ATTACHMENT E

PROJECT NAME: Culture Technology of Salmonids

FUNDING LEVEL: Year 1 - $54,563
Year 2 - $55,236

DURATION: 2 Years

ADMINISTRATIVE ADVISOR: Dr. Charles G. Scalet, Head, Wildlife and Fisheries Sciences, 202 Wildlife/Fisheries Science Building, South Dakota State University, Brookings, SD 57007-1696

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JUSTIFICATION

Salmonids (salmon and trout) are among the principal fishes currently being cultured in the North Central Region, the rainbow trout being the main species produced commercially (NCA-23 1987). Advancement of the salmonid aquaculture industry by applied studies of 1) triploid and monosex production and 2) less polluting diets was identified as a priority for research at the May 1989 joint meeting of the North Central Regional Aquaculture Center Industrial Advisory Council, Technical Committee, Extension Committee, and Board of Directors. Both rainbow trout and Atlantic salmon were identified as species of primary importance.

This industry, which is based on raceway and pond culture, is comparatively small and diffuse (e.g., compared to the Idaho trout industry), but it adds significantly to agricultural diversity in the region and to the total national production of trout (NCA-23 1987; WASC 1988). Marketing opportunities include the sale of fish for food (particularly to restaurants and for fresh fish and specialty products) and private stocking, plus sales through fee fishing operations and contractual production of fish for recreational fishing clubs, municipalities and government agencies (WASC 1988).

The two important areas of study outlined above need to be integrated in this regional project for a number of reasons. Proposed high density aquaculture will permit expansion and maximization of salmonid production using available water and facilities in the North Central Region. Strong evidence shows that sterile triploid fish, particularly females, have improved performance characteristics (Bye and Lincoln 1986; Purdom 1986a,b; Benfey et al. 1989) and, because of reduced agonistic behavior, may work especially well in high density aquaculture (see below). Clearly, less polluting diets will be required in order to meet existing water quality standards, especially as production and rearing densities increase.

Development of Triploid and Monosex Stocks

The production of triploid, triploid hybrid, and monosex stocks all have vast potential benefits to salmonid aquaculture (reviewed in Thorgaard 1986). Sterile triploids and triploid hybrids, especially females, do not go through sexual maturation and increase the profitability of large-fish production (Purdom 1986a; Donaldson 1986). Additionally, indirect evidence now indicates that triploids may have reduced competitive behavior (Cassani and Caton 1986a; Lincoln and Bye 1987), possibly providing them with an advantage in high density culture.

Clearly the development of chromosome set manipulation techniques for monosex culture and the production of sterile, triploid hybrid salmon may both prove to have important implications for culture in the North Central Region (see below). But, because of the funding limitations placed on this document, we are addressing related triploid hybrid proposals to other agencies (Washington Sea Grant, funded with G. Thorgaard; USDA CSRS, pending review; Southern Illinois University Office of Research and Development, funded). These projects will be closely coordinated with this study to maximize the benefit of the small amount of USDA funds available to individual investigators on this project. Our focus will be on the development of all-female diploid and triploid rainbow trout and Atlantic salmon for the immediate production opportunities in the North Central Region.

Application of some of these relatively new chromosome set manipulations to produce hybrid, sterile, or monosex salmonids by commercial fish farmers, especially in Europe, has already been shown to increase profitability (e.g., Bye and Lincoln 1986, Purdom 1986a; reviewed in Thorgaard 1986). Government sponsored research and extension has resulted with farmers converting approximately 80% of rainbow trout production in the United Kingdom to all-females or triploid all-females (Purdom 1986b); similar work in British Columbia resulted in the recent

conversion of more than 50% of the commercial production of chinook salmon to genetically manipulated all-females (Solar, Baker, and Donaldson, Department of Fisheries and Oceans, West Vancouver, B.C., presented at the annual meeting of the World Aquaculture Society, Los Angeles, California, 1989).

Unquestionably, similar applications will prove to increase profitability for U.S. producers. Clear Springs Trout Company, Buhl, Idaho, probably the largest producer of salmonids in this country with an estimated annual production of 20,000 t, reportedly documented better than a 10% increase in food conversion efficiency in a replicated study of diploid all-female rainbow trout (James Parsons, Clear Springs Trout Company, personal communication,). This was generally attributed to reduced agonistic behavior in the all-female test lots, and they are now phasing in monosex culture of pan-sized fish.

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

However, several major constraints currently prevent the industry-wide implementation of these techniques. The first relates to poor extension of these techniques in the U.S. resulting in a lack of understanding of genomic manipulations, primarily by small farmers. Also, although egg producers commonly advertise all-female eggs for sale in trade journals, a long-term survey by us (and others) shows that they are seldom, if ever, available. Basically the expertise to perform these manipulations has remained in the universities and has not been properly extended to the commercial sector. As a result, the potential increased profits remain limited to the few giant corporations that employ scientists to develop these techniques in house.

