AN ABSTRACT OF THE THESIS OF

JAMES CASS BAKER for the degree DOCTOR OF PHILOSOPHY
(Name of Student) (Degree)

in Fisheries and Wildlife presented on February 15, 1974
(Major Department) (Date)

Title: PROTEIN AND LIPID FACTORS IN THE BIOENERGETICS OF SWIMMING IN FISH (ONCORHYNCHUS KISUTCH)

Signature redacted for privacy.

Abstract approved by: Hugo Krueger

Wild juvenile coho salmon (Oncorhynchus kisutch) were subjected to various swimming tests including training for 11 hours at a mean velocity of 15 cm/sec and training plus swimming at water velocities of 15, 25, 35 and 45 cm/sec for 24 hours. All fish were successful except for three of 27 fish at the 35 cm/sec and 32 of 37 fish at 45 cm/sec.

With the high variability found in the wild population of salmon, it was obvious that statistical procedures more critical than comparisons of simple means would have to be employed if useful information was to be developed in order to establish experimental differences induced by swimming. Improved statistical treatment of the data was obtained by making use of generally accepted physiological interrelationships: (1) Weight, length and the biochemical parameters measured are interdependent; hence, water, ash, lipid and LFOM

(lipid free organic matter) are related to weight and length. (2) Lean body mass (fat free body mass) has a reasonably constant composition: a statement leading to the corollary that water, ash, nitrogen, protein content and LFOM are proportional to one another and to lean body mass. (3) Heat of combustion of organic matter is the sum of the heats of combustion of lipids and lipid free organic matter.

Stepwise multiple linear regression analyses were developed from data on controls for biochemical parameters as functions of W_o (initial weight), l_o (initial length) and T (time held in the laboratory).

$$\text{(Final weight) } W_f = -1.99 + 0.927\,W_o + 0.02\,l_o$$

$$\text{(Dry weight) DW} = 0.915 - 0.495\,W_o + 2.96 \times 10^{-5} T W_o - 0.011 W_o^3 + 0.157 W_o^2$$

$$\text{(Water) } H_2O = -1.413 + 0.660\,W_o + 0.027\,l_o - 1.896 \times 10^{-5}\,T W_o$$

$$\text{ASH} = -0.055 + 3.01 \times 10^{-5}\,l_o^2$$

$$\text{LIPID} = -0.074 + 0.077\,W_o + 1.94 \times 10^{-5} T W_o - 4.42 \times 10^{-7}\,l_o^3$$

$$\text{LFOM} = -0.059 + 0.174\,W_o$$

$$\text{NITROGEN} = -0.008 + 0.027\,W_o$$

$$\text{CALORIES} = -2454 + 2064\,W_o + 0.212\,T W_o - 0.005\,l_o^3 - 5.91\,W_o^3$$

The use of these equations reduced the standard deviation 50%, 72%, 68% and 68%, respectively for lipid, LFOM, nitrogen and caloric content per fish. These equations were then used to estimate the pre-

swimming levels for each parameter for any individual fish on the assumption that before swimming all salmon belonged to the same population as did the controls.

Fish swimming at 15 cm/sec had losses of 121 mg in water, 35 mg in LFOM, 4 mg in nitrogen and 433 calories per salmon during swimming, whereas the 25 cm/sec salmon had losses of 182 mg in H_2O, 33 mg in LFOM and 8.5 mg in nitrogen. Fish at the 35 cm/sec level had losses of 121 mg in water and 8 mg in nitrogen. In the 45 cm/sec group water loss was 115 mg, lipid loss was 16 mg and nitrogen loss was 4 mg. No other losses of statistical significance were noted. The losses described were not related in a linear fashion to the velocity of the water.

The relations between heats of combustion per fish and lipid and LFOM contents were altered by swimming as indicated by statistical equations developed for control and trained salmon and salmon after swimming at 15, 25, 35, and 45 cm/sec respectively:

$$H_C = 9182 \times \text{lipid/fish} + 4913 \times \text{LFOM/fish}$$

$$H_T = 9328 \times \text{lipid/fish} + 4868 \times \text{LFOM/fish}$$

$$H_{15} = 9816 \times \text{lipid/fish} + 4741 \times \text{LFOM/fish}$$

$$H_{25} = 10458 \times \text{lipid/fish} + 4670 \times \text{LFOM/fish}$$

$$H_{35} = 9518 \times \text{lipid/fish} + 4952 \times \text{LFOM/fish}$$

$$H_{45} = 9068 \times \text{lipid/fish} + 5042 \times \text{LFOM/fish}$$

In resume, no statistically significant difference in the derived heat of combustion of lipids and LFOM were noted between control salmon and salmon after training. Significant differences in the heats of combustion of lipids and LFOM developed between controls and fish after swimming at 15 cm/sec. Even greater differences were noted between controls and the salmon after swimming at 25 cm/sec.

Protein and Lipid Factors in the Bioenergetics of Swimming in Fish (*Oncorhynchus kisutch*)

by

James Cass Baker

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1975

APPROVED:

Signature redacted for privacy.

Professor of Physiology and Pharmacology
in charge of major

Signature redacted for privacy.

Head of Department of Fisheries and Wildlife

Signature redacted for privacy.

Dean of Graduate School

Date thesis is presented February 15, 1974

Typed by Illa W. Atwood for James Cass Baker

ACKNOWLEDGEMENTS

The author wishes to extend his most sincere appreciation to Dr. Hugo Krueger, Professor of Fisheries, Physiology and Pharmacology, for his advice and guidance during this investigation and for his considerable assistance in the preparation of this manuscript.

Special thanks are due to many members of the Departments of Agricultural Chemistry, Fisheries and Wildlife, Statistics, and Computer Science who provided assistance, advise and equipment.

To my wife Nancy who has aided me through this and past academic adventures I wish to offer special thanks; your patience and sacrifice has been appreciated.

This research was supported by U. S. Public Health Service Grants 1 T1 GM 1192 and 5 P10 ES00210.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CHO	Carbohydrate
DW	Dry Weight
D or Δ (delta)	Difference
G	Gain
H	Heat of Combustion; calories
L	Lipid
l_o	Initial length (fork length)
l_f	Final length (fork length)
LFOM	Lipid Free Organic Matter
N	Nitrogen
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
n	Number
n*	Number significant at $p \leq 0.05$
n**	Number significant at $p \leq 0.01$
n***	Number significant at $p \leq 0.001$
OM	Organic Matter
p	Probability of the occurrence of a difference by chance rather than as a consequence from the experimental procedure.
s	Standard Deviation

SE	Standard Error of Mean; $s/\sqrt{N - 1}$
T	Time in the laboratory
TCA	Tricarboxylic acid cycle
W	Wet Weight
W_o	Initial Weight
W_f	Final Weight
$\overline{X}$	Average values are indicated by a bar over the term, symbol of value; $\overline{N}$ is the average of N.

PROTEIN AND LIPID FACTORS IN THE BIOENERGETICS OF EXERCISE IN FISH (ONCORHYNCHUS KISUTCH)

INTRODUCTION

Brody (1945) considered bioenergetics, concerned with the energy transformations in living things, as a branch of general energetics. The basic concepts of energetics are generalized by the first and second laws of thermodynamics. The first law (the energy of the universe is conserved) deals with the mechanisms or rates of energy changes, but only with the initial and final energetic state of the system. The definiteness of the first law gives a sense of universal finality, and a firm basis for bioenergetic investigations even if the mechanisms of the reactions are unknown. The second law (the entropy of the universe increases) deals with the driving force of reactions, with chemical affinity, with energy losses involved in various reactions, and especially with limitations on the conversion of heat into work.

Winberg (1960 and 1961) stressed the need to apply these principles to the metabolism of active fish. In this same manuscript Winberg gave a good review of the literature up to that time. He made clear the need for well defined research, such that experimental conditions should be rigorously maintained and with an adequate use of clearly defined controls thereby making the information more

useful to the whole research community. Since the time of Winberg's (1960 and 1961) reviews many workers have investigated different aspects of active fish metabolism including the influence of acclimation, temperature, oxygen consumption and other metabolic studies. Some investigators have concentrated on the muscle systems and the physiology of these systems. Love (1970) provides an excellent review of this material.

Idler and Bitners (1958) described the changes in fat, protein (calculated on the basis of nitrogen determinations) and water in sockeye salmon, Oncorhynchus nerka, during spawning migration. Idler and Clemens (1958) described changes in water, fat, protein and also energy expenditures of migrating salmon. Niimi (1972) reported the changes in proximate analysis composition of largemouth bass, Micropteras salmoides, with starvation. Brett, Shellbourn and Shoop (1969) described the growth rates and body composition of fingerling sockeye salmon in relation to temperature and ration size. Of particular importance were the relationships developed between protein and water, and fat and water. The information developed will be considered more fully in a later section.

Many workers have investigated the levels of various metabolic components found in the plasma of fish under a variety of conditions. E. C. Black (1958) along with many co-workers (1958, 1959, 1960, and 1966) investigated the metabolic fate of carbohydrate intermediates

particularily glucose, lactic acid and pyruvic acid. Many other workers have reported low levels of glycogen and glucose in whole fish; Vinogradov (1953), Black (1958), Brett (1969), with Hochachka and Sinclair (1962) describing liver glycogen levels. The above studies were generally carried out in relationship to relatively short-term burst activity, spawning migration, or long-term feeding or starvation studies.

Ivlev (1945) and Gerking (1952) emphasize the importance of studying nitrogen metabolism particularly in juvenile fish for the problem of transfer of energy within a food chain. Krivobok (1958) has taken a very similar approach, in that he studied the nitrogen balance of fish in their environment. Gerking (1952, 1955a and 1955b) studied the protein metabolism in fish. The main emphasis of these studies was the use and fate of dietary protein but not active metabolic fates of protein. Duncan and Tarr (1958) described the changes in three protein fractions and one non-protein nitrogen fraction of the muscles of migrating sockeye salmon. As part of the same studies, Idler and Tsuyuki (1958) reported changes in urea nitrogen in migration. M. N. Kutty (1972) related ammonia excretion and respiratory quotient under routine and forced activity levels. Kutty concluded that Tilapia mossambica (Peters) utilized more protein the longer the exercise period. This factor coupled with anaerobic energy utilization

may be an advantage in preventing acidosis and conserving sodium in swimming fish.

Brett (1965) has provided information relating swimming velocity and oxygen consumption of Oncorhynchus nerka and attempted to calculate the caloric value of the work of swimming at elevated water velocities. In general, Brett found a ratio for maximum active metabolism to standard metabolism of 8:1 for a fish of 5 grams.

Swimming ability is an obvious requirement for survival of fish and this has been extensively investigated (Bainbridge, 1960 and 1962; Brett, 1965). However, there are many gaps in the information that make physiological and bioenergetic interpretations difficult. Several such gaps were brought to light during an investigation of fatty acid metabolism in salmon during swimming (Saddler, 1967).

Saddler (1967) had available data only on wet weight, lipid content and fatty acid contents of control and exercised salmon (Oncorhynchus kisutch) weighing approximately 8 g. At a velocity of 52 cm/sec the weight loss for swimming salmon was 830 mg. Lipids accounted for 54 mg and losses other than lipids were 776 mg. Of the 830 mg an interpretation was given by Krueger and co-workers (1968) that approximately 80% or 664 mg may have been water. Hence the protein plus carbohydrate losses would have been approximately 112 mg. They suggested that lipid loss represented 502 calories, the protein plus carbohydrate loss represented approximately 616

calories, and the total caloric loss 1118 calories. The similar loss calculated from oxygen consumption data of Brett (1964, 1965) on active metabolism during swimming of Oncorhynchus nerka was only 344 calories.

Thus the caloric cost of swimming estimated from material losses was three times as great as the cost calculated from oxygen consumption of Brett. However, the oxygen consumption data of Brett had been collected during one, and at the most two hours at high velocity, and the data of Saddler (1967) were based on swimming for 24 hours. Krueger and his co-workers suggested that perhaps, when a maximum effort was involved, each succeeding mile and each succeeding hour may have been more difficult and more costly to the salmon. Further, Brett (1964, 1965) found a ratio for maximum active metabolism to standard metabolism of 8:1 for a five gram fish. If the cost estimated from material losses could be supported by bioenergetic data, Krueger (1968) presumed that for prolonged exercise the ratio of active to standard metabolism might even reach a value of 24:1.

Thus the analysis of Krueger and his co-workers (1968) raised some important questions on the magnitude and source of calories lost during exercise of salmon and of the magnitude of the protein contribution to these losses. It is the objective of this thesis to provide information on lipid losses, protein losses, and the bioenergetics of

swimming in coho salmon to allow a more precise interpretation of the relations between oxygen consumption and caloric losses during swimming. (The data obtained for this thesis suggested that previous concepts of evaluating lipid and protein caloric loss had been oversimplified and that factors other than the decrease in lipid and protein masses needed to be considered.)

MATERIALS AND METHODS

Juvenile silver or coho salmon fingerlings (Oncorhynchus kisutch Walbaum) were seined from a wild population in the Yaquina River above Nashville, Oregon. The collection area was approximately one-half mile in length. In the attempt to produce a study which could be more directly applied to a natural situation, the use of wild fish had been chosen over the use of artificially propagated salmon. The fish were collected on three different dates: October 4, October 22, and December 4, 1969.

After the salmon were taken from the Yaquina River, fish between 65 mm and 80 mm in length were selected. These fish were transported to Oregon State University Oak Creek Fisheries Laboratory and were placed in three fifty gallon aquaria supplied from a common water source and recirculating pump to protect against accidental water flow failure. The aquaria were in a 15° C constant temperature room. The fish were fed an Oregon Moist Pellet diet twice daily, and any remaining foot was removed within one hour from the bottom of the aquaria by siphon. The aquaria were periodically cleaned and disinfected using Purina Brand Dairy Disinfectant. Care was taken to rinse the aquaria to prevent toxicant contamination. No evidence of toxicity was ever apparent with this type treatment. Food was discontinued 24 hours prior to the use of the fish in an

experimental procedure.

The Oregon Moist Pellet varies with the raw material available. The mean composition of the pellets (Crawford, OSU Dept. of Food Science, 1970, unpublished data) is 29% water, 13% fat, 10% ash and 36% protein. The minimum for protein (Oregon Pellet and Starter Mash Specifications, Fish Commission of Oregon, November 1970) is 25% and for fat 7%.

Swimming Performance

In November and December, 1969, 17 separate swimming tests were conducted using the captured wild fish. The group of fish, associated with a particular swimming test, were removed rapidly by hand net in the approximate numbers necessary for an experiment. For each test approximately 16 unmarked salmon were selected, in as random a manner possible. The fish thus selected were removed one by one and weighed and the fork length measured. Prior to the initial measurements an order was established to indicate which fish would be chosen as controls and which would be subjected to an exercise regime. Four to six were selected as controls and approximately 10 salmon were subjected to the performance procedure.

The initial measurement made on the salmon in these experiments were taken with the salmon alive. After removing excess water by patting the fish with a damp cloth, the fish were weighed. Lengths

were taken with the fish wet in a measuring trough.

The fish to be exercised were placed directly in the swimming tube. Control fish were placed in ice water until measurements of their weight and length were completed. Handling of the fish during measurement and introduction into the swimming apparatus was minimized and no anesthetic was used. The fish quickly became quiet after being introduced into the swimming apparatus (Figure 1) or into ice water. The control fish were sacrificed by a blow to the head, reweighed, remeasured and placed directly into a freezer. Subsequently the samples were freeze-dried and the analytical determinations carried out.

Swimming Tests

The swimming chamber was a modification of the apparatus used by Davis et al. (1963). The water velocity in the swimming chamber was adjusted to approximately 5 cm/sec, the minimum velocity allowing adequate maintenance of temperature control.

Three major changes in the apparatus were: (1) The removal of some bends in the tubing to improve the efficiency of the pump. (2) The use of an Anubar Flow Meter to determine more accurately the apparent water velocity. (3) The addition of electrical fields at both ends of the test chamber using a single loop of wire adhered to the inner surface of the swimming tube inside turbulence grids at both

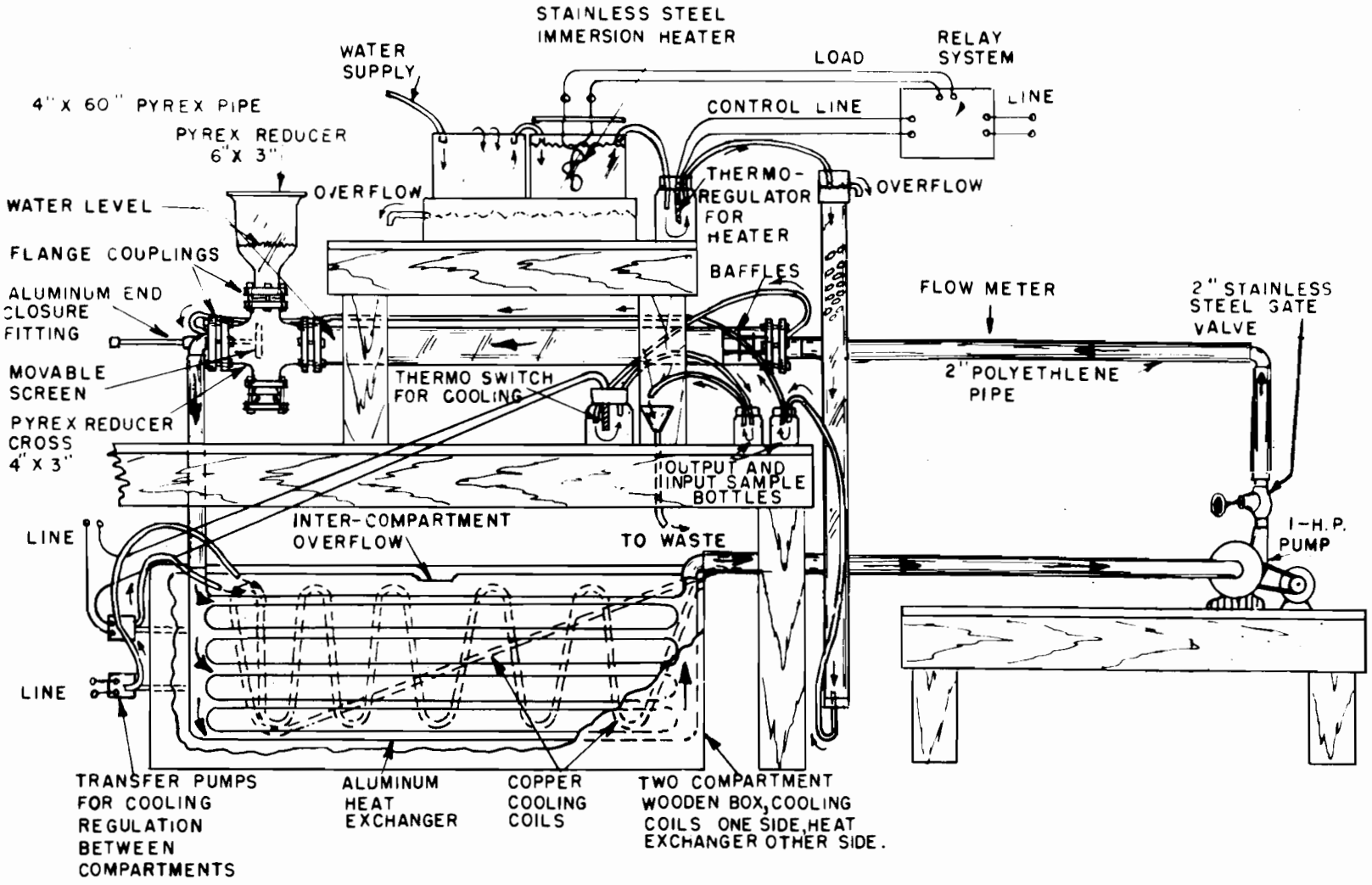


Figure 1. Swimming Apparatus.

ends of the test chamber. The electrical fields were maintained with a variable output D. C. generator to provide an electrical stimulus barrier during the training performance test. After the training period the use of the electrical field was discontinued. The temperature was controlled at 15^{o} C ± 1^{o} C for the duration of each swimming test. Oxygen was monitored periodically throughout the test period and was 10 ppm or higher. An exchange flow of approximately one liter of water per minute was maintained.

