

**Bonneville Power Administration  
Fish and Wildlife Program FY99 Proposal**

**Section 1. General administrative information**

**Develop open formula diets to yield quality smolts**

**Bonneville project number, if an ongoing project** 9148

**Business name of agency, institution or organization requesting funding**  
Abernathy Salmon Culture Technology Center

**Business acronym (if appropriate)** USFWS

**Proposal contact person or principal investigator:**

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**Subcontractors.**

<b>Organization</b>	<b>Mailing Address</b>	<b>City, ST Zip</b>	<b>Contact Name</b>

**NPPC Program Measure Number(s) which this project addresses.**

7.2, 7.2 A.6, 7.2 D, 7.4

**NMFS Biological Opinion Number(s) which this project addresses.**

NA

**Other planning document references.**

NA

**Subbasin.**

**Short description.**

Develop life stage diets for fish raised in supplementation/enhancement hatcheries to improve overall smolt quality and survival.

## Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
X	Anadromous fish		Construction		Watershed
	Resident fish		O & M		Biodiversity/genetics
	Wildlife	*	Production		Population dynamics
	Oceans/estuaries	X	Research		Ecosystems
	Climate		Monitoring/eval.		Flow/survival
	Other		Resource mgmt	*	Fish disease
			Planning/admin.	X	Supplementation
			Enforcement		Wildlife habitat en-
			Acquisitions		hancement/restoration

### Other keywords.

smolt quality, nutritional requirements, diets

## Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship

## Section 4. Objectives, tasks and schedules

### Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Determine if it is and at what level in the feed astaxanthin is an important immunomodulator and antioxidant	a	Feeding trial, Tissue analysis
2	Determine the efficacy of glucans as immunomodulators	a	Feeding trial, Disease challenge
3	Determine appropriate levels of lipid and carbohydrate in newly developed diets	a	Feeding trial, Tissue analysis
4	Determine possible mineral	a	Feeding trial,

	requirements for hatchery raised fish		Tissue analysis

**Objective schedules and costs**

Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	12/1999, 01/2000, 04/2000	05/2000, 12/2000, 05/2001	25
2	12/2000, 01/2001, 04/2001	05/2001, 12/2001, 05/2002	25
3	12/2001, 01/2002, 04/2002	05/2002, 12/2002, 05/2003	25
4	12/2002, 01/2003, 04/2002	05/2003, 05/2003, 05/2003	25

**Schedule constraints.**

Fish needed for the studies may not be available every year.

**Completion date.**

2003

**Section 5. Budget**

***FY99 budget by line item***

Item	Note	FY99
Personnel	GS-9	31,897.00
Fringe benefits		11,164.00
Supplies, materials, non-expendable property	Feed ingredients, chemicals, lab supplies for fish and feed analyses	30,000.00
Operations & maintenance	Maintenance of equipment, operating the well	14,000.00
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		
PIT tags	# of tags:	
Travel	Nutrition meeting	700.00
Indirect costs	19%	16,674.59

Subcontracts		
Other		
<b>TOTAL</b>		104,435.59

**Outyear costs**

<b>Outyear costs</b>	<b>FY2000</b>	<b>FY01</b>	<b>FY02</b>	<b>FY03</b>
Total budget	98,485.59	86,585.59	86,585.59	86,585.59
O&M as % of total	9.1%	10.4%	10.4%	10.4%

**Section 6. Abstract**

Nutrition research is essential when hatchery fish will be used for supplementation of weak wild and naturally spawning fish populations. In supplementation diets are needed that will maintain the health of the fish as well as produce fish that are Awild≡ in appearance and composition. Current hatchery production fish will also benefit from new diets. Habitat destruction has brought about the realization that there will be a greater dependance on hatchery raised stocks to replenish fish runs in the Columbia Basin and elsewhere. The importance of open formula diets in these rearing programs is twofold. In open formula diets the ingredients are known and can be specified. Also, because the formulas are open, they can be monitored through quality control programs. Thus there is a need to develop new open formula diets for use in hatchery production.

Specific areas of nutrition research will include diet protein/energy ratios, astaxanthin requirements, mineral requirements and the use of immunostimulants. Feeding trials will be conducted with four stocks of fish: Abernathy fall chinook, Carson spring chinook, Big Creek coho, Big Creek steelhead. The fish become available at different times so the studies will be staggered throughout the year. Growth, survival, feed conversion will be monitored during the trials. At the termination of the studies, the needed tissue samples will be collected and analyzed.

**Section 7. Project description**

**a. Technical and/or scientific background.**

Development of life stage diets for fry and juvenile fish raised in hatcheries is of critical importance to managers and culturists. Production of new, open formula feeds can help maximize post-release survival of hatchery fish by increasing similarity of hatchery fish to wild fish in appearance, physiology and proximate composition. An additional objective would be to maximize the health of the fish released from hatcheries (FWP 7.2A.6).

