Wedemeyer et al. 1990; Barton and Iwama 1991; and above cited references).

Both in nature and under culture conditions, fish are often exposed to a variety of stressors whose effects on physiological response mechanisms may be additive or synergistic (see reviews in Pickering 1981; also Pickering and Pottinger 1985; Barton et al. 1985, 1986; Patino et al. 1986; Thomas and Rice 1987; Wedemeyer et al. 1990; Barton and Iwama 1991). Whether due to single or multiple stressors, Schreck (1981) and Barton et al. (1986) have observed in rainbow trout and Pacific salmon that: (1) the physiological stress response and its deleterious consequences occur almost immediately, and (2) the resistance phase of the stress response (as measured by plasma cortisol, glucose and related end points) leads either to exhaustion or nearly perfect compensation, "although not necessarily the same performance capacity." Pickering and Stewart (1984), for example, found that brown trout subjected to chronic crowding stress, after exhibiting a transient rise (25 days) in plasma cortisol, continued to grow more slowly than uncrowded fish over a 110-day period. However, others have reported that chronic stress conditions evoke sustained elevation of blood cortisol levels in fish (e.g., Ainsworth et al. 1985; Pickering and Pottinger 1985, 1987a).

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" in fish, owing to prior exposure to other (known or unknown) stressors. This testing strategy is based on the concept that a high "stress load" would result in a "reduction of zones of tolerance to environmental extremes" and consequently in diminished "performance capacity," for example, in survival or growth (see also Schreck 1981; Barton and Iwama 1991).

Schreck (1981) proposed that the physiological stress response in fish may have a major "psychological" component. Thus, the intensity of response is likely to be governed by the extent to which a stressor causes fright, discomfort or pain. Like many fishes, salmonids often exhibit intraspecific aggression and hierarchical behavior which can be stressful and result in physical injury and lowered disease resistance (Keenleyside and Yamamoto 1962; Abbott and Dill 1985; Abbott/ et al. 1985; Peters et al. 1988, 1991). 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.

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. According to commercial trout producers (e.g., J. D. Erickson, Clear Springs Trout Company, Buhl, Idaho and H. Kettula, Seven Pines Fishery, Lewis, Wisconsin, personal communications), nipping behavior and subsequent fin damange (Abbott and Dill 1985) is a persistent problem in commercial rainbow trout culture at conventional raceway rearing densities (16 to 80 kg/m^3).

Very high rearing densities, however, may suppress agonistic behavior in rainbow trout. Buss et al. (1970) did not report any problems with aggressive behavior in rainbow trout raised to densities greater than 540 kg/m^3 in 6.3-L cylindrical hatching jars at a water flow rate of 2.3 L/minute. V. A. Mudrak of the Pennsylvania Fish Commission, Benner Spring Fish Research Station, Bellefonte, Pennsylvania, (personal communication) has since indicated that no obvious aggressive behavior was observed when rainbow trout were raised to a density of 136 kg/m^3 in a 4557-L silo, as described by Buss et al. (1970). Some researchers have reported that when salmonid rearing density is increased above a certain level, social hierarchies can break down, and the fish may grow as well or better than fish at lower densities (Keenleyside and Yamamoto 1962; Kawanabe 1969; Fagerlund et al. 1981). However, the exact rearing-density threshold at which the transition from aggressive to nonaggressive behavior occurs in different species and strains has apparently not been documented.

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. Experimentally, altered water quality is often treated as an inherent characteristic of increased rearing density (e.g., Fagerlund et al. 1981; Schreck et al. 1985; Pickering and Pottinger 1987a,b). Many investigators have assumed that water quality parameters (e.g., dissolved oxygen and ammonia concentrations) maintained above or below certain commonly accepted critical values are "good enough," despite obvious differences between treatment groups (e.g., Wedemeyer 1976; Fagerlund et al. 1981; Poston 1983). Accordingly, many experiments aimed at defining the density limits of various species have confounded rearing density with loading rate.

In some studies, researchers have attempted to compensate for differing water quality at various rearing densities by increasing flushing or flow rates (e.g., Wedemeyer 1976; Refstie and Kittelsen 1976; Refstie 1977; Poston 1983; Patino et al. 1986). A potential problem with this approach is that water flow itself can have widely varying effects on stress, both positive and negative, depending on circumstances (Pickering 1981; Woodward and Smith 1985). For example, in some instances, increased water flow could indirectly evoke a stress response by stimulating aggressive behavior (e.g., Gibson 1978; Cole and Noakes 1980).

Soderberg and Krise (1986) apparently resolved the problem of differential water quality and flow rate in experimental design by comparing the growth, condition and survival of juvenile lake trout raised at four different densities in cages suspended in common rearing tanks. Under these conditions, no significant differences in growth or condition were observed between lake trout stocked at density indices of 0.25, 0.5, 1.0 and 2.0. However, mortality was significantly higher at a density index of 2.0, due primarily to an epizootic disease outbreak. 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."

Kebus et al. (In Press), 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. Kjartansson et al. (1988) stocked 1.75-kg Atlantic salmon in 3-m-diameter tanks at density indices of about 0.16, 0.27 and 0.40, and used supplemental oxygen to maintain dissolved oxygen levels at approximately 12 ppm. They observed no effects of density on survival, growth or plasma cortisol levels. Blackburn778 and Clarke (1990), using supplemental oxygen "to remove the main limiting effect of loading," found that age-zero

coho salmon grew at the same rate when raised to final density indices of about 0.09, 0.17 and 0.37. Significantly, this was done using loading rates about three times higher than generally recommended for this species (Shepherd 1984).

Taken together, these findings clearly demonstrate that it is possible to raise salmonids at higher densities than is normally practiced. But before specific recommendations can be made to trout and salmon farmers in this regard, more research is needed to assess the effects of high rearing density on the stress responses and growth of fish to determine the upper limits on rearing density for practical production. With the exception of work by Kebus et al. (In Press), no definitive studies examining the effects of high rearing densities on the physiological stress response in salmonids, independent of water quality and flow, have been reported. Moreover, with the exception of the work of Patino et al. (1986), who examined the effects of rearing density and water flow on the functional development of coho salmon during smoltification, no controlled studies have been done to determine if high rearing density specifically causes physiological stress in salmonids reared in production raceways.

Stress and its varied effects are often identified as high priority areas 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. Recently, Goede and Barton (1990) 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. Such an indexing system could be extremely useful to practical fish culturists but needs to be systematically tested and validated before it is recommended for general use. One obvious way of doing this (as set forth by Goede and Barton 1990) is to contrast potential organismic indicators with established physiological measures of stress following prolonged exposure to various environmental (or experimental) conditions.

The goal of this part of the overall project is to determine if high rearing density affects the stress responses, survival and growth of cultured rainbow trout, independent of water quality and loading conditions. Through the use of challenge tests and measurements of appropriate physiological and organismic indicators of stress (see Adams7778 1990) we hope to define the upper density limit at which rainbow trout can be reared without subjecting them to chronic sub-lethal stress.

ANTICIPATED BENEFITS

The overall goal of this collaborative project is to enhance salmonid aquaculture in the North Central Region. The identified objectives are not intimately linked, but are necessary for continued growth and development. Improved feed conversion, less agonistic behaviour, and alleviation of the concerns regarding shipment of eggs across state lines may be some of the results of transferring the information necessary to produce triploid and monosex salmonids. Knowledge of the amounts of phosphorus and nitrogen absorbed by salmonids will help reduce the levels of those nutrients in effluents, thereby improving the regulatory atmosphere and possibly reducing feed costs. A better understanding of maximum stocking densities may allow current producers to increase the numbers of fish raised in a given amount of water and will help novice aquaculturists develop realistic business plans.

OBJECTIVES

The overall goal of this project is to optimize culture technologies of salmonids for the climatic and economic conditions existing in the North Central Region. Specific objectives to achieve this goal are:

1. To continue evaluations of all-female diploids, all-female triploids, and mixed-sex diploids through sexual maturity and to use broodstock developed in the region to produce all-female diploid and all-female triploid trout populations.

2. To continue development of less-polluting diets by:

    a. quantifying absorption of crude protein in rainbow trout fed commonly-available feedstuffs substituted at varying levels in the diet (evaluation of associative effects);
    b. develop baseline effluent values from several types of salmonid aquaculture facilities located in the region;
    c. to develop and field test a mass balance method to estimate phosphorus levels from feed sources in hatchery effluents; and
    d. quantify phosphorus absorption from common feedstuffs fed to Atlantic salmon.

3. 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.