There were five different levels of swimming performance tested:

1. Training Performance Level - 11 hours.
2. Training (11 hours) + swimming at 15 cm/sec up to 24 hours.
3. Training (11 hours) + swimming at 25 cm/sec up to 24 hours.
4. Training (11 hours) + swimming at 35 cm/sec up to 24 hours.
5. Training (11 hours) + swimming at 45 cm/sec up to 24 hours.

The lowest level of performance and a level to which all test groups were subjected was the training test. This test consisted of a series of increasing velocities in four steps from 5 to 15 centimeters per second (cm/sec) in the first 90 minutes of the test. The velocity of water flow was then held at 15 cm/sec for eight hours. The velocity was again increased in seven steps to 30 cm/sec in an additional 90

minutes. At this point the training performance test was complete, and 6 to 10 preselected salmon were removed from the swimming tube. These fish were transferred to ice water, and as soon as possible were killed by a blow to the head, weighed, measured and placed in a freezer. The total time involved in the training test was 11 hours.

For those fish to be subjected to further performance the water velocity was adjusted to 15, 25, 35, or 45 cm/sec in 60 minutes or less. Less time was taken where the velocity was decreased below the 30 cm/sec already attained and more time when the final test velocity was to be greater than 30 cm/sec. Each test at 15, 25, 35, and 45 cm/sec was limited in duration to 24 hours or until the fish failed to maintain a swimming position and were pinned by the water current to the rear retaining grid.

When a fish failed to swim against the flow of water or at 24 hours, it was removed from the swimming chamber, placed in ice water, sacrificed, measured, weighed and placed in a freezer. The elapsed time between removal from the swimming apparatus and placement in the freezer was normally two minutes or less. The fish were stored frozen until freeze drying, usually within 24 hours, was commenced. The initial length, initial weight and the time the fish were held in the laboratory prior to the experimental use was recorded for each unmarked fish. At the conclusion of each of the seventeen

swimming tests, the final lengths, final wet weights and times held in the laboratory were recorded and the fish appropriately identified.

At 45 cm/sec approximately 88% of the fish failed to swim the total performance test. At 35 cm/sec approximately 11% failed before the end of 24 hours. The time of each failure was recorded.

Analytical Procedures

Freeze Drying

The frozen fish were removed from the freezer, cut into small chunks and placed in a freeze drying apparatus in the laboratory of S. A. Fang, Department of Agricultural Chemistry, Oregon State University. The fish were held under a greatly reduced pressure for approximately 24 hours. The samples were removed and quickly weighed to determine the dry weight. The fish were then ground to a powder in a microfood mill with a number 6 screen. The resultant dry powder was collected, placed in a polystyrene vial with a snap cap and stored in the freezer over $CaSO_4$ dessicant until used for analysis.

The freeze drying procedure employed for these samples is considered 99 + % efficient with an operating pressure of 0.025 mm Hg (Fang, 1968), as well as minimally changing the chemical and structural characteristics of the dried material (Simatos and Rey, 1965) and (King, 1971). Simatos and Rey (1965) have reported 0.35 to

0.39 mg of non-freezable water per gram of dry weight. The non-freezable water may not be completely removed but no data was presented due the many difficulties in determining the amount of non-freezable water.

Ash

Approximately 10 mg of the residue after freeze drying were placed in preweighed porcelain crucibles and ashed uncovered in a large muffle furnace. The temperature was slowly increased to 600° C in approximately one hour. The 600° C temperature was maintained for 12 hours (Horwitz, 1965). These conditions were used to minimize the amount of carbon compounds that existed as carbonates. The error of this technique is approximately 0.5 mg per gram dry weight with an additional allowable 5% experimental error (Hogan, 1968). After cooling, the crucibles were transferred to a large desicator with $CaSO_4$ to reach a stable temperature and water content. The ash weight was then determined. Each sample was run in duplicate. If a large variation was noted, an additional sample was run if material was available. The average dry weight of the runs were used to determine the ash weight. The mean difference between duplicate samples was 2.9 mg per gram of dry weight with a standard deviation of 2.6 mg.

Lipid

Duplicate 100 mg samples of the residue after freeze drying were weighed and the lipids extracted using a modification of the method of Bligh and Dyer (1959). This extraction procedure is 98+ percent efficient (Folch, Lee and Stanley, 1957 and Sperry, 1955). A potential residue up to 5% of the lipid may be introduced by first freeze drying the samples (Folch and Lee, 1957). The dried material was placed in a 35 ml screw cap centrifuge tube with 15 ml of 2:1 (v/v) redistilled chloroform:methanol. The caps were sealed and the sample shaken for 15 minutes. The contents were filtered and the filtrate was collected directly into a 35 ml screw cap centrifuge tube with use of a slight vacuum. The original tube was rinsed with 15 ml of extracting solvent and the solvent run through the filter under a slight vacuum. Seven and seven-tenths (7.7) ml of water was then added, the tubes recapped, vigorously shaken and centrifuged. Transfer pipettes were used to remove the bottom layer containing the extracted lipid and chloroform. This layer was then added to a pretared aluminum pan. Care was taken not to transfer water to the pan. The chloroform layer was then evaporated to dryness on a hot plate set at 50° C. The residue was removed from the hot plate, allowed to reach room temperature and weighed. The mean difference between duplicate determinations was 4.3 mg per gram dry weight with a

standard deviation of 3.3 mg. No precautions were taken to prevent oxidation of lipids present.

Nitrogen

Nitrogen was determined using a modification of Colowick and Kaplans (1957) micro-Kjeldahl procedure. Duplicate samples of approximately 10 mg dry weight plus the digestion reagent, which contained approximately 100 mg of sucrose, 40 mg of mercuric oxide, 500 mg of potassium sulfate, and 1.5 ml of concentrated sulfuric acid were placed in a 30 ml micro-Kjeldahl flask. The contents of the flasks were digested on an electric heater until colorless for 30 minutes. The total time of the digestion was approximately 60 minutes. The cooled digest was washed into a micro-Kjeldahl still with five one milliliter portions of distilled water. Twenty-five milliliters of an aqueous mixture of 10% sodium hydroxide and 5% sodium thiosulfate were added to make the digest alkaline. This mixture was steam distilled. The distillate was collected in a 50 ml beaker containing 5 ml of 5% aqueous boric acid with the condensor tip beneath the surface of the liquid for the first five minutes of the distillation and above the liquid for the last two minutes. The tip of the condensor was rinsed with distilled water. The beaker containing the boric acid-distillate solution was titrated to a pH of 4.2 with a standard solution of potassium bi-iodate, by use of a type TTT 1b Radiometer

automatic titrator. Each milliliter of the potassium bi-iodate solution used was converted to an equivalent mass of nitrogen.

The potassium bi-iodate solutions were standardized against a 0.1 N NaOH standard solution prepared with carbon dioxide free distilled water. The titrator pH meter was standardized against a known pH 6.5 buffer solution. The entire procedure was standardized using measured quantities of dried para-aminobenzoic acid and a primary standard Sigma 121, high purity, recrystallized tris-hydroxymethyl-aminomethane (mol. wt. = 121.136 = eq. wt.) The mean difference between duplicate determinations was 3.5 mg per gram dry weight with a standard deviation of 2.5 mg.

Water and Lipid Free Organic Matter

Water is given by the difference between wet and dry weight of the fish. Organic matter (OM) is given by dry weight minus ash. Lipid free organic matter (LFOM) is given by organic matter minus lipid content.

Heat of Combustion

A Parr Micro-Oxygen Bomb Calorimeter was used to determine the heat of combustion of duplicate 100 mg samples of the ground freeze dried fish material. Pellets were made to the appropriate size in a pellet press. The bomb was loaded, fused with platinum wire and

filled with oxygen. The bomb was ignited with the Parr Ignition Unit. The heat content of the combusted sample was then determined by measuring the number of degrees Celsius the measured volume of the water bath increased. This process was standardized using standard benzoic acid pellets. The standard heat of combustion of benzoic acid according to Parr Instrument Company is 6318 calories. Determination of the heat of combustion of benzoic acid ranged from 6227 to 6431 calories with an average of 6318 cal and a standard deviation of 54 cal and a standard error of 16.3 for the mean. After the ignition the oxidized residue was rinsed and titrated with sodium thiosulfate to give the quantity of nitric acid formed during ignition and for which a correction is required (Parr Instrument Co., 1958 and 1960). Additional caloric determinations were performed if the difference between duplicate samples was greater than 100 calories. The mean difference between duplicate samples was 39.5 calories per gram of dry weight with a standard deviation of 23.7 calories.

DATA AND DISCUSSION

Characteristics of Control Population

The data from the control fish provided verification and extension of some previously available information; and the differences between control salmon and salmon exercised at different levels of performance provide some new insights into the physiology of exercise in fish and into the alterations produced by exercise. The data from control salmon will be considered first; then the data from the performance tested salmon and the differences between controls and performance tested salmon. The averages for the raw data collected from control and exercised salmon are tabulated in Tables I and II. A general description of the control population can be seen by a glance at Table III.

Variability of Control Population

The control population (Table III) was very variable. To establish an effect of exercise on factors of bioenergetic significance with simple means and standard deviations for even a sample size of 50 would require differences of at least 0.029, 0.012, and 0.074 grams per fish for lipids, nitrogen and LFOM at the 1% level of probability that the difference occurred by change; and 0.022, 0.009, and 0.056 grams at the 5% level. At the 1% level these differences require a

Table I. Averages of Data Obtained from Control and Exercised Coho Salmon.

Performance	Controls	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Number	98	41	28	26	27	37
Surviving number	98	41	28	26	24	5
Wet Weight, gm	4.547	4.423	4.730	4.309	4.521	4.183
Water, gm	3.554	3.456	3.693	3.409	3.529	3.255
Ash, mg	115	114	118	111	118	107
Organic matter, mg	877	853	919	823	894	822
Lipid, mg	142	141	154	136	153	129
LFOM, mg	736	712	765	687	745	696
Nitrogen, mg	118	118	124	107	113	108
Calories	4909	4768	5140	4627	5130	4677

Table II. Experimental Means and (Standard Deviations) of Weight, Length and Time Held in Laboratory of Fish Selected as Controls, to be Trained, or to be Trained and Exercised at Stated Velocities.

Variable	Control	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Number of Fish	98	41	28	27	27	38
Number Succeeding	98	41	28	27	24	3
Initial Length, mm	74.9 (5.3)	74.7 (4.2)	76.0 (3.7)	74.2 (5.1)	75.5 (4.8)	73.7 (3.6)
Final Length, mm	75.2 (5.7)	74.3 (4.2)	76.6 (3.7)	74.4 (5.4)	75.9 (5.1)	73.8 (3.9)
Initial Weight, g	4.571 (1.112)	4.429 (0.913)	4.937 (0.641)	4.574 (1.060)	4.687 (0.900)	4.323 (0.719)
Final Weight, g	4.547 (1.136)	4.423 (0.881)	4.730 (0.612)	4.309 (0.997)	4.521 (0.997)	4.183 (0.686)
Time at Use hrs	835 (356)	681 (343)	640 (250)	613 (335)	765 (414)	765 (390)

Table III. Data Describing Control Population of 98 Coho Salmon.

	Mean	Standard Deviation	Range	$\frac{s}{\bar{X}}$
Time held in Laboratory	635	356	39-1071 hours	.56
Initial Length	74.9	5.3	63-88 mm	.07
Final Length	75.2	5.7	65-88 mm	.08
Initial Wet Weight	4.571	1.11	2.24-8.22 g	.24
Final Wet Weight	4.547	1.14	2.28-8.02 g	.25
Final Dry Weight	0.922	0.287	0.54-1.73 g	.29
Chloroform Methanol Extractable Lipids	0.142	0.078	0.030-0.331 g	.55
Ash	0.115	0.026	0.067-0.189 g	.23
Water	3.564	0.87	2.05-4.81 g	
Nitrogen	0.118	0.032	0.065-0.197 g	.27
Lipid Free Organic Matter (LFOM)	0.736	0.201	0.370-1.286 g	
Caloric Content	4909	1625	2333-8991	.33

change of 20% in lipids, of 10% in nitrogen and 10% in LFOM. Caloric content would have to be altered by 600 cal per fish or more at the 1% level and 455 cal at the 5% level of experimental significance. The change required is 12% of the calories per fish at $p \leq 0.01$ and 9% at $p \leq 0.05$.

The variability problem can be emphasized by a glance at initial length and initial weight data. With respect to initial length all subsamples of Table II (controls and fish to be exercised at different levels) can be considered as random selections from the same population of fish with $p \leq 0.01$ taken as the area of exclusion of non-random samples.

But the following pairs of initial weights in Table II cannot be considered as derived from randomly selected samples of fish from the same population:

4.937 and 4.323	Δ	=	0.614	Standard error	0.18
4.937 and 4.429	Δ	=	0.508	Standard error	0.18

At a level of exclusion of $p \leq 0.01$, these pairs might be taken as indicating a statistically significant experimental difference and yet the difference is already present at the beginning of the experiment, before the salmon have been modified by training or training and exercise.

With the high variability of the wild population of salmon it is obvious that statistical procedures more critical than comparisons of

simple means would have to be developed if prolonged exercise (beyond training plus 24 hours) was to be limited in order to establish experimental differences induced by swimming. Improved statistical treatment of the data was obtained by making use of generally accepted physiological inter-relationships: (1) Weight, length and the biochemical parameters measured are interdependent; hence water, ash, lipid and LFOM are related to body weight and length. (2) Lean body mass (fat free body mass) has a reasonably constant composition: a statement leading to the corollary that water, ash, nitrogen, protein content and LFOM are proportional to one another and to lean body mass. (3) Heat of combustion of organic matter is the sum of the heats of combustion of lipids and lipid free organic matter. First will be discussed relationships to weight and length; second relations between nitrogen and LFOM and last relationships involving calories, LFOM, lipids and nitrogen.

Interrelationships with Initial Weight and Length

Dry weight, water, lipid, ash, LFOM and caloric content per fish can only be determined once and hence a given salmon cannot serve directly as its own control for these parameters. However, wet weight and body length could be determined several times if necessary. Tinsley, Krueger and Saddler (1973) had shown that the mass of specific fatty acids in control fish could be expressed as a function

of wet body weight, length and time held in the laboratory. As body length, body weight, and time held in the laboratory were known for our control fish, and in other fish prior to swimming, biochemical parameters of control fish, such as water, ash, lipid, nitrogen, LFOM and caloric content, could be expressed as simple functions of initial wet weight, initial length, and the time the fish were held in the laboratory; and the equations developed could later be used to estimate pre-exercise levels of the parameters in experimental fish.

The factors used to derive relationships for the control salmon population were: the time held in the laboratory T, T^2, initial length l_o, l_o^2, l_o^3; initial weight W_o, W_o^2, W_o^3; and TW_o. Equations were developed by a stepwise multiple linear regression analysis. The equations obtained are given in Table IV. In such an analysis (Tinsley, Krueger and Saddler, 1973), a regression is run for the dependent variable as a function of the independent variable with which it is most highly correlated. Subsequently, additional independent variables, in the order of decreasing correlation with the dependent variable, are entered into the regression equation in a stepwise fashion until an additional variable produced no further significant reduction in the residual sum of squares.

At each step correlations are developed for the deviations of each value of dependent variable from the predicting equation so far developed, with the corresponding values of each of the remaining

Table IV. Equations Describing Relationships of Salmon Composition to Initial Wet Weight, Initial Length and Time in the Laboratory.

$$W_f = -1.199 + 0.927\,W_o + 0.02\,l_o \quad (1)$$

$$DW = 0.915 - 0.495\,W_o + 2.96 \times 10^{-5}\,TW_o - 0.011\,W_o^3 + 0.157\,W_o^2 \quad (2)$$

$$H_2O = -1.413 + 0.660\,W_o + 0.027\,l_o - 1.896 \times 10^{-5}\,TW_o \quad (3)$$

$$ASH = -0.055 + 3.01 \times 10^{-5}\,l_o^2 \quad (4)$$

$$LIPID = -0.074 + 0.077\,W_o + 1.94 \times 10^{-5}\,TW_o - 4.42 \times 10^{-7}\,l_o^3 \quad (5)$$

$$LFOM = -0.059 + 0.174\,W_o \quad (6)$$

$$NITROGEN = -0.008 + 0.027\,W_o \quad (7)$$

$$CALORIES = -2454 + 2064\,W_o + 0.212\,TW_o - 0.005\,l_o^3 - 5.91\,W_o^3 \quad (8)$$

See section on Materials and Methods for distinctions between initial and final weights.

independent variables; the independent variable with the highest correlation coefficient is the next variable introduced into the equation. In this fashion a multiple regression is obtained which retains the independent variables which provide a significant input. Computing procedures for this type of analysis have been outlined by Efroymsen (1960).

The coefficients of the regression equations were chosen to keep the sums of the squares of the deviations at a minimum. The effect of this treatment was to provide a good fit to the data; however, the magnitude of the coefficient and the order of introduction of the variables would not necessarily have any biological significance. The equations developed were used to predict the magnitude of physiological parameters for other salmon from length, weight and time held in the laboratory, and thus allowed a pre-exercise estimation of the parameters in salmon forced to swim. These could then be compared with direct determinations of the parameters after swimming to obtain estimates of changes induced by physical activity.

Variability

The improvement in statistical treatment from raw means to means adjusted for weight, length and time held in the laboratory is shown by Table V. The variability of the uncorrected means of the lipid content was reduced 50%; of ash 58%; calories and nitrogen 68%;

Table V. Standard Deviations of Raw Means of Data Collected on Control Population of 98 Salmon and Deviations After Adjustments for Initial Weight (W_o), Initial Length (l_o), and Time (T) Salmon Were Held in Laboratory (Table IV).

Variable	Standard Deviation	Standard Deviation After Adjustments for l_o, W_o and T	Ratio
Final Wet Weight	1.137	0.133	.12
Dry Weight	0.287	0.080	.28
Water	0.868	0.122	.07
Ash	0.026	0.011	.42
Lipid	0.078	0.039	.50
LFOM	0.201	0.057	.28
Nitrogen	0.032	0.010	.32
Calories	1625	517	.32

LFOM 72% and water 78%. With a sample size of 50 and the equations of Table IV significant changes in calories, lipids, nitrogen, and LFOM at the $p \leq 0.01$ level were reduced to 3.9%, 10%, 3.2% and 2.9% of the respective averages; without adjustment for initial weight, length and time held in the laboratory the required changes were 12% for calories, 20% in lipids, 10% in nitrogen and LFOM.