As more and more weak and endangered stocks are moved into captive environments, it will be crucial to have feeds developed to meet their requirements (FWP 7.2, 7.2A.6). Studies to develop new open formula diets should be initiated. Goals are to produce

feeds that yield healthy smolts and that maximize post-release survival of juveniles to adulthood.

Pacific salmon, *Oncorhynchus* spp., obtain astaxanthin in natural prey throughout their life. Tacon (1981) suggested numerous positive effects of carotenoids in the diet. Generally this pigment is not added to fish feeds except to achieve consumer acceptance of farmed fish having the red-pink color of wild salmon. However, astaxanthin or cantaxanthin sprayed on a commercial formulation at 30 mg/kg feed enhanced growth of Atlantic salmon swim-up fry (Torrissen 1984). Christiansen et al. (1995a) found that levels of astaxanthin at 5.3 mg/kg or above in a semipurified diet improved survival and growth of first feeding Atlantic salmon (*Salmo salar*) fry. Burton (1989) determined that carotenoids, specifically  $\beta$ -carotene, acted as a chain-breaking antioxidant. He suggested that other carotenoids could have similar characteristics. Using diets supplemented with either a synthetic astaxanthin at 0.91 g/kg diet or a red yeast (*Phaffia rhodozyma*) containing astaxanthin at 9.8, 10.2 or 11.36 g/kg diet, Nakano et al. (1995) determined that rainbow trout (*Oncorhynchus mykiss*) had improved muscle pigmentation as well as improvement of liver function and increase of defensive potential against oxidative stress.

In addition, Christiansen et al. (1995b) showed a definite growth effect with the use of astaxanthin (60 mg/kg feed) in a semipurified diet. They also indicated that the astaxanthin could be having a positive effect on the antioxidant status of the fish as well as promoting better survival of the fish in a *Aeromonas salmonicida* challenge study. Bendich (1989) suggested that  $\beta$ -carotene as well as other carotenoids with nine or more conjugated double bonds may enhance immune function. Thompson et al. (1995) found no effect of astaxanthin on food conversion efficiency or growth rate. However, the rainbow trout were not first feeding fry. They fed these fish (8 g average weight) astaxanthin in the diet at 92.5 mg/kg and detected no immunostimulating effect.

The purpose of these studies would be to examine astaxanthin's ability to enhance the immune response, act as an antioxidant as well as give the fish a more natural coloration. Abernathy Salmon Culture Technology Center will be working with spring chinook, fall chinook, coho and steelhead.

Glucans administered by intraperitoneal injection or as a bath have been used to enhance the nonspecific immune response in Atlantic salmon, *Salmo salar*, coho, *Oncorhynchus kisutch*, channel catfish, *Ictalurus punctatus*, and brook trout, *Salvelinus fontinalis*, (Robertsen et al. 1990, Nikl et al. 1991, Chen and Ainsworth 1992, Anderson and Siwicki 1994). Engstad et al. (1992) and Jorgensen et al. (1993) found that the lysozyme levels in the blood of Atlantic salmon and rainbow trout, *Oncorhynchus mykiss*, respectively, increased after an intraperitoneal (i.p.) injection of a glucan from *Saccharomyces cerevisiae*. Matsuyama et al. (1992) also found an increase in serum lysozyme when yellowtail *Seriola quinqueradiata* were injected i.p. with  $\beta$ -1,3-glucans derived from *Schizophyllum commune* and *Sclerotium glaucanicum*.

Little work examining the effects of orally administered glucans on the fish's immune response has been done. Raa et al. (1992) achieved positive results feeding Macrogard, a

$\beta$ -1,3/1,6-glucan from yeast, to Atlantic salmon. Fish fed the glucan at 1 g/kg dry feed sustained a survival of approximately 60%. Whereas the fish fed the control diet had a 20% survival after a challenge with *Vibrio salmonicida*. Survivals were better (~85%) when the glucan fed fish were exposed to *V. anguillarum*. In addition, Siwicki et al. (1994) fed several immunostimulant preparations mixed into semipurified diets to rainbow trout. In the fish fed the immunostimulants there was an increase in oxidative radical release, myeloperoxidase activity, phagocytic indexes and potential killing activities of phagocytic cells including neutrophils. *Spirulina* also shows promise as an orally administered immunostimulant (Duncan and Klesius 1996). When *Spirulina* was fed at 2.7% of the diet for seven days the nonspecific immune response of channel catfish was enhanced.

This trial will examine the effect of orally administered immunostimulants on the nonspecific immune responses of fall chinook salmon, *Oncorhynchus tshawytscha*, juveniles. Lysozyme increases will be the response monitored.