Secondly, although the virtues of manipulated fish have been extolled by many authors (Donaldson 1986; Parsons et al. 1986; Purdom 1986a,b; Thorgaard 1986; Scheerer et al. 1987; Seeb et al. 1988; countless others) little detailed public information is available on the actual benefit (e.g., reduced cost of feed) of implementing this technology. Substantial information is available on the sterility of triploid females and the advantage that this confers on large-fish culture (e.g., Lincoln and Scott 1983; Benfey and Sutterlin 1984a; Bye and Lincoln 1986; Benfey et al. 1989; etc.), but additional study is needed to document feed conversion efficiencies in all-female diploids and triploids. Many farmers need very clear information on profit potential before they are willing to commit to such an enterprise.

Finally, a majority of the research and progress has been done with rainbow trout. Genomic manipulations with other salmonids, especially Atlantic salmon, needs additional study to clarify both potential benefits to producers and production protocols.

At the direction of the North Central Regional Aquaculture Center each proposal requires an extension component. Our goal is to develop and extend monosex culture of rainbow trout and Atlantic salmon to farmers in the Midwest.

Develop Less Polluting Diets

The atmosphere among regulatory agencies in the region is not encouraging toward aquaculture development. Commercial and public culture operations are facing closure and production limitations because of effluents released. Thus, addressing this topic is imperative for further development of salmonid aquaculture in the North Central Region. Three primary areas have to be considered in research of this nature--phosphorus, nitrogen and solids. Because of the limited scope of the program during the current fiscal year, and because phosphorus in effluents is the immediate problem, this objective will address dietary phosphorus dynamics only.

Reduction of phosphorus 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 phosphorus is generally considered to be the limiting nutrient in freshwater environments, phosphorus addition typically results in eutrophication (Wetzel, 1975). Therefore, regulatory agencies are imposing stricter requirements on aquaculturists and public facilities and threatening closure of facilities. Further, this has hindered the development and expansion of salmonid aquaculture in the region (eg., Minnesota iron pit facility, cage culture in the Great Lakes).

There are two sources of phosphorus 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 that was not

absorbed in the gastrointestinal tract as well as P turnover from the fish. Thus, if diets are formulated on an available P basis (i.e., maximizing P absorption), then P in feces will be reduced and effluent P 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, this information is not available for salmonids.

We cannot promote increased production stocking densities in the region with the current regulatory atmosphere and the fact that existing facilities are facing closure because of P 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 1 cannot be implemented.

RELATED CURRENT AND PREVIOUS WORK

Development of Triploid and Monosex Stocks

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 1983). 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).

Chromosome set manipulation recently played a central role in many salmonid genetics studies. Several experiments have examined inbreeding in gynogens (Thompson 1983; Taniguchi et al. 1986; Tabata and Gorie 1988), sterility in triploids (Lincoln and Scott 1983; Benfey and Sutterlin 1984b; Benfey et al. 1989), and enhanced viability of triploid hybrids (Scheerer and Thorgaard, 1983; Scheerer et al. 1987; Seeb et al. 1988).

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 (e.g., 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, 1986b). 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, 1986a, see below). All-female triploids do not produce secondary sexual characteristics and have improved meat production over diploids at sexual maturity (Benfey and Sutterlin, 1984a).

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; Benfey et al. 1989). Interestingly, when triploids are grown in common tanks with diploids, the triploids can show a reduced growth rate (Cassani and Caton 1986a; Lincoln and Bye 1987), strongly indicating that they have a 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 increased food conversion efficiency.

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 F1 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 (Piferrer et al., in press). 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 brood stock.

Develop Less Polluting Diets

Phosphorus (P) is an essential nutrient for all animals studied, and therefore has to be supplied in the diet. This element is classified as a macromineral because it is typically required in relatively high amounts. Deficiency symptoms include depressed weight gain, feed conversion and poor bone mineralization. Thus, the practical importance of adequate P is apparent.

Fish can absorb many elements directly from the water and meet all or part of their requirement. However, P absorption by trout directly from the water is minimal and this element must be provided in the diet (Phillips et al., 1958, 1960; cited in National Academy of Sciences, 1981).

Phosphorus is typically found in two forms--organic phytin P (or inositol hexaphosphoric acid), and various inorganic forms. Most vertebrates lack phytase, the enzyme that can liberate P from phytin, so this form is poorly utilized, and the poor utilization has been demonstrated by Ketola (1975a,b) for Atlantic salmon and rainbow trout. However, there is no quantitative evidence indicating the exact amounts of P available from typical feedstuffs used in salmonid diets. Inorganic forms provide more available P. Using inorganic forms of P, the dietary requirement has been estimated to be 0.7-0.8% of the diet for rainbow trout (Ogino and Takeda, 1978). Similarly, there is no quantitative evidence regarding the percentage of P available from inorganic forms. Most studies assume that all of the P from inorganic supplements is available, when this is not the case for other animals, nor for channel catfish (Lovell, 1978).

