THE EFFECT OF L-ASCORBIC ACID ON THE GROWTH OF SALMON AND TROUT

by

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ABSTRACT

As part of a continuing study into the growth and nutrition of salmonid fishes, a new fish diet was formulated and shown to supply their nutrient needs at a cost below that of a commercial ration. During the early stages of investigation, l-ascorbic acid (vitamin C) deficiency-like symptoms appeared among rainbow trout receiving the new diet. Stimulated by this finding, a series of experiments were conducted to elucidate the nutritional and microbial responses of four salmonid species to two l-ascorbic acid supplemented rations.

There was a marked nutritional response of the four species to l-ascorbic acid supplementation of their diet. For fish receiving 60 to 400 mg of l-ascorbic acid per kilo of diet, there was an increased survival, growth rate, fat storage, and tolerance to nitrogen embolism. The levels of l-ascorbic acid in the blood, liver, kidney and gill tissues were shown to reflect in a linear manner, the supplementation levels in the diet.

The microbial response of the fish to l-ascorbic acid supplementation of their diet was also marked. The counts of intestinal mesophilic bacteria were found to be significantly lower for fish receiving diets supplemented

with l-ascorbic acid.

As an initial aspect of a study into salmonid fish egg development and maternal nutrition, the l-ascorbic acid level in individual rainbow trout eggs was evaluated. The l-ascorbic acid isolated from these eggs was partially characterized by the spectral and paper chromatographic analysis of an osazone derivative.

The absence of information concerning several important aspects of the function of l-ascorbic acid in fish nutrition is noted and discussed.

The need for standardized rearing conditions in fish nutrition experiments provided the stimulus for the design of a new experimental self-cleaning fish tank that afforded a maximum weight of fish per unit volume of water by maintaining adequate dissolved oxygen levels with a venturi-type water admission device.

As part of a study being conducted by this laboratory into the rearing of axenic salmonid fish, an axenic fish rearing chamber was also designed and tested.

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INTRODUCTION

We cannot command nature except
by obeying her. Francis Bacon.

The science of nutrition has made rapid advances over the last fifty years; however, until recently, fish have been excluded from the limelight of nutritional investigation. The primary emphasis has been on the nutrition of man and his domesticated animals. With the advent of fish culture, and the realization of an increased demand for fish as a protein source for human consumption, the necessary stimulus was provided for studies into the nutritional requirements of fish.

The rearing of animals for an economic gain, be they terrestrial or aquatic, requires a detailed knowledge of their nutritional requirements if the adequate formulation of diets on a least cost basis from readily available feed ingredients is to be realized.

The aim of the domestic animal nutritionist, is to procure the means by which the full genetic potential for growth may be expressed within the narrow body temperature confines of the homeothermic animal in an atmosphere of twenty percent oxygen. The fish nutritionist is, however, presented with a poikilothermic animal that must function in an environment that offers a diminished oxygen supply for an

increased metabolic activity with increasing temperature. The situation is further complicated by the competition of microorganisms for this oxygen. An adequate definition of the nutritional requirements of fish will, therefore, be dependent upon a precise definition of the management conditions and their effect on the expression of the full genetic potential for growth.

The present work is a part of on going studies in the growth and development of salmonid fishes. Earlier work (Trust 1971, 1972, Trust and Wood 1973) has indicated that greater attention must be focussed upon the interactions between the salmonid fish's microbial environment and its nutritional environment.

This contribution had as its initial objective, the development of a suitable diet for salmonid fishes that could be formulated from constituents available within this province, and at a cost that was compatible with the objectives of the fish producer. A second objective, was to ascertain the extent of the interactions between the diet of the fish and its commensal microflora. As the studies progressed, it became apparent that the role of l-ascorbic acid (vitamin C) in the growth and development of the salmonid fish would have to be understood, at least in part, if the second objective was ever to be realized.

The work that has been completed is most conveniently presented under these subheadings:

(A), a salmonid diet; (B), the effect of l-ascorbic acid on the growth of four salmonid fishes; and (C), the l-ascorbic acid content of individual rainbow trout eggs.

SECTION A. A SALMONID DIET: INITIAL CONSIDERATIONS AND PRELIMINARY EXPERIMENTATION

I. Introduction

It was the aim of the initial work to establish a feed formulation that supplied the nutrient requirements and density needed for the optimal growth of several salmonid fishes.

A primary consideration must be the cost of such a diet. A commercial ration for fish may cost as much as eight hundred dollars per ton. Preliminary calculations in the autumn of 1972, suggested that a suitable ration could be formulated for slightly more than one hundred dollars per ton. With the rapid rise in market prices for feed ingredients, this estimate has since had to be revised to slightly less than three hundred dollars per ton.

The working hypothesis for the ration formulation was based on the fundamental concept that there is likely to be little or no difference between the needs of the biochemical systems of fish, and a domesticated species with an established nutritional requirement and a high growth rate, such as domestic poultry. Since the nutritional requirements of salmonid fish are tentatively known (Pearson 1968), it was our intention to formulate a ration of known composition to meet these tentatively known requirements as well as the documented nutritional requirements of poultry. This ration, which makes greater use of grain products than is usual in fish diets should result in a lower cost formulation for the feeding of hatchery fish.

II. The Nutritional Requirements of Salmonid Fish

A definitive treatment of the nutritional requirements of the salmonid fish is beyond the scope of this thesis. The reader is directed to a comprehensive collection of works on this subject by Halver (1972).

This section does, however, present comparative data on the nutritional requirements of salmonid fish and several domesticated species, viz., chickens, turkeys and swine. With the aid of this comparison, and from the results obtained from preliminary experimentation, a ration design suitable for salmonid fish will be presented.

(a) Energy and protein

The definition of a blend of diet constituents that will include the essential and non-essential amino acids required for normal growth and development within the cost framework imposed by the market place, is probably the most difficult task in feed formulation. The task is further complicated by the fact that total feed intake per unit time is a function of the metabolizable energy content of the diet. The amino acid requirement must, of necessity be the most important criterion upon which to base a feed formulation. If the formulation is to be specified exactly, there must be proper recognition of the balance that must exist between the protein and energy levels in the diet.

The protein requirements of chinook salmon *(Oncorhynchus tschawytscha)* were established by Delong *et al.* (1958). He concluded that the optimum protein level for chinook salmon was dependent upon water temperature, rising from 40 percent protein at 8.3 C (instantaneous relative growth rate [I.R.G.R.] = 0.72 percent per day), to 55 percent at 14.4 C (I.R.G.R. = 0.87 percent per day).

Most artificial fish diets contain approximately sevenety percent of their calories as protein (Halver 1972). These diets have probably been formulated to meet this requirement because the fish biologist has found that natural foods, as eaten, contain approximately seventy-two percent of their calories as protein. It has generally been assumed that salmonid fish utilize carbohydrates poorly (Phillips *et al.* 1948). However, since it has been subsequently shown that salmon can utilize relatively high levels of carbohydrates without physiological imbalance (Buhler and Halver 1961), it would be advantageous to design rations with higher levels of carbohydrates, thus affording a sufficient protein-sparing action to result in a greater relative utilization of the protein intake for body growth. The net result would be a lower protein intake which could substantially lower the cost of feeding fish and presumably reduce the sanitary problems assoc-

iated with the high nitrogen excretion that occurs in diets currently in use.

In general, an increase in the protein level of a diet necessitates an increase in the caloric level to provide energy for the effective metabolism of the additional protein. Likewise an increase in the caloric content of a diet must be compensated for by a similar increase in the protein level because feed intake will decrease, as domesticated animals, and presumably fish, eat to meet an energy requirement, not a protein requirement. Diets fed to hatchery trout contain from 1600 - 2600 kcal*kg^{-1} with corresponding protein levels (as fed) of 27 to 43 percent (Halver 1972). In all likelihood, fish adjust their feed intake to achieve the necessary daily intake of energy. It was shown that the energy required to produce a kilogram of trout (2000 - 4600 kcal kg^{-1} of fish) varied with the protein and caloric content of the diet, but was relatively independent of water temperature (Phillips and Brockway 1959). Presumably, as the water temperature increased so did the growth rate, and additional calories were supplied by an increased feed intake. A decrease in the water temperature would result in the opposite.

The protein requirement must be defined in

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*This figure represents metabolizable kilocalories.

relation to energy concentration (McKenzie 1964). Unfortunately, with the fish, minimum protein requirements for maximum growth rate in relation to the dietary energy levels have not been adequately described.

(b) Amino acids

The chinook salmon resembles young domestic animals in its total inability to synthesize the nine essential amino acids: histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. It is similar to the chick in its inability to synthesize arginine, and also seems to require dietary glycine supplementation for maximum growth. Like domesticated animals, the chinook salmon appears to be able to synthesize dispensable amino acids from arginine and glycine, but not from urea or diammonium citrate (Halver 1972).

The amino acid requirements of the chinook salmon, the rat, pig, and chick are presented in Table 1.* When the amino acid requirements are presented as percent of dietary protein, the similarity between the four species is remarkably close. The discrepancies that do exist may in part be explained by assuming that the stated requirements for the amino acids are not at or near the minimum protein levels needed for maximum growth (Halver 1972).

*The tables are located at the end of the results section.

(c) Vitamins

The salmonid fish require water-soluble and fat-soluble vitamins (Pearson 1968, Hishimoto and Okaichi 1969, Halver 1972). A comparison of the vitamin requirements for three species of salmonid fish and chickens, turkeys and swine is given in Table 2. It is worthy of note that the requirements for fish appear to be five to ten times greater than for domesticated animals and birds.

Fish, like man, appear to require l-ascorbic acid in their diets (McLaren *et al.* 1947, Kitamura *et al.* 1965).

(d) Minerals

The mineral requirements of fish have been studied to some extent (Halver 1964). Studies with radio-isotopes have shown that most, if not all, of the minerals needed for growth are absorbed directly into the body via the gills (Phillips *et al.* 1960).

Because of the limited information available on the mineral requirements of fish, all minerals essential for domesticated animals can be considered essential for fish until proven otherwise (Halver 1972).

(e) Fatty acids

Linoleic (18:2, ω6)*, linolenic (18:3, ω3) and

*(18 [number of carbons]: 2 [number of double bonds], 6 position of the double bonds from the methyl end of the fatty acid]).

arachidonic (20:4, ω6) comprise the essential fatty acids needed for fish growth. The tentative levels for linoleic and linolenic are 1 percent and 0.1 percent of the dry diet respectively (Nicolaides and Woodall 1962, Castell *et al.* 1972).

III. Materials and Methods

1. Tank design.

A description of the tank designed to hold the experimental fish is given in Appendix I.

2. Feed.

The diets formulated by this laboratory [Diet 1] were designated as UVIC-72 and UVIC-73. The quantity of ingredients for both rations were identical with the exception of canthaxanthin: UVIC-72 2.0 g per 1032 g of total ingredients, and in UVIC-73 0.5 g per 1030.5 g of total ingredients.

The common ingredients in both ration formulations were: wheat (150 g), oat groats (150 g), wheat germ meal (150 g), soya meal (150 g), Canadian herring meal (150 g), dry whey (150 g), brewers yeast (15 g), refined soya oil (80 g), cod liver oil (10 g), dicalcium phosphate (20 g), sodium chloride (5 g), and canthaxanthin. The grain products and dry whey were purchased from Buckerfield's Ltd., Victoria, B.C., and the brewers yeast from Viteway Bakery and Organic Foods

Centre Ltd., Victoria, B.C.

The wheat, groats, wheat germ, and soya meal were ground in a Thomas Mill (Arthur H. Thomas Co., Philadelphia, PA., USA) to pass a 0.025 inch screen. The herring meal was screened using an Endercott MK II Sieve Shaker (Endercott (test sieves) Ltd., London, S.W. 19, England) to remove eyes, scales and other undesirable objects. The dicalcium phosphate was ground to a fine powder using a mortar and pestle; to this was added yeast, sodium chloride, and canthaxanthin. All the ingredients were then thoroughly blended in a U.S. Stoneware feed mixer. The soya oil and cod liver oil were added and the mixture was kneaded by hand until of uniform consistency. The ration was frozen at -40°C until pelleted.

A commercial ration (Diet 2) was supplied by Moore-Clark Co., Salt Lake City, Utah, USA.

Before use, both diets were extruded through a 1/32 inch or a 1/16 inch die using a California Type CL-3 laboratory model pellet mill. The mill was preheated to the desired pelleting temperature of approximately 60°C by passing through it suitable quantities of Diet 2. After pelleting, the feed was allowed to stand at room temperature for no more than 12 h to cool and dry, and was then stored at 4°C until used.

The pellets were sterilized with ethylene oxide in tared vials by the method of Trust and Wood (1973). To test for sterility of the feeds after ethylene oxide treatment, 10 ml of Trypticase Soy broth (BBL) were added to each of two tubes containing 1 g of ethylene oxide-treated feed and incubated at 30°C for 120 h. Tubes were examined organoleptically for growth (Trust and Wood 1973).

3. Calculated proximate composition amino acid, vitamin and mineral composition of UVIC-72 & -73 rations.

Calculations were based upon composition tables of the N.R.C. (1971).

4. Analysed proximate composition.

The analyses of Diets 1 and 2 were conducted in the same way as the fish carcass analyses (Groves 1970, Horwitz 1970).

5. Amino acid analysis.

The amino acid composition of both diets was determined by AAA Laboratory, Seattle, Washington, USA.

6. Fish.

The initial evaluation of UVIC-72 (Diet 1), was conducted using juvenile rainbow trout (*Salmo gairdnerii*) and juvenile coho salmon (*Oncorhynchus kisutch*).

The rainbow trout used were of a mixed stock.

Stock I fish were reared from eggs obtained from spawning females from Lake Pennask, B.C., on July 12, 1972. On arrival at the laboratory, the eggs were stripped from the females, fertilized, and placed in a vertical flow hatchery. Details of the hatchery conditions were similar to those as described by Trust (1972). Hatching was completed by July 22, 1972. The alevins were reared in well water at a mean temperature of 14°C. Stock II fish were of similar age and size as stock I, but were obtained as fry from the Summerland hatchery, Summerland, B.C., in September, 1972. These fish had been suffering from "gill disease". The fish were held without feed in tanks at this laboratory. The "disease" disappeared within one week. Both stocks I and II were pooled and maintained on Diet 2 until October 24, 1972. These fish were used for experiment 1.

Juvenile coho salmon were obtained from stocks at this laboratory, and were maintained on Diet 2 until November 1, 1972. These fish were used for experiment 2 and experiment 3.

7. Body composition analysis

The chemical analysis of coho salmon carcasses was conducted by the methods of Groves (1970), Horwitz (1970), and Anderson (1973). It was found that moisture and fat composition could be obtained with fewer analytical manipulations by conducting these analyses

with fish which had been placed in tared 125 ml flasks.

8. Oxygen analysis of tank water.

The concentration of dissolved oxygen in the tank water was monitored throughout the experimental periods using the azide modification of the iodometric procedure as outlined in Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1965).

9. Bacteriological procedures.

Duplicate 1-ml water samples were collected from the tanks and duplicate serial dilutions were prepared in sterile well water. The viable bacteria present were enumerated on standard methods agar (Difco) by the Drop Plate Method (Miles and Misra 1938). Plates were incubated aerobically at 20°C for 96 hr.

10. Experiment 1.

Five representative groups each of 70 rainbow trout (mean weight of 1.4 g) were distributed into five tanks, and were fed UVIC-72 (Diet 1) and Diet 2 as follows: Tank 10, non-sterile Diet 2; Tank 11, ethylene oxide sterilized Diet 2; Tank 12, non-sterile Diet 1; and Tanks 13 and 14, ethylene oxide sterilized Diet 1.

The fish were fed at approximately 2 percent of their wet body weight per day, three times daily, and

were weighed by difference in water after each of three 15 day intervals and one 21 day interval.

11. Experiment 2.

Six representative groups of coho salmon (mean weight of 9.8 g) were distributed into six tanks as follows: Tank 16, no fish (control); Tank 17, 36 fish; Tank 18, 31 fish; Tank 19, 26 fish; Tank 20, 21 fish; Tank 21, 15 fish; and Tank 22, 10 fish. All the fish in these tanks were fed sterile Diet 1 two times daily at 1.5 - 2.0 percent of their wet body weight per day. Three stock tanks of approximately 35 fish each were maintained on sterile and non-sterile Diet 1 and sterile Diet 2, respectively.

Twenty days prior to commencing the experiment all the fish, with the exception of those in the stock tank receiving sterile Diet 2, were started on sterile Diet 1.

After 19 days the fish in Tanks 17 to 22 were anaesthetized with 100 ppm tricaine methanesulfate (MS-222), and individually weighed. Fish were removed from the stock tanks at the beginning and the end of the experiment for body composition analysis.

12. Experiment 3.

The fish used in Experiment 2 were pooled and distributed into two 0.91 meter diameter tanks

(approximate volume of 55 liters) located outdoors, as follows: Tank 1, 88 fish, sterile Diet 1; Tank 2, 86 fish, non-sterile Diet 1. The fish were fed two times daily at 1.0 - 1.5 percent of their wet body weight per day.

After 17 days the fish were anaesthetized with 100 ppm MS-222, and four fish were removed for carcass analysis when the experiment was terminated.

Tanks used in Experiments 1 and 2 were cleaned at biweekly intervals using a dilute hydrochloric acid solution. This method of cleaning effectively removed any algal growth. It was not necessary to clean the tanks in experiment 3 over the experimental period. The mean tank water temperature of Experiments 1 and 2 was 14.5°C, and for Experiment 3, 12°C.

IV. Results

(a) Diets

The calculated proximate composition of the UVIC-73 ration, Diet 1 (Appendix II), and the analysed proximate composition (Appendices VI - VII), were found to agree, and are compared in Table 3 to the analysed proximate composition for the commercial ration, Diet 2. Diet 2 had similar fat and gross energy levels, higher moisture and protein, and lower carbohydrate levels than Diet 1.

The total mineral (Appendix III) and vitamin

(Appendix V) levels of Diet 1 are presented in Table 4 and are compared to the mineral and vitamin requirements for chickens and poults. Of the minerals, only manganese is below the level required by chickens and poults, and of the vitamins, only niacin, which meets the requirement of chickens, is below that needed for poults.

The calculated (Appendix IV) and analysed amino acid levels of Diet 1 are compared in Table 5 to two other rations: Diet 2, and a test diet formulated by Halver (Appendix VIII). With the exception of arginine and methionine, Diet 1 supplies the required levels of amino acids for chinook salmon. Diet 1, with the exception of methionine, meets all the amino acid requirements on an as fed basis for broilers, poults and pigs (Table 6). When the sparing action of cystine is considered the dietary levels of methionine become borderline.

Both Halver's test diet on a dry diet basis, and Diet 2 on an as fed basis, supply the minimum tentative amino acid requirements for chinook salmon; however, Halver's diet on an as fed basis falls short of meeting these requirements.

When the amino acid levels are presented as grams of amino acid per 16 grams of protein nitrogen, there is a close similarity between Diet 1 and Diet 2.

However, the amino acid levels of both diets are slightly below the amino acid composition of whole hen egg protein, salmon roe, chum salmon meal and herring meal (Table 7).

(b) Experiments 1, 2 & 3

The results of growth Experiment 1 are presented in Table 8. There was a significant difference in the wet weight gain in grams per gram of feed intake, between fish on Diet 1 (0.69) and Diet 2 (1.13), but the differences between fish fed the sterile and non-sterile diets were not significant [two-tailed Student t test, 0.05 level, degrees of freedom equal 6]. The differences in the instantaneous relative growth rates for the two diets were not significantly different at the 0.05 level.

The mean oxygen level for all the tanks over the experimental period was 8.9 milligrams per liter, while a control tank with no fish had a dissolved oxygen level of 9.4 milligrams per liter (saturation at the mean water temperature of 14.5°C and 760 mm Hg is 10.3 mg/liter)

At the time the experiment was terminated, approximately 5 percent of the rainbow trout in Tanks 12, 13 and 14 (Diet 1) had developed scoliosis and lordosis accompanied by a darkened pigmentation of one-half the body along the anterior - posterior axis of symmetry.

These symptoms were first noticed six weeks after the experiment began, and the mortality was not significantly different from the control tanks on Diet 2.

The results of Experiment 1 show that the response to Diet 1 between coho salmon and rainbow trout was quite similar (Table 9). The effectiveness of feed utilization, as given in grams of wet gain per gram of feed intake, was not significantly different at the 0.05 level between the two species viz., 0.58 ± 0.07 for coho salmon in Tanks 17 - 22, and 0.68 ± 0.09 for the rainbow trout in Tanks 13 and 14. The instantaneous relative growth rates (I.R.G.R.) were, however, significantly different at the 0.05 level. The mean I.R.G.R. for rainbow trout on sterile Diet 1 was 1.72 ± 0.18 percent per day, while the value for the physiologically more mature coho salmon was 0.82 ± 0.11 percent per day.

The effectiveness of protein utilization for the coho salmon, given as grams of protein gain per gram of feed protein intake, was 0.35 ± 0.05 for fish in Tanks 17 - 22 (Diet 1). A similar value was not available for the rainbow trout.

The dissolved oxygen levels in Tanks 17 - 22 were monitored throughout the experimental period. A linear relationship between dissolved oxygen and live fish weight was determined (Fig. 1), and could be

described by the following equation:

$$Y = 9.4 - 0.0057X \quad S_E = \pm 0.37 \text{ mg } 0_2/\text{liter}$$

$$r = - 0.829$$

where Y is the concentration of dissolved oxygen in mg/liter, X is the live fish weight, S_E is the standard error of estimate of Y on X, and r is the correlation coefficient.

The tank water volume was at its mid-range of 29 liters for all oxygen determinations.

The aerobic microbial counts of the water in Tanks 17 to 22 varied from 1.66 to 10.8 x 10^4 organisms per ml. There was no significant difference between the sterilized and non-sterilized diet fed tanks. The water as it entered the tanks was found to be essentially free of any microorganisms by the detection method employed. A control tank with no fish had a count of 0.08 x 10^4 organisms per ml, and a control tank with no fish but receiving non-sterile feed had 5.48 x 10^4 organisms per ml of water. Similar counts were obtained from the stock tanks.

The growth response of the coho salmon in Experiment 3 was slightly better than that for Experiment 2. Although the growth rates were not significantly different, the efficiency of protein utilization was higher for fish fed on sterile and non-sterile Diet 1 in tanks 1 and 2 (0.52 and 0.43,

respectively) than for fish fed on sterile Diet 1 in tanks 17 to 22 (0.35 ± 0.05).

The overall response of fish in Tank 2 on non-sterile Diet 1 was below that for fish in Tank 1 on sterile Diet 1 (Table 10); however, considering the variation of the data for experiment 2, this difference is probably not significant.

An estimate of the amino acid intake and deposition as body protein was made using the data for Tank 1 coho salmon (Table 11). The amino acids available in the diet appear to meet the requirements for adequate protein deposition in the fish.

TABLE 1. Amino acid requirements of several species as percent of crude diet protein*

Essential Amino Acid	Chinook Salmon[1]	Rat[2]	Pig[3]	Chick[4]	Reference
arginine	6	1.5	1.5	6	[1]Klein and Halver (1970), [2]Mertz et al. (1952), [3]ibid, [4]Bird et al. (1960).
histidine	1.7	2.1	1.5	1.7	[1]Klein and Halver (1970), [2]Borman et al. (1946), [3]Mertz et al. (1955), [4]Bird et al. (1960).
leucine	3.9	7	4-5	7	[1]Chance et al. (1964), [2]Rose et al. (1949), [3]Eggert et et al. (1954), [4]Bird et al. (1960).
isoleucine	2.2-2.7[a]	4.0[b]	2.4-3.4[c]	3.0	[1]Chance et al. (1964), [2]Rose et al. (1949), [3]Becker (1957), [4]Bird et al. (1960).
valine	3.2	5-6	3.1	4.0	[1]Chance et al. (1964), [2]Rose et al. (1949), [3]Jackson et al. (1953), [4]Bird et al. (1960).
methionine	1.5[d]	-	1.4	-	[1]Halver et al. (1959), [3]Shelton et al. (1951).
lysine	5.0	5.2	4.7	-	[1]Halver et al. (1958), [2]Bressani and Mertz (1958), [3]Germann et al. (1958).
phenylalanine	5.1[e]	6-7	3.6	7.0	[1]Chance et al. (1964), [2]Rose and Womach (1946), [3]Mertz et al. (1954), [4]Bird et al. (1960).

TABLE 1. Continued

Essential Amino Acid	Chinook Salmon[1]	Rat[2]	Pig[3]	Chick[4]	Reference
threonine	2.3	3.1	3.0	3.0	[1]DeLong et al. (1962), [2]Frost (1950), [3]Beesen et al. (1953), [4]Block and Weiss (1956).
tryptophan	0.5	1.0	0.8	0.75	[1]Halver (1965), [2]Oesterling and Rose (1952), [3]Shelton et al. (1951), [4]West et al. (1952).

[a]leucine levels 1.5-2.3 % of dry diet

[b]12-13% protein in diet

[c]13.4-26.7% protein in diet

[d]in presence of adequate cystine (1.0% dry weight of diet)

[e]in presence of 0.4-0.0% tyrosine

*compare to Table 6

TABLE 2. Vitamin requirements of several species (in mg per kg of feed)

Vitamin ($mg\ kg^{-1}$ of diet)	Rainbow Trout[a,b]	Chinook Salmon[a,b]	Coho Salmon[a,b]	Poultry 0-8 wks[c]	Turkeys 0-8 wks[c]	Growing Swine[d]
Thiamine (B_1)	10-12	10-15	10-15	1.8	2.0	1.3
Riboflavin (B_2)	20-30	20-25	20-25	3.6	3.6	3.0
Pyridoxine (B_6)	10-15	15-20	15-20	3	4	1.5
Pantothenic acid	40-50	40-50	40-50	10	11	13.0
Niacin	120-150	150-200	150-200	27	70	22.0
Folic acid	6-10	6-10	6-10	0.55-1.2	0.9	-[g]
Cyanocobalamin (B_{12})	R[e]	0.015-0.02	0.015-0.02	0.009	0.003	22.0
Myo-Inositol	200-300	300-400	300-400	-[g]	-[g]	-[g]
Choline	R	600-800	600-800	1300	1900	1100
Biotin	1-1.2	1-1.5	1-1.5	0.09	0.3	-[g]
Ascorbic acid	100-150	100-150	50-80	-[f]	-[f]	-[f]
Vitamin A (I.U.)	2000-2500	R	R	1500	4000	2200
Vitamin D (I.U.)	-[g]	-[g]	-[g]	200	900	220
Vitamin E (I.U.)	R	40-50	R	10	10	-[g]
Vitamin K	R	R	R	0.53	0.7	-[g]

[a] Halver (1972)

[b] Fish fed at reference temperature of 15C with diets at about protein requirement

[c] N.R.C. 1971

[d] N.R.C. 1968

[e] Required, level not known

[f] Not required

[g] Not given

TABLE 3. Proximate composition of Diet 1 & Diet 2 as fed

Proximate:	Diet 1		Diet 2[e]
	Calculated[a]	Analysed[c]	
Dry matter (%)	91.5	92.9	90.7
Moisture (%)	8.5	7.1	9.3
Fiber (%)	2.0	2.0[b]	-[f]
Protein (%)	28.6	29.4	55.4
Fat (%)	12.1	12.0	11.9
Carbohydrate (%)	41.2	41.9	13.0
Ash (%)	7.6	7.6[d]	10.4
Gross energy (kcal/kg)	4489	4555	4806

[a]Refer to appendix II for details of the calculations.

[b]Calculated.

[c]Values presented are the mean percentages of the data in Appendices VI and VII.

[d]Calculated value.

[e]Moore-Clark commercial ration. Values presented are the mean percentages of the data in Appendices VI and VII.

[f]Not known.

TABLE 4. Mineral and vitamin requirements of chickens and turkeys (in percentage or amount per kg of feed)[a]

	Supplied by UVIC-73 ration[b]	Starting chickens (0-8 wk)	Starting poults (0-8 wk)
Calcium (%)	1.19	1.0	1.2
Phosphorus (%)	1.21	0.7	0.8
Iron (mg)	180	80	60
Magnesium (mg)	1000	500	500
Potassium (%)	0.75	0.2	0.4
Sodium (%)	0.21	0.15	0.15
Manganese (mg)	37	55	55
Zinc (mg)	48	50	50
Copper (mg)	42	4	6
Selenium (mg)	0.84	0.1	0.2
Vitamin A (IU)	4000	1500	4000
Vitamin D (IU)	>1000	200	900
Vitamin E (IU)	154.79	10	10
Biotin (mg)	0.33	0.09	0.3
Choline (mg)	2018	1300	1900
Cyanocobalamin (B_{12}) (mg)	31.8	0.01	0.003
Folic acid (mg)	1.28	0.55	0.9
Niacin (mg)	43.59	27	70
Pantothenic acid (mg)	22.21	10	11
Pyridoxine (B_6) (mg)	5.50	3	4
Riboflavin (B_2) (mg)	7.76	3.6	3.6
Thiamin (B_1) (mg)	8.20	1.8	2.0

[a] N.R.C. 1971.

[b] Refer to Appendix III and V.

TABLE 5. Amino acid composition of three diets (in % w/w)

	Diet 2[a]	Diet 1[b]		Test ration[e]		Tentative requirement for chinook salmon[h]
		A[c]	B[d]	A[f]	B[g]	
Alanine	3.38		1.46	2.2	0.6	
Arginine*	3.22	1.57	1.98	2.6	0.8	2.5
Aspartic acid	4.62		2.50	3.5	1.1	
Cysteine	0.00		0.00			
Cystine	0.55	0.52	0.41	0.2	0.07	1.0+
Glycine	3.78	1.33	1.40	3.9	1.3	
Glutamic acid	6.62		4.49	9.9	3.3	
Histidine*	1.36	0.61	0.71	1.3	0.4	0.7
Isoleucine*	2.14	1.35	1.19	2.5	0.8	1.5
Leucine*	4.03	1.92	1.94	3.9	1.3	1.0
Lysine*	3.95	2.00	2.02	4.0	1.3	2.1
Methionine*	0.91	0.51	0.34	1.2	0.4	0.5+
Phenylalanine*	2.30	1.10	1.20	2.2	0.7	1.7#
Proline	2.65		1.21	6.0	2.0	
Serine	2.94		1.34	2.8	0.9	
Threonine*	2.30	1.02	1.16	1.1	0.4	0.8
Tryptophan*	0.78	0.35	0.41	0.5	0.2	0.2
Tyrosine	1.65	0.96	0.92	2.5	0.8	0.4#
Valine*	3.00	1.30	1.46	3.0	1.0	1.5

[a] Moore-Clark Co., Salt Lake City, Utah, USA.

[b] UVIC-73 ration. Refer to Appendix IV.

[c] Calculated. Refer to Appendix IV.

[d] Analysed. Analysis performed by AAA Laboratory, Seattle, Wash., USA.

[e] Halver (1964). Amino acid composition calculated from Harvey (1956).

[f] Amino acid composition based on a dry diet basis. Refer to Appendix VIII.

[g] Amino acid composition based on an as fed basis.

[h] Halver (1964).

* = essential amino acid for chinook salmon.
+ = sparing action of cystine with methionine.
# = sparing action of tyrosine with phenyalanine.

TABLE 6. Amino acid requirements of several species*

Amino acid as % of diet	Broilers 0-6 wks[a]	Starting poults 0-8 wks[a]	Growing pigs 5-10 Kg[b]	Chinook Salmon[c]
Arginine	1.4	1.5	0.20	2.5
Glycine and/or serine	1.15	1.0	-	-
Histidine	0.46	0.55	0.27	0.7
Isoleucine	0.86	1.1	0.76	1.5
Leucine	1.6	1.9	0.90	1.0
Lysine	1.25	1.5	-	2.1
Methionine or Methionine	0.86	0.87	0.80	1.5
Cysteine	0.46	0.52	0.48	0.5
Phenylalanine or Phenylalanine	0.40	0.35	0.32	1.0
Tyrosine	1.5	1.80	0.50	2.1
Threonine	0.8	1.00	0.35	1.7
Tryptophan	0.7	0.80	0.15	0.4
Valine	0.8	1.00	0.70	0.8
	0.23	0.26	0.18	0.2
	1.0	1.2	1.20	1.3

[a] N.R.C. (1971)

[b] N.R.C. (1968)

[c] Halver (1964)

* Compare to Table 1

TABLE 7. Amino acid composition of hen egg protein, salmon roe, salmon meal, herring meal, UVIC-73 (Diet 1), and Moore-Clark commercial ration (Diet 2). Amino acid levels presented in g. per 16 g. N.

	Diet 1[a]	Diet 2[a]	Hen egg protein, whole[b]	Salmon roe[b]	Chum salmon meal[b]	Herring meal[c]
Nitrogen g per 100 g	4.83+	9.17+	13.70	1.46	11.10	11.45
Alanine	4.7	5.9	-	-	-	-
Arginine*	6.6	5.6	6.5	5.9	8.4	7.8
Aspartic acid	8.3	8.0	5.6	-	-	-
Cystine	1.4	1.0	2.2	-	-	1.0
Glutamic acid	14.9	11.5	13.2	-	-	-
Glycine	4.6	6.6	3.6	-	-	7.2
Histidine*	2.4	2.4	2.7	1.4	2.8	2.7
Leucine*	6.4	7.0	8.5	11.1	10.8	7.2
Isoleucine*	3.9	3.7	7.0	8.2	5.9	4.2
Lysine*	6.7	6.9	6.9	1.8	12.2	8.2
Methionine*	1.1	1.6	3.5	1.9	2.6	2.7
Phenylalanine*	4.0	4.0	5.4	6.3	5.3	3.4
Proline	4.0	4.6	4.3	-	-	-
Serine	4.4	5.1	7.2	-	-	-
Threonine*	3.8	4.0	4.7	4.7	5.4	4.0
Tryptophan*	1.4	1.4	1.8	0.7	1.0	0.9
Tyrosine	3.0	2.9	4.4	3.0	3.0	2.8
Valine*	4.8	5.2	8.1	7.5	6.8	7.9

[a] Amino acid analysis by AAA Laboratories, Seattle, Wash., USA.

[b] From Harvey (1956).

[c] Mean of six samples representative of 1960-61 B.C. herring season. Amino acid analysis by Wisconsin Alumni Research Foundation, Madison, Wisconsin, USA.

* = essential amino acid for salmonid fish.

+ = total nitrogen analysed by micro-Kjeldahl method of Horwitz (1970). Refer to Appendix VII. Nitrogen g per 100 g of diet as fed.

TABLE 8. Growth data for juvenile rainbow trout, experiment 1, fed two different diets[a,b]

Ration:	Diet 2-NS[c]	Diet 2-S[d]	Diet 1-NS	Diet 1-S	
Tank:	10	11	12	13	14
Period: 08-11-72 -22-11-72					
1. Number of fish -- Initial	69	70	69	70	69
Final	69	69	66	70	69
2. % survivors	100	99	94	100	100
3. Wet body weight (g) -- Initial[e]	96	98	103	98	101
Final[f]	131	136	136	128	132
4. Gain -- Wt (g)	35	38	33	30	31
5. Feed intake -- Wt (g)	34	35	39	39	39
6. Weight gained/g feed	1.03	1.09	0.85	0.77	0.80
7. I.R.G.R.[g]--%/day	2.17	2.34	1.99	1.91	1.91
Period: 22-11-72 -06-12-72					
1. Number of fish -- Initial	69	69	66	70	69
Final	69	69	66	70	69
2. % survivors	100	100	100	100	100
3. Wet body weight (g) -- Initial	131	135	132	128	132
Final	172	173	165	159	173
4. Gain -- Wt (g)	41	38	33	31	41
5. Feed intake -- Wt (g)	37	37	52	52	52
6. Weight gained/g feed	1.11	1.03	0.63	0.60	0.79
7. I.R.G.R.--%/day	1.94	1.78	1.56	1.55	1.89
Period: 6-12-72 -20-12-72					
1. Number of fish -- Initial	69	69	66	70	69
Final	68	68	66	70	69
2. % survivors	99	99	100	100	100
3. Wet body weight (g) -- Initial	172	173	165	159	173
Final	232	233	210	207	218
4. Gain -- Wt (g)	60	60	45	48	45
5. Feed intake -- Wt (g)	47	47	76	75	76
6. Weight gained/g feed	1.27	1.27	0.59	0.64	0.59
7. I.R.G.R.--%/day	2.14	2.13	1.72	1.88	1.65

TABLE 8. Continued

Ration:	Diet 2-NS[c]	Diet 2-S[d]	Diet 1-NS		Diet 1-S
Tank:	10	11	12	13	14
Period: 20-12-72 -10-01-73					
1. Number of fish -- Initial	68	68	66	70	69
Final	67	67	66	70	67
2. % survivors	99	99	100	100	97
3. Wet body weight (g) -- Initial	230	230	210	207	213
Final	318	300	299	279	297
4. Gain -- Wt. (g)	88	70	89	72	84
5. Feed intake -- Wt. (g)	69	69	126	114	133
6. Weight gained/g feed	1.28	1.01	0.71	0.63	0.63
7. I.R.G.R.--%/day	1.54	1.26	1.68	1.42	1.58
$\bar{X}$ weight gain g/g feed	1.17 ±0.11	1.10 ±0.10	0.70 ±0.10	0.66 ±0.07	0.70 ±0.10
$\bar{X}$ I.R.G.R. (% day^{-1})	1.94 ±0.25	1.88 ±0.41	1.74 ±0.16	1.69 ±0.21	1.76 ±0.15

[a]Diet 1 = UVIC-72

[b]Diet 2 = Commerical ration. Moore-Clark, Utah, USA.

[c]NS = Non-sterile.

[d]S = sterilized with ethylene oxide.

[e]Initial weights corrected for weight of losses from previous weighing.

[f]Final weights include the weight of losses.

[g]Instantaneous relative growth rate (Brody 1969).

TABLE 9. Estimates of mean protein, fat, and energy storage of coho salmon from tanks 17, 18, and 19 of experiment 2

Tank:		17	18	19
Group size	- Initial	36	31	26
	Final	36	31	26
Total wet body wt (g)[a]	- Initial	362.2	307.9	256.3
	Final	435.5	366.4	299.5
Fork length (cm)[b]	- Initial	9.8	9.7	9.7
	Final	10.2	10.0	10.0
Wet body wt (g)[c]	- Initial	10.1	9.9	9.9
	Final	12.1	11.8	11.5
Water (g)[d]	- Initial	7.57	7.42	7.42
	Final	9.07	8.85	8.62
Fat (g)[e]	- Initial	0.52	0.51	0.51
	Final	0.63	0.61	0.60
Protein (g)[f]	- Initial	1.69	1.66	1.66
	Final	2.02	1.97	1.92
Gain - Total wet body wt (g)		73.3	58.5	43.2
- Wt (g)		2.0	1.9	1.6
- Protein (g)		0.33	0.31	0.26
- Fat (g)		0.11	0.10	0.09
- Energy (kcal)[g]		2.90	2.70	2.32
Total feed intake (g)		108.1	89.7	72.1
Feed intake[h] (g)		3.00	2.89	2.77
Protein intake (g)		0.88	0.85	0.81
Fat intake (g)		0.36	0.35	0.33
Wt gained g/g feed		0.67	0.66	0.58
Protein gain g/g feed protein		0.38	0.36	0.32
Fat gain g/g feed fat		0.31	0.29	0.27
Energy gain (kcal)/energy consumed (kcal)		0.21	0.20	0.18
I.R.G.R.[i] (%/day)		0.95	0.92	0.79

[a,b,c] Refer to Appendix IX & X. The feeding period was from 13-11-72 to 01-12-72.

[d,e,f] Estimates of water, fat and protein made using mean values from coho salmon carcass analysis, Appendix XIV. Water = 74.96 % (w/w), fat = 20.69 % (d/w), and protein = 84.03 % (FFDM).

[g] Gross energy values were estimated on the basis of 5.67, 9.4 and 4.2 kcal/g for protein, fat and carbohydrate, respectively.

[h] UVIC-72 ration as fed: protein, 29.4 %; fat, 12.0 %; gross energy, 4.56 kcal/g.

[i] I.R.G.R. is the instantaneous relative growth rate (Brody 1969).