Levels of fat as well as composition of the fat are important. Dupree et al. (1979) reported that as the lipid content of the diet for channel catfish increased from 0% to 20%, the whole fish lipid levels increased from 3.8% to 13.2%. In a study using turbot, Caceres-Martinez et al. (1984) saw a negative effect in the fish tissue of excess dietary lipids. At the lowest protein level, 37.5%, the tissue lipid deposition increased as the lipid level in the feed increased from 10% to 20%. Use of a vegetable oil instead of animal fat prevented these abnormalities. Several investigators have shown that the fatty acid content of the lipids used in fish diets are important for good growth (Farkas, et al. 1977, Stickney and Andrews 1972, Heck and Calbert 1977, Farkas, et al. 1977, Stickney et al. 1984). The importance of fatty acids in the correct proportions in the diets is demonstrated in the study done by Lewis et al. (1985). Catfish fed various combinations of stearic, oleic, linoleic, and linolenic acids performed poorly. Castell et al. (1994) also showed the importance certain fatty acids in fish feeds. They found that arachidonic acid (20:4n-6) may be an essential fatty acid for juvenile turbot. Arachidonic acid is not given much attention in fish diets however, Bell et al. (1994) found in the 10 freshwater invertebrates (common prey for salmon) he analyzed for fatty acid composition that there were higher levels of 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3 and less 22:6n-3 than found in commercial diets used in smolt production. In a study done Lie et al. (1986), three different fats were tested in diets for cod. They used cod liver oil, Greenland halibut oil and peanut oil. The study indicated that the type and amount of fat used influenced fat deposition in the liver.

The liver is an indicator of how good the diet is for the fish. Fowler and Wood (1966) tested different supplemental dietary fats on chinook salmon fingerlings. The investigators found that when chinook salmon were fed a meat diet or an all-meat diet supplemented with hard animal fat as the principal lipid source fatty liver and degenerative changes of the spleen and hematopoietic part of the kidney occurred. Use of a vegetable oil instead of animal fat prevented these abnormalities.

Carbohydrates in the diet of salmonids is an important consideration, especially now that extrusion of feeds make the carbohydrate more digestible. Feeding high levels of digestible carbohydrate to salmon has resulted in increased liver size and glycogen content which is proportional to the carbohydrate fed (Wilson 1994). Starch inclusion in the diet of Atlantic salmon higher than 22% had negative effects on growth and feed utilization (Hemre et al. 1995). Inclusion of starch above 9% resulted in decreased starch digestibility. Ashley (1972) and Roberts and Bullock (1989) discuss the pathologies of excessive carbohydrates in the diet of fish.

Information on mineral requirements in fish is incomplete due to the fact that fish are capable of taking up minerals from the water through their gills and skin Lall (1989). Other complicating factors are the availability and digestibility of minerals in the diet (Shearer 1992, Li and Robinson 1996). Work is being done comparing the digestibility chelated and inorganic trace minerals (Bell and Cowey 1989, Li and Robinson 1996, Lim et al. 1996, Paripatananont and Lovell 1997). There have been mixed results in these studies probably depending on the composition of the feed and the alkalinity/hardness of the water. A study done by Felton et al. (1994) indicates that hatchery raised coho have significantly less copper and zinc content than wild smolts collected in the same watershed. Barrows (1997) showed that the use of a highly digestible chelate of copper improves fin condition in rainbow trout. Water at many hatcheries is low in minerals and diets may be marginally deficient in the minerals needed by fish. Immune function can also be affected by mineral deficiencies (Landolt 1989, Lim et al. 1996, El-Mowafi et al. 1997). For the hatchery fish to be more like their wild counterparts, mineral nutrition needs to be addressed.

**b. Proposal objectives.**

1. Determine if it is and at what level in the feed astaxanthin is an important immunomodulator and antioxidant
2. Determine the efficacy of glucans as immunomodulators
3. Determine appropriate levels of lipid and carbohydrate in newly developed diets
4. Determine possible mineral requirements for hatchery raised fish

**c. Rationale and significance to Regional Programs.**

The role of hatcheries in the Pacific Northwest and elsewhere is evolving such that they will become more than mitigation facilities. They will be supplementation and restoration hatcheries for threaten and endangered species (FWP 7.2). As such they need to produce fish that mimic, as closely as possible, the physical characteristics (e.g. size, coloration and body composition) of wild fish (FWP 7.2A.6). The feeds developed through this project would help reduce some of the perceived negative characteristics of hatchery raised fish (FWP 7.2D, 7.4). Without examining all the variables of the hatchery

influence it can not be determined if hatcheries are fit sources of fish for restoration and recovery of threaten and endangered fish populations.

For many years, fish culturists in the Pacific Northwest have used frozen moist diets to feed hard-to-raise species such as spring chinook salmon. Dry feeds have been used successfully in the culture of a number of fishes including trout, steelhead, Atlantic salmon and some species of Pacific salmon. Presently, there is one manufacturer of the moist frozen feed, and the only open-formula dry feed is made on a compaction mill (California pellet mill). Progress has been made in the salmon feed industry, and cooker-extruders are now being employed at the mills. There are several closed-formula extruded feeds available to hatcheries. However, these feeds have been developed for the commercial aquaculture industry where the desired product is a large fish, produced quickly. Historically hatcheries have also wanted big smolts for release, but thoughts on the Aideal smolt≡ are changing. A desire exists for hatcheries to produce >wild-type= fish. These fish would be >wild= in appearance and behavior as well as body composition. To meet this goal, new diets and feeding regimes need to be developed.

