THE INFLUENCE OF SALINITY ON PROTEIN REQUIREMENTS AND UTILIZATION BY RAINBOW TROUT, SALMO GAIRDNERI AND COHO SALMON, ONCORHYNCHUS KISUTCH

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ABSTRACT

THE INFLUENCE OF SALINITY ON PROTEIN REQUIREMENTS AND UTILIZATION BY RAINBOW TROUT, SALMO GAIRDNERI AND COHO SALMON, ONCORHYNCHUS KISUTCH

By

Ibrahim H. Zeitoun

Rainbow trout, *Salmo gairdneri* and coho salmon, *Oncorhynchus kisutch* juveniles maintained at 10 p.p.t. or 20 p.p.t. salinity were used in this research. Seven diets were tested from 30 to 60 per cent protein in five per cent increments. Gram weight gain, protein retention, total diet efficiency, protein efficiency ratio (P.E.R.) and net protein utilization (N.P.U.) methods were employed to evaluate the dietary protein levels. All the methods except that of total diet efficiency indicated that the minimum requirement of protein in the diet for rainbow trout at 10 p.p.t. and the coho salmon maintained at either salinity was approximately 40 per cent. Rainbow trout fingerlings at 20 p.p.t. exhibited a higher requirement (45 per cent). Total diet efficiency values reached a maximum for all tests at 50 per cent dietary protein. Salinity effects in coho salmon were negligible when the

fish were acclimated to the high salinity and salinity effects were not apparent if the osmoregulatory mechanism(s) were already developed. The techniques that have been established by nutritionists for estimating protein requirements of mammals and birds were successfully employed for fish. Analysis of variance and Duncan's multiple range tests were used to determine the significance of the average values.

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Dedication

To my beloved country, Egypt

and to my parents.

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INTRODUCTION

Fish nutrition is a developing branch of fisheries science which is concerned with the determination of nutritional requirements of fish for growth and other life processes. Included in this science is the interaction of these requirements with the various biotic and abiotic factors of the environment. Studies of the effect of environmental variables on body composition and quantitative nutritional requirements of fish are limited. Halver (1957) developed a test diet that could maintain fish without symptoms of nutritional deficiency. Brett (1971) conducted an experiment which established that Halver's diet (50 per cent protein) had the highest efficiency of the diets tested and that the protein-energy ratio (P/E) was more favorable than many other diets used in various laboratories to maintain and rear trout and salmon. This diet was modified and used satisfactorily in a series of projects aimed at determining the protein requirements of sockeye salmon, *Oncorhynchus nerka*, and rainbow trout, *Salmo gairdneri* (Halver, *et al*., 1964) and the chinook salmon, *O. tschawytscha* (DeLong, *et al*., 1958). Recently Cowey, *et al*., (1972) estimated the protein requirement of the young plaice, *Pleuronectes platessa* using freeze-dried cod muscle and shrimp meal. The essential amino acid requirements of the chinook salmon (Halver, 1957), the sockeye salmon (Halver and Shanks, 1960) and the channel catfish, *Ictalurus punctatus* (Dupree and Halver, 1970) were also determined. The above mentioned investigations focused on the determination of growth, defined as the increase in the weight of the fish. Brett, *et al*., (1969) studied the influence of temperature and the amount of diet consumed on the body composition of sockeye salmon, *O. nerka*. The importance of temperature as an abiotic factor influencing fish growth and metabolism has been discussed by Brown (1957), Winberg (1956), Warren and Davis (1967), and Paloheimo and Dickie (1966). DeLong, *et al*., (1958) found that the minimum protein requirement of chinook salmon fingerlings is dependent upon the water temperature. The requirement was around 40 per cent protein at 47 F and around 55 per cent at 58 F. Since a given diet can be transformed by the animal into different amounts and types of body material depending on biotic or abiotic factors (Kinne, 1960; Brown, 1957; Maynard and Loosli, 1969), an analysis of body composition of the experimental animals is a more accurate indicator of growth and efficiency of feed use than weight gain. A deposition of fat which may be associated with an increase in body weight may not be associated with true growth (Phillips, et al., 1957). Gerking (1955) applied the nitrogen retention method to evaluate protein metabolism as influenced by the rate of feeding in the bluegill, Lepomis macrochirus. Davies (1963) found a correlation between the efficiency of use of dietary energy (digestibility) by goldfish, Carassius auratus, and the dietary energy intake. A considerable number of studies have been conducted on the body composition of different species of the Salmonidae. These investigations attempted to link the influence of one or more of the biotic or abiotic factors to body composition (Parker & Vanstone, 1966; Vanstone & Markert, 1968; Brett, et al., 1969; Fessler & Wagner, 1969; Vanstone, et al., 1970; Groves, 1970). Brown (1957) discussed briefly the influence of sea water on the growth of salmon. She stated that salmonids grow better in sea water due to the greater food supply. Accordingly, it may be possible to influence the success of adaptation of fish to brackish water by modifying the diet composition. Phillips, et al., (1965) concluded that the protein of dehulled soybean meal was less efficiently utilized than that of fish or meat meals by brook trout, Salvelinus fontinalis fingerlings. Increasing the methionine content of low protein diets reduced the brook trout fingerlings' growth, decreased the percentages of body protein and fat and increased body water content and the total body sulfur (McCartney, 1969). Conte and Wagner (1965) indicated that ions other than chloride and sodium play a vital function in sea water homeostasis. The importance of protein molecules in the development of Donnan equilibria and osmoregulation was assumed to be great and was discussed by Potts and Parry (1963). The studies of Conte, et al., (1966) and Weisbart (1968) indicated that juvenile Salmonidae could be classified according to their ability to resist higher salinities. Chum salmon, O. keta, and pink salmon, O. gorbuscha, are euryhaline as fry while coho salmon, O. kisutch, and Salmo gairdneri have higher survival values than S. salar, S. clarki, and S. trutta of all sizes and at all studied salinities. However, Canagaratnam (1959) demonstrated the superiority of coho salmon, chum salmon, and sockeye salmon growth in higher osmotic media as compared to growth in media of lower osmolarity. Otto (1971) found that juvenile coho salmon median survival time underwent a seasonal fluctuation when maintained in high salinity water and the parrs were physiologically not ready to move downstream except after smoltation.

The goal of this research was to establish the quantitative protein requirement and nitrogen retention of rainbow trout and coho salmon as influenced by salinity. The findings may have application in many parts of the world where brackish water is available and fish protein production is needed. Also, these bioassays attempted to clarify the physiological and biochemical significance of various dietary protein levels on the fish employing several proven methods in animal nutrition. The procedures used were compared in order to rate their usefulness when applied to fish.

MATERIALS AND METHODS

The complete study was conducted at Bowman Bay Field Station, Anacortes, Washington and was supervised by the Western Fish Nutrition Laboratory (WFNL), U.S. Fish and Wildlife Service. The station is equipped with facilities for maintaining and handling experimental fish and is supplied with both sea water and fresh water which are pumped directly from Bowman Bay and Pass Lake, respectively. Both species used in this investigation were raised at WFNL in fresh water and fed artificial diets developed in the Laboratory (modified from Halver, 1957).

The purified-diet method was used in these experiments employing seven separate diets composed of 30-60 per cent protein in 5 per cent increments. The 60 per cent protein diet was eliminated from the coho salmon experiment for economic reasons. The various diet compositions, which were numerically labelled, are listed in Table 1. Regarding diets 269 and 270, the water: dry matter ratio was 1:1 instead of the 2:1 ratio of the rest of the diets. This modification was necessary to eliminate the excessive leaching of the low gelatin diets since the gelatin-binding efficiency of water soluble constituents of the diets is not satisfactory if the

Table 1.--Composition of different formulated diets

Basic composition	Dietary protein concentration, %[1]						
	30	35	40	45	50	55	60
Casein	22.8	26.6	30.4	34.2	38.0	41.8	45.6
Gelatin	7.2	8.4	9.6	10.8	12.0	13.2	14.4
Dextrin	48.0	43.0	38.0	33.0	28.0	23.0	18.0
Vitamin mixture	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Oil mixture	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Mineral mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calorie-Protein ratio, kcal/kg/% protein[2]	131	112	98	87	79	72	66
Dry matter: water in finished feed	1:1	1:1	1:2	1:2	1:2	1:2	1:2

[1]The Western Fish Nutrition Laboratory diet numbers which correspond to the diets providing the increasing protein levels are 269, 270, 271, 272, 8, 273, and 274, respectively.

[2]Based on a caloric density of 4 kcal/g for carbohydrate and protein, and 9 kcal/g for fat.

percentage of water in the diet is high. In addition, better physical structure of the diets was maintained if the water content of such diets was lowered. The use of purified diets made it possible to change the concentration of a given constituent with a minimum disturbance of other nutrient relationships.

Protein was supplied as casein and gelatin, carbohydrates as dextrin, and fat as a mixture (premix no. 4, Table 2) of corn oil, cod liver oil and alpha-tocopheryl acetate. Minerals were supplied as reagent grade salts (premix no. 5, Table 2), and vitamins as pure crystalline compounds (premix no. 1, Table 3).

Two salinities were used in the experiment, 10 p.p.t. and 20 p.p.t. Salinity was determined by using the conductivity method and plotting the curve of known salinity waters and their corresponding electrical resistance at known temperatures (Figure 1). The electrical resistance of water was checked twice daily to adjust the flow rate of the saline and fresh water supply going to the mixing cones as was necessary to maintain the resistance within ± 4.0 ohms of the estimated value of the curve. The curves were replotted as needed because of the effect of temperature on water conductivity. Water conductivities were determined using the YSI Model 31[1] conductivity meter.

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[1]Yellow Springs Instrument Co., Yellow Springs, Ohio.

Table 2.--Composition of the mineral mixture (Premix #5)

Ingredient	Grams
Salt mixture no. 2 USP X111*	100.000
$AlCl_3 \cdot 6H_2O$	0.015
Kl	0.015
CuCl	0.010
$MnSO_4 \cdot H_2O$	0.080
$CoCl_2 \cdot 6H_2O$	0.100
$ZnSO_4 \cdot 7H_2O$	0.300

*Salt mixture ingredients (Nutritional Biochemicals Corp., 1972 Diets manual, page 6, Cleveland, Ohio).

	Ingredient	Percentage
$CaH_4(PO_4)_2 \cdot H_2O$	Calcium biphosphate	13.58
$CaC_3H_5O_3 \cdot 5H_2O$	Calcium lactate	32.70
$Fe\ C_6H_5O_7 \cdot 3H_2O$	Ferric citrate	2.97
$MgSO_4$	Magnesium sulfate	13.20
KH_2PO_4	Potassium phosphate (dibasic)	23.98
$Na_2H_4(PO_4)_2 \cdot H_2O$	Sodium biphosphate	8.72
NaCl	Sodium chloride	4.35

Table 3.--Composition of the vitamin mixture (Premix #1)

Ingredient	Grams
Alpha-cellulose	8.000
Choline chloride	0.500
Inositol	0.200
Ascorbic acid	0.100
Niacin	0.075
Calcium pantothenate	0.050
Riboflavin	0.020
Menadione	0.004
Pyridoxine . HCl	0.005
Thiamine chloride . HCl	0.005
Folic acid	0.0015
Biotin	0.0005
Cyanocobalamin*	0.500 ml