Initial Wet Weight, Length and Time Held in Laboratory

An overall view of the multiple stepwise regression equations in Table IV indicates that the initial weight (W_o), in 7 of the 8 equations

listed, was more highly correlated with the parameters described than were length of the fish or time the fish were held in the laboratory. The exception was ash. Ash was more closely related to length of the fish than to body weight. Wet weight was the only factor in the equations for nitrogen and LFOM. The product of time in the laboratory and initial weight (TW_o) was the second factor in the equations for dry weight, lipids and calories and the third in the equation for water. W_o^3 appeared in the equations for dry weight and calories and W_o^2 in the equation for dry weight.

Time in the laboratory alone was not highly correlated with any of the eight parameters of Table IV. But (TW_o) as stated previously, appeared as the second factor in the equations for dry weight, lipid and calories and as the third factor in the equation for water.

Length (l_o) was not involved in the dry weight equation but was involved in equations for wet weight (W_f) and water. Length (l_o) appears in the formulation for organic matter (OM), and length squared (l_o^2) was the only factor involved in the equation for ash. Length cubed (l_o^3) appears in the equations expressing lipids, calories, and organic matter per fish.

The higher correlations between ash and initial length squared rather than between ash and wet weight was unexpected. The relationship between ash and length may mean that the skeletal weight of young salmon depends mainly on the length of the salmon, that skeletal

needs increase more rapidly than skeletal length, but that the skeletal needs are not closely related to variations in the dorso-ventral and sinistrodextral diameters due to variations in lipid content.

Final Length of Salmon

The length of each fish was measured as closely as possible to the nearest mm on removal from the holding tanks. Control fish were killed by a blow on the head and the length again measured. The predicting equation

$$l_f = -3.804 + 1.054\ l_o \qquad (9)$$

gave final lengths within 1% of the initial lengths over the range from 63 to 88 mm. Below 70 mm the final length was predicted as slightly lower and above 70 mm as slightly higher than the initial length. The standard deviation of the observed values from the predicted values was 1.33 mm (See Fig. 2).

Generally, the muscles were more relaxed after death and a final length slightly greater than the initial length was to be expected. Final lengths averaged approximately 0.3 mm longer than initial lengths; hence another predicting equation (operational rather than statistical) was:

$$l_f = 0.27 + l_o \qquad (10)$$

with the slightly higher standard deviation of 1.35. Over the range

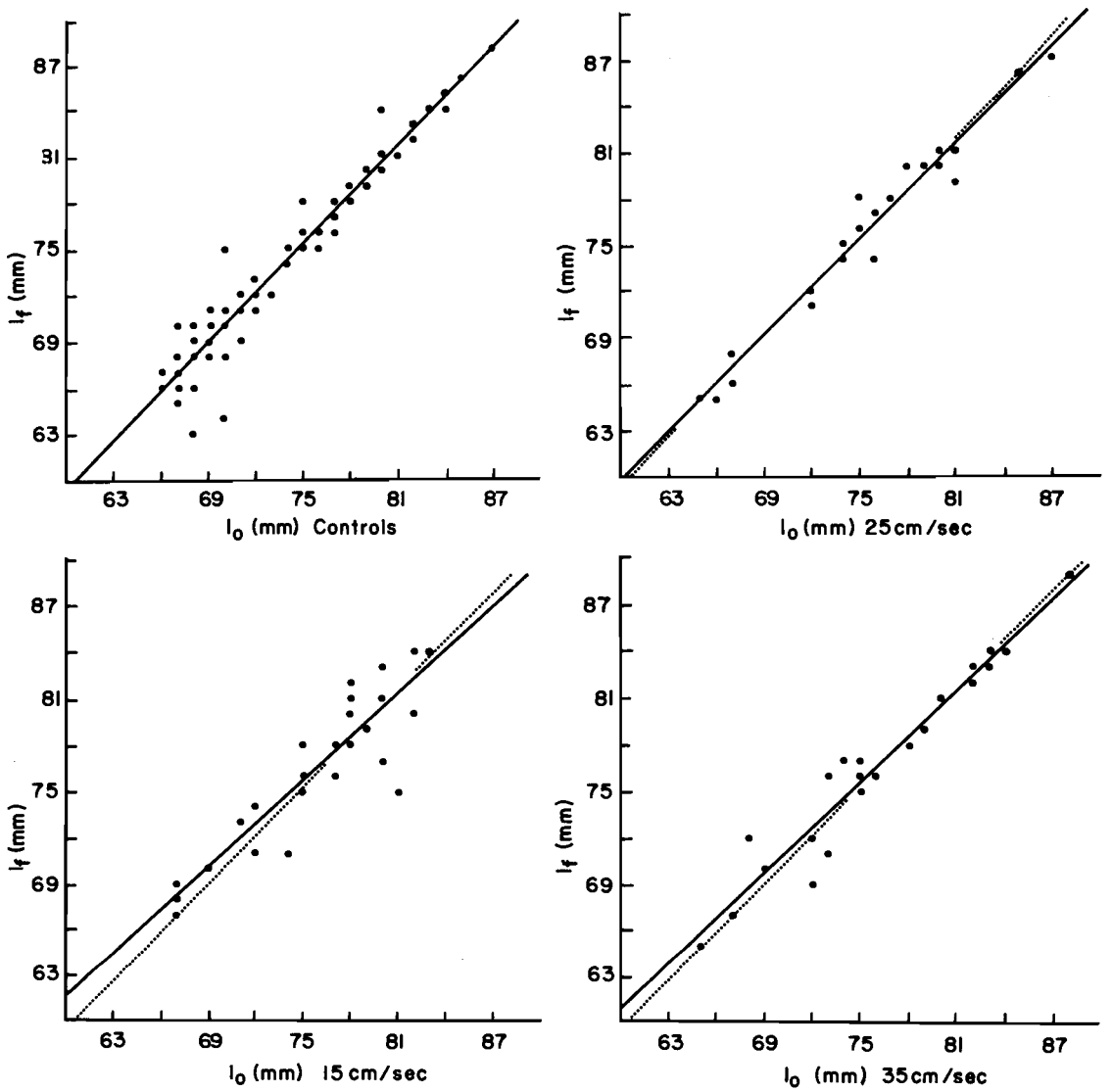


Figure 2. Plots of Final (Ordinate) vs Initial Lengths of Control Salmon or Salmon After Swimming at 15, 25, and 35 cm/sec. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the line expressing the best fit for controls.

from 63-88 mm in length, the lengths predicted by equations (9) and (10) were within 0.6 mm of one another.

Final Wet Weight

The average of the observed initial weights for the 98 control salmon was 4.571 g and the average of the observed final weights was 4.547 g. There was a mean loss of 24 mg between initial and final weights and the standard deviation from the mean loss was 133 mg. While the mean loss was 24 mg, because of the high standard deviation, equation (1) gave 43% of predicted values above the observed and 57% below the observed final values.

The use of equation (1) and Tables IV and V also yields 133 mg as the standard deviation of differences between predicted values of final wet weight and observed values of final wet weights in control salmon.

The relationship between observed initial and observed final weights in control salmon and in salmon after swimming is given in Figure 3. Visually the relationship in controls is essentially linear and is given by a 45^{o} line through the axis. Between two and five grams six initial weights were clearly above the final weights and near six grams two were below the final weights.

The line assuming a constant difference between initial and final weights is given by

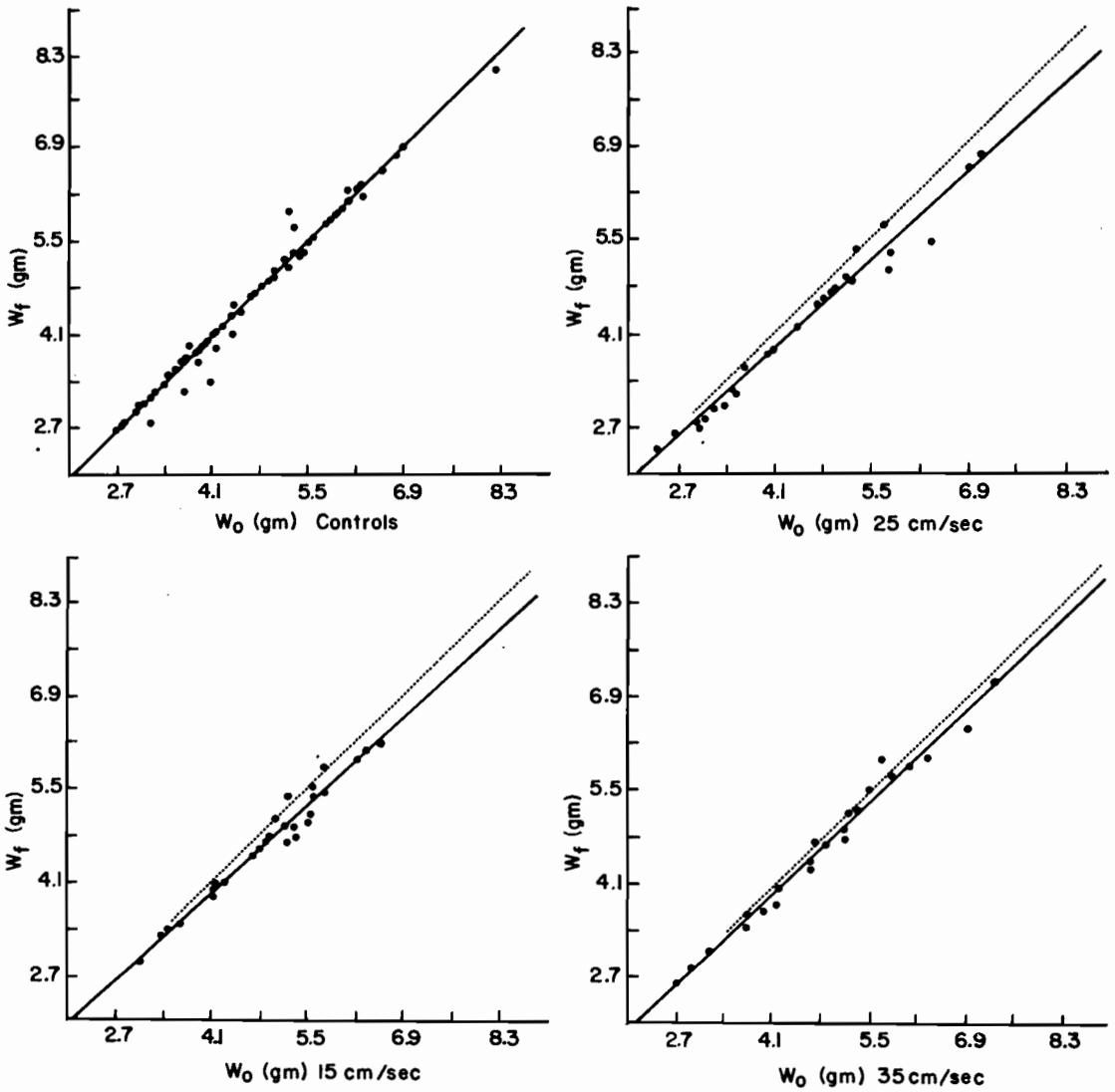


Figure 3. Plots of Final (Ordinate) Initial Weights of Control Salmon or Salmon After Swimming at 15, 25 and 35 cm/sec. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the line expressing the best fit for controls.

$$W_f = -0.024 + W_o \quad (11)$$

and the statistical line giving the best linear fit between initial and final values of weights is

$$W_f = -0.090 + 1.0144\ W_o \quad (12)$$

Equation (12) is associated with a standard deviation of 139, only slightly higher than the 133 mg associated with the more critical equation (1). Equation (12) runs at an angle of 45.4^o with the abscissa.

The final wet weight of control fish as a function of W_o, l_o and T, provides a population of final wet weights in which the mean is the same as before the adjustments. The standard deviation of the final wet weight, after adjustments for W_o, l_o and T, was reduced approximately 88% from 1.149 to 0.133 g (Table V).

Dry Weight

The mean dry weight of the controls was 0.992 g and varied from 0.535 g to 1.728 g. The dry weights of the control population of juvenile coho salmon are described by an equation including powers of initial weight (W_o, W_o^2 and W_o^3) and the product of the time held in the laboratory by the initial weight. The standard deviation of the salmon population was reduced 72% from 0.287 to 0.080 g by the application of the regression equation.

Water Content

Water is given by the difference between wet and dry weight. The mean water content of the control fish was 3.564 g and ranged from 2.05 g to 4.81 g. The standard deviation calculated for the 98 control fish was 0.868 g or 28% of the mean water mass. The ratio of water to wet weight averaged 0.782 and ranged from 0.761 to 0.793 with a standard deviation of 0.019. The ratio of the standard deviation of the water fraction to the mean fraction of water was 0.024. Hence, the mean water mass had an index of variability (standard deviation/water mass) of 28 percent while the water percentage had an index of variability of only 2.4 percent.

The multiple regression equation describing the water mass of the control salmon (Equation 2, Table IV) involves W_o, l_o, TW_o. The initial weight and length are the only adjustment factors which markedly decrease the standard deviation of observed from predicted values. The standard deviation of the water mass was reduced from 0.868 g to 0.142 g by adjustment for W_o alone; to 0.125 g by including l_o and 0.122 g with W_o, l_o and T.

Ash

Ash of control fish ranged 67 to 189 mg with an average of 115 mg and a standard deviation of 26 mg. Ash represents 2.53% of wet weight and 11.59% of dry weight. The standard deviation was reduced

to 11 mg by adjustment for length squared. (See also previous comment on ash under initial length.)

Lipid

The total lipid content is not as accurately described by a predicting equation as are water, ash, nitrogen and LFOM. W_o and TW_o were the first two factors introduced into the regression analysis for lipids, and the third was l_o^3. The mean lipid weight per fish of the control population was 142 mg with the standard deviation of 80 mg; this was reduced to 56 mg when adjustments were made for W_o, to 41 mg when TW_o was added and 39 mg on addition of l_o^3. Some of the variability in lipid content of control salmon was dependent on factors not measured in this experiment; these probably included individual variations in metabolism and in the ability to garner food (see Figure 4) (Brocksen, 1969; G. Chapman, 1969; and Warren and Davis, 1967).

LFOM

LFOM is given by the difference between organic matter and lipid. The mean LFOM mass per control salmon was 736 mg and ranged from 370 to 1286 mg. The standard deviation for the 98 control fish was 201 mg or 27% of the mean LFOM mass. The multiple regression equation describing the LFOM mass of control salmon (Equation 2, Table IV) involved only W_o. The standard deviation of

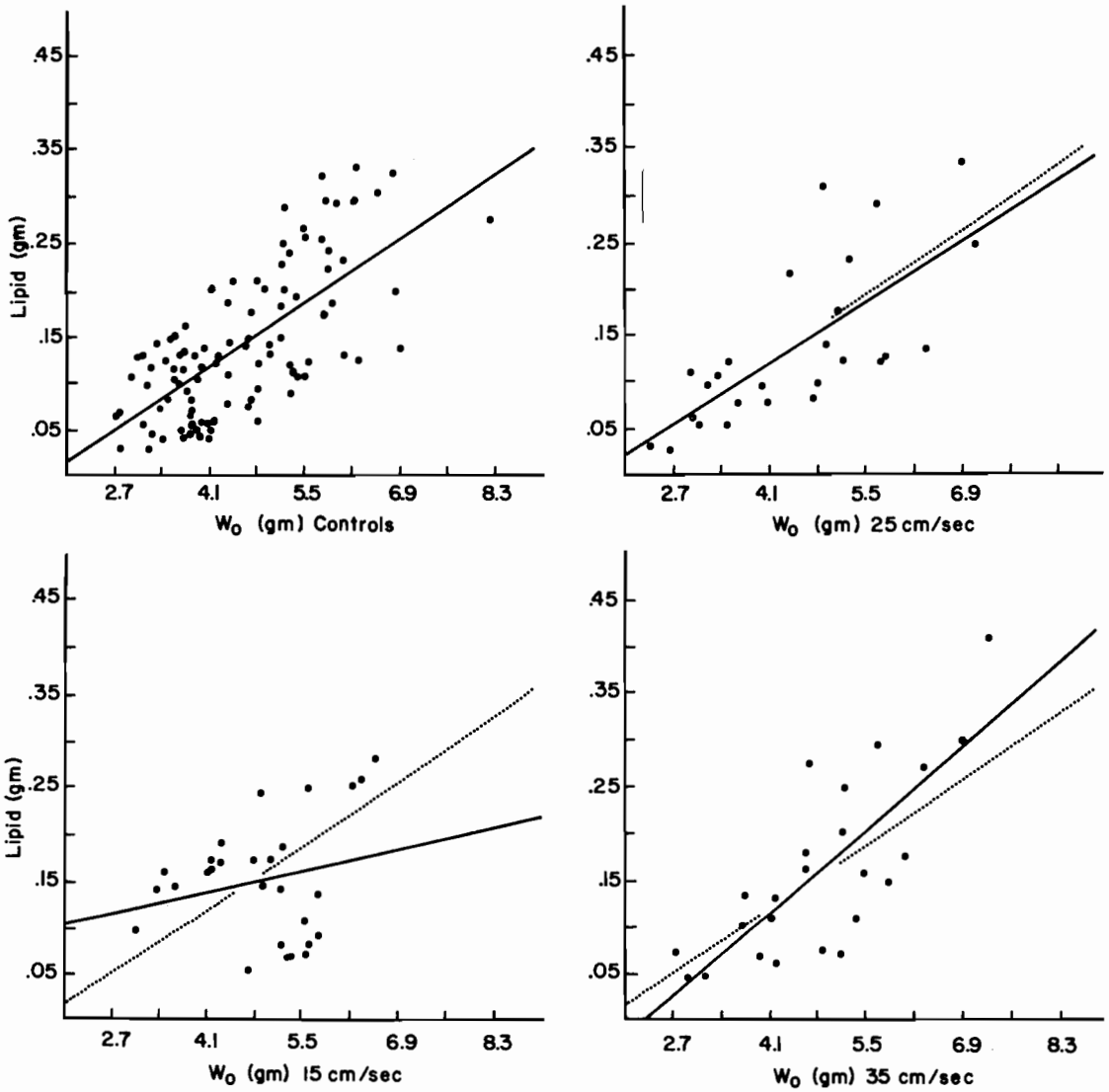


Figure 4. Plots of Lipid Weights Per Salmon (Ordinate) Against Initial Wet Weights. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the best fit for controls.

the LFOM mass was reduced from 201 to 57 mg by the adjustment for initial weight (see Figure 5).

Nitrogen

The mean content per control fish was 118 mg and ranged from 65 to 197 mg. The standard deviation calculated for the 98 control fish was 32 mg or 27% of the mean nitrogen content. Multiple regression equation (7) of Table IV describing the nitrogen mass per control salmon, involves only the initial weight. Initial length and time are not involved. The standard deviation of the nitrogen was reduced from 32 mg to 10 mg by the adjustment for initial weight (see Figure 6).