As more threatened and endangered stocks are moved into a hatchery rearing program, development of life stage diets (for fry and juvenile fish raised in hatcheries) is becoming a critical area of research. The new diets would be better adapted for fish at various stages of development. Specific areas of nutrition research would include diet protein/energy ratios, astaxanthin requirements, mineral requirements and the use of immunostimulants.

**d. Project history**

**e. Methods.**

Objective 1: Astaxanthin study

Feeding studies will be done using first feeding fry initially. Four different stocks of fish will be used: fall chinook salmon, *Oncorhynchus tshawytscha*, raised at the Abernathy SCTC, spring chinook (*O. tshawytscha*), Carson NFH stock, coho (*Oncorhynchus kisutch*), Big Creek, OR stock and steelhead (*Oncorhynchus mykiss*), Big Creek, OR. Fish will be stocked into 700 liter circular fiberglass tanks, 500 fish per tank. Four tanks were randomly assigned to each treatment. Well water (12°C) will be used at 19 liters/minute.

The feed used will be the open formula Abernathy diet with the appropriate levels of astaxanthin incorporated (5, 10, 15, 20, 25 mg/kg feed). The fish will be fed by hand four times a day, five days a week. On the weekends the fish will be fed with automatic feeders four times a day. The amounts of feed fed will be calculated by the method of Buterbaugh and Willoughby (1967). The fish will be weighed every two weeks and the amount feed adjusted accordingly. Ten fish will be sampled every month. Fish and feed

will be analyzed by Hoffman-LaRoche, N.J.

#### Objective 2: Immunostimulants study

For the glucan feeding trial all compounds will be added to a modified Abernathy diet. The respective percentages of the compounds will be substituted for mill run. The diets will be made at the Abernathy SCTC. The dosage period for each compound will be seven days.

Four separate feeding trials using each one of the stocks will be conducted. Juvenile fall chinook salmon, *Oncorhynchus tshawytscha*, raised at the Abernathy SCTC, spring chinook (*O. tshawytscha*), Carson NFH stock, coho (*Oncorhynchus kisutch*), Big Creek, OR stock and steelhead (*Oncorhynchus mykiss*), Big Creek, OR stock will be the stocks tested. As these fish will be available at slightly different times of the year, the feeding trials will be staggered. The fish in each trial will be tested for initial serum lysozyme, mucus lysozyme from the skin, nare and intestinal wall, and for gill sodium, potassium ATPase. After a one week acclimation period, groups of 250 fish will be weighed and sampled for skin mucus lysozyme, and transferred to 700 liter circular tanks, in 12-13EC well water, with flows at 19 liters/minute. Fish stocking densities will not exceed guidelines found for fall chinook salmon (Piper et al. 1982). Fish will be kept on natural photoperiod. Fish will be randomly assigned to the experimental tanks, with four replicates of the following treatment groups: control, fed Abernathy Control Diet, fish fed Levucell SB, fish fed *Schizochytrium*, and fish fed *Spirulina*.

At the start of the feeding study, an initial sample of 30 fish from the stock tank will be sampled for serum lysozyme, mucus lysozyme from the skin, nare and intestinal wall, and for gill sodium, potassium ATPase. All skin mucus samples for lysozymes will be collected from the lateral line area above the vent. Nare mucus will be obtained by inserting the loop into the nare and removing the loop after turning it 2 turn. Intestinal mucus will be collected by inserting the loop into the intestine to the first taper of the loop handle and drawing it out while gently scraping the intestinal wall by providing pressure on the outside of the fish. Blood will be obtained by severing the caudal peduncle and collected in Microtainer brand serum separation tubes (Becton Dickinson & Co. Rutherford, N. J. 07070). All samples will be frozen until analyzed. For all sampling, fish will be anesthetized using a 40 mg/L MS-222 solution buffered with sodium bicarbonate. The methods of collection and analysis were described by Schrock (1994).

After a one week feeding regime at 2% body weight, the fish will be fasted for 24 hours, then anesthetized with 40 mg/L MS-222, weighed and sampled for skin mucus lysozyme. After 20-30 minute recovery period the fish will be challenged. The fish will not be fed on the day of challenge, but feeding will be resumed the following day, and all groups will be fed Abernathy Control Diet at 2% body weight/day.