*Premix # 2

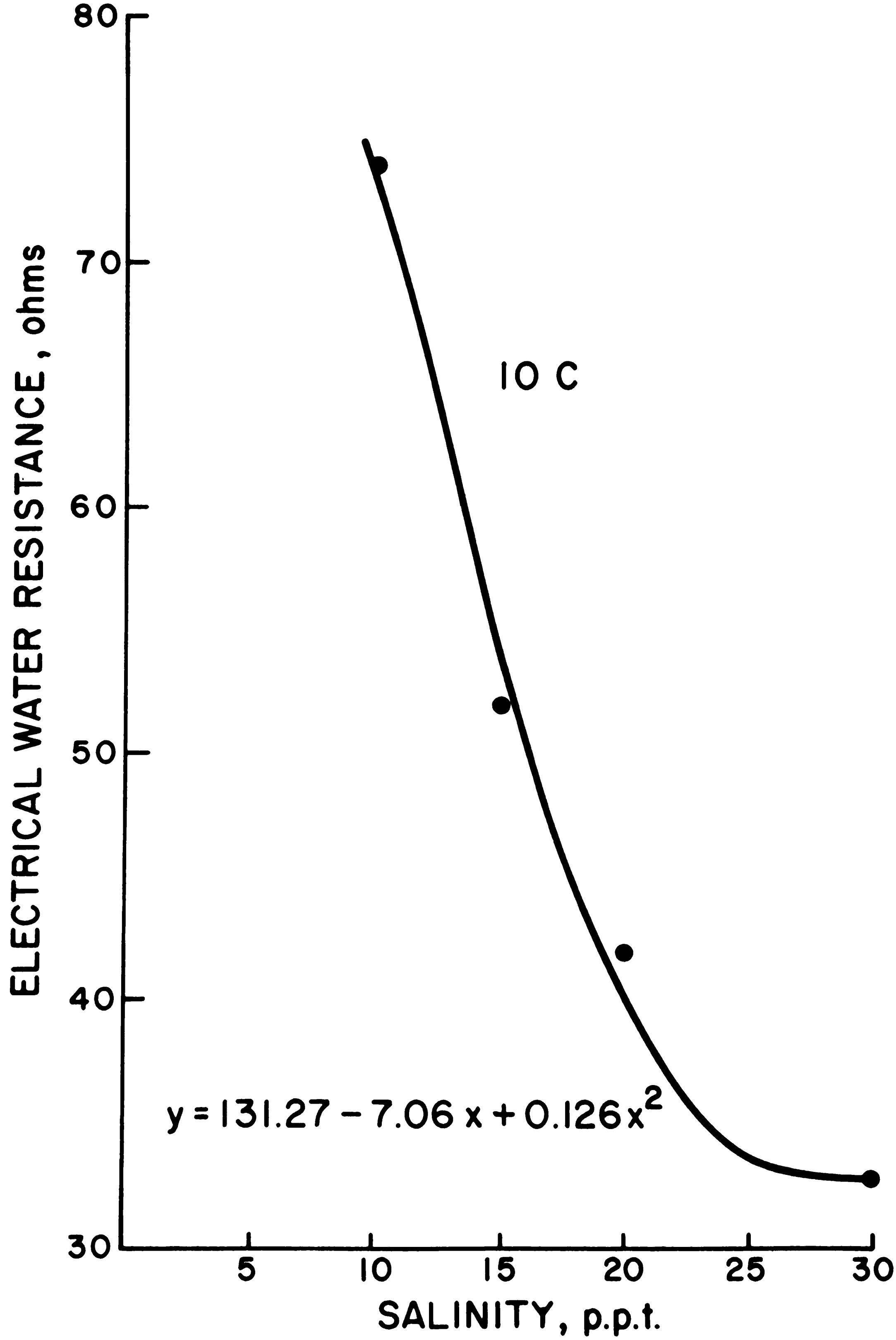
Vitamin B_{12} 10 mg/500 ml H_2O

Table 4.--Composition of the oil mixture (Premix #4)

Ingredient	Grams
Corn oil	7.0
Cod liver oil (3139 USP units vitamin A)	2.0
D,L-Alpha-tocopheryl acetate*	0.04

*Must be warmed in water bath before using.

Figure 1.--Standard curve relating salinity and electrical resistance of water at 10 C


80
70
60
50
40
30
ELECTRICAL WATER RESISTANCE, ohms
10 C
y = 131.27 − 7.06 x + 0.126x²
5
10
15
20
25
30
SALINITY, p.p.t.

Prior to the mixing of fresh and sea water, each was run through a "Crystaleen" fiberglass filter tank Model C-7[2] to remove suspended materials. Filtered water was then exposed to ultra violet light in an "Aquafine" liquid sterilizer Model MP 4 PVC[3] to inhibit bacterial growth and minimize disease problems. Fish were treated twice with 1 ppm Furanase[4] in the first week of the experiment in order to control infection and cure the fish of Sporocytophaga spp. Treatments were applied twice for one hour. Water flow was shut off during each application.

Duplications of the Gahimer, et al., (1971) cones were available for each tested diet at each salinity. The cones were modified in the coho salmon experiment to receive two water inlets instead of the one originally proposed (Figure 2). The modification was necessary to eliminate the complete dependence on one inlet which sometimes became blocked, causing the accidental suffocation of fish, particularly on stormy days when filtration efficiency dropped. In addition, an air pump was installed to supply additional air to the mixing cones. Water temperature was recorded daily from one cone in each salinity

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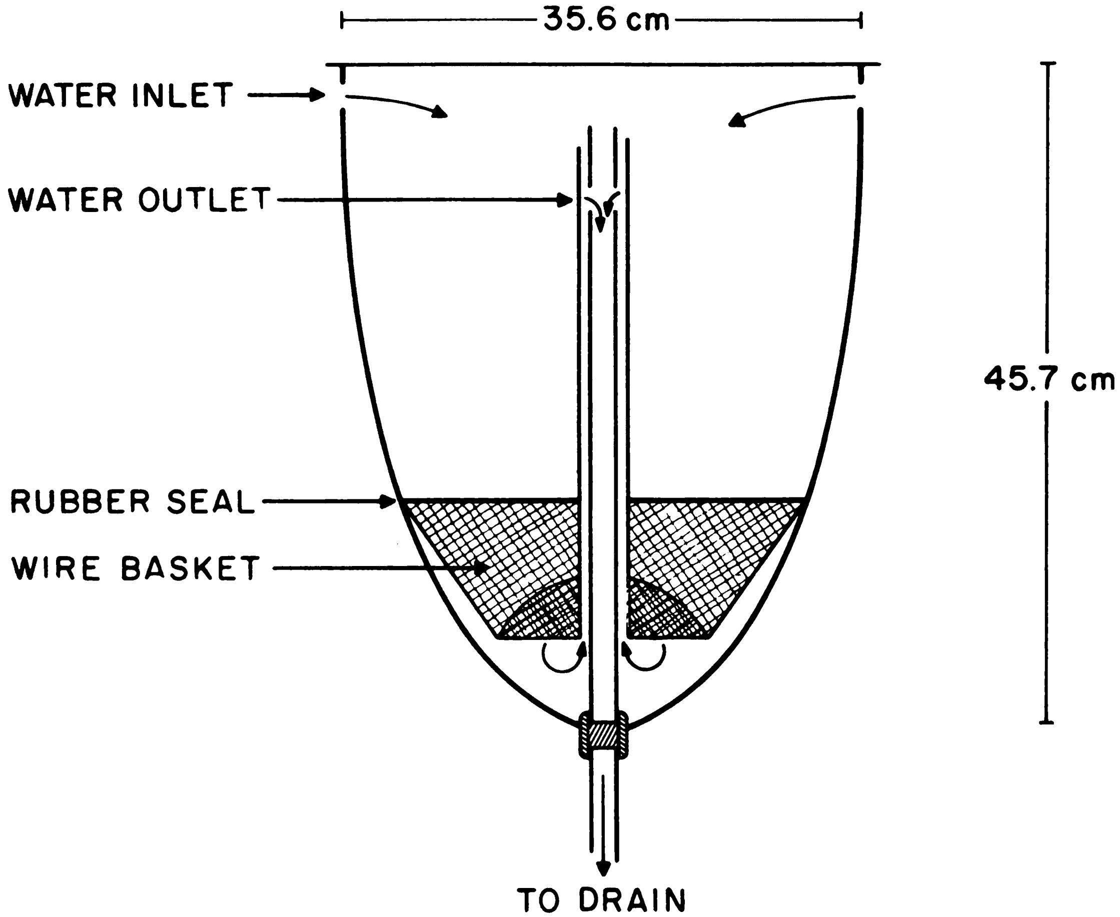
[2]Howard Construction Corp., 4547 N. Scottsdale Road, Scottsdale, Arizona.

[3]Aquafine Corp., 1230 Sunset Boulevard, Los Angeles, California.

[4]Dainippon Pharmaceutical Co., Ltd., Research Laboratories, Osaka, Japan.

Figure 2.--Illustration of the cones in which the fish were maintained during the test. Water volume was 25,380 ml and average water flow rate was 1300 ml/minute with the arrows indicating the flow direction


35.6 cm
WATER INLET
WATER OUTLET
45.7 cm
RUBBER SEAL
WIRE BASKET
TO DRAIN

level. Photoperiods and light intensity were maintained uniformly by placing a 10-watt light bulb over each cone. Fish were subjected to nine hours of illumination daily. A constant and uniform counterclockwise flow rate in all cones was maintained. Depending on fish size, each cone was stocked initially with 50 rainbow trout or 40 coho salmon fingerlings. Fish were acclimated to the new environment for 10 days during which time they were fed twice daily the same diet to be used in the tests. The construction of the cones allowed waste products to be easily eliminated.

Diets were prepared biweekly according to the Halver (1957) method and frozen until needed. Each diet was grated and fed twice a day, ad libitum, on a rigid time schedule. Grams of food consumed biweekly by each fish lot were determined. A total of ten weeks was used for each feeding experiment. Each diet was fed slowly to eliminate leaching of water soluble constituents and feeding stopped as soon as any portion reached the cone's basket. Dead fish were removed and mortality recorded daily. Weighing and counting of fish took place biweekly. The weighing technique followed that of Halver (1957). Diets and collective fish weights for each cone were measured to the nearest gram. Average fish weights were then estimated to the nearest tenth of a gram.

At the start and termination of each experiment, fish were starved for 48 hours after which representative samples from duplicate cones were taken for chemical analysis of the body.

After the last weighing period, fish were fed once and blood samples were collected and hematocrit values were measured twelve hours later. This was done to eliminate changes which might have taken place in the blood due to the influence of starvation, digestion or absorption. Blood was pooled in test tubes by severing the caudal peduncle of several fish to collect a sufficient amount. The blood was then centrifuged for 15 minutes and the serum was transferred to screw-capped plastic tubes and stored in a freezer for total serum protein determination. Very little or no hemolysis was observed. Body composition determinations for initial and final samples started by thawing the fish and calculating the wet weight for each fish. Fish were placed into tared large-mouth bottles and dried in ovens at 95 C to a constant weight. The moisture and dry matter were calculated and the dry fish were blended in a blender to form fine homogenous samples. Blending and homogenizing were facilitated by using isopropyl alcohol. Aliquots were allowed to dry in vacuum ovens at 55 C. Homogenates from the same cone were then placed together and mixed again using a mortar and pestle with isopropyl alcohol as a mixing agent. Then the samples were dried again and kept in screw-capped bottles under refrigeration (2-3 C).

Dried samples were analyzed for nitrogen by the micro-Kjeldahl method (A.O.A.C., 1970). Crude protein was estimated by multiplying nitrogen values by 6.25. Lipid contents were established by the Folch, et al., method (1957). Ash values were determined by burning the dry samples in a muffle furnace at 500 C.

Total serum protein was determined using the biuret method, applying the procedures of O'Brien, et al., (1968). Crystallized human plasma albumen[5] was used as a standard. Absorption values were read on a Beckman model B spectrophotometer at 545 mu. Statistical analyses were computed using the analysis of variance. Mean differences were compared using Duncan's multiple range test. Mean square errors (MSE) or standard errors ($\pm$SE) accompanied the tables and figures to identify the range of the means.

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[5]Dade Division, American Hospital Supply Corp., Miami, Florida.

RESULTS

In this experiment fish were maintained at two salinities in order to determine (1) the minimum requirements of dietary protein necessary to attain maximum weight gain and protein retention and, (2) the efficiency of growth and protein utilization. Each experiment was divided into two phases. The initial one was basically biological, involving the determination of weights of fish and diets and mortalities. The subsequent phase involved laboratory techniques. It is well understood that the main role of a successful dietary protein is to supply the body with a mixture of amino acids of appropriate proportions for maintenance and the synthesis of tissues (Maynard and Loosli, 1969). The success or failure of either species reared in each water salinity and fed various levels of protein was based on several accepted methods in animal nutrition.

