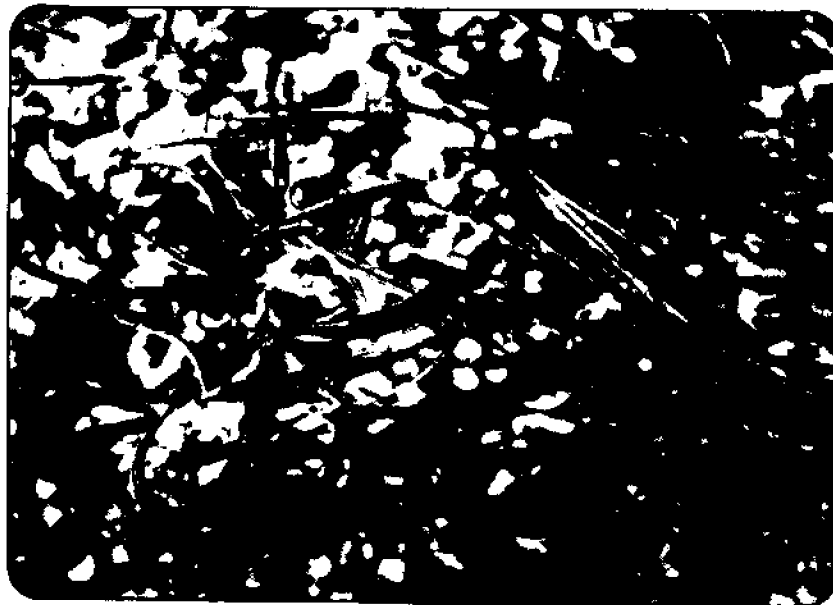


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Diet Development for Post-Larval Pink and Chum Salmon Held in Salt Water

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University of Alaska
Alaska Sea Grant Report No. 85-4
May 1985

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DIET DEVELOPMENT FOR POST-LARVAL PINK AND CHUM SALMON
HELD IN SALT WATER

by

C. L. Kerns
Marine Advisory Program
Fishery Industrial Technology Center

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Abstract

A four-year project was initiated to contribute to, and to stimulate the development of practical diets composed largely of locally produced fisheries products and by-products for pink (Oncorhynchus gorbuscha) and chum salmon (O. keta) fed in salt water at Alaskan temperature regimes.

The first year's efforts determined whether commercial Pacific salmon diets developed elsewhere for other species of Pacific salmon fed in fresh water at moderate water temperatures were satisfactory for pink salmon fry held in salt water at lower water temperatures. A feeding trial was held comparing the nutritional response of pink and chum salmon fry to two commonly used commercial salmon rations, OMP II and the Abernathy formulation of Silver Cup, with pollock (Theragra chalcogramma) roe as the control. The response of pink salmon fry fed pollock eggs was superior to that of fry given either diet. Fry fed Silver Cup had an instantaneous daily weight gain of 1.68 percent, fry given OMP II 1.99 percent, and the pollock egg-fed treatment, 3.43 percent. Calculated total marine survival, from fry release to returning adult (fishery interception plus hatchery return), of OMP II treatment fish was 3.1 percent. Pink salmon fry fed pollock eggs had an estimated marine survival of 7.0 percent. The marked disparity between nutritional responses to the two commercial feeds and that of the control group evidenced further dietary development efforts were warranted.

The second year's efforts delved into calorie/protein ratios. Three diets were used containing different levels of protein and energy: 62, 86, and 126 kcal of metabolizable energy for each 1 percent of protein in the feed. Dry ingredients and fresh, deboned black rockfish (Sebastes melanops) muscle tissue and viscera were varied inversely with stabilized salmon oil to produce the three feeds. The control ration was OMP II. Growth was similar to that reported for other salmonids. The intermediate level of protein (52 percent) and lipid (21.8 percent) diet produced the highest instantaneous daily weight (2.06 percent/day, 2.20 percent/day) and length (0.89 percent/day, 0.70 percent/day) gains in both pink and chum salmon fry, respectively. Statistically significant differences were found in the nutritional responses (weight and length gains, and condition factors) between the intermediate level fat and protein, and low fat (8.4 percent), high protein (62.4 percent), and high fat (35.3 percent) low protein, (41.3 percent), and control diets with pink salmon fry. There was a statistically significant difference between the 21.8 percent fat diet and the other diets in weight gains and condition factor but not in length gains for chum salmon fry.

The third year's efforts compared, on a production scale, the intermediate fat/protein level diet (Test 2) with two commercial diets. A mechanical malfunction necessitated the use of a substitute for the fresh fish component. Roe from Pacific cod (Gadus macrocephalus) was used instead of black rockfish flesh. The instantaneous daily weight gain of pink salmon fry fed OMP II was

60.6 percent and Biodiet 87.7 percent of the rate of weight gains of pink salmon fed the test diet, whereas during the previous year the ratio of rate of weight gains of fry fed OMP II and Test 2 was 73.8 percent. Chum salmon fry given OMP II had a weight increase rate 67.8 percent that of fry in the test diet treatment, and the Biodiet treatment fry 71.5 percent that of the Test 2 fry. During the previous year chum salmon fry fed OMP II had a weight increase rate 87.3 percent that of the Test 2 group fry.

Calculated marine survival from fry release to total adult return of the OMP II treatment adult return was 79.8 percent of the test diet treatment.

The fourth year's production-scale efforts utilized the intermediate level of lipid/protein diet (Test 2) using black rockfish as the wet component as in Year 2 tests. Relative performance of the test diet using rockfish flesh was reduced when compared with the same ration using cod roe. Pink salmon fry in the Biodiet treatment averaged 108.7 percent of the rate of weight gain over that of the test ration group; Biodiet supported a rate of weight gain 87.4 percent of that of Test 2 diet using cod eggs. Chum salmon fry given Biodiet responded with 107.8 percent of the rate of weight gain of the test diet treatment, whereas it was 71.5 percent of that of Test 2 with cod eggs. Calculated total marine survival of the pink salmon in the Biodiet treatment was 124.4 percent of the fish in the test diet group.

The data developed by this project, feeding 12 dietary treatments to approximately 7.22×10^6 salmon fry, strongly indicates that pollock or cod eggs in the diet of post-larval pink and chum salmon stimulates rapid development of digestive systems resulting in superior growth and marine survival. During years with favorable estuary conditions, the quality of feed given pink salmon fry apparently has less impact on marine survival rates than during other periods. During years with average estuary conditions, the quality of feed apparently is very important to marine survival. Differences in weight gain of chum salmon fry fed the same ration as pink salmon fry were found to be statistically highly significant for all dietary treatments. Subtle differences in the nutritional response of pink salmon fry to that of chum salmon fry fed the same ration were evident.

INTRODUCTION

MARINE SURVIVAL AND FEEDING

Short-term feeding of pink or chum salmon fry, coupled with releases from the hatchery timed to coincide with periods of optimal estuarian conditions, has demonstrated a positive effect on marine survival, fry to returning adult. Parker (1965) found that predation rates on pink salmon were higher on smaller, younger fry than on larger, older fry. In an experiment performed in Alaska, Martin, Heard, and Wertheimer (1981) found marine survival to be 3.1 percent for pink salmon released unfed, 4.6 percent for fry fed 30 days, 5.2 percent for fry fed 60 days, and 4.3 percent for fry fed 90 days. The average marine survivals of the 1978 and 1979 returns of adult pink salmon to Port San Juan Hatchery were 2.2 percent for unfed fry, and 6.2 percent for fed fry (W.H. Noerenberg, Prince William Sound Aquaculture Corporation, P.O. Box 1110, Cordova, AK, USA, 1979 personal communication).

In Japan, short-term feeding of chum salmon fry, coupled with proper release timing, has been demonstrated to be the causal mechanism for the improvement in marine survival (Mathews and Senn 1975; Akiyama *et al.* 1984; Shirahata in press). Prior to the 1966 brood year, chum salmon fry were released unfed; marine survival averaged approximately 1 percent (Kobayashi 1980). Subsequent to the inception of widespread feeding of fry, marine survivals have increased to nearly 3 percent (Ministry of Agriculture, Forestry and Fisheries 1981). The probable explanation according to Okada and Taniguchi (1971), is that while prey items are abundant in the coastal areas of northern Japan during the period of May and June, they are too large to be consumed by chum salmon weighing less than 1 to 2g. Brett (1965) found swimming speed to be directly proportional to length for sockeye salmon (*O. nerka*). Larger fry may be better able to evade predators as postulated by LeBrasseur (1969). Akiyama and Nose (1980) stated that the larger the fry at release, the longer they can sustain themselves because they have higher levels of deposited energy, yet a lower basal metabolic rate. Consequently, virtually all new pink and chum salmon hatcheries are constructed with the operational plan of short-term rearing. It is therefore important to develop optimal diet formulations for pink and chum fry.

AVAILABLE RATIONS

In Alaska, the two most widely used commercially available salmon feeds have been a frozen ration, Oregon Moist Pellet (OMP II), developed by Crawford and Law (1972); and a dry feedstuff, the Abernathy formulation (Fowler and Burrows 1971) marketed as Silver Cup (Murray Elevators). Both present problems to Alaskan salmon culturists: the former feed is difficult to keep frozen during transport, particularly when moved in small quantities to remote facilities, and the latter is not well accepted by fry at the low water temperatures that are the norm in Alaska during late winter and early spring.

PROTEIN/ENERGY RATIONS

While the literature on fish nutrition contains the results of dietary protein/energy studies with several salmonids, little work has been performed using pink and chum salmon fry, particularly those held in salt water.

Phillips and Brockway (1959) and Watanabe (1977) found it possible to reduce the protein level of hatchery diets for rainbow trout (Salmo gairdneri) by controlling the caloric content and form. Lee and Putnam (1973) conclude that at a dietary lipid level of 16 percent, some of the protein consumed apparently was used to synthesize body fats. Takeuchi, Watanabe and Ogino (1978), also studying rainbow trout, found that the optimum dietary levels of protein and fats is 35 percent protein and 15 to 20 percent lipids. Ringrose (1971) found brook trout (Salvelinus fontinalis) require 75 kcal of metabolizable energy for each 1 percent of protein in the ration ingested. Combs et al. (1962) stated the optimum diet of chinook salmon (O. tshawytscha) has 1 calorie of energy originating from protein for each 1 calorie derived from fats.

SECTION 1. POLLOCK EGGS IN THE DIET OF POST-LARVAL PINK SALMON

OBJECTIVES AND RATIONALE

The two most widely used salmon feeds used in Alaska (OMP II and the Abernathy formula of Silver Cup) are formulated for chinook and silver salmon fry held in fresh water. Therefore, it was necessary to conduct a feeding trial to compare those two feeds with a control to decide whether or not to proceed with development of other regimens for pink and chum salmon fry, ones perhaps more efficacious under Alaskan conditions. The control needed to be a naturally occurring substance that contained a balanced profile of nutrients. Pollock roe was chosen for the control due to the excellent growth response found by workers at Salkalin Island, U.S.S.R. chum salmon hatcheries (L.R. Donaldson, College of Fisheries, University of Washington, Seattle, WA, USA, 1978, personal communication; Kanid'ev 1968 trans. 1970). Carp (Cyprinus carpio) eggs were fed to largemouth bass (Micropterus salmoides) fry in New York state fish hatcheries during the 1930s (P. Tack, School of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan, USA, 1977, personal communication). Fish eggs are consumed by a number of different species of teleost fishes (See Discussion in Section 3.)

METHODS

Fish and Environmental Conditions

Approximately 30,000 post-larval pink salmon fry resulting from the 1977 brood year were drawn from a single day's emergence at the Port San Juan Hatchery located in Prince William Sound, a large embayment in Southcentral Alaska. The fry were divided into three lots by displacement weighing and randomly assigned to 1 m³ floating pens placed in salt water. Surface salinities ranged between 6 and 7 ppt; at 1 m the salinities were between 26 and 28 ppt. Surface temperatures were 6°C at the beginning of the 34-day test and 7°C at completion.