#### Disease challenge

Fish were challenged with *Vibrio anguillarum*. The culture will be prepared from

a lyophilized preparation. A vial of the culture will be added to 1 L of sterile 1% NaCl tryptic soy broth, and allowed to grow until the optical density reading at 405nm is > 0.800. Verification of the presence of viable *V. anguillarum* will be done by culturing of the broth on 1% NaCl tryptic soy agar plates at the time of the last tank challenge which should result in the growth of cream colored, round, raised colonies. Fish in the recovery buckets containing 13 L heated well water will be challenged with 10 ml of the inoculated broth for 1 hour under constant aeration. At exactly 1 hour, fish will be returned to their experimental tanks, and observed for signs of infection. At the time mortality reached 50% the remaining fish in each tank will be anesthetized and sampled for the following: serum lysozyme, mucus lysozyme from the skin, nare and intestine, and the kidney, spleen, and liver.

For statistical analysis, analysis of variance will be used to determine if there was a difference between treatments at the 0.05 level of significance. If the treatments are significantly different, the Student-Newman-Keuls method will be used to ascertain were the differences occurred (Ostle and Mensing 1975).

### **Objective 3: Lipid level study**

The fish to be used in the lipid level experiment will be fall chinook salmon fingerlings raised from eggs at the Abernathy SCTC as well as steelhead (Big Creek, OR stock). Two feeding studies will be conducted, one for each fish stock. Fish will be stocked into 700 liter circular fiberglass tanks, 250 fish per tank. Four tanks were randomly assigned to each treatment. Well water (12°C) will be used at 19 liters/minute.

The treatments will consist of the basic Abernathy diet with different levels of lipid used in the formulation. The levels of lipid used will be 9.0%, 11.4%, 19.9% and the control, 15.7%. Proximate analysis (AOAC 1990) of the feed ingredients for the experimental diets will be determined and the diets formulated. A small compaction-type pellet mill (California Pellet Mill, San Francisco, CA), without steam conditioning, will be used to prepare the diets. Feed will be made at the start of the experiment and stored at room temperature.

The fish will be fed by hand four times a day, five days a week. On the weekends the fish will be fed with automatic feeders four times a day. The amounts of feed fed will be calculated by the method of Buterbaugh and Willoughby (1967). The fish will be weighed every two weeks and the amount feed adjusted accordingly.

The data that will be reported for the feeding trial included average weight gain, gross feed conversion as well as liver glycogen and liver triglyceride levels. Liver analyses will be done by Biotech Research and Consulting, Inc., Corvallis, OR. Proximate analysis will be done on the fish and the feed. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments ( $P < 0.05$ ). Where differences are found, the treatments that are different from each other will be determined by the Student-Newman-Keuls method ( $P < 0.05$ ) (Ostle and Mensing 1975).

### **Objective 3: Semi-moist diet study**

In the open formula semi-moist diet study the fish used will be fall chinook salmon (*Oncorhynchus tshawytscha*) fingerlings, Abernathy SCTC stock, spring chinook (*O. tshawytscha*), Carson NFH stock, coho (*Oncorhynchus kisutch*), Big Creek, OR stock and steelhead (*Oncorhynchus mykiss*), Big Creek, OR stock. These fish, in separate studies, will be stocked into 700 liter circular fiberglass tanks, 250 fish per tank. Four tanks will be randomly assigned to each treatment. Well water (12°C) will be used at 19 liters/minute.

The treatments will consist of the three semi-moist formulations and the Abernathy Dry diet as a control. The formulations will have varying levels of carbohydrate added. Proximate analysis (AOAC 1990) of the feed ingredients for the experimental diets will be determined and the diets formulated. A Wenger X85 single screw cooker-extruder will be used to make the feeds. The diets will be made at the start of the experiment and stored at room temperature in plastic containers. The fish will be fed seven days a week with automatic feeders four times a day. The amounts of feed fed will be calculated by the method of Buterbaugh and Willoughby (1967). The fish will be weighed every two weeks and the amount feed will be adjusted accordingly. The quantities of feed fed to the different treatments will also be corrected for moisture in the feed.

The data reported for the feeding trial will include average weight gain, specific growth rate, gross feed conversion as well as liver glycogen and liver triglyceride levels. Liver analyses will be done by Biotech Research and Consulting, Inc., Corvallis, OR. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments ( $P < 0.05$ ). Where differences are found, the treatments that were different from each other will be determined by the Student-Newman-Keuls method ( $P < 0.05$ ) (Ostle and Mensing 1975).

### **Objective:4 Minerals**

The four stocks of fish that have been mentioned in the other studies will be used in this study. Fish will be stocked into 700 liter circular fiberglass tanks, 250 fish per tank. Four tanks were randomly assigned to each treatment. Well water (12°C) will be used at 19 liters/minute. The fish will be fed by hand four times a day, five days a week. On the weekends the fish will be fed with automatic feeders four times a day. The amounts of feed fed will be calculated by the method of Buterbaugh and Willoughby (1967). The fish will be weighed every two weeks and the amount feed adjusted accordingly.