Weight Gain Methods

The weight gain method depends on the fact that an inadequate supply of protein in the diet will reduce the weight gain or terminate growth completely. This method is sensitive, particularly in testing individual amino acid requirements in animals, but it has also been employed in evaluating the overall effect of protein (McLaughlan and Campbell, 1969). In these experiments percentage gain was employed instead of gram gain because average initial weights of the rainbow trout assigned to different diets were significantly different statistically (Table 5). Also, some fish nutritionists (Halver, 1957; and DeLong, et al., 1958 and 1962) have used the same parameter in their studies in determining some of the nutritional requirements of Oncorhynchus spp. Percentage gain was plotted against the percentage protein in the diet to determine the nitrogen growth index of each fish group (Figures 3 and 4). Nitrogen growth index is designated as the slope of the straight line of weight gain plotted against percentage protein in the diet (Allison, 1964). The nitrogen growth index for rainbow trout was 7.7 for those tested at 10 p.p.t. sea water and 7.6 for those at 20 p.p.t., while the nitrogen growth index for coho salmon at 10 and 20 p.p.t. were 3.5 and 2.5, respectively. Minimum protein requirements were estimated by using the method of Fisher, et al., (1957). A regression line (Y = aX + b) was fitted to the data over the ascending portion of the percentage gain response by the method of least squares. A straight line parallel to the x-axis was established by averaging the highest observed means which did not differ from each other as determined

Table 5.--Initial weights of coho salmon and rainbow trout fingerlings in grams

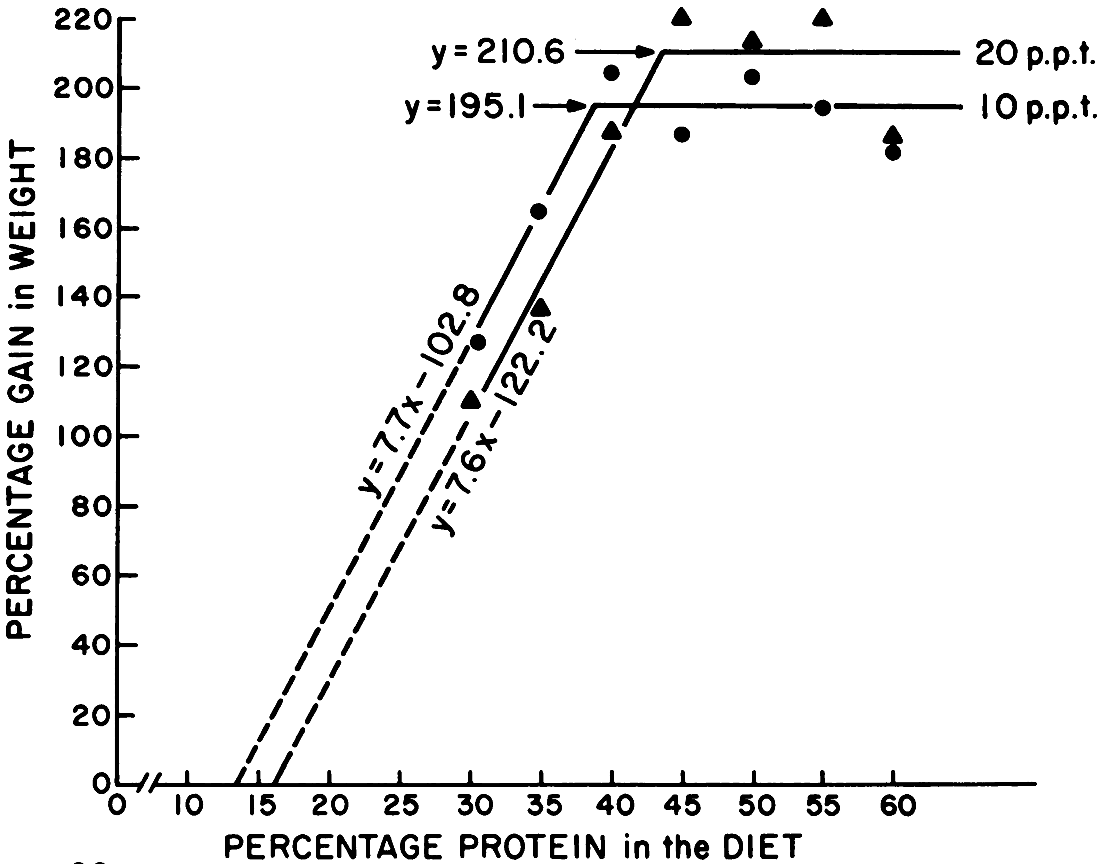
Species	Dietary protein concentration,%								Salinity, p.p.t.		
	30	35	40	45	50	55	60	±SE	10	20	±SE
Rainbow trout	6.5[a]	6.8[ab]	6.2[a]	6.3[a]	6.4[a]	6.3[a]	7.2[b]	0.15	6.5	6.5	0.08
Coho salmon	14.5	14.4	14.5	14.5	14.4	14.6		0.07	14.3	14.7[a]	0.04

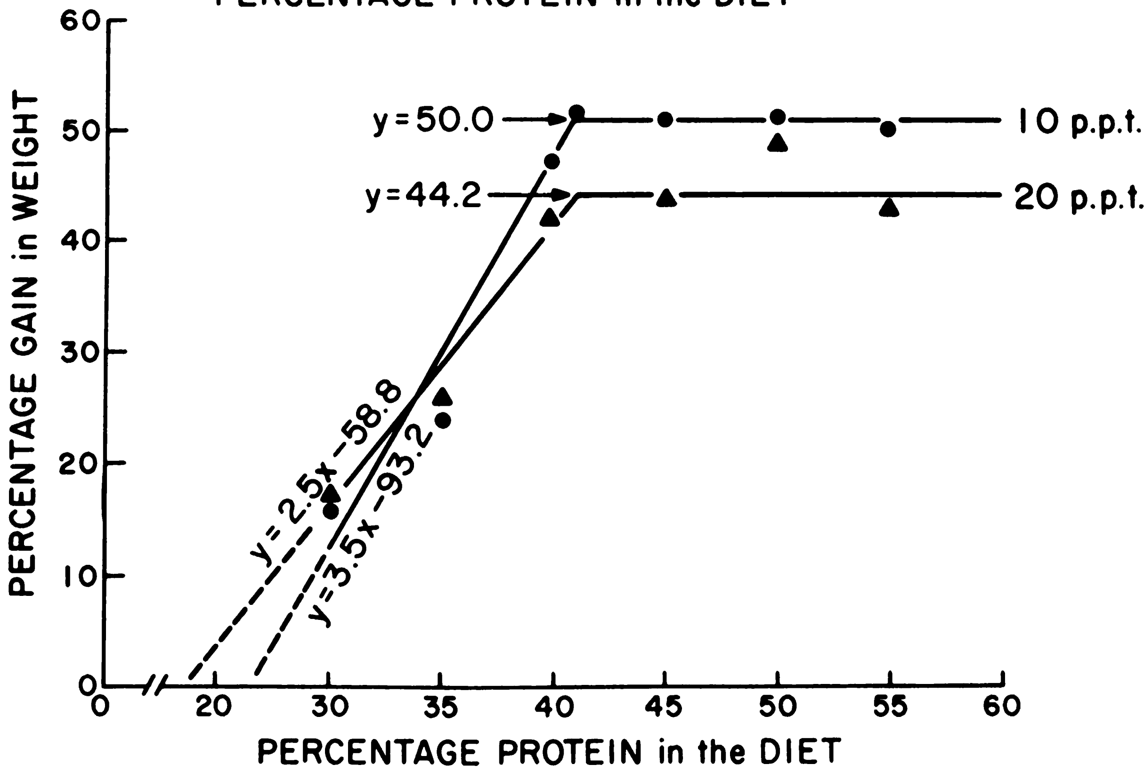
[a,b] Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

Figure 3.--Nitrogen growth index of the rainbow trout over a period of 10 weeks* [$\pm$SE = 10.10]

Figure 4.--Nitrogen growth index of the coho salmon over a period of 10 weeks* [$\pm$SE = 2.05]

*A regression line ($Y = aX + b$) was fitted to the data over the ascending portion of the percentage gain response. A straight line parallel to the x-axis was established by averaging the highest observed means which did not differ from each other as determined by the analysis of variance.


PERCENTAGE GAIN in WEIGHT
y = 210.6
20 p.p.t.
y = 195.1
10 p.p.t.
y = 7.7x − 102.8
y = 7.6x − 122.2
PERCENTAGE PROTEIN in the DIET


PERCENTAGE GAIN in WEIGHT
y = 50.0
10 p.p.t.
y = 44.2
20 p.p.t.
y = 2.5x − 58.8
y = 3.5x − 93.2
PERCENTAGE PROTEIN in the DIET

by the analysis of variance. The average value obtained was used as the Y determinant in establishing the position of this horizontal line. The point of interception of these two lines provided an estimate of the protein requirement. The line interceptions for rainbow trout indicated a minimum requirement of approximately 40 and 45% crude protein at 10 p.p.t. and 20 p.p.t. sea water, respectively. The coho salmon line interceptions suggested that 40% crude protein in the diet is the minimum dietary protein level for fish maintained in either of the two salinities to obtain the maximum weight gain.

Rainbow trout fed a 60% crude protein diet showed a decline in growth similar to that reported by DeLong et al., (1958) for chinook salmon that were fed 65% crude protein at 47 F. Also, Cowey, et al., (1972) demonstrated that high dietary protein repressed the growth rate of plaice.

The average daily gain and average daily feed for rainbow trout and coho salmon as influenced by percentage dietary crude protein and salinity are summarized in Tables 6 and 7. The average daily gain and average daily feed data resulted from calculating fish days for each two week group (Appendix A). Fish days for each group of fish was the sum of days each fish in the group survived. This was necessary to minimize the errors in accounting for the loss of diet used by the deceased fish

Table 6.--Average daily gain and average daily feed for rainbow trout for the ten week period at two week intervals

Average daily gain, mg	Dietary protein concentration, %								Salinity, p.p.t.		
	30	35	40	45	50	55	60	$\pm SE$	10	20	$\pm SE$
for 2 weeks	69^{a}	46^{a}	134^{b}	121^{b}	145^{b}	133^{b}	132^{b}	15	107	115	8
for 4 weeks	83^{a}	116^{b}	170^{c}	158^{c}	176^{c}	177^{c}	163^{c}	10	149	149	5
for 6 weeks	84^{a}	104^{a}	174^{b}	186^{b}	182^{b}	184^{b}	183^{b}	7	159	155	4
for 8 weeks	96^{a}	125^{b}	177^{c}	193^{c}	191^{c}	191^{c}	191^{c}	9	169	163	5
for 10 weeks	110^{a}	145^{b}	175^{c}	184^{c}	190^{c}	187^{c}	190^{c}	5	169	169	3
Average daily feed, mg											
for 2 weeks	139^{ab}	135^{ab}	150^{a}	132^{ab}	128^{b}	124^{b}	122^{b}	6	130	136	3
for 4 weeks	249^{a}	235^{a}	200^{b}	179^{ab}	165^{c}	167^{c}	161^{c}	7	194	194	4
for 6 weeks	271^{a}	260^{a}	207^{b}	204^{b}	177^{c}	175^{c}	177^{c}	6	212	208	3
for 8 weeks	262^{a}	261^{a}	209^{b}	208^{b}	184^{c}	178^{c}	183^{c}	6	213	211	3
for 10 weeks	259^{a}	256^{a}	209^{b}	204^{b}	184^{c}	175^{c}	182^{c}	6	209	210	3

[a,b,c] Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

Table 7.--Average daily gain and average daily feed for coho salmon for the ten week period at two week intervals

	Dietary protein concentration, %							Salinity, p.p.t.		
	30	35	40	45	50	55	±SE	10	20	±SE
Average daily gain, mg										
for 2 weeks	-16	-21	6	4	0	-4	7	-4	-6	4
for 4 weeks	1	20	34	36	25	24	11	19	27	6
for 6 weeks	10^a	16^a	58^b	58^b	46^b	47^b	5	43^a	35	3
for 8 weeks	20^a	30^a	65^b	73^b	68^b	66^b	4	61^a	46	2
for 10 weeks	35^a	51^b	97^c	99^c	102^c	96^c	3	83^a	77	2
Average daily feed, mg										
for 2 weeks	31	21	27	29	22	21	3	27	23	2
for 4 weeks	74^a	40^b	53^b	58^{ab}	49^b	41^b	6	56	49	3
for 6 weeks	111^a	63^b	77^b	80^b	65^b	61^b	7	83^a	70	4
for 8 weeks	122^a	81^b	96^b	92^b	81^b	74^b	6	101^a	81	4
for 10 weeks	141^a	106^b	114^b	110^b	100^b	94^b	5	120^a	101	3

[a,b,c] Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

as well as weight loss caused by the removal of the dead fish.

The weight gain data suggested that the fish were more sensitive to the dietary protein level than to the degree of salinity. Beyond the minimum requirements of protein, the growth of fish was not significantly different in either salinity. Apparently, the rainbow trout showed a clear response to the protein levels in the diets in the first two weeks of the test but the coho did not until after the fourth week. An inverse relationship could be established between the average daily feed and the percentage protein fed until the protein level for maximum growth was met.

To provide a useful way to compare growth of different size fish, the mean specific growth rate for each of the duplicate groups that received the same treatment was used, and since specific growth rate usually takes into account the time factor (Δt), this method was recommended by Brown (1957). Tables 8, 9, 10 and 11 summarized the specific growth rate and the initial and final average weights of coho salmon and rainbow trout maintained at two different salinities. Instantaneous growth rate or the specific growth rate (GR) was derived from the equation:

Table 8.--Initial and final average weights and the specific growth rate of rainbow trout at 10 p.p.t. after the ten week period

Item	Dietary protein concentration, %							
	30	35	40	45	50	55	60	±SE
Initial average weight, g	6.4	6.4	6.2	6.6	6.5	6.5	7.1	0.22
Final average weight, g	14.6	16.8	18.9	19.1	19.7	19.3	20.1	0.55
Specific growth rate	1.2	1.4	1.6	1.5	1.6	1.5	1.5	0.55

Table 9.--Initial and final average weights and the specific growth rate of rainbow trout at 20 p.p.t. after the ten week period

Item	Dietary protein concentration, %							
	30	35	40	45	50	55	60	±SE
Initial average weight, g	6.5	7.3	6.3	6.0	6.2	6.1	7.3	0.22
Final average weight, g	13.8	17.1	18.1	19.4	19.6	19.5	20.9	0.55
Specific growth rate	1.1	1.2	1.5	1.7	1.6	1.7	1.5	0.05

Table 10.--Initial and final average weights and the specific growth rate of coho salmon at 10 p.p.t. after the ten week period

Item	Dietary protein concentration, %						
	30	35	40	45	50	55	±SE
Initial average weight, g	14.3	14.1	14.3	14.4	14.2	14.3	0.10
Final average weight, g	16.6	17.5	21.6	21.8	21.5	21.4	0.35
Specific growth rate (X10)	2.1	3.0	5.9	5.9	5.9	5.8	0.20

Table 11.--Initial and final average weights and the specific growth rate of coho salmon at 20 p.p.t. after the ten week period

Item	Dietary protein concentration, %						
	30	35	40	45	50	55	±SE
Initial average weight, g	14.8	14.7	14.7	14.6	14.6	14.8	0.10
Final average weight, g	17.3	18.4	20.9	21.1	21.7	21.2	0.35
Specific growth rate (X10)	2.3	3.2	5.0	5.2	5.6	5.1	0.20

$$GR = \frac{\log_e W_t - \log_e W_o}{\Delta t}$$

where W_t and W_o were the average weights of fish at the end and beginning of the test, respectively, and Δt = 70 days on test.