Feed Handling and Preparation

The pollock eggs were kept frozen until just before use as was OMP II. The dry ration was stored at 10°C until use. Roe was placed in water at 100°C for 5 minutes to thaw and aid in the separation of eggs and skein. The roe was passed through a small vegetable grinder to separate individual eggs.

Feeding

The feeding rate was 2.53 percent of body weight/day (dry weight of feed to wet weight of fry) adjusted weekly. No adjustment was made for mortalities thus benefiting slower growing or higher mortality treatments by supplying more feed to the remaining fry. The three rations were fed 8 to 10 times/day by placing feed in a kitchen strainer and moving it back and forth on the water surface.

Marking

At the completion of the test 5,774 fry from the OMP II treatment and 5,831 fry from the pollock egg treatment were marked by removal of the left or right ventral fins, respectively. Fry from the Silver Cup treatment were not fin-clipped due to a lack of available marks. Fry from both marked treatment were held for 24 hours after marking as Parker, Black and Larkin (1963), four that newly marked pink salmon fry are more susceptible to predation than fry not marked. Mark recovery was by examination of returning adult fish during sales and spawning.

Analyses

Proximate and mineral content analyses were performed by the University of Alaska Agriculture Experiment Station at Palmer using standard procedures of the Association of Official Analytical Chemists (Horwitz 1975). Essential amino acid analyses were performed by a commercial laboratory (AAA Laboratory Mercer Island, Washington).

RESULTS OF CHEMICAL ANALYSES

Pollock eggs contained very high protein levels and low ash levels. They had very high levels of essential amino acids, with the exception of arginine and methionine (Tables 1 and 2), but low levels of lipids. During the drying process, prior to proximate analyses, lipid bled out of the eggs onto the foil container; consequently, the actual fat content probably was slightly higher than stated in Table 1. Eggs had a mean diameter of 474 microns (N=20).

A graph of length frequencies (Figure 1) indicates the large differential length gain to the dietary treatments. Figure 2 indicates the large differential in weight gains.

Response of Abernathy Treatment Fry

Fry fed the dry ration did not readily accept feed. Many either did not begin feeding or ate very little. The number of "pinheads" was high, and growth rate and feed conversions for this group were poor. Mortalities were in excess of twofold above other fry in the other dietary treatments (Tables 3 and 4). When observed approximately 1 hour after death, few Abernathy treatment fry were found to have autolyzed anterior ventral parietal walls indicating a low level of digestive enzyme activity. By the third week of the test, cannibalism was quite evident.

Table 1. Proximate and mineral composition of pollock eggs, averages of two values expressed on a percent dry weight

Item	Value
Crude protein ^a	83.8
Crude fat	3.2
Ash	2.0
Moisture ^b	77.0
Ca	0.08
P	1.65
K	0.22
Mg	0.07

^a(N x 6.25)

^bExpressed on an as-fed basis after blanching

=====

Table 2. Comparison of essential amino acid content of pollock eggs and essential amino acid requirements (EAAR) of chinook salmon and the percentage of EAAR supplied by pollock eggs

Amino acid	Eggs ^a	Eggs ^b	EAAR ^c	Eggs ^b / EAAR
Arginine	4.79	4.01	2.4	167.3
Histidine	2.24	1.88	0.7	268.2
Isoleucine	4.11	3.44	0.9	382.7
Leucine	7.25	6.08	1.6	379.7
Lysine	5.92	4.96	2.0	248.1
Met + Cys	3.17	2.66	0.5 ^d	531.3
Phe + Tyr	7.20	6.03	2.1 ^e	287.3
Threonine	4.19	3.51	0.9	390.1
Tryptophan	1.08	0.91	0.2	452.5
Valine	<u>4.65</u>	<u>3.90</u>	<u>1.3</u>	299.8
Sum of Amino Acids	44.60	37.38	12.6	

^aExpressed as g/100 g protein.

^bExpressed as percent of dry diet.

^cNAS (1973), expressed as percent of dry diet containing 40 percent protein

^dIn the absence of cystine.

^eIn absence of tyrosine.

Table 3. Mean lengths (mm), weights (g) and condition factors at the end of 34 days of pink salmon fry fed Silver Cup, OMP II or pollock roe

Diet	Length \pm SD ^a	Weight \pm SD ^b	K _D ^c
Silver Cup	35.1 \pm 3.63	0.312 \pm 0.1120	1.930
OMP II	36.7 \pm 4.26	0.346 \pm 0.1295	1.911
Pollock roe	45.0 \pm 3.41	0.564 \pm 0.1843	1.835

^aInitial length was 31.5 mm, N=100.

^bInitial weight was 0.176 g, N=100.

^c $100 \times \frac{\text{Weight}}{\text{Length}^3}$

=====

Table 4. Treatment means of instantaneous daily length (G_L) and weight gains (G_W), feed conversions (FC), protein efficiency ratios (PER), and mortalities of pink salmon fry fed OMP II, Silver Cup or pollock roe (N=100)

Diet	G _L ^a	G _W ^b	FC ^c	PER ^d	Mortalities (percent)
OMP II	0.45	1.99	1.87	1.02	26.1
Silver Cup	0.32	1.68	3.86	0.58	52.7
Pollock roe	1.05	3.43	0.78	1.54	18.0

^aG_L = (log_eFinal length - log_eInitial length)/time (days) x 100.

^bG_W = (log_eFinal weight - log_eInitial weight)/time (days) x 100.

^cDry weight of feed (g)/weight gain (g).

^dPER = weight gain (g)/protein fed (g).

Figure 1. Length frequencies of pink salmon fry, initial and final, fed Silver Cup or OMP II or pollock roe

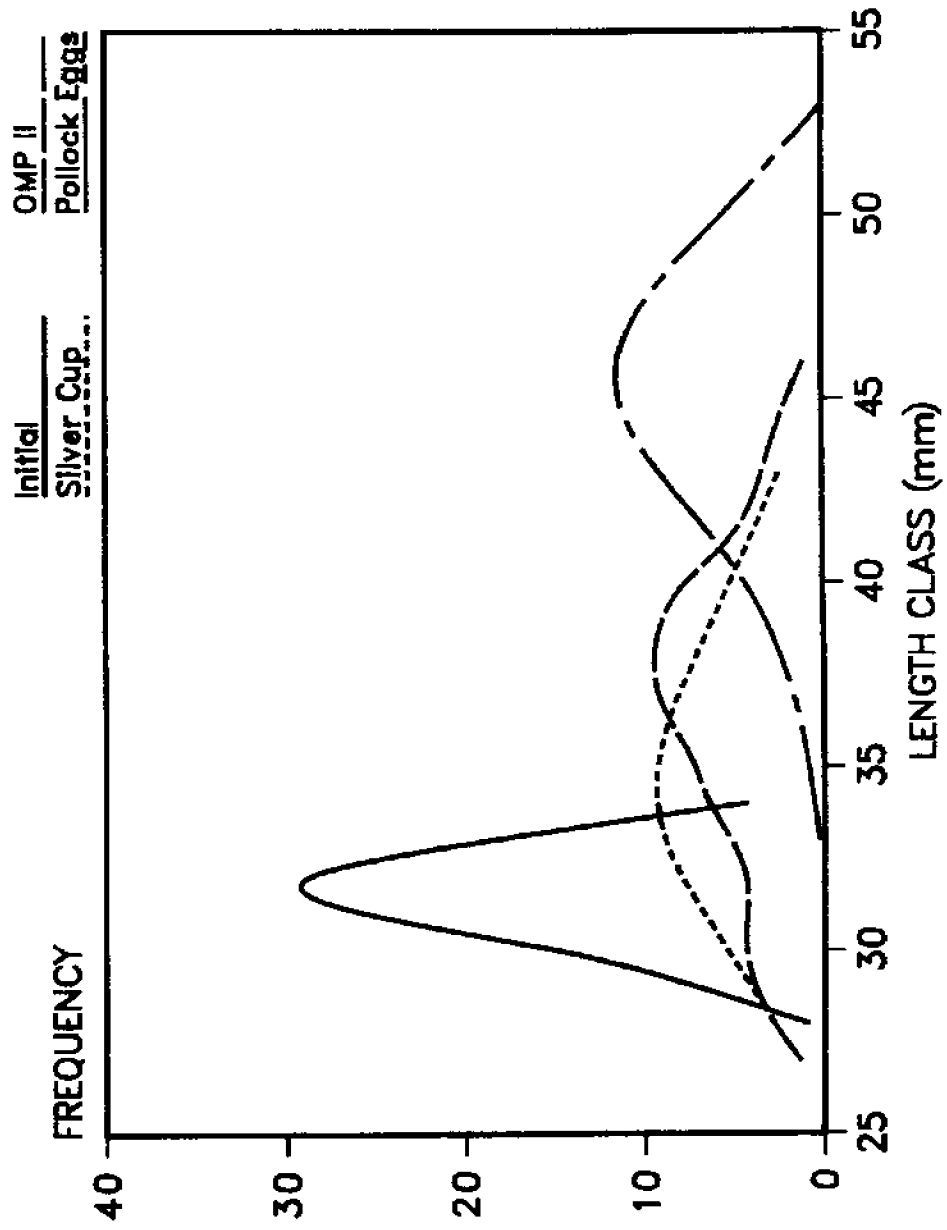
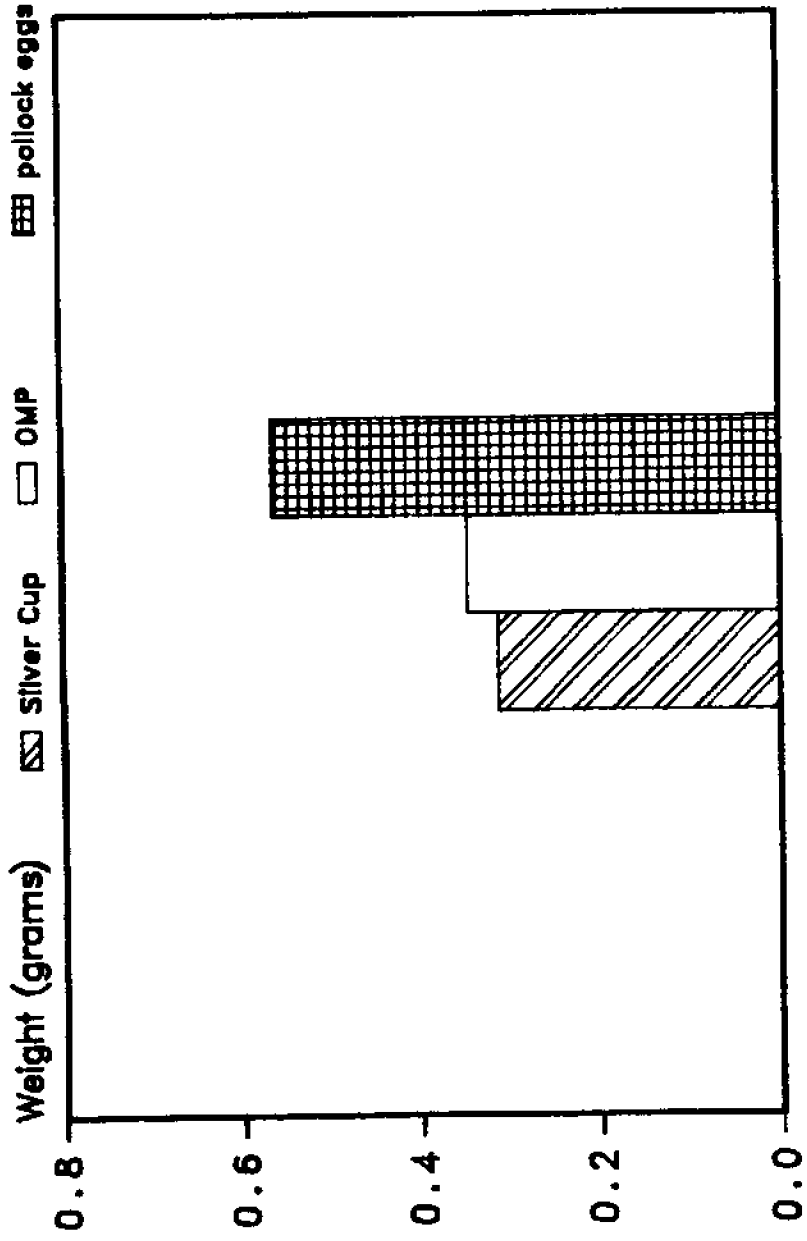


Figure 2. Final weights of pink salmon fry fed Silver Cup, OMP or pollock eggs.