TABLE 9 Continued. Estimates of mean protein, fat, and energy storage of coho salmon from tanks 20, 21, and 22 of experiment 2

Tank:		20	21	22
Group size	- Initial	21	15	10
	Final	21	15	10
Total wet body Wt (g)[a]	- Initial	216.4	151.0	103.7
	Final	246.0	175.8	121.8
Fork length (cm)[b]	- Initial	9.8	9.7	9.8
	Final	10.1	10.1	10.3
Wet body Wt (g)[c]	- Initial	10.3	10.1	10.4
	Final	11.7	11.7	12.2
Water (g)[d]	- Initial	7.72	7.57	7.80
	Final	8.77	8.77	9.15
Fat (g)[e]	- Initial	0.53	0.52	0.54
	Final	0.61	0.61	0.63
Protein (g)[f]	- Initial	1.72	1.69	1.73
	Final	1.95	1.95	2.03
Gain - Total wet body wt (g)		29.6	24.8	18.1
- Wt (g)		1.4	1.6	1.8
- Protein (g)		0.23	0.26	0.30
- Fat (g)		0.08	0.08	0.09
- Energy (kcal)		2.05	2.22	2.55
Total feed intake (g)		63.1	44.9	30.8
Feed intake[h] (g)		3.00	2.99	3.08
Protein intake (g)		0.88	0.88	0.91
Fat intake (g)		0.36	0.36	0.37
Wt gained g/g feed		0.47	0.54	0.58
Protein gain g/g feed protein		0.26	0.30	0.33
Fat gain g/g feed fat		0.22	0.22	0.24
Energy gain (kcal)/energy consumed (kcal)		0.15	0.16	0.18
I.R.G.R. (%/day)		0.67	0.77	0.84

TABLE 10. Estimates of mean protein, fat, and energy storage of coho salmon from experiment 3.

Tank:		1	2
Group size	- Initial	88	86
	Final	88	86
Total wet body wt (g)[a]	- Initial	1212.0	1186.1
	Final	1399.4	1347.2
Fork length (cm)[b]	- Initial	10.6	10.7
	Final	11.2	11.2
Wet body wt (g)[c]	- Initial	13.8	13.8
	Final	15.9	15.6
Water (g)[d]	- Initial	10.3	10.3
	Final	11.9	11.7
Fat (g)[e]	- Initial	0.72	0.72
	Final	0.82	0.81
Protein (g)[f]	- Initial	2.30	2.30
	Final	2.66	2.60
Gain - Total wet body wt (g)		187.4	161.1
Wt (g)		2.1	1.8
Protein (g)		0.36	0.30
Fat (g)		0.10	0.09
Energy (kcal)[g]		2.98	2.55
Total feed intake (g)		206.1	201.9
Feed intake[h] (g)		2.34	2.35
Protein intake (g)		0.69	0.69
Fat intake (g)		0.28	0.28
Wt gain g/g feed		0.90	0.88
Protein gain g/g feed protein		0.52	0.43
Fat gain g/g feed fat		0.35	0.32
Energy gain (kcal)/energy consumed (kcal)		0.28	0.24
I.R.G.R.[i] (%/day)[i]		0.83	0.72

[a,b,c] Refer to Appendix XI & XII. The feeding period was from 29-12-72 to 14-01-73.

[d,e,f] Estimates of water, fat and protein made using mean values from coho salmon carcass analysis, Appendix XIV. Water = 74.96% (w/w), fat = 20.69% (d/w), and protein = 84.03% (FFDM).

[g] Gross energy values were estimated on the basis of 5.67, 9.4 and 4.2 kcal/g for protein, fat and carbohydrate, respectively.

[h] UVIC-72; protein 29.4%; fat, 12.0%; gross energy, 4.56 kcal/g, as fed.

[i] I.R.G.R. is the instantaneous relative growth rate (Brody 1969). Calculated using mean wet weight gain.

TABLE 11. Estimated amino acid intake and deposition as body protein for coho salmon from tank 1, experiment 3

	Total amino acid intake in diet[a] (mg)	Amino acid deposited as body protein[b] (mg)	Calculated net amino acid utilization[c] (%)
Arginine	56.6	30.2	53.4
Histidine	20.7	10.1	48.8
Leucine	54.5	38.9	71.4*
Isoleucine	33.1	21.2	64.0
Lysine	57.3	43.9	76.6*
Methionine	9.7	9.4	96.9*
Phenylalanine	33.8	19.1	56.5
Threonine	32.4	19.4	59.9
Tryptophan	11.7	3.6	30.8
Tyrosine	25.5	10.8	42.4
Valine	40.7	24.5	60.2

[a]Calculated from a mean protein intake of 0.69 g. See Tables 5 and 6.

[b]Calculated using chum salmon meal values from Table 5.

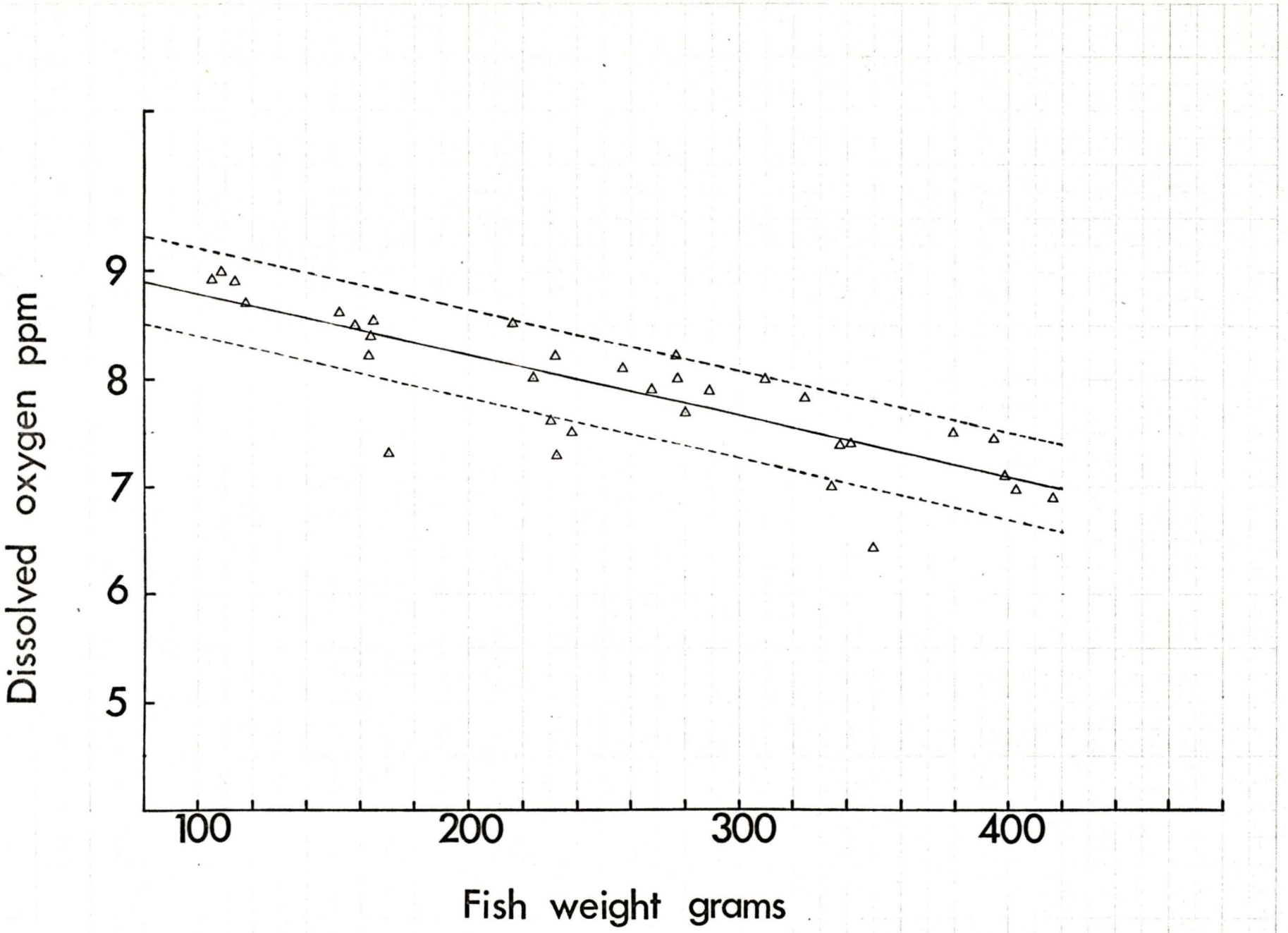
[c]Net amino acid utilization defined as: amino acid deposited as body protein (mg)/total amino acid intake in diet (mg).

*Possible limiting amino acids.

FIGURE 1. Dissolved oxygen (ppm) vs wet fish weight (g) for the coho salmon in experiment 2. The water and air flow rates were 150 and 500 cc min^{-1}, respectively, and oxygen determinations were conducted when the tank water volume was approximately 29 litres. The mean water pressure was 40 lbs in^{-2}. The regressed equation for the relationship was: $Y = 9.4 - 0.0057X$, $S_E \pm 0.39$ ppm

$r = -0.829$.

One standard error unit is indicated on the graph.


Dissolved oxygen ppm
9
8
7
6
5
100
200
300
400
Fish weight grams

V. Discussion

From a nutritional aspect, the fish has been regarded as representing a unique biological system. The general consensus has been that the fish require levels of protein and vitamins that are higher than the protein and vitamin requirements of other animals.

Our hypothesis, as stated in the introduction, questioned this disproportion.

For the most part, the nutritional requirements of fish are only tentative, and this adds to the difficulty in formulating a suitable ration of known composition. As a model, the chicken-turkey system was chosen, and a diet was formulated to meet their documented nutritional requirements. It was our opinion that this would provide a suitable basis upon which to formulate a ration for salmonid fish.

The growth data for rainbow trout and coho salmon, demonstrate that the UVIC-72 ration was capable of supplying the nutrient density needed for the growth of these fish. For the rainbow trout, there was no significant difference in growth rate over a nine week period between a commercial fish ration and the UVIC-72 ration, although there was a significant difference between the feed efficiency ratios. On the other hand, the feed efficiency ratios between coho salmon and rainbow trout were not significantly different, indicating the two species were equally able to utilize the

ration for growth purposes. The protein efficiency for the UVIC-72 ration was higher than reported values for the commercial ration (Groves 1970), indicating that a higher proportion of the protein consumed was directed towards body protein growth, and a lesser amount towards meeting the energy requirements of the fish.

The UVIC-72 ration was shown by analysis to contain levels of arginine and methionine that were below the specified levels for chinook salmon. However, estimates of the amino acid intake versus amino acid deposition as body protein (Table 11) [net amino acid utilization] indicate that the limiting amino acids may be lysine, leucine, and methionine even though the levels of lysine and leucine in the diet surpass the tentative required levels. Unfortunately, little work has been done with the limiting amino acid requirement of fish (cf. glycine requirement of chickens), and supplementation studies with the above amino acids should be conducted.

As a continuing study on the effect of ethylene oxide sterilization of feed on fish growth (Trust and Wood 1973), the preliminary investigations of the UVIC-72 ration were conducted with sterilized and non-sterilized rations. Over the experimental period of 9 weeks, no significant difference between the growth rates of rainbow trout on the sterile and non-sterile rations could be ascertained, confirming the results of Trust and Wood (1973).

The development of lordosis and scoliosis in the rainbow trout indicated that a possible deficiency of l-ascorbic acid existed in the UVIC-72 ration. The levels of the other vitamins, although far below the tentative required levels for various salmonid fish, appeared to be adequate for fish growth.

After a close examination of the literature, it was decided to investigate further the interactions between l-ascorbic acid and salmonid fish. The preliminary investigations of the UVIC-72 ration indicated that a suitable ration was now at our disposal for these studies.

SECTION B. THE EFFECT OF L-ASCORBIC ACID ON THE GROWTH OF FOUR SALMONID FISHES

I. Literature Review

It is the express purpose of this literature review to acquaint the reader, not only with the literature pertinent to the role of l-ascorbic acid in fish nutrition, but to present, in a brief fashion, a background on l-ascorbic acid that will encompass those points the author feels are essential to placing this role in its proper perspective.

A. Scurvy and vitamin C - a succinct history.

Since the beginning of recorded history, scurvy has been a scourge to mankind. The scorbutic condition was first described in the Ebers Papyrus which dates from about 1500 B.C. (Woodruff 1964). Hippocrates, the Roman armies, and the Crusaders were all too familiar with scurvy and its infamous symptoms. With the onset of long sea voyages, scurvy left its indelible mark upon the crews of Vasco de Gama, in 1497 and Jacques Cartier, in 1536.

Woodruff (1964), notes that it was not until the sixteenth century that a number of authors referred to the therapeutic use of scurvy grass, water cress and oranges in the prevention of this disease. In 1734, Bachstrom, gave the first clear account of the role played by fresh fruits and vegetables in the aetiology of scurvy. James Lind, a British naval surgeon,

conducted the first controlled experiments to evaluate the various remedies for scurvy. His famous work, *A Treatise of the Scurvy* , was published in 1753. Although Lind demonstrated the remedial value of citrus fruits in treating scurvy, their general therapeutic use was slow to meet acceptance, and it was not until 1795, that lime and lemon juice became a regular issue in the ration of the British navy, although these fruits had been under experimental investigation use since 1600.

Holst and Frölich (1904), opened the present era of experimental investigation into scurvy as a deficiency disease by producing scurvy in guinea pigs and in so doing introduced a suitable experimental animal for future studies. The antiscorbutic fraction of various citrus fruits was termed vitamin C by Drummond (1920).

A series of dissertations on the properties of the antiscorbutic moiety of lemon juice, were presented by Zilva (1923, 1924, 1925, 1927). He recognized that the vitamin could be isolated from lemon juice by precipitation with basic lead acetate, and that it was a nitrogen-free unstable substance with powerful reducing properties. Zilva (1927), associated the antiscorbutic activity of the vitamin with its reducing power, but found that freshly oxidized solutions still retained their physiological activity. Tillmans (1930, 1932), resolved the apparent anomaly when he found that

ascorbic acid could be reversibly oxidized to dehydro-ascorbic acid, and that both the oxidized and reduced forms possessed vitamin activity.

Szent-Györgi (1928, 1931, 1932a, 1932b, 1932c, 1933), isolated a crystalline, optically active compound with the formula $C_6H_8O_6$ from the adrenal cortex of oxen, and from oranges, cabbage and paprika. This compound was designated a "hexuronic acid" because it gave positive colour tests for sugars. After the demonstration of its antiscorbutic properties by Waugh and King (1932), Tillmans *et al.* (1932), and Szent-Györgi (1932a, 1932b), vitamin C or hexuronic acid, was termed l-ascorbic acid. The antiscorbutic factor isolated by Zilva was identical to l-ascorbic acid.

Reichstein *et al.* (1933) and Haworth *et al.* (1933), obtained unequivocal evidence that synthetic l-ascorbic acid was identical in physical and biological properties to that isolated from natural sources; hence all doubts about l-ascorbic acid being the true vitamin were abandoned.

B. <u>The chemistry of l-ascorbic acid.</u>

An excellent review on the chemical nature and constitution of l-ascorbic acid is available (Hay *et al.* 1967).

L-ascorbic acid or l-threo-hexono-1,4-lactone-2-ene, is an optically active ($[\alpha]_D^{20}$ + 24 (c = 1, H_2O)), odour-

less, white crystalline compound melting at 192°C and having the chemical formula $C_6H_8O_6$. It is soluble in water and ethanol, but practically insoluble in chloroform, ether, benzene, oils and fats.

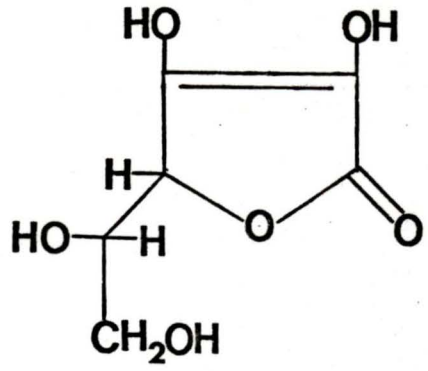
Normally it reacts as a monobasic acid liberating carbon dioxide from carbonates and bicarbonates with pK_1 4.25 in water (Bell 1961). A second ionization constant pK_2 11.79 (Nebbia 1959), has also been reported.

L-ascorbic acid also exists in nature as the oxidized form, l-dehydroascorbic acid $C_6H_6O_6$ (Figure 2). These compounds are in a state of reversible equilibrium in biological systems, and have the same biological activity (Woodruff 1964). Biological activity resides in the l-isomers only. Further oxidation yields biologically inactive 2,3-diketo-l-gulonic acid.

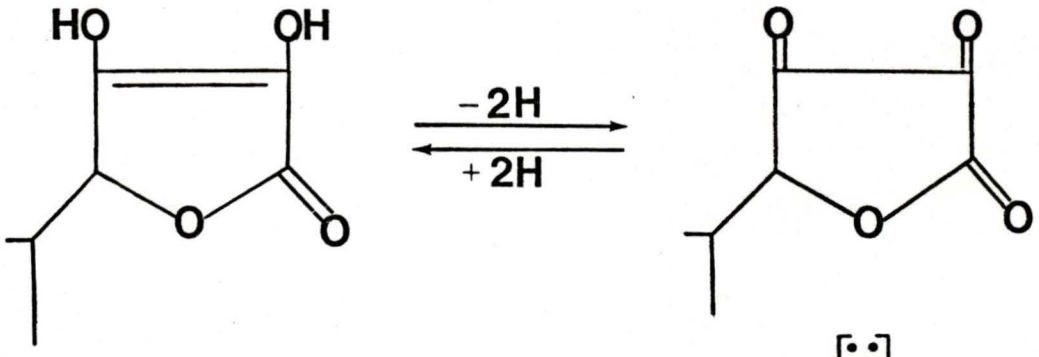
There are a wide range of published values for the extinction coefficients in aqueous solution at the absorption maxima of 265 nm at pH > 6.8 and 245 nm at pH < 1.5. Lawendel (1957), and Hewitt (1961), using strict anaerobic conditions and copper-free media, are in agreement with $E^{1\%}_{1cm}$ 936-945 at 265 nm and pH 6.5 or greater.

L-ascorbic acid is a powerful reducing substance as indicated by its ability to reduce Fehling's [$Cu^{++} \rightarrow Cu^{+}$] or Tollen's solution [$Ag^{+} \rightarrow Ag°$] at room temperature

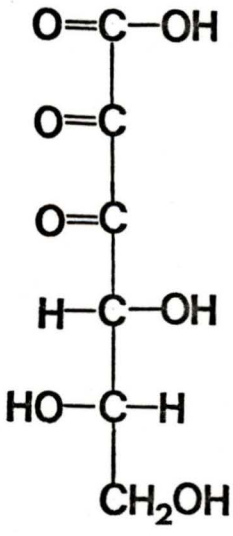
FIGURE 2. The structure of l-ascorbic acid (i), l-dehydroascorbic acid (ii), and 2,3-diketo-l-gulonic acid (iii). Note that the side chain at carbon 4 of the lactone ring has been abbreviated.


HO
OH
H
HO
H
O
O
CH2OH

[i]


HO
OH
O
O
−2H
+2H
O
O
O
O

[ii]


O=C–OH
O=C
O=C
H–C–OH
HO–C–H
CH2OH

[iii]

(Szent-Györgi, 1928). The E_o' of the l-ascorbic acid - l-dehydroascorbic acid system is +60 mV at pH 7.0 and 30°C (Ball, 1937), and +166 mV at pH 4.0 and 35°C (Assoc. Vit. Chem. 1951).

The aerobic oxidation of l-ascorbic acid, which is catalyzed by cupric ion, follows first order kinetics producing l-dehydroascorbic acid and hydrogen peroxide. The oxidation is inhibited by a number of chelating compounds such as EDTA, flavonoids, metaphosphoric acid and aryl thioureas (Hay *et al.* 1967). Ferrous and ferric ions are less effective than cupric ion in catalysing the oxidation of l-ascorbic acid in aqueous solution.

Most proteins and amino acids exert some protection over the oxidation of l-ascorbic acid either through the formation of stable l-ascorbic acid-protein, or cupric-protein complexes (Hay *et al.* 1967).

L-ascorbic acid is oxidized rapidly in the cold by two equivalents of chlorine, bromine, or iodine. The addition of hydrogen sulfide to the oxidized solution reverses the reaction, and regenerates the original vitamin (Hirst, 1933).

The rate of aerobic oxidation is pH - dependent, displaying maxima at pH 5.0 and 11.5. The nature of the oxidation products also show a dependence on pH. For example, oxalic acid is formed during the autooxidation of l-ascorbic acid in alkaline solutions (Burger 1951),

but not at pH 4.0. L-dehydroascorbic acid and hydrogen peroxide are also products of oxidation under alkaline conditions (Eddy 1953).

The photochemical oxidation of ascorbic acid can proceed under either aerobic or anaerobic conditions. The result of UV irradiation is dehydroascorbic acid and hydrogen peroxide. The presence of iron and oxygen greatly accelerates this oxidation (Baker 1955), and as one would expect, acidic solutions are less susceptible to photochemical oxidation.

Clearly, the important chemical properties of l-ascorbic acid are associated with the oxidizable dienol group which confers upon the compound considerable reducing power.

C. The chemical determination of l-ascorbic acid by the 2,4-dinitrophenylhydrazine method.

In the chemical determination of l-ascorbic acid, advantage is taken of the easily oxidizable hydrogens of the dienol group on carbon atoms two and three. A considerable number of methods are based on the oxidation - reduction measurements of this system, and excellent reviews are available (Merck 1956, Olliver 1967, Roe 1967).

Oxidation - reduction methods for l-ascorbic acid determinations are subjected to the limitations imposed by the presence of other reducing substances which may

react with the oxidizing agent. L-ascorbic acid may be inadvertently oxidized to an unknown extent prior to or during extraction from the tissues (Roe 1967). Since oxidation of the dienol group is a prerequisite step for the determination of l-ascorbic acid by the 2,4-dinitrophenylhydrazine (2,4-DNPH) procedure of Roe and Kuether (1943), their's was chosen as the method of preference for the present work.

When l-ascorbic acid is mildly oxidized, l-dehydroascorbic acid is formed. The latter compound undergoes spontaneous transformation to 2,3-diketogulonic acid slowly in neutral or mildly acid solutions and rapidly in alkaline medium (Roe 1967). These two compounds couple rapidly with 2,4-DNPH in 9 N H_2SO_4 forming an insoluble bis-2,3-DNPH-osazone derivative (Roe 1937). (See Figure 3). This osazone displays a stable brownish-red color with a prominent absorption at 510 - 540 nm when treated with 85 percent H_2SO_4.

The method is specific for the following reasons (Roe 1967):

(1) the osazone complex which is responsible for the colour is formed only with six-carbon and five-carbon sugars and sugar-like compounds,

(2) the rate of coupling between 2,4-DNPH and l-dehydroascorbic acid and 2,3-diketo-l-gulonic acid is much faster than with sugars and other

sugar-like compounds viz., reductones and reductic acid,

(3) interfering chromogen formation is avoided by working with dilute tissue extracts (usually 1:5 to 1:10), and by carrying out the coupling at 37°C.

The presence of a reducing reagent, such as thiourea, adds to the specificity of the method.

Extraction of l-ascorbic acid from tissues is accomplished using either 5% metaphosphoric acid - 10% acetic acid or 4 - 6% trichloroacetic acid (Roe 1967). Both solutions have been shown to be excellent preservatives of the vitamin. Metaphosphoric acid is the preferred solvent because of its ability to stabilize l-ascorbic acid in the presence of Cu^{++} and Fe^{+++} ions, and because of its enhanced protein precipitating properties over trichloroacetic acid.

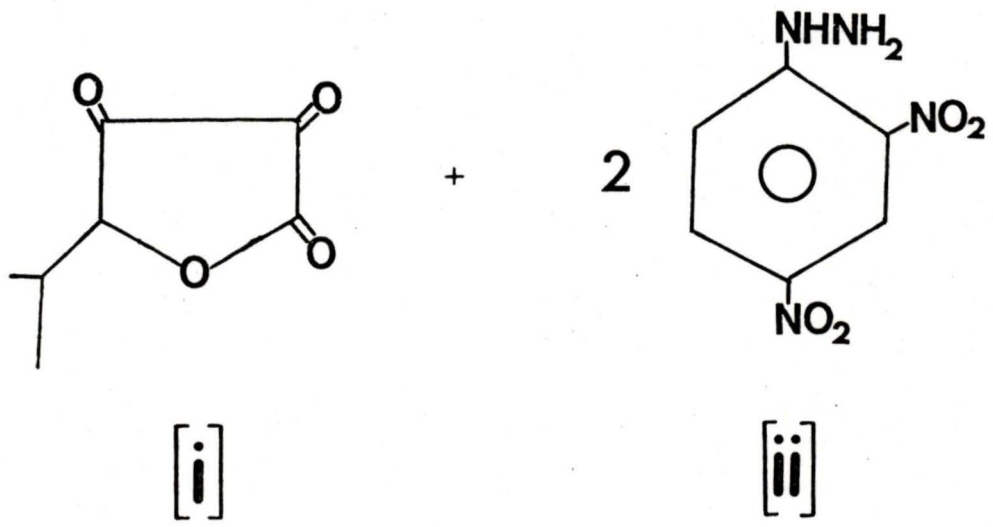
To accomplish the oxidation of l-ascorbic acid to l-dehydroascorbic acid prior to coupling, Norit (activated charcoal) is used. This oxidizing agent serves two functions (Mills and Roe 1947), (1) oxidation of l-ascorbic acid, (2) clarification of the tissue extract along with the removal of interfering pigments etc., which other oxidizing agents, such as bromine and 2,6-dichloroindolphenol, do not remove. Acetic acid or trichloroacetic acid must be present to prevent the absorption of the l-dehydroascorbic acid upon the Norit.

The temperature of coupling is critical, and adds greatly to the specificity of this method. Roe and Kuether (1943), established the temperature of 37°C for coupling of the oxidized l-ascorbic acid with 2,4-DNPH. At this temperature the rate of coupling of the oxidized vitamin is rapid, and the coupling of sugars slow (Roe 1967). Roe (1961), found no interference from glucose or fructose at concentrations up to 5 mg and 3 mg per ml, respectively, and glucuronic acid began to interfere at 0.25 mg per ml. Interference from the amounts of sugars or glucuronic acid found in plant and animal tissues does not occur when the required dilutions of the extract are made (Roe 1967).

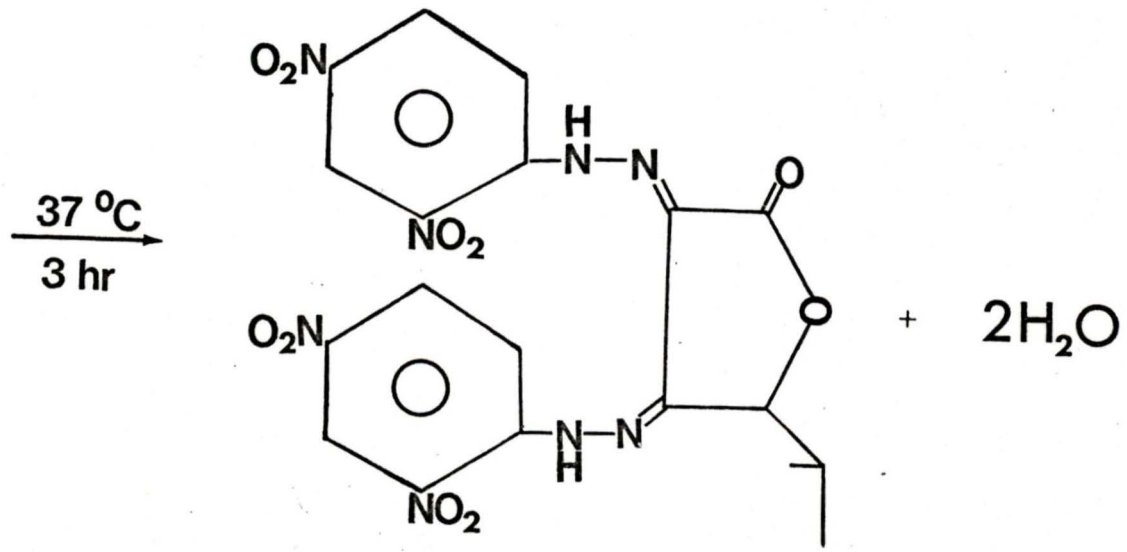
Several workers (Schaffert and Kingsley 1955 and Polk *et al.* 1960), modified the incubation temperature to 100°C. Coupling at this temperature will, however, yield erroneously high results with extracts of tissues containing sugars or other interfering substances. In animal tissues, the values with the coupling reaction at 100°C for kidney, liver and muscle were 32, 30 and 40 percent higher, respectively, than the values obtained by coupling at 37°C (Roe 1961).

A radiochemical evaluation of the 2,4-DNPH method has revealed that only 30 - 53 percent of the l-ascorbic acid present is really determined (Zloch *et al.*1971). This shortcoming itself does not cause more serious

FIGURE 3. The chemical reaction between 1-dehydro-ascorbic acid (i) and 2,4-dinitrophenyl-hydrazine (ii) to yield the bis-2,4-dinitrophenylhydrazine-osazone (iii). Note that the side chain at carbon 4 on the lactone ring has been abbreviated (refer to Figure 2).



[i] [ii]



[iii]

problems when compensated for by using a calibration curve which is constructed under the same conditions. It was confirmed that higher temperatures, although yielding a higher percent of oxidized l-ascorbic acid 2,4-DNPH-osazone, also gave erroneously high results due to the formation of other interfering osazones.

Further specificity for the determination of l-ascorbic acid is found in the colour forming reaction. This reaction involves solubilizing the 2,4-DNPH-osazone complex with 85 percent H_2SO_4. There is a molecular rearrangement of the bis-2,4-DNPH-osazone that yields a compound of unknown chemistry (Roe 1967), which absorbs maximally at 350 - 380 nm and 510 - 550 nm. The osazones of most potentially interfering substances are destroyed by the 85 percent H_2SO_4, since the acid splits the hydrazine linkage with the formation of the original products (Roe 1967).

D. The biochemical role of l-ascorbic acid.

In recent years, a number of more or less specific functions of l-ascorbic acid in intermediary metabolism have been studied. Reviews on this aspect of vitamin C are available (Merck 1956, and Mapson 1967).

The protrusive feature of l-ascorbic acid is the ease with which it may be oxidized and reversibly reduced. Almost all the terminal oxidases of plant and

animal tissues are capable of directly or indirectly catalyzing the oxidation of l-ascorbic acid (Mapson 1967). Reducing systems have been shown to exist in plant tissues. Szent-Györgi (1930), proposed an electron transport system employing glutathione and l-ascorbic acid as intermediates. This system is dependent upon dehydroascorbic acid reductase, an enzyme ubiquitous to plants, but absent from animal tissues. The oxidation of glutathione in crude kidney homogenates has been observed in reactions in which both diphosphopyridine nucleotide and l-ascorbic acid are involved (Stotz *et al.* 1937). A non-enzymatic reaction between glutathione and l-dehydroascorbic acid at pH 7.0 or above proceeds at an appreciable rate (Mapson 1967).

L-ascorbic acid is involved in a number of defined enzyme systems, e.g., hydroxylation to form norepinephrine, 5-hydroxylation of tryptophan, l-ascorbic acid-dependent diphosphopyridine nucleotide-oxidase, and tyrosine oxidation. In experimental scurvy, the activity of other enzyme systems is altered; these changes are probably adaptations to the altered metabolism induced by the scorbutic state (Woodruff 1964).

L-ascorbic acid plays a synergistic role with α-tocopherol (vitamin E) in the maintenance of intracellular antioxidants and free radical traps, both *in vitro* and *in vivo* (Mapson 1967). The conversion of folic acid to

folinic acid *in vitro* and *in vivo* requires l-ascorbic acid (Nichol and Welch 1950, and Shive *et al.* 1950). L-ascorbic acid is also involved with the formation of chondroitin sulfates, and is capable of forming stable sulfate derivatives (Baker *et al.* 1971).

The exact role of l-ascorbic acid in collagen formation is not known at the molecular level (Woodruff 1964). Various studies suggest, however, that the essential function relates to its ability to catalyze the hydroxylation of proline to hydroxyproline.

The most obvious lesions observed in scurvy are those related to a weakening of the intercellular substance of collagenous and fibrous connective tissue, and of cartilage, bone and dentine. Excellent and detailed descriptions of the symptoms characterizing the scorbutic condition are available (Youmans 1943, Woodruff 1964, and Chatterjee 1967).

The precise mechanism by which l-ascorbic acid is involved in the chemical reactions of the connective tissue cells remains to be elucidated.

E. L-ascorbic acid and fish.

Investigation into the l-ascorbic acid requirements of fish is in the early stages of development. It has only been determined in recent years that there is a a need and a role for l-ascorbic acid in the fish diet.

a. Feeding experiments.

The first report, which may have unwittingly established a link between l-ascorbic acid and fish nutrition, was made by McCay and Tunison (1934). They reported that brook trout (*Salvelinus fontinalis*) fed formalin-preserved meat developed lordosis (dorsal-ventral curvature of the spine) and scoliosis (anterior-posterior curvature of the spine) after one year on the diet. At this time, however, no attempt was made to correlate their work with the contemporary studies conducted by Szent-Györgi (1932a, 1932b) and Waugh (1932) on l-ascorbic acid deficiency in mammals.

McLaren *et al.* (1947), reported hemorrhagic liver, kidney and intestine in rainbow trout fed low vitamin C diets.

Investigations into the l-ascorbic acid requirements of fish made little progress until Kitamura *et al.* (1965), established that rainbow trout (*Salmo gardneri*) had a need for dietary supplementation with l-ascorbic acid. As early as 1962, this group of workers had observed scoliosis and lordosis in rainbow trout stocks fed artificial dried diet formulations. No l-ascorbic acid levels were recorded for this diet. It was soon established that the causative factor was a deficiency of

l-ascorbic acid. Rainbow trout fry were started, as soon as the yolk sac was absorbed, on a ration of 57 percent white fish meal, 30 percent α-wheat starch, 5 percent soy bean oil, 3 percent minerals and 5 percent vitamins. L-ascorbic acid was present at 2000 mg per kilo of diet. After a 53 day test period, the mortality rate among these fish was 13 percent, with only 0.8 percent of the survivors displaying deformities. Among fish receiving the identical ration, but with no l-ascorbic acid, the mortality rate was 27 percent with 25 percent of the surviving stock displaying deformities. Reversibility of the scorbutic condition was not investigated. Similar results were obtained with carp (*Cyprinus carpio*) and guppies (*Lebistes reticulatus*).

Short-term (16 - 20 weeks) feeding experiments by Halver (1957) and Coates (1958), with chinook (*Oncorhychus tshawytscha*) and coho salmon (*Oncorhychus kisutch*), indicated that normal growth responses and absence of mortality were observed when l-ascorbic acid was deleted from a test diet.

Shanks *et al.* (1962), reported scoliosis in rainbow trout fed a diet deficient in tryptophan. It is known, however, that the requirements of l-ascorbic acid in the guinea pig are increased considerably under tryptophan deficiency conditions.

In all likelihood, the scoliosis reported by Shanks *et al.*, may have arisen from l-ascorbic acid deficiency (Kitamura *et al.* 1965).

Halver *et al.*(1969), undertook a series of experiments to gain information on long-term, chronic l-ascorbic acid deficiency in salmon. The experiments were conducted in screening hatchery troughs using duplicate 200-fish groups of coho salmon and rainbow trout. The fish were held in spring water at 15°C. The levels of l-ascorbic acid added to the diet were 0, 50, 100, 200, 400 and 1000 mg per kilo of diet. The levels were, however, not measured immediately after feed mixing. The levels of blood and kidney ascorbic acid were measured after 24 weeks; however, no initial levels were reported.

Acute lordosis and scoliosis appeared only 20 to 30 weeks after fingerlings had been started on a diet deficient in l-ascorbic acid. After the 24 week experiment, only 35 out of the initial 200 trout started on the deficient diet survived the test period (Nut. Rev. 1971). Practically all the surviving trout on the deficient diet displayed acute lordosis and scoliosis. These symptoms were also noted for a small number of those trout receiving 50 mg/Kg.

TABLE 11a. Growth and l-ascorbic acid concentrations in blood and head kidney tissue of trout and salmon. Halver *et al.* (1969)

L-ascorbic acid in diet mg/Kg	Average weight at 24 weeks g	L-ascorbic acid concentration[b] Blood ug/g	Kidney ug/g	Growth rate[f] percent per day
Rainbow trout:				
0	2.4[d]	NG[a]	NG	1.2
50	9.6	34.4 ± 2.9	125	2.1
100	10.6	34.6 ± 1.3	137	
200	10.1	38.8 ± 3.3	132	2.3[c]
400	10.2	46.8 ± 6.2	162	
1000	10.8	51.0 ± 4.6	247	
Coho salmon:				
0	5.0[e]	22.3 ± 2.2	89	1.5
50	6.0	30.5 ± 1.2	132	
100	5.7	35.8 ± 1.6	265	
200	6.1	34.2 ± 2.3	183	1.6[c]
400	6.3	33.7 ± 2.0	225	
1000	6.0	37.8 ± 2.3	321	

[a] Not given.

[b] Average of five samples for blood (± S.D.) and two for head kidney tissue.

[c] Average growth rate for fish on diet supplementations 50 - 1000 mg/Kg.

[d] Average starting weight for duplicate 200-fish groups was 0.3 g.

[e] Average starting weight for duplicate 200-fish groups was 0.4 g.

[f] Instantaneous relative growth rate $K \equiv \frac{dw/dt}{w}$ (Brody 1964).

$$dw/dt = kW$$

$$\int_A^W dw/dt = kW = k\int_o^t dt$$

$$\ln W = \ln A + kt, \quad W = Ae^{kt}$$

There was no further improvement in the growth rate for trout receiving greater than 100 mg/Kg (see Table 11a). The minimum level of circulating l-ascorbic acid for adequate growth, as defined by Halver's rearing conditions, appeared to be 35 ug/g of whole blood.

For the coho, there was little difference in growth rate for the different levels of l-ascorbic acid supplementation (see Table 11a). In both the trout and salmon, the kidney levels of l-ascorbic acid reflected the supplementation level of the diet.

Wound healing experiments established that an increased rate of wound repair was commensurate with elevated levels of l-ascorbic acid in the diet.

Halver (1972), states that levels of l-ascorbic acid at 200 mg/kg of diet are adequate to maintain trout and salmon tissue l-ascorbic acid levels at saturation in fresh water systems at 10 - 15°C. However, the l-ascorbic acid tissue saturation levels for trout and salmon have yet to be determined.

b. Deficiency symptoms.

Deficiency symptoms of l-ascorbic acid in fish are generally related to impaired collagen formation (Halver *et al.* 1969, Halver 1972). Fish show

scoliosis, lordosis, internal hemorrhage, resorbed opercles, abnormal support cartilage in the gills, and hyperplasia of collagen and cartilage. Similar symptoms have been observed in the trout, salmon, yellow tail, carp, char and guppy (Kitamura *et al.* 1965, and Poston 1967).

Halver (1972), states that l-dehydroascorbic acid is inactive physiologically. There are no data that would substantiate this for fish, and it is certainly not the case with other animals (Youman 1943, Woodruff 1964, and Harris 1967). It is well known that l-dehydroascorbic acid is the only form of the vitamin that enters the tissues (Woodruff 1964).

Other work with l-ascorbic acid and fish has involved a study of the effects of darkness and l-ascorbic acid on calcium metabolism and gonadal maturation in the Killifish (Pang, 1971), and stress-induced l-ascorbic acid depletion of salmon and trout (Wedemeyer, 1969).

c. Tissue levels.

L-ascorbic acid levels were found to vary over a wide range in a number of fish tissues (Ikeda *et al.* 1963). Some literature values for l-ascorbic acid content in blood and kidney tissue are presented in Table 11b.

Halver *et al.* (1969), and Wedemeyer (1969), used the method of Polk *et al.* (1960), for the determination of total l-ascorbic acid. Since this method depends upon an incubation temperature of 100°C for 10 minutes to form the 2,4-DNPH-osazone, it is inadequate and gives l-ascorbic acid levels that may be erroneously high by 30 to 40 percent (Roe 1960, and Zloch 1971).

Ikeda *et al.* (1963), found that interfering osazones from extracts of fish tissues (carp and tuna), affect assessment of l-ascorbic acid. They found that the amino acids, histidine, tyrosine and tryptophan resulted in the highest interference. They recommended that the osazone should be chromatographed on an acid alumina column by the method of Mapson (1961), as this would separate the interfering osazones. Their recommendations are not wholly justified for the following reason: Ikeda *et al.* used 530 nm as the wavelength to measure the absorption of bis-2,4-DNPH-osazone instead of 540 as recommended by Roe (1943, 1967). The interference from other osazones is much greater at 530 nm and is almost negligible at 540 nm.

d. Radiochemical studies with l-ascorbic acid.