Instantaneous growth rates of both species maintained at the two different salinities gave the same results as the weight gain method.

Gross (or Total) Diet Efficiency

A parameter of particular interest to many fish physiologists is the gross efficiency of diet or gross efficiency of growth (Winberg, 1956; Brown, 1957; Kinne, 1960; Paloheimo & Dickie, 1966; and Brett, *et al.*, 1969). This measurement expresses the efficiency of the conversion of food to fish tissue. Gross (or total) diet efficiency was symbolized by Warren and Davis (1967) as:

$$E_t = G/I$$

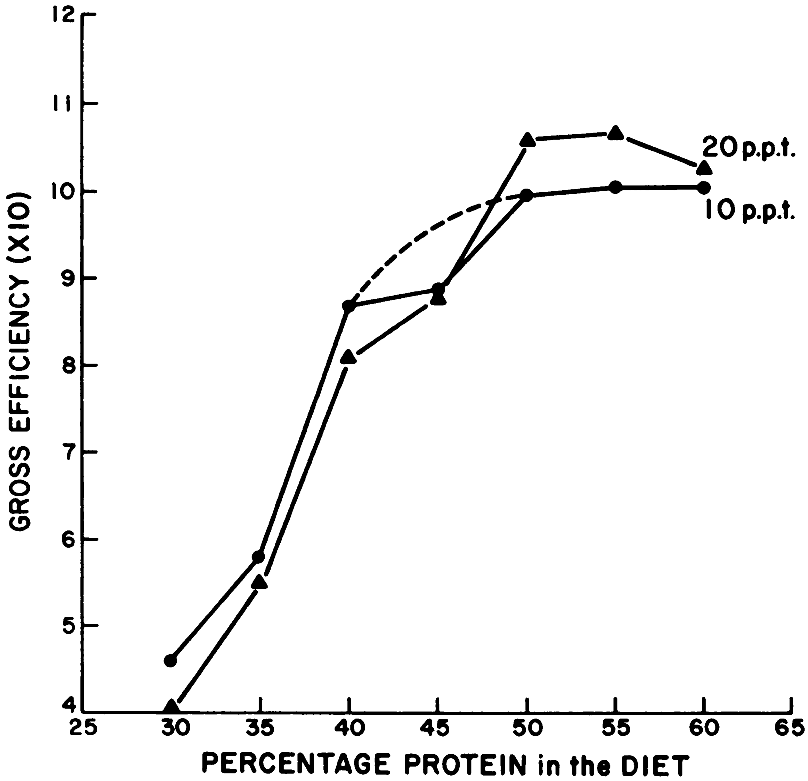
where E_t is the diet efficiency, G is the gain in weight and I is the diet intake.

In these tests, the gross diet efficiency calculations were based on the wet weight of the average fish and the average dry weight of the diet as fed. Therefore, the diet efficiency ratios tended to be higher than if both gain in weight and weight of the diet ingested were calculated on a dry basis (Figures 5 and 6). The diet efficiency ratios of the coho salmon and rainbow trout fingerlings established that salinity was of minor consequence while the dietary protein level was a major influence (Table 12). In contrast to the above, Kinne (1960) found that the growth efficiency of the desert pupfish, Cyprinodon macularius, increased with salinity. Over a sixteen week period and at 30 C the efficiency of food conversion reached a maximum at 15 p.p.t. salinity and decreased in the following order: 35 p.p.t. and then fresh water; and the diet efficiency ratios were 14.4, 10.6 and 8.8, respectively. The desert pupfish responded in a different fashion than did coho salmon or rainbow trout to saline media since the pupfish is basically a brackish water species.

The effect of the type of food on the conversion of dietary nitrogen to fish flesh was demonstrated by Pandian (1967a). He reported that the nitrogen conversion rate of Megalops cyprinoides was dependent on the quality of food ingested. The nitrogen conversion was 22.0% for fish fed prawn, Metapenaeus monoceros, whereas those fed mosquito fish, Gambusia affinis, converted 35.5% of nitrogen. He related this difference to the reduction of food intake on the prawn diet due to the bulk of the exoskeleton and not to its low digestibility. In the rainbow trout and coho salmon tests, where casein diets were fed, digestibility was assumed to be equal for the diets tested, and diet intake was not restricted.

Figure 5.--Relationship between gross efficiency and percentage protein in the diet for rainbow trout maintained at 10 p.p.t. and 20 p.p.t. salinity [$\pm$SE = 0.24]

Figure 6.--Relationship between gross efficiency and percentage protein in the diet for coho salmon maintained at 10 p.p.t. and 20 p.p.t. salinity [$\pm$SE = 0.69]


GROSS EFFICIENCY (X10)
12
11
10
9
8
7
6
5
4
25
30
35
40
45
50
55
60
65
20 p.p.t.
10 p.p.t.
PERCENTAGE PROTEIN in the DIET

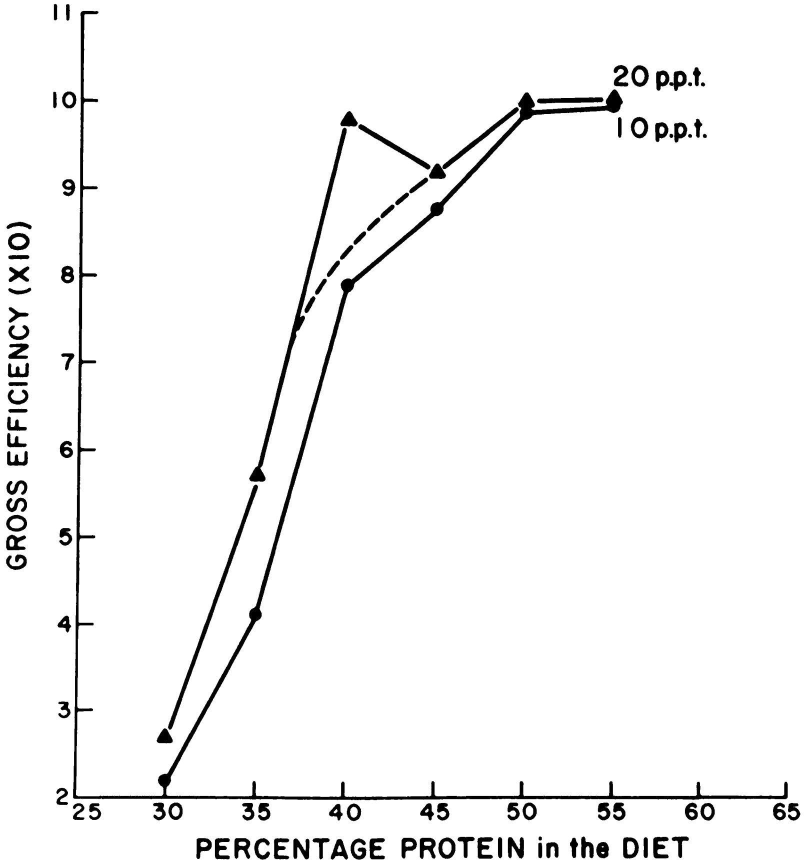

GROSS EFFICIENCY (X10)
11
10
9
8
7
6
5
4
3
2
25
30
35
40
45
50
55
60
65
20 p.p.t.
10 p.p.t.
PERCENTAGE PROTEIN in the DIET

Table 12.--Diet efficiency coefficients of rainbow trout and coho salmon fingerlings as influenced by the dietary protein level at the end of 10 weeks

Species	Dietary protein concentration,%								Salinity, p.p.t.		
	30	35	40	45	50	55	60	±SE	10	20	±SE
Rainbow trout	0.43^{a}	0.57^{b}	0.84^{c}	0.90^{d}	1.03^{e}	1.07^{e}	1.04^{e}	0.02	0.84	0.84	0.01
Coho salmon	0.25^{a}	0.49^{b}	0.88^{c}	0.90^{c}	1.02^{c}	1.03^{c}		0.05	0.72	0.80	0.03

[a,b,c,d,e]Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

Protein Efficiency Ratio

Protein efficiency ratios (P.E.R.), introduced by Osborne, et al., (1919), were employed in estimating minimum dietary protein requirements. P.E.R. is defined as the coefficient of grams gain per gram protein consumed. P.E.R. calculations are theoretically more precise than estimates of gross diet efficiency since dietary protein intake is the only item of the diet that was taken into account. Morrison and Campbell (1960) stated that P.E.R. for rats is a function of sex, age, genetics and length of feeding trial. Therefore, this information should be supplied in order to eliminate confusion when comparing data. In the present work, sex was difficult to determine because rainbow trout tested were yearlings while the coho salmon were slightly over a year old. Genetics of the fish would be considered uniform and the feeding trial lasted for a period of 10 weeks. P.E.R. figures were substantially influenced by the dietary protein level and not by the osmotic pressure of the medium. Table 13 presents the P.E.R. values as a function of percentage crude protein fed in the diet. P.E.R. reached the highest values around 40 and 45% dietary protein levels for both experimental species. Approximately 40 to 45 per cent crude protein in a diet for rainbow trout or coho salmon would be appropriate to support maximum gain. The data reported by Cowey, et al., (1972) on plaice showed

Table 13.--Protein efficiency ratios of coho salmon and rainbow trout fingerlings fed different levels of protein

Species	Dietary protein concentration, %								Salinity, p.p.t.		
	30	35	40	45	50	55	60	±SE	10	20	±SE
Rainbow trout	1.42[a]	1.62[b]	2.10[c]	2.01[c]	2.06[c]	1.95[c]	1.74[d]	0.04	1.86	1.83	0.02
Coho salmon	0.83[a]	1.40[b]	2.20[c]	2.01[c]	2.04[c]	1.87[c]		0.11	1.62	1.83	0.01

[a,b,c,d] Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

that protein efficiency ratio was highest at a 40 per cent dietary protein level although they estimated a minimum dietary protein requirement of 50 per cent for this species. These differences were not explained.

Biochemical Analysis of the Carcass

Phillips, et al., (1957) reported that increases in fish weight were not reliable indicators of true growth. Weight gain might be a result of fat deposition or other substances not directly related to true growth. Maynard and Loosli (1969) recommended the application of a slaughter test for the experimental animal. Proximate analyses, particularly crude protein determinations, were used in order to measure protein retention and net protein utilization. Total protein retention is a better indicator of the growth index than weight changes (Hegsted, 1964) although both methods tend to yield the same conclusion. Table 14 summarizes the percentage composition of the major constituents of rainbow trout and coho salmon, respectively, after a considerable period of acclimation to the two salinities studied but before differences in dietary protein levels were instituted. The proximate constituents of the carcass at the end of the experiment are listed in Tables 15 and 16 for both species maintained at 10 p.p.t. and 20 p.p.t. sea water. All determinations are expressed on a dry matter basis.