Response of OMP II Treatment Fry

Fry fed the frozen ration fared much better than did fry given Silver Cup. The fish readily accepted feed. Feed particles broke apart well in the gut, forming a slurry by the time it passed through the stomach (N=16). Weight and length gains, condition factor, feed conversion, and protein efficiency ratios (PER) were intermediate to that of the dry ration and pollock roe, and comparable to production lots not part of this experiment. Little parietal wall autolysis was noted. Cannibalism was evident by the third week of the feeding trial.

Response of Pollock Egg Treatment Fry

The group given pollock roe fed very actively. The weight and length gains of roe-fed fry were the highest recorded at the Port San Juan Hatchery during three years of feeding trials. Feed conversion was very high, indicating excellent feed utilization. Eggs readily disintegrated in the stomach (N=12). Pollock egg treatment fry demonstrated a far higher incidence of gut autolysis, 83 percent versus 2 percent for each of the other two dietary treatments, indicating that a high level of proteolytic digestive enzyme activity had developed. Little cannibalism was observed.

Mortalities

Mortalities in all treatments were high and were thought by personnel from the Alaska Department of Fish and Game Pathology Laboratory to be caused by marine diatoms Chaetoceros spp. clogging gill filaments. Martin, Heard and Wertheimer (1981) reported high mortalities of pink salmon fry held for extended periods as did Kennedy, Griffionen and Solmie (1977) from Chaetoceros spp.

Marine Survivals

The total marine survival was estimated to be 3.2 percent, calculated as a percentage of all pink salmon fry released in 1978 by the Port San Juan Hatchery that survived to be intercepted as adults by the commercial fishery plus those returning to the hatchery in 1979. Calculated total marine survival of fry fed OMP II was 3.1 percent; total marine survival of fry fed pollock roe was 7.0 percent, or 2.26 times higher than the OMP II-fed fry.

DISCUSSION

Silver Cup Treatment

The Abernathy treatment fry fared poorly. With the exception of moisture content, proximate analysis and ingredients of the two test rations do not vary appreciably. Physical consistency, however, does. Opening the gut of Abernathy treatment fry revealed either empty stomachs or virtually intact feed particles in the posterior portion of the stomach, but little if any material in the fore- and hindgut (N=40). The large particles exposed little surface area to the digestive process and did not appear to pass readily into the intestine. Trituration pressures evidently were insufficient to break apart the granules. The problem appeared to be caused by the tight physical agglomeration of the crumbles inherent in ring and die pelletizing and heat drying processes.

OMP II Treatment

Fry fed OMP II grew as well as production lots given the same ration. Instantaneous rate of daily weight gain of OMP II-fed fry was very close to that of the 2.04 percent/day weight gain of fry in production pens. Marine survival was also close to that of production lots that were released an average of about a month earlier. The mean out-migration date of fry resulting from naturally spawning pink salmon in Prince William Sound is May 1; the test fish were released on June 12. Unpublished data from prior time-of-release-studies at the Port San Juan Hatchery indicate mid-April to mid-May releases result in the highest marine survivals.

Digestive System Activity of Post-larval Salmonids

The digestive system of post-larval salmonids is not well developed. The muscle tissue of the stomach walls of post-larval pink salmon is thin. Tanaka (1969) reports that the stomach is absent in the larvae of *Salmo*, developing once fry are on exogenous feed. Kawai and Ikeda (1973) state that with rainbow trout, about 40 days are required on exogenous feeds to complete morphological and functional development of the digestive system. They stated that protein digestion is highly dependent upon a trypsin-like enzyme, maltase. They also found that newly emergent fry have extremely low maltase activity levels. Levels of pepsin-like and trypsin-like enzymes and activity levels of protease and amylase were found to parallel protein levels in the diet, and were highest in the dietary treatment fed the highest protein levels, a ration comprised of 80 percent fishmeal.

Growth of Fry Fed Pollock Eggs or Marine Zooplankton

Pink salmon fry fed pollock eggs had superior weight and length gains in comparison with the gains that marine zooplankton provided. Urquhart and Barnard (1979), in an experiment conducted during the same time period as this one and at a nearby location, held pink salmon fry from the Port San Juan hatchery in an area of high current velocities and zooplankton densities. The sole source of feed was zooplankton carried into the pen (the same type of pen used in this experiment; stocking density in their experiment, however, was an order of magnitude lower, and temperatures ranged slightly lower, 4.5°C to 6.5°C). Stomach analyses of fry sampled at weekly intervals showed that calanoid copepods, copepod eggs, and larval barnacles constituted the major portion of the diet. From the data they reported, instantaneous daily weight gain was 2.49 percent/day, and length gain 0.62 percent/day. By the calculation method used here, daily length gain for free-swimming pink salmon fry captured near the Port San Juan Hatchery was 0.66 percent/day.

Cautions

While the data presented in this paper indicates certain nutritional advantages gained by including pollock roe in the regimen of newly emergent pink salmon, a few words of caution are necessary. The pens were small; consequently, fry had more surface area on which to graze on ephyphitic algae than they would in production-sized lots. Nutrients lacking in the pollock roe may have been secured from algae or other feed particles flowing through the 3.0 mm mesh of the pen walls. There is no reason to suspect that pollock

eggs contain chitin, the principal component of exoskeletons of marine zooplankton, the main prey item of pink salmon fry once released into the marine environment. Pollock eggs should be supplemented with other feedstuffs containing chitin, in order to stimulate the production of chitinase. The lipid content of pollock roe is probably too low. Lee and Putnam (1973), working with rainbow trout, contend that at a dietary lipid level of 16 percent, some of the protein consumed apparently was being used to synthesize body fats. Combs *et al.* (1962) state that the optimum protein-to-energy ratio is 1:1 for chinook salmon. Consequently, it is recommended that fish eggs be supplemented with other ingredients high in marine fish oils.

Additionally, a practical ration should avoid utilizing just one ingredient, because quality variation can result in inimicable results. A ration that is a combination of a variety of ingredients results in a lowered dependency on the quality of any one feedstuff.

SECTION 2. DIETARY RESPONSE OF POST-LARVAL PINK AND CHUM SALMON TO THREE PROTEIN/ENERGY RATIOS

MATERIALS AND METHODS

Fish and Measurements

The chum and pink salmon fry originated from first and second generation stocks, respectively, transported approximately 144 km to the Port San Juan hatchery from Port Fildago. Initial size was estimated by taking a sample of 150 fry that were individually blotted dry before length and weight measurements were taken. The pink salmon fry directly out-migrated from incubator to saltwater pens on the day the feeding trial commenced; chum salmon fry were held in fresh water for two weeks and fed OMP II prior to being moved to saltwater. Displacement weighing was used to estimate numbers of fry. At the end of the experiment, 50 fry per replicate were blotted dry prior to length and weight measurements. Mortalities were counted daily.

Environmental Conditions

Surface salinities ranged from 12 ppt at the start to 23.5 ppt at the completion of the 28-day test; salinities at 1 m varied between 19 ppt and 23 ppt. At the start of the test temperatures ranged from 5.5°C at the surface to 9°C at 1 m; at the completion of the feeding trial the surface temperature was 9.5°C and at 1 m was 11°C. Biweekly sampling found dissolved oxygen levels to be near atmospheric saturation.

Experimental Design

For each species, two replicate groups of approximately 10,000 fry each were used for each of the four diets tested. Thus 16 replicate pens were required. Groups of fish were randomly assigned to 1 m³ floating pens in salt water.

Statistical analyses used were one way analysis of variance and Duncan's New Multiple Range. The calculations were made using the SPSS statistical software package. Treatment means of length, weight, and condition factor were compared to identify statistically significant differences. Duncan's

test was used at the $P < 0.05$ level. Regression analyses were performed on a TI 52 calculator.

Feed Formulation

The three test rations were composed of decreasing proportions of dry ingredients and fresh, deboned black rockfish muscle tissue and viscera, and increasing proportions of salmon oil. The resulting diets increased in energy content while decreasing in crude protein, ash and moisture (Table 5). Proximate analyses are in Table 6; values used in the calculations for Table 6 were protein -- 5.0 kcal/g; lipid -- 9.0 kcal/g; (Phillips 1972; Smith 1976).

Feed Preparation

Feed was prepared by first separately screening the salmon, herring and crab meals through a 0.21 mm screen; the overs were discarded. The meals were mixed together for 30 minutes in a concrete mixer. The other dry ingredients were mixed together for the same time period and then mixed with the meals, again for 30 minutes. The fresh fish was heated to 80°C and allowed to cool. The dry fraction, fresh fish, and oil were combined in a 400 liter vat, using a paddle, and mixed by passing through a corn mill (Corona D-4750) five times. On the last pass through the mill, burr plates were adjusted to produce a granule. Particle size was further reduced by passing the granules through a 0.7 mm Nitex screen. Few fines were evident. Test and control rations were kept frozen at -17°C.

Feeding

Feeding rate was 4 percent of body weight/day on a projected basis adjusted weekly that assumed an instantaneous daily weight gain (G_w) of 2.5 percent/day for all treatments for the trial period. Fish were fed by hand 8 to 10 times per day. No adjustments in the feeding rates were made due to mortalities. This feeding strategy was chosen to benefit the slower growing or higher mortality fish by supplying more feed, thereby enhancing the significance of observed differences in the nutritional response of fish to the different diets. Feeding rate was set to be in excess of demand to add to the significance of observed differences.

Laboratory Analyses

Pathological and histological examinations of sampled fish were made by Alaska Department of Fish and Game Pathology Laboratory personnel in Anchorage, Alaska. Proximate analyses were conducted at the University of Alaska Agriculture Experiment Station using standard procedures of the Association of Official Analytical Chemists (Horwitz 1975). Moisture determinations of rations, ingredients, and fry were made at 100°C for 24 hours in a Fisher Isotemp oven.

Table 5. Ingredients used in test rations. Values expressed on a percent dry weight basis

Ingredient	Diet		
	Test 1	Test 2	Test 3
Item			
Salmon meal	39.1	35.0	30.0
Herring meal	11.2	10.0	8.5
Crabmeal	11.2	10.0	8.5
Whey	7.8	7.0	6.0
Yeast	7.8	7.0	6.0
Salmon oil	---	14.5	30.0
Black Rockfish	19.4	13.0	7.5
Other ingredients ^{a,b,c}	3.5	3.5	3.5
Total	100.0	100.0	100.0

^aOther ingredients present in the final diet were choline chloride, 0.5 percent; fat incapsulated ascorbic acid, 0.3 percent; OMP II Vitamin pre-mix^b, 1.4 percent; and binder, 1.3 percent^c.