The diets used will be the Abernathy Dry diet with graded levels of each of the test minerals. Each mineral will be tested separately. The control diet will be the Abernathy diet with the standard mineral premix. The mineral controls will contain the minerals in the forms commonly found in mineral premixes. There will be 10 treatments and three replicates. The chelated minerals will be tested against the inorganic minerals

traditionally used in feeds. The minerals tested will include: copper, zinc, selenium.

The data reported for the feeding trial will include average weight gain, specific growth rate, gross feed conversion as well as whole body and liver mineral levels. Mineral analyses will be done by Covance, Madison, WI. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments ( $P < 0.05$ ). Where differences are found, the treatments that were different from each other will be determined by the Student-Newman-Keuls method ( $P < 0.05$ ) (Ostle and Mensing 1975).

**f. Facilities and equipment.**

Abernathy Salmon Culture Technology Center has 12 raceways, 8' X 80', 104 fiberglass circular tanks, 700 liters (1.2 m diameter, 0.6 m deep), 50 (16 tray) incubator stacks. Water sources include: creek water, 6000 gal/min winter, 3000-4000 gal/min summer, temperatures 4°C-16°C, winter-summer; two wells, old well, 250 gal/min, new well, 400-3200 gal/min, variable speed pump, temperature 12°C  $\nabla$  1°. In addition, Abernathy is equipped with a feed preparation laboratory and an analytical laboratory for proximate analyses of feeds and fish. The feed preparation laboratory has a California pellet mill capable of making a compacted pellet at a rate of up to 50 lbs/hour. The laboratory also has a Wenger X85 single screw cooker-extruder that can produce floating, sinking or semi-moist feeds at a rate of 150-600 lbs/hour as well as all the ancillary equipment needed to make fish feed.

Other facilities and equipment include: laboratory space adequate for fish sampling and tissue preparation, a walk-in freezer and refrigerator, a computer linked with the Wenger X85 for collecting data about each feed run and an office computer (486 CPU, 33MHz, 16B hard drive, 16MB RAM, 33.6Kbps modem) for data analysis and word processing.

**g. References.**

- Anderson, D. P. and A. K. Siwicki. 1994. Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. *Progressive Fish-Culturist* 56:258-261.
- Ashley, L. M. 1972. Nutritional *pathology*. In, *Fish nutrition*, J. Halver, ed. pp. 490-492. Academic Press, New York.
- Association of Official Analytical Chemists. 1990. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed. S. Williams (Editor). Association of Official Analytical Chemists, Arlington, Va. 1141 pp.
- Barrows, R. 1997. The effect of diet on fin erosion in rainbow trout. Presented at the Fish feed and Nutrition Workshop, Frankfort, KY, September 21-23, 1997.

- Bell, J. G. and C. B. Cowey. 1989. Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionine and selenocystine in Atlantic salmon (*Salmo salar*). *Aquaculture* 81:61-68.
- Bell, J. G., C. Ghioni and J. R. Sargent. 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. *Aquaculture* 128:301-313.
- Bendich, A. 1989. Carotenoids and the immune response. *Journal of Nutrition* 119:112-115.
- Burton, G. 1989. Antioxidant action of carotenoids. *Journal of Nutrition* 119:109-111.
- Buterbaugh, G. L. and H. Willoughby. 1967. A feeding guide for brook, brown, and rainbow trout. *Progressive Fish-Culturist* 29: 210-215.
- Caceres-Martin, C. M. Cadena-Roa and R. Metailler. 1984. Nutritional requirements of turbot (*Scophthalmus maximus*): I. A preliminary study of protein and lipid utilization. *Journal of the World Mariculture Society* 15:191-202.
- Castell, J. D., J. G. Bell, D. R. Tocher and J. R. Sargent. 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 128: 315-333.
- Chen, D. and A. J. Ainsworth. 1992. Glucan administration potentiates immune defense mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. *Journal of Fish Diseases* 15:295-304.
- Christiansen, R., O. Lie and O. J. Torrissen. 1995a. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First-feeding fry. *Aquaculture Nutrition* 1:189-198.
- Christiansen, R., J. Glette, O. Lie, O. J. Torrissen and R. Waagbo. 1995b. Antioxidant status and immunity in Atlantic salmon, *Salmo salar* L., fed semi-purified diets with and without astaxanthin supplementation. *Journal of Fish Diseases* 18:317-328.
- Duncan, P. L. and P. H. Klesius. 1996. Effects of feeding *Spirulina* on specific and nonspecific immune responses of channel catfish. *Journal of Aquatic Animal Health* 8:308-313.
- Dupree, H., E. Gauglitz, Jr., A. Hall and C. Houle. 1979. Effects of dietary lipids on the growth and acceptability (flavor) of channel catfish (*Ictalurus punctatus*). *Proc.*