Table 14.--Body composition of rainbow trout and coho salmon fingerlings after 10 days of adaptation to 10 p.p.t. or 20 p.p.t. sea water at the beginning of the experiment

Constituent	Salinity			
	10 p.p.t.	±SE	20 p.p.t.	±SE
Rainbow Trout				
Dry matter, % of fresh weight	20.9	1.24	21.3	0.66
Protein, % of dry matter	69.8	0.03	71.1	0.10
Lipid, % of dry matter	22.4	0.15	20.4	0.90
Ash, % of dry matter	8.2	0.04	8.1	0.01
Coho Salmon				
Dry matter, % of fresh weight	23.9	0.14	25.0	0.16
Protein, % of dry matter	62.7	0.27	62.3	0.17
Lipid, % of dry matter	26.3	0.59	27.5	0.12
Ash, % of dry matter	11.1	0.17	10.4	0.07

Table 15.--Major body constituents of rainbow trout fingerlings maintained at 10 p.p.t. or 20 p.p.t. and fed different percentages of protein

Constituent	Dietary protein concentration, %							Total df	MSE	Significance
	30	35	40	45	50	55	60			
				10 p.p.t.						
Dry matter, % of fresh weight	23.2	24.6	25.3	24.9	25.5	23.3	24.0	20	0.73	$P < 0.05$
Protein, % of dry matter	62.3	62.4	60.9	63.9	61.4	70.0	69.3	14	0.74	$P < 0.01$
Lipid, % of dry matter	29.9	32.8	30.0	28.4	31.9	22.5	23.8	17	2.40	$P < 0.01$
Ash, % of dry matter	7.5	6.8	7.6	8.2	6.4	8.5	8.2	13	0.16	$P < 0.01$
				20 p.p.t.						
Dry matter, % of fresh weight	26.2	25.3	25.5	24.6	25.4	25.6	25.1	19	0.90	NS
Protein, % of dry matter	64.0	62.9	64.1	65.4	63.9	62.9	64.1	13	0.68	NS
Lipid, % of dry matter	26.6	31.3	29.0	27.6	29.1	29.6	27.6	13	1.10	$P < 0.05$
Ash, % of dry matter	8.3	7.3	8.1	8.6	8.4	8.4	7.9	13	0.30	NS

Table 16.--Major body constituents of coho salmon fingerlings maintained at 10 p.p.t. or 20 p.p.t. and fed different percentages of protein

Constituent	Dietary protein concentration, %						Total df	MSE	Significance
	30	35	40	45	50	55			
				10 p.p.t.					
Dry matter, % of fresh weight	23.5	23.5	24.5	24.5	24.5	24.1	23	0.34	NS
Protein, % of dry matter	67.7	68.3	65.5	64.7	64.6	66.2	17	0.88	$P < 0.01$
Lipid, % of dry matter	21.8	22.2	24.2	25.6	25.3	23.0	17	0.61	$P < 0.01$
Ash, % of dry matter	11.0	10.1	9.9	9.9	9.9	10.6	17	0.11	$P < 0.01$
				20 p.p.t.*					
Dry matter, % of fresh weight	23.0	24.4	23.1	23.2	22.5	22.4	23	0.84	$P < 0.05$
Protein, % of dry matter	65.0	65.7	66.6	64.2	70.0	69.8	13	0.18	$P < 0.01$
Lipid, % of dry matter	24.0	25.4	23.4	24.8	19.2	19.1	15	1.54	$P < 0.01$
Ash, % of dry matter	10.9	10.6	10.1	10.3	10.7	11.4	16	0.65	$P < 0.05$

*Based on a 65 day feeding period.

The reported values of protein, lipids, and ash were the means of duplicate or triplicate determinations of the dry composite of all fish in each dietary treatment. Dry matter was determined on individual fish representing 10 per cent of the population in each dietary treatment. Standard error (±SE) or the mean square error (MSE) were computed to evaluate the overall range of the means. Cowey, et al., (1972) studied the plaice and found that increasing dietary protein levels were associated with increasing tissue protein levels. But in the studies with rainbow trout and coho salmon, while there were significant differences between the tissue protein levels produced by different diets, there was no linear relationship between them.

Apparent Net Protein Utilization and Protein Retention

Two major criticisms of the P.E.R. method are that (1) it does not allow for maintenance requirements, which are neglected by assuming that all the nitrogen intake is utilized for growth, and (2) it is based on the assumption that weight gain is constant in composition, which is not necessarily valid (Bender & Doell, 1957; and Allison, 1964). Other approaches have thus been used to evaluate the effect of differing dietary protein levels upon fish growth, namely net protein utilization (N.P.U.) and protein

retention. Average fish weights and protein values from the proximate analysis of the carcasses at the beginning and at the termination of the experiment were used to calculate these values.

The protein retention values were based on the following equation, which is similar to the procedure used by Gerking (1952) and Pandian (1967b).

$$\text{Protein retained} = \left[\begin{array}{c}\text{body protein at}\\ \text{end of the test,}\\ \text{gm}\end{array}\right] - \left[\begin{array}{c}\text{body protein at the}\\ \text{beginning of the}\\ \text{test, gm}\end{array}\right]$$

$$\text{Apparent N.P.U.} = \frac{\text{protein retained in grams}}{\text{protein consumed during the test in grams}} \times 100$$

The apparent N.P.U. values in these tests were not derived from the equation used by Miller and Bender (1955) which was:

$$\text{N.P.U.} = \frac{(\text{body N of test group}) - (\text{body N of nonprotein group})}{\text{N consumed by test group}} \times 100$$

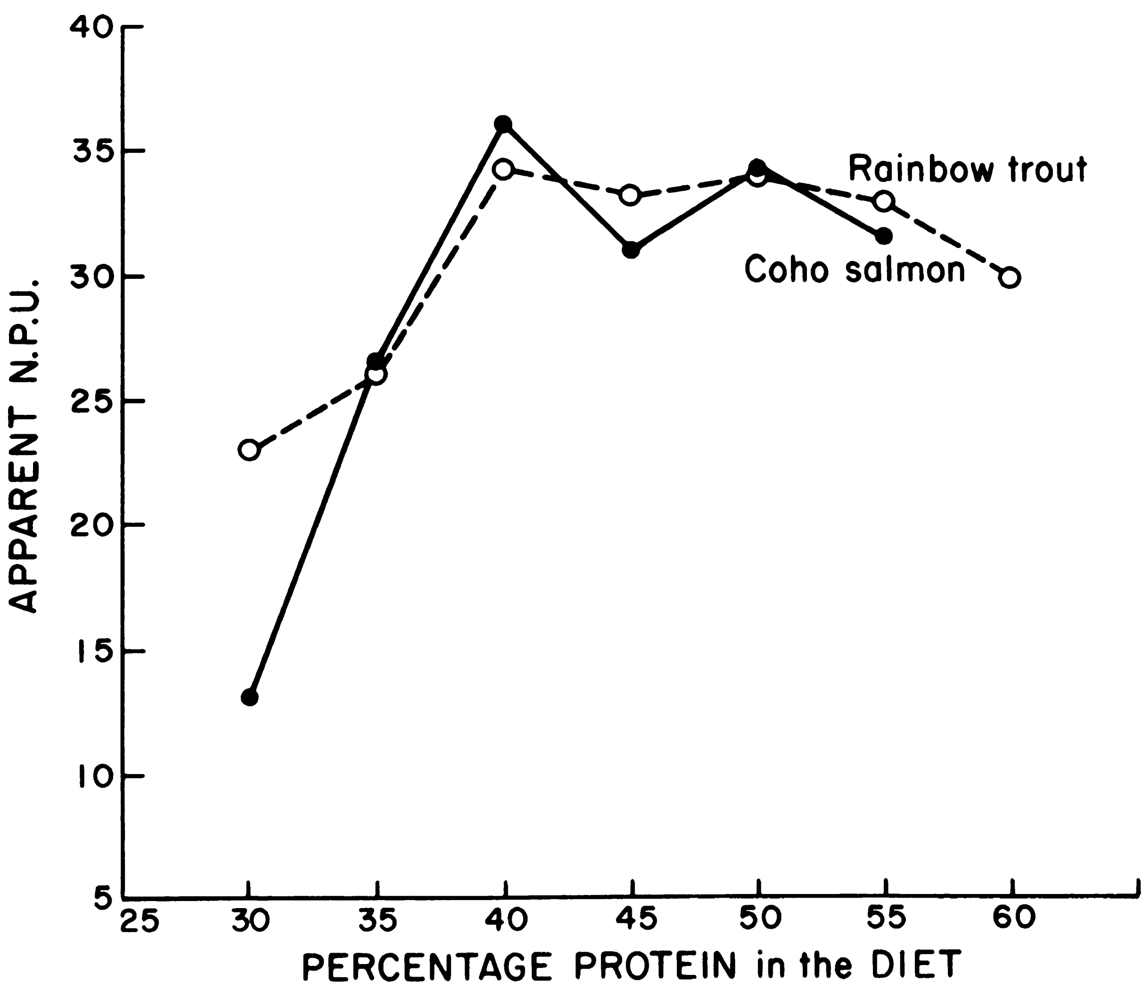
Since the test lacked a group of fish that had been fed a protein-free diet, the values resulting from these studies should not be considered the net values of protein utilization but rather the apparent net protein utilization.

Although N.P.U. (or apparent N.P.U.) has a theoretical advantage in allowing for maintenance requirements, the data derived from studies of rainbow trout and coho salmon did not alter the conclusions resulting from examinations of P.E.R. and growth index curves. Figure 7 demonstrates the effect of dietary protein on the apparent N.P.U. The values after the 40 per cent level of protein tend to plateau and exhibit only nonsignificant differences. The influence of protein levels in the diet was significant ($P \leq 0.05$) but the salinity effect was negligible. Analysis of the data indicated a significant interaction of salinity and dietary protein level upon apparent N.P.U. for the rainbow trout (Table 17) but not for coho salmon.

In the case of the rainbow trout, the mean value of apparent N.P.U. at 60 per cent dietary protein was significantly lower than the preceding values. A similar decline of apparent N.P.U. was observed in coho salmon at high dietary protein levels but this difference was not significantly different from previous values. It should be noted that the highest protein level used for coho salmon was only 55 per cent and higher levels might have further depressed apparent N.P.U. values. These findings are consistent with the depression of percentage gram gain at higher protein levels and are similar to observations of DeLong, et al., (1958) and Cowey, et al., (1972).

In regard to protein retention determinations, Figure 8 represents the pattern of fish reaction to both diets and salinity. Although the curves showed the same response to various diets as indicated by previous methods the data were expressed in terms of gram protein gain

Figure 7.--Apparent net protein utilization (N.P.U.) in relation to dietary protein levels for rainbow trout [$\pm$SE = 0.50] and coho salmon [$\pm$SE = 1.8]



40
35
30
25
20
15
10
5
APPARENT N.P.U.
Rainbow trout
Coho salmon
25
30
35
40
45
50
55
60
PERCENTAGE PROTEIN in the DIET

Table 17.--Apparent N.P.U. values of rainbow trout fingerlings fed different levels of protein and maintained at two different salinities [MSE = 1]

	Dietary protein concentration, %						
Salinity	30	35	40	45	50	55	60
10 p.p.t.	21.4^{a}	26.4^{b}	34.4^{c}	32.4^{c}	32.5^{c}	33.4^{c}	31.3^{c}
20 p.p.t.	24.6^{a}	25.8^{ac}	34.3^{b}	34.2^{b}	35.5^{b}	32.4^{b}	28.4^{c}

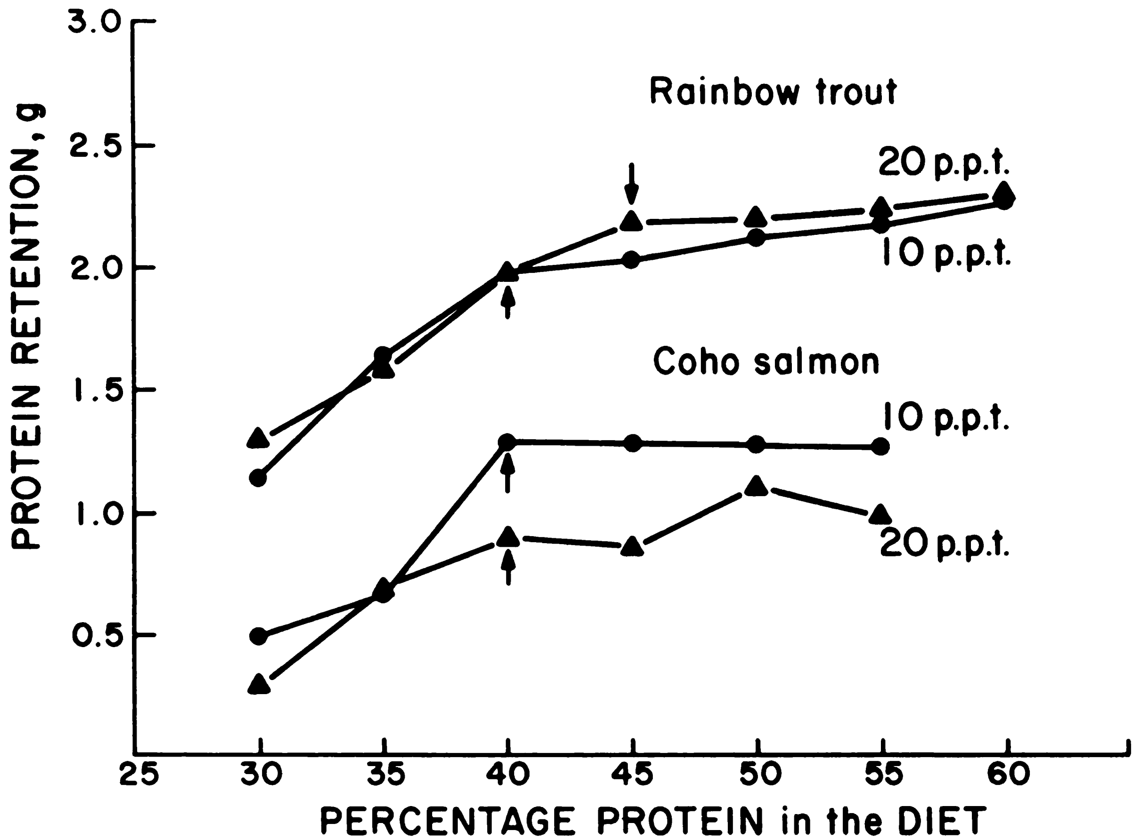
a,b,c Means on the same line under the same major heading followed by different superscripts are significantly (P < 0.05) different.