There is increasing interest in lowering the fish meal concentration in salmonid diets because this component is one of the most expensive ingredients in the U.S. and is a feedstuff subject to dramatic changes in price in any given year. The logical choices for substitution in salmonid diets are high-quality plant protein sources, such as soybean meal, peanut meal, cottonseed meal, and canola meal, or various animal by-product meals such as poultry by-product meal and meat and bone meals. All of these potential ingredients are less expensive than fish meal. It is difficult to formulate precise diets using animal by-product meals because of the variability in nutritional composition between lots. Therefore, the logical choices are high-quality plant feedstuffs. Some of this work has been initiated (Ketola, 1975a,b; funded project through the Western Regional Aquaculture Center; Westers, personal communication; Ketola et al., 1988) and more is forthcoming. One of the most important overall changes when substituting plant feedstuffs for animal feedstuffs is the form of P. Plant feedstuffs have a relatively high percentage of phytin P and relatively low concentration of inorganic P. Thus, knowledge of the P availabilities from typical salmonid feedstuffs as well as those currently under evaluation will be vitally important information for feed manufacturers and should help keep feed cost increases to a minimum. It is somewhat surprising that this information is not available for salmonids. Catfish feed manufacturers have P availability information values for typical feedstuffs (Lovell, 1978; Wilson et al., 1982) and this information is used to formulate those diets.

Dr. Don Garling, Michigan State University (MSU) has conducted preliminary studies pretreating high-quality plant protein sources with phytase as a means of increasing P availability for trout. Work completed to date indicates that pretreatment with phytase can reduce the need for P in the mineral premix. Rainbow trout were fed semipurified diets containing phytase pretreated soy protein and graded levels of inorganic P at 0, 25, 50, 75, or 100% of the recommended P supplement (National Academy of Sciences 1978, 1981) or were fed diets without phytase treated soy protein and 0 or 100% of the inorganic P was fed to four groups of rainbow trout fingerlings reared in 110-L flow-through aquaria for ten weeks. Fish were fed at a rate of 3.5% of their wet body weight per day divided into two equal feedings. Fish fed the diet without inorganic P supplementation and without phytase treated soy protein grew slowly and had high mortality rates. Growth was not significantly different between fish fed diets containing soy protein and 100% P supplementation or phytase treated soy protein diets. Reduction in the amount of P in trout feeds coupled with increased P availability should help reduce P in trout farm effluents.

OBJECTIVES

The overall goal of this regional research project is to optimize culture technologies of salmonids for the business constraints existing in the North Central Region. The specific objectives to achieve this goal are:

1. To produce all-female stocks of rainbow trout and Atlantic salmon for diploid and sterile triploid production.

2. To develop diets that are less polluting.

PROCEDURES

While specific laboratories will conduct experiments under one of the project objectives, frequent informal communication about interim progress will be strongly encouraged by the chair of the Salmonid Workgroup (currently, Dr. James E. Seeb). This will ensure that both the execution and any modifications of experiments proceed in a coordinated manner. Such frequent communication, in addition to annual Workgroup meetings, will be essential to the success of the project because the procedural details for many experiments under one objective are dependent on the progress of experiments under another objective. Researchers participating in this project will also be encouraged to coordinate publishing of results in scientific journals and extension publications in a timely manner.

Objective 1

The University of Minnesota (U MINN) will focus on the production characters of mono-sex diploid and triploid juvenile rainbow trout and the production of triploid Atlantic salmon. Southern Illinois University (SIU) will conduct experiments measuring feed conversion efficiency in mono-sex diploid and triploid rainbow trout and initiate development of mono-sex Atlantic salmon.

The two institutions will jointly coordinate food conversion and growth trials at commercial facilities volunteering space for longer-term, replicated studies. For example, Seven Pines Trout Hatchery, Lewis, Wisconsin, has offered to conduct trials, assist in the production of mono-sex sperm, and permit the unrestricted dissemination of resulting data and seed. Other growers will be incorporated as possible. This design will facilitate 1) the collection of long-term performance data that is impossible to obtain in university wet-labs, 2) the development of a Midwest mono-sex broodstock, and 3) the rapid extension of genomic manipulation technology to the commercial industry.

The general design in year one will be to initiate short-term performance studies at the universities and long-term production trials at Seven Pines (we will need to import mono-sex sperm, readily available from the United Kingdom, or possibly Canada) in order to initiate these studies in year one). This will expedite the production of fact sheets detailing the advantages of mono-sex (both diploid and triploid) production for rainbow trout growers. Additionally, we will initiate gynogenesis and sex-reversal experiments at SIU and Seven Pines in order to develop a Midwest source of mono-sex rainbow trout sperm.

Similarly, gynogenesis and sex-reversal experiments will be initiated to develop a source of mono-sex Atlantic salmon sperm which should be available for experiments by 1992. However, in year 2, we will schedule the production of mixed-sex triploid Atlantic salmon for MAD studies and the study of food conversion. Gametes from Atlantic salmon will be obtained from Mr. Bruce Cody, Minnesota Aquaculture Farms.