Ikeda and Sato (1964), established that 460 to 680 gram carp could synthesize l-ascorbic acid.

TABLE 11b. Literature values for l-ascorbic acid levels in fish blood and kidney

Fish	Reference	Method of assay	L-ascorbic acid level in diet mg/Kg	l-ascorbic acid concentration ug/g Blood	Kidney	Weight fish g	Water temp °C
Rainbow trout	Halver et al. 1969.	Polk et al. 1960.	50-1000	34-51	125-247	10-11	15
	Ikeda et al. 1963.	Ikeda et al. 1963.	NG[a]	NG	91	85	NG
Coho salmon	Halver et al. 1969.	Polk et al. 1960.	0-1000	22-38	89-321	5-6	15
	Wedemeyer 1969.	Polk et al. 1960.	NG	NG	89	NG	10
Carp	Ikeda et al. 1963.	Ikeda et al. 1963	NG	NG	85	250	NG

[a]Not given.

TABLE 11c. Comparative data on the turnover and body content of l-ascorbic acid-l-C^{14} in four species

Species	Half time (days)	Body pool (mg/100g)	Turnover time (days)	Turnover rate (mg/100g/day)
Carp[a]	3.8	16.1	5.5	2.9
Rat[b]	2.9	10.7	4.1	2.6
Guinea Pig[b]	4.0	5.4	5.8	0.9
Man[b]	16.0	2.2	23.0	0.1

[a]Ikeda and Sato (1965).

[b]Hellman and Burns (1958).

They injected ^{14}C-1 labeled glucose and isolated ^{14}C-6 labeled l-ascorbic acid. Injection of ^{14}C-6 labeled D-glucuoronolactone resulted in its conversion to l-ascorbic acid labeled primarily at carbon-1. The synthesis of l-ascorbic acid by the carp is, however, not adequate to meet its needs under conditions of elevated growth rate (Ikeda and Sato 1964).

Ikeda and Sato (1965), have also established the body pool of l-ascorbic acid in carp to be 16.1 mg per 100 g of wet body weight (WBW), the turnover time to be 5.5 days and a turnover rate of 2.9 mg per 100 g WBW per day. These data are of particular interest when compared to those for the rat, guinea pig and man (see Table 11c). The body pool of l-ascorbic acid in the carp is approximately 8 times that of man, and the turnover rate is approximately 30 times greater. The values for the rat, which can meet its requirements by synthesis, are quite close to those of the carp. No values are available for the trout or salmon.

Halver *et al.* (1971a), have demonstrated that intubation of catheterized rainbow trout with ^{14}C-1, ^{3}H-4 labeled l-ascorbic acid resulted in the excretion of one percent of the label in the urine during the following 72 hours. The free form of the vitamin was not excreted, although there was

evidence for a large number of degradation products. The pH of the urine was not given in this communication.

There is no information available on the ability of salmon or trout to synthesize l-ascorbic acid, even though the possibility has been suggested (Halver *et al.* 1969).

It has been demonstrated that intubation of rainbow trout and coho salmon with ^{35}S-labeled l-ascorbate-3-sulfate reduced scurvy symptoms (Halver *et al.* 1971b).

e. Conclusion.

Apparently, the requirement for l-ascorbic acid by fish depends upon the criterion used. The interactions between growth rate, physiological maturity, diet formulations, temperature, oxygen levels, algal and microbial populations and concentrations, will all affect this requirement, and have received no attention to date.

II. Materials and Methods

1. L-ascorbic acid analysis by the 2,4-dinitrophenylhydrazine procedure.

Two procedures using 2,4-DNPH were employed for the determination of l-ascorbic acid in blood and tissues: a macroprocedure employing either 5% HPO_3-10% HOAc* (for

*Acetic acid.

tissues and feeds) or 6% TCA[+] (for whole blood) as the solvent (Roe and Keuther 1943); and a microprocedure for whole blood employing 5% TCA as the solvent (Lowry *et al.* 1945). A detailed description of the reagent preparation and procedure is given by Roe (1967).

(a) Macroprocedure for the determination of l-ascorbic acid in fish tissues.

The tissue sample was homogenized in 10 to 20 volumes of ice-cold HPO_3-HOAc solvent using a Virtis tissue homogenizer (Virtis Research Equipment, Gardiner, New York), and allowed to sit at room temperature for 30 minutes. To the extract was added acid-washed Norit-211 (Fisher) in an amount approximately equal to 2% (w/v) of the tissue extract. The solution was shaken vigorously and allowed to sit for 5 minutes at room temperature. The solution was filtered through a Millipore (Swinnex-13) filtering apparatus attached to a 10 cc syringe. A Millipore OA filter with a pore diameter of 0.65 μ was used. This method of filtering was preferred to the recommended procedure which employed filter paper. If further analysis could not be conducted at this stage, the extract was stored at -20 C.

Triplicate 2 ml aliquots of the Norit-oxidized tissue filtrate were placed into 16 x 100 mm pyrex

[+]Trichloroacetic acid.

glass tubes. To two of the tubes were added 500 ul of the 2,4-DNPH reagent, while the third tube served as a blank. The three tubes were thoroughly mixed, covered with Parafilm* and placed in a water bath at 37 ± 0.1°C for 3 hours. The tubes were removed at the end of the incubation period and placed into an ice-water bath.

To each of the three tubes in the ice-water bath 2.5 ml of 85% H_2SO_4 were added dropwise and slowly from a burette. To the blank tube not containing any reagent, 500 ul of the 2,4-DNPH reagent were added. Each tube was shaken thoroughly under the ice-water, removed and mixed using a Vortex mixer. The tubes were then allowed to sit for 30 minutes at room temperature.

Aliquots of the solutions were transferred to glass cuvettes and the absorbances read at 540 nm using a Unicam SP 500 Series 2 Spectrophotometer.

(b) Macroprocedure for the determination of l-ascorbic acid in fish feeds.

With the exception of the following modifications, the procedure for the analysis of l-ascorbic acid in fish feeds was identical to that for fish tissues as described in part (a).

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*American Can Company, Marathon Products, Neenah, Wisconsin.

The feed samples (0.5g) were homogenized in 50 to 60 volumes of HPO_3-HOAc solvent using a mortar and pestle, and allowed to stand for 30 minutes at room temperature. After adding Norit, the tubes were shaken and then centrifuged at 3000 R.C.F.* for 10 minutes. The extracts were filtered through Whatman No. 42 ashless filter paper and analysed as in part (a).

(c) Macroprocedure for the determination of l-ascorbic acid in whole fish blood.

Blood samples were obtained by caudal peduncle amputation. The blood was drawn into a pasteur pipette and transferred to a tared pyrex glass tube containing 4 to 6 volumes of 6% TCA. To prevent coagulation of the blood during collection, the pipette was pre-rinsed with a 1 M potassium oxalate solution.

With a glass rod, clumps of blood were broken up and the solution stirred until a fine suspension was formed. The extract was allowed to stand for 10 minutes at room temperature. Norit was added in an amount approximately equal to 2% (w/v) of the blood extract, shaken and centrifuged at 5000 R.C.F. for 10 minutes. The extract was filtered through the millipore-syringe apparatus and the

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*Relative centrifugal force.

analysis conducted on 2 ml aliquots as in (a).

(d) Microprocedure for the determination of l-ascorbic acid in fish blood.

This procedure was an adaptation of the 2,4-dinitrophenylhydrazine method described in part (a). This method depended on $CuSO_4$, not Norit, to oxidize the l-ascorbic acid to its dehydro form.

To a 10 x 75 mm glass tube containing 200 ul of 5% TCA were added 50 ul* of whole fish blood. The contents were mixed thoroughly, the tube was capped with Parafilm and centrifuged at 12,000 R.C.F. for 10 minutes at 4 C using a Sorval RC2-B refrigerated centrifuge. A 150 ul aliquot of supernatant was transferred to another 10 x 75 mm tube containing 50 ul of 2,4-DNPH-$CuSO_4$-thiourea reagent, mixed and incubated at 37 ± 0.1°C for 4 hours.

After the incubation period, the tube was removed from the water bath and placed into an ice-water bath. To this tube 250 ul of ice-cold 65% H_2SO_4 were added and the contents mixed thoroughly. The tube was removed and allowed to stand for 30 minutes at room temperature. The solution was transferred to a 0.5 ml Pye-Unicam micro glass cuvette and the absorbance at 520 nm was recorded.

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*The blood was collected in a micro pipette that had been pre-rinsed with a 1 M potassium oxalate solution.

A blank consisted of 50 ul 2,4-DNPH-$CuSO_4$-thiourea reagent, 150 ul 5% TCA, and 250 ul of 65% H_2SO_4.

(e) Standard calibration curves.

A calibration curve for each of the three solvent assay procedures was prepared by dissolving 50 mg of l-ascorbic acid (Nutritional Biochemicals Corporation) in 50 ml of the solvent to be used in the assay. The solution was oxidized with bromine (Roe 1967), and a 10 ml aliquot was placed in a 500 ml volumetric flask and made to volume with the acid solvent selected.

A series of standard solutions ranging from 0.5 to 12 ug/ml were prepared by transferring the appropriate volume of the oxidized diluted standard to 100 ml volumetric flasks and making to volume with the appropriate acid solvent.

The analysis of the standard solutions was then conducted using the appropriate assay procedure.

2. Tank design.

A description of the tank designed to hold the experimental fish is given in Appendix I.

3. Feed.

Two diet formulations were employed, a ration formulated by this laboratory (UVIC-73) and designated

as Diet 1, and a commercial ration, Diet 2. A description of the proximate composition, ingredients, and pelleting procedure for the two diets have been described (Section A. (III). 2.).

Two batches of Diet 1 were prepared: batch I for the coho salmon and trout experiments for which the diet was supplemented with l-ascorbic acid at a level of approximately 400 mg/kg of diet, and batch II for the chum and kokanee salmon experiments for which the l-ascorbic acid supplementation levels were approximately 200 and 400 mg/kg of diet, respectively. The l-ascorbic acid supplementation level of Diet 2 coincided with that of the Diet 1 batches. L-ascorbic acid supplemented Diets 1 and 2 were designated as Diet 1-C and Diet 2-C.

The effect of ethylene oxide sterilization and two conditions of storage (-40°C and 23°C) on the level of l-ascorbic acid in the two diets was investigated using the assay procedures outline in Section B.(II).1.(b).

To determine the pH of sterile and non-sterile Diets 1 and 2, triplicate 1 gram samples of feed were added to 10 ml of pH 7.0 distilled water and the pH measured using a Fisher Accumet Model 220 pH meter.

4. Fish

The effect of l-ascorbic acid supplemented diets on the growth rates of salmonid fish was evaluated using juvenile coho salmon (Experiment 4), rainbow trout

(Experiments 5 and 6), chum salmon (Experiment 7), and kokanee salmon (Experiment 8).

The rainbow trout and coho salmon were obtained from experiments 1 and 3 (section A.).

The chum salmon (*Oncorhynchus keta*) were reared from eggs obtained from spawning females from the Goldstream River, Victoria, B.C., on November 17, 1972. On arrival at the laboratory, the eggs were stripped from the females, fertilized, and placed in a vertical flow hatchery. The details of the hatchery conditions were similar to those as described by Trust (1972). Hatching was completed by January 3, 1973. The alevins were reared in well water at a mean temperature of 14°C. The fry were maintained on sterile Diet 2 until May 23, 1973.

The kokanee salmon (*Oncorhynchus nerka*) were reared from eggs obtained from spawning females supplied by the B.C. Department of Recreation and Conservation on October 2, 1972. Hatching was completed by December 23, 1972. The alevins were reared in well water at a mean temperature of 14°C. The fry were maintained on sterile Diet 2 until May 23, 1973.

5. Bacteriological procedures.

(a) Feed.

The microbial burden of Diets 1 and 2 was determined at the beginning and end of Experiments 7 and 8. Feed samples were stored at -40°C until

submitted to bacteriological examination.

Standard bacteriological sampling techniques were followed in the procurement of samples (American Public Health Association 1965). A 1-g sample was transferred aseptically to a dilution tube containing 9 ml of buffered distilled water (pH 7.2). The tube was then shaken vigorously 25 times, and duplicate serial dilutions were prepared in buffered distilled water. The viable bacteria present in Diet 1 and Diet 2 were enumerated by the drop-plate method of Miles and Misra (1938). Standard Methods agar (Baltimore Biological Laboratories) plates were incubated aerobically at 4°C for 120 hr, 30°C for 48 hr, and anaerobically at 30°C for 72 hr. The aerobic and anaerobic spores were determined after treatment of the 10^{-1} dilution at 100°C for 5 minutes.

(b) Fish intestines.

The external abdominal wall was thoroughly scrubbed with a povidone-iodine solution (Bridine, British Drug House) that contained 1% available iodine. The peritoneal cavity was then opened using standard aseptic procedures. The intestine, stomach and oesophagus were removed and transferred to a sterile tared petri dish. It was usually necessary to pool the contents of two or more fish

to obtain a sample of suitable weight (2-10 grams). The gut tissues were transferred aseptically to a sterile homogenizing jar that had been pre-cooled to -20°C, and were diluted with ice-cold 1% (w/v) peptone water to give a 1:10 dilution. The sample was homogenized using an Osterizer blender (at full speed). Throughout the 1 minute homogenizing process the temperature of the gut suspension never exceeded 5°C.

Duplicate serial dilutions were prepared in 1% (w/v) peptone water. The viable mesophilic bacteria able to grow at 25°C on Standard Methods agar (BBL) were enumerated by the drop-plate method of Miles and Misra (1938). Anaerobiosis was obtained by BBL Hydrogen-Carbon dioxide Generator envelopes.

(c) Water.

The enumeration of the aerobic bacteria present in the tank water was conducted as in Section A.(II).9.

6. Experiment 4.

The coho salmon used in Experiment 3 (Section A.(II).12.) were pooled and distributed into five 0.91 meter diameter tanks and fed sterile Diet 2, sterile and non-sterile Diet 1 and Diet 1-C* as follows: Tank 1, 33 fish, sterile Diet 2; Tank 2, 35 fish, non-sterile Diet 1-C; Tank 4, 34 fish, non-sterile Diet 1; and Tank 5, 35

fish, sterile Diet 1. The fish were fed at 1.0 to 1.5 percent of their wet body weight per day.

The fish were weighed individually (see experiments 2 and 3 for a description of the method) after 15, 32, 45, 60 and 86 days. Fish were removed from each tank at various times throughout the experiment for body composition analysis.

The tanks were cleaned at biweekly intervals using a dilute hydrochloric acid solution.

7. Experiment 5.

The rainbow trout used in Experiment 1 (Section A. (II).10.) were distributed to eight tanks and fed sterile and non-sterile Diet 2, Diet 1, and l-ascorbic acid supplemented Diet 1 (Diet 1-C*) as follows: Tank 12, 64 fish, sterile Diet 2; Tank 13, 34 fish, non-sterile Diet 1; Tank 14, 31 fish, sterile Diet 1; Tank 15, 32 fish, sterile Diet 1; Tank 16, 30 fish, sterile Diet 1-C; Tank 17, 33 fish, sterile Diet 1-C; Tank 18, 34 fish, non-sterile Diet 1-C; and Tank 19, 69 fish, non-sterile Diet 2. The fish distributed to Tanks 12 and 19 were from Tanks 10 and 11 of Experiment 1 (Diet 2), while the remaining fish were pooled from Tanks 12, 13 and 14 of Experiment 1 (Diet 1), and were distributed to the remaining tanks. Fish displaying lordosis and/or

*Diet 1-C was supplemented with approximately 400 mg of l-ascorbic acid per kg of diet.

scoliosis were distributed equally among the fish receiving Diet 1 and Diet 1-C.

The fish were fed at approximately 2 percent of their wet body weight per day, three times daily, and were weighed by difference in water after one 11 day interval and after each of four 14 day intervals.

At the termination of the experiment, two fish from each tank were sacrificed, and the wet body weight, fork length and haematocrit were recorded for each fish.

The liver, kidney and blood from each of the two fish were pooled for the determination of l-ascorbic acid content by the 2,4-DNPH macroprocedure.

The microbial count of the tank water, and of a pooled intestinal sample from each of the two fish was determined.

The remaining fish were pooled and distributed to two 1.8 meter diameter circular tanks and were maintained on sterile Diet 2.

The tanks were cleaned at biweekly intervals with a dilute hydrochloric acid solution.

8. Experiment 6.

The pooled rainbow trout used in Experiment 4 were divided into three representative groups of large (26 - 39 g), medium (16 - 19 g) and small (10 - 11 g) fish. These fish were distributed into six tanks and fed sterile and non-sterile Diet 2 as follows: Tank 1,

32 fish (mean wet weight of 26-g), non-sterile; Tank 2, 36 fish (mean wet weight of 10-g), sterile; Tank 3, 35 fish (mean wet weight of 19-g), sterile; Tank 4, 37 fish (mean wet weight of 16-g), non-sterile; Tank 5, 31 fish (mean wet weight of 11-g), non-sterile; and Tank 6, 25 fish (mean wet weight of 39-g), sterile.

The fish were fed at approximately 1.5 percent of their wet body weight per day, three times daily, and were weighed by difference in water after 64 days, when the experiment was terminated.

At the termination of the experiment, two fish from each of Tanks 1 through 5 and one from Tank 6 were sacrificed. The wet body weight, fork length and haematocrit were recorded for each fish. Duplicate 50 ul blood samples were obtained from each fish for l-ascorbic acid analysis by the 2,4-DNPH microprocedure.

The microbial count of a pooled intestinal sample from the fish on sterile Diet 2, and of a pooled sample from fish on non-sterile Diet 2 was determined.

Tanks were cleaned at biweekly intervals using a dilute hydrochloric acid solution.

9. Experiment 7.

Eleven days prior to commencing the experiment, eight representative groups of 30 juvenile chum salmon (mean wet weight of 8.4 g) were distributed into eight tanks and equilibrated on the following rations: Tank

16, sterile Diet 2; Tank 17, sterile Diet 2-C; Tank 18, non-sterile Diet 2; Tank 19, non-sterile Diet 2-C; Tank 20, sterile Diet 1; Tank 21, sterile Diet 1-C; Tank 22, non-sterile Diet 1; and Tank 23, non-sterile Diet 1-C. Diets 1-C and 2-C were supplemented with approximately 200 mg of l-ascorbic acid per kg of diet.

At the termination of the equilibration period, four fish from each tank were sacrificed for l-ascorbic acid and body composition analysis. The wet body weight, fork length and haematocrit were recorded for each fish.

The remaining 26 fish per tank were weighed by difference in water and fed at approximately 1.5 percent of their wet body weight per day, three times daily, for 55 days. Ten days after commencing the feeding experiment, 2 fish were removed from each tank and the bacterial counts of the pooled intestinal samples were determined.

After 55 days the remaining fish were weighed by difference in water. For l-ascorbic acid analysis of the liver, kidney (macroprocedure), and blood (micro-procedure), four dark pigmented and 4 light pigmented fish were sacrificed from all the even numbered tanks (no l-ascorbic acid supplementation of the diet), and only 4 light pigmented fish from the odd numbered tanks (l-ascorbic acid supplementation of the diet). Four additional fish were removed from each tank for body

composition analysis and intestinal microbial counts.

10. Experiment 8.

Thirteen days prior to commencing the experiment, eight representative groups of 45 juvenile kokanee salmon (mean wet weight of 2.1 g) were distributed to eight tanks and equilibrated on the following ration scheme: Tank 8, sterile Diet 2; Tank 9, sterile Diet 2-C; Tank 10, non-sterile Diet 2; Tank 11, non-sterile Diet 2-C; Tank 12, sterile Diet 1; Tank 13, sterile Diet 1-C; Tank 14, non-sterile Diet 1; and Tank 15, non-sterile Diet 1-C. Diets 1-C and 2-C were supplemented with approximately 400 mg of l-ascorbic acid per kg of diet.

At the termination of the equilibration period 5 fish were sacrificed from each tank for body composition analysis. A 10 ul blood sample was collected from each of the fish and pooled for l-ascorbic acid microanalysis. The remaining fish were then weighed by difference in water and were fed to appetite three times daily. After 56 days the fish were weighed and the experiment terminated.

At the termination of the experiment, 5 fish were sacrificed from each tank. The wet body weight, fork length and haematocrit were recorded for each fish. A 50 ul blood sample was removed from each fish for l-ascorbic acid microanalysis, and the liver, kidney

and gill tissues from each of the five fish were pooled for analysis by the macroprocedure.

III. Results

(a) Feed studies.

The equations for the standard l-ascorbic acid assay curves are given in Table 12. With the exception of l-ascorbic acid concentrations from 5 - 6 ug/ml for the microprocedure, the macro and micro methods followed Beer's Law and were in good agreement with the literature values (Roe and Keuther 1943, Lowry *et al.* 1945).

The results in Tables 13 and 14 show that the l-ascorbic acid in Diets 1, 1-C, 2 and 2-C was unstable to sterilization and exposure to storage conditions at 25°C.

When Diet 2 and 2-C was allowed to cool and dry at 25°C after pelleting, the natural and supplemented levels of l-ascorbic acid decreased 21 - 29% during the first 12 hr. There was no significant decrease in the l-ascorbic acid levels of Diet 1 and 1-C under the same conditions. Gas sterilization for 24 hr and storage at 25°C for 7 days decreased the levels in Diet 2 and 2-C a further 23 - 60%, while the l-ascorbic acid levels of Diet 1 and 1-C only decreased by 12 - 18%.

The pH of the feed extracts increased slightly after ethylene oxide sterilization (Table 15) [signifi-

cantly different at 0.05 level]. A similar increase in the pH was observed when a control feed sample was subjected to a pressure of 1 mm Hg, but no ethylene oxide.

The mesophilic aerobic microbial count of Diet 1 (8.00×10^4/g) was substantially lower than for Diet 2 (120.00×10^4/g) (Table 16). The psychrophilic aerobic count was lower for Diet 1, although the aerobic spore count was higher than for Diet 2. For both diets the anaerobic mesophilic count was $\leq$ 600/g. Over the period of storage at -40°C, there was a 12 to 87% decrease in the mesophilic aerobic count of Diet 2 a 1, respectively.

(b) Experiment 4.

The results in Table 17 show that l-ascorbic acid supplementation of the UVIC-73 ration at 334 and 412 mg/kg of diet (Appendix XVI) had a marked effect on the efficiency of protein and fat conversion for the coho salmon.

For both supplemented and non-supplemented Diet 1, the protein efficiency was significantly greater than for the fish on Diet 2. However, the difference was significant at the 0.01 level for fish on the supplemented ration, Diet 1-C, and only at the 0.05 level for fish on the non-supplemented ration, Diet 1.

For fish on the supplemented ration, the conversion

of feed fat to body fat was significantly greater than for the fish on either non-supplemented Diet 1 or Diet 2.

The feed efficiency for Experiment 4 coho salmon on Diet 1 and Diet 1-C was not significantly different than that obtained for the fish in Experiment 2, but was significantly lower than for experiment 3 fish.

The energy efficiency and I.R.G.R. was not significantly different between fish on Diet 2 and those on Diet 1 or 1-C. However, the difference between Diet 1 and Diet 1-C fish was significant.

The mean tissue water, fat and protein levels for the coho salmon of Experiment 4 are presented in Table 18. The mean water content of the fish on Diet 2 was significantly higher than for fish on Diet 1 and Diet 1-C. Correspondingly, the mean fat levels of Diet 2 fed fish were lower than for fish fed on the other two rations. The mean protein levels were not significantly different for the fish in Experiment 4.

The body composition analysis for the coho salmon of Experiment 4 on Diet 1 corresponded to the analysis of the fish from Experiment 2 and 3.

Due to a malfunction in the well water pumping system, compressed air was added to the tank water. Only the fish in Tanks 2 and 3 which were receiving the supplemented ration, escaped from the severe symptoms

of nitrogen embolism that destroyed all the fish on the non-supplemented rations.

(c) Experiment 5.

The results in Table 19 show that l-ascorbic acid supplementation at 343 mg/kg (sterile Diet 1-C), and 412 mg/kg (non-sterile Diet 1-C) of diet, did not significantly alter the I.R.G.R. of the rainbow trout in Experiment 5.

The feed efficiency of fish on Diet 2 was significantly higher than for Diet 1 and Diet 1-C fed fish; however, the difference between fish fed on Diet 1 and Diet 1-C was not significant.

The mean l-ascorbic acid level in the blood, liver and kidney tissue of fish fed on non-supplemented Diet 1 and Diet 2 (the l-ascorbic acid level in both rations was 60 mg/kg of diet) were not significantly different (Table 20). However, the level in fish receiving the supplemented ration was approximately 5 to 7 times greater. The l-ascorbic acid level of the liver and kidney tissue was 4 to 8 times greater than the blood level, regardless of the diet consumed.

The results in Table 21 show that the mean haematocrit of Diet 2 fed fish (32.2%) was significantly lower than for Diet 1 and Diet 1-C fed fish (38.2 - 42.3%). The difference in haematocrit between fish receiving a supplemented or non-supplemented ration was

not significantly different.

Over the experimental period no further symptoms of l-ascorbic acid deficiency occurred in the non-supplemented diet fed fish. The scorbutic fish distributed among the tanks receiving Diet 2, were removed shortly after commencing the experiment. Due to the advanced nature of the deficiency, these fish were not recoverable.

The mesophilic aerobic bacterial counts of the tank water and intestines for the rainbow trout in Experiment 5 are presented in Table 22. Although the tank water counts ranged from 0.6 - 12.0 x 10^2 organisms/ml, and the feed counts from 100 - 10000 x 10^2 organisms/g (Table 16), the intestinal counts were very low.

(d) Experiment 6.

The growth results for the Experiment 6 rainbow trout are presented in Appendix XL. The feed efficiency and I.R.G.R. for Diet 2 fed fish was significantly lower than for the experimental 5 fish fed on the same ration.

The l-ascorbic acid level of whole blood from fish fed on sterile Diet 2 was significantly greater than that for fish fed on the non-sterile ration (Table 23). However, both l-ascorbic acid levels were greater than

the whole blood levels of fish fed on the same diet in Experiment 5.

No mesophilic aerobic or anaerobic bacteria were isolated from the intestines of fish fed on either sterile or non-sterile Diet 2.

(e) Experiment 7.

The effect of l-ascorbic acid supplementation of the diet on the feed efficiency and growth rate of the chum salmon in Experiment 7 was pronounced (Plate I). The results in Table 24 show that the feed efficiency and growth rate for fish receiving the supplemented rations (60 - 160 mg/kg) were markedly greater than for those fish receiving a non-supplemented diet (17 - 20 mg/kg). The fish receiving a supplemented ration were also shown to have a lower water and higher fat content (Appendix XLIV). The mean fat levels for fish on Diet 1 and Diet 1-C was significantly higher than for fish on Diet 2 and Diet 2-C.

Prior to commencing Experiment 7, a defect in the well water pumping system resulted in the addition of compressed air to the tank water. The symptoms of nitrogen embolism rapidly ensued. Although the mortality rate was low initially, the cases of eye blebs, subcutaneous nitrogen bubbles and eye haemorrhages had afflicted between 50 and 70 percent of the fish population. By the second week of feeding

there was a marked improvement in those fish receiving an l-ascorbic acid supplemented ration. The pigmentation of the fish had lightened to a pale green and the cases of eye blebs decreased (Plate II). However, the deterioration of the fish on the non-supplemented rations, particularly on Diet 2, continued. These fish initially displayed a darkening and paralysis of one side of the body along the anterior-posterior axis of symmetry. Later, areas of localized darkening occurred, but without paralysis. Severe eye blebs soon developed (Plate II), and were accompanied by haemorrhaging, necrosis and rejection of the eye(s), and ultimately death.

By the second week, scoliosis and lordosis had appeared among several of the fish receiving a non-supplemented ration, but were not necessarily accompanied by the above mentioned embolism symptoms (Plate III).

Other symptoms observed were: the dislodging of the ribs from the musculature during disection; the disintegration of the gill and gut tissues; and areas of scale denudation.

The mortality rates among the non-supplemented fish groups were from 27 to 54 percent, and were highest for those fish receiving Diet 2 (Table 24). For those fish receiving a l-ascorbic acid supplemented ration,

scoliosis and lordosis did not appear, and the instances of eye blebs were reduced and less severe.

The l-ascorbic acid level in blood, liver and kidney tissue increased linearly with increasing l-ascorbic acid supplementation levels of the diet (Table 25 and Figure 4). For fish not receiving a supplemented ration, the blood and tissue levels of l-ascorbic acid were significantly different between light and dark pigmented fish (Appendix XLIX).

The results in Table 26 show that l-ascorbic acid supplementation of both diets (62 - 159 mg/kg) lowered the intestinal mesophilic aerobic and anaerobic counts of feeding and 24 hr fasting fish from approximately 10^4-10^5 organisms/g to $<10^2$ organisms/g. Two aerobic colony types were isolatable from the intestines, and both were identified as *Pseudomonas* species. Both species were isolated from the tank water but not from the feed.

(f) Experiment 8.

L-ascorbic acid supplementation of Diet 1 and Diet 2 raised the growth rate of kokanee salmon (Table 27). The fat content of fish fed on Diet 1 and Diet 1-C was significantly greater than for fish fed on Diet 2 and Diet 2-C. Although the fat levels between fish fed on Diet 1 and Diet 1-C were not significantly different, a significant difference was obtained for Diet 2 and Diet 2-C fed fish (Appendix L).

The results in Table 28 show that the blood, liver,

kidney and gill l-ascorbic acid levels increased linearly with increasing dietary l-ascorbic acid supplementation. The kidney l-ascorbic acid levels tended to be more scattered than those for blood, liver or gill tissue (Figure 5). Fish fed on Diet 1 NS* (Tank 14) inadvertently received a supplemented ration for two or three days prior to sacrifice, and as a result, showed elevated l-ascorbic acid levels in all the tissues.

Nitrogen embolism symptoms did not appear among the kokanee fry, and only exceptionally large fish (7 - 8g) displayed the asymmetrical darkened pigmentation and paralysis. Among fish receiving non-supplemented Diet 2, approximately 25 percent displayed a darkening of the body pigmentation. These fish usually went off feed and eventually died.

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*Non-sterile.

TABLE 12. Equations[a] for l-ascorbic acid macro- and microprocedure standard curves

Procedure	Solvent	Equation
Macro		
	5% HPO_3-10% HOAc	$Y^* = 0.033C^b$ $S_{yx} = \pm 0.001$
	6% TCA	$Y^* = 0.044C$ $S_{yx} = \pm 0.010$
Micro		
	5% TCA	0-5 ug/ml : $Y^+ = 0.032C$ $S_{yx} = \pm 0.001$
		5-16 ug/ml : $Y^+ = 0.0287 + 0.0241C$ $S_{yx} = \pm 0.010$

*Y equals absorbance at 540 nm.

+Y equals absorbance at 520 nm.

[a]Data for the standard curves is presented in Appendix XV.

[b]C equals concentration of l-ascorbic acid ug/ml.

TABLE 13. Mean l-ascorbic acid levels in Diet 1-C fed to coho salmon and rainbow trout in experiment 4 and 5[a]

Diet	mg l-ascorbic acid per kg of feed
Non-sterile Diet 1-C	412 ± 5.91[b]
Sterile Diet 1-C	334 ± 11.1[b]
Non-sterile Diet 1	60.5 ± 0.901[c]
Non-sterile Diet 2	60.6 ± 2.75[c]

[a]Refer to Appendix XVI.

[b]Mean of 5 samples ± standard deviation.

[c]Mean of 2 samples.

TABLE 14. Mean l-ascorbic acid levels in in Diets 1, 1-C, 2 and 2-C for experiments 7 and 8

Diet	Tank No.	l-ascorbic acid mg/kg of diet			
		Immediately after pelleting[a]	After cooling and drying for 12 hr at 25C[b]	Sterilized	
				Stored at -40°C[c]	Stored at 25°C[d]
2 S[e]	8	31.6±8.21	-	17.0±1.95*+	
2-C[g] S[f]	9	372 ±9	294 ±33.4*	223 ±5.71*+	
2 NS[f]	10	31.6±8.21	-		
2-C NS	11	295 ±12	289 ±25.8		
1 S	12	21.9±1.68	23.1± 8.90	18.5±1.24*+	
1-C S	13	337 ±24	358 ±12.0	318 ±28.6*+	
1 NS	14	21.9±1.68	23.1± 8.90		
1-C NS	15	407 ±15	392 ±11.9		
2 S	16	31.6±8.21	20.8± 4.78†	17.0±1.95*+	*+#
2-C S	17	162 ±26	133 ± 7.57*	62.3±5.12*+	47.4±6.40
2 NS	18	31.6±8.21	20.8± 4.78†		*+
2-C NS	19	200 ±52	122 ± 3.19*		49.7±21.1
1 S	20	21.9±1.68	21.1± 1.91	18.5±1.24*+	*+#
1-C S	21	196 ±38	158 ± 17.8	136 ±11.6*+	112±10.8
1 NS	22	21.9±1.68	21.1± 1.91		*+
1-C NS	23	162 ±11	159 ± 13.6		131±10.5

[a]Appendix XVII, XVIII.

[b]Appendix XIX, XX. After drying and cooling, the feed was stored at -40°C for 94 days until submitted to analysis.

[c]After sterilizing for 24 hr. the feed was stored at -40°C.

[d]Samples were stored at 25°C for 1 week, then placed at -40°C until submitted to analysis.

[e]S equals sterile.

[f]NS equals non-sterile.

[g]C equals supplemented with l-ascorbic acid.

*Significantly different at the 0.01 level from values obtained immediately after pelleting.

+Significantly different at the 0.01 level from values obtained after cooling and drying.

#Significantly different at the 0.01 level from values obtained for sterilized feed stored at -40°C.

†Significantly different at the 0.05 level.

TABLE 15. pH of Diets 1, 1-C, 2 and 2-C

Diet		pH[a]
1	non-sterile	5.91
	sterile	6.19
	vacuum*	6.00
1-C	non-sterile	5.87
2	non-sterile	5.52
	sterile	5.70
	vacuum*	5.73
2-C	non-sterile	5.53

[a]Mean of three samples.

*A sample of feed was treated under the same conditions as the sterile sample, but with no ethylene oxide.

TABLE 16. Estimated microbial burden of Diet 1 and 2 at the beginning and end of experiments 7 and 8

	No. organisms x 10^2/g of diet			
	Diet 1 & 1C		Diet 2 & 2C	
Date:	2-06-73	13-08-73	2-06-73	13-08-73
Aerobic incubation (C)				
4	80	40	120	125
30	800	100	12000	10000
Anaerobic incubation				
30	4	4	6	5
Aerobic spores,				
30	120	110	40	38
Anaerobic spores,				
30	<1	<1	<1	<1

TABLE 17. A comparison of the feed, protein, fat and energy efficiency between Diet 1, 1-C, and Diet 2 for the coho salmon, experiment 4[a].

Diet:	Diet 2	Diet 1-C	Diet 1
Tank:	S 1	NS & S 2,3	NS & S 4,5
	N[c] =4	N = 10	N = 8
Efficiency[b]-			
Feed	0.71±0.15*	0.59±0.085	0.51±0.073*
Protein	0.22±0.040*[+]	0.33±0.049[+]	0.30±0.044*
Fat	0.19±0.048[+]	0.32±0.042[+#]	0.22±0.033[#]
Energy	0.19±0.039	0.20±0.028[+]	0.16±0.024[+]
I.R.G.R.[d] (%/day)	0.69±0.18	0.78±0.10*	0.64±0.11*

[a]Refer to Appendices XXIV to XXIX.

[b]Efficiency equals component gain/component consumed.

[c]Number of determinations.

[d]Instantaneous relative group rate.

*equals significantly different from each other at the 0.05 level.

+,# equals significantly different from each other at the 0.01 level.

TABLE 18. Mean water, fat, and protein for coho salmon experiment 4[a]

	Diet 2	Diet 1-C		Diet 1	
Ration:	S	NS	S	NS	S
Tank:	1	2	3	4	5
Water (%)	N[b]=13 76.39±1.58*	N=8 73.32±1.09+	N=11 74.51±1.83	N=19 74.98±1.35	N=8 75.03±1.11
Fat (% d/w)	N =13 15.32±5.61*	N=7 26.17±3.35	N=11 23.87±4.66	N=19 20.14±4.50+	N=8 20.04±4.52+
Protein (% FFDM)	N= 13 86.91±3.15	N=7 84.57±2.77	N=11 86.13±2.69	N=19 85.35±3.65	N=8 85.36±2.33

[a]Refer to Appendices XXXV and XXXVI.

[b]Number of determinations.

Water: *significantly different from 2, 3, 4 and 5 at 0.05 level.

+significantly different from 4 and 5 at 0.05 level.

Fat: *significantly different from 2, 3 and 4 at 0.05 level.

+significantly different from 2 and 3 at 0.05 level.

TABLE 19. Feed efficiency and I.R.G.R.[a] for rainbow trout on Diet 1, Diet 1-C, and Diet 2, Experiment 5[b]

Ration:	Diet 2 NS & S	Diet 1 NS & S	Diet 1-C NS & S
Tank:	19, 12	13, 14, 15	16, 17, 18
(i) Feed efficiency (wt gain g/g feed)	N[c]=10 0.95±0.15*	N=15 0.66±0.12	N=15 0.64±0.12
(ii) I.R.G.R. (%/day)	N =10 1.27±0.34	N=15 1.32±0.15	N=15 1.34±0.14

[a] I.R.G.R. is the instantaneous relative growth rate.

[b] Refer to Appendix XXXVII.

[c] Number of determinations.

*Significantly different at the 0.05 level (ν = 23 d.f.).

TABLE 20. Mean blood, liver and kidney l-ascorbic acid levels for rainbow trout from experiment 5[a]

Tank	Ration	l-ascorbic acid concentration			
		Feed mg/kg	Blood ug/g	Liver ug/g	Kidney ug/g
12,19	Diet 2	60	5.83±0.56	37.9±4.5	52.3±05.2
13,14 15	Diet 1	60	5.29±1.21	23.8±2.0	43.1±12.0
16,17 18	Diet 1-C	343-412	40.29±6.29	167 ±0.81	256 ±23.8

[a]Refer to Appendix XXXIX.

TABLE 21. Mean haematocrit values for rainbow trout on Diets 1, 1-C and 2, experiment 5[a]

Ration	Haematocrit (%)	N
Diet 2	32.3 ± 1.48*	4
Diet 1	42.3 ± 5.31	6
Diet 1-C	38.2 ± 3.39	6

[a]Refer to Appendix XXXVIII.

*Significantly different at 0.01 level ($\nu = 8$).

TABLE 22. Mesophilic aerobic bacterial count of tank water and intestines for rainbow trout at the termination of experiment 5

Tank	Ration	No. organisms Water	No. organisms Intestines
		$X10^2/ml$[a]	$X10^2/g$[b]
12	Diet 2 S	1.1	<0.1[c]
13	Diet 1 NS	1.4	NCO[d]
14	Diet 1 S	2.6	NCO
15	Diet 1 S	0.6	NCO
16	Diet 1-C S	4.0	NCO
17	Diet 1-C S	12.0	NCO
18	Diet 1-C NS	4.8	0.1
19	Diet 2 NS	4.0	<0.1

[a] ml of tank well water.

[b] g of intestine + contents (w/w).

[c] No growth in 10^{-1} dilution, but some growth on tissue smear.

[d] NCO equals no countable organisms in 10^{-1} dilution or from tissue smear.

TABLE 23. Mean blood l-ascorbic acid levels for rainbow trout at the termination of experiment 6

Ration	l-ascorbic acid ug/100 ml[a]	N
Diet 2 non-sterile	760 ± 99.4*	12
Diet 2 sterile	1045 ± 64.0	8

*significantly different at 0.01 level ($\nu = 18$).

[a]Analysis conducted on 50 ul blood samples. Refer to Appendix ILII.

TABLE 24. Growth data for chum salmon, experiment 7. Growth period 03-06-73 to 27-07-73

Ration	Diet 2		Diet 2-C		Diet 1		Diet 1-C	
	S	NS	S	NS	S	NS	S	NS
Tank	16	18	17	19	20	22	21	23
Group size - Initial	26	26	26	26	26	26	26	26
Final	18	10	21	18	23	17	24	24
% survivors[a]	77	46	88	77	96	73	100	100
% dark pigmented[b]	35	65	0	8	31	35	0	0
% eye blebs	8	19	8	8	19	19	0	0
% scoliosis and/or lordosis	16	12	0	0	12	23	0	0
Total wet wt (g)-Initial	239	228	239	217	234	225	230	241
uncorrected Final	265	103	459	375	332	245	483	491
corrected Final	357	292	509	419	370	309	517	524
Total weight gain (g)	118	64	270	202	136	84	287	283
Feed consumed (g)	184	116	322	255	269	201	416	396
gain g/g feed	0.64	0.55	0.84	0.79	0.51	0.42	0.69	0.71
I.R.G.R. (%/day)	0.36	0.29	1.58	1.66	0.85	0.93	1.49	1.44

[a] This value does not include the 2 fish per tank that were removed for intestinal counts.