- World Symp. on Finfish Nutrition and Fishfeed Technology, Hamburg 2:87-103.
- El-Mowafi, A. F. A., R. Waagbo and A. Maage. 1997. Effect of low dietary magnesium on immune response and osmoregulation of Atlantic salmon. *Journal of Aquatic Animal Health* 9:8-17.
- Engstad, R. E., B. Robertsen and E. Frivold. 1992. Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. *Fish & Shellfish Immunology* 2:287-297.
- Felton, S. P., R. Grace and M. Landolt. 1994. Significantly higher levels of zinc and copper found in wild compared to hatchery-reared coho salmon smolts *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms*. 18:233-236.
- Fowler, L. G. and E. M. Wood. 1966. Effect of type of supplemental dietary fat on chinook salmon fingerlings. *Progressive Fish Culturist*. 26:123-127.
- Heck, N. E. and H. E. Calbert. 1977. Use of animal fat in formulated diets for yellow perch. Proceedings of the eighth annual meeting world Mariculture Society; 1977 January 9-13; San Jose, Costa Rica. c1977:787-791.
- Hemre, G-I., K. Sanders, O. Lie, O. Torrissen, R. Waagbo. 1995. Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. *Aquaculture Research* 26:149-154.
- Jorgensen, J. B., G. J. E. Sharp, C. J. Secombest, and B. Robertsen. 1993. Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. *Fish & Immunology* 3:267-277.
- Lall, S. 1989. The Minerals. In, *Fish Nutrition*, 2nd edition, J. Halver, ed. pp.219-257. Academic Press, Inc., San Diego, New York.
- Landolt, M. L. 1989. The relationship between diet and the immune response of fish. *Aquaculture* 79: 193-206.
- Lewis, D. H., J. E. Marks and R. R. Stickney. 1985. Degenerative myopathy in channel catfish, *Ictalurus punctatus* (Rafinesque), maintained on rations containing purified fatty acids. *Journal of Fish Diseases* 8:563-565.
- Li, M. H. and E. H. Robinson. 1996. Comparison of chelated zinc and zinc sulfate as zinc sources for growth and bone mineralization of channel catfish (*Ictalurus punctatus*) fed practical diets. *Aquaculture* 146:237-243.
- Lie, O., E. Lied, and G. Lambertsen. 1986. Liver retention of fat and of fatty acids in cod (*Gadus mochua*) fed different oils. *Aquaculture* 59:187-196.

- Lim, C., P. H. Klesius and P. L. Duncan. 1996a. Immune response and resistance of channel catfish to *Edwardsiella ictaluri* challenge when fed various dietary levels of zinc methionine and zinc sulfate. *Journal of Aquatic Animal Health* 8:302-307.
- Lim, C. W. M. Sealey and P. H. Klesius. 1996b. Iron methionine and iron sulfate as sources of dietary iron for channel catfish *Ictalurus punctatus*. *Journal of the World Aquaculture Society* 27:290-296.
- Matsuyama, H., R. E. P. Mangindaan, T. Yano. 1992. Protective effect of schizophyllan and scleroglucan against *Streptococcus* sp. infection in yellowtail (*Seriola quinqueradiata*). *Aquaculture* 101:197-203.
- Mazur, C. N., D. A. Higgs, E. Plisetskaya and B. E. March. 1992. Utilization of dietary starch and glucose tolerance in juvenile chinook salmon (*Oncorhynchus tshawytscha*) of different strains in seawater. *Fish Physiology and Biochemistry* 10:303-313.
- Nakano, T., M. Tosa and M. Takeuchi. 1995. Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin. *J. Agric. Food Chem.* 43:1570-1573.
- Nikl, L., L. J. Albright, T. P. T. Evelyn. 1991. Influence of seven immunostimulants on the immune response of coho salmon to *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* 12:7-12.
- Ostle, B. And R. Mensing. 1975. *Statistics in research.* 596 pp. The Iowa State University Press, Ames.
- Paripatananont, T and R. T. Lovell. 1995. Chelated zinc reduces the dietary zinc requirement of channel catfish, *Ictalurus punctatus*. *Aquaculture* 133:73-82.
- Paripatananont, T and R. T. Lovell. 1997. Comparative net absorption of chelated and inorganic trace minerals in channel catfish *Ictalurus punctatus* diets. *Journal of the World Aquaculture Society* 28:62-67.
- Piper, R., I. McElwain, L. Orme, J. McCraren, L. Fowler and J. Leonard. 1982. *Fish Hatchery Management.* U. S. Department of Interior, Fish and Wildlife Service, Washington, D. C. pp. 517
- Raa, J., G. Roerstad, R. Engstad and B. Robertsen. 1992. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. In: *Diseases in Asian Aquaculture I.* M. Shariff, R. P. Subasinghe and J. R. Arthur, eds. pp. 39-50. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