<u>Experimental matings</u>

Three replicated crosses will be made to produce both mono-sex and control (mixed-sex) lots for the short-term experiments. For triploidy experiments, a portion of each mating will be heat-shocked in a 29°C water bath for 10 minutes to induce retention of the second polar body (Chourrout 1980; Scheerer and Thorgaard 1983), a protocol we found effective in both rainbow trout (Allendorf et al. 1986) and chum salmon (Seeb and Seeb 1986). This will also produce sufficient triploid Atlantic salmon for the experiments, but it may not be the optimal treatment for this species. If possible, now, or at a later date, experiments will be conducted to determine the optimal treatment protocols.

Eggs will be incubated in divided Heath trays (FAL/Heath Tray, Tacoma, WA). Prophylactic treatments of formalin will be administered to control fungus, and dead eggs will be removed and counted periodically after eye pigment is first observed. The yolk-sac fry from each lot will be transferred to separate tanks prior to the free-swimming stage and rate of survival and food conversion will be measured until 90 days after initiation of feeding at U MINN and until 270 days after the initiation of feeding at SIU.

Fish will be fed a standard salmon diet at a rate of 3% body weight per day. Growth measurements for replicate treatment means will be collected every two weeks after the initiation of feeding. When the SIU fish reach 5g they will be large enough to be tagged with an identifiable passively induced transponder (PIT tags). This will permit growth rate calculations for individual fish within each treatment. At this time, when all fish are identifiable, three replicates of pooled mixed sex, mono-sex diploids, and mono-sex triploids will be established. This will provide food conversion data on treatments reared in common.

Experiments at commercial facilities will be designed to fit their production scheme and yet maximize the quantity and quality of performance data for the fact sheets.

Gynogenesis, electrophoresis and flow cytometry

Gynogenesis will be performed following the techniques of Chourrout (1980). Heterologous sperm will be used in order to permit positive confirmation of successful gynogenesis (Allendorf et al. 1986). Horizontal starch-gel electrophoresis of proteins will be employed to test for any paternal alleles in the gynogenetic lots, the presence of which would indicate treatment failure.

Finally, flow cytometry (Allen, 1983) will be used to measure nuclear DNA content to confirm the ploidy of 20 randomly chosen individuals from each of the putative triploid groups.

Objective 2

The goal of this objective is to evaluate means of lowering P in effluents through reduction of fecal P concentrations. We propose a dual approach to this current problem. Dr. Don Garling (MSU) will continue to expand his evaluations of phytase pretreatment as a novel means of liberating P from phytin before plant feedstuffs are incorporated in diets. Additionally, Dr. Paul Brown (Purdue University) will quantify available P from typical feedstuffs used in salmonid diets, fed as the sole protein source and in compounded (multi-ingredient) diets. This information will allow more precise formulation of salmonid diets and reduce excreted P.

Phytase pretreatment studies will be conducted at Michigan State University (MSU) under the direction of Dr. Don Garling. Researchers at MSU will select a practical feed formulation high in plant protein sources (based on the current work of the Western Regional Aquaculture Center on alternative protein sources) or use the all plant diet recently developed by Dr. George Ketola, Tunison Laboratory of Fish Nutrition.

During year one, feedstuffs will be pretreated with phytase and incorporated into otherwise nutritionally complete practical diets. Inorganic P will be added to this diet at levels of 0, 50 or 100% of the recommended level (National Academy of Sciences, 1981).

Each diet will be fed to quadruplicate groups of juvenile rainbow trout (Shasta strain) for up to twenty weeks using standard fish rearing procedures. Experimental culture systems used at MSU will be 190-L tanks equipped with flow-through water supplied at a rate of 0.5 L/min. Temperature will be maintained at 12^{o}C.

Solids (uneaten feed and feces) will be removed daily. Once each week, solids will be collected three times during the course of the day using external standpipes modified for solids collection.

Phosphorus concentrations will be determined in initial samples of fish and from samples of fish at the termination of the study. Additionally, dietary P, and P in collected solids will be quantified by the method of Fiske and Subbarow (1925).

Weight gain, feed conversion, P in collected solids, P retention, total effluent P, and survival will be analyzed as a one-way analysis of variance using the appropriate statistical model.

During year 2, researchers at MSU will conduct similar studies proposed for year 1, but will grow triplicate groups of Shasta strain rainbow trout to marketable size in 950-L tanks. This tank system is equipped with flow-through water supply at 5 L/min. Solids removal and P quantification will follow similar procedures outlined for the first year studies, and the same data will be collected and analyzed.

Phosphorus availability studies will be conducted at Purdue University under the direction of Dr. Paul Brown. We will use the Shasta strain of rainbow trout as a model species and all studies will be conducted with 100-200 g fish.

While it might be beneficial to work with smaller fish, it is imperative that we conduct these studies with larger fish in order to collect adequate fecal samples for analyses.