[b] The percentage values represent the number of fish that developed symptoms/26.

TABLE 25. Initial and final blood, liver and kidney l-ascorbic acid levels for chum salmon experiment 7

Tank	Ration	l-ascorbic acid mg/kg of diet[a]	l-ascorbic acid ug/g					
			Blood		Liver		Kidney	
			Initial[b]	Final[c*]	Initial[b]	Final[d]	Initial[b]	Final[d]
16	Diet 2 S	17	1.25	10.2	4.44	6.51	5.74	15.2
17	Diet 2-C S	62	1.45	15.7	14.0	26.0	15.2	63.2
18	Diet 2 NS	21	1.84	12.0	8.24	12.6	15.1	11.6
19	Diet 2-C NS	122	2.40	15.0	10.9	37.0	9.04	82.7
20	Diet 1 S	19	2.36	9.14	1.61	3.83	11.2	11.1
21	Diet 1-C S	136	5.56	32.8	22.8	80.4	43.8	148
22	Diet 1 NS	21	2.26	8.42	3.01	8.34	13.1	9.80
23	Diet 1-C NS	159	4.21	33.3	30.1	86.1	36.6	158

[a]From Table 14.

[b]From Appendix XLVI.

[c]From Appendix XLVIII.

[d]From Appendix XLIX - N (light pigmented fish) values only.

*Mean values (ug/ml).

TABLE 26. Mesophilic bacterial count of the tank water and intestines for chum salmon, Experiment 7

Tank No	Ration	Intestinal count/g: l-ascorbic acid mg/kg of diet[a]	No. organisms x 10^2: Intestinal count/g: Aerobic: Initial	Aerobic: Final	Aerobic: Starved 24 hr	Anaerobic: Initial	Anaerobic: Final	Tank water/ml: Initial	Tank water/ml: Final
16	Diet 2S	17	82000	1200	1000	71000	2000	160	15.1
17	Diet 2-C S	62	NCO[b]	400	NCO	NCO	<1	96	14.1
18	Diet 2 NS	21	36000	20000	19000	38000	22500	30	12.5
19	Diet 2-C NS	122	40	NCO	NCO	NCO	<1	132	99
20	Diet 1S	19	40000	12000	11500	6500	9000	160	13.0
21	Diet 1-C S	136	NCO	440	NCO	NCO	<1	40	12.9
22	Diet 1 NS	21	5700	1200	1200	2600	1140	72	8.5
23	Diet 1-C NS	159	NCO	750	NCO	NCO	<1	28	10.7

[a] From Table 14.

[b] NCO = no countable organisms from intestinal tissue smear.

TABLE 27. Growth data for kokanee salmon, experiment 7. Growth period 06-06-73 to 30-07-73

Ration:	Diet 2		Diet 2-C		Diet 1		Diet 1-C	
	S	NS	S	NS	S	NS	S	NS
Tank:	8	10	9	11	12	14	13	15
Group size - Initial	40	40	40	40	40	40	40	40
Final	26	20	35	28	30	25	22	23
% survivors	65	50	88	70	75	63	55	58
Total wet wt (g) - Initial	90	80	86	81	80	79	82	79
Uncorrected Final	77	55	173	124	121	106	110	98
Corrected Final	193	166	261	218	212	202	208	194
Total wt gain (g)	103	86	175	137	132	123	126	115
Feed consumed (g)	61	51	90	76	105	99	105	95
gain g/g feed	1.7	1.7	1.9	1.8	1.3	1.2	1.2	1.2
I.R.G.R. (%/day)	0.58	0.58	1.51	1.42	1.27	1.38	1.62	1.40

TABLE 28. Initial and final blood, liver, kidney and gill l-ascorbic acid levels for kokanee salmon, experiment 8

Tank	Ration	l-ascorbic acid mg/kg of diet[a]	l-ascorbic acid concentration				
			Blood (ug/ml)		Final[d]		
			Initial[b]	Final[c]	Liver (ug/g)	Kidney (ug/g)	Gill (ug/g)
8	Diet 2 S	17	19.8	10.2	8.0	111	22.6
9	Diet 2-C S	223	19.9	22.5	123	125	130
10	Diet 2 NS	32	19.6	8.5	16.4	90.4	27.0
11	Diet 2-C NS	289	19.6	23.1	105	173	119
12	Diet 1 S	19	20.2	7.9	1.3	41.5	13.1
13	Diet 1-C S	318	23.4	26.8	146	208	141
14	Diet 1 NS	23	21.2	14.9*	64.3*	117*	85.7*
15	Diet 1-C NS	392	23.4	33.8	167	278	155

[a]From Table 14.

[b]From Appendix LI.

[c]From Appendix LIII.

[d]From Appendix LIV.

*These fish inadvertently received a supplemented ration prior to sacrifice.

FIGURE 4. Final l-ascorbic acid tissue levels for chum salmon vs diet supplementation levels in experiment 7.

Kidney: $Y = -7.54 \pm 1.01X \quad S_E \pm 16.1$ ug/g

Liver: $Y = -3.84 \pm 0.523X \quad S_E \pm 11.5$ ug/g

Blood: $Y = 6.52 \pm 0.152X \quad S_E \pm 4.96$ ug/g

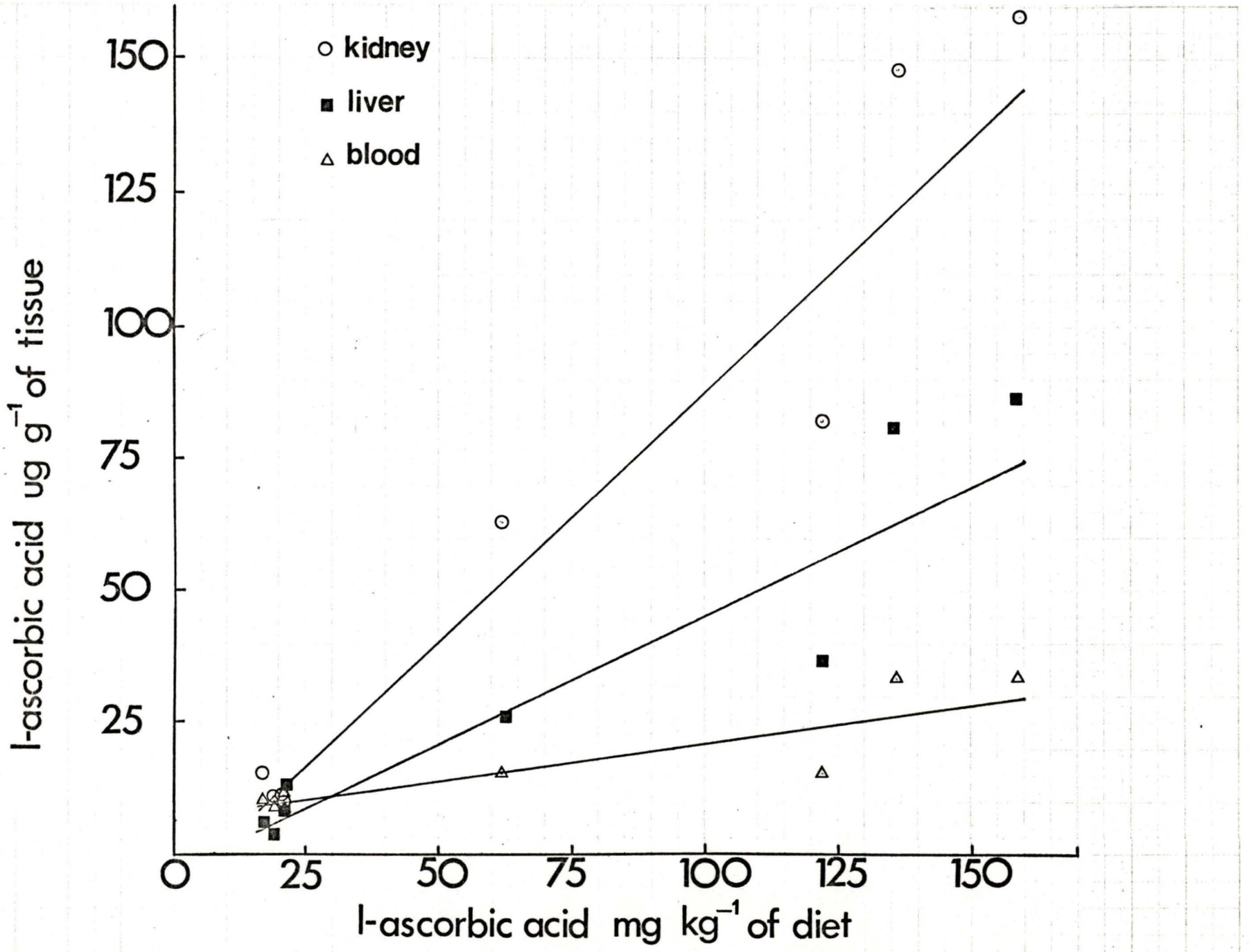

kidney
liver
blood
150
125
100
75
50
25
0
25
50
75
100
125
150
l-ascorbic acid ug g^{-1} of tissue
l-ascorbic acid mg kg^{-1} of diet

FIGURE 5. Final l-ascorbic acid tissue levels for kokanee salmon, vs diet supplementation levels in experiment 8.

Kidney: $Y = 14.6 \pm 0.607X$ $S_E \pm 24.6$ ug/g

Liver: $Y = 0.574 \pm 0.435X$ $S_E \pm 9.47$ ug/g

Gill: $Y = 16.0 \pm 0.384X$ $S_E \pm 14.9$ ug/g

Blood: $Y = 7.17 \pm 0.0642X$ $S_E \pm 0.01$ ug/g

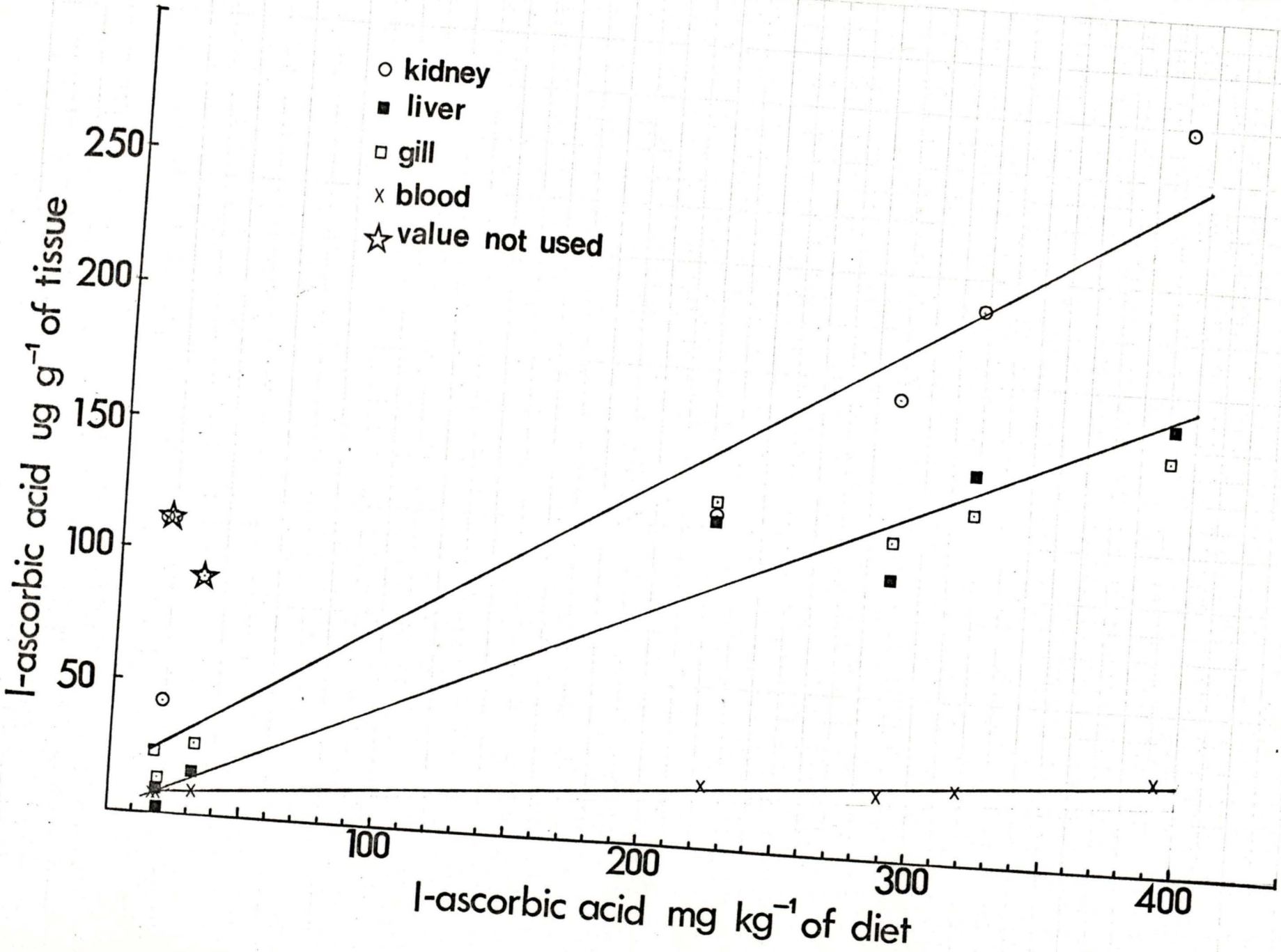

kidney
liver
gill
blood
value not used
250
200
150
100
50
l-ascorbic acid ug g⁻¹ of tissue
100
200
300
400
l-ascorbic acid mg kg⁻¹ of diet

PLATE I. Representative chum and kokanee salmon from each tank at the termination of experiments 7 and 8.

Fish from Tanks 12-15 and 20-23 received Diet 1, and fish from the remaining tanks received Diet 2. Fish from the odd numbered tanks received l-ascorbic acid supplementation at the following levels (mg/kg): Tank 9, 223; Tank 11, 289; Tank 13, 318; Tank 15, 392; Tank 17, 62; Tank 19, 122; Tank 21, 136; Tank 23, 159. For fish receiving the non-supplemented diets, the level was 17-23 mg/kg. [Refer to Table 14].

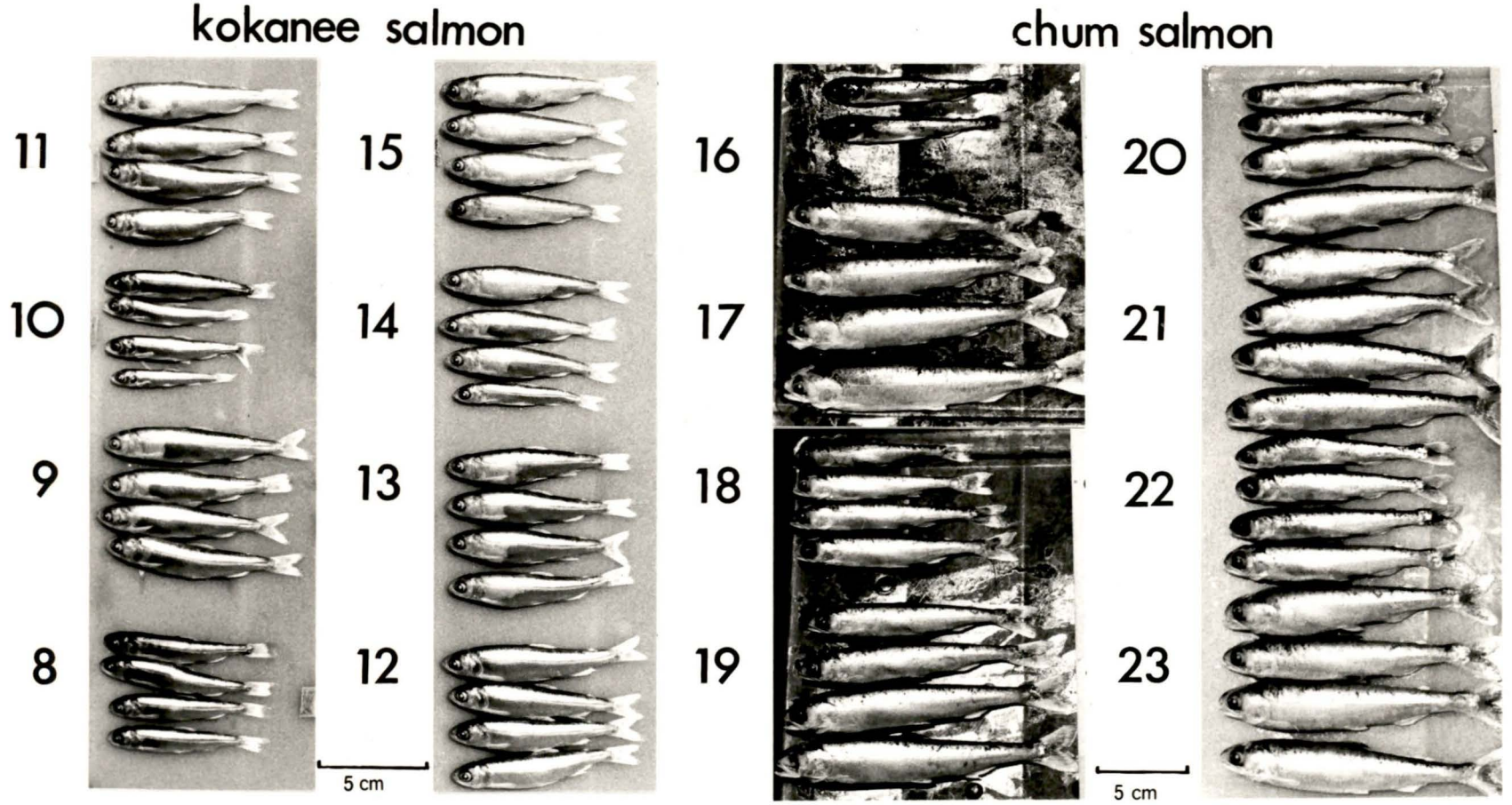

kokanee salmon
chum salmon
11
10
9
8
15
14
13
12
16
17
18
19
20
21
22
23
5 cm
5 cm

PLATE I

PLATE IIA. Chum salmon receiving non-supplemented, sterile Diet 2. The level of l-ascorbic acid in the diet was 21 mg/kg.

PLATE IIB. Chum salmon receiving l-ascorbic acid supplemented (62 mg/kg), sterile Diet 2. Compare to A, and note the lighter pigmentation.

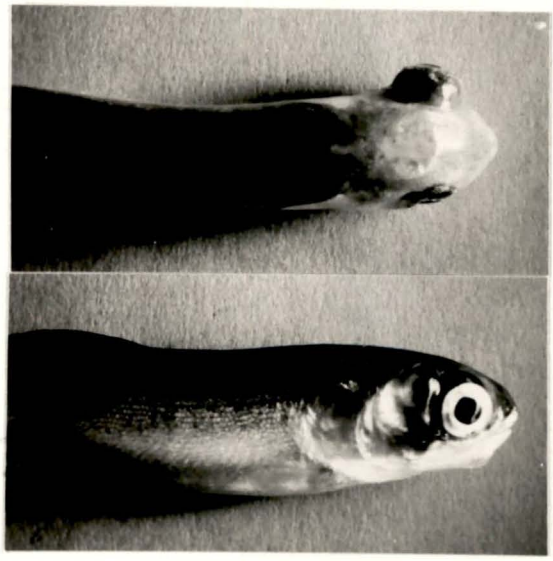
PLATE IIC. Typical symptoms of nitrogen embolism for a chum salmon receiving a non-supplemented diet (Diet 1, the level of l-ascorbic acid was 21 mg/kg). Note the haemorrhaging eye bleb. Actual size.

Plate IID. Typical symptoms of nitrogen embolism for a chum salmon receiving a l-ascorbic acid supplemented diet (Diet 1, 136 mg/kg). Note the nitrogen gas bubbles behind and below the eye, and the absence of an eye bleb. Actual size.

A

B

C



D

PLATE II

PLATE IIIA. Upper - chum salmon fed non-supplemented Diet 2 and showing spinal curvature typical of scoliosis. Middle - chum salmon on same diet as upper fish and showing spinal curvature typical of scoliosis and lordosis. Lower - chum salmon fed on l-ascorbic acid supplemented (62 mg/kg) Diet 2. Half actual size.

PLATE IIIB. Exposed spinal column of chum salmon fed on non-supplemented Diet 2 and showing spinal curvature typical of lordosis. Actual size.

PLATE IIIC. Exposed spinal column of chum salmon fed on the same ration as B and showing spinal curvature typical of scoliosis. Actual size.

A

B

C

PLATE III

IV. Discussion

L-ascorbic acid supplementation of the two fish diets produced marked responses in four species of salmonid fish: rainbow trout, coho salmon, chum salmon and kokanee salmon. Most noticeably, there was an increased survival rate, fat efficiency, fat storage, and in the case of chum salmon, an increased growth response. In contrast to the observations of Halver (1969), there was no increase in the growth rate of rainbow trout fed on diets with more than 100 mg of l-ascorbic acid per kg of diet. However, for rainbow trout fed on non-supplemented Diet 2, the whole blood l-ascorbic acid levels increased with decreasing growth rate.

The maximum mean circulating l-ascorbic acid level for the chum and kokanee salmon was approximately 33 ug/g of whole blood. The mean value for rainbow trout was higher, being 40 ug/g of whole blood. These values corresponded well to the literature values for rainbow trout and coho salmon (Halver 1969). Both the trout and salmon reflected the level of l-ascorbic acid in the two feeds similarly to the guinea pig and man.

The maximum mean kidney levels (158 - 278 ug/g) agreed with the values obtained for rainbow trout and coho salmon by Halver (1969), and as with the blood, reflected the l-ascorbic acid levels in the two feeds. The minimum mean l-ascorbic acid levels for blood and tissue (10-20 ug/g

for salmon, and 5-10 ug/g for rainbow trout) were, however, significantly lower than those levels reported by Halver (1969). The difference may in part be due to Halver's method of analysis which was more sensitive to interfering compounds. This may have resulted in erroneously high results when the l-ascorbic acid levels were low (Roe 1961).

There was a marked difference in the stability of l-ascorbic acid between Diet 1 and Diet 2. The lower rate of destruction of the vitamin in Diet 1 was probably due to the presence of reducing substances associated with the carbohydrate fraction. Halver (1969), did not measure the l-ascorbic acid content of his test diet at the time of feeding. Since the diet was fed wet (66% moisture), destruction of the vitamin may have occurred and the reported tissue concentrations of l-ascorbic acid may not reflect the true dietary intake levels. Howarth *et al.* (1972), have shown that for a pelleted guinea pig ration [lucerne meal 15, grass meal 25, skimmed milk powder 2, ground whole barley 9, ground whole oats 10, wheat bran 14.2, soya bean meal 3, soya bean oil 2, yellow maize meal 5, herring meal 1, meat and bone meal 1, mineral and vitamin mixture 3.8%, and crystalline l-ascorbic acid 1500 ug/g of diet] the destruction of l-ascorbic acid at 22-24°C and relative humidities of 30 - 75% was 45 - 95% after 8 wk, and when stored at 4°C and a relative humidity of 50%, the loss was 25 - 30% after 8 wk. They reported that pellets stored in

plastic bags at -20°C lost no vitamin C. It is important that similar studies be conducted on the stability of l-ascorbic acid in fish rations under normal hatchery storage conditions. It may be necessary to supplement rations with an l-ascorbic acid analog that is more stable.

Although the interactions between l-ascorbic acid and the intestinal microflora of man and some other mammals have been investigated, the interactions in fish have not been studied.

Primary interest has been focused on the decomposition of l-ascorbic acid by the intestinal microflora of man. Stepp (1936), remarked that changes in the level of l-ascorbic acid in the intestine were independent of changes in the other organs of the body, and that scurvy could be alleviated with smaller doses of vitamin C if it was injected into the blood instead of given *per os*. Preliminary *in vitro* studies have shown that enteric bacteria of the *Shigella-Escherichia* and *Klesiella-Aerobacter-Serratia* groups destroy l-ascorbic acid with great facility (Eddy and Ingram 1954, Young and James 1942, and Young and Rettger 1943).

It has also been shown that the presence of l-ascorbic acid delayed or suppressed the appearance of pantothenic acid deficiency in rats (Barboriak and Krehl 1957) probably by exerting a secondary effect mediated through a change in the intestinal microflora.

The study of the l-ascorbic acid-microbial interaction in the intestine of a fish presents a problem not encountered with man and other momeotherms. In the fish, maintained in water at a temperature of 12 to 15°C, the time of passage through the gastro-intestinal tract is approximately 24 hr (Brett 1970). In this time, only a few bacterial generations may have elapsed (Stanier *et al.* 1970), thereby limiting the role that the microorganisms may play in fish nutrition (cf. a 24-27 hr feed passage time in man, and intestinal microflora with shorter generation times. Moreover, little is known about the physical relationship between the bacteria and the fish intestine - are the bacteria present representative of a commensal flora, or are they merely transients?

The ability of l-ascorbic acid to lower the number of intestinal bacteria *in vivo* has not previously been reported, although the bacteriostatic and bactericidal effects *in vitro* have been reported (Eddy and Ingram 1953). The intestinal tract of man and other mammals is relatively longer than that of fish, and any antimicrobial action is more likely to occur in the most anterior portion of the intestine and escape detection when normal microbiological techniques are used for sample procurement.

There are three possible mechanisms by which a decrease in the number of viable organisms in the intestine of fish can be envisaged. Firstly, l-ascorbic acid may

exert a bactericidal action; secondly, it may effect a microbial mediated antagonism; and thirdly, it may effect a host mediated antagonism.

The bactericidal effect seems unlikely to play an important role because its action would be limited to the most anterior sections of the alimentary tract, namely the stomach and pyloric caeca. This antimicrobial action must occur in the alimentary tract, as there was no effect on the viable microbial count of the supplemented fish rations. Feeding fish sterile diets did not appreciably alter the microbial count.

L-ascorbic acid may induce a microbial-mediated antagonism by introducing conditions (e.g., reducing the oxidation-reduction potential) that are favourable for a microorganism(s) producing an inimical agent(s) that inhibit(s) the growth of other microbial species within or on the walls of the intestinal lumen. The bactericidal and microbial mediated antagonism are both dependent upon the absorption time for l-ascorbic acid from the gastro-intestinal tract, which has yet to be ascertained.

A rigorous investigation of the microorganisms present in the fish intestine must be conducted, as only the aerobic and anaerobic mesophilic bacteria were enumerated. The techniques used could easily result in the complete destruction of strict anaerobes. Moreover, the media selected for enumeration may have been nutritionally inade-

quate for certain bacterial species.

The third, and perhaps most plausable explanation for the observed decrease in the intestinal counts of fish receiving a l-ascorbic acid supplemented feed, may be a host-mediated antagonism. Since l-ascorbic acid increases the integrity of the intercellular ground substance, and hence the integrity of the gastro-intestinal tract tissues, there may be a decreased tendency for the bacteria to attach to the intestinal cell walls. This may be mediated either through secretion of compounds that obstruct this attachment, or by the secretion of inimical agent(s) by the intestinal cells. Preliminary studies did not show the intestinal tissues of rainbow trout or chum salmon to prevent the growth of *Escherichia coli* or the *Pseudomonas* species isolated from the chum salmon.

The increased tolerance of fish to nitrogen embolism when receiving a l-ascorbic acid supplemented diet is very important. Since early in this century, the fish culturalist has been periodically plagued by nitrogen embolism among his fish stocks. A comprehensive treatise on the causative agent and symptoms has been presented by Marsh and Gorham (1905). Recently, reports have presented details of recurrences of this condition among hatchery fish stocks (Harvey and Smith 1962), and of river systems with associated dam projects (Beiningen and Ebel 1970).

The apparent increased tolerance of fish to

nitrogen embolism is probably due to the strengthening of the intercellular ground substance, particularly in the capillary net work. There is less hemorrhaging and the distension of the eyes is reduced; as a consequence, the opportunity for microbial infection is markedly decreased.

V. Conclusion

The relationship between l-ascorbic acid and fish nutrition is more complex than was originally thought. The understanding of this relationship is complicated by our lack of essential information regarding turnover rates of the vitamin in salmonid fish tissues at various physiological ages. Furthermore, the relationship between l-ascorbic acid and tolerance to nitrogen embolism, and between l-ascorbic acid and the intestinal microorganisms of fish present a fertile area for further investigation. The use of the fish as a model for the further study of l-ascorbic acid in human nutrition should not be overlooked.

SECTION C. THE L-ASCORBIC ACID CONTENT OF INDIVIDUAL RAINBOW TROUT EGGS

I. Introduction

The role between maternal nutrition and egg development in salmonid fish has not been investigated. The hatchability of eggs, and the survival of alevins will be dependent upon the availability of adequate levels of nutrient components during egg development.

The initial steps in such an investigation must of necessity involve a quantitation of the nutrient components in the female fish and her developing eggs. As a continuing study of work initiated in this laboratory into the vitamin levels of individual salmonid fish eggs, the l-ascorbic acid levels of rainbow trout (*Salmo gardnerii*) eggs were determined prior to, and at the time of spawning.

II. Materials and Methods

1. Fish.

Seven male and thirteen female spawning rainbow trout (mean wet weight of 340 g) from Pennask Lake, B.C., were supplied by the B.C. Department of Recreation and Conservation. Upon arrival at the laboratory, the fish were placed into a 1.83 m diameter holding tank. Four females and two males were sacrificed within two hours after arrival. Blood samples (0.5 g) were obtained by caudal peduncle amputation for l-ascorbic acid analysis, and added to tared tubes containing 2 ml of 6% TCA. The abdomen was opened using standard aseptic procedures. The two ovaries with egg contents

intact, were removed and placed into sterile 1000 ml beakers. Egg samples were dissected from the fore, mid and hind thirds of the ovary [anterior-posterior location of ovary in abdominal cavity], placed into sterile 100 ml beakers, covered with a damp paper towel, placed in ice and stored at 4°C until submitted to l-ascorbic acid microanalysis. Liver and kidney tissue samples (0.5 - 1.0 g) were placed into 10 ml of 5% HPO_3-10% HOAc solvent and submitted to l-ascorbic acid macroanalysis.

The remaining fish were held for a further 19 days at which time four gravid females extruded their roe and died. The surviving five males and five females were immediately sacrificed. Blood samples (1.0 g) were obtained by heart puncture for l-ascorbic acid analysis, and placed into tared tubes containing 5 ml of 6% TCA. The abdomen was opened and a portion of the eggs were removed, fertilized and placed in the egg hatchery (Trust 1972). The remaining eggs were removed and stored as described above until submitted to l-ascorbic acid macroanalysis.

2. L-ascorbic acid analysis.

The liver and kidney l-ascorbic acid levels were determined by the macroprocedure employing 5% HPO_3-10% HOAc as the solvent, and outlined in Section B. (II). 1. (a). The blood l-ascorbic acid levels were

determined by the macroprocedure employing 6% TCA, Section B. (II). 1. (c). The l-ascorbic acid content of individual rainbow trout eggs was determined by the microprocedure described in Section B. (II). 1. (d).

From each of four females, 20 individual eggs were dissected from the fore, mid and hind thirds of the ovary, individually weighed and then extracted with 500 ul of ice-cold 5% TCA. Each egg was crushed with a glass rod until a fine pink-coloured suspension was formed. The suspension was centrifuged at 10,000 rpm for 20 minutes using a Sorvall RC2-B refrigerated centrifuge. A 150 ul aliquot of supernatant was analyzed for l-ascorbic acid content as outlined in Section B. (II). 1. (d). If the analysis could not be conducted at this stage, the 150 ul aliquot was stored at -20°C until submitted for analysis.

To confirm the results of the microprocedure, the macroprocedure using HPO_3-HOA_c as solvent was employed. Ten eggs chosen from the same section of the ovary were pooled, weighed and extracted with either 5 or 10 ml of HPO_3-HOAc solvent. The eggs were crushed thoroughly with a glass rod until a fine suspension was obtained, and the analysis conducted as described in Section B. (II). 1. (a).

3. Egg moisture and fat composition

Moisture and fat were determined on five pooled 20 egg samples from each female fish by the method of Horwitz (1970).

4. Partial characterization of the crude bis-2,4-dinitrophenylhydrazine osazone from rainbow trout eggs.

Fifty eggs were extracted with 10 ml of the HPO_3-HOAc solvent, and the 2,4-dinitrophenylhydrazine osazone(s) [designated as compound I] was prepared as described in Section B. (II). 1. (a). After the incubation period for 3 hr at 37°C, the solution was centrifuged at 20,000 rpm for 30 min (Sorvall RC2-B refrigerated centrifuge) to aggregate the insoluble osazones from the 9N H_2SO_4-2,4-dinitrophenylhydrazine solution. The precipitate was washed with 0.1 N H_2SO_4 to remove most of the 2,4-dinitrophenylhydrazine reagent. The washed osazones were then dissolved in a small portion of ethyl acetate. A similar procedure was followed for the procurement of the bis-2,4-dinitrophenylhydrazine osazone of l-ascorbic acid.

The crude egg osazone, compound I, was subjected to spectroscopic analysis between 300 and 550 nm, and paper chromatography by the method of Mapson (1961). The solvent for the spectral study was ethyl acetate, and for the paper chromatography an aqueous phenol

(10% w/v) - acetic acid (10% v/v) - glycerol (10% v/v) solvent was employed. A control using the osazone derivative of synthetic l-ascorbic acid was also analysed.

III. Results

The results in Table 30 show that there was no significant change in the liver and kidney tissue l-ascorbic acid levels over the experimental period. However, the results in Table 31 show that there was a significant difference between the mean whole blood l-ascorbic acid concentration of the fish upon arrival at the laboratory (2.91 ± 0.59 ug/g), and after holding for 19 days (4.39 ± 0.96 ug/g). The l-ascorbic acid levels in the blood and tissues of male and female rainbow trout were not significantly different.

The data in Table 32 show that the l-ascorbic acid concentration in the eggs as determined by either the micro or macroprocedures, did not differ significantly. A comparison of the results in Tables 32 and 33 show that the initial l-ascorbic acid levels in the eggs were significantly higher than the final levels.

The mean wet weights, fat free dry matter, and l-ascorbic acid content of the individual trout eggs are presented in Appendix LVII and summarized in Tables 34 and 35. If the 0.01 level of significance is chosen as the criterion upon which to reject the null hypothesis that the

mean l-ascorbic acid levels are not significantly different, then the following results may be noted: (1) within individual ovaries, there was a uniformity between the wet egg weight, fat free dry matter, and l-ascorbic acid content between the fore and mid sections, and the fore and hind sections; (2) between ovaries of different fish, there tended to be a uniformity between the fore sections, but not between the mid or hind sections. The individual egg l-ascorbic acid levels were significantly different from the kidney and liver l-ascorbic acid levels. The mean range of l-ascorbic acid concentrations between the eggs of four fish were between 5.27 - 7.35 ug/wet egg (89 - 151 ug/g of wet egg).

The 2,4-dinitrophenylhydrazine osazone of l-dehydroascorbic acid and compound I in ethyl acetate, displayed maxima in the spectral regions 300-390 nm, 435-550 nm. The 2,4-dinitrophenylhydrazine reagent displayed a maximum absorption at 340 nm (Figure 6).

Two compounds were separated when compound I was analysed by paper chromatography: a red compound with R_f 0.66 and a yellow compound with R_f 0.84. The yellow compound was shown to have the same R_f value as 2,4-dinitrophenylhydrazine. The red osazone of l-dehydroascorbic acid separated into an open-chain compound (orange-yellow form with R_f 0.43), and a closed-ring lactone compound (red form with R_f 0.66) in accordance with Mapson (1961)(Figure 7).

TABLE 29. Sex, fork length, and body, liver and kidney weights for spawning rainbow trout from Pennask Lake, B.C.

Fish No.[a]	Sex	Wet Body wt (g)	Fork Length (cm)	Wet Liver[b] (g)	Wet Kidney[b] (g)
1	F	333	34.9	0.92	0.66
2	M	295	32.4	0.96	0.54
3	F	400	30.5	0.83	0.77
4	F	278	30.8	1.35	0.75
5	M	380	35.6	1.13	0.96
6	F	370	30.8	0.71	0.95
7	M	376	31.8	0.99	0.69
8	M	335	31.8	0.64	1.07
9	M	274	27.9	0.87	0.88
10	F	404	31.5	1.09	0.73
11	M	311	29.2	0.99	0.87
12	F	356	29.0	0.88	0.44
13	F	270	27.2	1.00	0.55
14	F	338	28.2	0.41	0.68
15	F	366	29.0	0.83	0.50
16	M	337	29.0	0.89	0.72

[a]Fish 1-6, sacrificed 22-06-73.
Fish 7-16, sacrified 11-07-73.

[b]Wt of tissue taken for analysis.

TABLE 30. L-ascorbic acid levels in the liver and kidney tissue[a] of spawning rainbow trout from Pennask Lake, B.C.

	Liver			Kidney		
Fish No.	Absorbance* 540 nm	ug/ml from standard curve	ug/g of tissue[b]	Absorbance* 540 nm	ug/ml from standard curve	ug/g of tissue[c]
1	0.210	6.33	68.8	0.183	5.51	83.5
2	0.159	4.79	49.9	0.189	5.69	105.4
3	0.265	7.98	96.2	0.218	6.57	85.3
4	0.168	5.06	37.5	-	-	-
5	0.159	4.79	42.4	0.198	5.96	62.1
6	0.225	6.78	95.5	0.242	7.29	76.7
7	0.230	6.93	70.0	0.215	6.48	93.9
8	0.222	6.69	104.5	0.295	8.89	83.0
9	0.130	3.92	45.0	0.163	4.91	55.8
10	0.147	4.43	40.6	0.228	6.87	94.1
11	0.173	5.21	52.6	-	-	-
12	0.259	7.80	88.7	0.208	6.27	142.4
13	0.130	3.92	39.2	0.271	8.16	148.4
14	0.183	5.51	134.4	0.151	4.55	66.9
15	0.267	8.04	96.9	0.175	5.27	105.4
16	0.343	10.33	116.1	0.420	12.65	175.7

[a]Tissue samples extracted with 10 ml of HPO_3-HOAc solvent. See Table 29 for tissue weights.

[b]For fish 1-6, mean = 65.1±23.9 ug/g of liver.
for fish 7-16, mean = 78.8±32.4 ug/g of liver.

[c]For fish 1-6, mean = 82.6±14.0 ug/g of kidney.
For fish 7-16, mean = 107.3±37.8 ug/g of kidney.

*Mean absorbance of duplicate samples.

TABLE 31. L-ascorbic acid levels in the whole blood of spawning rainbow trout from Pennask Lake, B.C.

Fish No	Sample No	Wt blood[a] (g)	Absorbance 540 nm	l-ascorbic acid ug/ml from standard curve	l-ascorbic acid ug/g of whole blood
2	1	0.50	0.030	0.690	2.76
	2	0.36	0.026	0.598	3.32
	3	0.31	0.024	0.552	3.56
	4	0.37	0.035	0.805	4.35
					$\bar{X}$ 3.50±0.57
3	1	0.52	0.024	0.552	2.12
	2	0.45	0.023	0.529	2.35
	3	0.49	0.027	0.621	2.53
	4	0.35	0.022	0.506	2.89
					$\bar{X}$ 2.47±0.28
4	1	0.38	0.027	0.621	3.27
	2	0.31	0.025	0.575	3.71
	3	0.45	0.026	0.598	2.66
					$\bar{X}$ 3.21±0.43
5	1	0.54	0.023	0.529	1.96
	2	0.30	0.015	0.345	2.30
	3	0.43	0.023	0.529	2.46
	4	0.57	0.038	0.874	3.07
					$\bar{X}$ 2.45±0.40
6	1	0.56	0.032	0.736	2.63
	2	0.46	0.033	0.759	3.30
	3	0.45	0.033	0.759	3.37
	4	0.47	0.028	0.644	2.74
					$\bar{X}$ 3.01±0.33
7		1.53	0.047	1.08	3.53
8		1.00	0.038	0.874	4.37
9		1.71	0.062	1.43	4.17
10		2.44	0.094	2.16	4.43
11		3.27	0.110	2.53	3.87
12		1.24	0.052	1.20	4.82
13		1.24	0.031	0.713	2.87
14		0.55	0.031	0.713	6.48
15		1.21	0.052	1.20	4.94
16		-	-		$\bar{X}$ 4.39±0.96

[a]For fish 2-6, blood was obtained by caudal peduncle amputation and added to tared tubes containing 2 ml 6% TCA.
For fish 7-16, blood was obtained by heart puncture and added to tared tubes containing 5 ml of 6% TCA.