PROCEDURES

Development of Triploid and Monosex Stocks (Objective 1)

Southern Illinois University-Carbondale (SIUC) will continue experiments evaluating survival, growth, feed conversion, gonad production, fillet production, and dress-out percent of all-female diploid, all-female triploid, and mixed-sex diploid rainbow trout through sexual maturity. This work will only be for the first year of this project. Robert J. Sheehan will serve as the Principal Investigator, but James E. Seeb of the Alaska Department of Fish and Game has agreed to continue as an active participant in the project. The Alaska Department of Fish and Game entered into a cooperative agreement with SIUC during the second year of the first salmonid project to conduct studies at the Fort Richardson State Fish Hatchery, Anchorage, Alaska. Both Seven Pines Trout Farm and Alaska Fish and Game will continue as cooperators in the third of experiments of the study. Experiments to be conducted at SIUC, described below, will also be conducted at Fort Richardson State Fish Hatchery, but methods may be modified slightly at the latter site.

Experiments paralleling those being conducted at SIUC will also be done at Seven Pines Trout Farm and the Fort Richardson State Fish Hatchery. However, SIUC experiments will be highly controlled, replicated, laboratory experiments, whereas work at the sites of the two cooperators will be under practical fish culture conditions. This approach, which combines highly controlled laboratory experimental work and the enlistment of cooperators for evaluations under practical fish culture conditions, will provide a large amount of valuable information for a relatively small investment from the North Central Regional Aquaculture Center.

Growth performance of mixed-sex diploid, all-female diploids, and all-female triploid rainbow trout will be monitored from 180 days through sexual maturity during the year 3 work proposed herein. Twelve raceways will be used, with fish from each of the three genetic treatments evaluated in quadruplicate raceways.

Rainbow trout will be reared at a constant water temperature of 15 ^{o}C and at a density index typical of commercial fish culture situations in the Midwest. Standard feeding rates for rainbow trout will be used (Piper et al. 1986), which take into account that feed rate (as a percent of body weight) requirements are inversely related to fish size and directly related to temperature. Feeding rates will be adjusted every two weeks as the fish grow. Purina Trout Chow will be the diet used.

Water quality will be maintained by a water flush; water flows will exceed those determined by the following equation (Brannon7778 1991):

$$N = (0.25)/(0.00143 \times O_x)$$

and:

$$p = R/N,$$

where:

N = L/min required/kg of food fed,
0.25 = kg O_2 to metabolize 1 kg of food,
0.00143 = conversion constant (assumed to be about 1.3),
O_x = DO differential (inlet DO - outlet DO),
p = kg of food fed, and
R = total flow in L/min.

Rainbow trout from 4 to 7 of the matings for a given genetic treatment will be placed in equal numbers in each quadruplicate raceway. Each fish will be injected with a passive integrated transponder (PIT tag), permitting identification of individuals from the different matings. Thus, progeny from each of the 4 to 7 matings for a given genetic treatment will be evaluated together in each of the quadruplicate raceways so that differences in performance between matings can be determined and separated from tank effects.

The following data analyses will be done to compare the effects of the three genetic treatments. Instantaneous growth rates will be compared by Analysis of Variance (ANOVA), using a repeated

measures statistical design. Dressout % (eviscerated, head on), gonadal somatic index (weight of gonad/total fish weight), liver somatic index (weight of liver/total fish weight), fillet weight (weight of fillet/total fish weight), and food conversion (dry weight of food fed/wet weight of fish gain) will be analyzed through ANOVA at the end of the study. Main effects to be evaluated in the models will be genetic treatment and female. Variation in responses due to male genetic contributions will also be explored through ANOVA. Should initial sizes of fish in the three genetic treatments be significantly different, then Analysis of Covariance will be used to calculate growth with initial size as the covariate, since growth is a function of size in fish.

An SIUC research assistant will be on site at the Fort Richardson State Fish Hatchery beginning January, 1992 and throughout the remainder of Year 2 of the previous proposal to facilitate cooperative work. A research assistant will also be stationed at the Hatchery for about 6 months of this proposed project. One trip to Seven Pines Trout Farm has been planned to coordinate work conducted at that site.

Development of Less-Polluting Diets (Objective 2)

Researchers at MSU will feed triplicate groups of rainbow trout an experimental low P diet based on the results of our first salmonid research project (MSU modification of Ketola's T2M diet with phytase treated soy meal) or the practical trout feed fed by the Michigan Department of Natural Resources, Fish Division (MDNR). A third group of salmonids (chinook or coho salmon) will be fed the practical feed fed by the MDNR. Fish will be fed at a rate of 3% of the wet weight per day divided into 2 equal feedings (8:00 and 16:00 hours).

Trout will be reared from approximately 50 mm to 225 mm and salmon from 50 mm to MDNR stocking size. All fish will be reared in specially modified semi-oval 380 1 tanks which permit routine collection of solids. Tanks will be supplied with aerated well water at 12.5 ± 1 ^{o}C. Fish will be maintained on the ambient light:dark photoperiod. Initial fish stocking rates (density) and loadings will be at levels recommended by the MDNR for their hatcheries (Westers 1979). Rates will be maintained by removal of fish every 28 days during the growing cycle. Fish removed to maintain levels near optimum density will be analyzed for P levels. P levels in the effluent will be estimated by the follow equation:

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

where:

P_{ef} = P in effluent
P_{fed} = P in feed
P_f = P in feces
P_{tm} = P in fish at the time measured during the grow-out cycle
P_{ti} = P in fish at the beginning of the grow-out cycle

Fecal samples will be collected weekly. Effluent water samples will be collected 48 hours before each 28 day weight sample and density readjustment. Effluent water sampling will begin 1 hour before the first feeding and continue at 30 minute intervals for 24 hours. Storage and analysis of fecal waste and effluent P will be accomplished by the method of Fiske and Subbarow (1925). Body samples will be analyzed for total P by standard AOAC methods (AOAC 1980).

Laboratory data will be compared to samples taken at the same frequency from one or two fish hatcheries in Michigan. All samples will be taken and analyzed as described above except P effluent concentrations. Effluent concentrations from normal hatchery monitoring will be used.

Researchers at Purdue University will participate in the hatchery monitoring, specifically at the Twin Branch hatchery, Mishawaka, IN, and Curtis Creek Trout Rearing Station, Curtis Creek, IN. Twin Branch is a recirculating hatchery design (approximately 85% recirculating) equipped with a settling pond and is the primary hatchery for stocking Indiana's portion of southern Lake Michigan. Curtis Creek is a flow-through raceway hatchery responsible for rearing trout. Water samples will be collected one day each month from March through November of each year of this project. Samples will be collected 30 minutes prior to feeding then every 30 minutes after feeding for 6 hours. Phosphorus, nitrogenous products, and other minerals will be measured with standard field testing equipment or acidified and brought back to campus for measurement with our atomic absorption spectrophotometer. Collecting samples over time at both types of hatchery will allow comparisons of the different hatchery designs and changes in effluent wastes as loading and feed allotment increases.

Additional controlled, replicated nutrient absorption studies with rainbow trout will be conducted at Purdue that involve quantifying crude protein absorption from compounded diets containing varying sources and levels of commonly-available ingredients. Current diets fed to salmonids include several ingredients

including blood meal, corn gluten meal, soybean meal, wheat products, and several types of fish meal. Apparent absorption of crude protein will be determined by substituting graded levels of the target feedstuff into otherwise nutritionally-complete diets. Substitution levels will be 10, 20, 30 or 40% in diets containing sufficient essential amino acids to meet the known requirements of salmonids (NAS 1983). Fecal samples will be collected with the tanks described by Cho et al. (1982) that have been verified as appropriate for these analyses.

Other protocols (dietary premixes, diet mixing and handling protocols, fish stocking and monitoring, fecal collection and analyses) will be identical to methods currently in use with the first salmonid project.

Juvenile Atlantic salmon or eyed eggs will be transported to Purdue and acclimated to our experimental conditions. Those fish will be grown to an appropriate size (6 inches) and stocked into fecal collection tanks currently in place and functional. Triplicate groups of fish will be fed diets identical to those currently under evaluation with rainbow trout in our laboratory. Those diets contain a single source of phosphorus, chromic oxide, fish oil, and a nutritionally-complete vitamin premix. The supplemental mineral premix is calcium- and phosphorus-free to avoid potential mineral interactions.

Analytical procedures will be identical to those described in the previous proposal for chromium and crude protein analyses will be conducted by standard Kjeldahl analysis using existing equipment.

Data from this objective will be statistically analyzed by appropriate methods. Studies involving feeding fish in controlled situations will be analyzed by parametric methods, while those studies involving effluent monitoring at selected sites will be analyzed by nonparametric methods.