Nutrient availability studies involve chemical analyses of feed and fecal samples (Note: nutrient digestibility and availability are synonomous terms, digestibility is typically used with macronutrients such as protein, lipid, etc., while availability is typically used with micronutrients such as essential amino acids, minerals, vitamins, etc.). Using inert indicators of availability (most often chromic oxide), the ratios of indicator to nutrient in feed vs. fecal samples allows calculation of the percentage of nutrient that "disappeared" (presumably absorbed) as the diet traversed the gastrointestinal tract. This is termed the apparent availability. However, the gastrointestinal tract is the primary excretory route for some nutrients of cellular origin, so the nutrient concentration in feces is comprised of unabsorbed nutrient from the diet and endogenously-derived secretions into the gastrointestinal tract. We can account for the endogenously-derived material by feeding a nutrient-free diet and the availability estimate is then termed true availability. With P, it is important to account for this endogenous secretion because the primary buffer in the small intestine is phosphate-based. Therefore, P secretions can be large and apparent availability estimates are not as precise as true availability estimates. To date, there are no true P availability estimates for any fish species. We propose attempting true availability estimates. Virtually all feedstuffs contain P, so formulating an otherwise nutritionally complete diet completely devoid of P appears to be unrealistic. However, we can formulate a very low P diet using a small amount of casein and gelatin (10-12% of the diet), plus crystalline L-amino acids such that the resulting combination meets the requirements of salmonids (National Academy of Sciences, 1981). Other components in the diet (carbohydrate, lipid and cellulose) will be P-free, and we will add a P-free mineral premix and nutritionally complete vitamin premix. The low P diet will be randomly evaluated with the other experimental diets and P excretion will be quantified.

Clearly, there are unique problems when conducting availability studies with fish. One of the most studied aspects has been a reliable method of collecting feces and avoiding nutrient leaching (Smith, 1971; Smith and Lovell, 1972; Smith and Lovell, 1973; Austreng, 1978; Windell et al.,1978; Choubert et al., 1979; Smith et al., 1980). Several fecal collection methods have been used, but one that has been used most with salmonids has been the fecal collection system designed by Cho and others, which is a fecal settling method. This system has been verified as appropriate and reliable for determining protein, lipid and energy digestibilities for rainbow trout (Cho et al., 1982) and amino acid availabilities for catfish (Wilson et al., 1981). As the first portion of this study, we propose evaluation of anal cannulation (Windell et al., 1978) compared to fecal settling as methods for use in determining P availability. If the fecal settling method provides reliable estimates of P availability, this method will be used because it is a better method for quantifying nutrient availability for large numbers of feedstuffs and is a much less stressful method for the fish.

Typical availability studies will be used. These include a preliminary period of six days in which consistent consumption of the experimental diets is attained, followed by a four day fecal collection period. All evaluations will be conducted sequentially and all feedstuffs will be replicated at least three times in a randomly selected manner.

All experimental diets will be formulated, mixed, and pelleted in our laboratory and stored frozen prior to their use. All diets will contain a nutritionally complete vitamin premix and a P-free, but otherwise nutritionally complete, mineral premix (National Academy of Sciences, 1981), marine fish lipid sources, and chromic oxide as the indicator. Feedstuffs evaluated will include menhaden meal, sardine meal, herring meal, soybean meal (mechanically extracted), full-fat soybean meal, peanut meal, canola meal, poultry by-product meal, blood meal, cottonseed meal, and phytase pretreated plant protein sources supplied from MSU. All feedstuffs will be evaluated as the sole P source in the diet, a typical means of evaluating nutrient availabilities. Level of incorporation in the diet will vary depending on protein content of the feedstuff. Spinelli et al. (1983) identified an interaction of protein and phytin, thus holding protein level constant at 35% of the diet seems appropriate. Feedstuffs will be substituted at the expense of cellulose. In addition to this typical method of determining nutrient availabilities (as the single source in the diet), we propose quantifying P availability from compounded, or multi-ingredient, diets. Nutrient availabilities from compounded diets are not necessarily the sum of availabilities from the various feedstuffs. Dietary associative effects (changes in digestibility on addition of feedstuffs) is a recognized phenomenon (Schneider and Flatt, 1975), yet has received little attention in fish nutrition studies. Recently, Brown et al. (in press) demonstrated that dietary associative effects significantly alter consumption and apparent dry matter digestibility values for red swamp crayfish. Specifically, we will quantify P availability from several open formula salmonid diets that we will mix and pellet in our laboratory.

Phosphorus and chromium concentrations of diets and resulting feces will be determined by the methods of Fiske and Subbarow (1925) and Brown et al. (1985), respectively, following acid digestion. Availability estimates will be calculated by standard formula (Maynard et al., 1979).

Evaluation of single feedstuffs will take place during year 1, and evaluation of compounded diets will be done during year 2. All data will be analyzed as a one-way analysis of variance.

Summary

Data collected by SIU, U MINN, MSU, and Purdue investigators and MDNR on an ongoing basis, and the findings published in a timely manner in appropriate peer-reviewed national or international scientific journals. Extension information will be published through regional and station bulletins, in collaboration with the NCRAC Aquaculture Extension Work Group.