TABLE 32. Comparison of macroprocedure and microprocedure for the analyses of l-ascorbic acid in rainbow trout eggs[a]

Fish No. Egg position in ovary	mean l-ascorbic acid concentration			
	ug/g wet egg		ug/g egg FFDM	
	Macro	Micro	Macro	Micro
1 Fore	108±2	113±8	206±5	216±16
3 Mid	101±1	104±3	188±4	195±6*
4 Mid	119±6	128±10	230±10	247±23
6 Hind	153±6	151±7	288±11	284±14

*Significantly different at 0.05 level.

[a]Refer to Appendices LVI and LVII, and Table 34.

TABLE 33. Mean l-ascorbic acid content of pooled rainbow trout eggs[a]

Fish No.	Mean l-ascorbic acid concentration	
	ug/g wet egg	ug/g of egg FFDM
10	93 ± 6[b]	163 ± 8
12	93 ± 11	154 ± 19
14	101 ± 6	175 ± 10
15	114 ± 3	200 ± 5

[a]Refer to Appendix LVI.

[b]Mean and standard deviation of 10 samples. Refer to Appendix

TABLE 34. Mean wet weight, fat free dry matter, and l-ascorbic acid content of rainbow trout eggs[a,b]

Fish No.	Egg location in ovary	Wet Egg Wt (mg)	FFDM (mg)	l-ascorbic acid		
				ug/wet egg	ug/g of wet egg	ug/g egg FFDM
1	Fore	635±27	333±14	7.17± .37	113±8	216±16
	Mid	620±10	325±6	7.34± .18	118±4	225±7
	Hind	612±15	321±8	7.19± .21*	118±4*	224±8*
3	Fore	676±15	360±8	7.24± .23	107±3	201±5
	Mid	685±19	365±10	7.13± .22	104±3	195±6
	Hind	766±52	409±27	6.83±1.05*	89±16*	167±31*
4	Fore	491±16	253±8	6.65±0.43	136±9	262±17
	Mid	510±22	263±11	6.52±0.43*	128±10*	247±23*
	Hind	526±26	271±13	5.27±0.39*	100±7*	196±15*
6	Fore	502±20	267±11	6.68±1.08*	133±19*	243±40*
	Mid	517±17	275±9	6.76±0.32	131±8	245±14
	Hind	487±24	259±13	7.35±0.31	151±7	284±14

*N = 19, otherwise N = 20.

[a]For individual egg data, refer to Appendix LVII.

[b]For egg composition data, refer to Appendix LV.

TABLE 35. Analysis of egg l-ascorbic acid levels presented in Table 34

Fish No. & Egg location in ovary[a]	Wet egg wt	Egg FFDM	l-ascorbic acid ug/wet egg	l-ascorbic acid ug/g wet egg	l-ascorbic acid ug/g egg FFDM
1F & 1M	+	*	NS	*	*
& 1H	*	+	NS	*	NS
1M & 1H	NS	NS	NS	NS	NS
3F & 3M	NS	NS	NS	+	+
& 3H	+	+	NS	+	+
3M & 3H	+	+	NS	+	+
4F & 4M	+	+	NS	*	+
& 4H	+	+	+	+	*
4M & 4H	*	+	+	+	*
6F & 6M	*	*	NS	NS	NS
& 6H	*	*	+	+	+
6M & 6H	+	+	+	+	+
1F & 3F	+	+	NS	+	+
& 4F	+	+	+	+	+
& 6F	+	+	NS	+	+
3F & 4F	+	+	+	+	+
& 6F	+	+	*	+	+
4F & 6F	NS	+	NS	NS	NS
1M & 3M	+	+	+	+	+
& 4M	+	+	+	+	+
& 6M	+	+	+	+	+
3M & 4M	+	+	+	+	+
6M	+	+	+	+	+
4M & 6M	NS	+	NS	NS	NS
1H & 3H	+	+	NS	+	+
& 4H	+	+	+	+	+
& 6H	+	+	+	+	+
3H & 4H	+	+	+	*	+
6H	+	+	*	+	+
4H & 6H	+	+	+	+	+

[a] F = fore
M = mid
H = hind

*=significantly different at 0.05 level
+=significantly different at 0.01 level
NS=not significantly different.

FIGURE 6. The 300-550 nm spectral absorption curve in ethyl acetate for the bis-2,4-dinitrophenylhydrazine osazone of l-dehydroascorbic acid (a), the 2,4-dinitrophenylhydrazine derivative isolated from rainbow trout eggs (b), and 2,4-dinitrophenylhydrazine (c).

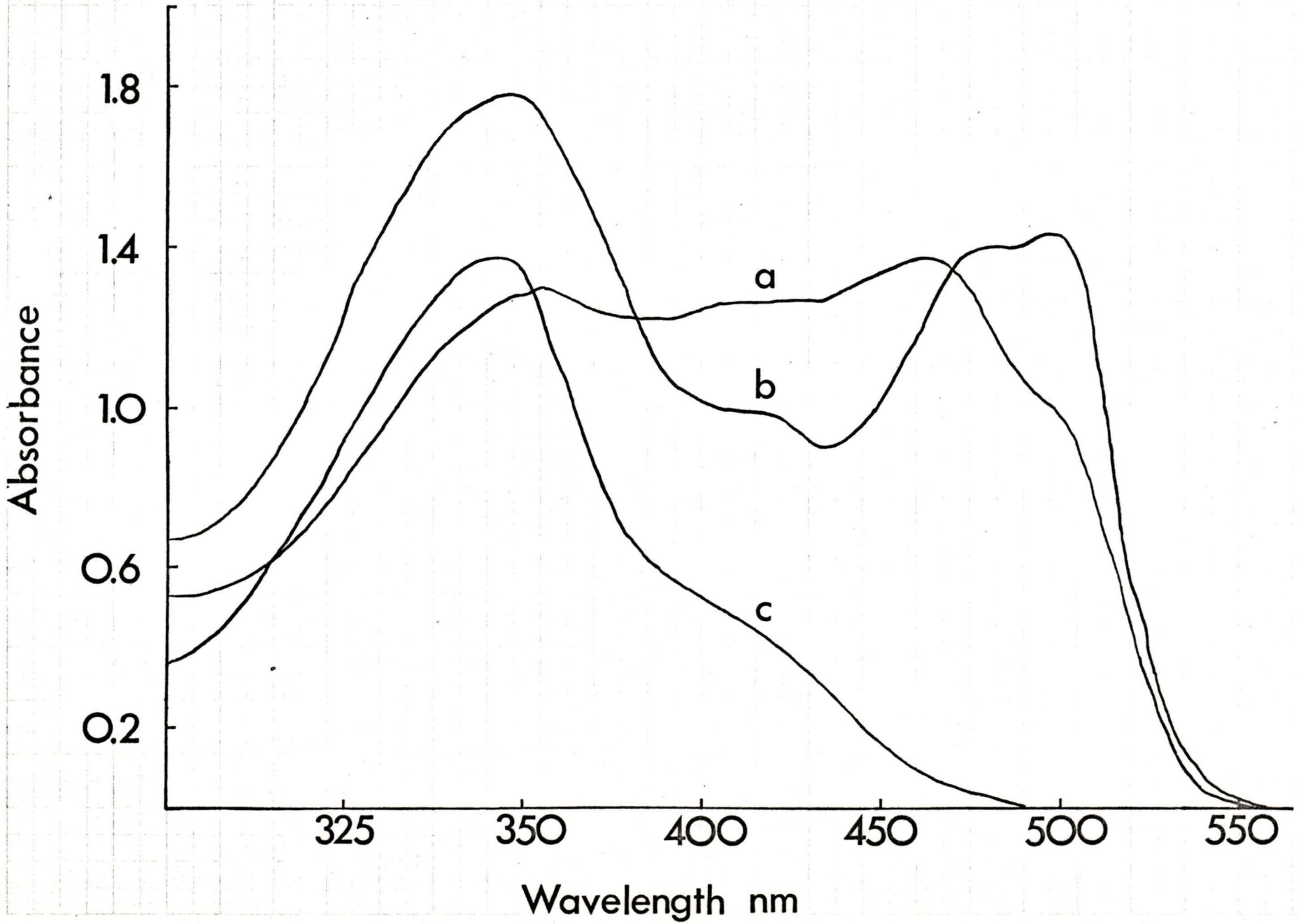
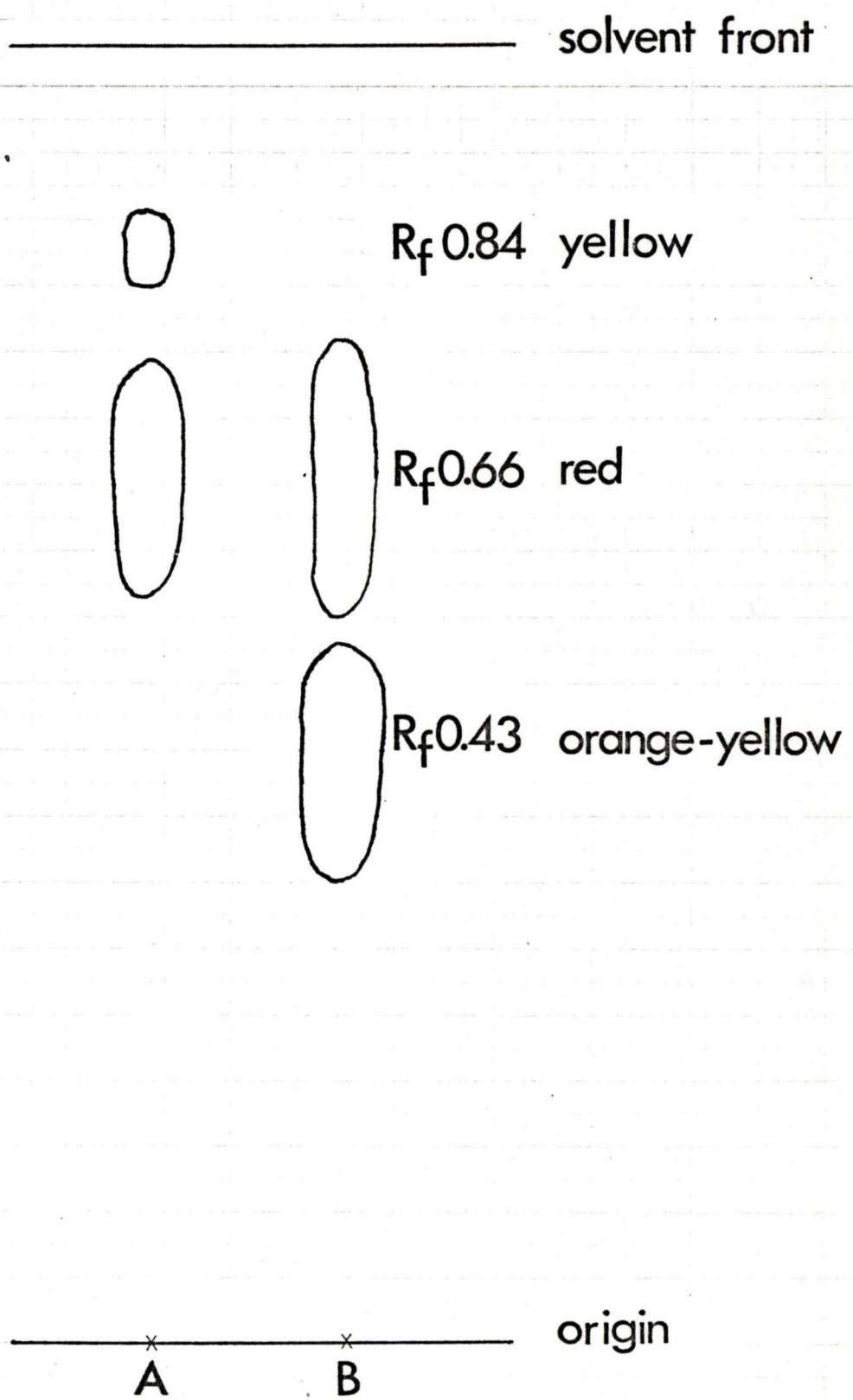

1.8
1.4
1.0
0.6
0.2
Absorbance
a
b
c
325
350
400
450
500
550
Wavelength nm

FIGURE 7. Paper chromatogram of the 2,4-dinitrophenylhydrazine osazone of dehydro-l-ascorbic acid (B) and the 2,4-dinitrophenylhydrazine derivative isolated from rainbow trout eggs (A). Solvent, water - phenol - acetic acid - glycerol (70:10:10:10).


solvent front
Rf 0.84 yellow
Rf 0.66 red
Rf 0.43 orange-yellow
origin
A
B

IV. Discussion

This report presents for the first time l-ascorbic acid concentrations of individual rainbow trout eggs. Moreover, this is also the first report of the l-ascorbic acid microprocedure for blood (Lowry *et al.* 1945), being successfully adapted to tissues.

The results of the partial characterization of compound I, indicated that it was the 2,4-dinitrophenylhydrazine osazone of l-dehydroascorbic acid. Unlike similar studies conducted with rat urine (Probst and Schultze 1950), the concentrations of interfering substances were low in the rainbow trout egg. It would have been advantageous to purify the derivative on an acid activated Al_2O_3 column prior to spectral characterization, however, this was not done since this was not a rigorous characterization, and there was no indication by paper chromatography of significant levels of interfering substances.

The l-ascorbic acid levels in rainbow trout eggs were found to be similar to the Atlantic salmon (*Salmo salar*) egg levels of 140 ug/g of wet egg (Fixsen and Roscoe 1938).

In man and the guinea pig, pregnancy appears to increase the requirement for l-ascorbic acid. The concentrations of the vitamin are high in the placenta, and fetal levels are higher than maternal levels, suggesting an active transfer mechanism (Woodruff 1964). Whether or not a similar increased demand for l-ascorbic acid in gravid fish

occurs is not known. Although the l-ascorbic acid levels of the individual eggs from the first four females were significantly higher than the maternal tissue levels, it can not be said that this offers proof for an active transfer mechanism since the l-ascorbic acid levels in the ovary tissue and the past nutritional history of the fish were unknown.

The low initial blood levels of l-ascorbic acid can probably be attributed to the stress conditions encountered during transport from Pennask Lake. It has been shown that the l-ascorbic acid levels in the blood of salmonid fish drop significantly under stress conditions (Wedemeyer 1969).

The stability of the tissue levels of l-ascorbic acid over a 19 day fasting period would indicate that spawning rainbow trout may have some l-ascorbic acid synthetic capacity. The significant decrease in the egg l-ascorbic acid concentration over the 19 day period may be associated with the separation of the eggs from the ovarian tissue at the time of spawning. Considering the maternal tissue levels of l-ascorbic acid, it is unlikely that there was a depletion of the egg l-ascorbic acid by the female.

To appreciate the significance of the observed variation among rainbow trout eggs from different females, the role between maternal l-ascorbic acid levels and egg development, hatchability and survival of the alevins must be ascertained. It is also necessary to know the l-ascorbic

acid levels in the ovarian tissue during egg development, and the fate of l-ascorbic acid during embryonic development and alevin development up to the time the yolk-sac is absorbed and active feeding begins.

The elucidation of the maternal-egg nutritional role should prove to be an exciting research area.

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APPENDIX I. Experimental fish tank designs for conventional and axenic fish

(a) An experimental conventional fish tank design.

To date, there has been no attempt to standardize the rearing conditions for experimental fish. It has been customary for fish nutrition experiments to be conducted in hatchery troughs. These are adequate for the following reasons: firstly, the design affords a large surface area for the attachment and colonization by microorganisms; secondly, the aeration systems introduce large numbers of organisms from the atmosphere into the trough water; and thirdly, an excessive amount of time is spent in cleaning the tanks. Reports of daily cleanings are not uncommon (Halver 1969). The response, or lack of response, of fish to nutritional investigation will be governed by these management conditions.

An initial objective of this work was to refine and test an experimental self-cleaning fish tank designed in this laboratory that afforded a maximum weight of fish per unit volume of water by maintaining adequate dissolved oxygen levels, constant temperature, and control over the introduction of microorganisms from the atmosphere.

The plexiglass tank design specifications are given

in Figure 8.

Adequate dissolved oxygen levels (refer to Figure 1, Section A) were achieved by introducing the well water into the tank through a 0.75 mm diameter orifice contained in the venturi apparatus. The water [40 lbs per in^2 for experiments 1 - 6, and 16 - 20 lbs per in^2 for experiments 7 and 8] entered the tank at a controlled rate of 150 - 200 cc min^{-1}. Providing the bottom end of the plexiglass venturi tube was submerged, air was drawn through the apparatus at a rate of 500 - 800 cc min^{-1}. The water circulation rate was 7.5 cm sec^{-1}.

By means of the plexiglass siphon, the tank water volume cycled between a minimum of 25 liters and a maximum of 33 liters every 40 - 50 minutes. During the tank siphoning cycle, a negative pressure was created above the tank water (the water was leaving the system at a rate of 2.6 liters min^{-1}). Air was drawn through a removable glass-wool filter in the clear plexiglass lid. During tank filling, however, a positive pressure was created, and the air above the tank water was recycled by the venturi apparatus.

Feed was introduced by way of the removable glass-wool filter in the tank cover.

Outdoor tanks (0.9 m and 1.8 m diameter) were constructed of fiber glass (Plate IV). These tanks

were partially covered by galvanized steel lids and operated on the same principle as the smaller indoor tanks just described.

(b) An experimental axenic fish tank design.

To understand the role of the fish microflora in fish growth, it will be necessary to conduct nutritional studies with axenic (germ-free) fish. As an initial step towards this objective, Trust and Wood (1973), have shown that fish diets can be effectively sterilized without significant damage to the feed, and without harmful nutritional effects. Trust (1973), has also demonstrated the successful axenic procurement and fertilization of salmonid fish eggs.

As a further contribution to this area of study, an axenic fish rearing chamber was designed and tested.

The rearing chamber consisted of a 4 litre Pyrex glass reaction vessel with an attached glass side-arm (Plate IV). Sterile water was introduced from a 4 litre glass reservoir, and waste-water was collected in a sterile 4 litre flask. The water inlet and outlet attachments were made by a test tube filling apparatus (Pyrex), which allowed for the aseptic replacement of the water reservoir and waste-water receiving flask.

Air was filtered through a Sartorius Membrane Filter (0.45 μ). Glasswool served as the pre-filtering medium.

Feed was introduced through an opening in the top of the rearing chamber. The water reservoir, waste-water receiving flask and main chamber were autoclaved separately for 20 min at 15 lbs. The apparatus was housed in a water-cooled respirometer which was maintained at 16°C.

On October 24, 1972 milt and eggs were obtained from spawning male and female kokanee salmon[a] by standard aseptic procedures (Trust 1973). The eggs were fertilized aseptically and 25 eggs were placed into each of four 95 x 62 mm anaerobic culture dishes (Pyrex) containing 50 - 75 ml of sterile well water, and incubated in a refrigerator-incubator at 12°C. The water was changed at weekly intervals.

Hatching was completed by the 21-12-72. Two of the four fish lots became contaminated with a *Pseudomonas* species. One lot was treated with tetracycline (100 ug/ml) and neomycin (10 ug/ml), and the other served as a non-sterile control. For the antibiotic treated fish lot, no aerobic or anaerobic growth on Standard Methods agar (BBL) at 25°C could be demonstrated by the drop plate method of Miles and Misra (1938).

On February 10, 1973 sterile alevins were placed

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[a]Eggs were supplied by the B.C. Department of Recreation and Conservation.

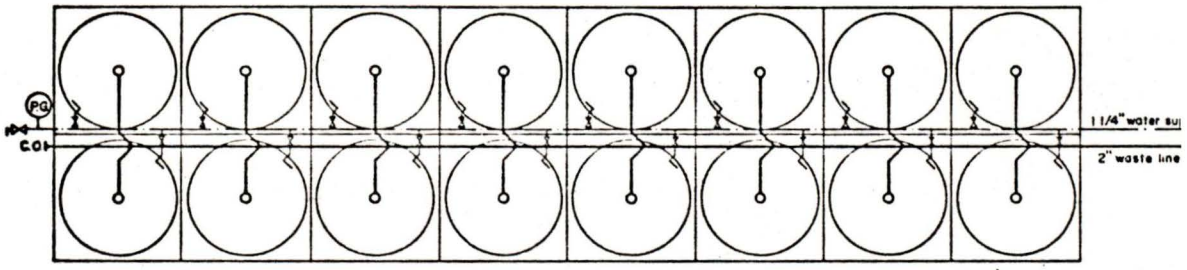
into the axenic rearing chamber which contained a 1% peptone-well water mixture, and 4 fish were maintained in the sterile culture dishes containing the same medium. A control consisted of 4 fish in non-sterile well water.

Sterile feed (Diet 2) was introduced to each lot of fish, however, none would eat. All the fish died by the 20-02-73. No microorganisms could be isolated from the sterile systems up to six months later by the detection procedure employed.

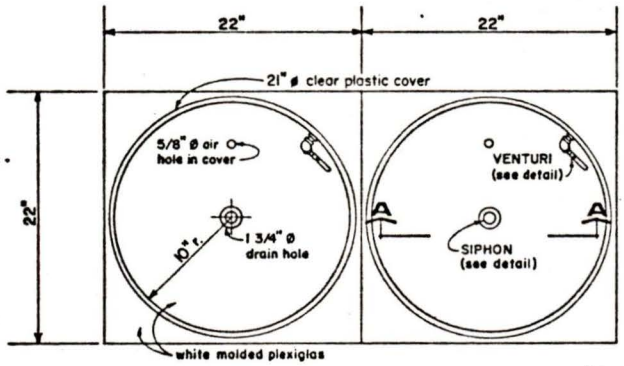
The failure of the fish to respond to the feed was probably due to the time delay between the absorption of the yolk sac and the introduction to feed.

The present tank design will need to be modified considerably to meet the needs of nutritional experimentation, however, it has been demonstrated that axenic fish can be reared to the alevin stage.

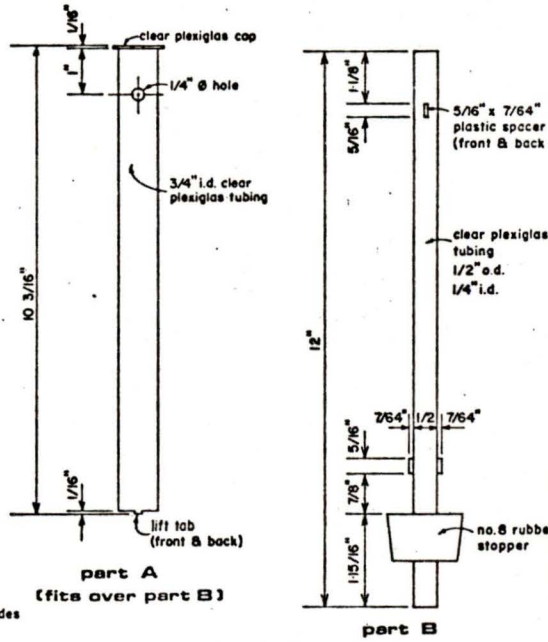
APPENDIX FIGURE 8. The experimental conventional fish tank design.



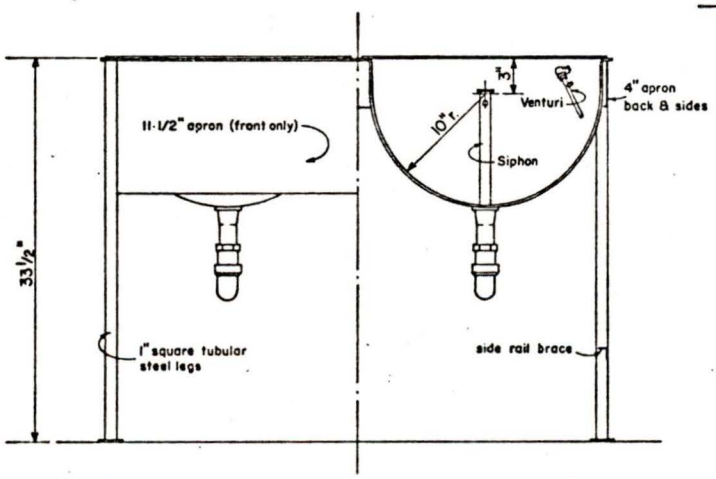
Plumbing Plan



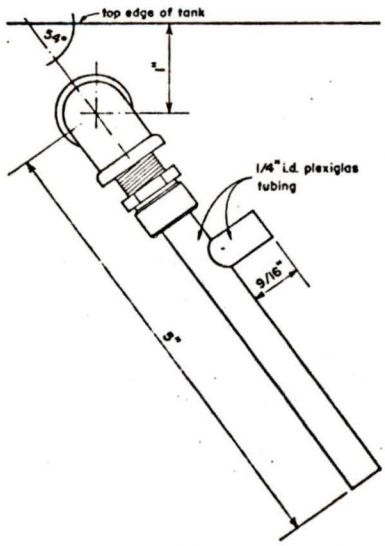
Plan



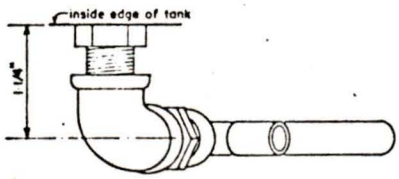
Siphon Details



Front Elevation & Section A-A



Venturi - elevation



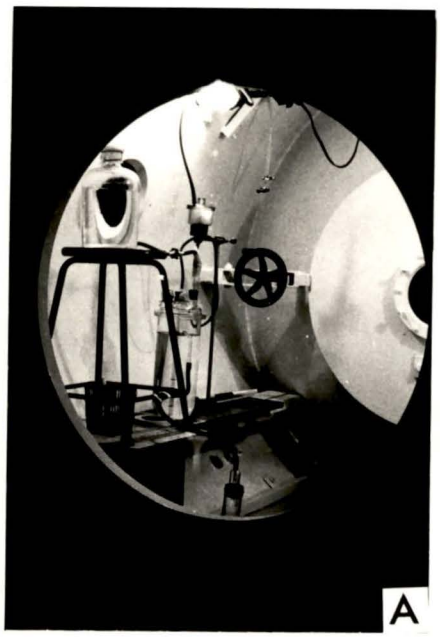
Venturi - plan

APPENDIX PLATE IV. A. Axenic fish rearing tank assembly as viewed from outside the respirometer chamber.

APPENDIX PLATE IV. B. Rearing chamber. Arrows indicate the direction of air flow.

APPENDIX PLATE IV. C. Outdoor tank arrangement.

APPENDIX PLATE IV. D. View of water reservoir and air filter for the axenic fish rearing tank assembly.



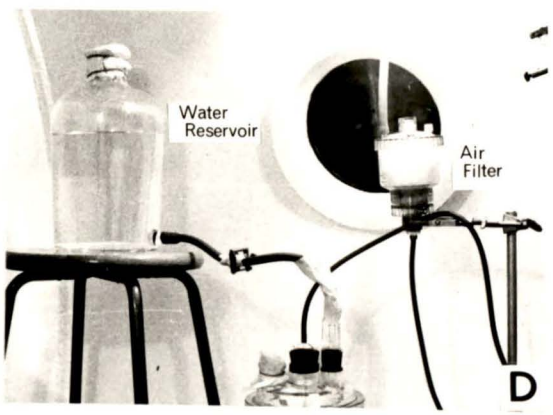


PLATE IV

APPENDIX II. Calculated composition of UVIC-73 ration[a]. Energy and proximate.

		WHEAT	OAT GROATS	WHEAT GERM MEAL	SOYA MEAL	FISH MEAL	DRY WHEY	BREWERS YEAST	SOYA OIL[b]	COD LIVER OIL	SALT	DICAL[c]	CANTHA-XANTHIN	TOTAL
INGREDIENT	(g)	150.0	150.0	150.0	150.0	150.0	150.0	15.0	80.0	10.0	5.0	20.0	0.5	1030.5
ENERGY														
metabolizable energy (kcal)		463	468	463	363	446	303	36	720	90	-	-	-	3352
gross energy (kcal)[d]		-	-	-	-	-	-	-	-	-	-	-	-	4625
PROXIMATE														
dry matter	(g)	134.0	136.0	135.0	135.0	138.0	136.0	14.0	80.0	10.0	5.0	20.0	0.5	943.5
moisture	(g)	16.0	14.0	15.0	15.0	12.0	14.0	1.0	-	-	-	-	-	87.0
fiber	(g)	4.0	6.0	4.5	4.2	1.5	0.3	0.5	-	-	-	-	-	21.0
protein	(g)	19.5	23.7	39.3	76.3	105.9	23.5	6.7	-	-	-	-	-	294.9
fat[e]	(g)	2.5	3.0	10.5	1.5	15.7	1.2	0.2	80.0	10.0	-	-	-	124.6
carbohydrate[f]	(g)	-	-	-	-	-	-	-	-	-	-	-	-	424.3
ash[e]	(g)	3.7	3.0	6.5	8.4	15.7	15.4	1.0	-	-	5.0	20.0	-	78.7

[a]Calculated from Nutrient requirements of domestic animals. Number 1. National Research Council. 1971.

[b]Refined Crisco Oil. Contains antioxidants BHT; BHA; dimethylpolysiloxane.

[c]Dicalcium phosphate.

[d]Gross energy value of ration is based on 5.67, 9.4, and 4.2 kcal/g for protein, fat, and carbohydrate, respectively.

[e]Commercial solvents corporation. Feed ingredient analysis table. 1973.

[f]Carbohydrate calculated as [100 - (+ protein + fat + ash + moisture + fiber)].

APPENDIX III. Calculated composition of UVIC-73 ration. Minerals.

	WHEAT	OAT GROATS	WHEAT GERM MEAL	SOYA MEAL	FISH MEAL	DRY WHEY	BREWERS YEAST	SALT	DICAL	TOTAL
INGREDIENT (g)	150	150	150	150	150	150	15	5	20	
MINERALS										
calcium (mg)	75	120	105	390	4410	2320	20	-	4800	12240
phosphorus (mg)	600	735	1560	930	3300	1480	215	-	3700	12520
iron (mg)	7.50	57.0	16.5	-	84.0	24.0	1.5	-	-	1905
magnesium (mg)	150	135	150	405	-	195	35	-	-	1070
potassium (mg)	750	510	600	3030	750	1800	258	-	-	7698
sodium (mg)	90	75	75	15	750	720	11	428	-	2164
manganese (mg)	4.92	4.29	20.23	6.82	1.49	0.69	0.09	-	-	38.53
zinc (mg)	2.10	21.50	2.10	6.75	16.50	-	0.58	-	-	49.53
copper (mg)	6.75	9.60	13.20	5.44	1.26	6.46	0.50	-	-	43.21
selenium[a] (mg)										0.87

[a]Rey (1973). The selenium value of Diet 2 (Moore-Clark) was found to be 2.28 ± 0.1 ppm.

APPENDIX IV. Calculated composition of UVIC-73 ration. Amino acids.

		WHEAT	OAT GROATS	WHEAT GERM MEAL	SOYA MEAL	FISH MEAL	DRY WHEY	BREWERS YEAST	TOTAL
INGREDIENT	g	150	150	150	150	150	150	15	
AMINO ACID									
arginine*	g	1.05	1.05	2.40	4.80	6.00	0.60	0.33	16.23
glycine	g	0.37	0.97	0.97	3.15	7.50	0.45	0.26	13.67
histidine*	g	1.05	0.45	0.75	1.65	1.95	0.30	0.17	6.32
isoleucine*	g	1.05	0.82	1.80	3.75	4.80	1.35	0.32	13.89
leucine*	g	1.35	1.50	1.65	5.10	7.65	2.10	0.48	19.83
lysine*	g	0.67	0.15	2.40	4.30	10.95	1.65	0.45	20.57
methionine*	g	0.30	0.30	0.45	0.90	3.00	0.30	0.01	5.26
cystine	g	0.37	0.36	0.75	1.00	2.40	0.45	0.08	5.41
phenylalanine*	g	1.05	0.97	1.20	3.30	3.90	0.60	0.27	11.29
tyrosine	g	0.90	1.36	1.36	2.10	3.50	0.45	0.23	9.90
threonine*	g	0.63	0.72	1.20	2.55	3.90	1.20	0.32	10.52
tryptophan*	g	0.27	0.30	0.45	0.90	1.35	0.30	0.08	3.65
valine*	g	0.90	1.02	1.65	3.60	4.80	1.05	0.35	13.37

*Essential amino acid for salmonid fish.

APPENDIX V. Calculated composition of UVIC-73 ration. Vitamins.

		WHEAT	OAT GROATS	WHEAT GERM MEAL	SOYA MEAL	FISH MEAL	DRY WHEY	BREWERS YEAST	VIT D/A	TOTAL
INGREDIENT	g	150	150	150	150	150	150	15	9.6	
vitamin A	i.u.	-	-	-	-	-	-	-	>4000	>4000
vitamin D	i.u.	-	-	-	-	-	-	-	>1000	>1000
vitamin E	i.u.	1.60	3.60	149.05	0.49	0.05	-	-	-	154.79
biotin	mg	0.016	0.033	0.033	0.048	0.063	0.060	0.083	-	0.336
choline	mg	110.0	180.0	451.5	414.0	600.6	265.4	58.3	-	2079.8
cyanocobalamin (B_{12})	mg	-	-	-	-	32.80	0.004	-	-	32.8
folic acid	mg	0.060	0.050	0.030	0.540	0.360	0.135	0.146	-	1.321
niacin	mg	7.63	4.21	7.09	3.24	13.33	1.68	6.71	-	43.89
pantothenic acid	mg	1.87	3.46	1.68	2.17	1.71	10.35	1.65	-	22.89
pyridoxine (B_6)	mg	0.61	0.33	1.95	1.20	0.56	0.37	0.65	-	5.67
riboflavin (B_2)	mg	0.15	0.27	0.76	0.46	1.35	4.48	0.53	-	8.00
thiamin (B_1)	mg	0.92	1.05	4.19	0.36	-	0.56	1.37	-	8.45

APPENDIX VI. The composition by analysis of UVIC-72 (diet 1) and Moore-Clark commercial ration, bag number 1 (diet 2).

Ration:	Diet 1[a]	Diet 2
Dry matter (%)	92.98 ± 0.11*	91.70 ± 0.08
Moisture (%)	7.02 ± 0.03	8.30 ± 0.04
Fat % (w/w)[b]	12.11 ± 0.09	12.47 ± 0.07
Fat % (d/w)[c]	13.04 ± 0.09	13.70 ± 0.18
Protein[+] % (FFDM)[d]	35.29 ± 0.33	67.54 ± 1.13
Protein % (w/w)	28.55 ± 0.32	53.55 ± 0.77

[a] Diet 1 and diet 2 are non-sterile.

[b] (w/w) = wet weight.

[c] (d/w) = dry weight.

[d] (FFDM) = fat free dry matter.

* = standard deviation for three samples of each ration.

+ = %, (N x 6.25).

APPENDIX VII. The composition by analysis of UVIC-73 (diet 1) and Moore-Clark commercial ration, bag number 2 (diet 2).

Ration:	Diet 1-NS[a]	Diet 1-S[b]	Diet 2-NS	Diet-2-S
Dry matter (%)	92.76±0.11	92.94±0.07	89.73±0.43	90.43±0.05*
Moisture (%)	7.24±0.11	7.07±0.07	10.29±0.42	9.58±0.05
Fat % (w/w)	11.89±0.16	11.66±0.53	11.33±0.22	11.28±0.22
Fat % (d/w)	12.82±0.16	12.90±0.16	12.59±0.24	12.47±0.24
Protein % (FFDM)	37.29±0.43		72.57±0.44	
Protein % (w/w)	30.22±0.35		57.33±0.13	

[a] NS = non-sterile.

[b] S = sterilized with ethylene oxide.

* = standard deviation for five samples of each ration.

APPENDIX VIII. Halver's complete test diet for salmonid fish[a].

Complete test diet:

	grams	% (w/w)	% (d/w)
casein	38	12.7	38
gelatin	12	4.0	12
corn oil	7	2.3	7
cod liver oil	2	0.7	2
white dextrin	28	9.3	28
α-cellulose mixture	9	3.0	9
α-cellulose	8		
vitamins	1		
	9		
mineral mix	4	1.3	4
water	200	66.7	
total weight of diet as fed	300		
Protein[b]			49

Vitamin mix:

	mg
Thiamin·HCL	5
Riboflavin	20
Pyridoxine·HCL	5
Choline chloride	500
Nicotinic acid	75
Ca-pantothenate	50
Inositol	200
Biotin	0.5
Folic acid	1.5
L-ascorbic acid	100
Vitamin B_{12}	0.01
Menadione (K)	4
α-tocopherol acetate	40

Mineral mix: mg

USP XII #2 plus

$AlCl_3$	15 mg
$ZnSO_4$	300 mg
CuCl	10 mg
$MnSO_4$	80 mg
KI	15 mg
$CoCl_2$	100 mg

per 100 g of salt mixture.

[a]From Halver (1969). Instructions for preparation of feed are given.

[b]Calculated from tables in Harvey (1956).

APPENDIX IX. Initial wet body weight and fork length data for coho salmon, experiment 2.