^bVitamins in milligrams per kilogram of diet: Vitamin B12, 3,969; riboflavin, 3,528; niacin, 12,569; d-pantothenic acid, 7,056; menadione, 395; folic acid, 849; pyridoxine, 1,177; thiamine, 1,574; d-biotin, 39.7; inositol, 17,640. Vitamins in international units per kilogram of diet: E, 33,516.

^cAlginate, Protinal F150

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Table 6. Proximate composition in percent dry weight and calculated bioenergetic characteristics of test diets or OMP II

Item	Test 1	Test 2	Test 3	OMP II
Crude Protein	62.4	52.8	41.3	52.2
Lipid	8.4	21.8	35.3	17.9
Ash	11.9	9.1	8.6	11.3
Moisture ^a	29.0	24.6	19.0	29.1
kcal/g protein	6.2	8.6	12.6	N.A.
kcal/g of feed	3.87	4.54	5.27	4.22
kcal/l percent protein	62:1	86:1	126:1	81:1
calories from Lipid :				
calories from Protein	1:4.2	1:1.4	1:0.7	1:1.6

^aAs fed.

RESULTS

Of the four diets tested, the ration containing intermediate level of calories and protein (21.8 percent fat, 52.8 percent protein), Test 2, produced the highest weight and length gains with both pink and chum salmon fry (Tables 7 and 8; and Figure 3). There was a highly significant effect of dietary treatment found between weight gains for pink salmon fry fed the 21.8 percent fat diet ($P < 0.0000$) and the other diets; a significant effect was found between chum salmon treatments ($P < 0.0498$) fed Test 2 and the other diets. Effect of diet on length gains of pink salmon fry given the 21.8 percent fat diet was found to be highly statistically significant ($P < 0.0000$); while lengths did not differ for chum salmon fry ($P < 0.2983$). Differences in condition factors (KD) were found to be highly significant ($P < 0.0000$) for both pink and chum salmon fry. With pink salmon fry, however, the 8.4 percent fat diet produced the highest KD and the 35.8 percent fat diet the lowest, with the means of the 21.8 percent fat feed and OMP II with the highest and lowest weight gain, respectively, being in the same subset. With respect to condition factor, fry fed Test Diet 1, with no added lipid, fared as well as fry consuming Test Diet 3, which contained the maximum quantity of oil that could be physically forced in.

Fat and Glycogen Deposits

As the percentage of lipid in the rations increased, more fatty tissues were found under both gross and microscopic examination. The fry examined prior to the commencement of feeding had more extensive fat deposits than did the fry fed the low fat diet and OMP II. Glycogen appeared in tissues of fish from all rations, but not excessively so, even in the groups fed the diet with the highest lipid levels. Liver vacuolation first appeared in fry given the 21.1 percent fat ration and was more extensive in the pink salmon than in chum salmon fry. There appeared to be no increase in liver vacuolation in the fish fed the 35.3 percent fat ration over the fry fed the 21.1 percent ration for both species, although pink salmon fry did show abundant vacuolation in the epithelium of the pyloric caeca.

DISCUSSION

An increase in the metabolizable energy:protein ratio generally leads to an increase of fat deposits (Ringrose 1971; Lee and Putnam 1973; Watanabe 1977). Akiyama, Yagisawa and Nose (1981) fed a semi-purified ration to chum salmon an initially weighting of 0.9 g each at 5 percent, 10 percent, and 14 percent added fat at two temperatures. At 9.4°C no substantial difference in growth rate occurred; at 16.3°C the rations containing 10 percent and 14 percent added fat were almost identical. They concluded that the optimal level of added fat for chum salmon fry at the lower temperature was 5 percent, and at the higher temperature, 10 percent. Their data indicated that the rate of dietary fat deposition in chum salmon may be significantly lower than in rainbow trout. I did not find that to be the case in this experiment. This may be due to the use of smaller fry, from a different stock, fed different rations. In developing a practical ration for salmonid fry that are to be released into a uncontrolled marine environment, the comment by Akiyama and Nose (1980) on the value of stored energy contributing to increased marine survival should be considered. In this experiment, increasing levels of lipid in the rations used clearly increased lipid depositions.

Table 7. Mean lengths (mm) and weights (g) at the end of 28 days^a of pink salmon fry fed test diets or OMP II

Diet	Replication	Length \pm SD ^b	Weight \pm SD ^b
Test 1	1	35.7 \pm 3.21	0.3368 \pm 0.0883
	2	36.7 \pm 2.68	0.3543 \pm 0.0754
	Mean	36.2 \pm 2.99 A	0.3453 \pm 0.0824 A
Test 2	1	37.2 \pm 2.86	0.3542 \pm 0.0790
	2	37.7 \pm 2.72	0.3769 \pm 0.0780
	Mean	37.5 \pm 2.79 B	0.3657 \pm 0.0789 A
Test 3	1	36.8 \pm 2.66	0.3619 \pm 0.0751
	2	37.3 \pm 2.15	0.3342 \pm 0.0597
	Mean	37.1 \pm 2.42 B	0.3480 \pm 0.0689 A
OMP II	1	35.7 \pm 3.06	0.3156 \pm 0.0788
	2	35.6 \pm 2.29	0.3133 \pm 0.0612
	Mean	35.7 \pm 2.69 A	0.3145 \pm 0.0703 B

^aAverage initial length was 29.2 mm and average initial blotted weight was 0.2053 g.

^bMeans of lengths or of weights followed by the same letter were found to be homogeneous subsets by Duncan's New Multiple Range Test following a one-way analysis of variance.

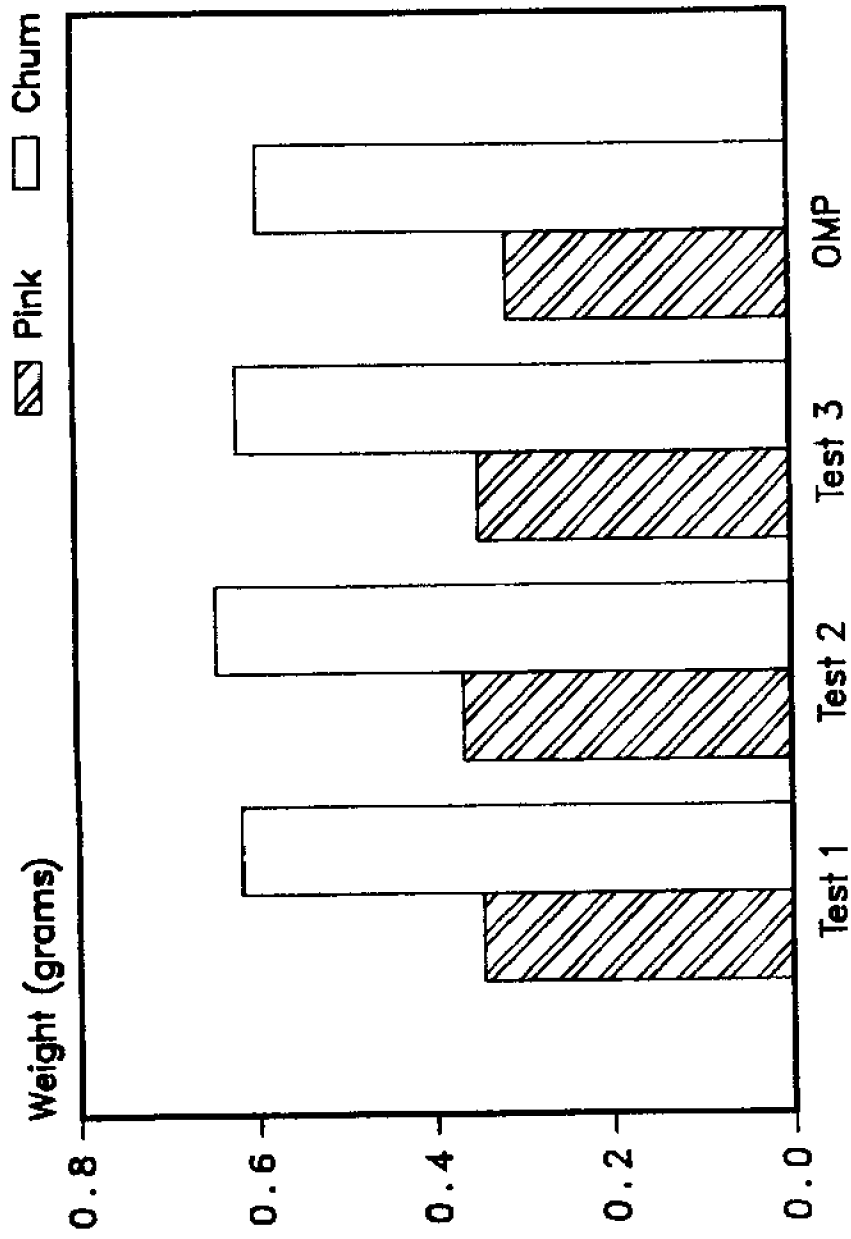
Table 8. Mean lengths (mm) and weights (g) at the end of 28 days^a of chum salmon fry fed test diets or OMP II

Diet	Replication	Length \pm SD ^b	Weight \pm SD ^b
Test 1	1	42.5 \pm 3.24	0.6014 \pm 0.1282
	2	42.9 \pm 3.18	0.6252 \pm 0.1384
	Mean	42.0 \pm 3.22 A	0.6176 \pm 0.1333 AB
Test 2	1	42.7 \pm 2.68	0.6114 \pm 0.1118
	2	43.6 \pm 3.03	0.6755 \pm 0.1291
	Mean	43.1 \pm 2.89 A	0.6434 \pm 0.1244 A
Test 3	1	42.8 \pm 2.05	0.6493 \pm 0.0944
	2	42.9 \pm 2.99	0.5887 \pm 0.1217
	Mean	42.9 \pm 2.55 A	0.6190 \pm 0.1125 AB
OMP II	1	42.8 \pm 2.72	0.6278 \pm 0.1143
	2	42.1 \pm 3.29	0.5615 \pm 0.1279
	Mean	42.5 \pm 3.03 A	0.5947 \pm 0.1252 B

^aAverage initial length was 35.41 mm, average initial blotted weight was 0.3472 g.

^bMeans of lengths or of weights followed by the same letter were found to be homogeneous subsets by Duncan's New Multiple Range Test following a one way analysis of variance.

Figure 3. Final weights of pink and chum salmon fry fed test diets or OMP.



Species Differences

Pink salmon appeared to consume smaller amounts of feed, particularly during their first five days in salt water. After this initial period, they fed quite actively, whereas chum salmon fed actively from the start -- a difference perhaps due to the two weeks of fresh water feeding the latter species had prior to the commencement of the test. Chum salmon fry had an average instantaneous daily weight gain which was 0.29 percent/day greater than that of pink salmon (Table 9); however, length gains averaged 0.15 percent/day less for chum salmon than pink salmon (Table 9). Protein efficiency ratios (PER) were lower for pink salmon than for chum, reflecting the slower weight gains among pink salmon fry. Given the same diet, weight and length gains were similar, but of a different magnitude between species ($r=0.985$). Mortalities were higher for pink salmon than for chum salmon.

Whole Body Proximate Analyses

Proximate analyses of the fry reflected dietary components closely in chum salmon fry, but less so among pink salmon fry (Table 10). Body lipid levels varied directly with dietary fat levels: for chum salmon fry, $r=0.997$; for pink salmon fry, $r=0.989$. Carcass protein and water contents were directly proportional to dietary protein levels with post-larval chum salmon ($r=0.974$, and $r=0.999$, respectively) but not for pink salmon fry ($r=0.382$ and $r=0.504$, respectively). Carcass ash content varied directly with feed ash content for chum salmon fry ($r=0.998$), but not for pink salmon fry ($r=0.585$).

Feed Conversions

Feed conversions closely reflected weight gain, as all treatments were fed equal rates. Protein Efficiency Ratios (PER) and feed conversions reflected excess feeding rates. PERs were considerably lower than other workers have found (Akiyama, Yagisawa and Nose 1981), indicating the formulations used may have contained less than completely digestible proteins for pink and chum salmon during post-larval life stage.