Heats of Combustion

Multiple regression equation (8) for the total caloric content per salmon included terms for W_o, TW_o, l_o^3 and W_o^3. The mean total heat of combustion per fish was 4909 calories, with a range from 2333 to 8991, and a standard deviation of 1625 calories. The deviation was reduced to 517 by correcting for weight, time and initial length. The use of W_o reduced the deviation from 1124 to 671 or 59%. The addition of TW_o caused a further reduction to 553 calories or an additional 7%. The addition of length cubed gave an adjusted deviation of 534 or an additional one percent reduction; and the addition of weight cubed

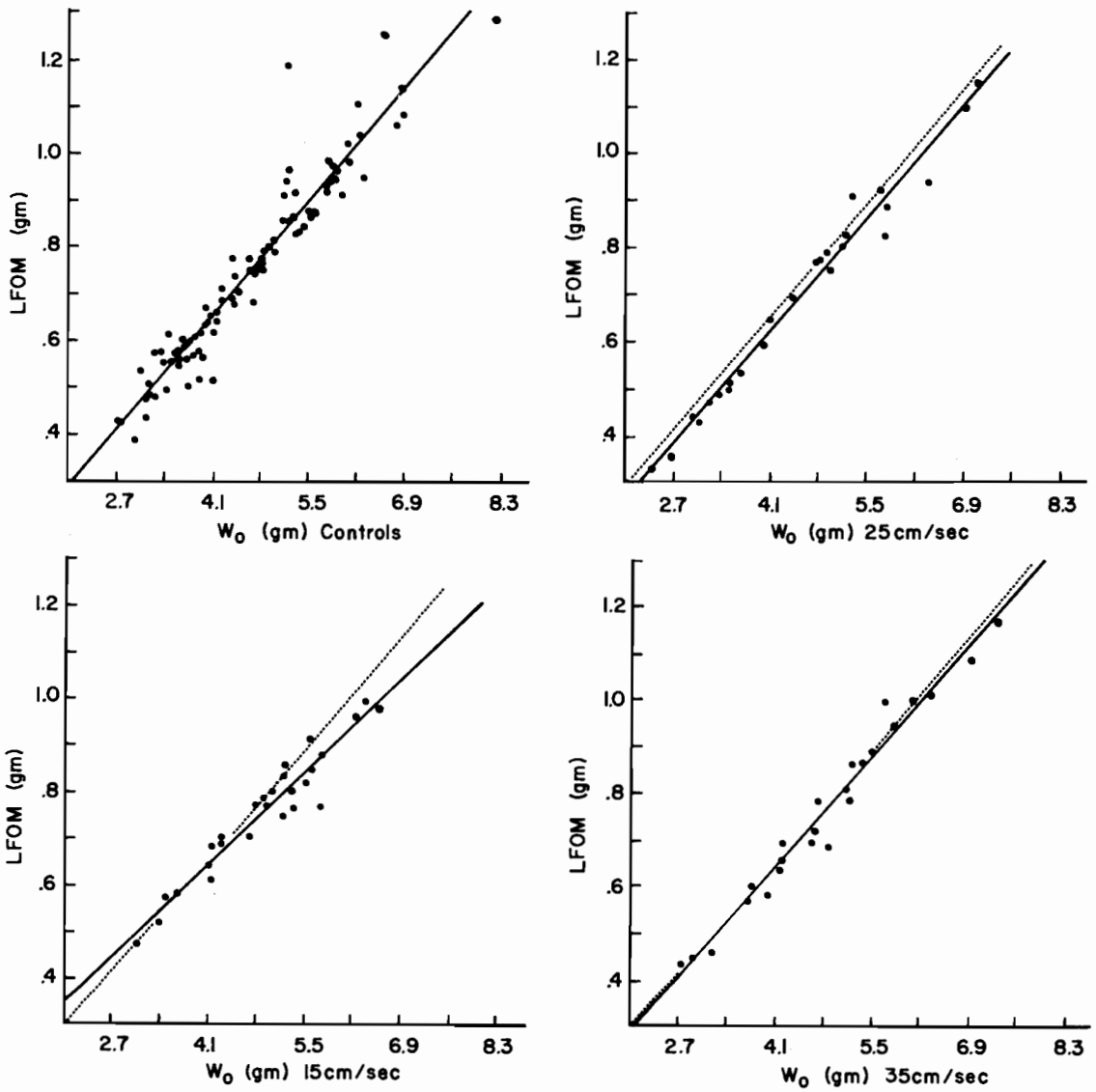


Figure 5. Plots of LFOM Per Salmon (Ordinate) Against Initial Wet Weights. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the best fit for the controls.

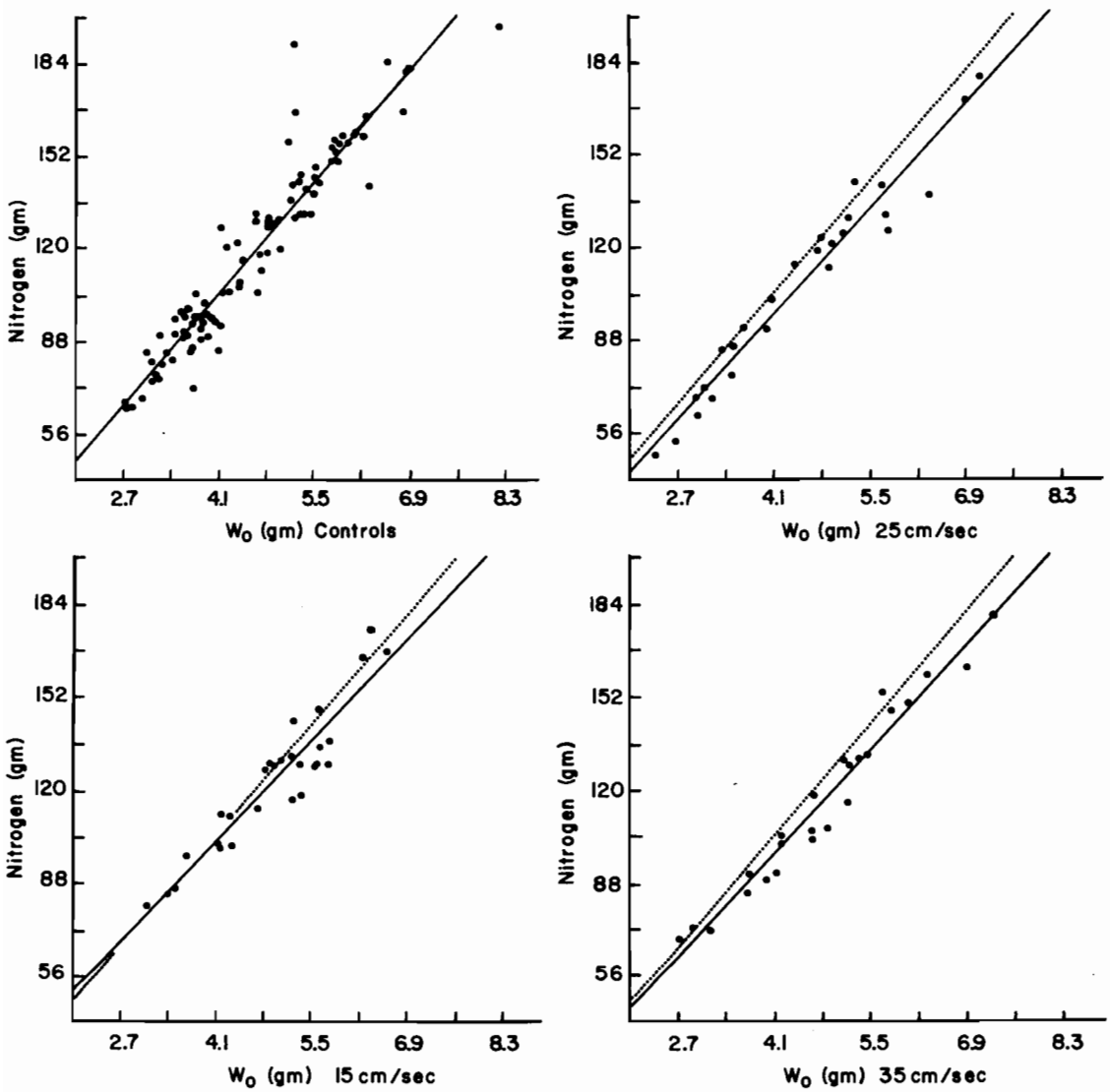


Figure 6. Plot of Nitrogen Per Salmon (Ordinate) Against Initial Wet Weights. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the best fit for controls.

yielded the deviation of 517 or another additional one percent reduction from the 1625 unadjusted standard deviation of the heat of combustion per fish. Most of the decrease in variability with the use of regression equation (8) came from body weight and a very large fraction of the remainder from the addition of the product of weight by time held in the laboratory (see Figure 7).

Relationships Between Nitrogen and LFOM

Many relationships can be developed among the eight parameters considered in Table III, but from the standpoint of bioenergetics, the important relationships are those between LFOM, nitrogen, lipids, and calories. Several simple statistical treatments are available for the relationship between nitrogen and lipid free organic matter. The usual treatment is to assume that nitrogen and LFOM are related linearly as

$$\text{LFOM} = A + B\,(\text{nitrogen}) \qquad (13)$$

and to evaluate A and B so that the sum of the squares of deviations of observed values will be a minimum.

The second and third evaluations are related to the generalization that the composition of the fat free protoplasmic mass, also termed the lean body mass, is constant, and that LFOM and nitrogen are components of the lean body mass.

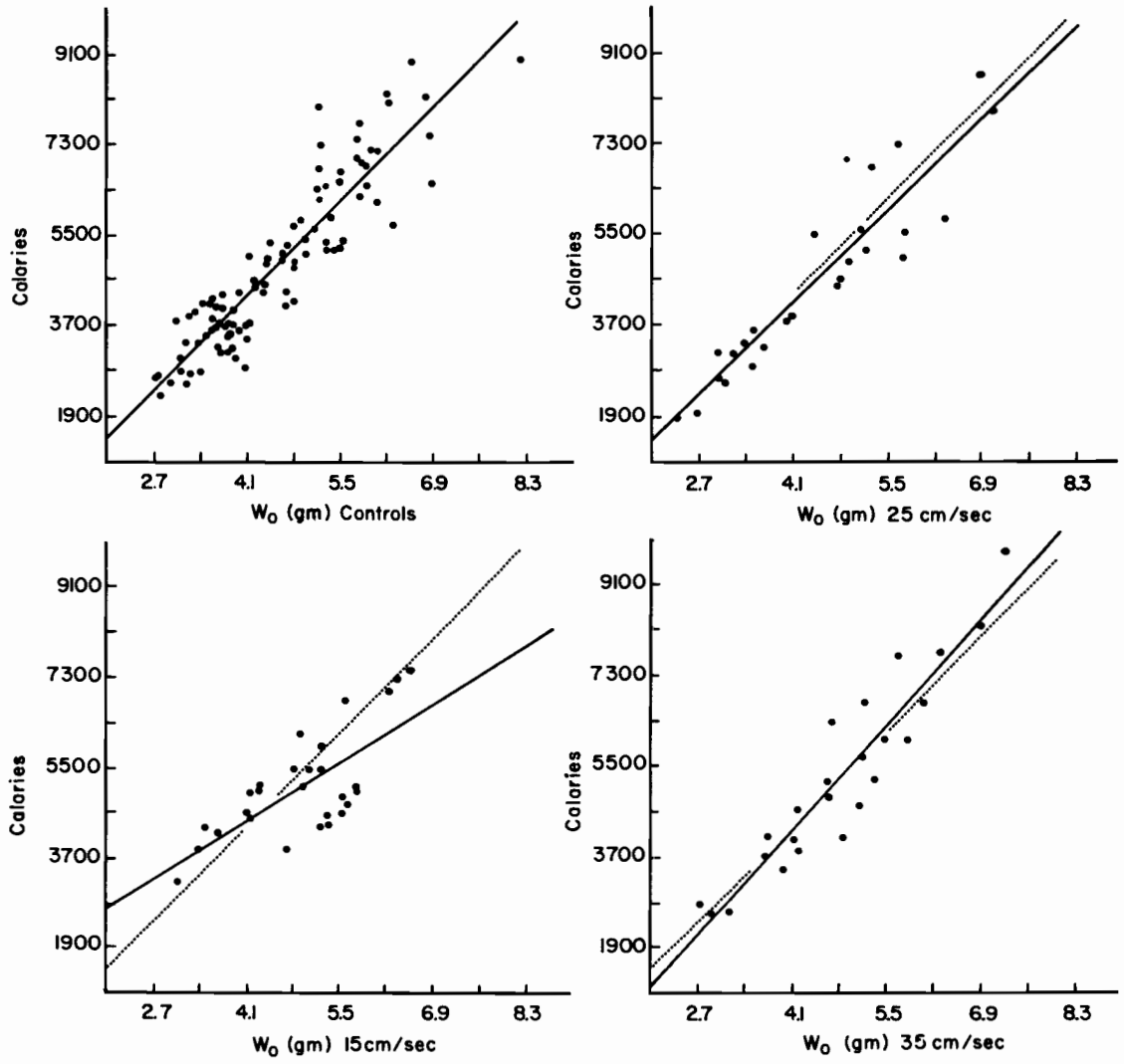


Figure 7. Plots of Heat of Combustion Calories Per Salmon (Ordinate) Against Initial Wet Weights. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the best for controls.

Then

$$\text{LFOM} = K \times \text{Nitrogen} \tag{14}$$

and the ith fish will fit this relationship with a small error.

$$\text{LFOM}_i = K \times \text{Nitrogen}_i + e_i \tag{15}$$

Hence,

$$\sum_1^n \text{LFOM} = K \times \sum_1^n \text{Nitrogen} + \sum_1^n e = K \sum_1^n \text{Nitrogen} \tag{16}$$

if the sum of the errors is set to be zero. Also

$$\frac{\sum \text{LFOM}}{n} = K \sum \frac{\text{Nitrogen}}{n} \tag{17}$$

and

$$\overline{\text{LFOM}} = K \times \overline{\text{Nitrogen}} \tag{18}$$

Thus, if the linear equation, expressing the relationship between LFOM and nitrogen, runs through the origin and the point indicating the mean of nitrogen and LFOM, the sums of the errors in equation (16) will be zero.

A variation on equation (14) leads to the third possibility of establishing the relationship between nitrogen and LFOM:

$$\text{LFOM}_i/\text{Nitrogen}_i = A_i \tag{19}$$

and

$$\sum_1^n \text{LFOM}_i/\text{Nitrogen}_i = \sum_1^n A_i \tag{20}$$

$$\frac{\sum_{1}^{n} \text{LFOM}_i/\text{Nitrogen}_i}{n} = \frac{\sum_{1}^{n} A_i}{n} \qquad (21)$$

and

$$\overline{\text{LFOM}_i/\text{Nitrogen}_i} = \overline{A_i} \qquad (22)$$

With equation (16) the relationship sought is the equation of the line through the origin and through $(\overline{\text{Nitrogen}}, \overline{\text{LFOM}})$. With equation (22) the relationship sought is the average ratio of LFOM/Nitrogen.

Evaluation of the constants in equations (13), (16) and (22) gave the following relationship between LFOM and nitrogen:

		Standard Deviation	
LFOM = 0.0165 + 6.11 Nitrogen		42.7 mg	(13a)
LFOM = 6.250 x Nitrogen		43.0 mg	(18a)
LFOM = 6.246 x Nitrogen		43.0 mg	(22a)
LFOM = 6.264 x Nitrogen		43.1 mg	(22b)

The ratio 6.25 was derived from average values of LFOM and nitrogen; 6.246 from the average of the ratios LFOM:nitrogen; and 6.264 from the reciprocal of the average ratio of nitrogen to LFOM. Equations (13a), (18a), (22a) and (22b) yield values of nitrogen within one mg of each other over range of one to eight gram salmon.

In Figure 8 are plotted 98 points for LFOM (abscissa) and nitrogen (ordinate) from control salmon. The line corresponding to equation (13a) is also given.

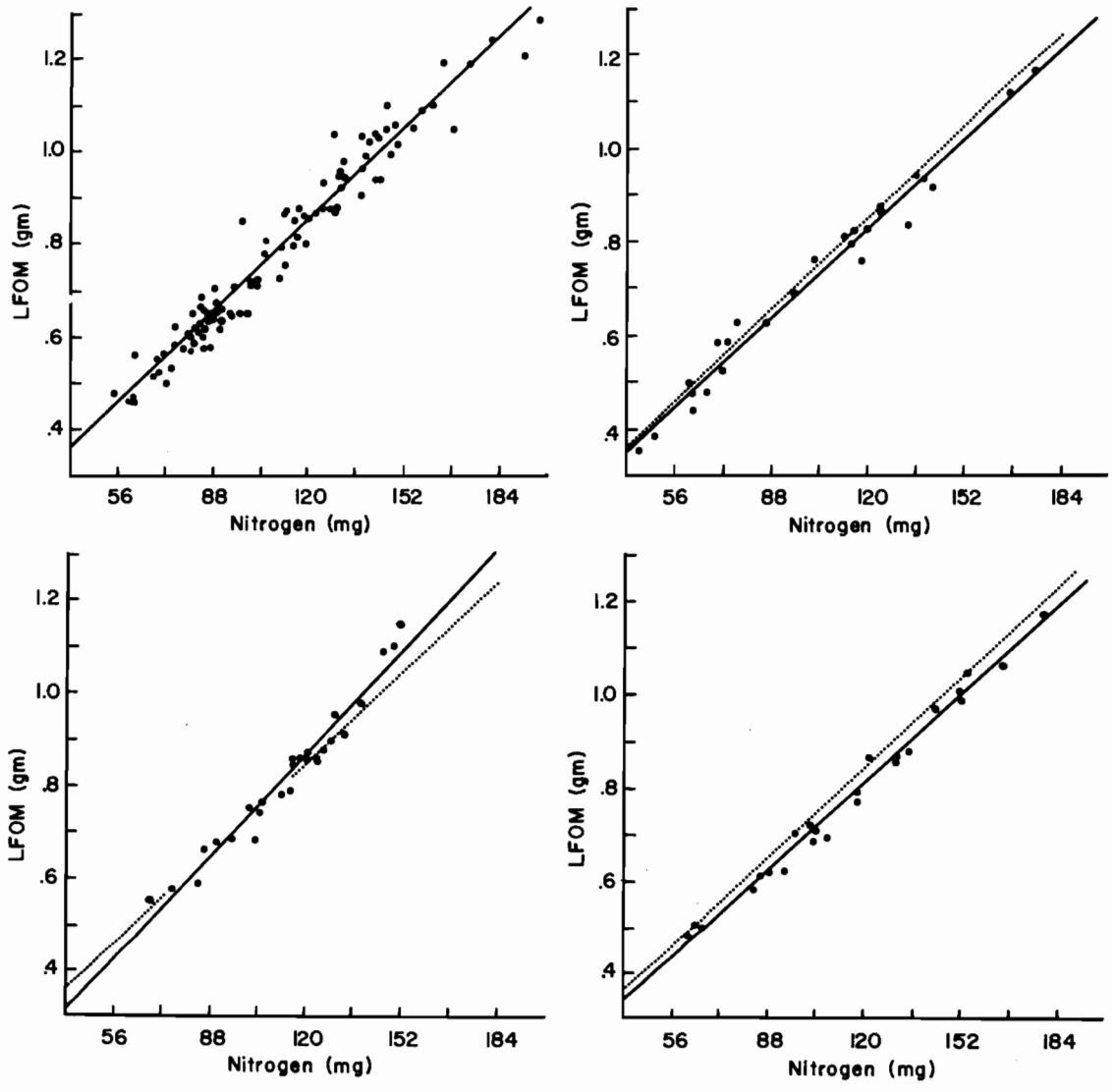


Figure 8. Plots of LFOM Per Salmon (Ordinate) Against Nitrogen Per Salmon. The upper left quadrant gives data from control salmon and the lower left, upper right and lower right quadrants give data respectively per salmon after swimming at 15, 25 or 35 cm/sec. The solid line in each graph is the statistical best fit of the points. The dotted line in three quadrants is a duplication of the best fit for controls.