Tank:	17		18		19		20		21		22	
	W.W.[a]	F.L.[b]	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	9.9	9.7	9.2	9.6	8.6	9.3	7.8	9.1	8.4	9.2	10.2	9.6
	9.0	9.4	8.8	9.2	8.4	9.2	10.0	9.7	7.9	9.1	11.5	10.1
	11.0	9.8	10.0	9.6	9.6	9.6	11.6	10.1	8.7	9.5	12.1	10.2
	10.7	9.9	10.2	9.8	9.7	9.6	11.3	10.1	9.6	9.6	11.3	10.3
	11.0	10.2	11.9	10.3	11.6	10.1	10.7	10.0	9.3	9.5	7.3	9.0
	9.9	10.0	12.0	10.4	8.9	9.5	9.5	9.4	9.3	9.6	10.9	9.9
	9.0	9.6	11.1	10.1	11.1	10.1	11.3	9.9	10.5	9.8	10.4	10.0
	7.6	9.1	8.5	9.2	9.6	9.6	7.2	8.9	11.7	10.4	9.4	9.5
	8.4	9.3	10.1	9.7	9.4	9.7	9.8	9.8	9.9	9.6	9.9	9.7
	10.1	9.9	10.4	10.0	11.4	10.2	9.1	9.6	9.9	9.8	10.7	9.9
	8.4	9.6	11.0	10.0	13.2	10.6	12.2	10.4	10.7	10.3		
	11.7	10.2	10.4	10.0	9.2	9.6	9.4	9.5	12.0	10.3		
	10.6	9.9	9.8	9.6	11.4	10.1	12.5	10.4	9.9	9.6		
	10.4	9.9	10.5	9.8	10.6	10.0	12.3	10.3	10.8	9.7		
	9.6	9.7	10.0	9.8	9.0	9.5	10.3	9.7	12.4	10.2		
	14.5	10.8	9.9	9.8	9.9	9.7	8.8	9.3				
	10.4	10.0	10.6	10.1	7.9	9.1	9.7	9.6				
	9.1	9.5	8.8	9.4	9.1	9.5	10.1	10.0				
	7.4	9.1	9.8	9.7	9.0	9.4	10.1	9.7				
	9.2	9.6	9.1	9.6	10.3	9.7	12.0	10.3				
	10.0	9.7	11.7	10.1	8.9	9.5	10.7	9.8				
	10.9	9.9	10.9	9.9	11.3	9.8						
	9.7	9.6	8.9	9.4	10.4	9.8						
	10.4	9.9	8.4	9.4	8.8	9.5						
	10.7	9.9	8.5	9.4	10.5	9.6						
	11.1	10.0	10.0	9.8	8.5	9.1						
	13.3	10.6	8.2	9.1								
	8.2	9.2	9.5	9.7								
	8.8	9.5	9.3	9.6								
	9.5	9.4	11.5	10.1								
	9.9	9.4	8.9	9.4								
	8.7	9.5										
	10.5	10.0										
	12.8	10.2										
	10.6	9.8										
	9.2	9.5										
ΣWW (g)	362.2		307.9		256.3		216.4		151.0		103.7	
$\overline{XWW}$ (g)	10.1±1.5g		9.9±1.1		9.9±1.2		10.3±1.4		10.1±1.3		10.4±1.3	
$\overline{XFL}$ (cm)	9.8±0.4cm		9.7±0.3		9.7±0.3		9.8±0.4		9.7±0.4		9.8±0.4	

[a] W.W. = wet weight (g)

[b] F.L. = fork length (cm)

APPENDIX X. Final wet body weight and fork length data for coho salmon, experiment 2

Tank:	17		18		19		20		21		22	
	W.W.[a]	F.L.[b]	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	10.1	9.6	9.8	9.6	11.7	10.0	11.2	9.9	12.5	10.1	11.1	10.0
	10.9	9.7	11.3	10.2	10.3	9.8	12.3	10.4	14.3	10.6	12.6	10.0
	10.8	10.1	10.2	9.5	14.6	10.8	10.9	10.1	10.2	10.0	12.4	10.4
	10.8	10.0	11.8	10.0	10.4	9.9	13.9	10.9	11.8	10.0	11.5	10.4
	10.5	10.0	10.9	10.0	13.2	10.5	12.4	10.1	11.0	9.9	13.6	10.6
	9.7	9.6	12.9	10.4	9.5	9.5	7.8	8.9	12.0	10.5	14.0	10.6
	9.0	9.5	11.4	9.9	11.5	9.8	11.4	10.0	11.3	9.9	13.6	11.0
	11.1	9.6	13.3	10.4	13.7	10.5	12.7	10.3	12.7	10.6	13.5	10.9
	13.6	10.5	11.9	10.0	11.6	9.9	10.6	9.8	13.8	10.7	8.6	9.3
	10.4	9.6	12.9	10.4	10.4	9.6	11.5	10.2	9.4	9.5	10.9	9.7
	13.5	10.5	13.4	10.3	9.9	9.6	10.8	10.0	11.9	10.5		
	12.0	10.0	11.8	10.1	10.3	9.6	13.4	10.6	11.0	10.1		
	12.7	10.3	12.0	10.4	12.5	9.9	13.7	10.6	12.1	10.0		
	12.3	10.3	13.1	10.5	13.6	10.3	11.2	10.0	11.2	10.1		
	12.6	10.2	14.3	10.6	12.8	10.5	12.9	10.6	10.6	9.6		
	13.0	10.4	10.4	9.5	12.1	10.4	9.0	9.5				
	12.7	10.4	10.3	9.8	9.7	9.5	11.1	9.7				
	15.3	11.2	13.3	10.6	10.3	9.7	10.0	9.5				
	12.5	10.1	12.5	9.5	11.9	10.0	13.4	10.1				
	11.6	10.0	12.5	9.8	10.7	9.7	15.0	11.0				
	15.0	10.6	11.8	10.2	9.8	9.7	10.8	10.1				
	15.7	11.0	10.1	9.6	11.2	10.1						
	8.9	9.4	11.1	9.8	11.4	9.8						
	10.0	9.8	12.9	10.2	11.7	10.2						
	10.2	9.9	14.3	10.6	11.1	10.1						
	12.7	10.4	11.4	10.0	13.6	10.2						
	13.3	10.4	11.3	9.9								
	14.5	10.9	11.4	9.9								
	12.9	10.5	11.5	10.0								
	12.2	10.2	10.2	9.9								
	16.7	11.2	10.4	9.8								
	11.3	9.9										
	12.5	10.3										
	11.5	9.9										
	10.7	9.8										
	12.3	10.2										
ΣWW (g)	435.5		366.4		299.5		246.0		175.8		121.8	
$\overline{X}$WW (g)	12.1±1.8		11.8±1.2		11.5±1.4		11.7±1.7		11.7±1.2		12.2±1.6	
$\overline{X}$FL (g)	10.2±0.5		10.0±0.3		10.0±0.3		10.1±0.5		10.1±0.4		10.3±0.5	

[a] W.W. = wet body weight (g)

[b] F.L. = fork length (cm)

APPENDIX XI. Initial wet body weight and fork length data for coho salmon, experiment 3

Tank:	1				2			
	W.W.[a]	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	15.4	11.0	17.4	11.2	19.7	11.3	15.1	11.1
	16.9	11.5	11.1	10.1	18.2	12.0	14.8	11.2
	11.7	10.4	12.2	10.3	19.3	12.0	14.7	10.8
	19.2	11.6	14.1	10.8	16.8	11.7	14.4	10.9
	13.2	10.6	12.6	10.3	16.5	11.7	13.5	10.7
	14.8	10.8	17.0	11.2	15.8	11.3	13.0	10.5
	12.3	10.6	18.0	11.3	16.6	11.7	10.0	9.7
	15.3	11.1	20.0	11.7	16.4	11.5	13.2	10.8
	12.4	10.2	12.3	10.1	18.2	11.8	12.3	10.5
	13.8	10.6	14.0	10.0	15.1	11.4	13.7	10.5
	13.1	10.6	15.8	11.0	15.2	11.1	11.2	10.4
	14.7	11.5	12.2	10.2	16.1	11.5	10.8	10.0
	16.0	11.2	11.6	10.1	13.2	10.5	15.0	10.7
	16.0	11.1	11.5	10.3	12.0	10.5	13.7	10.7
	12.7	10.5	13.0	10.4	11.4	10.2	13.4	10.5
	12.0	10.2	10.7	9.8	14.0	11.0	12.0	10.4
	13.2	10.8	12.3	10.2	11.5	10.0	15.9	11.3
	12.5	10.4	13.1	11.0	11.7	10.0	9.5	9.7
	10.3	9.5	15.6	11.0	9.6	10.0	20.0	11.9
	12.8	10.4	12.7	10.5	9.0	9.5	13.9	10.7
	14.5	10.8	12.8	10.6	13.6	10.9	13.3	10.7
	11.4	10.2	11.3	10.5	13.1	10.5	16.1	11.1
	12.4	10.5	10.0	9.8	12.3	10.2	14.3	10.5
	15.7	10.9	15.0	10.7	10.5	10.0	17.0	11.4
	15.3	10.8	13.2	10.5	12.3	10.0	15.2	10.8
	18.2	11.5	14.1	10.8	11.0	10.0	12.0	10.2
	15.4	10.8	11.9	10.6	11.7	10.0	12.3	10.3
	16.2	11.0	11.6	10.1	9.5	9.8	13.8	10.9
	14.7	10.6	12.4	10.3	10.7	9.8	12.3	10.5
	13.8	10.4	13.5	10.5	15.4	10.9	13.2	10.9
	15.1	10.9	11.7	10.2	15.8	11.4	13.0	10.9
	13.5	10.6	14.5	11.0	13.4	10.9	13.0	10.6
	13.5	10.6	15.2	11.2	16.0	11.0	11.4	9.8
	11.3	10.0	13.0	10.9	11.8	10.5	16.7	11.1
	12.1	10.1	12.8	10.5	12.9	10.4	15.5	11.1
	18.0	11.6	16.1	11.2	12.8	10.4	13.9	11.0
	10.5	9.8	12.6	10.1	12.4	10.2	16.2	11.4
	12.3	10.3	14.4	10.9	13.0	10.5	16.3	11.1

APPENDIX XI. Continued

Tank:	1				2			
	W.W.[a]	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	15.5	10.8	13.3	10.6	12.1	10.1	12.3	10.4
	13.9	10.5	11.3	10.3	15.6	10.9	15.1	11.0
	13.4	10.4	16.7	10.9	11.4	10.5	12.4	10.5
	13.8	10.5	10.0	9.8	16.3	11.0	16.0	11.0
	17.7	11.5	11.8	10.5	14.1	10.8	10.6	9.9
	14.2	10.9	14.9	11.0				
ΣWW (g)	1212.0				1186.1			
$\bar{X}$WW (g)	13.8±2.1				13.8±2.4			
$\bar{X}$FL (g)	10.6±0.5				10.7±0.6			

APPENDIX XII. Final wet body weight and fork length data for coho salmon, experiment 3

Tank:	1				2			
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	13.7	10.9	17.3	11.6	13.3	10.8	17.6	11.5
	15.2	11.4	19.3	12.1	14.2	11.1	13.8	10.5
	17.8	11.5	16.7	11.4	19.8	12.0	13.6	10.6
	17.1	11.4	15.4	11.0	20.1	12.4	14.0	10.7
	15.3	11.3	13.8	11.0	17.8	11.9	18.7	11.7
	18.6	11.8	13.8	10.6	13.8	10.7	15.4	11.1
	14.2	10.8	14.5	11.0	12.7	10.5	17.5	11.8
	15.4	11.1	17.3	11.8	15.8	11.2	18.8	11.8
	17.8	11.3	14.0	10.6	13.7	10.8	12.2	11.4
	20.8	12.2	22.7	12.5	13.9	10.9	12.4	10.5
	16.1	11.0	17.9	11.6	12.7	10.4	13.3	10.4
	15.3	11.1	13.1	10.1	15.6	11.2	15.5	10.9
	19.2	12.2	13.2	10.0	12.9	10.6	15.0	10.9
	15.7	11.4	14.8	11.1	16.4	11.6	23.8	12.8
	15.8	11.3	12.3	10.2	13.8	10.8	17.8	11.7
	15.6	11.2	16.7	11.6	11.6	10.3	20.1	12.6
	12.9	11.0	17.7	12.0	13.2	10.7	17.2	11.6
	14.2	10.6	18.1	11.5	10.4	9.9	15.8	11.2
	14.7	11.0	16.6	11.4	16.6	11.8	13.6	10.6
	12.5	10.3	14.9	11.0	15.2	11.4	16.7	10.1
	17.4	11.5	16.1	11.4	17.4	11.2	13.1	10.5
	14.6	10.9	17.1	11.6	15.8	11.1	18.2	11.8
	19.9	12.4	14.4	10.9	13.2	10.7	14.7	11.2
	19.0	12.0	13.7	10.7	14.5	11.3	13.2	10.8
	15.2	11.3	16.1	11.4	20.2	12.2	14.2	11.1
	17.2	11.5	16.8	11.3	15.8	11.3	12.0	10.4
	14.2	10.5	16.0	11.1	19.2	12.1	18.7	12.2
	17.4	11.6	13.3	10.7	17.6	11.6	16.5	11.2
	16.2	11.4	18.9	11.9	19.2	12.3	12.6	11.4
	14.5	11.0	16.3	11.7	13.8	12.7	16.9	11.1
	15.6	11.1	18.9	12.0	21.9	12.5	16.6	10.9
	15.1	11.0	13.8	11.0	14.5	11.0	23.0	12.1
	13.7	10.8	13.9	10.8	13.1	10.6	19.6	11.8
	13.6	10.9	16.9	11.1	15.1	11.2	16.0	11.1
	13.2	10.5	15.2	11.0	15.4	11.3	15.2	11.0
	13.1	10.6	19.7	12.3	17.5	11.4	17.6	11.8
	12.0	10.0	13.0	10.5	15.5	11.0	15.4	11.1
	14.2	10.9	15.1	11.1	17.4	11.6	14.0	10.8
	16.6	11.4	13.2	10.7	17.8	11.6	15.0	10.9
	14.9	11.0	19.8	12.0	15.2	11.2	11.7	9.9

APPENDIX XII. Continued

Tank:	1				2			
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	16.2	11.2	14.4	10.8	16.8	11.2	14.9	11.1
	15.8	11.4	13.9	10.6	16.3	11.5	11.4	10.0
	18.6	12.1	17.1	11.6	14.2	11.0	13.0	10.5
	20.5	12.0	19.1	11.4				

ΣWW (g)	1399.4	1347.2
$\overline{X}$WW (g)	15.9 ± 2.2	15.6 ± 2.7
$\overline{X}$FL (cm)	11.2 ± 0.6	11.2 ± 0.6

APPENDIX XIII. Carcass composition of coho salmon. Experiments 2 & 3: Tank no. & date of sacrifice

Tank no.	Fish sample no.	Date of sacrifice
representative	17, 18, 19	12-11-72. Start of experiment 2.
stock fed on UVIC-72-S[a] (Diet 1)	20, 21, 22, 23 24, 25, 26	1-12-72. End of experiment 2.
stock fed on UVIC-73-NS[b] (Diet 1)	30, 31, 32, 33	1-12-72. End of experiment 2.
stock fed on Moore-Clark commercial ration-S (Diet 2)	34, 35, 36, 37	1-12-72. End of experiment 2.
19 UVIC-72-S (Diet 1)	39, 40, 41, 42, 43, 44, 45	1-12-72. End of experiment 2
1 & 2 UVIC-72-S, 72NS (Diet 1)	49, 49A, 50, 50A	15-01-73. End of experiment 3.

[a] S = sterile

[b] NS = non-sterile

APPENDIX XIV. Carcass composition of coho salmon. Experiments 2 & 3

Sample no.	Wet weight (g)	Fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat % (w/w)	Fat % (d/w)	Protein* (g)	Protein+ (g)	Protein* % (FFDM)
17	10.26	9.5	7.73	75.34	0.42	4.09	16.60	1.71	1.81	81.04
18	8.58	9.1	6.50	75.36	0.39	4.55	18.75	1.42	1.46	84.02
19	7.78	8.8	5.76	74.04	0.41	5.27	20.30	1.26	1.35	78.26
$\bar{X}$	8.87	9.1	6.66	74.91	0.41	4.64	18.55	1.46	1.54	81.77
20	13.81	10.5	10.29	74.44	0.85	6.15	24.08	2.29		85.45
21	10.93	9.4	8.15	74.57	0.68	6.22	24.46	1.80		85.71
22	9.76	9.4	7.40	75.82	0.45	4.61	19.07	1.63		85.34
23	9.31	9.2	7.12	76.48	0.38	4.08	17.35	1.56		86.19
24	14.21	10.4	10.62	74.03	0.98	6.90	26.56	2.37		87.45
25	11.08	9.8	8.28	74.73	0.67	6.05	23.93	1.84		86.38
26	11.96	9.9	9.08	75.92	0.41	3.43	14.24	2.01		81.38
$\bar{X}$	11.58	9.8	8.71	75.14	0.63	5.35	21.38	1.93		85.41
30	16.58	11.4	12.42	74.91	0.83	5.01	19.95	2.79		83.78
31	13.18	10.4	9.78	74.20	0.77	5.84	22.65	2.18		82.89
32	14.15	10.8	10.35	73.14	0.92	6.50	24.21	2.31		80.21
33	10.61	9.9	7.90	74.46	0.60	5.66	22.14	1.74	1.85	82.46
$\bar{X}$	13.63	10.6	10.1	74.18	0.78	5.75	22.24	2.26		82.34
34	14.61	10.7	11.02	75.43	0.71	4.86	19.78	2.46		85.42
35	15.71	10.8	11.78	74.98	0.94	5.98	23.92	2.64		88.29
36	13.57	10.4	10.43	76.86	0.45	3.32	14.33	2.33		86.61
37	14.41	10.8	10.82	75.09	0.70	4.86	19.50	2.42	2.45	83.73
$\bar{X}$	14.58	10.7	11.01	75.59	0.70	4.76	19.38	2.46		86.01
39	12.44	10.4	9.07	72.91	0.92	7.40	27.30	2.01		82.04
40	9.51	9.3	7.22	75.92	0.44	4.63	19.21	1.59		85.95
41	10.10	10.0	7.63	75.54	0.40	3.96	16.19	1.68		81.16
42	11.13	10.1	8.31	74.66	0.55	4.94	19.50	1.84		81.06
43	9.10	9.0	6.90	75.82	0.45	4.95	20.45	1.51		86.29
44	12.30	9.8	9.17	74.55	0.68	5.52	21.73	2.04		83.27
45	11.76	9.5	8.74	74.32	0.70	5.95	23.18	1.94		83.62
$\bar{X}$	10.91	9.7	8.15	74.82	0.59	5.34	21.09	1.80		83.34

APPENDIX XIV. Continued

Sample no.	Wet weight (g)	Fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat % (w/w)	Fat % (d/w)	Protein* (g)	Protein+ (g)	Protein* % (FFDM)
49	12.88	10.4	9.74	75.62	0.63	4.89	20.06	2.17		86.45
49A	16.37	11.3	12.12	74.04	1.06	6.48	24.94	2.72		85.27
50	12.34	10.3	9.40	76.18	0.56	4.54	19.05	2.09		87.82
50A	12.51	10.4	9.64	77.06	0.48	3.84	16.72	2.14		89.54
$\bar{X}$	13.53	10.6	10.23	75.73	0.68	4.94	20.19	2.28		87.27

*Protein (g) calculated from Groves (1970): $P = 0.204W^{1.038} \pm \begin{matrix} 7.5\% \\ 7.3\% \end{matrix}$

for fish no. 17-33, 39-50A, the $\bar{X}$ water (% w/w) = 74.96±0.50,
the $\bar{X}$ fat (% d/w) = 20.69±1.25,
the $\bar{X}$ protein (% FFDM) = 84.03±2.04.

+Analysed (N x 6.25).

APPENDIX XV. Absorbances for
1-ascorbic acid standard curves

A. Macroprocedure for fish tissues and feeds.
Solvent = 5% HPO_3 - 10% HOAc.

1-ascorbic acid ug/ml	0.5	2	4	8	10	12	15
Absorbance[+]	0.018	0.066	0.132	0.262	0.329	0.391	0.484
540 nm.	0.018	0.068	0.132	0.262	0.324	0.384	0.479
	0.016	0.067	0.131	0.255	0.344	0.406	0.480
	0.016	0.069	0.129	0.260	0.343	0.412	0.474
	0.020	0.076	0.134	0.244	-	-	-
	0.022	0.078	0.129	0.250	-	-	-
	0.019	0.066	0.138	0.257	0.316	0.374	-
	0.020	0.067	0.134	0.254	0.312	0.369	-
	0.017	0.063	0.129	0.258	0.326	0.386	-
	0.016	0.063	0.129	0.250	0.323	0.386	-
Mean absorbance	0.018	0.068	0.132	0.255	0.327	0.389	0.480
K*	0.036	0.034	0.033	0.032	0.033	0.032	0.032
$\overline{K}$	0.033						

B. Macroprocedure for fish blood.
Solvent = 6% TCA.

1-ascorbic acid ug/ml	0.5	1	2	4	6	8	10	12
Absorbance	0.026	0.050	0.090	0.172	0.254	0.330	0.422	0.506
540 nm.	0.025	0.046	0.090	0.174	0.252	0.335	0.422	0.506
	0.022	0.041	0.084	0.177	0.255	0.342	0.424	0.508
	0.021	0.041	0.084	0.180	0.254	0.340	0.425	0.508
	-	-	-	-	-	-	0.420	-
	-	-	-	-	-	-	0.421	-
Mean absorbance	0.024	0.045	0.087	0.176	0.254	0.337	0.422	0.507
K	0.047	0.045	0.044	0.044	0.042	0.042	0.042	0.042
$\overline{K}$	0.044							

APPENDIX XV. Continued

C. Microprocedure for fish blood.
Solvent = 5% TCA.

l-ascorbic acid ug/ml	0.5	1	2	5	8	10	12	16
Absorbance 520 nm.	0.017	0.035	0.062	0.144	0.218	0.257	0.302	-
	0.016	0.031	0.062	0.154	0.237	0.283	0.324	0.434
	0.016	0.039	0.060	0.150	0.228	0.261	0.301	0.410
Mean absorbance	0.016	0.035	0.061	0.149				
K	0.033	0.035	0.031	0.030				
$\overline{K}$	0.032							

For l-ascorbic acid concentrations of 8-16 ug/ml,
Absorbance = 0.0287 + 0.0241 x concentration ug/ml

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*Absorbance = K·C·L·
Where L = 1, $K = \frac{\text{Absorbance}}{C}$

+Slit width = 0.055 mm
Temperature = 23 C.
Pye-Unicam SP 500 Series 2 Spectrophotometer.

APPENDIX XVI. L-Ascorbic acid levels in sterile and non-sterile Diet 1-C*, batch I, fed to coho salmon and rainbow trout in experiments 4 and 5

Sample	Absorbance at 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed
UVIC-73	0.231	6.96	417
(Diet 1-C)	0.231	6.96	417
non-sterile	0.222	6.69	401
	0.228	6.87	412
	0.229	6.90	414
$\bar{X}$[a]			412 ± 5.91
UVIC-73	0.182	5.48	329
(Diet 1-C)	0.190	5.72	343
sterile	0.180	5.42	326
	0.179	5.39	323
	0.195	5.87	352
$\bar{X}$			334 ± 11.1
Diet 1	0.033	0.994	59.6
non-sterile	0.034	1.02	61.4
$\bar{X}$			60.5 ± 0.901
Diet 2	0.032	0.964	57.8
non-sterile	0.035	1.05	63.3
$\bar{X}$			60.6 ± 2.75

[a]Mean ± standard deviation.

*0.50 g samples of feed were extracted with 30 ml of HPO_3-HOAc solvent. Samples were taken at the end of the experimental feed period.

$$\text{mg l-ascorbic acid per kg of diet} = \frac{\text{ug/ml from curve}}{1000\ \text{ug/mg}} \times 30\ \text{ml} \times \frac{1000\ \text{g/kg}}{0.50\ \text{g}}$$

APPENDIX XVII. L-Ascorbic acid levels in Diet 1 and 1-C[a] immediately after pelleting for experiments 7 and 8

Ration Tank No.	Absorbance 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed	% Recovery
Diet 1				
12	0.016	0.482	24.1	
	0.013	0.392	19.6	
	*0.151	4.55		103
14	0.016	0.482	24.1	
	0.015	0.452	22.6	
	*0.154	4.64		
20	0.013	0.391	19.6	
	0.014	0.422	21.1	
	*0.153	4.61		98
22	0.015	0.452	22.6	
	0.014	0.422	21.1	
	*0.153	4.61		105
$\bar{X}$[b]			21.9 ± 1.68	
Diet 1-C				
13	0.240	7.23	361	
	0.208	6.27	313	
			337 ± 24	
15	0.280	8.43	422	
	0.260	7.83	392	
			407 ± 15	
21	0.155	4.67	233	
	0.105	3.16	158	
			196 ± 38	
23	0.100	3.01	151	
	0.114	3.43	172	
			162 ± 11	

[a]Duplicate 0.5 g samples of feed were extracted with 25 ml of HPO_3-HOAc solvent.

[b]Mean ± standard deviation.

*A 2 ml aliquot of a 2 mg/ml l-ascorbic acid solution was added to 0.5 g of sample and made up to 25 ml with HPO_3-HOAc solvent.

APPENDIX XVIII. L-Ascorbic acid levels in Diet 2, and 2-C[a] immediately after pelleting for experiments 7 and 8

Ration	Tank No.	Absorbance 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed	% Recovery*
Diet 2	8	0.018	0.542	27.1	
		0.018	0.542	27.1	
		*0.155	4.67		103
	10	0.015	0.452	22.6	
		0.018	0.542	27.1	
		*0.153	4.61		103
	16	0.022	0.663	33.1	
		0.023	0.693	34.6	
		*0.158	4.76		102
	18	0.020	0.602	30.1	
		0.034	1.02	51.2	
		*0.159	4.79		99
$\bar{X}$[b]				31.6 ± 8.21	
Diet 2-C	9	0.241	7.26	363	
		0.253	7.62	381	
$\bar{X}$				372 ± 9	
	11	0.201	6.05	303	
		0.190	5.72	286	
$\bar{X}$				295 ± 12	
	17	0.090	2.71	136	
		0.125	3.77	188	
$\bar{X}$				162 ± 26	
	19	0.098	2.95	148	
		0.167	5.03	252	
$\bar{X}$				200 ± 52	

a,b,*For an explanation, refer to Appendix XVII.

APPENDIX XIX. L-Ascorbic acid levels in pelleted Diet 1 and 1-C[a] after cooling and drying at 25°C for 12 hr.[b]

Ration	Tank No.	Absorbance 540 nm	ug/ml from curve	mg l-ascorbic acid per kg of feed	Mean
Diet 1	12, 14[c]	0.012	0.361	18.1	
		0.015	0.452	22.6	
		0.031	0.934	46.7	
		0.015	0.452	22.6	
		0.010	0.301	15.1	
		0.014	0.422	21.1	
		0.020	0.602	30.1	
		0.012	0.361	18.1	
		0.010	0.301	15.1	
		0.014	0.422	21.1	23.1 ± 8.90
	20,22	0.013	0.392	19.6	
		0.015	0.452	22.6	
		0.015	0.452	22.6	
		0.012	0.361	18.1	
		0.015	0.452	22.6	21.1 ± 1.91
Diet 1-C	13	0.245	7.38	369	
		0.239	7.20	360	
		0.228	6.87	343	
		0.230	6.93	346	
		0.248	7.45	373	358 ± 12.0
	15	0.261	7.86	393	
		0.246	7.41	370	
		0.260	7.83	392	
		0.265	7.98	399	
		0.269	8.10	405	392 ± 11.9
	21	0.110	3.31	166	
		0.086	2.59	130	
		0.110	3.31	166	
		0.096	2.89	146	
		0.120	3.61	181	158 ± 17.8
	23	0.096	2.89	145	
		0.114	3.43	172	
		0.102	3.07	154	
		0.097	2.92	146	
		0.118	3.55	178	159 ± 13.6

[a] Feed samples (0.5 g) were extracted with 25 ml of HPO_3-HOAc solvent.

[b] Samples were stored at -40°C until submitted to analysis.

[c] Samples pooled for analysis

APPENDIX XX. L-Ascorbic acid levels in pelleted Diet 2 and 2-C[a] after cooling and drying at 25°C for 12 hr.[b]

Ration	Tank No.	Absorbance 540 nm	ug/ml from curve	mg l-ascorbic acid per kg of feed	Mean
Diet 2	16, 18[c]	0.011	0.331	16.6	
		0.010	0.301	15.1	
		0.017	0.512	25.6	
		0.013	0.392	19.6	
		0.018	0.542	27.1	20.8 ± 4.78
Diet 2-C	9	0.168	5.06	253	
		0.184	5.54	277	
		0.235	7.08	354	
		0.194	5.84	292	
		0.196	5.91	295	294 ± 33.8
	11	0.221	6.66	333	
		0.201	6.05	303	
		0.184	5.54	277	
		0.180	5.42	271	
		0.174	5.24	262	289 ± 25.8
	17	0.089	2.68	134	
		0.094	2.83	142	
		0.079	2.38	119	
		0.088	2.65	133	
		0.090	2.71	136	133 ± 7.57
	19	0.080	2.41	121	
		0.081	2.44	122	
		0.084	2.53	127	
		0.081	2.44	122	
		0.078	2.35	127	122 ± 3.19

[a,b,c]For an explanation, refer to Appendix XIX.

APPENDIX XXI. L-Ascorbic acid levels in sterilized[a] Diets 1 and 2

Ration	Tank No.	Absorbance 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed	Mean
Diet 1	12, 20[b]	0.013	0.392	19.6	
		0.012	0.361	18.1	
		0.013	0.392	19.6	
		0.011	0.331	16.6	18.5 ± 1.24
	8, 16	0.009	0.271	15.1	
		0.008	0.241	18.1	
		0.005	0.151	15.1	
		0.010	0.301	19.6	17.0 ± 1.95

[a]Sterilized for 24 hr. with an ethylene oxide (20%) - carbon dioxide (80%) gas mixture.

[b]Samples pooled for analysis.

APPENDIX XXII. L-Ascorbic acid levels in sterilized Diets 1-C and 2-C[a]

Ration	Tank No.	Absorbance 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed	Mean
Diet 1-C	13	0.239	7.20	360	
		0.225	6.78	339	
		0.200	6.02	301	
		0.205	6.17	309	
		0.185	5.57	279	318 ± 28.6
	21	0.110	3.31	166	
		0.079	2.38	119	
		0.085	2.56	128	
		0.091	2.74	137	
		0.088	2.65	133	136 ± 11.6
Diet 2-C	9	0.151	4.55	227	
		0.150	4.52	226	
		0.150	4.52	226	
		0.141	4.25	212	
		0.150	4.52	226	223 ± 5.71
	17	0.035	1.08	54.2	
		0.041	1.23	61.7	
		0.045	1.36	67.8	
		0.045	1.36	67.8	
		0.040	1.20	60.2	62.3 ± 5.12

[a]Refer Appendix XXI.

APPENDIX XXIII. L-Ascorbic acid levels in sterile and non-sterile Diets 1-C and 2-C after storage at 25°C for 7 days

Diet	Tank No.	Absorbance 540 nm	ug/ml from standard curve	mg l-ascorbic acid per kg of feed	Mean
Diet 1-C sterile	21	0.079	2.38	119	
		0.080	2.41	120	
		0.062	1.87	93.4	
		0.076	2.29	114	112 ± 10.8
Diet 1-C non-sterile	23	0.098	2.95	148	
		0.084	2.53	127	
		0.084	2.53	127	
		0.080	2.41	120	131 ± 10.5
Diet 2-C sterile		0.037	1.11	55.7	
		0.032	0.961	48.0	
		0.025	0.753	37.7	
		0.032	0.961	48.0	47.4 ± 6.40
Diet 2-C non-sterile		0.019	0.572	28.6	
		0.025	0.753	37.7	
		0.032	0.961	48.0	
		0.056	1.69	84.3	49.7 ± 21.1

APPENDIX XXIV. Estimates of mean protein, fat, and energy storage of coho salmon from experiment 4. Feeding period, 17-01-73 to 31-01-73

Ration:		Diet 2 S	Diet 1-C NS	Diet 1-C S	Diet 1 NS	Diet 1 S
Tank:		1	2	3	4	5
Group size	Initial	33	35	35	34	35
	Final	33	35	35	34	35
Total wet body weight (g)[a]	Initial	526.1	543.8	554.4	526.5	553.4
	Final	561.1	609.0	612.3	593.0	619.7
Fork length (cm)[b]	Initial	11.2	11.2	11.2	11.3	11.2
	Final	11.6	11.5	11.7	11.7	11.7
Wet body wt (g)[c]	Initial	15.94	15.54	15.84	15.49	15.81
	Final	17.00	17.40	17.49	17.44	17.71
Water (g)[d]	Initial	12.18	11.49	11.71	11.59	11.83
	Final	12.99	12.86	12.93	13.05	13.25
Fat (g)[e]	Initial	0.58	1.01	1.03	0.78	0.80
	Final	0.61	1.14	1.14	0.88	0.90
Protein (g)[f]	Initial	2.72	2.60	2.66	2.67	2.72
	Final	2.91	2.91	2.93	3.01	3.05
Gain - Total wet body wt (g)		35	65.2	57.9	66.5	66.3
- Wt (g)		1.06	1.86	1.65	1.95	1.90
- Protein (g)		0.19	0.31	0.27	0.34	0.33
- Fat (g)		0.03	0.13	0.11	0.10	0.10
- Energy (kcal)[g]		1.16	2.98	2.56	2.87	2.71
Total feed intake (g)		68.5	113.9	114.8	109.2	114.7
Feed intake[h] (g)		2.08	3.25	3.28	3.21	3.28
Protein intake (g)		1.15	0.96	0.96	0.94	0.96
Fat intake (g)		0.25	0.39	0.39	0.38	0.39
Energy intake (kcal)		8.69	14.8	15.0	14.6	15.0
Wt gained g/g feed		0.51	0.57	0.50	0.61	0.58
Protein gain g/g feed protein		0.16	0.32	0.28	0.36	0.34
Fat gain g/g feed fat		0.12	0.33	0.28	0.26	0.26
Energy gain (kcal)/energy consumed (kcal)		0.13	0.20	0.17	0.20	0.18
I.R.G.R.[i] (%/day)		0.45	0.77	0.68	0.77	0.76

[a,b,c] Refer to Appendices XXX and XXXI.

Refer to Appendix XXIX for a description of the other symbols.

APPENDIX XXV. Estimates of mean protein, fat and energy storage of coho salmon from experiment 4. Feeding period 02-02-73 to 17-02-73

Ration:		Diet 2 S	Diet 1-C NS	Diet 1-C S	Diet 1 NS	Diet 1 S
Tank:		1	2	3	4	5
Group size	Initial	32	34	34	33	34
	Final	32	34	34	33	34
Total net body wt (g)[a]	Initial	545.0	593.8	596.7	577.3	603.8
	Final	632.0	682.0	700.5	650.7	679.3
Fork length (cm)[b]	Initial	11.6	11.5	11.7	11.7	11.7
	Final	12.0	12.1	12.4	12.2	12.3
Wet body wt (g)[c]	Initial	17.03	17.46	17.55	17.49	17.76
	Final	19.75	20.06	20.60	19.72	19.98
Water (g)[d]	Initial	13.01	12.91	12.97	13.09	13.29
	Final	15.09	14.83	15.23	14.76	14.95
Fat (g)[e]	Initial	0.62	1.14	1.15	0.88	0.90
	Final	0.71	1.31	1.34	1.00	1.01
Protein (g)[f]	Initial	2.91	2.92	2.94	3.02	3.06
	Final	3.38	3.36	3.45	3.39	3.44
Gain - Total net body wt (g)		87	88.2	103.8	73.4	75.5
- Wt (g)		2.72	2.60	3.05	2.23	2.22
- Protein (g)		0.47	0.44	0.51	0.37	0.38
- Fat (g)		0.09	0.17	0.19	0.12	0.11
- Energy (g)[g]		3.51	4.09	4.68	3.23	3.18
Total feed intake (g)		96.2	133.8	134.6	126.8	136.4
Feed intake[h] (g)		3.00	3.94	3.96	3.84	4.01
Protein intake (g)		1.66	1.16	1.16	1.13	1.18
Fat intake (g)		0.36	0.47	0.48	0.46	0.48
Energy intake (kcal)		14.4	18.0	18.1	17.5	18.3
Wt gain g/g feed		0.91	0.66	0.77	0.58	0.55
Protein gain g/g feed protein		0.28	0.38	0.44	0.33	0.32
Fat gain g/g feed fat		0.25	0.36	0.40	0.26	0.23
Energy gain (kcal)/energy consumed (kcal)		0.24	0.23	0.26	0.18	0.17
I.R.G.R.[i] (%/day)		0.95	0.87	0.98	0.74	0.73

[a,b,c] Refer to Appendices XXXI and XXXII.

APPENDIX XXVI. Estimates of mean protein, fat and energy storage of coho salmon from experiment 4. Feeding period 18-02-73 to 02-03-73

Ration:		Diet 2 S	Diet 1-C NS	Diet 1-C S	Diet 1 NS	Diet 1 S
Tank:		1	2	3	4	5
Group size	Initial	30	32	32	31	32
	Final	26	32	30	24	32
Total wet body weight (g)[a]	Initial	600.8	639.1	668.0	609.6	642.0
	Final	654.3	714.3	734.0	649.4	691.8
Fork length (cm)[b]	Initial	12.1	12.1	12.4	12.2	12.2
	Final	12.6	12.6	12.8	12.5	12.7
Wet body weight (g)[c]	Initial	20.03	19.97	20.91	19.66	20.06
	Final	21.81	22.32	22.94	20.95	21.62
Water (g)[e]	Initial	15.30	14.76	15.46	14.71	15.01
	Final	16.66	16.50	16.96	15.68	16.18
Fat (g)[f]	Initial	0.72	1.30	1.36	0.99	1.01
	Final	0.79	1.46	1.50	1.06	1.09
Protein (g)[g]	Initial	3.43	3.35	3.50	3.39	3.46
	Final	3.73	3.73	3.84	3.61	3.73
Gain - Total net gain (g)		53.5	75.2	65.0	39.8	49.8
- Wt (g)		1.78	2.35	2.03	1.29	1.56
- Protein (g)		0.30	0.38	0.34	0.22	0.27
- Fat (g)		0.07	0.16	0.14	0.07	0.08
- Energy (kcal)[g]		2.36	3.65	3.25	1.91	2.28
Total feed intake (g)		79.5	124.0	126.0	96.9	115.1
Feed intake[h] (g)		2.65	3.88	3.94	3.13	3.60
Protein intake (g)		1.47	1.14	1.16	0.92	1.06
Fat intake (g)		0.32	0.47	0.47	0.38	0.43
Energy intake (kcal)		12.7	17.7	18.0	14.3	16.4
Wt gain g/g feed		0.67	0.61	0.52	0.41	0.43
Protein gain g/g feed protein		0.20	0.33	0.29	0.24	0.25
Fat gain g/g feed fat		0.22	0.34	0.30	0.18	0.19
Energy gain(kcal)/energy consumed (kcal)		0.19	0.21	0.18	0.13	0.14
I.R.G.R.[i] (%/day)		0.66	0.84	0.70	0.49	0.55

[a,b,c]Refer to Appendices XXXII and XXXIII.

APPENDIX XXVII. Estimates of mean protein, fat and energy storage of coho salmon from experiment 4. Feeding period 04-03-73 to 17-03-73

Ration:		Diet 2	Diet 1-C		Diet 1	
		S	NS	S	NS	S
Tank:		1	2	3	4	5
Group size	Initial	26	32	30	24	32
	Final	26	32	30	24	32
Total wet body wt (g)[a]	Initial	572.1	714.3	695.9	522.4	691.8
	Final	631.6	789.6	785.9	564.4	748.9
Fork length (cm)[b]	Initial	12.6	12.6	12.9	12.7	12.7
	Final	13.9	13.0	13.4	13.1	13.1
Wet body wt (g)[c]	Initial	22.00	22.32	23.20	21.77	21.62
	Final	24.29	24.67	26.20	23.52	23.40
Water (g)[d]	Initial	16.81	16.50	17.15	16.29	16.18
	Final	18.56	18.24	19.37	17.60	17.51
Fat (g)[e]	Initial	0.80	1.46	1.51	1.10	1.09
	Final	0.87	1.61	1.71	1.19	1.18
Protein (g)[f]	Initial	3.76	3.73	3.89	3.75	3.73
	Final	4.16	4.13	4.39	4.05	4.03
Gain - Total net gain (g)		59.5	75.3	90.0	42.0	57.1
- Wt (g)		2.29	2.35	3.00	21.75	1.78
- Protein (g)		0.40	0.40	0.50	0.30	0.30
- Fat (g)		0.07	0.15	0.20	0.09	0.09
- Energy (kcal)[g]		2.93	3.68	4.72	2.55	2.55
Total feed intake (g)		77.7	138.1	135.2	89.5	124.5
Feed intake[h] (g)		2.99	4.32	4.51	3.73	3.89
Protein intake (g)		1.66	1.27	1.33	1.10	1.14
Fat intake (g)		0.36	0.52	0.54	0.45	0.47
Energy intake (g)		14.4	19.7	20.6	17.0	17.7
Wt gain g/g feed		0.77	0.54	0.67	0.47	0.46
Protein g/g feed protein		0.24	0.31	0.38	0.27	0.26
Fat g/g feed fat		0.19	0.29	0.37	0.20	0.19
Energy gain (kcal)/energy consumed (kcal)		0.20	0.19	0.23	0.15	0.14
I.R.G.R.[i] (%/day)		0.71	0.73	0.87	0.54	0.57

[a,b,c]Refer to Appendices XXXIII and XXXIV.

APPENDIX XXVIII. Estimates of mean protein, fat and energy storage of coho salmon from experiment 4. Feeding period 18-03-73 to 12-04-73

Ration:		Diet 1-C NS	Diet 1-C S
Tank:		2	3
Group size	Initial	31	27
	Final	29	27
Total wet body wt (g)[a]	Initial	769.6	710.4
	Final	922.5	851.3
Fork length (cm)[b]	Initial	13.0	13.4
	Final	13.8	14.3
Wet body wt (g)[c]	Initial	24.82	26.31
	Final	29.76	31.53
Water (g)[d]	Initial	18.35	19.45
	Final	22.00	23.31
Fat (g)[e]	Initial	1.62	1.72
	Final	1.94	2.06
Protein (g)[f]	Initial	4.15	4.40
	Final	4.99	5.28
Gain - Total weight gain (g)		152.9	140.9
- Wt (g)		4.94	5.22
- Protein (g)		0.84	0.88
- Fat (g)		0.32	0.34
- Energy (kcal)[g]		7.76	8.19
Total feed intake (g)		291.8	280.6
Feed intake[h] (g)		9.41	10.4
Protein intake (g)		2.77	3.06
Fat intake (g)		1.13	1.25
Energy intake (kcal)		42.9	47.4
Wt gain g/g feed		0.52	0.50
Protein gain g/g feed protein		0.30	0.29
Fat gain g/g feed fat		0.28	0.27
Energy gain (kcal)/energy intake (kcal)		0.18	0.17
I.R.G.R.[i] (%/day)		0.71	0.69

APPENDIX XXIX. Footnotes to Appendices XXIV - XXVIII

[d,e,f] Estimates of water, fat and protein made using mean values from coho salmon carcass analysis. Refer to Table 18 and Appendices XXXV and XXXVI.

Fish on Diet 1-C - water (73.92% w/w), fat (25.02% d/w), protein (85.66% FFDM).

Fish on Diet 1 - water (74.84% w/w), fat (20.09% d/w), protein (85.66% FFDM).

Fish on Diet 2 - water (76.39% w/w), fat (15.32% d/w), protein (85.66% FFDM).