- Roberts, R. J. and A. M. Bullock. 1989. Nutritional pathology. In, Fish Nutrition, 2nd edition, J. Halver, ed. p. 430. Academic Press, Inc., San Diego, New York.
- Robertsen, B., G. Roerstad, R. Engstad and J. Raa. 1990. Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. *Journal of Fish Diseases* 13:391-400.
- Schrock, R. M. 1994. Quantifying non-specific disease response in adult and juvenile salmon. In Proceedings of an International Fish Physiology Symposium, Physiology Section, American Fisheries Society and the Fish Physiology Association. Vancouver, B. C. July 16-21, 1994.
- Shearer, K. D, A. Maage, J. Opstvedt and H. Mundheim. 1992. Effects of high-ash diets on growth, feed efficiency, and zinc status of juvenile Atlantic salmon (*Salmo salar*). *Aquaculture* 106:345-355.
- Siwicki, A. J., D. P. Anderson and G. L. Rumsey. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology* 41:125-139.
- Stickney, R. R. and J. W. Andrews. 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. *Journal of Nutrition* 102:249-258.
- Stickney, R. R., R. B. Mcgeachin and E. H. Robinson. 1984. Effect of dietary linoleic acid level on growth, food conversion and survival of channel catfish. *Journal World Mariculture Society* 15:186-190.
- Tacon, A. 1981. Speculative review of possible carotenoid function in fish. *Progressive Fish-Culturist* 43:205-208.
- Thompson, I., G. Choubert, D. F. Houlihan, C. J. Secombes. 1995. The effect of dietary vitamin A and astaxanthin on the immunocompetence of rainbow trout. *Aquaculture* 133:91-102.
- Torrissen, O. J. 1984. Pigmentation of salmonids--effects of carotenoids in eggs and start-feeding diet on survival and growth rate. *Aquaculture* 43:185-193.
- Wilson, R. P. 1994. Utilization of dietary carbohydrate by fish. *Aquaculture* 124:67-80.

## **Section 8. Relationships to other projects**

## Section 9. Key personnel

**Ann L. Gannam**  
**Abernathy Salmon Culture Technology Center**  
**Longview, WA 98632**

### Education

1988 Ph.D. in Fish Nutrition /Aquaculture, Auburn University, Auburn, Alabama.  
1980 Masters of Science in Biology, University of Southern Mississippi, Hattiesburg, Miss  
1976 Bachelor of Science in Zoology, University of Georgia, Athens, Georgia.

### Related Experience

1992-present Nutritionist, USFWS, Abernathy Salmon Culture Technology Center. Responsibilities include developing new diets, conducting feeding trials and working on feed problems at the federal hatcheries in Region 1. Am also responsible for feed mill inspections (fish feed quality control) to insure compliance with specifications for feeds made for the federal government in Region 1.

1989-1992 Assistant Professor, Fisheries, University of Arkansas Pine Bluff, Department of Agriculture. Was an adjunct assistant professor at the University of Arkansas Fayetteville. Responsibilities included teaching fisheries courses as well as conducting research in fish. Conducted fish nutrition studies addressing alternative protein options for channel catfish, lipid concerns in hybrid striped bass, cost effective feed for golden shiners and temperature/growth for tilapia.

9/1988-2/1989 Research Associate in fish nutrition at the University of Arkansas Pine Bluff. Responsibilities included equipping and maintaining the nutrition laboratory. Did preliminary studies to determine the feasibility of using sunflower seed meal as a substitute for soybean meal in catfish diets. Consulted with catfish and baitfish farmers about problems concerning fish feeding and nutrition.

Gannam, A. L., P. R. Waterstrat, R. Pascho and C. McKibben. 1994. An evaluation of feather meal as a feed ingredient and immunomodulator in fall chinook salmon (*Oncorhynchus tshawytscha*). Poster presented at the International Fish Physiology Symposium, Vancouver, BC, July 16-21, 1994.

Gannam, A. L. and M. Muel. 1995. Effects of dietary iron on disease resistance in fall chinook salmon. Abstract, World Aquaculture Meeting, San Diego, CA, February 1-4 1995.

Schrock, R. M. and A. Gannam. 1996. Comparison of three glucan preparations as feed additives in juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) challenged with *Vibrio anguillarum*. Presented at the American Fisheries Society Fish Health Section Meeting, Madison, WI, August 6-9 1996.

Gannam, A. L., R. M. Schrock and M. W. Hack. 1997. The use of three glucan preparations as feed additives in diets for fall chinook salmon, *Oncorhynchus tshawytscha*. Poster presentation at the World Aquaculture Meeting, Seattle, WA, February 19-23 1997.

Gannam, A. L. 1997. Development of open formula diets and new feeding strategies: A progress report. 48th Annual Pacific Northwest Fish Culture Conference, Glenden Beach, OR, December 2-4,

1997.

## **Section 10. Information/technology transfer**

Information generated from this project may be disseminated through peer-reviewed technical journals such as Journal of Nutrition, Journal of Food Science, Aquaculture, The Progressive Fish-Culturist and the Journal of the World Aquaculture Society; the Technology Transfer Series and BPA reports. In addition, feed mills will be notified of positive results and recommendations will be made for feed manufacture.