Calories, Lipid and LFOM

The heat obtained with oxygen bomb combustion procedures is almost entirely dependent on the content of organic matter. Organic matter of fish residues may be divided into a lipid fraction (L) and a non-lipid fraction (LFOM). Caloric content can then be expressed as a function of lipid and LFOM contents:

$$\text{Total Calories} = \text{Lipid Calories} + \text{LFOM Calories.} \tag{23}$$

or as stated in more general terms:

$$H = A \cdot L + B \cdot LFOM \tag{24}$$

where A is the heat of combustion of a gram of lipid and B is the heat of combustion of a gram of LFOM and L and LFOM are the weights of lipid and LFOM per fish or per gram of fish dry weight.

From control fish there were 98 separate salmon where H, L, and LFOM were available. If reasonable values of A and B are known, then for the ith fish

$$H_i = A \cdot L_i + B \cdot LFOM_i + error_i \tag{25}$$

and

$$\sum H_i = A \sum L_i + B \sum LFOM_i + \sum errors_i \tag{26}$$

If A and B are so chosen that $\sum errors = 0$, then

$$\sum H_i = A \sum L_i + B \sum LFOM \quad (27)$$

and

$$\overline{H} = A \cdot \overline{L} + B\ \overline{LFOM} \quad (28)$$

Equation (28) can be satisfied by an infinite series of pairs of values for A and B. However, if the additional restriction is placed that the sum of the 98 squares of the errors, between the values of H_i predicted by equation (28) and the corresponding observed values, should be a minimum, approximations of the true values of A and B can be obtained by a variety of analytical procedures. The solution obtained was

$$H = 8990\ L + 4927\ LFOM \quad (29)$$

with a residual standard deviation of 271 calories for the observed points from the plane described by equation (29). The standard deviation of the coefficient 8990 was 208 calories. The standard deviation of the coefficient 4927 was 40 calories. (Equations derived for control and exercised fish are given in Tables VI and VII.) The 8990 calories per gram is a statistical evaluation of the average heat of combustion of the chloroform-methanol extractable lipid from a freeze-dried preparation and the 4927 calories the average heat of combustion per gram of the associated LFOM.

The lipid and ash free dry weight referred to as LFOM is mainly

Table VI. Equations Describing Relationship Between Calories, Lipid and LFOM. The Statistical Evaluations Give Calories Per Gram of Lipid and Calories Per Gram LFOM. Data are Based on Lipid and LFOM Per Gram Dry Weight of Fish.

H_C	=	8990	x	lipid/g D.W.	+	4927	x	LFOM/g D.W.	(30)
H_T	=	9088	x	lipid/g D.W.	+	4880	x	LFOM/g D.W.	(31)
H_{15}	=	9843	x	lipid/g D.W.	+	4740	x	LFOM/g D.W.	(32)
H_{25}	=	10,343	x	lipid/g D.W.	+	4681	x	LFOM/g D.W.	(33)
H_{35}	=	9396	x	lipid/g D.W.	+	4944	x	LFOM/g D.W.	(34)
H_{45}	=	9303	x	lipid/g D.W.	+	4997	x	LFOM/g D.W.	(35)

Table VII. Equations Describing Relationship Between Calories, Lipid and LFOM. The Statistical Evaluations Give Calories Per Gram of Lipid and Calories Per Gram LFOM. Data are Based on Lipid and LFOM Per Fish. The Data of Table VII are Weighted Proportionately to the Weight of Each Fish.

H_C	=	9182	x	lipid/fish	+	4913	x	LFOM/fish	(36)
H_T	=	9328	x	lipid/fish	+	4868	x	LFOM/fish	(37)
H_{15}	=	9816	x	lipid/fish	+	4741	x	LFOM/fish	(38)
H_{25}	=	10458	x	lipid/fish	+	4670	x	LFOM/fish	(39)
H_{35}	=	9518	x	lipid/fish	+	4952	x	LFOM/fish	(40)
H_{45}	=	9068	x	lipid/fish	+	5042	x	LFOM/fish	(41)

a protein and carbohydrate fraction. The analyses performed in these experiments did not include direct experimental determination of the carbohydrate fraction. The carbohydrate fraction of whole fish is small with reported values ranging from 0.3% (Black, 1958 and 1960; Dean and Goodnight, 1964) to 0.6% (Fraser, Lo and Dyer, 1966) with a value of 3 to 4% for livers from normally fed fish (Hochachka and Sinclair, 1962). As, in the case of small fish, all the material available was needed for other determinations, particularly heats of combustion, hence carbohydrates were not determined.

If the expected amount of carbohydrate present in these juvenile coho salmon was assumed to be 0.58 g % as reported by Fraser, Lo and Dyer (1966) and the caloric value of this carbohydrate fraction was 4100 calories per gram, approximately 24 calories per gram of fish would be derived from the carbohydrate fraction. This amounts to approximately 2% of the caloric content of the salmon.

On this basis equation (30) can be adjusted to account approximately for the carbohydrate fraction. (Corrections should also be made for amino acids and for TCA cycle related metabolites.)

$$H = 8990\ L + (4927\ LFOM - 24) + 4100\ CHO \qquad (30a)$$

$$H = 8990\ L + 4903\ Protein + 24\ Calories. \qquad (30b)$$

Calories, Lipid and Nitrogen

The caloric content per fish of control salmon may also be expressed as a function of lipid and nitrogen content:

$$H = 9553 \times L + 30,189 \times \text{Nitrogen} \qquad (42)$$

with a standard error of the coefficients of 460 and 609 calories respectively, and a standard deviation and standard error of 249 and 25 calories for the observed H values from the plane described by equation (29).

The terms LFOM and nitrogen in equations (36) and (42) are related in a ratio of 6.25. Correspondingly one would expect that the caloric content per gram of nitrogen would be 6.25 times the caloric content of 4913 calories per gram of LFOM or 30,706 cal/g N. The 30,189 cal/g N derived from equation (30) is low by 517 cal/g. The 517 calories or 1.6% deviation reflects the fact that LFOM and nitrogen have a correlation coefficient of 0.977.

Further, the statistical approach for the nitrogen equation would tend to distribute the calories from non-nitrogeneous LFOM between both lipids and nitrogen and give rise to a higher estimate for lipids and a lower estimate for LFOM as 6.25 x nitrogen.

In control fish, the estimated average caloric content per gram of chloroform-methanol extractable lipid was 9182 calories. This is higher than the 8729 calories per gram of ether extracted lipid at the

Oak Creek Laboratory (Chadwick, Chapman et al.; unpublished data.) Their data were derived from the guppy (Lesbistes reticulatus) and the cichlid fish (Cichlastoma bimaculatum). The difference may lie in the possibility that chloroform-methanol extracts greater amounts of high caloric content materials such as fatty acid free residual lipids which may contain sterols from freeze-dry preparations than ether does from an oven-dried preparation or/and there may be species and nutritional differences. Brody (1945) gave a higher heat of combustion of domestic animals of 9300 calories.

The calculated heat of combustion of a gram of lipid for controls in Table VI was 8990 calories. When the data on heats of combustion were weighted for total lipid per fish, a coefficient of 9182 calories per gram was obtained (Table VII). This suggests a greater proportion of extractable sterols were present in the heavier fish. (A list giving the heats of combustion of some physiological compounds can be found in Table IX.)

Alterations in Salmon with Swimming

Fingerling silver salmon had been forced to swim against water velocities of 15, 25, 35 or 45 cm/sec after a training period of 11 hours to give a range of performances including only training, a velocity where the fingerling fish could swim without apparent difficulty and a velocity that only a few fingerling were able to maintain.

Table VIII. Equations Describing Relationship Between Calories, Lipid and Nitrogen. The Statistical Evaluations Give Calories Per Gram of Lipid and Calories Per Gram of Nitrogen. Data are Based on Total Lipid and Total Nitrogen Per Fish. The Data Used are Weighted Proportionately to the Weight of Each Fish.

H_C	=	9,553	lipid/fish	+	30,189	Nitrogen/fish	(42)
H_T	=	10,388	lipid/fish	+	28,011	Nitrogen/fish	(43)
H_{15}	=	8,805	lipid/fish	+	30,419	Nitrogen/fish	(44)
H_{25}	=	10,907	lipid/fish	+	29,502	Nitrogen/fish	(45)
H_{35}	=	9,896	lipid/fish	+	32,096	Nitrogen/fish	(46)
H_{45}	=	9,022	lipid/fish	+	32,463	Nitrogen/fish	(47)

Table IX. Heats of Combustion of Some Physiological Compounds. [International Critical Tables(1929), Kharasch (1928), Brody (1945)].

Compound	Mol. wt.	Kcal/mole	cal/gm
Fatty Acid			
8:0	144	1151	7990
12:0	200	1770	8850
14:0	228	2084	9139
16:0	256	2388	9330
18:0	284	2702	9514
18:1	282	2667	9457
20:0	312	3026	9689
22:0	340	3338	9809
22:1	338	3290	9724
22:2	336	3255	9688
22:6	328	3098	9445
Cholesterol	386	3959	10247
Glycerol	92	395.6	4300
Glycerol esters			
Tributyrate	302	1940	6423
Trilaurate	638	5701	8936
Dibrassidate	732	6947	9490
Tribrassidate	1052	10225	9720
Amino Acids			
Alanine	89	388.5	4360
Aspartic acid	133	384.9	2892
Glutamic acid	147	542.4	3687
Leucine	131	855.6	6526
Lysine	146	756.0	5233
Serine	105	343.7	3270
Valine	117	700.8	5989
Carbohydrate			
Glucose	180	673.0	3750
Glycogen-starch	$(172)_n$	722.4	4200
Citric acid	192	475.8	2478
Lactic acid	90	329.4	3660
Cetyl Alcohol ($C_{16}H_{34}O$)	242	606.0	2504

Metabolic costs during experiments with swimming salmon are expected to be related to the velocity of swimming, the duration of swimming, the initial weights and the initial composition of the salmon. The last two factors could not be controlled as rigidly as desired and hence the occurrence of swimming losses were frequently not establishable and their possible presence was masked by the random and non-random effects of initial weight and composition of fingerling salmon.

The unadjusted averages of data collected on salmon forced to swim at different performance levels have been presented in Tables I and II. On the bases that an individual fish represents a living unit and that many of the few significant differences induced by swimming disappear when control and post-swimming data are compared on the basis of a gram of wet weight, a gram of dry weight or per kilocalorie of fish, the larger fraction of the data is presented on a per fish basis and later summarizing data per gram of wet weight and per gram of dry weight are given in Tables X and XI.

Because there were statistically significant differences between control salmon and salmon chosen for swimming performance testing (even before they were tested), discussions of alterations induced by swimming will depend on data obtained with the equations of Table IV to provide pre-swimming information. Such predicted pre-swimming

Table X. Wet Weight Composition of Salmon. Basic Unadjusted Data Collected on Control and Exercised Coho Salmon Expressed in Terms of Grams Per Gram of Wet Weight.

Variable	Control	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Wet Weight	1.00	1.00	1.00	1.00	1.00	1.00
Dry Weight	0.218	0.217	0.220	0.218	0.222	0.222
H_2O (gm)	0.782	0.783	0.780	0.782	0.778	0.778
Ash (mg)	0.025	0.026	0.025	0.026	0.026	0.026
Lipid (mg)	0.031	0.031	0.033	0.031	0.032	0.030
LFOM (gm)	0.162	0.160	0.162	0.161	0.164	0.166
Nitrogen (mg)	0.026	0.027	0.026	0.025	0.025	0.026
Calories	1069	1064	1094	1070	1114	1112

Table XI. Dry Weight Composition of Salmon. Basic Unadjusted Data Collected on Control and Exercised Coho Salmon Expressed in Terms of Grams Per Gram of Dry Weight.

Variable	Control	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Wet Weight (gm) Final	4.584	4.574	4.566	4.613	4.467	4.508
Dry Weight (gm)	1.00	1.00	1.00	1.00	1.00	1.00
H_2O (gm)	3.626	3.648	3.569	3.619	3.521	3.536
Ash (mg)	0.119	0.121	0.114	0.123	0.117	0.117
Lipid (mg)	0.136	0.141	0.148	0.137	0.141	0.132
LFOM (gm)	0.745	0.738	0.739	0.741	0.742	0.751
Nitrogen (mg)	0.119	0.123	0.119	0.115	0.113	0.116
Calories	4892	4880	4953	4881	4993	4982

values and associated post-swimming data are given in Tables XII, XIII and XIV.

With data available for 98 control salmon and 28 to 41 at each of the performance levels studied, the level of probability separating experimental and chance differences was taken as $p = 0.01$. Differences indicating a level of $0.01 \leq p \leq 0.05$ are usually not considered significant unless other experimental evidence supports their validity.

Performance

As described in an earlier section, the exercise levels to which juvenile coho salmon were subjected included training and exposure to 15, 25, 35 and 45 cm/sec of water velocity. The fish subjected to the training regime and those swimming at 15 and 25 cm/sec were capable of successful performance for the complete test period. Three of the 27 fish at 35 cm/sec were unable to perform and failed in the first four hours of the twenty-four hour test; twenty-four fish in this group were successful. Only five of the thirty-seven fish in the 45 cm/sec group were able to maintain performance for the full twenty-four hour exercise period. Twenty-nine fish failed to swim in the first three hours. Three more failed within a total of five hours.

Final Length

While there were some small differences between the initial

Table XII. Controls and Post-Exercise Experimental Post-Exercise Means (Upper) and Predicted Means for Pre-Exercise Data (Lower). Weights are in grams.

Variable	Controls	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
No. of Fish	98	41	28	26	27	37
Wet Weight	4.547	4.423	4.730	4.309	4.521	4.183
		4.408	4.907	4.531	4.664	4.320
Dry Weight	0.992	0.967	1.036	0.934	1.012	0.928
		0.967	1.102	0.977	1.044	0.955
Water (gm)	3.554	3.456	3.693	3.409	3.529	3.255
		3.449	3.814	3.591	3.650	3.370
Ash (mg)	115.1	113.7	117.6	111.0	117.8	106.7
		114.0	120.0	110.6	121.2	109.4
Organic Matter	0.877	0.853	0.919	0.823	0.894	0.822
		0.850	0.988	0.881	0.923	0.835
Lipid (mg)	141.9	140.7	154.1	136.1	152.7	128.9
		135.8	171.3	140.0	160.3	144.9
LFOM (gm)	0.736	0.712	0.765	0.687	0.745	0.696
		0.710	0.800	0.720	0.750	0.699
Nitrogen (mg)	117.7	118.3	123.8	106.6	112.9	107.6
		113.8	127.7	115.1	120.9	111.5
Calories	4909	4768	5140	4627	5130	4677
		4722	5573	4775	5199	4769

Table XIII. Mean Differences After Exercise. (Observed-Predicted) With Calculated "t" Value. Positive Values are Gains, Negative Values are Gains, Negative Values are Losses During Exercise. Weight Values are in mg.

Variable	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
No. of Fish	41	28	26	27	38
Wet weight, mg	+15	-177***	-222***	-143.4***	-137.1***
	0.40	3.495	3.51	3.208	3.356
Final length, mm	+0.62	+0.31	-0.04	+0.25	-0.05
	1.58	0.51	0.16	0.76	0.20
Dry weight, mg	-0.3	-65.8*	-42.5**	-32.4*	-27.3
	0.297	2.72	2.76	2.10	1.71
Water, mg	+6.9	-121.4**	-182***	-121.1***	-115.4**
	0.235	3.07	3.44	3.35	3.04
Ash, mg	-0.3	-2.4*	+0.4	-3.4	-2.7*
	0.234	2.38	4.0	1.56	2.67
AFDW, mg	-3.2	-68.8**	-58.3***	-28.5*	-13.0
	0.204	2.96	(3.32)	2.03	0.82
Lipid, mg	+4.9	-17.2	-3.9	-7.6	-16.0*
	0.617	1.69	0.46	0.85	2.16
LFDW, mg	-2.3	-43.4**	-37.8**	-14.8	-6.8
	0.224	3.06	3.07	1.46	0.61
LFOM, mg	+1.5	-34.7**	-32.7**	-11.0	-2.7
	0.162	2.80	3.07	1.20	0.27
Nitrogen, mg	+4.5*	-3.9*	-8.5***	-8.0***	-3.9*
	2.42	2.45	3.85	3.68	2.02
Calories	+46.5	-432.9*	-148.1	-68.9	-114.1
	0.463	2.68	1.22	0.59	1.07

* 5% level of significance ** 1% level of significance *** 0.1% level of significance

Table XIV. Significant Material Losses in Juvenile Coho Salmon as a Result of Exercise. The Calculated "t" Values are Given Below Each Loss. Losses are in mg.

Variable	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Wet Weight		-177***	-222***	-143***	-137***
		3.50	-3.51	-3.20	-3.36
Dry Weight		-65.8*	-42.5**	-32.4*	
		2.72	-2.46	2.10	
Water		-121.4**	-181.5***	-121.1***	-115.4**
		-3.07	-3.44	-3.35	-3.04
Lipid					-16.0*
					-2.16
LFOM		-34.7**	-32.7**		
		-2.80	-3.07		
Nitrogen	+4.5*	-3.9*	-8.5***	-8.0***	-3.9*
	+2.42*	-2.45	-3.85	-3.68	-2.02
Calories		-432.9* cal			
		-2.68			

* 5% level of significance ** 1% level of significance *** 0.1% level of significance

lengths of the groups of salmon exercised at different levels of performance (Table I) and also small differences in the final lengths, the groups were not statistically different as far as initial and final lengths were concerned.

The statistical relationship between l_o and l_f for 98 controls was given by equation (9) and that for 81 salmon placed to swim at water velocities of 15, 25 and 35 cm/sec is given by equation (48):

$$\text{Controls:} \qquad l_f = -3.804 + 1.054\, l_o \qquad (9)$$

$$\text{After swimming:} \qquad l_f = -1.658 + 1.027\, l_o \qquad (48)$$

The differences between these two equations are not statistically significant over the range from 63 to 88 mm. Equation (9) predicts final lengths from 0.4 mm below to 0.8 mm above the initial lengths. Equation (48) predicts final length from 0.3 mm below to 0.7 mm above the initial lengths and over the range from 63 to 88 mm values predicted by either equation vary less than 0.4 mm from values predicted by the other. In the controls, on the average, there was no significant difference between initial and final lengths; and the details of this discussion of final length requires the conclusion that initial and final lengths of swimming fish were not altered by 35 hours in the swimming apparatus.