Mortalities

Mortalities were minimal at first but increased at the end of the test (Table 9). Fry in the OMP II treatment had the highest mortality rate -- 28.7 percent, most of which occurred during the last week of the feeding trial, and was thought to reflect their location in the pen array as much as dietary treatment. A marine diatom that clogged gill filaments identified as being from the *Chaetosolis* group was thought by personnel from the Alaska Department of Fish and Game Pathology Laboratory to be the cause. Almost all of the mortalities occurred in pens located on the seaward edge of the pen complex. Martin, Heard and Wertheimer (1981) reported high mortalities of pink salmon fry held for extended periods in marine waters as has Kennedy, Griffioen and Solmie (1977) from *Chaetoceros* spp.

Table 9. Treatment means of instantaneous daily weight (G_W) and length (G_L) gains, mortality rates, feed conversions, Bam's Condition factors (K_D), and PER of pink or chum salmon fry fed Test Diet 1, 2, or 3, or OMP II

Diet	G_W^a	G_L^b	Mort. (%)	Feed Conversion ^c	$K_D \pm SD^{d,e}$	PER ^f
Pink salmon						
Test 1	1.86	0.77	6.3	2.17	1.924 \pm 0.008 A	0.74
Test 2	2.06	0.89	5.5	1.89	1.897 \pm 0.004 B	1.00
Test 3	1.88	0.86	4.6	2.13	1.890 \pm 0.004 C	1.14
OMP II	1.52	0.72	28.7	2.78	1.897 \pm 0.008 B	0.69
Chum salmon						
Test 1	2.06	0.61	<1	1.90	1.990 \pm 0.000 A	0.84
Test 2	2.20	0.70	2.6	1.73	1.993 \pm 0.000 A	1.09
Test 3	2.07	0.69	<1	1.89	1.981 \pm 0.003 B	1.28
OMP II	1.92	0.65	<1	2.07	1.970 \pm 0.000 C	0.92

^a $G_W = (\log_e \text{Final weight} - \log_e \text{Initial weight}) / \text{time (in days)} \times 100$

^b $G_L = (\log_e \text{Final length} - \log_e \text{Initial length}) / \text{time (in days)} \times 100$

^cDry weight of feed(g)/weight gain(g).

^d $100 \times \frac{\text{Weight}^{-3}}{\text{Length}}$

^eMeans of condition factors followed by the same letter were found to be homogeneous subsets by Duncan's New Multiple Range Test following a one way analysis of variance.

^fProtein Efficiency Ratio (PER)=weight gain (g)/protein fed (g)

Table 10. Whole body proximate composition in percent dry weight of pink chum salmon fry fed Test Diet 1, 2, or 3; or OMP II^a

Diet	Water ^b	Protein	Lipid	Ash
Pink salmon				
Test 1	80.7	71.9	8.1	10.0
Test 2	79.4	68.8	12.1	9.6
Test 3	80.0	70.6	14.5	9.9
OMP II	80.7	70.6	10.4	10.9
Chum salmon				
Test 1	81.7	75.6	7.9	10.6
Test 2	80.7	70.6	12.3	9.5
Test 3	79.6	68.1	18.0	9.2
OMP II	80.3	71.9	10.0	10.0

^aMeans of two replications of 25 fry each.

^bWet weight.

Marine Survival

No estimate of the relative marine survival of dietary treatment fry is available because fry marking was not performed. Total marine survival was estimated to be 6.6 percent from fry release to adult return to the commercial fishery and to the hatchery of all fry released from Port San Juan Hatchery during the spring of 1979.

SECTION 3. PRODUCTION-SCALE TESTING OF AN INTERMEDIATE LEVEL OF PROTEIN AND LIPID RATION

OBJECTIVES

The purpose of the third year's test was to determine on a production scale if the nutritional response of post-larval pink and chum salmon to the standard production ration was significantly different from that of a newly introduced closed formula ration or from the test diet comprised of mainly locally derived fisheries byproducts.

METHODS

Experimental Design

The fry used were brood year 1979 F2 generation pink and P1 generation chum salmon originating from Ewan and Port Fildago area stocks respectively, and transferred to the Port San Juan hatchery located on Evans Island, Prince William Sound, Alaska. Pink salmon fry had directly outmigrated from their incubators to saltwater pens on the day the feeding trial commenced; chum salmon were held in fresh water for an average of 14 days and fed OMP II and pasteurized cod roe at 0.5 percent of body weight prior to being piped to salt water. A total of 3.6×10^6 pink salmon fry were divided into lots of approximately 4×10^5 fry for each of three replications for each diet by displacement weighing. All fry were transferred via pipeline. The three treatment replications for chum salmon diet treatments were restricted to 10,000 each due to the small numbers available. At the beginning of the feeding trial, a sample of 100 fry of each species were individually blotted dry, and length/weight measurements taken. At the end of the test, length/weight measurements were taken from 100 fry from each replication using the same procedures as with the initial measurements. Mortalities were counted daily.

Marking

Slightly more than 10,000 pink salmon fry from each replication of the OMP II-fed fry and the test ration-fed fry were marked by removal of the left ventral fin (OMP II treatment, 30,962 total) and right ventral fin (test group, 30,775 total). In a separate experiment of release timing, two additional lots -- early, and mid-release -- of pink salmon fry were marked (the two dietary test treatments served as the late release). The early and mid-release groups were fed OMP II and cod roe. Pink salmon fry from the Biodiet treatment and chum salmon fry were not fin-clipped due to a lack of available marks.

Mark recoveries were by sampling adult fish, cost recovery and broodstock, that returned to the hatchery in the following summer.

Environmental Conditions

Pink salmon fry were held in 20 m³ pens; chum were held in 1 m³ floating pens. Densities were the same as used in production lots, 4.0kg/m³. Surface salinities ranged from 11 ppt at the start to 25 ppt at the completion of the test. Salinity at 1 m varied between 19 ppt and 27 ppt for the 24 day (pink salmon) and 18 day (chum salmon) test. Initial temperatures were 5.5°C at the surface and 9°C at 1 m. At completion, the surface temperature was 9.5°C and 13.0°C at 1 m. Biweekly sampling found dissolved oxygen levels to be at or near atmospheric equilibrium.

Feed Formulation

Three dietary treatments were used: OMP II, the control ration; Biodiet (Bioproducts, Inc.), a closed formula intermediate moisture feed; and a test ration. The test ration was formulated the same as the previous year's Test Ration 2 with two exceptions. Due to a mechanical failure, black rockfish muscle tissue and viscera were unavailable. Pasteurized roe from Pacific cod was substituted equally on a dry weight basis and potassium sorbate was added (Table 11, Table 12). Dry weights of the various diet components were determined using a Fisher Isotemp oven; samples were kept at 100°C for 24 hrs.

Feed Preparation

The test ration was prepared by first screening the salmon, herring and crab meals through a 1.19 mm screen. The overs (16.0 percent, 16.6 percent, and 25.9 percent of the initial weight respectively) were discarded. The meals were mixed together for 30 minutes in a concrete mixer. The other dry ingredients were mixed together for the same length of time, then mixed with the meals again for 30 minutes. The cod roe was thawed overnight at 10°C, and heated until it reached 80°C. Then the binder and potassium sorbate were added and the mixture allowed to cool. Mixing of the major components was by combining the dry fraction, roe, and oil in a Hobart 10 hp grinder using progressively smaller grinder plates. After the fifth pass through the grinder the ration was cooled by spreading it on polyethelene sheeting. A Corona D-4750 corn mill was used to produce a granule. The feed was sized by passing it through a 0.7 mm Nitex screen. The test ration was placed in plastic-lined kraft bags, purged with compressed gaseous nitrogen, and kept at 10°C.

Feeding Rates

Feeding rates were the same as those in production lots and ranged from 1 percent to 4.5 percent of body weight/day on a programmed basis adjusted weekly that assumed an initial G_w of 0.75 percent/day and increased to 3.75 percent/day for pink salmon and 1.0 percent to 4.0 percent for chum salmon at the end of the trial. Feeding rates were judged by the fish culturists to be ad libitum. Fish were manually fed 20 times/day. No adjustments in the feeding rates were made due to mortalities. This feeding strategy presented an advantage to slower growing fry or treatments with higher mortalities as these groups received relatively more feed than the other treatments.

Table 11. Composition of test ration

Ingredient	Percent Dry Weight
Salmon meal	35.12
Herring meal	10.06
Crab meal	10.06
Brewers' yeast	7.01
Whey	7.01
OMP II Vitamin pre-mix ^a	1.70
Choline chloride ^b	0.50
Alginate ^b	1.20
Potassium sorbate	0.40
Salmon oil	15.94
Cod roe	11.00
Total	100.00

^aVitamins in mg/kg of diet: Vitamin B12, 3,969; riboflavin, 3,528; niacin, 12,569; d-pantothenic acid, 7,056; menadione, 395; folic acid, 849; pyridoxine, 1,177; thiamine, 1,574; d-biotin, 39.7; inositol, 17,640; fat encapsulated ascorbic acid, 3,000. Vitamins in international units/per kg of diet: E, 33,516.

^bAlginate, Protinal F150

Table 12. Proximate analysis of the test ration and cod roe expressed on a percent dry weight basis

Item	Test Ration	Cod Roe
Crude protein	52.8	88.09
Fat	22.20	1.85
Ash	15.50	2.70
Moisture ^a	31.0	81.61

^aExpressed on an as-fed basis.

Analyses

Proximate composition determinations were conducted by the University of Alaska Agriculture Experiment Stations at Palmer using the standard procedures of the Association of Official Analytical Chemists (Horwitz 1975). Statistical tests used were one-way and nested one-way analysis of variance and Duncan's New Multiple Range, and Tukey's honestly significant difference (Steele and Torrie 1960) performed on the University of Alaska Honeywell main frame computer using the SPSS statistical software package to determine if differences in means of weight, length, and condition factor were statistically significant. Duncan's and Tukey's tests were used at the $P < 0.05$ level.

RESULTS

Of the three diets tested, the test ration produced the highest instantaneous daily weight and length gains, and condition factor for both pink and chum salmon fry (Tables 13, 14 and 15; and Figure 4), with chum salmon demonstrating the most pronounced response. Highly significant differences in weight and length gains, and condition factor were found with both pink and chum salmon fry fed the test diet when compared to the other two treatments ($P < 0.0000$).

Comparison With Previous Year's Feeding Trial

Pink salmon fry fed OMP II demonstrated minimal differences during both years with the exception of rate of increase in length (Table 15). Pink salmon fry emerged 4.4 mm greater in length than those from the prior year (an inherent stock difference between even and odd year fish is suggested) but demonstrated a much lower rate of increase in length once given exogenous feed. Differences between years for test ration were marked, with feed conversions being twice as favorable with the previous year's formulation as well as rate of weight gain being improved.

Chum salmon fed OMP II had consistent increases in length and feed conversion between years; the increase in weight was greater during this year. The dietary response of test diet treatments during this year was improved over that of the previous year: Improvement in rate of weight gain and in feed conversion were especially notable, with rate of length increase also improved. Again, chum were observed to feed much more actively.

Proximate Analyses

The nutritional response as indicated by proximate analysis of both pink and chum salmon fry fed Biodiet was intermediate to that of fry fed OMP II and the test diet, with pink salmon fry fairing somewhat better (Table 16). Chum salmon fry fed Biodiet had a higher ash and lower lipid and protein composition relative to fry fed the other two rations, whereas pink salmon fry fed Biodiet had slightly higher lipid, and slightly lower ash content to fry fed even the test diet.