FACILITIES

Objective 1

This research will be conducted in part at the U MINN fisheries wet lab which includes: a 284m^2 fish culture lab supplied with filtered well water (7.6 liters/sec., 10^oC, 6 on-line iron filters) and outfitted with on-line air compressor, on-line chiller unit, hot water heat exchangers, UV-sterilization filter, back-up electrical generator, tool shop, air compressor, and dechlorination filters (for use of city water in emergencies). Heath-Techna tray incubators, circular fiberglass tanks and automatic feeders are available for culture of salmonids in this study. Water supply for these incubators and tanks is connected to a 24 hr. alarm system monitoring water level and temperature.

U MINN facilities for water chemistry analyses, and other analyses needed for incubating eggs and rearing juveniles of salmonid hybrids are located in four labs (203m^2) of the Dept. of Fisheries and Wildlife. Major equipment includes: hoods, refrigerators, one -80^oC freezer (0.49m^3), one walk-in freezer (5.3m^3), electronic and triple-beam balances, one spectrophotometer, pH and oxygen meters, Winkler D.O. apparatus, on-line water de-ionizer, drying oven, muffle furnace, table-top centrifuges, microscopes, and a Beckman MDL J2-21 centrifuge. Access to the mainframe computer and numerous microcomputers, printers, software, and accessories are available for data analysis and reporting.

Genetic analyses will be performed at the Genetics Lab in the Cooperative Fisheries Research Laboratory, Southern Illinois University, Carbondale. The laboratory is equipped to perform allozyme analyses, mitochondrial DNA analyses, induced polyploid experiments, and chromosomal studies. Major laboratory equipment includes a Beckman L7-65 ultracentrifuge with vertical rotor; Beckman high speed J-21 centrifuge and rotor; Beckman refrigerated table-top centrifuge; microfuge; 11 power supplies with constant current and constant voltage capabilities; two 19.6 ft^3-80^oC freezers; automatic gel cooling system with capacity for 10 gels at -2^oC; Gene-Vac chemical vacuum pump; gel dryer; computer interface to analytical balance, digitizer pad, and pit tag reader; and four IBM compatible personal computers with installed genetic analysis software. Short-term rearing experiments will be conducted in tanks at SIU's 10,000 ft^2 wet-lab facility.

Objective 2

Purdue University has a wet laboratory equipped with numerous tanks, air supply, and temperature controls necessary for conducting this research. The analytical laboratory features the necessary UV-VIS spectrophotometer and associated supplies for conducting phosphorus and chromium determinations. Feed mixing and pelleting equipment are also present.

Michigan State University has the necessary wet lab, tanks and water supply to conduct the proposed studies. Feeds will be formulated in cooperation with researchers in the western region or the Tunison Laboratory of Fish Nutrition. Feeds will be manufactured and phosphorus determinations conducted with the help of MSU Department of Animal Science.

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ATTACHMENT E

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Illinois	James E. Seeb Southern Illinois University	Fish Genetics
	Gary D. Miller Southern Illinois University	Salmonid Aquaculture
Indiana	Paul B. Brown Purdue University	Fish Nutrition
Michigan	Donald L. Garling, Jr. Michigan State University	Aquaculture/Extension
	James A. Copeland Michigan Dept. of Natural Resources	Fish Production/Technology
	Harry Westers Michigan Dept. of Natural Resources	Fish Production/Technology
Minnesota	Anne R. Kapuscinski University of Minnesota	Fish Genetics

ATTACHMENT E

INDIVIDUAL BUDGETS FOR PARTICIPATING INSTITUTIONS

Illinois

Southern Illinois University
James E. Seeb (Objective 1)

Minnesota

University of Minnesota
Anne R. Kapuscinski (Objective 1)

Indiana

Purdue University
Paul B. Brown (Objective 2)

Michigan

Michigan State University
Donald L. Garling (Objective 2)

ATTACHMENT E

PROPOSED SALMONID BUDGET FOR SOUTHERN ILLINOIS UNIVERSITY

(Seeb)

Objective 1

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)						
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc	1	0.50	1	0.50	$9,600	$10,080
b. Other Professionals						
c. Graduate Students						
d. Prebaccalaureate Students					$1018	$1,510
e. Secretarial-Clerical						
f. Technical, Shop, and Other ...						
Total Salaries and Wages					$10,618	$11,590
B. Fringe Benefits (11.1% of 2a + 267/mm)					$2,669	$2,772
C. **Total Salaries, Wages and Fringe Benefits**					$13,287	$14,312
D. Nonexpendable Equipment					$1,200	$0
E. Materials and Supplies					$1,500	$2,000
F. Travel - Domestic (*Including Canada*)					$2,500	$2,500
G. Other Direct Costs					$1,000	$1,000
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$19,487	$19,812
TOTAL PROJECT COSTS					$39,299	

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

ATTACHMENT E

PROPOSED SALMONID BUDGET FOR
UNIVERSITY OF MINNESOTA

(Kapuscinski)