The latter deduction is very important in that it provides data to support the assumptions made by Tinsley, Krueger and Saddler

(1973) that length of experimental salmon was not altered by 35 hours of swimming. Saddler (1967) had not taken initial lengths of his salmon, but he did record final length. On the basis of time held in feeding tanks and of final lengths, Tinsley, Krueger and Saddler developed equations to predict pre-swimming levels of lipids and levels of fatty acids in salmon chosen for swimming.

Final Wet Weight

The 11 hour adjustment or training period induced no significant change in wet weight. But net weight losses (Tables XIII and XIV) of 177, 222, 143 and 137 mg were noted at 15, 25, 35 and 45 cm/sec water velocities in the glass swimming tube. The relationship between swimming velocities and wet weight losses was not linear. The wet weight losses were significant at the $p \leq 0.001$ level.

Dry Weight

Training induced no significant change in dry weight. Average losses of 66, 43, 32 and 27 mg per fish were recorded at velocities of 15, 25, 35 and 45 cm/sec. The loss in dry weight seems to decrease with the intensity of performance. As only the 43 mg loss was significant at the one percent level and the 66 mg and 32 mg losses only at the 5% level, dry weight losses were more variable and sometimes relatively smaller than wet weight losses (Tables XIII).

Water

The mean water loss for salmon (Table XIV) was statistically significant at the $p \leq 0.001$ level for fish after swimming at 25 and 35 cm/sec and at the $p \leq 0.01$ level at 15 and 45 cm/sec. There was no linear relationship of water loss to level of performance, the greatest water loss occurring at 25 cm/sec. Control salmon, trained salmon, and salmon after swimming at 15 and 25 cm/sec, had a water content near 78.2% while the fish after swimming at 25 cm and 45 cm/sec, were only slightly lower at 77.8%. At 25 cm/sec, the salmon lost relatively more dry matter and at 45 cm/sec slightly more water (Tables X, XI, and XII).

The average loss of water mass per salmon during swimming at 15, 25, 35 and 45 cm/sec, was 121, 182, 121 and 115 mg; and amounted to 3.2, 5.1, 3.3 and 3.4% of the water present initially.

The respective water losses were 68.6, 82.0, 84.5 and 84.2% of the total weight losses. Water loss seems to have been an increasing fraction of the total weight loss as the level of performance was increased.

Idler and Clemens (1958) described the changes in water content of migrating sockeye salmon. Their findings are quite complicated over the duration of the migratory journey. Overall losses of proximate analysis components is influenced by a relative gain in water content. Figure 2 in the paper of Idler and Clemens indicates

major differences between male and female salmon. At various times there were large increases in water content as well as large losses at other times or sampling stations. Several authors obtained similar data with both migration and starvation studies (Love, 1970). Losses were reversed with the resumption of feeding. Differences in fish species, duration of the studies, sex, sexual maturity, size and other influences make very difficult the comparisons of the data of others with the data in this thesis.

Data presented by Idler and Clemens (1958) converted to units similar to those used in this thesis suggest a maximum gain in water of 43 mg per fish for the duration of the swimming tests to a loss of 9 mg per fish might have been attributable to depletion changes. The methods of data analysis used herewith do not support nor contradict changes which might be expected due to changes introduced by the depletion of constituent components. From data of Niimi (1972) it can be calculated that a total of 21 mg of water per fish would have been lost if expressed on the basis of the data presented in this thesis. It is suggested that water losses and water balance are a much greater problem to juvenile coho salmon as used in this study than for mature migrating sockeye salmon or largemouth bass.

Lipid

Lipid losses during swimming were obtained at all levels of

performance, but due to the great variability of the lipid data, even after correction for length, initial weight and time held in the laboratory, the differences were not statistically significant even at the $p = 0.05$ level except for the salmon after swimming at 45 cm/sec. At 45 cm/sec the average loss of lipid was 16 mg per salmon. The lipid per gram of dry weight increased from 136 mg in controls to 148 mg in fish after swimming at 15 cm per sec. (Additional comments on lipid will be made under the title of Calories, Lipids and LFOM.)

Data developed from Idler and Clemens (1958) suggest that if salmon in this study used lipids at a similar rate as migrating sockeye salmon losses of approximately 37 mg per fish would have been expected. Losses from 4 to 17 mg of lipid per fish were obtained in this study. Similarly, data calculated from starving bass (Niimi, 1972) for salmon in the present study would require a loss of only 3.4 mg of lipid per fish. One could conclude from these different data that lipids do contribute to the metabolic expenditures of migrating, starving or swimming fish.

LFOM

Losses in LFOM were observed at all levels of performance tested and the losses at 15 and 25 cm per sec. were significant well beyond the $p = 0.01$ level. Losses at 35 and 45 cm per sec were not

statistically significant. The recorded loss in LFOM decreased as the performance level was raised from 15 to 45 cm per sec (Tables XI-XIV). Changes in LFOM per gram of wet weight and per gram of dry weight were not very marked.

Losses of only 4 mg (0.9 mg/g fish) protein in exercised salmon would be expected on the basis of the data of Idler and Clemens (1958) and Niimi (1972). The greater protein losses of 35 and 33 mg (7.4 and 7.7 mg/g fish) per fish at 15 and 25 cm/sec found in this study suggests that the observed differences may be the result of a higher intensity of activity in the exercised salmon. In support of this explanation, Kutty (1972) showed an increasing loss of nitrogen with increasing duration of activity, ranging from 1.8 mg (0.4 mg/g fish) nitrogen after one hour to 5.8 mg (1.3 mg/g fish) nitrogen after six hours. These nitrogen losses are equivalent to 11 to 36 mg protein loss.

Nitrogen

The main bioenergetic difference obtained between salmon after training and control salmon was in the nitrogen per fish (Tables XIII and XIV). Strange to say, while the probability of the difference having occurred by chance was 1 in 64, the difference was an increase of 4.5 mg of nitrogen per fish. As the possibility of a dietary increase in nitrogen seems remote under the experimental conditions chosen,

and as other data lend little statistical or physiological support for verification of the increase, and as the difference was not statistically significant at the $p \leq 0.01$ level, inadequate sampling may have been involved. This is verified by the fact that the 22 salmon selected as controls, along with the salmon simultaneously selected for training had an average content of 3.0 mg above the average of 118 mg of nitrogen found in the total control sample.

Additional support of a special selection problem with the trained fish is given by the average time in the laboratory of 635 hours (Table II) for controls and 681 hours for the trained salmon. These differences were not statistically significant but a difference of almost two days more in the feeding tanks has a definite biological significance. Also, the mass of nitrogen per gram of dry weight was unusually high at 123 mg in salmon after training. Hence, because of the low statistical significance of the 4.5 mg difference in nitrogen per fish, of the 3.0 mg higher nitrogen content of concomitant controls, and of the 46 hours longer feeding period, it is felt that the gain of 4.5 mg nitrogen by the salmon during training was a selection artifact.

Losses of nitrogen were obtained at all four velocities studied. The losses of 8.5 and 8.0 mg sustained at velocities of 25 and 35 cm/sec were statistically significant at the $p \leq 0.001$ level and the losses of 4 mg at both 15 cm and 45 cm/sec were statistically

significant at the $p \leq 0.05$ level (Table XIII). The nitrogen per gram of wet weight after swimming (Table X) was less in salmon after swimming at 25, 35 and 45 cm/sec than in controls.

As stated in the section covering changes in LFOM, Kutty (1972) suggested increasing losses of nitrogen with increasing time of activity. This and other data were insufficient to extrapolate to 24 hours of forced activity. Further discussion of the relationships between nitrogen and LFOM is included in a later section.

Heats of Combustion and Caloric Losses

Losses in caloric content per fish with swimming (Table XIII) were recorded at all levels but statistically significance was reached only at 15 cm per sec and then not even at the $p = 0.01$ level.

The average caloric content per fish after swimming varied from the 4909 cal in controls to 5140 cal in salmon after swimming at 15 cm per second and 4627 cal at 25 cm per second. The differences were little dependent on the intensity of effort in swimming but were due mainly to differences in initial weight and the period the salmon fingerlings were retained in the laboratory (Tables I and II). For similar reasons calories per gram of wet weight after swimming were above the average of 1080 for controls at 15, 35 and 45 cm/sec and reached values of 1094, 1114 and 1112 respectively. Likewise, the average calories per gram of dry weight (Table XI)

was 4892 for controls but 4953, 4993 and 4982 after swimming at 15, 35 and 45 cm/sec, at 25 cm/sec the value was 4881 calories. Again the higher values were dependent on initial weights and time in the laboratory and not on the swimming or swimming velocity.

Calories, Lipid and LFOM

Our most important contribution to the physiology of swimming in fish was the suggestion that the heats of combustion of the methanol-chloroform extractable lipid was increased and the heat of combustion of LFOM was decreased (Table VI) during swimming. The heat of combustion per salmon for controls averaged 4909 calories at an average weight of 4.547 grams per salmon. The standard deviation of the heat of combustion was 1625 cal, and this was reduced to 517 calories by adjustment for length, initial weight and time held in the laboratory (Table V). Equation (36) of Table VII:

$$H = 9182\ L \times 4913\ LFOM \qquad (36)$$

fitted the control data very closely and the standard deviation of the control values from the plane described was only 90 calories.

When equation (36) is applied to caloric, lipid and LFOM data from salmon after swimming, the predicted average heat of combustion was very close to the observed heat of combustion for salmon after training or after training plus swimming for 24 hours at 25

cm/sec. At 15 cm/sec, equation (36) gave a predicted average heat of combustion 33* calories too low and at 35 and 45 cm/sec, values of 68*** and 74*** calories too high. In three of the five performance levels, equation (36) does not fit the heat of combustion-lipid-LFOM data although it fits the data from controls very well.

The standard deviations of the H values from the planes described in equations (30-35) (Table VI) are 271, 274, 254, 271, 280 and 253 calories for controls, trained, 15, 25, 35 and 45 cm/sec; and for the equations (36-41) (Table VII) are respectively 90, 87, 43, 51, 96 and 80 calories per gram of lipid. Because of the better fit, mainly, the equations of Table VII are used in further discussions. Making assumptions in a manner similar to the multiple regression equation for the control data, the heats of combustion of lipids increased from 9182 to 9328, 9816 and 10,458 from controls to trained and to salmon after swimming at 15 and 25 cm/sec and then dropped to 9518 and 9068 at 35 and 45 cm/sec. The alteration in the heats of combustion of the lipids were not linear with velocity. Heats of combustion of the LFOM decreased from 4913 to 4868, 4741 and 4670 from controls to trained salmon and salmon after swimming at 15 and 25 cm/sec and then increased to 4952 and 5042 in salmon at 35 and 45 cm/sec.

Lipids contributed 26.5% to the calories remaining in control salmon, 27.6 in trained salmon and 29.4, 30.7, 28.4 and 25.0%

for salmon after swimming at 15, 25, 35 and 45 cm/sec. LFOM contributed 73.7, 72.3, 70.6, 69.3, 71.4 and 75.0% of the calories respectively.

Lipid

An increase in the heat of combustion of the lipid extracted from salmon after swimming would require that low caloric lipid had been expended and/or that high caloric lipid material had been released from the LFOM fraction, and that during swimming, the caloric properties of the extractable lipids and of the LFOM had been altered. The alterations in lipid and LFOM heats of combustion imply that during exercise there is a liberation or formation of high caloric content material in chloroform-methanol extractable lipid material from LFOM, i.e. a liberation or formation of lipid material not extractable from control salmon.

Chadwick and Chapman (1968) and others at Oregon State University Oak Creek Fisheries Laboratory have shown heats of combustions of approximately 8729 cal/g for lipid fractions obtained by ether extraction of the dry matter (oven dried) from guppy (Lebistes reticulatus). Brody (1945) gave the heats of combustion for fats containing 16 to 18 carbon fatty acids as 9400 cal/g. The 8729 cal/g value would indicate a lipid residue low in high molecular weight fatty acids and complex lipids. The 9182 cal/g derived in equation (36)

would reflect a lipid with an increased content of more high molecular weight fatty acids plus sterols or complex lipids.

An increase in the heat of combustion per gram of lipid can be explained by an absolute or relative disappearance of low molecular weight fatty acids, by partial saturation of some double bonds, and an increase in the absolute or relative amounts of sterols with high heats of combustion. The 9182 and 9328 calories of equations (36) and (37) are reasonable values for a mixture of triglycerates with heats of combustion ranging from 8936 to 9800 calories with about 19% sterols (see Saddler, 1967). A heat of combustion of cholesterol was determined by Bertholet and Andre in 1899 to be 10,247 cal/g. This figure was modified later by Markley (1947) to be approximately 10,300 cal per gram of cholesterol.

Saddler (1967) using 7.9 g salmon swimming at 52 cm/sec indicates greater fractional losses of 14 and 16 carbon fatty acids (24%), than of the 18 and 20 carbon fatty acids (21%), and of the 22 carbon fatty acids (8%). Therefore, the heat of combustion of the resulting lipids would be increased. A 9816 cal/g lipid obtained in equation (38) describes a lipid mixture in which the content of sterols would have to be increased three-fold with the fatty acid composition remaining fixed as in the control composition, or a concurrent change in fatty acid composition would have to occur at the same time if the sterol increase were less than three-fold. Saddler (1967) suggests

an increase of 60% in sterols would be possible. If this increase were the case, then the resulting sterol free lipid would contain a lipid mixture with a heat of combustion of approximately 9600 cal/g lipid. A lipid mixture with a 9600 cal/g heat of combustion could not contain significant masses of compounds smaller than 16 or 18 carbon fatty acids. These changes in fatty acid content due to exercise are within approximately 100 calories/g of the estimated heats of combustion of exercised salmon fatty acids (Saddler, 1967). The 10,458 cal/g of equation (39) would require an even less heterogeneous mixture of high molecular weight, saturated fatty acids and sterols with few lipids of low molecular weight.

It is difficult to explain any heat of combustion of lipids above 9660 calories per gram. This figure is derived from literature values of 9514 cal per gram of stearic acid, possibly 9474 for glyceryl tristearate and 10,300 cal per gram of cholesterol with fatty acid free residual lipid containing sterols estimated at 20% of the lipid extract (Table XIX). Idler and Bitners (1958) have shown a 26% increase of total cholesterol and a 36% increase of free cholesterol in plasma during the migration of sockeye salmon (O. nerka). Idler and Tsuyuki (1958) in similar studies found high levels of free cholesterol at 261 mg% and total cholesterol of 572 mg% in migrating salmon. The normal levels of total cholesterol in warm blooded animals is 150-200 mg% (Starling, 1930). Thus, an increased sterol content

and altered fatty acid composition of fish lipids could explain most of the observed increases in the heats of combustion of lipid extracts.

A chemical explanation for the high heat of combustion may lie in the possibility that some lipid was lost in the methanol-water layer discard. If total lipid values per fish are higher than those recorded from the choloroform extraction, and the higher lipid values had been used, the equations would have given lower values for the heats of combustion of the lipids (Table IX).

LFOM

As the characteristic of the lipids apparently changed with an increase in the caloric content per gram from 9182 in controls to 10,458 calories after swimming, the caloric content of the LFOM dropped from 4913 calories per gram to 4868 with training, and to 4741 and 4670 at 15 and 25 cm/sec. The caloric content increased at 35 cm/sec to 4952 calories and to 5042 calories at 45 cm/sec (Table VII). There were significant losses in LFOM of 35 and 33 mg after swimming at 15 and 25 cm/sec. An 11 mg loss of LFOM at 35 cm/sec, and a 3.9 mg loss at 45 cm/sec (both not statistically significant) were obtained. The ratios of LFOM lost to nitrogen lost were 8.9, 3.8, 1.4 and 0.8 at 15, 25, 35 and 45 cm/sec. The nitrogen losses were relatively greater than the LFOM losses at 25, 35 and 45 cm/sec, and at 15 cm/sec the LFOM loss was relatively greater

when compared to control data.

LFOM is a complex mixture of nitrogenous compounds and non-nitrogenous compounds. The nitrogen containing compounds include proteins, organic bases, nucleic acids, amino acids, purines, pyrimidines and nitrogen waste products. The non-nitrogenous fraction of LFOM includes components of events in glycolysis, the TCA cycle, other carbohydrate residues from deamination of amino acids, carbohydrates and perhaps a lipid fraction not extractable by a mixture of chloroform-methanol. The non-extractable lipid fraction might include phospholipid compounds and sterols.

Nitrogen and LFOM

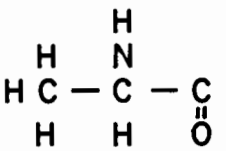
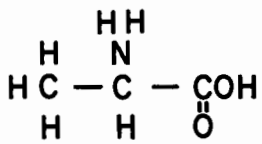
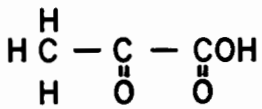
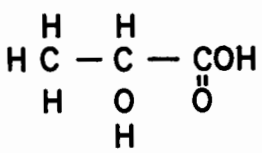
The ratio of LFOM:nitrogen was 6.25 in controls with a standard deviation of 43 mg for LFOM per salmon. The ratio in salmon after training was 6.026**. While this value differs from controls, the low final ratio was possibly partly due to the unusually high level of nitrogen in the salmon before training (see p. 67). The ratio of 6.198 in salmon after swimming 24 hours at 15 cm/sec was not statistically different from controls. Ratios of 6.447*, 6.606*** and 6.478* after velocities of 25, 35 and 45 cm/sec, reflected the loss of nitrogen relatively greater than that of LFOM. Again, the changes in the ratio of LFOM:nitrogen were not linearly related to velocity or level of performance required.

Non-protein nitrogen of blood plasma (Sterling, 1930) of man is given as 0.03%. Brett (1969) reported similar concentrations of non-protein nitrogen in salmon. This suggests that the non-protein nitrogen of a five gram fish could lose only 1.5 mg of non-protein nitrogen and that loss of more than 0.5 mg of nitrogen from a salmon during exercise would require the breakdown of protein. If the protein lost was from intracellular membranes such as mitochondria or endoplasmic reticulum, fatty acids, sterols and amino acids would be released. Such a release could account for the increased heats of combustion of the lipid extracted by chloroform-methanol from salmon after swimming.

Alanine

Table XV reflects some of the mass and caloric alterations to be expected as protein, the main component of LFOM, is metabolized. The data in this study describes the loss of nitrogen with a less than proportional quantity of LFOM being lost due to exercise. This would suggest the hydrolysis of protein with the deamination of the resulting amino acids and the retention of the carbon fragments. These changes would lead to a decreased caloric content with a possible increase in the mass of LFOM. A peptide containing the amino acid alanine has been chosen as a descriptive example. The skeleton of alanine included between two peptide linkages, has a molecular

Table XV. Chemical and Bioenergetic Changes in Alanine (Large Calories).