Table 13. Mean final lengths (mm) and weights (g) at the end of 24 days of pink salmon fry fed OMP II, Biodiet or the test diet

Diet	Replication	Length \pm SD ^{a,b}	Weight \pm SD ^{a,b}
OMP II	1	33.9 \pm 2.44	0.2360 \pm 0.0717
	2	34.6 \pm 2.43	0.2497 \pm 0.0650
	3	35.2 \pm 2.37	0.2705 \pm 0.0715
	Mean	34.6 \pm 2.46 A	0.2521 \pm 0.0707 A
Biodiet	1	37.1 \pm 2.56	0.3311 \pm 0.0765
	2	34.8 \pm 2.80	0.2657 \pm 0.0657
	3	35.6 \pm 2.39	0.2870 \pm 0.0748
	Mean	35.8 \pm 2.52 B	0.2946 \pm 0.0772 B
Test	1	37.1 \pm 2.92	0.3274 \pm 0.0851
	2	36.5 \pm 2.52	0.3194 \pm 0.0762
	3	36.4 \pm 2.59	0.3015 \pm 0.0757
	Mean	36.7 \pm 2.69 C	0.3161 \pm 0.0796 C

^aAverage initial length was 33.6 mm each and average initial blotted weight was 0.1782 g each.

^bMeans of lengths and weights followed by the same letter were found to be homogeneous subsets.

Table 14. Mean final lengths (mm) and weights (g) at the end of 18 days of chum salmon fry fed OMP II, Biodiet or test diet

Diet	Replication	Length \pm SD ^{a,b}	Weight \pm SD ^{a,b}
OMP II	1	41.8 \pm 2.22	0.5205 \pm 0.1053
	2	42.5 \pm 2.94	0.5217 \pm 0.1151
	3	41.8 \pm 2.68	0.5150 \pm 0.1229
	Mean	42.0 \pm 2.65 A	0.5190 \pm 0.1143 A
Biodiet	1	42.3 \pm 2.69	0.5288 \pm 0.1203
	2	42.4 \pm 2.67	0.5338 \pm 0.1231
	3	42.2 \pm 2.73	0.5358 \pm 0.1324
	Mean	42.3 \pm 2.69 A	0.5328 \pm 0.1250 A
Test	1	44.4 \pm 1.82	0.6519 \pm 0.0934
	2	44.5 \pm 2.14	0.6572 \pm 0.1049
	3	44.3 \pm 2.16	0.6535 \pm 0.0989
	Mean	44.4 \pm 2.04 B	0.6542 \pm 0.0989 B

^aAverage initial length was 37.6 mm each and average initial blotted weight was 0.3184 g each.

^bMeans of weights followed by the same letter were found to be homogeneous subsets.

Table 15. Treatment means of instantaneous daily weight (G_W) and length gains (G_L) mortalities, feed conversions, Bam's condition factors (KD), and PER of pink and chum salmon fry fed OMP II, Biodiet or test diet^a

Diet	G_W^b	G_L^c	Mort. (%)	Feed Conversion ^d	$K_D + SD^{e,f}$	PER ^g
Pink Salmon						
OMP II	1.45	0.12	<1	2.04	1.809 ± 0.074 A	0.96
Biodiet	2.09	0.26	<1	1.31	1.841 ± 0.068 B	1.33
Test	2.39	0.37	<1	1.11	1.844 ± 0.077 B	1.57
Chum Salmon						
OMP II	2.71	0.61	<1	1.84	1.901 ± 0.079 A	1.07
Biodiet	2.86	0.65	1.0	1.54	1.902 ± 0.066 A	1.13
Test	4.00	0.92	1.2	1.11	1.949 ± 0.044 B	1.56

^aTreatment means of 100 fry from each of the three replications.

^b $G_W = (\log_e \text{Final weight} - \log_e \text{Initial weight}) / \text{time (in days)} \times 100$

^c $G_L = (\log_e \text{Final length} - \log_e \text{Initial length}) / \text{time (in days)} \times 100$

^dDry weight of feed(g)/weight gain(g).

^e $100 \times \frac{\text{Weight}^{-3}}{\text{Length}}$

^fMeans followed by the same letter are homogeneous subsets.

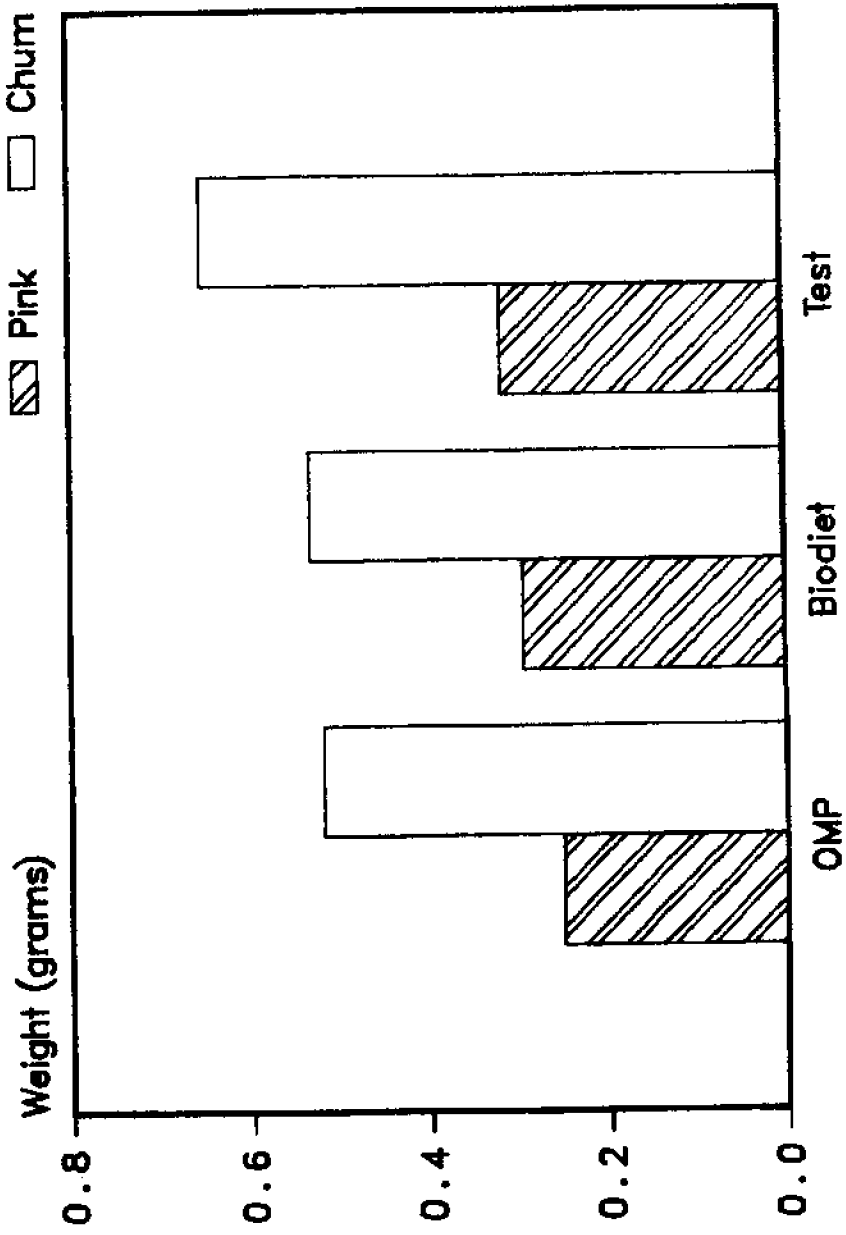
^gProtein Efficiency Ratio (PER) = weight gain (g)/protein fed (g)

Table 16. Whole body proximate composition of pink and chum salmon fry, initial and final, fed OMP II, Biodiet or test diet^a

<u>Species/Diet</u>	<u>Crude Protein</u>	<u>Lipid</u>	<u>Ash</u>
Pink Salmon			
Initial	83.12	5.22	4.60
OMP II	79.39	6.18	5.80
Biodiet	75.42	9.77	5.89
Test	74.79	9.07	6.21
Chum Salmon			
Initial	81.65	6.32	4.67
OMP II	77.38	8.57	6.69
Biodiet	76.81	7.62	8.21
Test	73.48	12.91	6.24

^a Means of two replications of 25 fry each. Results expressed as mean of three replications for each dietary treatment.

Figure 4. Final weights of pink and chum salmon fed OMP or Biodiet or test diet.



Marine Survivals

Of the return of 905,938 adult pink salmon to Port San Juan Hatchery, 35.36 percent of the total (320,362 fish) were checked for missing fins. Marked fish recovery of pink salmon fed OMP II was 253 and test ration, 315. Returns to the hatchery, including an expansion factor for the adult fish present but not checked for missing fins, was estimated to be 2.31 percent for fish fed OMP II and 2.89 percent for those fed the test ration. Alaska Department of Fish and Game personnel estimated the fisheries interception rate on the returning adult fish prior to reaching the hatchery to be 60 percent for an apparent marine survival of 5.78 and 7.24 percent for OMP II-fed and test diet-fed pink salmon lots, respectively. The other two mark groups, the early and mid-releases, had an apparent marine survival of 7.73 percent and 7.67 percent, respectively. The overall return of 10.5 percent was the highest recorded at Port San Juan Hatchery.

DISCUSSION

The only differences between the formulation of the test diet fed in 1980 and Test Diet 2 used in 1979, were the substitution of cod roe for black rockfish flesh and viscera, and the inclusion of a low level of potassium sorbate. Other authors have reported successes using ova from teleost fish in the diet of largemouth bass fry (Micropterus salmoides), a species notoriously difficult to adapt to formulated rations (Brandenburg, Ray and Lewis 1979; Willis and Flickinger 1981) and for smallmouth bass (Micropterus dolomieu) (Willis and Flickinger 1982).

Fish Eggs as a Prey Item in Fish Diets

Fish eggs have been reported in the diets of a wide variety of fish species: Johnson (1983) found sub-yearling chinook salmon to feed on fish eggs. Cooney et al. (1978) reported trace occurrences in the gut of pink salmon. Magomedov and Murzabikova (1978) found that chum salmon feed on fish eggs. Wells (1980) commonly found fish ova in the gut of yellow perch (Perca flavescens) and spottail shiners (Notropis hudsonius). Elrod et al. (1981) also found fish eggs in the gut of yellow perch and white perch (Morone americana). Talent (1976) found fish ova to be important in the diet of leopard sharks (Triakis semifasciata) <90 cm in length. Given the similarities in the morphological development of larval teleost fish, nutritional requirements may well be similar for post-larval bony fish. Additionally, the bioavailability of proteins in fish eggs may be greater than the proteins in fish flesh and meals due to the higher level of complexing as well as the insolubilizing effect of processing temperatures on fish meals.

Fin Clips

Fin removal had a probable adverse effect on marine survival of all mark treatments. The literature on differential mortality due to fin removal marking is varied: Brynildson and Brynildson (1967) report no significant difference between clipped and unclipped brown trout (Salmo trutta). Nicola and Cordone (1973) found survival reductions of up to 80 percent with rainbow trout. Ricker (1976) reported greatly reduced survivals of fin-clipped pink salmon. Handicap ratios of unclipped to clipped lots in his study ranged from

1.119 to 1.575. The handicap ratios of instantaneous rate of total mortality, Z (Ricker 1975), between the estimated returns from unmarked and marked lots were early-release 1.136, mid-release 1.140, OMP II-fed 1.266, and test diet-fed 1.166. Fin removal procedures are difficult to precisely standardize because of differences in the experience levels of the individuals involved, unknown regeneration rate between different clips, and variant quality control standards.