Effects of High Rearing Density on Stress, Growth and Production (Objective 3)

Research to characterize the effects of high rearing densities on the stress responses, survival and growth of juvenile rainbow trout will be done collaboratively by investigators from the University of Wisconsin-Madison (UW-Madison) and the University of Nebraska-Lincoln (UN-L). The UW-Madison will conduct laboratory experiments in tanks. The UN-L, with the assistance of the Nebraska Game and Parks Commission, will conduct field trials in production raceways to test laboratory findings. Analytical procedures used to evaluate physiological stress will be done primarily by UW-Madison researchers. Selected routine procedures will also be run by UN-L investigators. Potential organismic indicators of stress (Goede and Barton 1990) will be contrasted with established physiological measures on fish under both experimental laboratory (UW-Madison) and production (UN-L) conditions.

Principal hypotheses for Objective 3 are: (1) Stress due to aggressive behavior will decline at density indices above 0.5. (2) Stress due to physical crowding alone will increase at density indices above 2.0. (3) Therefore, density indices of 1.0 to 2.0 will be optimal for high-density minimum-stress culture (independent of water quality and flow). Alternative hypotheses are: (1) stress levels will increase progressively with rearing density, irrespective of density-related suppression of aggressive behavior; and (2) rearing density will have no effect on stress responses. We will also test the null hypotheses that (1) high rearing density has no effect on the stress responses of rainbow trout subjected to additional acute stressors such as handling, and (2) organismic indicators do not provide an efficacious means of evaluating stress levels in trout.

To the extent possible, all our investigations will be done with Kamloops rainbow trout provided by the Nebraska Game and Parks Commission and the U.S. Fish and Wildlife Service. The source of eggs for these fish will be the Ennis National Fish Hatchery, Ennis, Montana. Depending on circumstances and needs, some studies at the UW-Madison may be done using Kamloops trout from Wisconsin sources.

Stress responses in fish can be readily influenced by a variety of factors, including prior treatment (Barton 1988; Barton and Iwama 1991). Because of this, particular attention will be focused on environmental control and good husbandry procedures, both before and during experiments and production trials. Culture and fish handling methods that minimize stress will be employed. Fish will be acclimatized to laboratory or ambient hatchery rearing conditions for a minimum of 3 weeks before assignment to experimental tanks or raceways. After assignment, unless otherwise indicated by experimental design, fish will be acclimated to control conditions for 2 to 3 weeks before the initiation of experimental treatments. Every effort will be made to either eliminate or account for extraneous variables. Blood samples will be collected using standard procedures (Houston 1990).

Laboratory tank experiments will employ both the basic and multifactorial variations of the experimental design of Soderberg and Krise (1988) and Kebus et al. (In Press) to evaluate the effects of different rearing densities. For each trial, fish of similar size will be randomly assigned to four plastic or polyester-mesh cages suspended in each of three or four cylindrical (1.2- or 1.8-m-diameter) fiberglass rearing tanks. Each of the four cages in each rearing tank will be stocked at four different density indices, giving

a total of three or four replicate groups at each density. Because each tank will contain one replicate of each density, all tanks in each trial will contain the same total density (kg/m^3) of fish. Cage dimensions will be determined by tank size and experimental need.

In each experiment, water supplied to the rearing tanks will be aerated (via packed columns and/or air stones) or supplemented with pure oxygen as needed to maintain total dissolved gas pressure and oxygen in tanks slightly below saturation levels. Total dissolved gas pressure, dissolved oxygen, pH and total ammonia-nitrogen will be monitored at appropriate intervals, using a Weiss or Sweeney "saturometer," a calibrated dissolved-oxygen probe and standard procedures (AOAC 1980; APHA et al. 1985), as applicable. Photoperiod will be 16 h light/8 h dark or ambient. Experiments will be conducted at 10, 13 and/or 15 ± 0.5 °C. Fish will be fed a set ration based on percent body weight two to three times daily, using commercially-available trout feed.

UW-Madison researchers will examine density indices ranging from 0.25 to 4.0 at loading levels ranging from 0.5 to 3.0 kg of fish/L/minute, utilizing a 3 x 4 factorial randomized complete-block design, with four rearing densities at each of three loading levels, repeated three times. Initial trials will test density indices of 0.25, 0.5, 1.5 and 2.5 at loading levels of 0.5, 1.5 and 3.0 kg/L/minute. The water flow into each tank will be adjusted to provide the different loading rates. The power of this design is that it provides a means of defining the upper density limits on rearing juvenile rainbow trout at different loading levels, and will permit us to determine if interactions exist between these two parameters.

The duration of laboratory experiments will be 3 to 15 weeks, depending on the purpose of each experiment (e.g., the assessment of short- or long-term stress responses). A minimum of two long-term experiments will be conducted in tanks. Experiments involving the comparison of growth responses will be 9 to 15 weeks long. Feeding levels and water flow rates will be adjusted every 2 weeks to account for fish growth. At the ends of experiments and at 2-week intervals in long-term experiments, the total weight of each replicate treatment group will be determined, in addition to the lengths and weights of individual fish sampled from each treatment group. Based on these determinations, fish will be added to or removed from each cage to maintain the density indices being tested.

Several standard measures of primary, secondary and tertiary stress responses will be employed in both tank and raceway studies. The type and number of measures used will depend on the purpose of particular experiments (see Adams 1990). Levels of cortisol in blood samples will be measured by an enzyme-linked immunosorbant assay (ELISA) recently developed and validated by the UW Aquaculture Program (Kayes 1988; Barry et al. 1991). Plasma glucose and chloride titers will be determined by procedures recently validated for use in rainbow trout and lake trout (Barry et al. 1991). Hematocrits, plasma osmolality and protein levels (Wedemeyer and McLeay 1981), leucocrits, and possibly differential blood cell counts (Wedemeyer and Yasutake 1977; Pickering and Pottinger 1987b) will also be evaluated as measures of stress. Length and weight gain, specific growth rate, condition factor, feed conversion, and incidence of disease, morbidity and mortality will be examined, as appropriate. Potential organismic indicators of stress, of the type described by Goede and Barton (1990), will be systematically evaluated and contrasted with physiological measures of stress in both our tank and raceway investigations.

To assess "stress load" in fish subjected to different density indices and loadings in laboratory tanks, fish will be challenged at the ends of experiments with an acute handling stressor. The physiological responses (e.g., cortisol, glucose and chloride titers) of these fish over time (e.g., 0, 1, 3, 6, 24, 48 hours, and 7 days post-stress) will be compared with unchallenged controls.

Previous research done at the UW-Madison to evaluate the effects of time of day, fish handling techniques, anesthetization, and blood-collection methods on the stress responses of rainbow trout and lake trout has demonstrated that with appropriate precautions, the introduction of procedural artifacts into the type of experiments we propose can be minimized or eliminated (Kebus et al. In Press; Barry et al. 1991). Similar fish handling, anesthetization, and blood-collection procedures will be used in both tank and raceway studies.

The primary focus of the Nebraska component of the project will be to evaluate the long-term stress-related effects of different (intermediate and high) rearing densities on rainbow trout under production conditions, and to systematically test (and if possible validate) potential organismic indicators of stress (see Goede and Barton 1990) as possible tools for use by practicing fish culturists. The production trials will be run at the Calamus State Fish Hatchery near Burwell, Nebraska, utilizing findings generated by short- and intermediate-term (3- to 9-week) tank experiments done at the UW-Madison.

Because a large experiment could potentially put much of the state's annual production of Kamloops trout at risk, the Nebraska part of the project will be done in two phases: a small-scale pilot study utilizing cylindrical tanks and the basic experimental design of Soderberg and Krise (1986) and Kebus et al. (In Press), and one or more subsequent full-scale raceway trials (the exact number depending on time and

the availability of fish and other resources). The pilot study will parallel the intermediate- to long-term (9- to 15-week) experiments to be done at the UW-Madison, but will examine only four rearing densities at one loading level.

The primary purpose of the pilot study will be to evaluate the potentially confounding effects of differences in water chemistry (especially pH, hardness and alkalinity), personnel and fish culture practices between the Nebraska and Wisconsin research sites, and thereby help minimize the risk of catastrophic failures (due to mistaken assumptions) during the raceway trials. To the extent possible, the feeding schedule and other husbandry practices that we plan to use with the raceway trials will also be employed in the pilot study. Each raceway trial will examine the effects of a minimum of two different rearing densities, with a minimum of three replicate raceways per treatment, will begin with fish at about 65-mm total length (TL), and will be continued until they reach a stocking size of about 270-mm TL. If sufficient numbers of fish are available, we plan to evaluate the effects of at least three different rearing densities under raceway culture conditions.