	Molecular Weight	Molar Heat of Combustion	Heat of Combustion Per Gram
	71	395.5	5.57
			4.91*
	89	388.5	4.36
	88	279.1	3.17
	90	329.4	3.66

* The LFOM of controls had a heat of combustion of 4.91 calories.

equivalent of 71 and the alanine released by hydrolysis, a molecular weight of 89 with a heat of combustion of 388.5 calories per mole (Table IX). If each peptide bond has a free energy of formation of 7 large calories (Leninger, 1970), the heat of combustion of the molar equivalent of the alanine skeleton in protein would be 388.5 + 7 or 395.5 calories. On a per gram basis, the skeleton would have a heat of combustion of 5.57 large calories and alanine of 4.36 calories. LFOM from salmon residues, a mixture of proteins and molecules with lower heats of combustion than protein, had an average heat of combustion of 4.91 large calories per gram. Nitrogen is 15.7% of the mass of the alanine. A polypeptide composed of (n) alanine units would be approximately 19.7% nitrogen; one molecule of water was withdrawn in the formation of each peptide linkage of the alanine polypeptide. When proteins are hydrolized, due to addition of water, the mass of the amino acids released is greater than that of the protein. With the ratio of LFOM:N of 6.25, the average amino acid skeleton in salmon proteins would be in the vicinity of 87.25 and the addition of 18 for the water molecule taken up in hydrolysis would yield 105.25. On hydrolysis, there is a possible increase of 20.6% in the weight of the protein hydrolyzed.

When alanine is converted by oxidative deamination, the molar heat of combustion of alanine of 388.5 calories is reduced to the 279.1 molar heat of combustion of pyruvic acid. Conversion of pyruvic acid

to lactic acid yields an increase in the molar heat of combustion to 329.4 calories.

Considerable energy is released on going from alanine to pyruvic acid and less from alanine to lactic acid via pyruvic acid. On leaving protein the mass of the amino acids, and their end products through pyruvate or lactate lead to an increase in mass of the products but to decrease in the heats of combustion per gram from 5.57 to 4.36 to alanine; from 4.36 to 3.66 from alanine to lactate and 4.36 to 3.17 from alanine to pyruvate.

Reconciliation

A series of relationships have been developed to reconcile the changes that occur among the parameters of juvenile salmon. By defining X as the content per fish of a parameter, and letting A be equal to the content of the parameter per unit of weight (The unit can be for wet weight, dry weight, lipid weight, LFOM weight or others.) before swimming and B equal to the content of the parameter per gram of wet weight after swimming, algebraically:

$$\Delta(\text{mass of fish}) = W_o - W_f = D \tag{49}$$

$$\Delta X = A \times W_o - B \times W_f \tag{50}$$

Substituting,

$$\Delta X = A(W_f + D) - B \times W_f \tag{51}$$

Simplifying,

$$\Delta X = (A - B)W_f + A \times D \qquad (52)$$

The term AD of equation (52) represents the loss in a particular parameter associated with the loss in weight on the assumption that the composition of the fish had not changed. (A - B) represents the change in the composition of X per unit of the remainder. (A - B) times W_f would then represent a loss in a particular parameter which is due to the change in composition of the exercised fish if B is less than A. If B is greater than A, then the (A - B) would describe a relative gain in the parameter in the remaining mass. If the changes in the mass of a variable are in direct proportion to the initial mass only, the total change in the variable would be accounted for in the AD term of equation (52).

The heat of combustion per gram of the LFOM decreased during training, and there was an increase in the LFOM mass to yield a loss of 32 calories associated with the change in composition and a gain of 7 calories with the increased mass. There was a net loss of 25 calories and the loss in LFOM calories gave a net calculated gain of 40 calories (next to last line of Table XV). The calories predicted equation (8) for the fish selected for training (before swimming) were 4722 and the observed calories after swimming 4768 or a difference (gain) of 47 (last line of Table XVI).

Table XVI. Reconciliation of Changes Among LFOM, Lipid and Calories During Exercise. Gains in LFOM, Lipid or Total Calories are Indicated by Positive Values. Losses in LFOM, Lipid or Total Calories are Indicated by Negative Values. All Values are Calories Per Fish.

	Trained	15 cm/sec	25 cm/sec	35 cm/sec	45 cm/sec
Caloric changes with changed composition of lipid	+20	+ 98	+174	+51	- 15
Caloric changes from change in lipid mass	+45	-158	- 36	-70	-147
Total caloric changes for lipids	+65	- 60	+138	-19	-162
Caloric changes with changed composition of LFOM	-32	-132	-167	+29	+ 90
Caloric changes from change in LFOM mass	+ 7	-170	-160	-54	- 25
Total caloric changes for LFOM	-25	-302	-327	-25	+ 65
LFOM plus lipid changes	+40	-362	-189	-44	- 97
Caloric changes based on heats of combustion (pre- and post exercise)	+47	-433	-148	-69	-114

Similar descriptions can be read from Table XVI for fish selected to swim at 15, 25, 35 and 45 cm/sec.

Reconciliation of changes in the fish mass and composition, particularly for lipids and LFOM, can be developed to describe the caloric changes brought about by exercise (Table XVI). No attempt has been made to attach statistical significance to the data of Table XVI or to use only statistically significant data. We were merely trying to see how closely our derived information and concepts fitted to data available in this thesis. Tables XII and XVI indicated that in the trained salmon the heat of combustion of the lipids and the mass of lipids was higher than in controls. Associated with the changed heat of combustion (which requires a change in composition), was an increase of 20 calories and with the change in lipid mass, an increase of 45 calories to give an increase of 65 calories in the lipid per salmon after swimming as compared with control salmon (Table XVI). A consideration of the wide variability of the data for calories, lipid and LFOM and the generally small differences between data in the last two lines of Table XVI suggests that the manipulations used and the concepts developed fit reasonably well the data presented.

Velocity and Performance

Although difficult to evaluate and interpret, data on lipid, LFOM, nitrogen and calories per fish and the derived data on heats

of combustion per gram of lipid and per gram of LFOM are internally consistent at each level of performance. The statistically significant data on alterations induced by swimming have been emphasized. Some alterations, not statistically significant, have been pointed out and used. Undoubtedly among the items studied, there were many alterations not established, but one can conclude that if alterations existed they were not large.

Tables XVII and XVIII give a summary of the alterations observed in salmon after swimming 11 hours during a training period plus 24 hours more at 15, 25, 35 or 45 cm/sec. Data on training alone are not given because the changes were small and not statistically significant. Table XVII gives basic data at all velocities, but at 35 and 45 cm/sec, information is given separately for fish swimming for 24 hours, those unable to swim for 24 hours, and all of the fish placed to swim at 35 and 45 cm/sec. The breakdown data are used to develop Table XVIII comparing failing and swimming salmon.

Presumably, the metabolic cost of swimming for juvenile coho salmon should be related to the velocity and kinetic energy attained, the duration of activity and the distance traveled. Using the relationship that kinetic energy is equal to one-half the mass times the velocity squared, one would expect the energy expenditure to have an ascending curvilinear relationship with the velocity (Averett 1968; Bainbridge 1960 and 1962; Brett 1964, 1965 and 1967; and Winberg

Table XVII. Summary of Alterations Induced by Swimming at Velocities of 15, 25, 35 and 45 cm/sec.

	15 cm/sec	25 cm/sec	35 cm/sec			45 cm/sec		
			All	Succeeding	Failing	All	Succeeding	Failing
Number of Salmon	28	26	27	24	3	37	5	32
Equivalent Distance Traveled, km	18.8	27.6	34.0	36.9	9.3	14.7	46.2	9.8
Loss in Water, mg	121	181	121	120	132	115	275	90
Loss in Lipids, mg	17.2	3.9	7.6	4	36.7	16	16.4	16.0
Loss in LFOM, mg	35	33	11	14	gain 13	2.7	41	gain 3.2
Loss in Nitrogen, mg	3.9	8.5	8.0	8.3	5.5	3.9	11.4	2.8
Loss in Calories	433	148	69	56	172	114	299	85
Heat of Combustion								
Lipids (Control 9182), cal/g	9816	10,458	9518			9068		
LFOM (Control 4913), cal/g	4741	4641	4952			5042		

Table XVIII. Basic Average Data Per Fish Comparing Salmon Failing and Succeeding in Swimming Against Water Velocities of 35 and 45 cm/sec.

Number	35 cm/sec		45 cm/sec	
	Succeeding	Failing	Succeeding	Failing
Predicted Initial Length	76.3	69.1	80.8	72.6
Final Length	76.8	69.0	80.6	72.7
Predicted Initial Wet Wt.	4.814	3.464	5.491	4.137
Final Wet Wt.	4.673	3.309	5.157	4.031
Predicted Initial H_2O	3.770	2.694	4.305	3.224
Final H_2O	3.650	2.562	4.030	3.134
Predicted Initial Lipid	.165	0.123	.157	0.143
Final Lipid	.161	0.086	.141	0.121
Predicted LFOM	.781	0.554	.893	0.669
Final LFOM	.767	0.567	.852	0.672
Predicted Calories	5372	3813	5881	4595
Final Calories	5316	3641	5582	4536
Predicted Nitrogen	.125	0.089	.143	.107
Final Nitrogen	.117	0.084	.131	.104

1960). At 15, 25, 35 and 45 cm/sec, velocities energy expenditures in the ratio of 1:2.8:5.4:9.0 would be expected while the velocities were in the ratio of 1:1.7:2.3:3. A much more complex relationship was obtained experimentally with losses of 433, 148, 56 and 299 calories for fish successfully swimming for 24 hours against the test velocities.

Since the caloric data obtained indicates much more complex relationships between the levels of performance and the changes occurring as a result of this performance, a paragraph should be added on observed behavior at the different velocities. All fish kept themselves lined up parallel the axis of the water stream but heading into the stream. Essentially, each fish remained in the same horizontal and vertical position in the tube. During training and at 15 cm/sec, there was considerable side movement of the tail, but with regular opercular movements. At 25 cm/sec, the extensive sideward displacements of the tail were replaced by a rapid vibrating movement of small amplitude and opercular movements were still present. At 35 and 45 cm/sec, easily discernible movements of the body and tail as well as opercular movements had disappeared and the gills would have been irrigated by the flow of the stream of water through the mouth and gills. The fish used by Bainbridge (1958a) exhibited similar changes in behavior with changes in velocity.

The main work done by a fish on the environment during

swimming seems to be a displacement of water. Considerably more random lateral displacement and opercular movement seems to occur at 15 cm/sec and still less at 35 and 45 cm/sec.

Some comments by M. James Lighthill (1969) may have a bearing on our interpretation: " . . . a fish can maintain a steady speed only if it can move its body and fins so as to produce a net forward force or thrust exactly balancing the resistance of water." " . . . When any object starts pushing the water with a speed (w), the water just in front and just behind immediately starts moving at the same speed. The change is immediate because liquids have a great capacity for avoiding alterations in their density. Water a bit farther in front, or farther behind, is not pushed forward at the full speed (w) but at some reduced speed."

Anguilliform motion appears to have been the swimming mechanism of the earliest fishes. In anguilliform motion viscous drag is counteracted by a thrust derived from momentum-shedding at a deep vertical trailing edge. The first major modification of the basic anguilliform method is known as carangiform swimming. A film by Sir James Gray, shows that a wave passes backwards whose emplitude increases rather fast from almost zero at the mid-point of the fish's length to a large value at the tail. This modified undulatory motion confined to the neighborhood of the tail is known as carangiform motion. For anguilliform motion there is immediate transfer

of lateral momentum to the water from the pushing of fish cross-sections.

In carangiform motion the fluctuating water momentum may produce fluctuating lateral forces on the fish, and so generate bodily recoil movements from side to side. These would impair efficiency by generating a lot of extra wake disturbance. Fishes using carangiform have found two methods for minimizing fluctuating water momentum. The effect is minimized by reducing the fluctuating side-force due to rate of change of water momentum and by the adoption of a rather large depth of cross-section in the central region of the body. The fins in the fish's plane of symmetry; the dorsal, ventral and caudal fins are for propulsion and for stability against side-to-side movements.

The frequency of oscillation changes with size, and in fact depends almost exclusively on the length of the fish. Tiny tropical fishes, like the tetras, exhibit the same motion as do a carp, but enormously more rapid. Films reveal the great flexibility of the caudal fin in these freshwater fishes; as it moves from side to side, it always curves concavely towards its direction of motion (Lighthill, 1969).

Velocity: 15 cm/sec.

On the basis that a piece of wood would have been carried

downstream during a similar procedure, while the salmon remained nearly stationary in the swimming tube, it can be interpreted that the salmon swam 18.8 kilometers over the 35 hour procedure (Table XVII). During this time 121 mg of water were lost. Nitrogen loss was 3.9 mg and LFOM 35 mg. Somewhat more LFOM was lost than nitrogen. Perhaps about 10 mg of non-nitrogenous materials were burned and/or excreted.

Of the 433 calories lost (Table XVI), approximately 158 were associated with the loss of lipid; 17 were associated with the loss of LFOM and 132 with a decreased heat of combustion of LFOM; a gain of 98 calories was associated with the increased heat of combustion of the lipids. (There is an overall discrepancy of 71 calories, 158 + 132 + 170 - 98 equals 362 instead of 433 calories). The 71 calorie discrepancy is largely due to the fact that we did not have equal precision in predicting initial weight, average lipid, LFOM and calories per fish. Also the A and B in the equation: H = A Lipid + B LFOM had a measurable standard error of the mean. Some small fraction of the discrepancy could be due to inherent errors in the analytical methods used.

Velocity: 25 cm/sec.

After swimming 27.6 kilometers during 35 hours involving final water velocities of 25 cm/sec, 181 mg of water were lost per salmon;

lipid losses were 3.9 mg and LFOM losses were 33 mg. Nitrogen losses were 8.5 mg and relatively much higher than the LFOM suggesting that much of amino acid metabolism was halted beyond the deamination stage.

Of the 148 calories lost, 36 calories were lost as lipid; 160 mg were lost with the LFOM; and a further 167 cal loss with the decreased heat of combustion of the LFOM. The increased heat of combustion of lipids led to a storage of 174 calories. (There is an overall discrepancy of 41 calories: 36 + 160 + 167 - 174 equals 189 instead of 148 calories). The losses at 15 cm/sec were not statistically significantly different from losses at 25 cm/sec for water lipid, LFOM and nitrogen. Similarly no statistically significant differences were found for the items at 15 and 35 cm/sec.

The percentage of water decreased in the salmon swimming at 25 cm/sec to 77.8% from a figure of 78.2%. Not enough is known of the change in composition of the cations, but the decrease in water suggests an increase in the material component of osmotic pressure within the salmon. This may have counteracted and decreased the forces tending toward further water losses.

Velocity: 35 cm/sec

Some juvenile coho salmon presented with a current of water at 35 and 45 cm/sec did not swim the full 24 hours. These failing fish

showed changes of much greater magnitude than fish capable of swimming for 24 hours at these velocities. Only three fish failed within the first two hours at 35 cm/sec; therefore, no adequate statistical information could be developed for this group. The data developed for the three salmon that failed along with comparable significant data for the 32 failing fish at 45 cm/sec indicate that changes of magnitude developed when the fish fails to hold position in high velocity currents (Tables XVII and XVIII).

Because all fish survived at velocities of 15 and 25 cm/sec but some failed at 35 and some at 45 cm/sec, and because there were large differences between the surviving and failing fish, it is difficult to make adequate comparisons of fish swimming at 15 and 25 cm with those swimming at 35 and 45 cm/sec. The best comparison seems to be of the fish that were able to swim for 24 hours at 35 and 45 cm/sec with the fish at 15 and 25 cm/sec and to make a separate comparison for succeeding against failing salmon and to make whatever possible statements comparing failing salmon at 35 cm/sec with failing salmon at 45 cm/sec. The available data are given in Tables XVII and XVIII.

The average length of the salmon chosen to swim at 35 cm/sec was 75.9 mm. The surviving fish had an average length of 76.8 and those failing to swim 69.0; this suggests that the fish that failed were relatively short. The surviving fish had an initial weight of 4.814

grams and the 3 failing fish had an initial weight to 3.309 grams. There was a very effective separation into light and heavier salmon.

The 24 fish succeeding in swimming at 35 cm/sec, traveled an equivalent distance of 36.9 km. The water loss for these fish was 120 mg per salmon. The lipid loss of the salmon succeeding at 35 cm/sec was 4.0 mg, the LFOM loss 14 mg, and the nitrogen loss 8.5 mg, indicating a relative excess of nitrogen loss and hence, simple deamination of some amino-acids, possibly with a conversion of the keto-acids to lactic acid analoges via reaction with NADH. Of the 56 calories lost 37 can be accounted for by loss of lipid and 69 by loss of LFOM with possibly a gain of 55 calories in the heat of combustion of residual lipid (37 + 69 - 55 equals 51 instead of 56 calories).

The failing fish at 35 cm/sec, traveled only an equivalent distance of 9.3 kilometers but water loss was lighter at 132 mg on the average in the failing fish and 120 in the succeeding fish. Lipid loss at 36.7 mg per salmon was 9 times as high in the failing as in the succeeding salmon. There was a gain of 13 mg in the LFOM of the three failing fish compared with a loss of 14 mg in the LFOM of the succeeding fish. However, there was a loss of 5.5 mg nitrogen in the failing fish. The loss in calories of the failing salmon was high at 172 but with only three failing salmon, the data available have little statistical significance.

Velocity: 45 cm

The swimming procedure divided the 37 fish swimming at 45 cm/sec into five large fish with an average length of 80.6 mm an average initial weight of 5.49 grams and capable of holding against the 45 cm/sec velocity for 24 hours; and 32 unsuccessful fish with an average length of 72.7 mm and an average initial weight of 4.14 grams. The successful salmon traveled an average equivalent distance of 46.2 kilometers. The loss in water was very high at 275 mg per salmon. The loss in lipids was 16.4 mg.

The succeeding salmon lost 41 mg of LFOM and 11.4 mg of nitrogen to suggest again simple deamination of some amino acids, as 11.4 mg of nitrogen is approximately equal to 71 mg of LFOM. Of the 299 calories lost, 151 were possibly derived from the lipid loss and 201 from the LFOM loss with a 60 cal loss in the heat of combustion of residual lipid counteracted by a gain of 110 calories in the heat of combustion of the LFOM (151 + 201 + 60 - 110 equal 302 instead of 299 calories).

The 32 salmon that failed to swim for 24 hours traveled an equivalent distance of 9.8 km and lost an average of 90 mg of water. Inspections of water loss data (Table XVII) indicates that this is relatively high for less than five hours swimming at a high velocity. The water loss was 2.79% of the initial water, while the succeeding fish after 46.2 kilometers had lost 6.38% of their water.

The failing salmon lost 16.0 mg of lipid but gained 3.2 mg of LFOM. The loss in lipids suggests a loss of 144 calories and gain in LFOM of 16 cal while the observed loss was 85 calories. The 43 calories not yet accounted for were probably small gains in the heats of combustion of the residual lipid and residual LFOM.

Comments on Brett, Krueger and Saddler

These experiments were designed to clarify and amplify some of the observations of Saddler (1967) and some deductions by Krueger and others (1968) from the data of Saddler and of Brett (1964, 1965). The data here presented are not directly comparable with those of Saddler because his juvenile salmon (Oncorhynchus kisutch), were somewhat longer and heavier (Table XIX). With heavier salmon Saddler was able to use test velocities of 52, 54, 56, and 59 cm/sec, whereas the highest velocity tested in this study was 45 cm/sec. In Saddler's experiments, at each velocity the forced exercise again divided the salmon into a group that was able to swim at a given velocity for shorter periods of time and a group that was able to swim for longer periods of time. The shorter and lighter salmon were less competent at a given velocity than were the longer and heavier salmon. The percentage of the salmon failing to swim for 24 hours was 29% at 52 cm/sec and 79% at 59 cm/sec.