Table 18.--Apparent N.P.U. values of coho salmon fingerlings fed different levels of protein and maintained at two different salinities [MSE = 13]

	Dietary protein concentration, %					
Salinity	30	35	40	45	50	55
10 p.p.t.	16.0	24.3	35.7	34.3	34.7	33.7
20 p.p.t.	10.4	28.8	36.4	27.5	33.8	29.4
Mean	13.2^{a}	26.5^{b}	36.1^{c}	30.9^{bc}	34.2^{c}	31.5^{bc}

a,b,c Means on the same line under the same major heading followed by different superscripts are significantly (P < 0.05) different.

Figure 8.--Relationships between protein retention and dietary protein levels for rainbow trout [$\pm$SE = 0.08] and coho salmon [$\pm$SE = 0.05] maintained at 10 p.p.t. and 20 p.p.t. salinity



3.0
2.5
2.0
1.5
1.0
0.5
PROTEIN RETENTION, g
Rainbow trout
20 p.p.t.
10 p.p.t.
Coho salmon
10 p.p.t.
20 p.p.t.
25
30
35
40
45
50
55
60
PERCENTAGE PROTEIN in the DIET

and thus could be used to rate the protein levels of the diets by eliminating the interference of the alterations in the body composition of the test animal. The observed decline of the N.P.U. at high dietary protein levels might be a result of the drop in the efficiency of utilization of the protein. A portion of the dietary protein is expected to be deaminated to supply the body with the necessary energy for growth and maintenance. The deamination process requires a high expenditure of energy. Therefore, 50 or 55 per cent dietary protein would be the threshold limit of protein incorporated in a casein diet after which any increase in the protein level will decrease the efficiency of protein utilization.

Maintenance

The maintenance requirement is defined as the level at which the animal will neither lose nor gain weight, in other words, when percentage growth equals zero. From this definition, an attempt was made to estimate the protein level required for maintenance. The nitrogen growth index curves were manipulated by extending the ascending line downward until they met the x-axis which represented the percentage dietary protein at zero growth. This manipulation was based on the assumption that weight gain and percentage protein intake were directly related until the point of inflection. Hegsted and Chang (1965a) related gain in weight of rats to nitrogen intake and found that they were linearly related from zero growth until maximum growth was reached. Although the minimum protein requirements for growth of the two groups of coho salmon at 10 p.p.t. and 20 p.p.t. sea water were not substantially different, the two groups differed slightly in the estimated maintenance protein requirement which was around the 25 per cent level, whereas the maintenance protein level of the two groups of rainbow trout fingerlings was close to 15 per cent crude protein. It appeared from this approach that the osmotic pressure of the medium exerted little influence over the maintenance requirement and could be neglected. Thus, estimation of the maintenance protein requirement for fish at either of the two salinities would be valid.

Mortality

Mortality was determined on a daily basis. Dead fish were removed at the time of their discovery. The amount of diet they used before death was subtracted from the total diet used by the whole group. This was facilitated by determining the average daily feed which is dependent on the number of fish days. Rainbow trout maintained at 20 p.p.t. sea water showed a significantly higher mortality rate than those maintained at 10 p.p.t. and the values were 9.4 and 2.1 per cent, respectively. The coho salmon mortalities were not significantly different between the two salinities, 2.1 and 2.7 per cent. The protein level in the diet had no significant effect on the percentage mortality at either salinity level. Appendix A summarizes the biweekly number of deceased fish in relation to the treatment and salinity. It was apparent that mortality started in the first week of the experiment in spite of the acclimation period.

Hematology

In the case of rainbow trout, hematocrit determinations were significantly higher for fish maintained at 20 p.p.t. than those at 10 p.p.t. Total serum protein values of rainbow trout were not influenced by either salinity or dietary protein level.

Contrary to these results, the coho salmon exhibited higher total serum protein values for those fish maintained at the lower salinity level. The mean values of total serum protein and hematocrits for coho salmon and rainbow trout are listed in Table 19. However, salinity comparisons were not complete since the fish maintained at 20 p.p.t. receiving the 30 per cent protein diet were not tested and those receiving the 35 per cent diet were suffering from hypoxia. Oxygen deficiency or the capture of fish may affect the blood composition particularly the relative proportion of plasma protein fractions (Bouck & Ball, 1965 and 1966).

Table 19.--Total serum protein and hematocrit as influenced by salinity and dietary protein level for rainbow trout and coho salmon

	Dietary protein concentration, %								Salinity, p.p.t.		
	30	35	40	45	50	55	60	±SE	10	20	±SE
Rainbow trout											
Total serum protein, g/100 ml	2.6	2.4	2.6	2.6	2.4	2.7	2.4	0.1	2.5	2.6	0.06
Hematocrit, %	38.0	34.5	36.5	36.0	40.5	38.0	37.0	1.4	35.3	39.1[a]	0.8
Coho salmon											
Total serum protein, g/100 ml	1.9[a]*	2.0[b]	2.3[c]	2.4[d]	2.4[d]	2.5[e]		0.03	2.4[a]	2.1	0.02

*Average of 1.7 was assumed for fish at 20 p.p.t., based on a least square estimate of this mean.

[a,b,c,d,e]Means on the same line under the same major heading followed by different superscripts are significantly ($P < 0.05$) different.

Hematocrit was not determined for the coho salmon because the values would not be valid as hypoxia would increase the hematocrit values (Schiffman & Fromm, 1959).

DISCUSSION

The experiments were designed to provide statistical parameters for determining the difference between responses of fish maintained at two different salinities and also to measure the sensitivity of fish to different dietary protein levels. The results have potential usefulness when applied to the culture of fingerlings of Salmonidae or other species in brackish water. Comparative methods to evaluate the nutritive value of protein were employed in order to determine the best procedure to evaluate future nutritional bioassays.

Proteins are the basic constituents of the cells, tissues, and organs. They play a vital role in growth and all the regulatory mechanisms in the animal body. The minimum protein requirements for growth are important to minimize costs of fish rearing since protein is the most expensive item in formulating artificial diets. Minimal requirements of protein, as salinity in the rearing tanks changed, had not been previously determined. DeLong, et al., (1958) found that a rise in temperature increased the minimal protein requirements of chinook salmon.

The amount of protein required for growth was used to support three important functions: body maintenance, formation and growth of tissues, and allowance for losses in metabolism. The amount of the protein that is required for the tissue formed may be estimated from proximate analysis data (Maynard and Loosli, 1969).

The output of the tests established that growth was accomplished with each level of protein in the diets. In addition, an inadequate percentage protein intake did not block growth, terminate it, or exhibit any protein deficiency symptoms in the fish. Each group responded relative to the level of protein tested as long as such level was above the maintenance requirements and the protein biological value appropriate.

The percentage weight gain and protein retention methods established that salinity influenced the protein requirements of rainbow trout fingerlings but not those of the coho salmon. In the case of the rainbow trout, the percentage of dietary protein for maximum growth increased as the salinity increased. The problem was to determine with accuracy the minimum requirement of protein which produced the maximum weight gain or protein retention. Therefore, the values given for the protein requirement were approximated to the nearest dietary protein level tested.

The rise of protein requirements with salinity seemed to be a function of the osmotic pressure of the medium. The importance of proteins in regulating the internal fluids of the animals against a hypertonic medium through the binding of some ions in indiffusible complexes and thereby inducing a Donnan equilibrium was discussed by Potts and Parry (1963). However, this mechanism accounted for only a small part of the overall mechanisms of hyperosmoregulation.

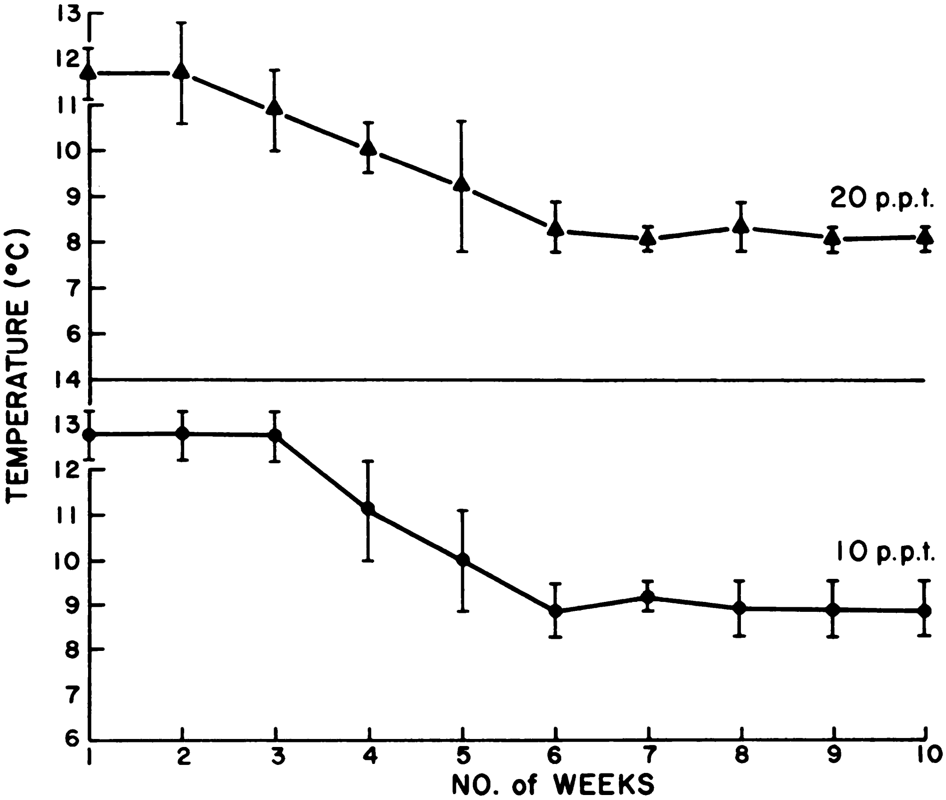
Concurrently, the rainbow trout at 20 p.p.t. salinity showed a substantially higher percentage of mortality than those at 10 p.p.t. Apparently, rainbow trout fingerlings at higher salinities expended more energy to maintain their osmotic equilibrium, resulting in greater stress and frequently death. In the case of the coho salmon, the stress induced by the two salinities studied was minimal and mortalities were low and not statistically different. Otto (1971) found that the highest growth values of coho salmon juvenile were obtained at salinities of 5 to 10 p.p.t. throughout the presmolt period. Canagaratnam (1959) stated that coho salmon fry grew faster in water of 12 to 18 p.p.t. than in fresh water. Otto and McInerney (1970) concluded that the preferred salinity for the presmolts of coho salmon increased from 7-8 p.p.t. in June to 13-14 p.p.t. in February. Salinity preference was determined by introducing a salinity