SECTION 4. FURTHER PRODUCTION-SCALE TESTS SUBSTITUTING DEBONED BLACK ROCKFISH FLESH AND VISCERA FOR COD EGGS IN THE TEST DIET

OBJECTIVES

The purpose of the fourth year's test was to determine if on a production scale significant effects could be detected between the nutritional responses of post-larval pink and chum salmon fed Biodiet and the test ration using fresh fish for the wet fraction.

METHODS

Experimental Design

The fry used were F3 generation pink and P1 generation chum salmon originating from Ewan and Port Fildago area stocks respectively, transferred to Port San Juan Hatchery. Pink salmon fry had directly outmigrated from their incubators to salt water pens on the day the feeding trial commenced; chum salmon were held in fresh water for an average of five days and fed Biodiet 0.5 percent of body weight prior to being piped to salt water. A total of 3.2×10^6 pink salmon fry were divided into eight lots of 4×10^5 fry for each of four replications for each of the two diets tested. Numbers of fry were estimated by displacement weighing. The fry were piped to the 20 m³ pens. The three treatment replications for the two chum salmon diet treatment were restricted to 10,000 each due to the small numbers of fry available; they were held in 1 m³ floating pens. Dietary treatment was randomly assigned. At the end of the test, sample length and weight determinations were made on 100 patted-dry fry per replication.

Environmental Conditions

Surface salinities ranged from 10 ppt at the start to 27 ppt at the completion of the test. Salinity at 1 m varied between 19 ppt and 27 ppt for the 20 day (pink salmon) and 16 day (chum salmon) test. Initial temperatures were 7°C at the surface; at completion surface temperature was 9.5°C. Biweekly sampling found dissolved oxygen levels to be near or at atmospheric saturation.

Marking

A total of 112,007 pink salmon fry from the Biodiet-fed treatment and 110,605 fry the test ration-fed fry were marked by removal of left ventral fin (Biodiet) and adipose fin (test). In a unrelated test of release timing, 111,044 pink fry were marked by removal of the right ventral fin. Chum salmon fry were not fin-clipped due to a lack of available marks. Mark recoveries were by sampling adult fish, cost recovery and broodstock, that returned to the hatchery during the following summer.

Feed Formulation

The rations tested were Biodiet and the previous year's test ration. The test ration was formulated the same as was the previous year's test ration with one exception: Fresh black rockfish muscle tissue and viscera were used instead of cod roe. Dry weights of the various diet components were determined using a Fisher Isotemp oven; samples were kept at 100°C for 24 hrs. (See Tables 17 and 18.)

Feed Preparation

The test ration was prepared by first screening the salmon, herring and crab meals through a 1.0 mm screen. The overs (8.7 percent, 8.8 percent, and 27.8 percent of the initial weight respectively) were discarded. The meals were mixed together for 30 minutes in a concrete mixer. The other dry ingredients were mixed together for the same length of time and then mixed with the meals again for 30 minutes. The black rockfish were ground whole and passed through a deboning attachment on a 10 hp Hobart meat grinder. The fresh fish was heated until it reached 80°C, then the binder and potassium sorbate added and the mixture allowed to cool. Mixing of the major components was by combining the dry fraction, fresh fish and oil in the grinder using progressively smaller grinder plates. After the fifth pass through, the ration was cooled by spreading it on polyethelene sheeting. A Corona D-4750 corn mill was used to produce a granule. The feed was sized by passing it through a 0.7 mm Nitex screen. The test ration was placed in plastic-lined kraft bags and purged with compressed nitrogen gas. The feed was stored at 10°C until use.

Feeding

Feeding rates were the same as those used in production lots. Rates ranged from 1 percent to 4.5 percent of body weight/day on a programmed basis, adjusted weekly. Feeding rates were judged by the fish culturists to be ad libitum. Fish were manually fed 4 to 12 times/day. No adjustments in the feeding rates were made due to mortalities. This feeding strategy presented an advantage to slower growing fry or treatments with higher mortalities as they received more feed than the other treatments.

Analyses

Proximate analyses were performed at the University of Alaska Agricultural Research Station using standard procedures of the Association of Official Analytical Chemists (Horwitz 1975). Statistical analyses used were one-way and nested one-way analysis of variance performed on the University of Alaska Honeywell computer using the SPSS statistical software package to determine if differences in means of weight, length, and condition factor were statistically significant. Tukey's test of least significant difference for multiple comparison was used to compare the differences among marked groups of fish.

Table 17. Composition of the test ration

<u>Ingredient</u>	<u>Percent Dry Weight</u>
Salmon meal	35.12
Herring meal	10.06
Crab meal	10.06
Brewers' yeast	7.01
Whey	7.01
OMP II Vitamin pre-mix ^a	1.70
Choline ^b chloride	0.50
Alginate ^b	1.20
Potassium sorbate	0.40
Salmon oil	15.94
Black rockfish flesh	<u>11.00</u>
Total	100.00

^a Vitamins in mg/kg of diet: Vitamin B12, 3,969; riboflavin, 3,528; niacin, 12,569; d-pantothenic acid, 7,056; menadione, 395; folic acid, 849; pyridoxine, 1,177; thiamine, 1,574; d-biotin, 39.7; inositol, 17,640; fat encapsulated ascorbic acid, 3,000. Vitamins in international units/kg of diet: E, 33,516.

^bBinder, Protinal F150

Table 18. Proximate analysis of the test ration or Biodiet expressed on a percent dry weight basis

<u>Item</u>	<u>Test Ration</u>	<u>Biodiet</u>
Crude protein	55.38	57.2
Fat	22.20	20.32
Ash ^a	15.50	13.1
Moisture ^a	21.0	20.2

^aExpressed on an as-fed basis.

RESULTS

Statistical Analyses

Of the two diets tested, Biodiet produced more favorable nutritional responses. Instantaneous daily weight and length gains (Tables 19 and 20; and Figure 5) were better, and condition factors were marginally better for Biodiet-fed chum salmon fry. Condition factors were marginally better for test diet-fed pink salmon fry. Protein Efficiency Ratios and feed conversions reflected weight and length gains (Table 21). Proximate analyses demonstrated little differences (Table 22). Chum salmon again demonstrated the most pronounced dietary response. Pink salmon fed Biodiet were found to have a statistical difference in weight gain ($P < 0.0095$), statistical significance in condition factor ($P < 0.0278$), and no difference in length ($P < 0.3313$). Chum salmon fry given Biodiet did not demonstrate statistical differences in either weight gain ($P < 0.059$), or for condition factor ($P < 0.4837$), but did in regards to length ($P < 0.0213$). Return rates for the early release treatment were significant at the $P = 0.05$ level over that of the test diet treatment, but not over the Biodiet lot. There was no statistical difference between the return rates of the Biodiet-fed treatment and that of the test diet-fed group.

The situation was reversed in the interspecific feeding responses of fry tested with that of the previous year. Pink salmon for all treatments initially fed more actively than did chum salmon fry. The shorter fresh water period chum fry were exposed to during this year's test was thought to be the causative factor.

Mark Recovery

Of the 1.951×10^6 adult pink salmon that returned to Port San Juan Hatchery during 1982, 2.515×10^5 fish were checked for missing fins. Marked fish recovery of pink salmon fed Biodiet was 140 and test ration, 111. Survival to the hatchery (expanded to account for fish not checked for marks) was 0.97 percent for fish fed Biodiet and 0.78 percent for those fed the test ration. Alaska Department of Fish and Game personnel estimated commercial fisheries interception rate on the returning adult fish prior to reaching the hatchery to be 62 percent for an overall marine survival estimate of 2.55 percent and 2.05 percent for the Biodiet and test diet treatments, respectively. Apparent marine survival of the early release test lot was 3.42 percent, demonstrating the importance of time of release as well as feeding. Marking handicap ratios of the instantaneous mortality rate, Z (Ricker 1975), between unmarked and marked salmon fry were 1.296 for the early release lot, 1.408 for the Biodiet treatment, and test diet group, 1.493. Overall total marine survival, fry to returning adult (fishery + hatchery) of all fry released was estimated to be 7.4 percent.

Ricker (1976) reported reduced survival of fin clipped pink salmon; handicap ratios for instantaneous mortality rates of marked to unmarked fry ranged from 1.119 - 1.575. Martin, Heard and Wertheimer (1981) found a substantially lower handicap ratio, 1.07, between marked and unmarked unfed fry; the authors concluded that the release timing of the two particular lots of pink salmon fry corresponded with optimum estuarian conditions.

Table 19. Mean final lengths (mm) and weights (g) at the end of 20 days of pink salmon fry fed Biodiet or test diet

Diet	Replication	Length \pm SD ^a	Weight \pm SD ^a
Biodiet	1	38.9 \pm 3.54	0.3346 \pm 0.0884
	2	37.6 \pm 2.65	0.2905 \pm 0.0712
	3	39.3 \pm 2.81	0.3354 \pm 0.0773
	4	39.7 \pm 2.84	0.3529 \pm 0.0863
	Mean	38.9 \pm 3.07	0.3283 \pm 0.0833
Test	1	38.1 \pm 2.24	0.2945 \pm 0.0553
	2	38.6 \pm 2.35	0.3063 \pm 0.0679
	3	38.7 \pm 2.30	0.3199 \pm 0.0550
	4	39.3 \pm 2.84	0.3186 \pm 0.0606
	Mean	38.7 \pm 2.32	0.3148 \pm 0.0796

^aAverage initial length was 32.7 mm each and average initial blotted weight was 0.1940 g each (N = 134).

Table 20. Mean final lengths (mm) and weights (g) at the end of 16 days of chum salmon fry fed Biodiet or test diet

Diet	Replication	Length \pm SD ^a	Weight \pm SD ^a
Biodiet	1	43.0 \pm 5.52	0.5337 \pm 0.1214
	2	43.1 \pm 2.98	0.5378 \pm 0.1199
	3	42.3 \pm 2.87	0.5119 \pm 0.1199
	Mean	42.8 \pm 3.99	0.5278 \pm 0.1205
Test	1	41.8 \pm 3.02	0.5037 \pm 0.1134
	2	42.2 \pm 2.81	0.5079 \pm 0.1076
	3	42.5 \pm 2.64	0.5183 \pm 0.1115
	Mean	42.2 \pm 2.83	0.5100 \pm 0.1107

^aAverage initial length was 37.9 mm each and average initial blotted weight was 0.3254 g each (N = 147).

Table 21. Treatment means of instantaneous daily weight (G_W) and length (G_L) gains, mortalities, feed conversions (FC), Bam's condition factors, and PER of pink or chum salmon fry fed Biodiet or test diet^a

Diet	G_W^b	G_L^c	Mort. (%)	Feed Conversion ^d	$K_D \pm SD^e$	PER ^f
Pink salmon						
Biodiet	2.63	0.87	<1	1.78	1.761 ± 0.059	2.10
Test	2.42	0.84	<1	1.96	1.752 ± 0.063	1.97
Chum Salmon						
BioDiet	3.03	0.76	1.0	1.53	1.881 ± 0.095	2.27
Test	2.81	0.67	1.2	1.66	1.886 ± 0.063	2.17

^aAverages of treatment means.

^b $G_W = (\log_e \text{Final weight} - \log_e \text{Initial weight}) / \text{time (in days)} \times 100$

^c $G_L = (\log_e \text{Final length} - \log_e \text{Initial length}) / \text{time (in days)} \times 100$

^dDry weight of feed(g)/weight gain(g).