Objective 1

	Year 1 No.	Year 1 FTEs	Year 2 No.	Year 2 FTEs	Year 1	Year 2
A. Salaries and Wages						
1. No. of Senior Personnel & FTEs[1]						
a. (Co)-PI(s)	1	0.02	1	0.02	$0	$0
b. Senior Associates						
2. No. of Other Personnel (Non-Faculty) & FTEs						
a. Research Assoc./Postdoc						
b. Other Professionals						
c. Graduate Students						
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other	1	0.19	1	0.19	$4,469	$4,573
Total Salaries and Wages					$4,469	$4,573
B. Fringe Benefits (25% of F)					$1,118	$1,143
C. **Total Salaries, Wages and Fringe Benefits**					$5,587	$5,716
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,065	$1,066
F. Travel - Domestic (*Including Canada*)					$3,424	$3,642
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$10,076	$10,424
TOTAL PROJECT COSTS					$20,500	

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

ATTACHMENT E

PROPOSED SALMONID 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	0.10	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	0.25	1	0.25	$4,500	$4,950
d. Prebaccalaureate Students	1	1.00	1	1.00	$1,920	$1,920
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$6,420	$6,870
B. Fringe Benefits					$205	$220
C. **Total Salaries, Wages and Fringe Benefits**					$6,625	$7,090
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$4,675	$4,210
F. Travel - Domestic (*Including Canada*)					$1,200	$1,200
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$12,500	$12,500
TOTAL PROJECT COSTS					$25,000	

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

ATTACHMENT E

PROPOSED SALMONID BUDGET FOR MICHIGAN STATE UNIVERSITY

(Garling)

Objective 2

	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)	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	$10,500	$11,000
d. Prebaccalaureate Students						
e. Secretarial-Clerical						
f. Technical, Shop, and Other						
Total Salaries and Wages					$10,500	$11,000
B. Fringe Benefits					$0	$0
C. **Total Salaries, Wages and Fringe Benefits**					$10,500	$11,000
D. Nonexpendable Equipment					$0	$0
E. Materials and Supplies					$1,500	$1,000
F. Travel - Domestic (*Including Canada*)					$500	$500
G. Other Direct Costs					$0	$0
TOTAL PROJECT COSTS PER YEAR (*C through G*)					$12,500	$12,500
TOTAL PROJECT COSTS					$25,000	

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

ATTACHMENT E

CULTURE TECHNOLOGY OF SALMONIDS

Budget Summary for Each Participating Institution at 54.6K for the First Year

	SIU	U MINN	PURDUE	MSU	TOTALS
Salaries and Wages	$10,618	$4,469	$6,420	$10,500	$32,007
Fringe Benefits	$2,669	$1,118	$205	$0	$3,992
Total Salaries, Wages and Benefits	**$13,287**	**$5,587**	**$6,625**	**$10,500**	**$35,999**
Nonexpendable Equipment	$1,200	$0	$0	$0	$1,200
Materials and Supplies	$1,500	$1,065	$4,675	$1,500	$8,740
Travel	$2,500	$3,424	$1,200	$500	$7,624
Other Direct Costs	$1,000	$0	$0	$0	$1,000
TOTAL PROJECT COSTS	**$19,487**	**$10,076**	**$12,500**	**$12,500**	**$54,563**

Budget Summary for Each Participating Institution at 55.2K for the Second Year

	SIU	U MINN	PURDUE	MSU	TOTALS
Salaries and Wages	$11,590	$4,573	$6,870	$11,000	$34,033
Fringe Benefits	$2,722	$1,143	$220	$0	$4,085
Total Salaries, Wages and Benefits	**$14,312**	**$5,716**	**$7,090**	**$11,000**	**$38,118**
Nonexpendable Equipment	$0	$0	$0	$0	$0
Materials and Supplies	$2,000	$1,066	$4,210	$1,000	$8,276
Travel	$2,500	$3,642	$1,200	$500	$7,842
Other Direct Costs	$1,000	$0	$0	$0	$1,000
TOTAL PROJECT COSTS	**$19,812**	**$10,424**	**$12,500**	**$12,500**	**$55,236**

ATTACHMENT E

RESOURCE COMMITMENT FROM INSTITUTIONS[1]

(Salaries, Supplies, Expenses and Equipment)

Institution/Item		Year 1	Year 2
Southern Illinois University			
Salaries and Benefits: SY @ 0.10 FTE		$4,531	$4,984
TY @ 0.50 FTE		$6,984	$0
Waiver of overhead @ 44%		$5,066	$2,193
	TOTAL PER YEAR	**$16,581**	**$7,177**
University of Minnesota			
Salaries and Benefits: SY @ 0.02 FTE		$1,192	$1,192
SY @ 0.05 FTE		$1,288	$1,288
Supplies, Expenses and Equipment:		$3,608	$3,608
	TOTAL PER YEAR	**$6,088**	**$6,088**
Purdue University			
Salaries and Benefits: SY @ 0.10 FTE		$5,625	$5,625
Supplies, Expenses and Equipment:		$19,000	$13,000
	TOTAL PER YEAR	**$24,625**	**$18,625**
Michigan State University			
Salaries and Benefits: SY @ 0.05 FTE		$2,812	$2,812
Supplies, Expenses and Equipment		$14,400	$14,800
	TOTAL PER YEAR	**$17,212**	$17,612
	GRAND TOTAL	**$97,187**	**$87,656**

[1]Since cost sharing is not a legal requirement and due to the difficulty in accounting for small items, documentation will not be maintained.