[g] Gross energy values were estimated on the basis of 5.67, 9.4 and 4.2 kcal/g for protein, fat and carbohydrate, respectively.

[h] Diet 1 as fed: protein (29.4%), fat (12.0%), energy (4.56 kcal/g).

Diet 2 as fed: protein (55.4%), fat (11.9%), energy (4.81 kcal/g).

[i] I.R.G.R. = instantaneous relative growth rate.

APPENDIX XXX. Coho salmon wet body weight and fork length data for experiment 4. Feed period commencing 17-01-73 to 31-01-73.

Tank:	1		2		3		4		5	
	W.W.[a]	F.L.[b]	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	18.7	12.2	17.4	11.4	12.0	10.0	14.1	11.1	15.2	11.4
	16.5	11.2	15.5	11.0	14.2	10.9	19.7	12.0	17.8	11.5
	12.6	11.4	17.5	11.6	16.6	11.4	20.1	12.4	17.1	11.4
	16.9	11.1	17.7	11.6	14.9	11.0	17.8	11.9	15.3	11.3
	16.7	10.9	15.2	11.2	16.1	11.2	13.8	10.7	18.5	11.8
	22.9	12.1	16.8	11.2	15.8	11.4	12.7	10.5	14.2	10.8
	19.6	11.8	16.3	11.5	18.5	12.1	15.8	11.2	15.4	11.1
	16.0	11.1	14.2	11.0	20.5	12.0	13.7	10.8	17.8	11.3
	15.3	11.0	17.7	11.5	17.3	11.6	13.9	10.9	20.8	12.2
	17.6	11.8	13.8	10.5	19.3	12.1	12.7	10.4	16.1	11.0
	15.4	11.1	11.1	10.0	16.7	11.4	15.5	11.2	15.3	11.1
	14.0	10.8	13.7	10.6	15.4	11.0	12.9	10.6	19.1	12.2
	15.0	10.9	14.0	10.7	13.8	11.0	16.3	11.6	15.7	11.4
	11.7	9.9	18.7	11.7	13.8	10.6	13.7	10.8	15.7	11.3
	14.9	11.1	15.3	11.1	14.5	11.0	11.7	10.3	15.6	11.2
	11.3	10.0	17.5	11.8	17.3	11.8	13.3	10.7	12.9	11.0
	15.9	11.1	18.9	11.8	14.0	10.6	10.5	9.9	14.2	10.6
	13.3	10.7	12.2	11.4	22.7	12.5	16.7	11.8	14.7	11.0
	18.9	11.9	12.4	10.5	17.9	11.6	15.1	11.4	12.5	10.3
	16.3	11.7	13.3	10.4	13.1	10.1	17.3	11.2	17.3	11.5
	18.9	12.0	15.5	10.9	13.2	10.0	15.8	11.1	14.5	10.9
	13.7	11.0	14.9	10.9	14.8	11.1	13.2	10.7	19.9	12.4
	13.9	10.8	23.8	12.8	12.3	10.2	14.5	11.3	19.0	12.0
	16.9	11.1	17.8	11.7	16.7	11.6	20.3	12.2	15.2	11.3
	15.2	11.0	20.1	12.6	17.7	12.0	15.7	11.3	17.2	11.5
	19.7	12.3	17.1	11.6	18.1	11.5	19.1	12.1	14.2	10.5
	12.9	10.5	15.8	11.2	16.6	11.4	17.6	11.6	17.3	11.6
	15.1	11.1	13.5	10.6	14.9	11.0	19.3	12.3	16.1	11.4
	13.1	10.7	10.7	10.1	16.1	11.4	13.7	12.7	14.5	11.0
	19.8	12.0	13.1	10.5	17.1	11.6	21.9	12.5	15.6	11.1
	14.4	10.8	18.3	11.8	14.4	10.9	14.5	11.0	15.1	11.0
	13.9	10.6	14.7	11.2	13.7	10.7	13.1	10.6	13.7	10.8
	19.1	11.4	13.2	10.8	16.1	11.4	15.1	11.2	13.6	10.9
			14.2	11.1	11.6	10.0	15.4	11.3	13.2	10.5
			11.9	10.4	16.7	11.3			13.1	10.6
Σ	526.1		543.8		554.4		526.5		553.4	
$\bar{X}$	15.94	11.2	15.54	11.2	15.84	11.2	15.49	11.3	15.81	11.2

[a] Wet body wt (g)

[b] Fork length (cm)

APPENDIX XXXI. Coho salmon wet body weight and fork length data for experiment 4. Feed period ending 17-02-73 to 31-01-73.

Tank:	1		2		3		4		5	
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	21.1	11.8	14.9	11.0	17.8	11.8	17.9	11.5	17.1	11.5
	17.7	11.5	19.5	12.0	15.3	13.0	20.7	12.6	17.3	11.8
	17.5	11.7	18.6	11.9	20.2	12.6	19.7	11.7	18.9	12.0
	15.1	10.9	28.1	13.3	16.3	11.5	18.1	12.0	15.6	11.1
	20.5	12.6	18.2	11.5	16.5	11.3	14.5	11.0	20.1	11.9
	16.4	11.3	21.9	13.0	23.5	12.5	21.0	12.8	16.7	11.8
	20.3	12.5	17.9	11.0	18.5	11.8	18.1	11.8	16.6	11.6
	14.7	10.8	18.6	11.9	20.1	11.9	22.4	13.1	18.5	11.5
	18.3	12.0	18.9	12.2	15.3	11.5	19.4	12.1	19.1	12.0
	16.3	11.2	18.7	11.5	18.1	11.9	23.5	12.9	16.9	11.8
	14.1	10.9	15.1	10.9	14.9	11.5	16.3	11.3	16.2	11.1
	15.1	11.2	20.2	12.0	13.6	10.5	16.3	11.7	22.8	12.5
	16.6	11.5	14.1	11.0	18.0	12.2	15.9	11.0	17.0	11.7
	16.7	11.6	16.9	11.5	17.2	11.4	13.9	10.9	17.5	11.9
	14.9	11.2	17.1	11.5	17.1	11.8	15.1	11.2	17.6	11.6
	16.9	12.0	21.5	12.2	13.7	10.4	16.0	11.4	17.6	11.6
	13.0	10.5	14.9	11.2	14.3	10.8	18.3	12.0	19.3	11.9
	15.2	11.0	15.1	10.9	17.3	11.5	13.8	10.8	14.7	11.3
	16.1	11.7	12.5	10.4	16.9	11.6	19.7	11.9	13.7	10.8
	13.3	10.8	13.3	10.9	13.5	10.5	21.8	12.5	20.5	12.2
	23.1	12.9	12.7	10.5	17.8	11.3	17.2	11.7	20.9	12.5
	17.7	11.5	19.3	11.6	18.5	11.2	22.7	12.5	23.0	12.5
	20.4	12.1	19.9	12.2	18.1	11.5	18.7	12.0	16.8	11.7
	18.5	12.0	19.9	11.7	19.5	12.6	15.7	11.3	19.8	12.0
	20.2	12.8	19.8	12.2	21.0	12.4	16.7	11.4	17.7	11.6
	17.4	11.6	18.0	11.5	16.4	11.3	14.3	11.0	18.8	12.0
	17.6	11.5	16.1	11.0	19.5	12.4	19.5	12.2	16.7	11.5
	14.7	11.4	20.6	12.0	17.9	11.9	14.1	11.2	16.2	11.3
	13.5	11.0	17.1	11.4	17.9	11.9	16.9	11.6	16.1	11.5
	21.3	12.7	15.4	11.5	18.8	12.0	17.5	11.4	20.9	12.8
	13.8	10.9	14.4	10.9	19.5	12.1	14.8	10.9	15.4	11.2
	*16.1	11.8	16.1	11.5	15.7	11.2	12.0	11.0	14.6	11.0
	17.0	11.4	15.4	11.2	19.1	12.0	14.8	11.0	16.0	11.5
			13.1	10.6	18.9	11.8	*15.7	11.1	17.2	12.0
			*15.2	10.8	*15.6	10.9			*15.9	10.8
Σ	561.1		609.0		612.3		593.0		619.7	
$\bar{X}$	17.00	11.6	17.40	11.5	17.49	11.7	17.44	11.7	17.71	11.7
Σ^{a}_{CORR}	545.0		593.8		596.7		577.3		603.8	
$\bar{X}_{CORR}$	17.03	11.6	17.46	11.5	17.55	11.7	17.49	11.7	17.76	11.7

*Sacrificed for body composition analysis.

[a]Corrected sum and mean.

APPENDIX XXXII. Coho salmon wet body weight and fork length data for experiment 4. Feed period ending 02-02-73 to 17-02-73.

Tank:	1		2		3		4		5	
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	24.3	13.4	21.0	12.8	22.5	12.9	21.9	12.9	16.7	11.5
	20.2	12.0	20.7	12.3	19.7	11.9	21.6	12.6	25.5	12.9
	20.0	12.0	31.1	14.0	21.1	12.5	20.4	12.3	21.3	12.5
	21.3	12.1	21.3	12.2	22.5	13.0	16.1	11.6	23.4	12.6
	25.1	13.1	25.1	13.5	22.3	12.8	16.9	11.8	18.9	12.0
	19.9	12.0	24.3	12.5	28.7	13.6	24.1	13.0	18.5	12.0
	18.1	11.2	20.4	12.3	18.1	11.7	23.3	13.0	23.9	13.0
	24.3	12.4	23.5	12.8	20.6	12.0	17.4	11.6	18.9	12.3
	25.0	13.0	18.7	12.3	20.5	11.9	19.9	12.5	18.3	12.1
	20.7	12.4	23.7	12.8	23.3	13.1	13.7	10.6	21.5	12.6
	18.7	12.0	24.1	12.8	16.8	11.4	16.1	11.2	19.0	12.6
	26.4	13.2	20.0	12.1	20.9	12.9	22.1	12.8	20.1	12.6
	21.9	13.0	23.5	12.7	22.3	12.8	19.5	12.4	21.7	12.6
	18.9	12.0	17.5	11.8	23.7	12.7	16.0	11.4	22.5	12.6
	16.3	11.6	19.6	12.1	20.0	12.4	17.2	11.5	17.5	11.6
	15.4	11.1	16.7	11.0	19.7	12.0	18.8	12.1	17.3	11.4
	16.8	11.4	15.1	11.1	18.7	12.4	20.5	12.1	18.5	11.5
	20.1	11.9	17.1	11.9	15.0	11.3	17.7	11.6	15.1	11.2
	21.2	12.4	17.5	11.8	21.0	12.3	20.5	12.5	19.1	12.5
	17.7	11.7	17.2	11.4	19.7	11.9	25.5	13.8	18.3	12.0
	23.8	13.0	17.1	11.4	19.3	12.5	22.9	12.7	21.5	12.5
	20.6	12.5	23.1	13.0	18.8	12.1	24.7	13.2	19.2	11.8
	20.3	11.8	13.9	10.8	22.3	13.2	16.1	11.6	25.7	13.0
	19.8	12.0	18.3	12.0	20.9	12.3	16.1	11.4	19.3	12.5
	16.1	11.2	16.3	11.3	23.7	13.2	20.3	12.5	19.6	12.3
	17.3	11.5	20.9	12.3	18.3	12.5	24.9	13.3	20.5	12.4
	20.4	12.0	20.5	11.8	26.3	13.0	22.3	12.4	19.1	12.3
	14.4	11.3	15.7	11.4	29.9	12.8	18.2	12.0	21.5	12.5
	18.1	11.7	22.6	12.6	20.9	12.6	19.3	12.1	22.1	12.3
	17.7	12.1	15.1	11.1	20.5	12.6	19.3	12.2	22.5	12.8
	*15.1	10.9	20.5	11.9	20.5	12.5	16.3	11.5	18.3	12.1
	*16.1	11.5	17.0	11.5	10.5	10.1	*22.7	13.2	16.7	11.9
			*20.5	12.0	*15.4	11.8	*18.4	11.8	*19.5	12.2
			*22.4	12.6	*16.1	11.2			*17.8	12.5
Σ	632.0		682.0		700.5		650.7		679.3	
$\overline{X}$	19.75	12.0	20.06	12.1	20.60	12.4	19.72	12.2	19.98	12.3
Σ_{CORR}	600.8		639.1		669.0		609.6		642.0	
$\overline{X}_{CORR}$	20.03	12.1	19.97	12.1	20.91	12.4	19.66	12.2	20.06	12.3

APPENDIX XXXIII. Coho salmon wet body weight and fork length data for experiment 4. Feed period ending 18-02-73 to 02-03-73.

Tank:	1		2		3		4		5	
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	29.3	14.0	28.7	14.0	22.3	13.0	26.1	13.5	23.3	12.8
	27.7	13.8	26.7	13.0	23.7	13.4	23.1	13.2	20.0	12.4
	21.9	12.5	27.9	13.6	23.5	12.4	25.4	13.5	22.8	13.0
	26.6	12.9	23.9	12.9	21.3	12.4	29.3	14.3	20.8	12.8
	19.5	12.5	19.1	11.8	20.1	12.1	23.1	13.2	21.7	12.8
	22.7	12.8	22.2	12.1	22.9	12.9	18.5	12.3	17.7	12.0
	21.7	12.3	23.3	12.7	22.8	12.9	19.4	12.4	21.9	12.8
	21.7	12.5	25.6	13.1	24.3	13.3	25.2	13.2	19.3	12.5
	22.3	12.4	27.2	13.3	25.5	13.6	17.9	12.0	22.1	12.3
	21.9	12.4	23.5	12.7	21.5	12.3	24.9	12.8	20.3	12.6
	19.5	12.0	26.3	13.1	22.5	12.6	20.4	12.6	20.1	12.3
	26.3	13.5	24.8	13.0	21.8	13.0	21.5	12.8	23.7	13.1
	20.8	12.9	23.3	12.9	22.8	12.4	22.9	13.2	21.2	13.0
	22.5	12.5	22.2	12.9	27.1	13.7	18.3	11.6	21.5	12.5
	21.1	12.6	21.1	12.5	22.2	13.0	20.5	12.5	23.7	13.0
	16.9	11.6	17.9	11.6	22.3	12.8	17.3	11.9	22.6	12.7
	19.1	11.8	18.5	11.7	18.9	11.9	15.6	11.2	24.1	13.0
	16.1	11.8	17.4	11.7	16.7	11.2	25.8	13.7	16.3	11.7
	28.5	13.8	23.3	13.2	20.1	12.9	22.0	12.5	20.5	12.8
	21.1	12.6	17.9	11.8	24.9	13.5	23.5	12.8	26.6	13.5
	21.4	12.9	19.2	12.4	27.1	13.6	22.9	13.0	21.4	12.6
	18.5	12.0	15.1	11.0	25.9	13.5	22.1	12.9	25.3	13.5
	24.1	12.9	17.6	11.5	33.2	14.1	16.5	11.6	19.1	11.8
	24.1	13.2	20.3	12.2	29.3	13.4	20.5	12.2	20.5	12.6
	18.9	12.0	19.0	12.2	24.3	13.3	*24.9	13.4	22.5	13.1
	17.9	12.2	19.1	12.0	25.6	12.9	*16.2	11.3	19.7	12.5
	*25.9	13.3	23.8	12.9	23.3	13.3	*20.3	12.6	19.0	12.4
	*21.8	13.0	21.2	12.6	23.1	12.6	*17.0	11.8	27.5	13.5
	*16.6	11.5	19.4	12.1	16.5	11.6	*17.6	12.0	23.1	13.0
	*17.9	12.2	26.6	13.4	20.4	12.6	*16.7	11.3	25.5	13.2
			18.5	11.6	*18.9	11.9	*14.0	11.0	19.6	12.2
			33.7	14.2	*19.2	12.4			18.4	11.8
Σ	654.3		714.3		734.0		649.4		691.8	
$\bar{X}$	21.81	12.6	22.32	12.6	22.94	12.8	20.95	12.5	21.62	12.7
Σ_{CORR}	572.1				695.9		522.4			
$\bar{X}_{CORR}$	22.00	12.6			23.20	12.9	21.77	12.7		

APPENDIX XXXIV. Coho salmon wet body weight and fork length data for experiment 4. Feed period ending 04-03-73 to 17-03-73.

Tank:	1		2		3		4		5	
	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.	W.W.	F.L.
	23.5	12.9	27.3	13.4	28.0	13.8	24.1	12.8	24.3	13.3
	22.9	12.9	25.5	13.3	37.1	14.8	17.2	11.5	23.5	13.3
	20.5	12.0	37.7	14.7	23.9	12.9	25.1	13.6	27.3	13.5
	24.1	13.2	26.2	13.2	30.5	14.2	25.7	13.6	21.5	13.0
	23.1	12.5	34.1	14.5	21.6	12.4	28.3	14.0	29.5	13.9
	23.3	12.8	29.2	13.9	27.6	13.2	32.8	14.6	25.5	13.2
	31.1	14.2	23.0	13.0	25.7	13.2	26.9	13.4	23.7	13.0
	32.8	14.6	22.8	12.6	25.5	13.4	23.1	12.8	23.3	12.9
	30.0	13.4	26.5	13.4	26.1	13.1	22.0	13.0	21.5	13.0
	24.0	12.9	25.7	13.8	25.1	13.5	17.9	12.1	22.9	13.2
	28.7	13.9	19.9	11.9	24.3	12.9	17.1	11.8	23.5	13.4
	24.1	13.0	25.7	13.1	25.1	13.4	19.3	12.0	25.9	13.6
	32.1	14.6	22.1	12.5	25.3	13.2	25.9	13.8	24.2	13.2
	20.9	12.8	20.6	12.2	26.1	13.0	21.8	12.8	23.7	12.9
	25.1	13.3	21.1	12.4	24.9	12.9	22.1	12.8	20.7	12.7
	25.9	13.3	16.5	11.4	27.3	14.1	27.1	13.8	25.9	12.7
	18.5	12.0	19.3	11.9	31.7	13.8	24.7	13.9	21.5	12.8
	19.5	12.6	28.2	13.5	31.3	14.3	19.2	12.4	22.8	13.3
	27.7	13.9	26.7	13.1	27.6	13.8	27.1	13.8	25.3	13.3
	21.2	12.0	32.1	14.5	25.5	13.8	24.3	13.0	29.5	13.9
	22.0	12.7	29.7	13.4	18.6	12.0	19.6	12.4	22.0	13.0
	23.5	13.0	21.5	12.7	31.2	14.5	23.9	13.2	26.2	13.5
	23.0	13.4	19.7	12.1	21.5	12.4	23.9	13.1	21.2	12.5
	18.3	12.0	27.5	13.3	29.5	14.2	25.3	13.4	27.4	13.9
	23.9	13.0	20.5	12.4	23.3	12.7			23.5	13.0
	21.9	13.1	31.6	14.1	27.7	13.8			23.7	13.0
			26.1	13.3	18.4	11.5			20.7	12.8
			20.9	12.1	30.1	14.3			20.1	12.3
			21.3	12.6	22.5	13.0			19.3	12.2
			20.7	12.5	22.9	13.2			17.3	12.0
			19.9	12.0					22.2	13.2
			*20.0	11.7					19.3	12.3
Σ	631.6		789.6		785.9		564.4		748.9	
$\bar{X}$	24.29	13.1	24.67	13.0	26.20	13.4	23.52	13.1	23.40	13.1

APPENDIX XXXV. Coho salmon carcass analysis for experiment 4.

Index to fish number, tank number and date of sacrifice

Tank:	1	2	3	4	5
Diet	Diet 2 S	Diet 1-C NS	Diet 1-C S	Diet 1 NS	Diet 1 S
Date/Fish no.					
01-02-73	51	52	53	54	55
17-02-73	56, 57	58, 59	60, 61	62, 63	64, 65
23-02-73	68		66, 67	69. 70 71, 72	
26-02-73	73, 74 75			76, 77	
17-03-73	78, 79	80	81, 82 83	84, 85 86	87, 88
3-04-73	89			90	
11-04-73	91			92, 93, 94	
12-04-73	29, 46	95, 105 106, 107	102, 103 104	96, 97 98	99, 100 101

APPENDIX XXXVI. Body composition data for coho salmon. Experiment 5

Fish No.	Wet body weight (g)	Fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)	Protein^{+} (g)	Protein (% FFDM)
51	15.71	11.3	12.06	76.77	0.49	3.12	13.42	2.70	85.44
56	14.42	10.6	11.02	76.42	0.60	4.16	17.65	2.46	87.86
57	15.39	11.0	11.86	77.06	0.56	3.64	15.86	2.66	89.56
68	17.28	11.4	12.88	74.54	0.92	5.32	20.91	2.90	83.33
73	24.84	12.8	18.82	75.76	0.90	3.62	14.95	4.29	83.79
74	15.71	11.1	11.78	74.98	0.82	5.22	20.87	2.64	84.89
75	20.63	12.8	16.45	79.74	0.11	0.53	2.63	3.73	91.65
78	20.91	12.9	16.14	77.19	0.40	1.91	8.39	3.66	83.75
79	23.35	12.8	18.16	77.77	0.52	2.23	10.02	4.14	88.65
89	25.33	13.5	18.96	74.85	1.27	5.01	19.93	4.33	84.90
91	19.50	12.6	15.26	78.26	0.57	2.92	13.44	3.45	94.01
29	25.23	13.5	19.13	75.82	1.05	4.16	17.21	4.37	86.53
46	31.05	13.8	22.95	73.91	1.93	6.22	23.83	5.27	85.41
$\bar{X}$				76.39		3.70	15.32		86.91
52	14.47	10.5	10.80	74.64	0.82	5.67	22.34	2.41	84.56
58	21.46	11.9	15.64	72.88	1.70	7.92	29.21	3.55	86.17
59	19.68	12.0	14.11	71.70	1.58	8.03	28.37	3.18	79.70
80	18.99	11.6	13.74	72.35	1.44	7.58	27.43	3.10	81.36
95	23.67	12.8	17.85	75.41	1.17	4.94	20.10	4.06	87.31*
105	22.66	12.3	16.60	73.26				3.77	
106	28.81	13.8	21.13	73.34	2.01	7.13	26.17	4.84	85.36
107	24.13	12.9	17.63	73.06	1.92	7.96	29.54	4.01	87.55
$\bar{X}$				73.32		7.03	26.17		84.57

*Analysed value is 86.35% FFDM.

$^{+}$Protein (g) calculated from Groves (1970):

$$P = 0.204W^{1.038} \pm \begin{matrix} 7.5\% \\ 7.3\% \end{matrix}, \quad \begin{matrix} \text{where } P=\text{protein(g)} \\ \text{where } W=\text{water(g)} \end{matrix}$$

APPENDIX XXXVI. Continued

Fish No.	Wet body weight (g)	Fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)	Protein^{+}(g)	Protein (% FFDM)
53	14.67	10.7	10.78	73.48	1.09	7.43	28.02	2.40	85.71*
60	14.63	11.2	11.39	77.85	0.30	2.05	9.26	2.55	86.73
61	15.32	10.4	11.40	74.41	0.99	6.46	25.26	2.55	87.03
66	18.25	11.7	14.04	76.93	0.76	4.16	18.05	3.17	91.88
67	18.48	11.2	13.43	72.79	1.40	7.58	27.72	3.03	83.01
81	28.98	14.0	21.84	75.36	1.39	4.80	19.47	5.01	87.13
82	21.92	13.1	16.69	76.14	0.91	4.15	17.40	3.79	87.73
83	21.98	12.4	16.06	73.07	1.41	6.41	23.82	3.64	80.71
102	39.18	15.2	28.90	73.76	2.58	6.58	25.10	6.70	87.01
103	33.47	15.0	24.89	74.37	1.81	5.41	21.10	5.74	84.79
104	35.66	14.1	25.46	71.40	3.34	9.37	32.75	5.88	85.71
$\bar{X}$				74.51		5.85	22.54		86.13
54	15.12	10.9	11.12	73.55	1.14	7.54	28.50	2.49	87.06
62	21.66	12.8	15.82	73.04	1.40	6.88	25.51	3.58	82.30
63	17.71	11.8	13.38	75.55	0.80	4.52	18.48	3.01	85.27
69	15.73	11.0	11.68	74.25	0.96	6.10	23.70	2.62	84.79
70	19.60	12.1	14.77	75.36	0.91	4.64	18.84	3.33	84.94
71	23.82	12.9	17.72	74.39	1.20	5.04	19.67	4.03	82.24
72	15.91	11.0	11.77	73.98	0.86	5.41	20.78	2.64	80.49
76	15.73	11.6	12.45	79.15	0.28	1.78	8.54	2.79	93.00
77	16.39	11.2	12.28	74.92	1.06	6.47	25.79	2.75	90.16
84	24.68	13.4	18.33	74.27	1.35	5.47	21.26	4.18	83.60
85	23.11	13.1	17.17	74.30	1.26	5.45	21.21	3.90	83.33
86	24.16	13.2	18.21	75.50	1.15	4.76	19.33	4.14	86.25
90	17.51	11.8	13.32	76.07	0.75	4.28	17.90	3.00	87.21
92	24.89	13.0	18.43	74.05	1.56	6.27	24.15	4.20	85.71
93	26.44	13.4	19.59	74.09	1.56	5.90	22.77	4.48	84.69
94	37.18	14.4	27.92	75.09	1.71	4.60	18.47	6.46	85.56

*Analysed value was 84.97% FFDM.

APPENDIX XXXVI. Continued

Fish No	Wet body weight (g)	Fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)	Protein^{+}(g)	Protein (% FFDM)
96	33.30	14.4	24.70	74.17	1.16	3.48	13.49	5.69	76.48
97	19.46	12.0	14.88	76.46	0.86	4.42	18.78	3.37	90.59
98	29.10	14.0	22.22	76.36	1.07	3.68	15.55	5.11	87.95
$\bar{X}$				74.98		5.09	20.14		85.35
55	15.24	10.5	11.22	73.62	1.07	7.02	26.62	2.51	85.08
64	17.11	11.8	12.86	75.16	0.76	4.44	17.88	2.89	82.81
65	19.12	11.7	14.22	74.37	1.24	6.49	25.31	3.21	87.70
87	19.04	12.3	14.19	74.53	1.10	5.78	22.68	3.20	85.33
88	21.85	12.9	16.27	74.46	1.20	5.49	21.51	3.69	84.25
99	22.84	13.1	17.32	75.83	0.82	3.59	14.86	3.94	83.83
100	23.22	13.4	18.00	77.52	0.68	2.93	13.03	4.10	90.31
101	31.01	14.1	23.19	74.78	1.44	4.64	18.41	5.33	83.54
$\bar{X}$				75.03		5.05	20.04		85.36

Refer to Appendix XXIX for a summary of the mean water, fat and protein values for fish on Diets 1, 1-C, and 2.

APPENDIX XXXVII. Growth data for the rainbow trout of experiment 5

Diet:		Diet 2		Diet 1			Diet 1-C		
		NS	S	NS	S	S	S	S	NS
Tank:		19	12	13	14	15	16	17	18
Period: 15-01-73 to 25-01-73									
Group size -	Initial	69	64	34	31	32	30	33	34
	Final	69	64	34	31	32	30	33	34
Total wet wt (g)	Initial	316	298	152	138	147	145	140	148
	Final	388	370	173	161	171	171	158	170
Feed intake (g)		61	60	43	45	44	45	45	45
Wet gain (g)		72	72	21	23	24	26	18	22
Wt gain g/g feed		1.17	1.21	0.49	0.51	0.54	0.58	0.40	0.49
I.R.G.R. (%/day)		1.86	1.96	1.19	1.38	1.38	1.51	1.11	1.27
Period 25-01-73 to 08-02-73									
Group size -	Initial	69	64	34	31	32	30	33	34
	Final	69	64	34	31	32	30	33	34
Total wet wt (g)	Initial	388	370	173	161	171	171	158	170
	Final	452	431	206	204	210	206	197	219
Feed intake (g)		73.5	73.5	60.3	59.9	59.7	59.6	60.1	60.6
Wet gain (g)		64	61	33	43	39	35	39	49
Wt gain g/g feed		0.87	0.83	0.55	0.72	0.65	0.59	0.65	0.81
I.R.G.R. (%/day)		1.09	1.09	1.25	1.70	1.47	1.33	1.57	1.81
Period 08-02-73 to 22-02-73									
Group size -	Initial	69	64	34	31	32	30	33	34
	Final	69	63	34	29	32	28	32	33
Total wet wt. (g)	Initial	452	431	206	204	210	206	197	219
	Final	529	509	246	236	255	242	239	258
Feed intake (g)		92.0	87.6	62.4	61.2	61.1	61.6	62.7	62.7
Wet gain (g)		77	78	40	32	45	36	42	39
Wt gain g/g feed		0.84	0.89	0.64	0.52	0.74	0.58	0.67	0.62
I.R.G.R. (%/day)		1.12	1.19	1.27	1.04	1.39	1.15	1.38	1.17

APPENDIX XXXVII. Continued

Diet:		Diet 2		Diet 1			Diet 1-C		
		NS	S	NS	S	S	S	S	NS
Tank:		19	12	13	14	15	16	17	18
Period: 22-02-73 to 08-03-73									
Group size -	Initial	69	63	34	29	32	28	32	33
	Final	69	63	34	29	32	28	32	33
Total wet wt (g)	Initial	529	505	246	231	255	237	239	255
	Final	635	590	303	283	307	286	287	314
Feed intake (g)		95.6	88.9	66.5	65.4	68.4	65.1	63.3	68.0
Wet gain (g)		106	85	57	52	52	49	48	59
Wt gain g/g feed		1.11	0.96	0.86	0.80	0.76	0.75	0.76	0.87
I.R.G.R. (%/day)		1.30	1.11	1.48	1.45	1.32	1.33	1.31	1.97
Period: 08-03-73 to 22-03-73									
Group size -	Initial	69	63	34	29	32	28	32	33
	Final	69	63	33	29	31	28	32	33
Total wet wt (g)	Initial	635	590	303	283	307	286	287	314
	Final	715	684	346	338	368	339	326	364
Feed intake (g)		105.5	110.4	74	74.7	74.7	74.7	74.7	74.7
Wet gain (g)		80	94	43	55	61	53	39	50
Wt gain g/g feed		0.76	0.85	0.56	0.74	0.82	0.71	0.52	0.67
I.R.G.R. (%/day)		0.88	1.08	0.97	1.30	1.30	1.22	0.92	1.03

APPENDIX XXXVIII. Tissue weight data for the rainbow trout sacrificed at the termination of experiment 5

Tank	Diet	Wet body wt (g)	Fork length (cm)	Sex	Haematocrit (%)	Pooled intestine weight (g)	Pooled wet liver wt (g)	Pooled wet kidney wt (g)	Pooled blood wt (g)
12	Diet 2	31.41	14.7	M	32	5.39	0.631	0.351	0.550
	S	26.30	13.2	F	33				
13	Diet 1	32.82	13.8	M	42	5.74	0.758	0.461	0.557
	NS	31.21	14.2	F	33				
14	Diet 1	35.54	15.1	F	40	5.15	0.920	0.283	0.468
	S	22.76	12.8	M	48				
15	Diet 1	37.24	14.6	F	42	7.33	0.985	0.363	0.537
	S	28.38	13.8	F	49				
16	Diet								
	1-C	38.32	14.8	F	43	4.94	0.925	0.393	0.430
	S	26.52	13.3	M	42				
17	Diet								
	1-C	24.03	13.0	F	39	6.02	0.868	0.260	0.345
	S	21.54	12.4	F	35				
18	Diet								
	1-C	23.46	12.9	F	35	5.05	0.809	0.365	0.434
	NS	28.70	14.1	F	35				
19	Diet 2	33.37	14.2	F	34	5.38	0.526	0.275	0.562
	NS	24.48	13.5	F	30				

APPENDIX XXXIX. L-Ascorbic acid levels in the blood, kidney and liver of the rainbow trout at the termination of experiment 5.

Tank No	Absorbance 540 nm	ug/ml from standard curve	mg/100 g
(i) whole blood l-ascorbic acid levels[a]			
12	0.042	0.966	0.527
13	0.048	1.10	0.594
14	0.043	0.989	0.634
15	0.028	0.644	0.360
16	0.198	4.55	3.18
17	0.212	4.87	4.24
18	0.294	6.76	4.67
19	0.052	1.20	0.638
(ii) wet kidney l-ascorbic acid levels[b]			
12	0.067	2.02	5.75
13	0.048	1.45	3.14
14	0.056	1.69	5.96
15	0.046	1.39	3.82
16	0.310	9.34	23.8
17	0.250	7.53	29.0
18	0.292	8.80	24.1
19	0.043	1.30	4.71
(iii) wet liver l-ascorbic acid levels[b]			
12	0.070	2.11	3.34
13	0.066	1.99	2.62
14	0.065	1.96	2.13
15	0.078	2.35	2.39
16	0.510	15.4	16.6
17	0.482	14.5	16.7
18	0.450	13.6	16.8
19	0.074	2.23	4.24

[a]Blood added to tared tubes containing 3 ml 6% TCA.

[b]Tissue added to tared tubes containing 10 ml HPO_3-HOAc solvent. Wt of tissues are given in Appendix XXXVIII.

APPENDIX XL. Growth data for the rainbow trout of experiment 6 from 27-05-73 to 29-07-73

Diet 2 Ration:		NS	S	S	NS	NS	S
Tank:		1	2	3	4	5	6
Group size -	Initial	32	36	35	37	31	25
	Final	31	36	33	36	31	24
Total wet wt (g)	Initial	818	349	682	607	351	987
	Final*	1385	581	1099	1002	543	1739
Wt gain (g)		585	232	454	417	192	780
Feed intake (g)		877	314	691	623	295	1157
Gain g/g feed		0.67	0.74	0.66	0.67	0.65	0.67
I.R.G.R. (%/day)		0.82	0.79	0.84	0.78	0.68	0.74

*corrected for losses.

APPENDIX XLI. Tissue weight data for the rainbow trout sacrificed at the termination of experiment 6

Tank	Fish No.	Wet body wt (g)	Fork length (cm)	Sex	Haematocrit (%)	Pooled wet wt of intestines (g)
1	1.	33.65	14.8	F	15	
	2.	51.45	16.4	M	36	
						Tanks 1,4,5: 10.80, 11.22
2	1.	28.70	13.4	F	32	
	2.	24.25	13.8	F	17	
						Tanks 2,3,6: 13.31, 6.75
3	1.	55.80	17.0	M	22	
	2.	28.25	14.1	M	34	
4	1.	44.70	16.0	M	34	
	2.	32.15	15.1	M	19	
5	1.	34.20	14.2	M	22	
	2.	23.75	13.6	F	20	
6	1.	79.45	19.0	F	18	

APPENDIX XLII. L-Ascorbic acid level in the blood[a] of rainbow trout at the termination of experiment 6.

Tank	Fish No.	Absorbance 520 nm	ug/100 ml	Mean
1	1	0.040	623	
		0.036	561	678 ± 89.2
	2	0.048	748	
		0.050	779	
2	1	0.075	1168	
		0.068	1059	1098 ± 41.9
	2	0.069	1075	
		0.070	1090	
3	1	0.066	1028	
		0.061	950	993 ± 29.9
	2	0.065	1012	
		0.063	981	
4	1	0.046	717	
		0.050	779	740 ± 25.7
	2	0.046	717	
		0.048	748	
5	1	0.061	950	
		0.056	872	861 ± 61.6
	2	0.054	841	
		0.050	779	
6	1	0.060	935	

[a] 50 ul blood samples

APPENDIX XLIII. Tissue weight data for initial chum salmon analysis, experiment 7

Tank	Fish No.	Wet body wt (g)	Fork length (cm)	Haem-atocrit (%)	Pooled Blood (g)	Pooled Liver (g)	Pooled Kidney (g)
16	1	9.52*	10.1	38			
	2	10.01	10.4	40	0.55	0.19	0.21
	3	8.88	10.1	44			
	4	8.29	9.9	38			
				$\bar{X}$[a] = 40 ± 2			
17	1	8.07	9.7	40			
	2	7.36	9.6	28	0.54	0.44	0.23
	3	9.68*	10.5	40			
	4	9.14	10.3	35			
				$\bar{X}$ = 36 ± 5			
18	1	8.31	9.4	17			
	2	6.97	9.1	42	0.45	0.19	0.12
	3	7.15	9.2	42			
	4	5.62	8.5	43			
				$\bar{X}$ = 36 ± 11			
19	1	5.95	8.6	45			
	2	10.37	10.9	10	0.44	0.41	0.32
	3	10.59	10.6	36			
	4	8.94	10.0	31			
				$\bar{X}$ = 31 ± 13			
20	1	6.97	9.1	36			
	2	7.99*	9.6	42	0.35	0.45	0.27
	3	8.15	9.5	34			
	4	5.67	9.1	33			
				$\bar{X}$ = 36 ± 3			
21	1	8.07	9.9	37			
	2	9.11	10.3	34	0.43	0.45	0.22
	3	7.31	9.1	33			
	4	7.83*	10.1	16			
				$\bar{X}$ = 30 ± 8			
22	1	12.28	10.8	41			
	2	9.31*	9.5	38	0.55	0.16	0.22
	3	9.22	9.9	34			
	4	6.95	9.6	36			
				$\bar{X}$ = 37 ± 3			
23	1	7.72	9.9	42			
	2	7.92*	9.4	29	0.48	0.52	0.28
	3	6.59	9.0	43			
	4	9.36	10.0	43			
				$\bar{X}$ = 39 ± 6			

*Fish used for body composition analysis.

[a]Mean haematocrit ± standard deviation.

APPENDIX XLIV. Initial body composition data for chum salmon, experiment 7

Fish No.[a]	Wet Body Wt (g)	Mean Fork Length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)
16.1 17.3	19.20	10.3	13.82	71.98	0.27	1.41	5.02
20.2 21.4	15.82	9.9	11.64	73.58	0.28	1.77	6.70
22.2 23.2	17.23	9.5	12.86	74.64	0.27	1.57	6.18

[a]Refer to Appendix XLIII.

APPENDIX XLV. Final body composition data for chum salmon, experiment 7

Tank & Fish No.	Total Wet Body Wt (g)	Mean Fork Length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)
16 A[a]	24.41	11.3	20.12	82.43	0.19	0.78	4.42
B	25.48	9.9	22.74	89.25	0.03	0.12	1.09
17 A	62.08	15.1	47.42	76.39	2.62	4.22	17.87
B	32.77	12.3	26.07	79.56	0.89	2.71	13.28
18 A	11.94	9.8	10.13	84.84	0.01	0.08	0.55
19 A	54.37	14.2	41.62	76.55	2.32	4.27	17.91
B	45.24	13.0	35.11	77.61	1.78	3.93	17.57
20 A	45.41	13.2	34.34	75.62	2.77	6.10	25.02
B	42.85	12.0	33.53	78.25	1.73	4.04	18.56
21 A	20.68	13.4	15.69	75.87	1.21	5.85	24.25
B	39.28	12.3	29.86	76.02	2.35	5.98	24.95
22 A	23.99	11.4	19.22	80.12	0.50	2.08	10.48
B	18.43	10.4	15.05	81.62	0.21	1.14	6.21
23 A	49.48	13.6	36.63	74.03	3.59	7.26	27.94
B	43.42	12.9	32.97	75.93	2.59	5.96	24.78

[a]Each sample represents 2 pooled fish.

APPENDIX XLVI. L-Ascorbic acid levels in the blood, liver and kidney of chum salmon at the start of experiment 7.

Tank	Absorbance 540 nm	ug/ml from standard curve	mg/100g
(i) Blood[a]			
16	0.015	0.345	0.125
17	0.017	0.391	0.145
18	0.018	0.414	0.184
19	0.023	0.529	0.240
20	0.018	0.414	0.236
21	0.052	1.20	0.556
22	0.027	0.621	0.226
23	0.044	1.01	0.421
(ii) Liver[b]			
16	0.007	0.211	0.444
17	0.051	1.54	1.40
18	0.013	0.392	0.824
19	0.037	1.11	1.09
20	0.006	0.181	0.161
21	0.085	2.56	2.28
22	0.004	0.120	0.301
23	0.130	3.92	3.01
(iii) Kidney[b],			
16	0.010	0.301	0.574
17	0.029	0.873	1.52
18	0.015	0.452	1.51
19	0.024	0.723	0.904
20	0.025	0.753	1.12
21	0.080	2.41	4.38
22	0.024	0.723	1.31
23	0.085	2.56	3.66

[a]Whole blood added to tared tubes containing 2.0 ml 6% TCA.

[b]Tissue samples frozen in N_2(l) and stored for 48 hr. at -20C then extracted with 4 ml HPO_3- ^{2}HOAc solvent. For tissue and blood weights refer to Appendix XLIII.