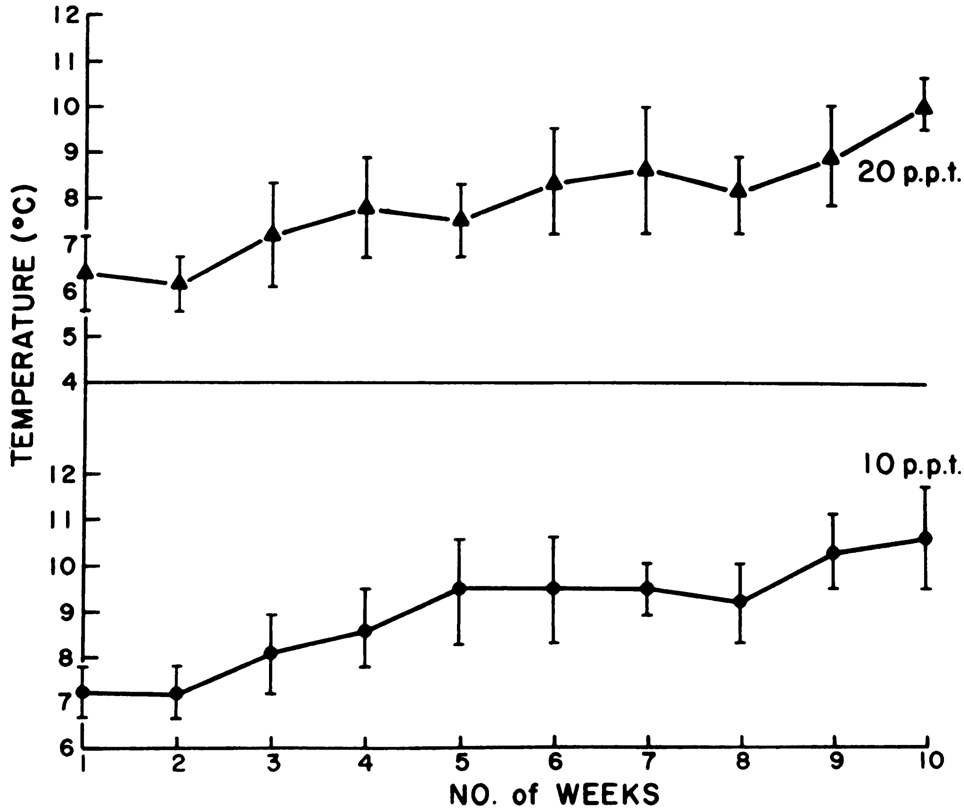
gradient and observing turning frequency as the gradient approached the fish and by observing evidence of an alarm response. The results of Kepshire and McNeil (1972) and Bullivant (1960) showed that chinook salmon fingerlings grew better at intermediate salinities (17-18 p.p.t.) and in fresh water than in 100% sea water. These authors attributed the poorer performance in 100% sea water to the greater expenditure of energy by the fry to maintain osmotic equilibrium. Conte and Wagner (1965) and Conte, et al., (1966) reported that seaward migration of steelhead trout and coho salmon is preceded by sea water adaptation. Also, euryhalinity mechanisms developed earlier in coho salmon than in steelhead trout. These mechanisms were a function of size and independent of age. Therefore, the increased dietary protein requirements with increased salinity (based on prevention of mortality) may be necessary to overcome the stress of maintaining osmolarity of body fluids since weight gain and protein retention of the two groups maintained in the two salinities did not differ substantially. Stress diminished as the fish grew larger and this was demonstrated in the coho salmon which underwent smolt transformation. The initial weights of the coho salmon were more than twice those of the rainbow trout and the initial total lengths were considerably greater in the case of the coho salmon (11.2 ± 0.3 versus 7.5 ± 0.2). There was no gain in weight until after the second week of the test for the coho salmon in spite of the availability of food. This may be attributed to the temperature influence on appetite described by Brett, et al., (1969) with sockeye salmon. Reduction of metabolic rate of poikilothermic animals was directly related to temperature (Fry, 1957). However, these results agreed with those of Vanstone and Markert (1968) who noticed that laboratory-reared coho salmon grew in a steadily decreasing rate in early winter. Therefore, the timing of the test was not proper from the standpoint of supporting optimum growth and late winter or spring would be more appropriate seasons because the experiments were dependent on natural water and consequently on natural water temperatures. The temperature gradients were plotted weekly (Figures 9 and 10). Unfortunately, a precise comparison of the two species is not possible because of the different age groups and because the two experiments were conducted under completely different thermal conditions. Temperatures of the water for the coho salmon were more or less close to those of the rainbow trout only after the fourth week of the experiment.

The weight gain and protein retention data indicated that beyond the minimum protein required for maximum gain in weight and protein retention, the values leveled off or decreased, without significant differences between dietary protein levels. The biological capability

Figure 9.--Weekly thermal range for the rainbow trout at 10 p.p.t. and 20 p.p.t. salinity

Figure 10.--Weekly thermal range for the coho salmon at 10 p.p.t. and 20 p.p.t. salinity


TEMPERATURE (°C)
20 p.p.t.
10 p.p.t.
NO. of WEEKS


TEMPERATURE (°C)
20 p.p.t.
10 p.p.t.
NO. of WEEKS

to achieve the maximum weight gain and protein retention appeared to be species specific and environmentally dependent. Gerking (1952) termed the point at which no further growth would take place as the growth potential of the species. Pandian (1967b) related the same term to the protein conversion rate value beyond which the values declined. Protein retained in each fish is considered as the net protein resulting from the difference between protein consumed and protein excreted. Warren and Davis (1967) designated the same caloric difference as the scope for growth.

The daily consumption of protein in grams per kilogram of weight gain by rainbow trout and coho salmon fed diets with varying protein concentration is shown in Table 20. The data show that maximum gain with minimum protein intake was achieved by both species on diets containing 40 per cent protein. The values were 477 and 465 g protein/day/kg gain for rainbow trout and coho salmon, respectively. At dietary protein concentrations of 35 and 30 per cent, appreciably greater amounts of protein were consumed per day per kg of weight gain. This is a reflection of both a higher feed intake on low dietary protein concentrations and poorer efficiency of dietary protein utilization. For rainbow trout raised in 20 p.p.t. salinity, the minimum protein intake per kilogram gain was achieved on the 50 per cent protein diet. However,

Table 20.--Protein consumed per day in grams per kilogram of gain by fish maintained at 10 p.p.t. or 20 p.p.t. and fed with varying protein concentration

	Dietary protein concentration, %						
	30	35	40	45	50	55	60
			Rainbow Trout				
10 p.p.t.	675	597	459	514	497	517	568
20 p.p.t.	738	635	495	483	471	510	581
Mean	704	616	477	499	484	514	575
			Coho Salmon				
10 p.p.t.	1336	846	507	510	505	534	
20 p.p.t.	1117	616	422	489	475	538	
Mean	1227	731	465	500	490	536	

this value was not significantly different from those on 45 or 40 per cent protein. These results agreed with those obtained from the nitrogen index curves which showed that the rainbow trout exhibited a higher protein requirement at 20 p.p.t. than at 10 p.p.t.

Total diet efficiency values responded directly to the quantity of protein in the diets. They increased as the amount of protein intake increased. These results coincided with the findings of Pandian (1967a) who attributed the decline in the conversion efficiency to the reduction in the quantity of food intake. The salinity had a nonsignificant effect on the efficiency of food conversion with a tendency for higher values for those fish maintained at a higher salinity. Kinne (1960) reported that growth rates of the euryhaline teleost Cyprinodon macularius and efficiencies of food conversion were higher at 35 p.p.t. than in fresh water, while Otto (1971) found that the growth rates, food intake, and gross food conversion efficiency had the highest values at salinities of 5 to 10 p.p.t. throughout the presmolt period of the coho salmon. The efficiency of food conversion is dependent on many factors and general statements should not be made. Conditions favorable to one fish would not necessarily be favorable to another. The best response is believed to depend on the environment to which the fish has been adapted and in which it has been reared.

Higher food conversion efficiency took place in fish hatched and grown in the same salinity than in fish transferred into another salinity after hatching (Kinne, 1962). Maximum gross efficiency of the diet output in the case of rainbow trout and coho salmon fingerlings was identical. The maximum was reached at 50 per cent dietary protein. This implied that diet no. 8 would be the most favorably balanced diet in spite of the indications that maximum weight gain, specific growth rate and protein retention were reached below the 50 per cent protein level. Brett (1971) obtained 48 per cent for the gross conversion of Halver's (1957) 50 per cent protein diet, which was the highest among many other diets that were compared. Although the available data were meaningful, they could not be compared with other data simply because the calculations of the coefficients were based on the wet weight gain of the fish and the dry weight of the ration. Since body composition is dependent on the food quality, physiological status of the animal, growth rate, and environmental factors (Love, 1957 and 1970; Maynard and Loosli, 1969), water content of the gain was variable. Therefore, the dry weights of fish gain were recommended and used by Brown (1957), Kinne (1960 and 1962), Otto (1971), and Brett, et al., (1969). The application of wet weight of fish gain was for three reasons: (1) the results could be applied by the common fish farmers and culturists with no laboratories as a quick and practical method to compare the efficiency of diets; (2) the evaluations of the overall dietary protein and protein intake were conducted using more precise methods whose values were presented earlier in the text; and (3) the method and the formula were already in use at the Western Fish Nutrition Laboratory. Dry weights of gain could be calculated by multiplying the average dry matter of the body composition by the average weight of the fish at the beginning and at the end of the test.

It was clear that by increasing the level of protein of the diet above maintenance level, the diet efficiency coefficients increased, approaching a maximum level at which the efficiency stabilized or declined. It has been previously stated that the quantity of the diet consumed was related to the gross efficiency of growth, reaching a maximum value at the maximum diet intake when the net efficiency was constant (Warren and Davis, 1967).

Paloheimo and Dickie (1966), after reviewing some valuable growth articles, stated that the logarithm of gross efficiency dropped as the quantity of food increased. In such a case the decline would be a function of food quality and not food quantity. Gross efficiency of growth of sockeye salmon fingerlings was temperature dependent (Brett, *et al.*, 1969). A decline of the logarithm of gross efficiency took place at the progressively higher dietary intakes associated with increasing temperatures. The highest values occurred between 5 and 10 C.

The inflection point of dietary efficiency of both the coho salmon and the rainbow trout took place at and beyond the 50 per cent dietary protein level. This level of inflection was 5 to 10 per cent higher than the conclusions from percentage weight gain and protein retention. The importance of a proper balance between energy and protein was discussed by Ringrose (1971) for brook trout. He found that the calorie-to-protein ratio (C/P) of 75 kcal/kg/% protein was approximately optimum for producing maximum growth and feed efficiency. The C/P ratios of the diets used in the rainbow trout and coho salmon tests ranged between 131.0 in the case of diet no. 269 and 66 for diet no. 273. Based on the apparent optimum dietary protein levels it could be said that C/P of 98.0 and 87.0 were the optimum ratios for producing maximum weight gain for the coho salmon and rainbow trout fingerlings. Biological Value of protein is a term used to define the percentage of protein absorbed and utilized by the body of the animal. It is dependent on the amounts and proportions of the essential amino acids available to the body. Tryptophan was included as an indispensable amino acid for the growth of rainbow trout (Shanks, et al., 1962). In addition, the knowledge that gelatin is deficient in tryptophan would tend to cause the belief that there may have been some variation of the protein biological value of the tested diets.

Some protein reserve in the body of fish may be helpful in countering stressful conditions. Excess protein, however, would be wasted through burning or transformation to fat and deposition as fat tissues since storage is very limited. Also, protein would be used as energy if the supplied energy was insufficient or the protein fed was low in quality (Phillips, 1969). Therefore, it was desirable to maintain an appropriate calorie-to-protein ratio in the diet which would promote optimum efficiency of energy utilization because carbohydrates have a sparing action on proteins. Phillips and Brockway (1959) stated that high dietary protein level would increase the metabolic rate of the fish to get rid of the toxic nitrogenous products which resulted from the deamination processes. Although the protein efficiency ratio (P.E.R.) and net protein utilization (N.P.U.) gave essentially the same relative ratings of the various levels of dietary protein as were obtained by the gain in weight and protein retention methods, the need for some information on the efficiency of the protein utilization by fish in relation to the quantity and quality of protein intake would be valuable.

The P.E.R. method was reported to be more reproducible than that of N.P.U., which was believed to be a more complex and difficult procedure (Chapman, et al., 1959; Derse, 1960; and Middleton, et al., 1960). Apparently the determinations of MSE showed that apparent N.P.U. values varied with the level of the dietary protein tested more than did the P.E.R. determinations. However, P.E.R. and N.P.U. outcomes showed similar responses that increased with dietary protein until they reached maximum values at the best balanced diet. Therefore, it was concluded that P.E.R. might be correlated with N.P.U. since this correlation has been frequently established in mammals (Bender, 1956; Bender & Doell, 1957; and Henry, 1965).

Chapman, et al., (1959) studied six protein sources of different qualities with rats and reported that P.E.R. determinations were more widely spread between low and high quality proteins than were either the net protein ratio or the net protein utilization.

From the results, P.E.R. values tended to level off when the maximum values of 2.1 and 2.2 for rainbow trout and coho salmon, respectively, were reached at approximately the 40 per cent protein level, although a slight decline was noticeable at higher levels of protein intake. Rippon (1959) found that rats which received .87 g protein/day and those which received 1.47 g/day did not show a significant difference in the calculated P.E.R. This suggested that both intakes were higher than the minimum level of protein intake that gave maximum weight gain. The reported values of P.E.R. by Cowey, et al., (1972) for the plaice were relatively lower than the values calculated for rainbow trout and coho salmon. The high values of P.E.R. might be due to the higher digestibility of casein diets than the freeze-dried cod muscle and shrimp meal diets used by Cowey, et al., (1972).

Obviously the protein efficiency ratio procedure was subject to a few handicaps such as: (1) it does not allow for maintenance by not including a control group of animals fed on a protein-free diet and, (2) it assumes that the increase in weight of the body is proportional to the protein retained, which is not always a valid assumption. In spite of the above mentioned limitations, this method is generally applicable to fish bioassay and the results rated the overall nutritive values of proteins in the diets better than the total diet efficiency method. Efficiency of conversion of dietary protein to tissue protein seemed to level off after the maximum growth was obtained or when the species growth potential (Gerking, 1952) was attained. Excessive protein intake will be eliminated since the capacity of the body to store protein is limited. The results of the studies of Morrison and Campbell (1960) established that P.E.R., which is correlated to N.P.U. (Bender & Doell, 1957), varied not only with the quantity and the quality of the dietary protein, but also with the duration of the test and the sex of the experimental animal. They suggested that the factors studied should be standardized if reproducible results were desirable.

To compensate for the maintenance allowance which was not considered in the P.E.R. calculations, Bender and Doell (1957) proposed a modified method by including a control group fed on a protein-free diet. This new method was called the net protein ratio (N.P.R.) and could be converted to a percentage scale (termed protein retention efficiency, P.R.E.) by multiplying N.P.R. by sixteen. The factor sixteen was derived by determination of the percentage protein in new tissue of the growing rat. No serious attempt was made to calculate the N.P.R. and the P.R.E. in this study since the equation requires a non-protein fed group.