Depending on experimental needs and the size and number of trout available, the production trials will be done either in 1.2- or 1.8-m-diameter cylindrical tanks (the pilot study), or in 0.61-m-wide x 6.1-m-long x 0.61-m-deep, 0.91-m-wide x 6.1-m-long x 0.91-m-deep, 1.8-m-wide x 20-m-long x 1.2-m-deep, or 2.4-m-wide x 27-m-long x 1.2-m-deep raceways, the last two sizes of which are equipped with removable baffles at 2.4-m intervals. All production experiments will utilize 13 ^{o}C well water, with the flow or turnover rates through all rearing units adjusted to a set value calculated (and verified) to limit dissolved un-ionized ammonia concentrations at the highest rearing densities well within accepted limits. Pure oxygen supplementation through sealed packed columns will be employed as needed to maintain dissolved oxygen concentrations across treatment groups as constant as possible, at a level slightly below saturation. Fish will be hand fed a set ration based on percent body weight, two times a day. The amount fed each rearing unit will be adjusted every 2 weeks, based on projected growth and monthly subsample determinations of weight gain. Density indices being tested will be maintained as constant as possible throughout each production trial by the addition or removal of fish from experimental units.

In the Nebraska component of the project, stress challenge tests (employing a standardized acute handling stressor) will be administered to samples of fish from each treatment group at points approximately midway through, and about a month before, the end of each production experiment. In addition to evaluating physiological end-points resulting from these tests, the long-term (post-challenge) survival, disease-incidence and growth of challenged fish in raceways will be compared with that of unchallenged fish. The general health of fish throughout the production trials will be monitored closely with the assistance of the State Hatchery Biologist, who is based at the Calamus facility and is responsible for disease inspections statewide.

While the focus of the proposed project is not on aggressive behavior, some assessment of it is needed to fully test our hypothesis that stress levels at different rearing densities is at least partly related to variations in the degree of aggressive behavior. To evaluate the latter, observations will be made of the relative levels of nipping or fighting behavior exhibited by fish under different experimental conditions. Experimental populations of salmonids that have developed social hierarchies often have body length and weight frequency distributions that are skewed towards smaller sizes (Fagerlund et al. 1981; Schreck et al. 1985); and according to some investigators (see Purdom 1974; Jobling and Wandsvik 1983), levels of "within-group" hierarchical behavior and/or competition can often be estimated by comparing changes over time in the coefficient of variance for the length, weight, condition factor and/or growth rates of fish in each treatment group. Our studies will utilize these indirect measures of aggressive behavior in addition to direct observation.

Whenever possible, parametric statistical methods will be used to analyze numerical data from both laboratory tank and production experiments. Nonparametric statistics will be employed when the application of parametric methods is found to be inappropriate or unfeasible. Data collected by UW-Madison and UN-L investigators will be collated on an ongoing basis, and the findings published in a timely manner in appropriate peer-reviewed national or international scientific journals. Extension information outlining the practical implication and benefits of the completed research will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

FACILITIES

Development of Triploid and Monosex Stocks (Objective 1)

Southern Illinois University has an 18-raceway, temperature- controlled, water reuse system that will be dedicated to this work. The services of the SIU Fish Health, Water Quality and Genetics Laboratories

will also be available to support work on this project. The capabilities of these labs and other support facilties and services available were described in the previous proposal.

Fort Richardson State Fish Hatchery has all support facilities and services available through the Alaska Department of Fish and Game. This hatchery is the primary facility for the state's genetics research program.

Develpoment of Less-Polluting Diets (Objective 2)

Purdue University has the necessary wet laboratory and fecal collection tanks for conducting the proposed studies. Additionally, a new, fully equipped Nutrition Laboratory has been established that contains all the necessary analytical equipment for completing these studies. A new wet laboratory will begin construction in March 1992. If that faciltiy is completed prior to initiation of these studies, it will be used in this research.

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

Effects of High Rearing Density on Stress, Growth and Production (Objective 3)

Hormonal and other physiological measures, and laboratory tank experiments on rearing density and stress will be done by the UW Aquaculture Program of the UW-Madison, under the direction of Jeffrey A. Malison and Terence P. Barry. The experiments 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).

Studies on the effects of rearing density on trout stress responses and growth under production conditions will be done by UN-L investigators, under the direction of Terrence B. Kayes and in collaboration with the Nebraska Game and Parks Commission. The production experiments will be conducted at the Calamus State Fish Hatchery, a 59-hectare facility located immediately downstream of the (2023-hectare) Calamus Reservoir, near Burwell, Nebraska. Physical resources available at "the Calamus" include: 11 0.20-hectare and 40 0.40-hectare fish production ponds; 8 1.8-m-wide x 20-m-long x 1.2-m-deep and 16 2.4-m-wide x 27-m-long x 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 x 6.1-m-long x 0.61-m-deep and 8 0.91-m-wide x 6.1-m-long x 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 the Calamus include: reservoir water (with a seasonal temperature variation from about 4 ^oC in winter to about 22 ^oC in summer) supplied to all the indoor and outdoor facilities via a 91-cm-diameter main; and about 11-m^3/minute and 1.1-m^3/minute water flow from eight wells supplying 13 ^oC and 11 ^oC water, respectively, from two separate aquifers, to all the raceways and indoor facilities. A very large pure-oxygen supply system is in place at the Calamus, and oxygen supplementation in individual tanks and raceways can be achieved through the use of sealed packed-columns. 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 to the campus laboratory, or essential equipment can be moved to the Calamus laboratory.

REFERENCES

Abbott, J.C., and L.M. Dill. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 42:1702-1706.

Abbott, J.C., R.L. Dunbrack, and C.D. Orr. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behavior 92:241-253.

Adams, S.M. 1990. Biological indicators of stress in fish. American Fisheries Symposium 8. American Fisheries Society, Bethesda.

Ainsworth, A.J., P.R. Bowser, and M.H. Beleau. 1985. Serum cortisol levels in channel catfish from production ponds. Progressive Fish-Culturist 47:176-181.

Allen, S.K., and S.L. Downing. 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. Journal of Experimental Marine Biology and Ecology 102:197-208.

Allendorf, F.W., and R.F. Leary. 1984. Heterozygosity in gynogenetic diploids and triploids estimated by gene-centromere recombination rates. Aquaculture 43:413-420.

AOAC (Association of Official Analytical Chemists). 1980. Official methods of analysis, 13th edition. Washington, DC.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1985. Standard Methods for the Examination of Water and Wastewater, 16th edition. American Public Health Association, Washington, DC.

Barry, T.P., T.B. Kayes, J.A. Malison, and C.H. Amundson. 1991. Validation of a highly sensitive microplate enzyme-linked immunoassay (ELISA) for measuring serum cortisol in fish, and a comparison of primary physiological stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Journal of the World Aquaculture Society 22:14A.

Barton, B.A. 1988. Endocrine and metabolic responses of fish to stress. International Association of Aquatic Animal Medicine Proceedings 19:41-55.

Barton, B.A., and C.B. Schreck. 1987. Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 62:299-310.

Barton, B.A., G.S. Weiner, and C.B. Schreck. 1985. Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. Canadian Journal of Fisheries and Aquatic Sciences 42:710-717.

Barton, B.A., C.B. Schreck, and L.A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society 115:245-251.

Barton, B.A., and G.K. Iwama. 1991. Physiological changes in fish from stress on aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Disease 1:3-26.

Benfey, T.J., and A.M. Sutterlin. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture 36:359-367.

Bergheim, A., and A.R. Selmer-Olsen. 1978. River pollution from a large trout farm in Norway. Aquaculture 14:267-270.

Blackburn, J., and W.C. Clarke. 1990. Lack of density effect on growth and smolt quality in zero-age coho salmon. Aquacultural Engineering 9:121-130.

Brannon, E.L. 1991. Rainbow trout culture. Pages 21-55 *in* R.R. Stickney, editor. Culture of salmonid fishes. CRC Press, Boca Raton, Florida.

Brown, P.B. In Press. Comparison of fecal collection methods for determining phosphorus absorption in rainbow trout. 4th International Symposium on Fish Feeds and Nutrition, Biarritz, France.

Brown, P.B., E.H. Robinson, A.E. Clark, and A.L. Lawrence. 1989. Apparent digestible energy coefficients and associative effects in practical diets for red swamp crayfish. Journal of the World Aquaculture Society 20:122-126.

Buss, K., D.R. Graff, and E.R. Miller. 1970. Trout culture in vertical units. Progressive Fish-Culturist 32:187-191.