^e $100 \times \frac{\text{weight}^{-3}}{\text{Length}}$

^fProtein Efficiency Ratio (PER) = Weight gain (g)/Protein fed (g)

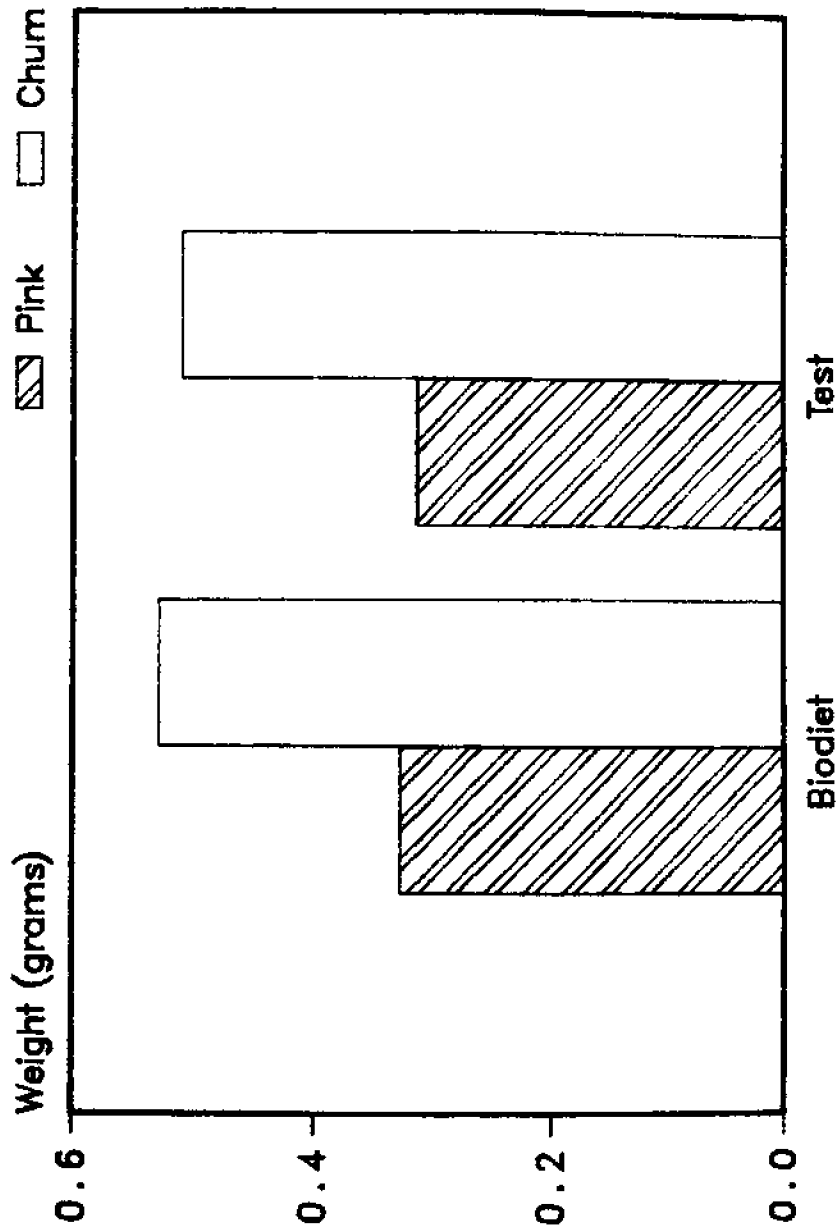
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Table 22. Whole body proximate composition of pink and chum salmon fry, initial and final, fed Biodiet or test diet^a

Diet	Protein	Lipid	Ash
Pink Salmon			
Initial	82.21	5.32	4.54
Biodiet	76.46	9.23	6.95
Test	74.79	9.07	6.21
Chum Salmon			
Initial	82.56	5.63	4.76
BioDiet	79.18	6.06	7.39
Test	78.83	6.15	7.66

^aMeans of two replications of 25 fry each. Results expressed as mean of the four replications for each dietary treatment.

Figure 5. Final weights of pink and chum salmon fry fed Biodiet or test diet.



DISCUSSION

The only difference in formulation of the test ration between this year's efforts and the previous year's was the substitution of fresh fish flesh for cod eggs. The fresh fish/egg component was included at a relatively low level (11 percent dry weight). A large relative difference was found, however, in nutritional response ($G_M = -0.51$) with pink salmon fry and with chum salmon fry ($G_M = -1.36$) between the two formulations and years, and Biodiet. A direct comparison between the two year's formulation is necessary before firm conclusions can be drawn.

CONCLUSIONS

Year 01. Heat processed pollock roe (and probably that of other teleost fishes) may provide a nearly balanced regimen to biochemically readily-digestible nutrients to post-larval pink salmon superior to that of naturally occurring marine zooplankton, and formulated practical rations. Feeding pollock roe to newly emergent pink salmon fry evidently induces a rapid development of the digestive system. Diets of post-larval pink salmon should be as high in easily digestible protein as is practical.

Year 02. Based upon the evidence presented here, the response to a given calorie:protein ratio of post-larval pink and chum salmon can be assumed to be analogous with that of other salmonids. Approximately 50 percent of the dietary calories should come from lipids, and 50 percent from proteins, with the lipid level approaching 20 percent at crude protein levels above 50 percent. As evidenced by body lipid positions, pink salmon fry may require slightly lower dietary fat levels than do chum salmon fry.

Year 03. Although the test ration-fed treatment had a 25.3 percent survival improvement over that of the control group, the increase in return was not commensurate with increases in weight or body lipid content, length gain, or decreases in feed conversion efficiencies between the two marked treatments. The influence of factors beyond the period of care by hatchery personnel, principally favorable nearshore conditions, were thought to be more important than the particular ration fed. It may well be that during years of optimal estuarine conditions the particular regimen employed is not as important as it is during less favorable years.

Year 04. The slight increases in rate of weight gain (8.7 percent) and length gain (3.6 percent) that the Biodiet treatment enjoyed over that of the test diet lot resulted in an apparent 26.1 percent increase in marine survival, which was statistically significant ($P < 0.01$). If recovery of marked fish is indicative of relative marine survival and no differential mortality occurs between different marks (a view not shared by Ricker 1976), a qualitative differential exists in the value of regimens that exceeds measured differences at time of release during years of average marine productivity.

Overall. Chum salmon fry were found to gain weight at a more rapid rate than pink salmon fry in all nine tests in which pink and chum salmon fry were given the same rations. (See Appendices 1 to 8.) Chum salmon fry had an instantaneous daily weight gain that average 129.56 percent of that of pink salmon fry given the same diets, which was statistically highly significant ($P < 0.01$). The rate of length gain, however, was variable. During 1979, chum fry averaged 82.4 percent of the instantaneous daily length gain of pink salmon; in 1980, it was 334.0 percent; and in 1981, 83.6 percent. Regression analysis found the correlation of the rate of length gain to be poorly related to rate of weight gain ($r = 0.5553$) for pink salmon fry. Chum salmon fry, however, had a somewhat higher correlation: $r = 0.7901$. Condition factor was not found to be closely related to instantaneous daily length gain with pink ($r = -0.1079$) or chum ($r = -0.3758$) salmon fry. Condition factor was not found to be closely related to rate of weight gain either: pink ($r = -0.4688$) or chum salmon fry ($r = -0.1980$).

There appear to be subtle differences in nutritional response between pink and chum salmon fry given the same ration. Pink salmon fry appear to require slightly lower fat levels in their diets than do chum salmon fry. Deposits of fat and glycogen were found to be higher in pink salmon fry than in chum salmon fry when fed diets containing the same fat levels.

Marking pink and chum salmon fry by fin removal probably results in less than totally reliable information on relative marine survival rates, and is a questionable indicator of absolute marine survival rates.

Diet quality of is of particular importance during years of less than optimal estuarian conditions.

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Appendices

Appendix I. Rate of length and weight gains for all years for pink salmon fry

Year/Diet	G _L	G _W
80/OMP II	0.12	1.45
80/Biodiet	0.26	2.09
78/Silver Cup	0.32	1.68
80/2w/eggs	0.37	2.39
78/OMP II	0.45	1.99
79/OMP II	0.72	1.52
79/1	0.77	1.86
81/2w/fish	0.84	2.42
79/3	0.86	1.88
81/Biodiet	0.87	2.63
79/2w/fish	0.89	2.06
78/eggs	1.05	3.43

Appendix II. Rate of length and weight gains for all years for chum salmon fry

Year/Diet	G _L	G _W
79/1	0.61	2.06
80/OMP II	0.61	2.71
79/OMP II	0.65	1.92
80/Biodiet	0.65	2.09
81/2w/fish	0.67	2.81
79/3	0.69	2.07
79/2w/fish	0.70	2.20
81/Biodiet	0.76	3.03
80/2w/eggs	0.92	4.00

Appendix III. Duncan's multiple range test for homogeneous subsets for pink salmon fry lengths for all years

Subset 1 Year/Diet Mean	80/OMP 34.6	79/OMP 35.7
Subset 2 Year/Diet Mean	80/Bio 35.8	79/1 36.2
Subset 3 Year/Diet Mean	79/1 36.2	80/2 36.7
Subset 4 Year/Diet Mean	80/2 36.7	79/3 37.1
Subset 5 Year/Diet Mean	79/3 37.1	79/2 37.5
Subset 6 Year/Diet Mean	81/2 38.7	81/Bio 38.9

Appendix IV. Duncan's multiple range test for homogeneous subsets for pink salmon fry weights for all years

Subset 1 Year/Diet Mean	80/OMP 0.2521		
Subset 2 Year/Diet Mean	80/Bio 0.2946		
Subset 3 Year/Diet Mean	79/OMP 0.3145	81/2 0.3148	80/2 0.3161
Subset 4 Year/Diet Mean	81/Bio 0.3283		
Subset 5 Year/Diet Mean	79/1 0.3453	79/3 0.3480	79/2 0.3657

Appendix V. Duncan's multiple range test for homogeneous subsets for pink salmon fry condition factor for all years

Subset 1				
Year/Diet	81/2			
Mean	1.752			
Subset 2				
Year/Diet	81/Bio			
Mean	1.761			
Subset 3				
Year/Diet	80/OMP			
Mean	1.809			
Subset 4				
Year/Diet	80/Bio	80/2		
Mean	1.841	1.844		
Subset 5				
Year/Diet	79/3	79/OMP	79/2	
Mean	1.890	1.897	1.897	
Subset 6				
Year/Diet	79/1			
Mean	1.924			

Appendix VI. Duncan's multiple range test for homogeneous subsets for chum salmon fry lengths for all years

Subset 1						
Year/Diet	80/OMP	81/2	80/Bio	79/1	79/OMP	
Mean	42.0	42.2	42.3	42.5	42.5	
Subset 2						
Year/Diet	81/2	80/Bio	79/1	79/OMP	81/Bio	79
Mean	42.2	42.3	42.5	42.5	42.8	42
Subset 3						
Year/Diet	79/1	79/OMP	81/Bio	79/3	79/2	
Mean	42.5	42.5	42.8	42.9	43.1	
Subset 4						
Year/Diet	80/2					
Mean	44.4					

Appendix VII. Duncan's multiple range test for homogeneous subsets for chum salmon fry weights for all years

Subset 1				
Year/Diet	81/2	80/OMP	81/Bio	
Mean	0.5100	0.5190	0.5278	
Subset 2				
Year/Diet	81/Bio	80/Bio		
Mean	0.5278	0.5328		
Subset 3				
Year/Diet	79/OMP	79/1	79/3	79/2
Mean	0.5947	0.6133	0.6190	0.6434
Subset 4				
Year/Diet	79/2	80/2		
Mean	0.6434	0.6542		

Appendix VIII. Duncan's multiple range test for homogeneous subsets for chum salmon fry condition factor for all years

Subset 1			
Year/Diet	81/Bio	81/2	
Mean	1.881	1.886	
Subset 2			
Year/Diet	80/OMP	80/Bio	
Mean	1.901	1.902	
Subset 3			
Year/Diet	80/2		
Mean	1.949		
Subset 4			
Year/Diet	79/OMP		
Mean	1.970		
Subset 5			
Year/Diet	79/3	79/1	79/2
Mean	1.981	1.990	1.993

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