Net protein utilization (N.P.U.) determinations which were proposed to measure the percentage of food protein that was retained by the fish were subjected in the rainbow trout and coho salmon tests to criticism identical to that mentioned for P.E.R. calculations, which was the lack of data from a control group fed a protein-free diet. Hence, the values of N.P.U. in this article would not be regarded as absolute since maintenance allowance of protein was not taken into account. Therefore, the term apparent net protein utilization was proposed as more appropriate. The apparent values of N.P.U.

were defined as the percentage protein retained in a given period of protein intake. Accordingly, these values tended to be numerically lower than would be true if a control group had been included. Chapman, et al.,(1959) estimated the P.E.R. for weaning rats fed 10 per cent protein as casein was equal to 2.5 in a four week assay. The N.P.U. for the same period was 72.2. The results in these two experiments established that P.E.R. and N.P.U. without any doubt varied with the tested level of protein. The findings of Morrison, et al., (1963) and Henry (1965) were similar to this conclusion. Rippon (1959) demonstrated that P.E.R. values were highly related to the Biological Value of the proteins and Bender (1956) reported a good correlation between P.E.R. and N.P.U. Therefore, the Biological Value of any protein would vary with the level of protein tested. The same conclusion was given by Maynard and Loosli (1969). Miller and Payne (1961) claimed that diets in which more than half of the energy was supplied in the form of proteins were unable to support growth in rats. Also, they reported that a decline in the net protein utilization was observed with increases in the protein concentration of the diets if the dietary protein was above the maintenance level. Recently, Cowey, et al., (1972) claimed identical findings with the plaice. The results of the rainbow trout and coho salmon tests did not confirm those of Miller and Payne (1961) and

Cowey, et al., (1972) but correspond to those of Njaa (1962) who implied that N.P.U. leveled off at high protein levels. Morrison, et al., (1963) failed to support the claim of Miller and Payne (1961), and they concluded that the decrease in N.P.U. with an increase of protein concentration of the diet found by Miller and Payne (1961) might be a result of diet deficiency in auxiliary growth factors. In addition, Hegsted and Chang (1965b) reported that N.P.U. decreased only after the maximal growth was achieved.

The relationship of net protein utilization and Biological Value of a given protein was expressed as B.V. = N.P.U./TD by Miller and Bender (1955) where TD referred to the true digestibility of the protein ingested which was independent of the percentage protein in the diet in rats (Forbes, et al., 1958) and in rainbow trout (Nose, 1967). The digestibility of Halver's (1957) diet that was applied in these tests was proven to be higher than two other natural and two other formulated diets (Brett, 1971). Therefore, the findings of these experiments should be adjusted by any commercial firm in order to meet the natural needs of fish at different water salinities to attain maximum growth and healthy conditions. The data under investigation were subjected to one important handicap which was the significant difference of the initial weights. This problem was minimized by employing the instantaneous growth rate method or applying the percentage gain values instead of using absolute gram gain in determining the growth curves. Hegsted and Chang (1965a) stated that log-dose response curves with gain were sigmoid in nature and recommended their use instead of the more complex procedures. In fisheries science, information on the efficiency of protein utilization was limited. Gerking (1952 and 1955) designated the term percentage utilization of protein for growth instead of the widely used term "biological value" which refers to that percentage of dietary protein retained by fish from the absorbed protein portion. Whatever the term that he may have used, he found that as the body weight of fish increased, the ability of fish to utilize the nitrogen component of the food for growth decreased. Pandian (1967b) applied the term "efficiency of protein conversion" to the definition of biological value. The tested fish were the euryhaline fishes Megalops cyprinoides and Ophiocephalus striatus. He reported a conclusion identical to that of Gerking (1952). In both experiments, natural foods were tested and no formulated diets were employed. Determinations of Biological Value of the protein were criticized by Hegsted and Chang (1965a) since they overestimated the nutritive values of low quality proteins. In mammals, Forbes, et al., (1958) found that the biological value decreased with the increase of the concentration of protein in the diets.

No comparable data were available in fisheries science and few individuals have attempted to apply to fishes the techniques used in mammalian and avian studies. All the methods that were used in these two bioassays rated the various protein levels in the same order and there was no serious difference in the output obtained. One of the most interesting points that emerged was the deviation of the total diet efficiency inflection point from those resulting from the rest of the methods. Therefore, the total diet efficiency method would be the least accurate method to be recommended to evaluate the dietary protein level. Generally speaking, the amount of protein in the diet exerts a biological influence on the growth rate. Low protein levels cause lower growth rate, growth efficiency, and protein conversion. Any relative increase in these is related directly to the increase of the protein level until the species specific growth potential is reached and after which growth or conversion will stabilize or decline.

The significant increase in the hematocrit values for the rainbow trout at 20 p.p.t. rather than at 10 p.p.t. was related to the loss of some water from the blood in the process of homeostasis in higher salinity media. The insignificant effect of various dietary proteins on the total serum protein and hematocrit values of the rainbow trout implied that the fish, due to the limited ability to store protein, responded similarly to the protein intake as long as it was above the maintenance level of protein.

In spite of the marked significance of total serum protein of the coho salmon as influenced by the concentration of protein intake the data could not be decisively discussed due to the disaster the test experienced on the 65th day with the loss of a few fish by asphyxia. However, it was deemed advisable to present such data if needed for future comparison.

Wedemeyer and Chatterton (1970 and 1971) attempted to establish normal ranges for rainbow trout and coho salmon. They stated that 2 to 6 g/100 ml and 1.4 to 4.3 g/100 ml were the normal total serum protein values for rainbow trout and coho salmon respectively while the hematocrit values as determined for the coho salmon juveniles ranged between 32.5 and 52.5 per cent. Consequently, the figures obtained in this study indicated that the tested coho salmon and rainbow trout juveniles were within the normal ranges proposed.

The environmental salinity might induce a positive effect on the protein content of the blood. Love (1970) stated that the specific gravity of the blood is dependent on the amount of protein in euryhaline fish. The changes that would occur in the fish when transformed from one salinity to another were discussed thoroughly by Winberg

(1956) and Love (1970). These changes in the rate of metabolism, blood composition and urine flow revert to the original patterns after a short period of adaptation to the new environment. These findings might account for the insignificant difference of the total serum protein values of the rainbow trout in the two salinities.

SUMMARY

The study was conducted at Bowman Bay Field Station, Anacortes, Washington of the Western Fish Nutrition Laboratory, U.S. Fish and Wildlife Service.

Juvenile rainbow trout, *Salmo gairdneri* and coho salmon, *Oncorhynchus kisutch* were used to determine protein requirements as influenced by salinity of 10 p.p.t. or 20 p.p.t.

The purified-diet method was employed, using seven protein levels that were supplied as casein and gelatin. The range of the dietary protein levels was 30 to 60 per cent in 5 per cent increments.

Evaluation of dietary protein levels and the reactions of the fish to environmental salinity was based on several techniques developed by mammal and bird nutritionists as well as methods which have been used by fish physiologists and nutritionists.

Percentage weight gain, gram protein gain (protein retention), gross or total efficiency of growth, specific growth rate, protein efficiency ratio (P.E.R.) and net protein utilization (N.P.U.) were criteria employed.

All the criteria, except for gross efficiency, indicated that 40 per cent protein in the diet was the

minimum value to support maximum growth of the two groups of coho salmon and that of rainbow trout at 10 p.p.t. salinity, while 45 per cent dietary protein concentration was the minimum level for the rainbow trout raised in 20 p.p.t. salinity.

Growth leveled off after reaching a maximum figure followed by a decline if the protein level of the diet exceeded 55 per cent.

An inverse relationship was established between gain in weight and feed/gain.

The ability of the fish to withstand higher osmotic pressure than that of their bodies depends largely upon the size, age, and physiological status of the fish .

Dietary protein levels for maintenance were predicted for both the rainbow trout and coho salmon by extending the growth index curves downward to meet the zero growth level. Maintenance levels were close to 15 and 25 per cent dietary protein for rainbow trout and coho salmon, respectively.

The calorie-to-protein ratio of the diets that produced maximum gain ranged between 98.0 and 87.0 kcal/kg/% protein.

Analyses of variance and Duncan multiple range tests were used to determine the significance of the data. Either standard error (±SE) or mean square error (MSE) accompanied each table or figure to give pertinent information about the range of the means.

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APPENDIX

Table 1.--Number of fish days of rainbow trout at 10 p.p.t.

Dietary protein concentration, %	1st 2 weeks	2nd 2 weeks	Total	3rd 2 weeks	Total	4th 2 weeks	Total	5th 2 weeks	Total
30	700	700	1400	700	2100	700	2800	700	3500
30	699 (1)	686	1385	686	2071	686	2757	686	3443
35	700	700	1400	700	2100	700	2800	700	3500
35	684 (2)	672	1356	672	2028	672	2700	672	3372
40	714	714	1428	714	2142	714	2856	714	3570
40	697 (2)	672	1369	672	2041	672	2713	672	3385
45	700	700	1400	700	2100	700	2800	700	3500
45	693 (1)	686	1379	686	2065	686	2751	686	3437
50	678 (4)	644	1322	644	1966	644	2610	644	3254
50	699 (1)	674 (1)	1373	672	2045	672	2717	672	3389
55	700	700	1400	699 (1)	2099	686	2785	686	3471
55	700	700	1400	700	2100	700	2800	700	3500
60	700	700	1400	7000	2100	700	2800	700	3500
60	687 (1)								

The number of dead fish is indicated between parentheses.

Table 2.--Number of fish days of rainbow trout at 20 p.p.t.

Dietary protein concentration, %	1st 2 weeks	2nd 2 weeks	Total	3rd 2 weeks	Total	4th 2 weeks	Total	5th 2 weeks	Total
30	698 (2)	661 (1)	1359	657 (1)	2016	627 (3)	2643	597 (1)	3240
30	700	700	1400	689 (4)	2089	632 (2)	2721	597 (1)	3318
35	700	699 (1)	1399	686	2085	686	2771	674	3445
35	710 (2)	672 (4)	1382	621 (3)	2003	588	2591	588	3179
40	700	700	1400	700	2100	700	2800		
40	685 (3)	657 (1)	1342	644	1986	644	2630	644	3274
45	680 (2)	672	1352	661 (1)	2013	658	2671	658	3329
45	682 (4)	658	1340	634 (2)	1974	627 (1)	2601	590 (2)	3191
50	688 (1)	686	1374	686	2060	676 (1)	2736	672	3408
50	687 (1)	686	1373	674 (1)	2047	660 (1)	2707	658	3365
55	686 (2)	672	1358	664 (1)	2022	658	2680	658	3338
55	687 (2)	672	1359	660 (1)	2019	658	2677	658	3335
60	687 (1)	686	1374	674 (1)	2048	672	2720	672	3392
60	687 (1)	679 (1)	1366	671 (1)	2037	658	2695	658	3353

The number of dead fish is indicated between parentheses.

Table 3.--Number of fish days of coho salmon at 10 p.p.t.

Dietary protein concentration, %	1st 2 weeks	2nd 2 weeks	Total	3rd 2 weeks	Total	4th 2 weeks	Total	5th 2 weeks	Total
30	560	560	1120	560	1680	560	2240	560	2800
30	560	560	1120	546 (1)	1666	532 (1)	2198	532	2730
35	560	560	1120	560	1680	560	2240	560	2800
35	560	546 (1)	1106	532 (1)	1638	532	2170	532	2702
40	560	560	1120	560	1680	560	2240	560	2800
40	560	560	1120	560	1680	560	2240	560	2800
45	560	560	1120	560	1680	560	2240	560	2800
45	560	560	1120	560	1680	560	2240	560	2800
50	560	560	1120	556 (4)	1676	504	2180	504	2684
50	560	560	1120	560	1680	560	2240	560	2800
55	560	560	1120	560	1680	560	2240	558 (2)	2798
55	560	560	1120	560	1680	560	2240	560	2800

The number of dead fish is indicated between parentheses.

Table 4.--Number of fish days of coho salmon at 20 p.p.t.

Dietary protein concentration, %	1st 2 weeks	2nd 2 weeks	Total	3rd 2 weeks	Total	4th 2 weeks	Total	5th 2 weeks	Total
30	560	560	1120	560	1680	560	2240	560	2800
30	560	556 (1)	1116	546	1662	546	2208	546	2754
35	560	560	1120	560	1680	560	2240	560	2800
35	560	560	1120	560	1680	560	2240	560	2800
40	560	560	1120	574 (+1)	1694	574	2268	574	2842
40	553 (2)	402 (6)	955	448	1403	448	1851	448	2299
45	560	560	1120	560	1680	560	2240	560	2800
45	560	560	1120	554 (1)	1674	546	2220	546	2766
50	560	560	1120	560	1680	560	2240	560	2800
50	560	560	1120	557 (1)	1677	525 (2)	2202	518	2720
55	560	560	1120	560	1680	560	2240	560	2800
55	560	560	1120	560	1680	560	2240	560	2800

The number of dead fish is indicated between parentheses.