Bye, V.J., and R.F. Lincoln. 1986. Commercial methods for the control of sexual maturation in rainbow trout (*Salmo gairdneri* R.). Aquaculture 57:299-310.

Cassani, J.R., and W.E. Caton. 1986. Growth comparisons of diploid and triploid grass carp under varying conditions. Progressive Fish-Culturist 48:184-187.

Cho, C.Y., S.J. Slinger, and H.S. Bayley. 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology 73B:25-41.

Cole, K.S. 1976. Social behavior and social organization of young rainbow trout, *Salmo gairdneri*, of hatchery origin. Master of Science thesis, University of Guelph, Guelph, Ontario.

Cole, C.S., and D.L.G. Noakes. 1980. Development of early social behavior of rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). Behavioral Processes 5:97-112.

Cowey, C.B., and C.Y. Cho, editors. 1991. Nutritional strategies in management of aquaculture wastes, Proceedings of the International Symposium. Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada.

Donaldson, E.M. 1986. The integrated development and application of controlled reproduction techniques in Pacific salmonid aquaculture. Fish Physiology and Biochemistry 2:9-24.

Donaldson, E.M., and G.A. Hunter. 1982. Sex control in fish with particular reference to salmonids. Canadian Journal of Fisheries and Aquatic Sciences 39:99-110.

Ejike, C., and C.B. Schreck. 1980. Stress and social hierarchy rank in coho salmon. Transactions of the American Fisheries Society 109:423-426.

Fagerlund, U.H.M., J.R. McBride, and E.T. Stone. 1981. Stress-related effects of hatchery rearing density on coho salmon. Transactions of the American Fisheries Society 110:644-649.

Fenderson, O.C., and R.M. Carpenter. 1971. Effects of crowding of the behavior of juvenile hatchery and wild landlocked Atlantic salmon (*Salmo salar* L.). Animal Behavior 19:439-447.

Ferguson, M.M., D.L.G. Noakes, and D. Romani. 1983. Restricted behavioral plasticity of juvenile lake charr, *Salvelinus namaycush*. Environmental Biology of Fishes 8:151-156.

Fiske, C.H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. Journal of Biological Chemistry 66:375-400.

Fujino, K., M. Kamakura, R. Mito, and K. Arai. 1987a. Overdominance of survivals at phosphogluconate dehydrogenase locus in triploid Pacific abalone populations. Nippon Suisan Gakkaishi 53:1759-1764.

Fujino, K., S. Okumura, and H. Inayoshi. 1987b. Temperature tolerance differences among normal diploid and triploid Pacific abalones. Nippon Suisan Gakkaishi 53:15-21.

Gibson, R.J. 1978. The behavior of juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) with regard to temperature and water velocity. Transactions of the American Fisheries Society 107:703-712.

Goede, R.W., and B.A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pages 93-108 *in* S.M. Adams, editor. Biological indicators of stress in fish. American Fisheries Symposium 8, Bethesda, Maryland.

Harris, J. 1981. Federal regulation of fish hatchery effluent quality. Pages 157-161 *in* L.J. Allen and E.C. Kinney, editors. Proceedings of the bio-engineering symposium for fish culture. American Fisheries Society, Bethesda, Maryland.

Hinshaw, R.N. 1973. Pollution as a result of fish cultural activities. U.S. Environmental Protection Agency, Report EPA-R3-73-009, Washington, DC.

Houston, A.H. 1990. Blood and circulation. Pages 273-334 *in* C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Johnson, O.W., W.W. Dickhoff, and F.M. Utter. 1986. Comparative growth and development of diploid and triploid coho salmon, *Oncorhynchus kisutch*. Aquaculture 57:329-336.

JSA (Joint Subcommittee on Aquaculture of the Federal Coordinating Council on Science, Engineering and Technology). 1983. Trout Species Plan. Pages 146-160 *in* National aquaculture development plan, volume 2. Washington, D.C.

Jobling, M., and A. Wandsvik. 1983. Effect of social interactions on growth rates and conversion efficiency of arctic charr, *Salvelinus alpinus* L. Journal of Fish Biology 22:577-584.

Kayes, T. 1988. Assessment of low-level gas supersaturation as stressors in lake trout and rainbow trout (R/AQ-14). Pages 227-245 *in* University of Wisconsin 1988-90 proposal to the National Sea Grant College Program, volume 2. University of Wisconsin Sea Grant College Program, Madison.

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

Keenleyside, M.H., and F.T. Yamamoto. 1962. Territorial behavior of juvenile Atlantic salmon (*Salmo salar* L.). Behavior 19:139-169.

Kendra, W. 1991. Quality of salmonid hatchery effluents during a summer low-flow season. Transactions of the American Fisheries Society 120:43-51.

Ketola, H.G. 1985. Mineral nutrition: Effects of phosphorus in trout and salmon feeds on water pollution. *In* C.B. Cowey, A.M. Mackie and J.G. Bell, editors. Nutrition and feeding in fish. Academic Press, Orlando.

Kjartansson, H., S. Fivelstad, J.M. Thomassen, and M.J. Smith. 1988. Effects of different stocking densities on physiological parameters and growth of adult Atlantic salmon (*Salmo salar* L.) reared in circular tanks. Aquaculture 73:261-274.

Komaru, A., and K.T. Wada. 1989. Gametogenesis and growth of induced triploid scallops *Chlamys nobilis*. Nippon Suisan Gakkaishi 55(3):447-452.

Korzeniewski, K., Z. Banat, and A. Moczulska. 1982. Changes in water of the Uniesc and Skotawa rivers, caused by intensive trout culture. Polish Archives of Hydrobiology 29:683-691.

Leatherland, J.F., and C.Y. Cho. 1985. Effect of rearing density on thyroid and interrenal gland activity and plasma and hepatic metabolite levels in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 27:583-592.

Liao, P.B. 1970. Pollution potential of salmonid fish hatcheries. Water and Sewage Works 117:291-297.

Lincoln, R.F. 1981. Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice x flounder (*Platichthys flesus*) hybrids. Journal of Fish Biology 19:415-426.

Lincoln, R.F., and V.J. Bye. 1987. Growth rates of diploid and triploid rainbow trout (*Salmo gairdneri* R.) over the spawning season. Page 134 *in* D.R. Idler, L.W. Cum and J.M. Walsh, editors. Proceedings of the Third International Symposium on the Reproductive Physiology of Fish. Memorial University of Newfoundland, St. John's, Newfoundland.

Lincoln, R.F., and A.P. Scott. 1984. Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 25:385-392.

Maule, A.G., R.A. Tripp, S.L. Kaattari, and C.B. Schreck. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endrocinology 120:135-142.

NAS (National Academy of Sciences). 1983. Nutrient requirements of fish and shellfish, revised edition. National Academy Press, Washington, DC.

NCA-23 (Aquaculture Subgroup of the NCA-23 Fish and Wildlife Committee). 1987. North Central Regional Aquaculture Center. A report to the North Central Regional Research Committee and North Central Regional Agriculture Experiment Station Directors.

Noakes, D.L.G., and J.F. Leatherland. 1977. Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). Environmental Biology of Fishes 2:131-136.

Parker, P. 1988. Who are the investors behind the boom in American salmon farms? National Fisherman (July):24-25.

Patino, R., C.B. Schreck, J.L. Banks, and W.S. Zaugg. 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Transactions of the American Fisheries Society 115:828-837.

Peters, G., M. Faisal, T. Lang, and I.I. Ahmed. 1988. Stress caused by social interaction and its effect on susceptibility to *Aeromonas hydrophila* infection in rainbow trout, *Salmo gairdneri*. Diseases of Aquatic Organisms 4:83-89.

Peters, G., A. Nubgen, A. Raabe, and A. Mock. 1991. Social stress induces structural and functional alterations of phagocytes in rainbow trout (*Oncorhynchus mykiss*). Fish and Shellfish Immunology 1:17-32.

Pickering, A.D., and A. Stewart. 1984. Acclimation of the interrenal tissue of the brown trout *Salmo trutta* L., to chronic crowding stress. Journal of Fish Biology 24:731-740.

Pickering A.D., and T.G. Pottinger. 1985. Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. Journal of Fish Biology 27:611-619.

Pickering, A.D., and T.G. Pottinger. 1987a. Poor water quality suppresses the cortisol responses of salmonid fish to handling and confinement. Journal of Fish Biology 30:363-374.

Pickering, A.D., and T.G. Pottinger. 1987b. Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. Journal of Fish Biology 30:701-712.

Pickering, A.D., editor. 1981. Stress and fish. Academic Press, New York.