ATTACHMENT E

SCHEDULE OF COMPLETION OF OBJECTIVES

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

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

ATTACHMENT E

LIST OF PRINCIPLE INVESTIGATORS

Paul B. Brown, Purdue University

Donald L. Garling, Jr., Michigan State University

Anne R. Kapuscinski, University of Minnesota

James E. Seeb, Southern Illinois University

ATTACHMENT E

VITA

Paul B. Brown
Assistant Professor
Department of Forestry and Natural Resources
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EDUCATION

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

POSITIONS

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

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Membership Concerns Committee, Walleye Technical Committee, Culture Section
World Aquaculture Society
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., A. Emery, A.L. Lawrence, and E.H. Robinson. In press. Digestible energy values for red swamp crayfish and evaluation of dietary associative effects in practical feeds. Journal of the World Aquaculture Society.

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

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Hubbard, D.M., E.H. Robinson, P.B. Brown, and W.H. Daniels. 1986. Optimum ratio of dietary protein to energy for red crayfish (*Procambarus clarkii*). Progressive Fish-Culturist 48:233-237.

ATTACHMENT E

VITA

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Fish Culture and Fisheries Extension Specialist
Department of Fisheries and Wildlife
Michigan State University
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EDUCATION

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

POSITIONS

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

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 gaidneri*). 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. Presented at the Annual Meeting of the World Aquaculture Society, January 4-9, Honolulu.

Masterson, M.F., and D.L. Garling. 1986. Effect of feed color on feed acceptance and growth of walleye (*Stizostedion vitreum* v.) fingerlings. Progressive Fish-Culturist 48:306-309.

Ostrowski, A.O., and D.L. Garling. 1986. Dietary androgen-estrogen combinations in growth promotion in fingerling rainbow trout. Progressive Fish-Culturist 48:268-272.

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

ATTACHMENT E

VITA

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EDUCATION

B.A. Swarthmore College 1976
M.S. Oregon State University 1980
Ph.D. Oregon State University 1984

POSITIONS

Associate Professor/Extension Specialist (Aquaculture), University of Minnesota (1989-Present)
Assistant Professor/Extension Specialist (Aquaculture), University of Minnesota (1984-1989)
Instructor/Project Leader/Research Assistant, Oregon State University (1980-1984)
Research Assistant, Oregon State University (1977-1980)
Aquaculture Research Technician, Weyerhaeuser Company (1976-1977)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society: Fish Culture Section, Genetics Section, NCD Fish Genetics Technical Committee
Genetics Society of America
International Association of Genetics in Aquaculture (Charter Member)
Society for the Study of Evolution
World Aquaculture Society
Sigma Xi, Phi Kappa Phi, Phi Sigma, Gamma Sigma Delta

SELECTED PUBLICATIONS

Yoon, S.J., E.M. Hallerman, M.L. Gross, Z. Liu, J.F. Schneider, A.J. Faras., P.B. Hackett, A.R. Kapuscinski, and K.S. Guise. In press. Transfer of the gene for neomycin resistance into goldfish (*Carrassius auratus*). Aquaculture

Kapuscinski, A.R. In Press. Integration of Transgenic Fish into Aquaculture. Food Reviews International (Invited Paper)

Hallerman, E.M., J.F. Schneider, M.L. Gross, A.J. Faras, P.B. Hackett, K.S. Guise, and A.R. Kapuscinski. 1989. Enzymatic dechorionation of goldfish, walleye and northern pike eggs. Transactions of the American Fisheries Society 117:456-460.

Phillips, R.B., and A.R. Kapuscinski. 1988. High frequency of translocation heterozygotes in odd year populations of pink salmon (*Oncorhynchus gorbuscha*). Cytogenetics and Cell Genetics 48:178-182.

ATTACHMENT E

VITA

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EDUCATION

B.S. University of Puget Sound 1974
M.S. University of Washington 1982
Ph.D. University of Washington 1987

POSITIONS

Assistant Professor, Southern Illinois University-Carbondale (1988-present)
Research Assistant Professor, University of Idaho, Moscow (1987-1988)
Graduate Assistant, University of Washington, Seattle (1982-1986)
Fish Biologist, Washington Department of Fisheries, Olympia (1978-1980)
Fish Biologist, Pacific Fisheries Research, Seattle (1976-1978, 1980-1982)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
American Genetics Association
American Society of Ichthyologists and Herpetologists
Genetics Society of America
International Association for Genetics in Aquaculture
Sigma Xi

SELECTED PUBLICATIONS

Seeb, J.E., and G.D. Miller. In press. The integration of alloenzyme analyses and genomic manipulations for fish culture and management. *In* D.H. Whitmore, editor. Application of electrophoresis and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, Florida.

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