APPENDIX XLVII. Tissue weight data for final chum analysis, experiment 7

Tank	Fish No	Wet body wt (g)	Fork length (cm)	Haematocrit (%)	Pooled wet liver wt (g)	Pooled wet kidney wt (g)
16N[a]	1	19.95	12.3	39		
	2	23.30	13.6	49	1.11	0.74
	3	31.45	15.0	38		
	4	33.70	14.6	50		
				$\bar{X}$[c] = 44 ± 6		
16D[b]	5	11.35	11.6			
	6	19.35	12.5	38	0.47	0.46
	7	9.00	10.6	28		
	8	12.50	10.8	39		
				$\bar{X}$ = 35 ± 5		
17	1	22.80	12.4	47		
	2	29.50	15.0	36	1.10	0.96
	3	18.65	12.0	43		
	4	26.30	14.5	39		
				$\bar{X}$ = 41 ± 4		
18N	1	10.05	9.9	28		
	2	14.50	11.4	20	0.36	0.39
	3	7.15	9.2	20		
	4	7.70	9.2	26		
				$\bar{X}$ = 24 ± 4		
18D	5	17.40	11.8	48		
	6	12.22	12.0	14	0.54	0.56
	7	11.27	11.5	17		
	8	11.07	10.9	29		
				$\bar{X}$ = 27 ± 13		
19	1	24.25	13.3	38		
	2	17.95	11.1	47	0.96	0.79
	3	26.80	13.2	41		
	4	22.75	13.4	44		
				$\bar{X}$ = 43 ± 3		
20N	1	18.60	12.8	37		
	2	29.55	14.2	40	1.18	0.64
	3	18.40	12.6	38		
	4	17.65	12.3	39		
				$\bar{X}$ = 39 ± 1		
20D	5	12.00	11.7	36		
	6	19.00	12.6	42	0.69	0.49
	7	11.95	10.9	34		
	8	13.00	11.5	37		
				$\bar{X}$ = 37 ± 3		

APPENDIX XLVII. Continued

Tank	Fish No	Wet body wt (g)	Fork length (cm)	Haematocrit (%)	Pooled wet liver wt (g)	Pooled wet kidney wt (g)
21	1	23.25	13.8	36		
	2	31.00	14.9	37	1.69	0.86
	3	26.70	14.0	44		
	4	27.35	14.5	40		
				$\bar{X}$ = 39 ± 3		
22N	1	23.70	13.8	41		
	2	27.55	14.6	40		
	3	24.40	13.6	42	1.12	0.63
	4	17.85	12.1	33		
				$\bar{X}$ = 39 ± 4		
22D	5	12.40	11.4	40		
	6	12.35	10.4	44	0.46	0.31
	7	8.05	10.3	42		
	8	9.00	10.2	37		
				$\bar{X}$ = 41 ± 3		
23	1	30.40	14.4	40		
	2	19.75	12.7	38	1.26	0.76
	3	22.10	13.0	40		
	4	24.35	14.2	42		
				$\bar{X}$ = 40 ± 1		

[a]Normal light pigmented fish similar to control fish pigmentation.

[b]Dark pigmented fish.

[c]Mean haematocrit ± standard deviation.

APPENDIX XLVIII. L-Ascorbic acid level in the blood of chum salmon at the termination of experiment 7.

Tank	Fish No	ABS @ 520 nm	ug/ml from standard curve	mg/100 ml whole blood	
16	1*	0.070	2.18	1.09	
		0.064	1.99	0.997	
	2	0.075	2.34	1.17	
		0.070	2.18	1.09	
	3	0.076	2.37	1.18	
		0.095	2.96	1.48	
	4	0.050	1.56	0.779	
		0.060	1.87	0.935	
	5	0.054	1.68	0.841	
		0.069	2.15	1.07	
	6	0.060	1.87	0.935	
		0.065	2.02	1.01	
	7	0.067	2.09	1.04	
		0.059	1.84	0.919	
	8	0.056	1.74	0.872	
		0.059	1.84	0.919	
					$\bar{X}$ = 1.02 ± 0.162
17	1	0.114	3.55	1.78	
		0.110	3.43	1.71	
	2	0.101	3.15	1.57	
		0.100	3.12	1.56	
	3	0.086	2.68	1.34	
		0.105	3.27	1.64	
	4	0.090	2.80	1.40	
		0.099	3.08	1.54	
					$\bar{X}$ = 1.57 ± 0.137
18	1	0.075	2.34	1.17	
		0.081	2.52	1.26	
	2	0.080	2.49	1.25	
		0.076	2.37	1.18	
	3	0.085	2.65	1.32	
		0.085	2.65	1.32	
	4	0.060	1.87	0.935	
		0.073	2.27	1.14	
	5	0.072	2.24	1.12	
		0.082	2.55	1.28	
					$\bar{X}$ = 1.20 ± 0.110

*Duplicate 50 ul samples.

APPENDIX XLVIII. Continued

Tank	Fish No	ABS @ 540 nm	ug/ml from standard curve	mg/100 ml whole blood	
19	1	0.092	2.87	1.43	
		0.081	2.52	1.26	
	2	0.120	3.74	1.87	
		-	-	-	
	3	0.096	2.99	1.50	
		0.096	2.99	1.50	
	4	0.092	2.87	1.43	
		0.095	2.96	1.48	
					$\overline{X}$ = 1.50 ± 0.171
20	1	0.065	2.02	1.01	
		0.065	2.02	1.01	
	2	0.068	2.12	1.06	
		0.054	1.68	0.841	
	3	0.062	1.93	0.966	
		0.060	1.87	0.934	
	4	0.046	1.43	0.717	
		0.053	1.65	0.826	
	5	0.041	1.28	0.639	
		0.045	1.40	0.701	
	6	0.066	2.06	1.03	
		0.059	1.84	0.919	
	7	0.065	2.02	1.01	
		0.068	2.12	1.06	
	8	0.059	1.84	0.919	
		0.063	1.96	0.981	
					$\overline{X}$ = 0.914 ± 0.129
21	1	0.193	6.82	3.41	
		0.180	6.28	3.14	
	2	0.195	6.90	3.45	
		0.181	6.32	3.16	
	3	0.188	6.61	3.30	
		0.192	6.78	3.39	
	4	0.184	6.44	3.22	
		0.180	6.28	3.14	
					$\overline{X}$ = 3.28 ± 0.122
22	1	0.056	1.74	0.872	
		0.050	1.56	0.779	
	2	0.055	1.71	0.857	
		0.046	1.43	0.717	
	3	0.047	1.46	0.732	
		0.055	1.71	0.857	
	4	0.056	1.74	0.872	
		0.058	1.81	0.903	
	5	0.052	1.62	0.810	
		0.056	1.74	0.872	
	6	0.051	1.59	0.794	
		0.055	1.71	0.857	

APPENDIX XLVIII. Continued

Tank	Fish No	ABS @ 540 nm	ug/ml from standard curve	mg/100 ml whole blood	
	7	0.057	1.78	0.888	
		0.060	1.87	0.935	
	8	0.054	1.68	0.841	
		0.057	1.78	0.888	
					$\bar{X}$ = 0.842 ± 0.0583
23	1	0.190	6.69	3.35	
		0.181	6.32	3.16	
	2	0.188	6.61	3.30	
		0.188	6.61	3.30	
	3	0.182	6.36	3.18	
		0.194	6.86	3.43	
	4	0.200	7.11	3.55	
		0.192	6.78	3.39	
					$\bar{X}$ = 3.33 ± 0.120

APPENDIX XLIX. L-Ascorbic acid level in the liver and kidney of chum salmon at the termination of experiment 7

Tank		Liver[a] absorbance 540 nm	ug/ml from standard curve	mg/100 g	Kidney[a] Absorbance 540 nm	ug/ml from standard curve	mg/ 100 g
16	N[c]	0.024*	0.723	0.651	0.072	2.25	1.52
	D[b]	0.005	0.151	0.166	0.015	0.452	0.491
17		0.095*	2.86	2.60	0.403	12.1	6.32
18	N	0.030	0.904	1.26	0.030	0.904	1.16
	D	0.002	0.060	0.056	0.028	0.843	0.753
19		0.118*	3.55	3.70	0.434	13.1	8.27
20	N	0.015*	0.452	0.383	0.047	1.42	1.11
	D	0.021	0.633	0.458	0.024	0.723	0.74
21		0.451*	13.6	8.04	0.421*	12.7	14.8
22	N	0.031*	0.934	0.834	0.041	1.23	0.980
	D	0.006	0.181	0.196	0.018	0.542	0.874
23		0.360*	10.8	8.61	0.403*	12.1	15.8

[a]For tissue weights see Appendix XLVII.

[b]Liver and kidney samples from dark pigmented fish.

[c]Liver and kidney samples from light pigmented fish.

*Tissues homogenized in 10 ml of HPO_3 - HOAc solvent; all others in 5 ml.

APPENDIX L. Initial and final body composition data for kokanee salmon, experiment 8

Tank	Wet body wt (g)	Mean fork length (cm)	Water (g)	Water (%)	Fat (g)	Fat (% w/w)	Fat (% d/w)
Initial[a]:							
8,9	17.05	5.4	13.14	77.07	0.98	5.75	25.06
10,11	11.91	5.2	9.50	79.76	0.46	3.86	19.09
12,13	13.33	5.3	10.57	79.29	0.51	3.83	18.48
14,15	15.66	5.4	11.95	76.31	0.98	6.26	26.42
Final[b]:							
8	9.49	6.7	7.32	77.13	0.47	4.95	21.66
9	15.21	8.1	10.32	67.67	1.34	8.79	27.18
10	6.67	6.2	5.23	78.41	0.28	4.20	19.44
11	19.59	7.7	14.51	74.07	1.56	7.96	30.71
12	18.68	7.7	13.60	72.81	1.77	9.48	34.84
13	17.29	7.4	12.71	73.51	1.69	9.78	36.90
14	12.76	6.9	9.34	73.12	1.15	9.01	33.63
15	16.52	7.4	12.04	72.88	1.55	9.38	34.60

[a]10 fish pooled.

[b]4 fish pooled.

APPENDIX LI. Initial l-ascorbic acid level in the blood of kokanee salmon, experiment 8[a]

Tank	Absorbance 520 nm	ug/ml from standard curve	mg/100 ml whole blood
8	0.127	3.96	1.98
9	0.128	3.99	1.99
10	0.126	3.93	1.96
11	0.126	3.93	1.96
12	0.130	4.05	2.02
13	0.150	4.67	2.34
14	0.136	4.24	2.12
15	0.152	4.74	2.34

[a]Analysis conducted on 5 pooled 10 ul blood samples from 5 fish per tank.

APPENDIX LII. Tissue weight data for final kokanee salmon analysis, experiment 8

Tank	Fish No	Wet body wt (g)	Fork length (cm)	Haematocrit	Pooled net liver wt (g)	Pooled net kidney wt (g)	Pooled net gill wt (g)
8	1	5.06	8.1	37			
	2	3.78	7.6	-[a]	0.15	0.05	0.24
	3	4.52	7.4	-			
	4	4.11	7.3	-			
	5	3.14	6.5	-			
9	1	7.85	8.8	39			
	2	6.88	8.4	36			
	3	7.93	8.5	37	0.39	0.15	0.40
	4	7.90	8.9	34			
	5	7.66	8.6	35			
				$\bar{X}^a = 36 \pm 2$			
10	1	6.22	8.3	36			
	2	4.40	7.6	32	0.11	0.04	0.29
	3	3.68	7.2	-			
	4	3.45	7.2	-			
	5	2.68	6.4	-			
11	1	6.22	8.2	38			
	2	7.43	8.3	49	0.32	0.12	0.41
	3	7.32	8.8	44			
	4	5.34	6.1	40			
	5	5.76	8.2	39			
				$\bar{X} = 42 \pm 4$			
12	1	5.65	8.0	43			
	2	6.04	8.4	38	0.47	0.09	0.46
	3	5.80	8.0	37			
	4	5.89	8.1	36			
	5	5.91	8.3	38			
				$\bar{X} = 38 \pm 2$			
13	1	8.70	9.6	44			
	2	7.18	8.6	40	0.35	0.10	0.49
	3	6.61	8.5	38			
	4	5.89	8.3	35			
	5	6.13	8.2	36			
				$\bar{X} = 39 \pm 3$			
14	1	5.22	7.6	40			
	2	8.83	9.1	40	0.45	0.14	0.52
	3	7.13	8.5	40			
	4	8.21	9.0	36			
	5	5.83	8.0	-			
				$\bar{X} = 39 \pm 2$			

[a]Mean haemocrit ± standard deviation.

APPENDIX LII. Continued

Tank	Fish No	Wet body wt (g)	Fork length (cm)	Haematocrit	Pooled net liver wt (g)	Pooled net kidney wt (g)	Pooled net gill wt (g)
15	1	7.22	8.4	39			
	2	6.08	7.8	42	0.32	0.10	0.42
	3	5.40	7.9	35			
	4	4.43	7.4	37			
	5	5.46	8.2	-			
				$\bar{X}$ = 38 ± 3			

APPENDIX LIII. L-Ascorbic acid level in the blood of kokanee salmon at the termination of experiment 8

KOKANEE BLOOD

Tank	Fish No	ABS @ 520 nm	mg/ml from curve	mg/100 gm whole blood	Mean
8	1	0.074	2.31	1.15	
	2	0.070	2.18	1.09	
	3	0.050	1.56	0.779	
	4	0.062	1.93	0.966	
	5	0.069	2.15	1.07	
					1.02 ± 0.130*
9	1	0.164	5.61	2.81	
	2	0.150	4.67	2.34	
	3	0.124	3.86	1.93	
	4	0.132	4.11	2.06	
	5	0.134	4.17	2.09	
					2.25 ± 0.312
10	1	0.059	1.84	0.919	
	2	0.049	1.53	0.763	
	3	0.053	1.65	0.826	
	4	0.058	1.81	0.903	
	5	-	-	-	
					0.853 ± 0.0632*
11	1	0.169	5.82	2.91	
	2	0.148	4.61	2.31	
	3	0.135	4.21	2.10	
	4	0.130	4.05	2.02	
	5	0.141	4.39	2.20	
					2.31 ± 0.316
12	1	0.056	1.74	0.872	
	2	0.049	1.53	0.763	
	3	0.046	1.43	0.717	
	4	0.055	1.71	0.857	
	5	0.048	1.50	0.748	
					0.791 ± 0.0608*
13	1	0.140	4.36	2.18	
	2	0.163	5.57	2.79	
	3	0.184	6.44	3.22	
	4	0.179	6.24	3.12	
	5	0.133	4.14	2.07	
					2.68 ± 0.473*
14	1	0.104	3.24	1.62	
	2	0.107	3.33	1.67	
	3	0.103	3.21	1.60	
	4	0.090	2.80	1.40	
	5	0.076	2.37	1.18	
					1.49 ± 0.182*

APPENDIX LIII. Continued

Tank	Fish No	ABS @ 520 nm	mg/ml from curve	mg/100 gm whole blood	Mean
15	1	0.193	6.82	3.41	
	2	0.198	7.02	3.51	
	3	0.196	6.94	3.47	
	4	0.182	6.36	3.18	
	5	0.190	6.69	3.35	
					3.38 ± 0.116*

APPENDIX LIV. L-Ascorbic acid level in the liver, kidney, and gill tissue[a] of kokanee salmon at the termination of experiment 8

Tank No	(Liver) ABS @ 540	mg/ml curve	mg/100g	(Kidney) ABS @ 540	mg/ml curve	mg/100g
8	0.010	0.301	0.803	0.046	1.39	11.1
9	0.398	12.0	12.3	0.156	4.70	12.5
10	0.015	0.452	1.64	0.030	0.904	9.04
11	0.280	8.43	10.5	0.172	5.18	17.3
12	0.005	0.151	0.128	0.031	0.934	4.15
13	0.425	12.8	14.63	0.173	5.4	20.8
14	0.240	7.23	6.43	0.136	4.10	11.7
15	0.443	13.3	16.7	0.231	6.96	27.8

	(Gill) ABS @ 540	mg/ml curve	mg/100g
8	0.045	1.36	2.26
9	0.431	13.0	13.0
10	0.065	1.96	2.70
11	0.405	12.2	11.9
12	0.050	1.51	1.31
13	0.573	17.3	14.1
14	0.370	11.1	8.57
15	0.540	16.3	15.5

[a]Tissues extracted with 4 ul of HPO_3/HOAc solvent.

APPENDIX LV. Moisture and fat content of rainbow trout eggs

Fish No.	Sample No.[a]	Water (%)	Fat (% d/w)	Mean egg FFDM (% d/w)
1	1	58.43	11.31	
	2	58.54	9.58	
	3	58.10	11.11	
	4	59.57	12.07	
		$\bar{X}$ 58.56±0.60	$\bar{X}$ 11.02±0.90	88.98
3	6	57.60	13.36	
	7	57.25	10.03	
	8	58.95	11.24	
	9	61.99	-	
	10	61.70	-	
		$\bar{X}$ 59.50±2.00	$\bar{X}$ 11.54±1.38	88.46
4	11	57.11	10.76	
	12	57.51	10.39	
	13	58.76	10.07	
	14	57.26	-	
		$\bar{X}$ 57.66±0.65	$\bar{X}$ 10.41±0.28	89.59
6	16	59.21	8.94	
	17	59.13	9.17	
	18	58.98	8.99	
	19	59.73	9.00	
	20	59.67	8.67	
		$\bar{X}$ 59.35±0.30	$\bar{X}$ 8.95±0.16	91.05
10	21	62.62	10.65	
	22	63.38	10.64	
	23	63.36	10.90	
	24	62.54	10.73	
	25	63.74	10.85	
		$\bar{X}$ 63.13±0.47	$\bar{X}$ 10.75±0.10	89.25
12	26	66.76	11.38	
	27	67.59	11.00	
	28	66.91	11.57	
	29	69.83	11.30	
	30	66.99	10.76	
		$\bar{X}$ 67.62±1.14	$\bar{X}$ 11.20±0.29	88.80
14	31	64.65	9.04	
	32	64.58	8.83	
	33	64.27	8.58	
	34	64.91	9.21	
	35	64.54	8.92	
		$\bar{X}$ 64.59±0.21	$\bar{X}$ 8.92±0.21	91.08

APPENDIX LV. Continued

Fish No.	Sample No.[a]	Water (%)	Fat (% d/w)	Mean egg FFDM (% d/w)
15	36	64.29	10.56	
	37	63.54	10.19	
	38	63.51	10.39	
	39	63.24	10.52	
	40	63.93	10.72	
		$\bar{X}$ 63.70±0.37	$\bar{X}$ 10.48±0.18	89.52

[a]20 eggs per sample.

Mean egg FFDM (% d/w) = 89.59±0.98.

APPENDIX LVI. L-ascorbic acid content of pooled rainbow trout eggs

Fish No.	Sample No.[a,b]	Wet Egg Wt (g)	FFDM[c] (g)	Absorbance[d] 540 nm	ug/g from standard curve	ug/g wet egg	ug/g egg FFDM
1	1	0.6500	0.3410	0.451	13.58	105	199
	2	0.3257	0.3257	0.454	13.67	110	210
	3	0.6078	0.3189	0.444	13.37	110	210
	4	0.6157	0.3230	0.436	13.13	106	203
						$\bar{X}$= 108±2	$\bar{X}$= 206±5
3	1	0.6810	0.3630	0.461	13.89	102	191
	2	0.6678	0.3560	0.443	13.34	100	187
	3	0.6788	0.3618	0.459	13.83	102	191
	4	0.6744	0.3595	0.445	13.40	99	181
						$\bar{X}$= 101±1	$\bar{X}$= 188±4
4	1	0.4980	0.2573	0.401	12.08	121	235
	2	0.4848	0.2504	0.381	11.48	118	229
	3	0.5168	0.2670	0.378	11.39	110	213
	4	0.5113	0.2641	0.423	12.74	125	241
						$\bar{X}$= 119±6	$\bar{X}$= 230±10
6	1	0.5051	0.2686	0.525	15.81	157	294
	2	0.4959	0.2637	0.481	14.49	146	275
	3	0.5015	0.2667	0.492	14.82	149	281
	4	0.5115	0.2720	0.548	16.51	161	303
						$\bar{X}$= 153±6	$\bar{X}$= 288±11
10	1	0.746	0.471	0.253	7.62	102	162
	2	0.752	0.425	0.231	6.96	93	164
	3	0.758	0.429	0.248	7.45	99	174
	4	0.782	0.442	0.247	7.44	95	168
	5	0.792	0.448	0.239	7.20	91	161
	6	0.763	0.432	0.249	7.50	98	174
	7	0.775	0.438	0.222	6.69	86	153
	8	0.749	0.424	0.235	7.08	95	167
	9	0.808	0.457	0.236	7.11	88	156
	10	0.799	0.452	0.223	6.72	84	149
						$\bar{X}$= 93±6	$\bar{X}$= 163±8
12	1	0.842	0.510	0.315	9.49	113	186
	2	0.885	0.536	0.295	8.89	100	166
	3	0.947	0.574	0.240	7.23	76	126
	4	0.935	0.566	0.259	7.80	83	138
	5	0.899	0.545	0.294	8.86	99	163
	6	0.911	0.552	0.232	6.99	77	127
	7	0.913	0.553	0.279	8.40	92	152
	8	0.895	0.542	0.310	9.34	104	172
	9	0.899	0.545	0.279	8.40	93	154
	10	0.879	0.527	0.269	8.10	93	154
						$\bar{X}$= 93±11	$\bar{X}$= 154±19

APPENDIX LVI. Continued

Fish No	Sample[a,b] No.	Wet Egg Wt (g)	FFDM[c] (g)	Absorbance[d] 540 nm	ug/g from standard curve	ug/g wet egg	ug/g egg FFDM
14	1	0.733	0.424	0.269	8.10	111	191
	2	0.763	0.442	0.270	8.13	107	184
	3	0.745	0.431	0.230	6.93	93	161
	4	0.742	0.429	0.265	7.98	108	186
	5	0.748	0.433	0.265	7.98	107	184
	6	0.754	0.436	0.237	7.14	95	164
	7	0.765	0.443	0.247	7.44	97	168
	8	0.729	0.422	0.251	7.56	104	179
	9	0.742	0.429	0.239	7.20	97	168
	10	0.758	0.439	0.240	7.23	95	165
						$\bar{X}$= 101±6	$\bar{X}$= 175±10
15	1	0.727	0.415	0.280	8.43	116	203
	2	0.724	0.413	0.281	8.46	117	205
	3	0.743	0.424	0.288	8.67	117	205
	4	0.741	0.423	0.281	8.46	114	200
	5	0.718	0.410	0.281	8.46	118	206
	6	0.744	0.425	0.286	8.61	116	203
	7	0.586	0.334	0.210	6.33	108	190
	8	0.731	0.417	0.272	8.19	112	196
	9	0.753	0.430	0.281	8.46	112	197
	10	0.734	0.419	0.271	8.16	111	195
						$\bar{X}$= 114±3	$\bar{X}$= 200±5

[a] 10 eggs pooled per sample.

[b] Samples from fish 1-6 extracted with 5 ml HPO_3-HOAc solvent. Samples from fish 10-14 extracted with 10 ml HPO_3-HOAc solvent.

[c] Calculated using analysed moisture and a mean FFDM (% d/w) = 89.59. Refer to Appendix I.

[d] Mean absorbance of duplicate samples.

APPENDIX LVII. L-ascorbic acid content of individual rainbow trout eggs

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
1 Fore*	1	0.0680	0.0357	0.372	14.24	7.12	105	199
	2	0.0641	0.0336	0.359	13.71	6.79	106	202
	3	0.0626	0.0328	0.382	14.65	7.33	117	223
	4	0.0691	0.0363	0.382	14.65	7.32	106	202
	5	0.0608	0.0319	0.358	13.65	6.83	112	214
	6	0.0615	0.0323	0.339	12.86	6.43	105	199
	7	0.0644	0.0338	0.372	14.23	7.12	111	211
	8	0.0610	0.0320	0.392	15.06	7.53	123	235
	9	0.0683	0.0358	0.321	12.12	6.06	89	169
	10	0.0656	0.0344	0.380	14.56	7.28	111	212
	11	0.0616	0.0323	0.375	14.36	7.18	117	222
	12	0.0631	0.0331	0.374	14.32	7.16	113	216
	13	0.0622	0.0326	0.390	14.98	7.49	120	230
	14	0.0624	0.0327	0.388	14.90	7.45	119	228
	15	0.0665	0.0349	0.389	14.94	7.47	112	214
	16	0.0588	0.0308	0.386	14.81	7.41	126	240
	17	0.0611	0.0321	0.390	14.98	7.49	123	233
	18	0.0629	0.0330	0.385	14.77	7.39	117	224
	19	0.0638	0.0335	0.381	14.61	7.31	115	218
	20	0.0630	0.0331	0.378	14.48	7.24	115	219
1 Mid	1	0.0612	0.0321	0.387	14.86	7.43	121	231
	2	0.0612	0.0321	0.384	14.73	7.37	120	229
	3	0.0616	0.0323	0.369	14.11	7.06	115	218
	4	0.0618	0.0324	0.394	15.15	7.58	123	234
	5	0.0639	0.0335	0.394	15.15	7.58	119	226
	6	0.0620	0.0325	0.382	14.65	7.33	118	225
	7	0.0616	0.0323	0.380	14.56	7.28	118	225
	8	0.0624	0.0327	0.390	14.98	7.49	120	229
	9	0.0627	0.0329	0.378	14.48	7.24	115	220
	10	0.0612	0.0321	0.380	14.56	7.28	119	227
	11	0.0607	0.0318	0.391	15.02	7.51	124	236
	12	0.0603	0.0316	0.372	14.23	7.12	118	225
	13	0.0619	0.0325	0.395	15.19	7.60	123	233
	14	0.0605	0.0317	0.390	14.98	7.49	124	236
	15	0.0626	0.0328	0.383	14.69	7.35	117	224
	16	0.0640	0.0336	0.385	14.77	7.39	115	220
	17	0.0615	0.0323	0.380	14.56	7.28	118	225
	18	0.0632	0.0332	0.380	14.56	7.28	115	219
	19	0.0629	0.0330	0.359	13.69	6.85	109	207
	20	0.0633	0.0332	0.379	14.52	7.26	115	219

*Egg samples taken for macro analysis.

APPENDIX LVII. Continued

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
1 Hind	1	0.0634	0.0333	0.378	14.48	7.24	114	217
	2	0.0615	0.0323	0.369	14.11	7.06	115	219
	3	0.0621	0.0326	0.358	13.65	6.83	110	210
	4	0.0600	0.0315	0.368	14.07	7.04	117	223
	5	0.0572	0.0300	0.368	14.07	7.04	123	235
	6	0.0619	0.0325	0.373	14.27	7.14	115	220
	7	0.0600	0.0315	0.389	14.94	7.47	125	237
	8	0.0614	0.0322	0.382	14.65	7.33	119	228
	9	0.0608	0.0319	0.375	14.36	7.18	118	225
	10	0.0607	0.0318	0.370	14.15	7.08	117	223
	11	0.0644	0.0338	0.375	14.36	7.18	111	211
	12	0.0601	0.0315	0.380	14.56	7.28	121	231
	13	0.0600	0.0315	0.394	15.15	7.58	126	240
	14	0.0603	0.0316	0.378	14.48	7.24	120	229
	15	0.0608	0.0319	0.380	14.56	7.28	120	228
	16	0.0607	0.0318	0.361	13.78	6.89	114	217
	17	0.0622	0.0326	0.380	14.56	7.28	117	223
	18	0.0634	0.0333	0.395	15.19	7.60	120	228
	19	0.0606	0.0318	0.362	13.82	6.91	114	217
	20	0.0619	0.0325	-	-	-	-	-
3 Fore	1	0.0691	0.0368	0.372	14.23	7.12	103	193
	2	0.0687	0.0366	0.389	14.94	7.47	109	204
	3	0.0674	0.0359	0.373	14.27	7.14	106	199
	4	0.0672	0.0358	0.379	14.52	7.26	108	203
	5	0.0679	0.0362	0.378	14.48	7.24	107	200
	6	0.0677	0.0361	0.361	13.78	6.89	102	191
	7	0.0642	0.0342	0.358	13.65	6.83	106	200
	8	0.0645	0.0344	0.351	13.36	6.68	104	194
	9	0.0662	0.0353	0.370	14.15	7.08	107	201
	10	0.0681	0.0363	0.381	14.61	7.31	107	201
	11	0.0693	0.0369	0.378	14.48	7.24	104	196
	12	0.0673	0.0359	0.378	14.48	7.24	108	202
	13	0.0687	0.0366	0.388	14.90	7.45	108	204
	14	0.0666	0.0355	0.388	14.90	7.45	112	210
	15	0.0679	0.0362	0.390	14.98	7.49	110	207
	16	0.0666	0.0355	0.389	14.94	7.47	112	210
	17	0.0684	0.0365	0.395	15.19	7.60	111	208
	18	0.0668	0.0356	0.378	14.48	7.24	108	203
	19	0.0677	0.0361	0.374	14.32	7.16	106	198
	20	0.0712	0.0380	0.389	14.94	7.47	105	197

APPENDIX LVII. Continued

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
3 Mid*	1	0.0704	0.0375	0.385	14.77	7.39	105	197
	2	0.0723	0.0385	0.389	14.94	7.47	103	194
	3	0.0666	0.0355	0.372	14.23	7.12	107	185
	4	0.0666	0.0355	0.361	13.78	6.89	103	194
	5	0.0697	0.0372	0.370	14.15	7.08	102	193
	6	0.0688	0.0366	0.381	14.61	7.31	106	200
	7	0.0692	0.0368	0.374	14.32	7.16	103	195
	8	0.0670	0.0357	0.371	14.19	7.10	106	199
	9	0.0691	0.0368	0.389	14.94	7.47	108	203
	10	0.0662	0.0352	0.368	14.07	7.04	106	200
	11	0.0684	0.0365	0.382	14.65	7.33	107	201
	12	0.0664	0.0354	0.370	14.15	7.08	107	200
	13	0.0673	0.0359	0.368	14.07	7.04	105	196
	14	0.0640	0.0341	0.358	13.65	6.83	107	200
	15	0.0712	0.0380	0.392	15.06	7.53	106	198
	16	0.0700	0.0373	0.355	13.53	6.77	97	181
	17	0.0677	0.0361	0.365	13.94	6.97	103	193
	18	0.0687	0.0366	0.369	14.11	7.06	103	193
	19	0.0700	0.0373	0.355	13.53	6.77	97	182
	20	0.0697	0.0371	0.379	14.52	7.26	104	196
3 Hind	1	0.0680	0.0362	0.379	14.52	7.26	107	201
	2	0.0813	0.0433	0.248	9.09	4.55	56	105
	3	0.0888	0.0473	0.368	14.07	7.04	79	149
	4	0.0834	0.0445	0.201	7.14	3.57	43	80
	5	0.0764	0.0407	0.370	14.15	7.08	93	174
	6	0.0831	0.0442	0.375	14.36	7.18	86	162
	7	0.0737	0.0393	0.350	13.32	6.66	90	169
	8	0.0724	0.0386	0.399	15.35	7.68	106	199
	9	0.0759	0.0405	0.390	14.98	7.49	98	185
	10	0.0679	0.0371	0.380	14.56	7.28	107	196
	11	0.0734	0.0391	0.385	14.77	7.39	101	189
	12	0.0777	0.0414	0.400	15.39	7.70	99	186
	13	0.0771	0.0411	0.355	13.53	6.77	87	165
	14	0.0808	0.0431	0.308	11.58	5.79	72	134
	15	0.0776	0.0414	0.377	14.44	7.22	93	174
	16	0.0731	0.0390	0.381	14.61	7.31	100	187
	17	0.0751	0.0400	0.355	13.53	6.77	91	169
	18	0.0789	0.0421	0.390	14.98	7.49	95	178
	19	0.0694	0.0370	-	-	-	-	-
	20	0.0785	0.0418	0.388	14.90	7.45	95	178

APPENDIX LVII. Continued

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
4 Fore	1	0.0474	0.0245	0.354	13.49	6.75	142	276
	2	0.0510	0.0263	0.371	14.19	7.10	139	270
	3	0.0506	0.0261	0.351	13.36	6.68	132	256
	4	0.0471	0.0243	0.368	14.07	7.02	149	289
	5	0.0484	0.0250	0.379	14.52	7.26	150	290
	6	0.0505	0.0261	0.365	13.94	6.97	138	267
	7	0.0515	0.0266	0.358	13.65	6.83	133	257
	8	0.0500	0.0258	0.371	14.19	7.10	142	275
	9	0.0476	0.0246	0.345	13.11	6.56	138	267
	10	0.0484	0.0250	0.301	11.29	5.65	117	226
	11	0.0497	0.0257	0.330	12.49	6.25	126	243
	12	0.0487	0.0252	0.363	13.86	6.93	142	275
	13	0.0492	0.0254	0.325	12.28	6.14	125	242
	14	0.0482	0.0249	0.355	13.53	6.77	140	272
	15	0.0480	0.0248	0.348	13.24	6.62	138	267
	16	0.0471	0.0243	0.300	11.25	5.63	119	232
	17	0.0481	0.0248	0.350	13.32	6.66	138	269
	18	0.0483	0.0250	0.350	13.32	6.66	138	266
	19	0.0486	0.0251	0.342	12.99	6.50	134	259
	20	0.0530	0.0274	0.361	13.78	6.89	130	251
4 Mid*	1	0.0514	0.0266	0.339	12.86	6.43	125	242
	2	0.0487	0.0252	0.361	13.78	6.89	141	273
	3	0.0505	0.0261	0.344	13.07	6.54	129	251
	4	0.0506	0.0261	0.341	12.95	6.48	128	248
	5	0.0488	0.0252	0.340	12.91	6.46	132	256
	6	0.0510	0.0263	0.349	13.28	6.64	130	252
	7	0.0512	0.0264	0.349	13.28	6.64	130	252
	8	0.0490	0.0253	0.353	13.44	6.72	137	266
	9	0.0508	0.0262	0.358	13.65	6.83	134	261
	10	0.0500	0.0258	0.328	12.41	6.21	124	240
	11	0.0501	0.0259	0.355	13.53	6.77	135	261
	12	0.0519	0.0268	-	-	-	-	-
	13	0.0479	0.0247	0.350	13.32	6.66	139	270
	14	0.0519	0.0268	0.268	9.92	4.96	96	166
	15	0.0531	0.0274	0.352	13.40	6.70	126	245
	16	0.0500	0.0258	0.352	13.40	6.70	134	260
	17	0.0584	0.0301	0.361	13.78	6.89	118	229
	18	0.0499	0.0258	0.357	13.61	6.81	136	264
	19	0.0512	0.0264	0.321	12.13	6.07	118	229
	20	0.0534	0.0276	0.341	12.91	6.46	121	234

APPENDIX LVII. Continued

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
4 Hind	1	0.0534	0.0276	0.275	10.22	5.11	96	185
	2	0.0558	0.0288	0.301	11.30	5.65	102	225
	3	0.0524	0.0271	0.285	10.63	5.32	101	196
	4	0.0539	0.0278	0.275	10.22	5.11	95	184
	5	0.0524	0.0271	0.318	12.00	6.00	115	221
	6	0.0500	0.0258	-	-	-	-	-
	7	0.0475	0.0245	0.281	10.47	5.24	110	214
	8	0.0542	0.0280	0.291	10.88	5.44	100	194
	9	0.0536	0.0277	0.265	9.81	4.91	92	177
	10	0.0531	0.0274	0.254	9.35	4.68	88	171
	11	0.0534	0.0276	0.288	10.76	5.38	101	195
	12	0.0509	0.0263	0.273	10.14	5.07	97	193
	13	0.0565	0.0292	0.299	11.22	5.61	99	192
	14	0.0531	0.0274	0.308	11.59	5.80	109	215
	15	0.0541	0.0279	0.289	10.80	5.40	99	194
	16	0.0507	0.0262	0.300	11.26	5.63	111	215
	17	0.0512	0.0264	0.260	9.60	4.80	94	182
	18	0.0533	0.0275	0.283	10.55	5.28	99	192
	19	0.0559	0.0289	0.286	10.68	5.34	96	185
	20	0.0456	0.0236	0.239	8.73	4.37	96	185
6 Fore	1	0.0488	0.0259	0.329	12.46	6.23	128	241
	2	0.0500	0.0266	0.389	14.95	7.48	150	281
	3	0.0500	0.0266	0.360	13.75	6.88	138	259
	4	0.0534	0.0284	0.402	15.49	7.75	145	273
	5	0.0490	0.0261	0.352	13.41	6.71	137	191
	6	0.0525	0.0279	0.388	14.91	7.46	142	267
	7	0.0521	0.0277	-	-	-	-	-
	8	0.0507	0.0270	0.390	14.99	7.50	148	278
	9	0.0492	0.0261	0.409	15.78	7.89	160	302
	10	0.0474	0.0252	0.190	6.69	3.35	71	133
	11	0.0449	0.0239	0.285	10.63	5.32	118	222
	12	0.0505	0.0269	0.300	11.26	5.63	111	209
	13	0.0503	0.0267	0.369	14.12	7.06	140	264
	14	0.0515	0.0274	0.359	13.71	6.86	133	250
	15	0.0478	0.0254	0.345	13.12	6.56	137	258
	16	0.0489	0.0260	0.289	10.80	5.40	110	208
	17	0.0521	0.0277	0.373	14.29	7.15	137	192
	18	0.0496	0.0263	0.350	13.33	6.67	134	254
	19	0.0526	0.0280	0.400	15.41	7.71	146	275
	20	0.0517	0.0275	0.378	14.49	7.25	140	264

APPENDIX LVII. Continued

Fish No. and Egg location in ovary	Egg No.	Wet egg wt (g)	FFDM (g)	Absorbance 520 nm	ug/ml from standard curve	ug wet egg	ug/g wet egg	ug/g FFDM
6 Mid	1	0.0530	0.0282	0.332	12.59	6.30	119	223
	2	0.0508	0.0270	0.350	13.33	6.67	131	247
	3	0.0482	0.0256	0.349	13.29	6.65	138	260
	4	0.0530	0.0282	0.390	14.99	7.50	141	266
	5	0.0521	0.0277	0.362	13.83	6.92	133	250
	6	0.0524	0.0279	0.368	14.08	7.04	135	252
	7	0.0490	0.0261	0.361	13.79	6.90	141	264
	8	0.0529	0.0281	0.362	13.83	6.92	131	246
	9	0.0553	0.0294	0.330	12.50	6.25	113	213
	10	0.0517	0.0275	0.349	13.29	6.65	129	242
	11	0.0500	0.0266	0.333	12.63	6.32	126	238
	12	0.0516	0.0274	0.355	13.54	6.77	131	247
	13	0.0500	0.0266	0.338	12.83	6.42	128	241
	14	0.0508	0.0270	0.341	13.03	6.52	128	224
	15	0.0550	0.0292	0.378	14.49	7.23	132	248
	16	0.0524	0.0279	0.345	13.12	6.56	116	235
	17	0.0525	0.0279	0.362	13.83	6.92	132	248
	18	0.0514	0.0273	0.350	13.33	6.67	130	244
	19	0.0505	0.0269	0.376	14.41	7.21	143	268
	20	0.0518	0.0275	0.360	13.75	6.88	133	250
6 Hind*	1	0.0483	0.0257	0.362	13.83	6.92	143	269
	2	0.0522	0.0278	0.392	15.07	7.54	144	271
	3	0.0500	0.0266	0.400	15.41	7.71	154	290
	4	0.0500	0.0266	0.400	15.07	7.54	151	283
	5	0.0510	0.0271	0.375	14.37	7.19	141	265
	6	0.0505	0.0269	0.380	14.58	7.29	144	271
	7	0.0488	0.0259	0.396	15.24	7.62	156	294
	8	0.0509	0.0271	0.402	15.49	7.75	152	286
	9	0.0488	0.0259	0.385	14.78	7.39	151	285
	10	0.0511	0.0272	0.392	15.07	7.54	147	277
	11	0.0422	0.0224	0.352	13.41	6.71	159	300
	12	0.0460	0.0245	0.398	13.61	6.81	148	278
	13	0.0495	0.0263	0.375	14.37	7.19	145	273
	14	0.0465	0.0247	0.398	15.32	7.66	164	310
	15	0.0482	0.0256	0.355	13.54	6.77	140	264
	16	0.0478	0.0254	0.378	14.49	7.25	152	285
	17	0.0469	0.0249	0.395	15.20	7.60	162	305
	18	0.0500	0.0266	0.389	14.95	7.48	150	281
	19	0.0500	0.0266	0.390	15.00	7.50	150	282
	20	0.0450	0.0239	0.388	14.91	7.46	166	312

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Trust, T. J., and R. W. Coombs. 1973. Antibacterial activity of

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