Piper, R.C., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler, and J.R. Leonard. 1982. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, D.C.

Poston, H.A. 1983. Effect of population density of lake trout in cylindrical jars on growth and oxygen consumption. Progressive Fish-Culturist 45:8-13.

Purdom, C.E. 1974. Variation in fish. Pages 347-355 *in* F.R. Hardin Jones, editor. Sea fisheries research. John Wiley and Sons, New York.

Purdom, C.E. 1986. Genetic techniques for control of sexuality in fish farming. Fish Physiology and Biochemistry 2:3-8.

Refstie, T. 1977. Effect of density on growth and survival of rainbow trout. Aquaculture 11:329-334.

Refstie, T., and A. Kittelsen. 1976. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture 8:319-326.

Scheerer, P.D., G.H. Thorgaard, and J.E. Seeb. 1987. Performance and developmental stability of triploid tiger trout (*Salmo trutta* x *Salvelinus fontinalis*). Transactions of the American Fisheries Society 116:92-97.

Schreck, C.B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. Pages 295-322 *in* A.D. Pickering, editor. Stress and fish. Academic Press, New York.

Schreck, C.B., R. Patino, C.K. Pring, and J.R. Winton. 1985. Effects of rearing densities on indices of smoltification and performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 45:345-358.

Seeb, J.E., and G.D. Miller. 1989. The integration of allozyme analysis and genomic manipulations for fish culture and management. Pages 265-279 *in* D. Whitmore, editor. Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Inc., Boca Raton, Florida.

Senn, H., J. Mack, and L. Rothfus. 1984. Compendium of low-cost Pacific salmon and steelhead trout production facilities and practices in the Pacific Northwest. U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, Oregon.

Shepherd, B.G. 1984. The biological design process used in the development of federal government facilities during Phase 1 of the salmonid enhancement program. Canadian Technical Report on Fisheries and Aquatic Science Number 1275.

Soderberg, R.W., and W.F. Krise. 1986. Effects of rearing density on growth and survival of lake trout. Progressive Fish-Culturist 48:30-32.

Symons, P.E.K. 1970. The possible role of social and territorial behavior of Atlantic salmon parr in the production of smolts. Technical Report of the Fisheries Research Board of Canada Number 206.

Thomas, R.E., and S.D. Rice. 1987. Effect of water-soluble fraction Cook Inlet crude oil on swimming performance and plasma cortisol in juvenile coho salmon (*Oncorhynchus kisutch*). Comparative Biochemistry and Physiology 87C:177-180.

Thompson, D. 1983. The efficiency of induced diploid gynogenesis in inbreeding. Aquaculture 33:237-244.

Thorgaard, G.H. 1986. Ploidy manipulation and performance. Aquaculture 57:57-64.

Visscher, L., and W. Godby. 1987. Oxygen supplementation: a new technology in fish culture. Information Bulletin Number 1. U.S. Fish and Wildlife Service, Region 6, Denver.

Visscher, L., and W.P. Dwyer. 1990. Oxygen supplementation: a new technology in fish culture, volume 2. Information Bulletin Number 2. U.S. Fish and Wildlife Service, Region 6, Denver, Colorado.

WASC (Wisconsin Aquaculture Study Committee). 1988. Wisconsin aquaculture: a state plan. Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison.

Wedemeyer, G.A. 1976. Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. Journal of the Fisheries Research Board of Canada 33:2699-2702.

Wedemeyer, G.A., and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stressors. Pages 248-275 *in* A.D. Pickering, editor. Stress and fish. Academic Press, New York.

Wedemeyer, G.A. and W.T. Yasutake. 1977. Clinical methods for the assessment of the effects of environmental stress in fish health. U.S. Fish and Wildlife Service Technical Paper 89, Washington, D.C.

Wedemeyer, G.A., B.A. Barton, and D.J. McLeay. 1989. Stress and acclimation. Pages 451-489 *in* C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Wedemeyer, G.A., B.A. Barton, and D.J. McLeay. 1990. Stress and acclimation. Pages 451-489 *in* C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Westers, H. 1979. Fish culture practices for the State of Michigan. Michigan Department of Natural Resources, Fish Division. Lansing, MI.

Westers, H., and K.M. Pratt. 1977. Rational design of hatcheries for intensive salmonid culture, based on metabolic characteristics. Progressive Fish-Culturist 39:157-165.

Wetzel, R.G. 1983. Limnology, second edition. Saunders College Publishing, Philadelphia.

Wiesmann, D., H. Scheid, and E. Pfeffer. 1988. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 69:263-270.

Wilson, R.P. 1989. Amino acids and protein. Pages 112-153 *in* J.E. Halver, editor. Fish nutrition. Academic Press, New York.

Woodward, J.J. and L.S. Smith. 1985. Exercise training and the stress response in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 26:435-447.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	Robert J. Sheehan Southern Illinois University-Carbondale	Aquaculture/Genetics
Indiana	Paul B. Brown Purdue University	Aquaculture/Nutrition
Michigan	Donald L. Garling Michigan State University	Aquaculture/Nutrition
Nebraska	Terrence B. Kayes University of Nebraska-Lincoln	Aquaculture Production/Fish Physiology and Nutrition/Aquaculture Extension
Wisconsin	Jeffrey A. Malison University of Wisconsin-Madison	Aquaculture/Physiology/ Endocrinology
	Terence P. Barry University of Wisconsin-Madison	Aquaculture/Physiology/ Endocrinology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Southern Illinois University-Carbondale
Robert J. Sheehan

Purdue University
Paul B. Brown

Michigan State University
Donald L. Garling

University of Nebraska-Lincoln
Terrence B. Kayes

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

PROPOSED PROJECT BUDGET FOR
SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE (SIUC)

(Sheehan)

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			$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			$10,914	$0
d. Prebaccalaureate Students	1	0.20			$2,288	$0
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$13,202	$0
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$13,202	$0
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$2,000	$0
F. Travel - Domestic (*Including Canada*)					$3,500	$0
G. Other Direct Costs					$1,298	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$20,000	$0
TOTAL PROJECT COSTS					$20,000	

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

BUDGET JUSTIFICATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

A. Salaries and Wages. Graduate Assistant (0.50 FTE) to assist in experiments conducted at the Alaska Department of Fish and Game, Fort Richardson State Fish Hatchery and a Prebaccalaureate Student (0.20 FTE) to assist PI and Graduate Student thesis research in experiments at SIUC.

F. Travel. Trip to Fort Richardson State Fish Hatchery (Anchorage, Alaska) for PI ($1,300); Costs for research assistant travel to and from Fort Richardson State Fish Hatchery ($1,600); Travel to Work Group Meeting ($300); and one trip to Seven Pines Trout Farm in Lewis, Wisconsin ($300)

G. Other Direct Costs. Telecommunication, repairs, parts and maintenance of equipment, photocopying, and postage/freight costs.

PROPOSED PROJECT BUDGET FOR
PURDUE UNIVERSITY

(Brown)

Objective 2

	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						
b. Other Professionals						
c. Graduate Students	1	0.50	1	0.50	$10,900	$11,250
d. Prebaccalaureate Students	1	0.10	1	0.10	$1,400	$1,500
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$12,300	$12,750
B. Fringe Benefits					$261	$266
C. **Total Salaries, Wages and Fringe Benefits**					$12,561	$13,016
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$2,000	$2,000
F. Travel - Domestic (*Including Canada*)					$1,500	$1,500
G. Other Direct Costs					$1,439	$984
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

A. Salaries and Wages. One M.S.-level Graduate Student (0.50 FTE) will be employed on the project. Responsibilities of the student will include fish acquisition and acclimation, fish husbandry, diet manufacturing, scientific feeding of fish, analytical analyses and monitoring water quality from the selected hatcheries. A Prebaccalauerate Student (0.10 FTE) will be hired to assist PI and Graduate Student.

E. Materials and Supplies. This category represents costs of feedstuffs, dietary ingredients, and chemical reagents.

F. Travel. Costs included represent travel-related expenses to attend regaular meetings of the work group and to professional meetings to present our research findings.

G. Other Direct Costs. Telephone, photocopying, FAX and computer costs.

PROPOSED PROJECT BUDGET FOR
MICHIGAN STATE UNIVERSITY (MSU)

(Garling)

Objective 2

	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	1	0.10	$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	$12,500	$13,750
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$12,500	$13,750
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$12,500	$13,750
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$250	$250
F. Travel - Domestic (*Including Canada*)					$1,000	$1,250
G. Other Direct Costs					$2,375	$2,612
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$16,125	$17,862
TOTAL PROJECT COSTS					$33,987	

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

BUDGET JUSTIFICATION FOR MICHIGAN STATE UNIVERSITY

A. Salaries and Wages. One Graduate Student (0.50 FTE) to care for fish, collect hatchery effluent samples, prepare sample for P analysis.

E. Materials and Supplies. Chemicals for P analysis preparation, miscellaneous fish culture supplies, and fish food.

F. Travel. To obtain fish and P samples (Year 1) and partial travel to NCRAC Work Group meetings (Year 1 and Year 2).

G. Other Direct Costs. Utilities for the MSU Aquaculture Laboratory.

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

(Kayes)

Objective 3

	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						
b. Other Professionals						
c. Graduate Students	1	0.49	1	0.49	$9,100	$9,605
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$9,100	$9,605
B. Fringe Benefits					$1,200	$1,260
C. **Total Salaries, Wages and Fringe Benefits**					$10,300	$10,865
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,000	$1,100
F. Travel - Domestic (*Including Canada*)					$980	$1,180
G. Other Direct Costs					$200	$300
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$12,480	$13,445
				TOTAL PROJECT COSTS	$25,925	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF NEBRASKA-LINCOLN

A. Salaries and Wages. A Graduate Student (0.49 FTE) is needed to assist (1) Nebraska Game and Parks Commission personnel with the set up of experiments and general fish husbandry, and (2) the PI with the conduct of experiments, stress challenge tests, and analyses of physiological and organismic indicators of stress.

E. Materials and Supplies. Biochemicals, reagents and laboratory supplies (e.g., glassware, buffers, alcohol, tissue fixatives, microscope slides, dissection equipment) are needed to conduct blood and organismic analyses. Net pens, nets, other wet laboratory supplies, dry ice, insulated containers, etc. are needed to conduct the experiments at UN-L, and transport blood and tissue samples to the UW-Madison. Fish and fish feeds will be provided by the Nebraska Game and Parks Commission.

F. Travel. Travel funds requested are required to meet fleet car and part of the meal costs for six to nine round trips per year from the UN-L campus to the Calamus State Fish Hatchery (a distance of about 230 miles). The cost of long-term stays, lodging (usually), and additional trips to the Calamus hatchery will be covered by other mechanisms.

G. Other Direct Costs. About $100/year is needed for telephone, FAX, postage and photocopying. An additional $100-$200/year is required for overnight express shipment of frozen blood and tissue samples to the UW-Madison.

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

(Malison and Barry)

Objective 3

	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)	2	0.12	2	0.12	$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	$13,524	$14,200
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$13,524	$14,200
B. Fringe Benefits (for 2c)					$1,420	$1,491
C. **Total Salaries, Wages and Fringe Benefits**					$14,944	$15,691
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,500	$1,500
F. Travel - Domestic (*Including Canada*)					$525	$525
G. Other Direct Costs					$200	$200
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$17,169	$17,916
TOTAL PROJECT COSTS					$35,085	

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

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-MADISON

A. Salaries and Wages. A Graduate Student (0.50 FTE) is needed to: (1) obtain and transport fish and provide fish husbandry; (2) conduct short-term and long-term experiments; and (3) conduct analyses on plasma cortisol, glucose, chloride, and organismic indicators of stress.

E. Materials and Supplies. Biochemicals, reagents and laboratory supplies (e.g., tubes, microtiter plates, etc.) are needed to conduct analyses of plasma cortisol, chloride and glucose. Fish feed, net pens and wet laboratory supplies are required for fish husbandry and to conduct fish culture experiments.

F. Travel. Needed for travel to the University of Nebraska-Lincoln to obtain and transport fish.

G. Other Direct Costs. Telephone, FAX, postage and photocopying costs.

CULTURE TECHNOLOGY OF SALMONIDS

Budget Summary for Each Participating Institution at $83.3K for the First Year

	SIUC	Purdue	MSU	UN-L	UW	TOTAL
Total Salaries and Wages	$13,202	$12,300	$12,500	$9,100	$13,524	$60,626
Fringe Benefits	$0	$261	$0	$1,200	$1,420	$2,881
Total Salaries, Wages and Benefits	$13,202	$12,561	$12,500	$10,300	$14,944	$63,507
Nonexpendable Equipment	$0	$0	$0	$0	$0	$0
Materials and Supplies	$2,000	$2,000	$250	$1,000	$1,500	$6,750
Travel	$3,500	$1,500	$1,000	$980	$525	$7,505
Other Direct Costs	$1,298	$1,439	$2,375	$200	$200	$5,512
TOTAL PROJECT COSTS	**$20,000**	**$17,500**	**$16,125**	**$12,480**	**$17,169**	**$83,274**

Budget Summary for Each Participating Institution at $66.7K for the Second Year

	SIUC	Purdue	MSU	UN-L	UW	TOTAL
Total Salaries and Wages	$0	$12,750	$13,750	$9,605	$14,200	$50,305
Fringe Benefits	$0	$266	$0	$1,260	$1,491	$3,017
Total Salaries, Wages and Benefits	$0	$13,016	$13,750	$10,865	$15,691	$53,322
Nonexpendable Equipment	$0	$0	$0	$0	$0	$0
Materials and Supplies	$0	$2,000	$250	$1,100	$1,500	$4,850
Travel	$0	$1,500	$1,250	$1,180	$525	$4,455
Other Direct Costs	$0	$984	$2,612	$300	$200	$4,096
TOTAL PROJECT COSTS	**$0**	**$17,500**	**$17,862**	**$13,445**	**$17,916**	**$66,723**

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

Institution/Item		Year 1	Year 2
Southern Illinois University-Carbondale			
Salaries and Benefits: SY @ 0.10 FTE		$6,160	$0
Waiver of Overhead		$8,800	$0
	Total	$14,960	$0
Purdue University			
Salaries and Benefits: SY @ 0.05 FTE		$5,130	$6,000
Supplies, Expenses and Equipment		$23,500	$23,500
	Total	$28,630	$29,500
Michigan State University			
Salaries and Benefits: SY @ 0.05 FTE		$5,275	$5,475
Supplies, Expenses, Equipment, and Waiver of Overhead		$15,250	$15,250
	Total	$20,525	$20,725
University of Nebraska-Lincoln			
Salaries and Benefits: SY @ 0.05 FTE		$3,240	$3,370
Supplies, Expenses, Equipment, and Waiver of Overhead		$4,636	$4,927
	Total	$7,876	$8,297
Nebraska Game and Parks Commission			
TY @ 0.05 FTE, Fish, Facilities, Fish Feeds, Chemicals, Analytical and Wet Laboratory Supplies, and Waiver of Overhead		$32,500	$34,200
University of Wisconsin-Madison			
Salaries and Benefits: SY @ 0.12 FTE		$4,095	$4,095
Supplies, Expenses, and Equipment		$16,045	$16,045
	Total	$20,140	$20,140
	Total per Year	$124,631	$112,862
	GRAND TOTAL	**$237,493**	

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

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1. Completed in Year 1.

Objective 2. Initiated in Year 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

Donald L. Garling, Michigan State University

Terrence B. Kayes, University of Nebraska-Lincoln

Jeffrey A. Malison, University of Wisconsin-Madison

Robert J. Sheehan, Southern Illinois University-Carbondale

VITA

Terence P. Barry
Assistant Researcher
University of Wisconsin Aquaculture Program
103 Babcock Hall, 1605 Linden Drive
University of Wisconsin-Madison
Madison, WI 53706

Phone: (608)263-1242
FAX: (608) 262-6872

EDUCATION

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

POSITIONS

Assistant Researcher, University of Wisconsin Aquaculture Program, University of Wisconsin-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
Asian Fisheries Society
World Aquaculture Society

SELECTED PUBLICATIONS

Barry, T.P., T.B. Kayes, J.A. Malison, and C.H. Amundson. 1991. Validation of a highly sensitive microplate enzyme-linked immunoassay (ELISA) for measuring serum cortisol in fish, and a comparison of primary physiological stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Journal of the World Aquaculture Society 22:14A.

Barry, T.P., K. Aida, T. Okumura, and I. Hanyu. 1990. The shift from C-19 to C-21 steroid synthesis in spawning male common carp, *Cyprinus carpio*, is regulated by the inhibition of androgen production by progestogens produced by spermatozoa. Biology of Reproduction 43:105-112.

Barry, T.P., A.J.G. Santos, K. Furukawa, K. Aida, and I. Hanyu. 1990. Steroid profiles during spawning in male common carp. General and Comparative Endocrinology 80:223-231.

Barry, T.P., and G.V. Callard. 1990. Identification and stage-related synthesis of 11-deoxycorticosterone (DOC) by the dogfish (*Squalus acanthias*) testis. Bulletin of the Mount Desert Island Biological Laboratory 29:131-132.

Barry, T.P., K. Aida, and I. Hanyu. 1989. The effects of 17α,20β-dihydroxy-4-pregnen-3-one on the in vitro biosynthesis of 11-ketotestosterone by testicular fragments of the common carp, *Cyprinus carpio*. Journal of Experimental Zoology 251:117-120.

Barry, T.P., and E.G. Grau. 1986. Estradiol-17β and thyrotropin-releasing hormone stimulate prolactin release from the pituitary gland of a teleost fish in vitro. General and Comparative Endocrinology 62:306-314.

VITA

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

Phone: (317) 494-4968
FAX: (317) 494-0409

EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Membership Concerns Committee (National) 1985-present; Walleye Technical Committee; Fish Culture Section 1985-present; Walleye Technical Committee (North Central Division) 1988-present, Fish Culture Section, Indiana Chapter
World Aquaculture Society, United States Chapter
International Association of Astacology
American Institute of Fishery Research Biologists
American Association for the Advancement of Science
Sigma Xi, Gamma Sigma Delta

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. 1989. Comparison of 26 and 30 percent protein feeds for channel catfish. Progressive Fish-Culturist 51:149-151.

Brown, P. B., D. A. Davis, and E. H. Robinson. 1988. An estimate of the dietary lysine requirement of juvenile red drum. Journal of the World Aquaculture Society 19:109-112.

Robinson, E. H., D. LaBomascus, P. B. Brown, and T. L. Linton. 1987. Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. Aquaculture 64:267-276.

Robinson, E. H., S. D. Rawles, P. B. Brown, H. E. Yette, and L. W. Green. 1986. Dietary calcium requirement of channel catfish *Ictalurus punctatus*, reared in calcium-free water. Aquaculture 53:263-270.

Brown, P. B., R. J. Strange, and K. R. Robbins. 1985. Protein digestibility coefficients for yearling channel catfish fed various high-protein feedstuffs. Progressive Fish-Culturist 47:94-97.

VITA

Donald L. Garling
Professor and Fish Culture and
Fisheries Extension Specialist
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI 48824

Phone (517) 353-1989
FAX: (517) 336-1699

EDUCATION

B.S. University of Dayton, 1970
M.S. Eastern Kentucky University, 1972
Ph.D. Mississippi State University, 1976

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture and Fisheries Educators Sections
Beta Beta Beta
Sigma Xi
Gamma Sigma Delta

SELECTED PUBLICATIONS

Garling, D. L. 1991. NCRAC research programs to enhance the potential of yellow perch aquaculture in the region. Pages 253-255 *in* Proceedings of the North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. Michigan Department of Natural Resources, Wolf Lake Fish Hatchery, Mattawan, Michigan.

Westmaas, A., W. Young, and D. Garling. 1991. Induction of polyploids in bluegill and chinook salmon. Pages 110-112 *in* Proceedings of the North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. Michigan Department of Natural Resources, Wolf Lake Fish Hatchery, Mattawan, Michigan.

Machado, J. P., T. G. Bell, D. L. Garling, Jr., N. R. Kevern, and A. L. Trapp. 1989. Effect of carbon monoxide and exposure on gas-bubble trauma in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 46:74-80.

Westerhoff, R., D. L. Garling, and H. A. Tanner. 1988. Development of techniques to produce triploid chinook salmon for stocking the Great Lakes. Journal of the World Aquaculture Society 19:73A.

Garling, D. L., and L. A. Helfrich. 1984. Making plans for commercial fish culture in Michigan. Michigan Cooperative Extension Service Bulletin No. 3-1775. Michigan State University, East Lansing.

VITA

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

Phone: (402) 472-8183
FAX: (402) 472-2964

EDUCATION

B.A. Chico State College, 1968
M.A. California State University at Chico, 1972
Ph.D. University of Wisconsin-Madison, 1978

POSITIONS

Associate Professor, Dept. of Forestry, Fisheries and Wildlife, University of Nebraska-Lincoln (1990-present)
Assistant Director and Associate Scientist, University of Wisconsin Aquaculture Program, University of Wisconsin-Madison (1979-1990)
Project Biologist, Aquaculture Research Laboratory, University of Wisconsin-Madison (1974-1979)
EPA Trainee, Laboratory of Limnology, University of Wisconsin-Madison (1970-1972)
Instructor, Department of Biological Sciences, Chico State College (1968-1970)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Society of Zoologists: Divisions of Comparative Endocrinology, Comparative Physiology and Biochemistry, Ecology, and Comparative Immunology
American Fisheries Society: Fish Culture, Bioengineering, Fish Health, Water Quality, and Early Life History Sections
World Aquaculture Society

SELECTED PUBLICATIONS

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

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

Malison, J.A., T.B. Kayes, B.C. Wentworth, and C.H. Amundson. 1988. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Canadian Journal of Fisheries and Aquatic Sciences 45:1942-1948.

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

VITA

Jeffrey A. Malison
Assistant Director
University of Wisconsin Aquaculture Program
103 Babcock Hall, 1605 Linden Drive
University of Wisconsin-Madison
Madison, WI 53706

Phone: (608) 263-1242
FAX: (608) 262-6872

EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Fisheries Society
American Society of Zoologists
World Aquaculture Society

SELECTED PUBLICATIONS

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

Malison, J.A., and J.A. Held. In Press. Effects of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (*Perca flavescens*) to intensive culture conditions. Aquaculture.

Barry, T.P., T.B. Kayes, J.A. Malison, and C.H. Amundson. 1991. Validation of a highly sensitive microplate enzyme-linked immunoassay (ELISA) for measuring serum cortisol in fish, and a comparison of primary physiological stress responses in rainbow trout (*Onchorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). Journal of the World Aquaculture Society 22:14A.

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

Malison, J.A., T.B. Kayes, B.C. Wentworth, and C.H. Amundson. 1988. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Canadian Journal of Fisheries and Aquatic Sciences 45:1942-1948.

VITA

Robert J. Sheehan
Assistant Director, Cooperative Fisheries Research Lab and
and Associate Professor, Department of Zoology
Southern Illinois University-Carbondale
Carbondale, IL 62901

Phone: (618) 453-6089
FAX: (618) 536-7761

EDUCATION

B.S. Northeastern Illinois University, 1973
M.A. Southern Illinois University-Carbondale, 1976
Ph.D. Southern Illinois University-Carbondale, 1984

POSITIONS

Associate Professor, Department of Zoology, Southern Illinois University-Carbondale (1992-present)
Assistant Professor, Department of Zoology, Southern Illinois University-Carbondale (1986-1992)
Assistant Professor, Department Fisheries and Wildlife Science, Virginia Polytechnic Institute & State University (1984-1986)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Early Life History, Exotic Fishes, Fish Culture, Fisheries Educators, and Water Quality Sections

SELECTED PUBLICATIONS

Bodensteiner, L.R., R.J. Sheehan, W.M. Lewis, and P.S. Wills. In Press. Effects of long-term repetitive formalin treatments during winter on channel catfish fingerlings. Journal of Aquatic Animal Health.

Krum, H.N., and R.J. Sheehan. In Press. Development of a magnetic activity-detection system. Animal Behaviour.

Sheehan, R.J., L.R. Bodensteiner, W.M. Lewis, P.S. Wills, and A.M. Brandenburg. In Press. Flowing water: an effective treatment for ichthyophthiriasis. Transactions of the American Fisheries Society.

Sheehan, R.J., P.S. Wills, and W.T. Davin. 1991. Crayfish production: a promising enterprise for the Midwest. Pages 219-225 *in* Proceedings of the North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. Michigan Department of Natural Resources, Wolf Lake Fish Hatchery, Mattawan, Michigan.

Sheehan, R.J., L.R. Bodensteiner, W.M. Lewis, D.E. Logsdon, and S.D. Scherck. 1990. Long-term survival and swimming performance of young of the year fishes at low temperatures: Links between physiological capacity and winter habitat requirements. Pages 98-108 *in* R. Sauer, editor. Proceedings of the Restoration of Midwestern Stream Habitat Symposium, Rivers and Streams Technical Committee, North-Central Division, American Fisheries Society, Minneapolis, Minnesota, December 4-5, 1990.

Sheehan, R.J., R.J. Neves, and H.E. Kitchel. 1989. Fate of freshwater mussels transplanted to formerly polluted reaches of the Clinch and North Fork Holston Rivers, Virginia. Journal of Freshwater Ecology 5:139-149.