Wet weight losses obtained by Saddler (830, 940, 410, and 480

Table XIX. Average Values for Length, Weight, Weight of Lipid, Percent of Lipid, and Weight of Fatty Acid Methyl Esters of Coho Salmon Used for Swimming Performance Experiments (Taken from Saddler, Table 37, and Supplemented)

Swimming Velocity cm/sec	Length cm	Weight grams	Weight of Lipid		Weight of Ester mg	Fat F/D	Other Lipids	
			mg	%			(D-F)/D	D-F
Control of Salmon								
0	8.06	5.22	233	4.38	189.2	.812	.188	43.8
Salmon Swimming Less Than 24 Hours								
52	7.79	4.16	177	4.20	145.0	.819	.181	32.0
54	7.60	4.40	187	4.58	152.4	.815	.185	34.6
56	7.94	4.58	224	4.83	174.7	.780	.220	49.3
59	7.82	4.48	203	4.45	161.8	.797	.202	41.2
Salmon Swimming for 24 Hours								
52	7.98	4.44	180	4.07	150.3	.835	.165	29.7
54	7.98	4.69	238	5.06	195.8	.823	.177	42.2
56	8.30	5.51	313	5.75	247.6	.791	.209	65.4
59	8.25	5.14	299	5.82	237.1	.831	.169	61.9
Total Salmon Swimming								
52	7.91	4.39	179	4.10	148.8	.831	.169	30.2
54	7.74	4.28	206	4.76	168.7	.819	.181	37.3
56	8.03	4.81	245	5.04	191.5	.781	.218	53.5
59	7.90	4.74	223	4.74	177.7	.796	.203	45.3
A	B	C	D	E	F			

mg) were considerably higher than losses in this study (177 to 222 mg). Involved in the differences were higher initial wet weight and higher water velocities. The data of this thesis indicated relatively greater losses in water mass than in losses of other compartments. Hence, this would suggest that the greater fraction of losses reported by Saddler (1967) were water.

In the experiments of Baker average lipid loss varied from 4 to 17 mg per salmon. At 52 cm/sec the average lipid loss of the salmon of Saddler was 54 mg or three times the greatest loss obtained by Baker. The total loss of 830 mg wet weight obtained by Saddler implies a loss of 776 mg of water plus LFOM and suggests a greater protein loss than was obtained by Baker. The greater lipid and protein losses by the salmon of Saddler can be interpreted as incompatible with the losses described by Baker.

Krueger and his coworkers (1968) estimated that the lipid free water content of coho salmon would be 80% of the lipid free wet weight. In the experiments reported in this thesis, the average water content ranged from 0.803 to 0.817, a range which suggests that an estimate of 80% for the water content of the salmon of Saddler was reasonable, providing the wet weight difference between the salmon of Saddler and of Baker did not carry with it an alteration in the basic composition (lipid, water, LFOM and ash).

But direct bioenergetic comparisons between the data of

Saddler (1967) and Baker are difficult. First there are seasonal and geographic differences; then there are variations in the weight-behavior-metabolism complex; then variations in the details of swimming studied including preliminary training, velocities chosen, duration of swimming and the precision of analytical procedures. There may be many other important differences not yet recognized. However, it is clear that proteins and/or lipids may be involved at a given velocity and some other combination of protein and lipid at another velocity.

Data by Brett (1965) on the oxygen consumption of a 5.2 gram Oncorhynchus nerka over a two-hour period suggest that minimum and maximum caloric expenditures of energy would be:

	ml Oxygen Used in 35 hours		Calorie Equivalent		Calories lost observed by Baker (herewith)
	Mini-mum	Maxi-mum	Mini-mum	Maxi-mum	
15 cm/sec	14 ml	26 ml	68	136	433
25 cm/sec	19 ml	28 ml	93	141	148
35 cm/sec	25 ml	31 ml	126	155	69 (56)
45 cm/sec	34 ml	38 ml	171	189	114 (299)

The calories lost as observed by Baker (herewith) include heat production from metabolism including oxidation and the calories excreted. The calories lost calculated from oxygen used are equivalent only to heat production. Hence to the data calculated from Brett

should be added 5 calories per mg of nitrogen lost, presumably as ammonia (Krueger et al., 1968). The caloric losses calculated from data of Brett and the losses reported by Baker are of the same order of magnitude but there are extensive differences.

Two papers available after the completion of this study and thesis, by Brett (1973) and Brett and Glass (1973) suggest a reasonable agreement with much of the basic data of Baker concerning depletion of the constituent components of fish. Brett (1973) found that excitement, spontaneous activity and oxygen debt reduction after handling elevated the oxygen consumption rate over that for the minimum locomotor demand at low velocities by 300% and even as much as 500%. He suggests that at low velocities it might take up to eight days for fingerling fish to settle down to steady swimming. The elevated oxygen consumption after handling becomes less with increasing swimming velocities.

Brett and Glass (1973) investigating the metabolic rates and critical swimming speeds of sockeye salmon in relation to size and temperature show that the ability of these fish to swim against a specified current is greatest at 15 C for the achievement of a critical swimming speed, a maximum of 60 minutes at a set sustained velocity. Brett and Glass's study suggests that five gram fish are capable of oxygen consumption in excess of 900 mg O_2 per kilogram per hour between the temperatures of 13 and 20 C. The optimum

temperature of 15 C for salmon produces a maximum metabolic rate, a rate which is much higher for small fish than for large fish. The onset of fatigue at critical swimming speeds results in repeated burst activity by the salmon which was shown by Brett (1972) to drive the metabolic demand for oxygen to 10 times the active metabolic rate.

Perhaps the lack of any kind of linear relationships between material losses and swimming velocity may be influenced by both behavioral and temperature dependent physiological phenomena.

The exercise experiments should be amplified by making extensive histological studies of control salmon and salmon spent or fatigued by swimming at high velocities. Further studies should be made of changes in salmon subsequent to swimming against high velocities when not fed, or fed, or with additions of the more prevalent amino-acids to the ambient water. More detailed metabolic studies should also be made.

Selection of Salmon and Experimental Procedures

In retrospect, too many factors were allowed to influence the experimental design and too many unrecognized factors created additional problems. An attempt to provide data of ecological value led to the choice of salmon collected from streams rather than hatchery salmon. A limited number of juvenile salmon were available for

sampling. The low number available made it impossible to select individual fish for experiments in a statistically random fashion. The availability of only a few fish at any one time also may have influenced this study by necessitating the use of fish collected at three different collection dates and held in the laboratory for variable periods of time. The behavioral patterns of juvenile coho salmon suggest that the stronger fish occupy favorable upstream positions while pushing the less competitive young salmon further and further down stream. This probably explains why the population present in the sample area was quite diverse and allows the possibility that the members of the populations are derived from many different separate redd areas. However, a certain similarity of competitiveness might be found among the members of a specific sample area.

In spite of the cost of the equipment, it is suggested that, if these experiments are to be amplified at some future date, three to six parallel swimming set-ups should be provided and run simultaneously with the maximum out-of-phase relationships between simultaneous or parallel runs of less than three hours. Pre-swimming data should be collected on all fish and should include at a minimum two determinations of length, weight and volume taken in the sequence: length, weight, volume, length, weight, and volume. Hatchery fish should be used to decrease individual variability.

In the design of the experiment only the simplest questions should be asked and the design set to answer these questions. Additional data or procedures may be added that do not require an alteration in design or interfere with obtaining an answer to the initial questions. If more complex answers are desired, several research projects should be tied together in a series of experiments designed or controlled by a single member of the staff.

SUMMARY

Wild juvenile coho salmon (Oncorhynchus kisutch) were subjected to various swimming tests including training for 11 hours at a mean velocity of 15 cm/sec and training plus swimming at water velocities of 15, 25, 35 and 45 cm/sec for 24 hours. All fish were successful except for three of 27 fish at the 35 cm/sec and 32 of 37 fish at 45 cm/sec.

With the high variability found in the wild population of salmon, statistical procedures more critical than comparisons of means were obtained by making use of generally accepted physiological interrelationships: (1) Weight, length and the biochemical parameters measured are interdependent. (2) Lean body mass (fat free body mass) has a reasonably constant composition. (3) Heat of combustion of organic matter is the sum of the heats of combustion of lipids and of lipid free organic matter.

Stepwise multiple linear regression analyses were developed from data on controls for biochemical parameters as functions of W_o (initial weight), l_o (initial length) and T (time held in the laboratory). The use of these equations reduced the standard deviation 50%, 72%, 68% and 68% respectively for lipid, LFOM, nitrogen and caloric content per fish. These equations were then used to estimate the pre-swimming levels for each parameter for any individual fish

on the assumption that before swimming all salmon belonged to the same population as did the controls. Fish swimming at 15 cm/sec had losses of 121 mg in water, 35 mg in LFOM, 4 mg in nitrogen and 433 calories per salmon during swimming. Whereas the 25 cm/sec salmon had losses of 182 mg in H_2O, 33 mg in LFOM and 8.5 mg in nitrogen. Fish at 35 cm/sec level had losses of 121 mg in water and 8 mg in nitrogen. In the 45 cm/sec group, water loss was 115 mg, lipid loss was 16 mg and nitrogen loss was 4 mg.

No other losses of statistical significance were noted. The losses described were not related in a linear fashion to the velocity of the water.

In resume, no statistically significant difference in the derived heats of combustion were noted between control salmon and salmon after training. Significant differences in the heats of combustion of lipids and LFOM, developed between controls and fish after swimming at 15 cm/sec. Even greater differences were noted between controls and the salmon after swimming at 25 cm/sec. The differences developed at 35 cm/sec were less than those at either 15 or 25 cm/sec.

The increase in the heat of combustion of the lipid extracted from salmon after swimming would require that low caloric lipid had been expended and/or that high caloric lipid material had been released from the LFOM fraction, and that during swimming, the

caloric properties of the extractable lipids and of the LFOM had been altered.

The juvenile salmon apparently have a minimum caloric loss at velocities around 35 cm/sec when swimming against a current of water with a constant velocity. At water velocities below this, the salmon had more freedom of random movement as well as the necessity of ventilating the gills using opercular muscles. At velocities higher than approximately 35 cm/sec, the attempt to hold position against a set current became more and more difficult with more and more of the metabolic resources being mobilized and lost at a faster rate per hour or per kilometer traveled. At a 35 cm/sec velocity the water current may have been sufficient to deliver oxygen to blood and eliminate the need for the fish to actively ventilate the gills.

Physical trauma from the force of the water may be a factor in failure of the smaller fish to swim for 24 hours against a 35 or 45 cm/sec water velocity. The energy costs of swimming were relatively high, about 6% of the total calories per day, at a 15 cm/sec velocity but dropped to 2% at 25 cm/sec and to one percent at 35 cm/sec for the successful salmon. But the cost increased to 3% of the total calories available in a salmon at 45 cm/sec for the successful salmon. The very high loss in lipids in the failing fish suggests a disruption of intracellular structure and of readily available sources of energy. Failing salmon seemed unable to mobilize sufficient

materials for metabolic needs over long periods of time.

Since the caloric losses were based on heat of combustion data, they included all losses in energy including glucose metabolism, oxidation of fats, hydrolysis of proteins and oxidation of amino acids. Only 40% of this energy would be available on the basis of accepted concepts involving ATP, it seems that more conservative methods of utilizing energy may be involved (a definite statement would require an evaluation of the work done by the fish on the environment expressed in gram centimeters or pressure-volume determinations of work done). A current concept (McClare, 1973) suggests that energy transducing units are molecular machines. These machines are characterized by energy transfer through vibrationally excited states involving cyclical energization and deenergization with work being done during deenergization and with resonance as a key component. Such an interpretation would allow energy transfer from almost any chemical reaction even those involving the release of amino acids from proteins or the release of nitrogen from amino acids.

BIBLIOGRAPHY

Averett, R. C. 1968. Influence of temperature on energy and material utilization by juvenile coho salmon. Ph.D. thesis. Corvallis, Oregon State University. 74 numb. leaves.

Bainbridge, R. 1958a. The speed of swimming fish as related to size and the frequency and amplitude of tailbeat. Journal of Experimental Biology 35:109-133.

_____. 1958b. The locomotion of fish. New Scientist 4:476-478.

_____. 1961. Problems of fish locomotion. Symposium of the Zoological Society of London. Proceedings 5:13-32.

_____. 1962. Training, speed and stamina in trout. Journal of Experimental Biology 39:537-556.

Berthelot, M. M. and G. Andre. 1899. Nouvelles recherches sur les chaleurs de formation et de combustion de divers composes azotes et autres. Annals of Chemistry and Physics 17(17): 433-441.

Black, E. C. 1958. Energy stores and metabolism in relation to muscular activity in fishes following exercise. In P. A. Larkin (ed.) The investigation of fish-power problems. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B. C. pages 51-67.

Black, E. C., G. T. Mamming, and K. Hayaski. 1966. Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate, and lactate in venous blood of Rainbow Trout (Salmo gairdnerii) during and following severe exercise. Journal of the Fisheries Research Board of Canada 23(6):783-795.

Black, E. C., A. G. Robertson, A. R. Hanslip, W. G. Chiu. 1960. Alterations in glygogen, glucose and lactate in rainbow and Kamloops trout, Salmo gairdneri, following muscular activity. Journal of the Fisheries Research Board of Canada 17:487-500.

Black, E. C., Wing-Gay Chiu, F. D. Forbes and A. Hanslip. 1959. Changes in pH, carbonate and lactate of blood of yearling Kamloops trout (Salmo gairnerii) during and following severe

muscular activity. Journal of the Fisheries Research Board of Canada 16(4):391-402.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21:1183-1225.

_____. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (Oncorhynchus nerka). Journal of the Fisheries Research Board of Canada 22:1491-1501.

_____. 1967. Swimming performance of sockeye salmon (Oncorhynchus nerka) in relation to fatigue, time, and temperature. Journal of the Fisheries Research Board of Canada 24: 1731-1741.

_____. 1973. Energy expenditure of sockeye salmon, Oncorhynchus nerka, during sustained performance. Journal of the Fisheries Research Board of Canada 30(12 pt 1):1799-1809.

Brett, J. R. and N. R. Glass. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (Oncorhynchus nerka) in relation to size and temperature. Journal of the Fisheries Research Board of Canada 30(3):379-387.

Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. Journal of the Fisheries Research Board of Canada 26(9): 2363-2384.

Brody, Samuel. 1945. Bioenergetics and growth. New York, Hafner Publishing Company. 1023 p.

Chapman, D. W. and T. C. Bjornn. 1969. Distribution of salmonids in streams, with special reference to food and feeding. In Symposium on Salmon and Trout in Streams. H. R. MacMillan Lectures in Fisheries. ed. T. G. Northcote. The University of British Columbia, Vancourver, B. C. p. 153-176.

Chadwick, G., G. Chapman and others. 1968. Heats of combustion of fish tissue and lipids. Unpublished data from the Oak Creek Fisheries Laboratory, Corvallis, Oregon State University.

Colowick, Sidney P. and Nathan O. Kaplan (eds.). 1957. Methods in enzymology. Vol. 3. New York, Academic Press. 1154 p.

Dahlberg, Michael L. 1963. Influence of dissolved oxygen and carbon dioxide on sustained swimming speed of juvenile large mouth bass and coho salmon. Master's thesis. Corvallis, Oregon State University. 57 numb. leaves.

Davis, G. E., J. Foster, C. E. Warren and P. Doudoroff. 1963. The influences of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. Transactions of the American Fisheries Society 92(2):111-124.

Efroymsen, M. A. 1960. Multiple regression analysis. In: Mathematical Methods for Digital Computers (A. Ralston and H. Wilf, eds.). pp. 191-203. New York, John Wiley and Sons, Inc.

Fang, S. C. 1968. Associate Professor, Oregon State University, Department of Agricultural Chemistry. Personal communication. Corvallis, Oregon, September 1968.

Fish Commission of Oregon. 1970. Oregon pellet and starter mash specifications. November, 1970.

Folch, J. and M. Lee. 1951. Proteolipids, a new type of tissue lipoproteins. Journal of Biological Chemistry 191:807-817.

Folch, J., M. Lee and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 226:497-509.

Fraser, Doris L., E. Lo and W. J. Dyer. 1966. Nucleotide interference with glycogen estimation in white and red muscle of cod. Journal of the Fisheries Research Board of Canada 23: 921-924.

Gerking, S. D. 1952. The protein metabolism of sunfishes of different ages. Physiological Zoology 25:358-372.

Gerking, S. D. 1955a. Influence of rate of feeding on body composition and protein metabolism of Bluegill Sunfish. Physiological Zoology 28(4):267-282.

Gerking, S. D. 1955b. Endogenous nitrogenous excretion of Bluegill Sunfish. Physiological Zoology 28(4):283-289.

Hochachka, P. W. and A. C. Sinclair. 1962. Glycogen stores in trout tissues before and after stream planting. Journal of the Fisheries Research Board of Canada 19:127-136.

Hogan, Louise. 1968. Oregon State University, Department of Agricultural Chemistry. Personal communication. Corvallis, Oregon. September 1968.

Horwitz, W. (ed.) 1965. Official methods of analysis of the association of official agricultural chemists. 10th ed. George Banta Co., Inc. Menasha, Wis. p. 273.

Idler, D. R. and I. Bitners. 1958. Biochemical studies on sockeye salmon during spawning migration. II. Cholesterol, fat, protein and water in the flesh of standard fish. Canadian Journal of Biochemistry and Physiology 36(8):793-798.

Idler, D. R. and H. Clemens. 1958. The energy expenditure of Frazer River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. In: International Pacific Salmon Fisheries Commission Annual Report. 1, 1937/38+. 80 p.

Idler, D. R. and H. Tsuyuki. 1958. Biochemical studies on sockeye salmon during spawning migration. I. Physical measurements, plasma cholesterol, and electrolyte levels. Canadian Journal of Biochemistry and Physiology 36(8):783-791.

Ivlev, V. S. 1945. "The biological productivity of waters." Usp. sovrem. Biol. 19:98-120. In: Fisheries Research Board of Canada Translation Series No. 394.

Johnston, David W. 1970. Caloricdensity of avian adipose tissue. Comparative Biochemistry and Physiology 34:827-832.

Kharasch, M. S. 1928. Heats of combustion of organic compounds. Bureau of Standards Journal of Research 2:359-430.

King, C. J. 1970. Freeze drying of foods. In: CRC Critical Reviews in Food Technology, ed. by T. E. Furia, Cleveland, Ohio, The Chemical Rubber Company, Vol. 1(3). p. 379-478.

Krueger, H. M., J. B. Saddler, G. A. Chapman, I. J. Tinsley, and R. R. Lowry. 1968. Bioenergetics, exercise and fatty acids in fish. American Zoologist 8:119-129.

Kutty, M. N. 1972. Respiratory quotient and ammonia excretion in Tilapia mossambica. Marine Biology 16:126-133.

Lighthill, M. James. 1969. How do fish swim. Endeavor 29(107): 77-83.

Love, R. M. 1970. The chemical biology of fishes. London, Academic Press. p. 547.

Markley, Klare S. 1947. Fatty acids. New York, Interscience Publishers, Inc. p. 668.

McClare, Colin (King College London). 1973. Proceedings of New York Academy of Sciences Conference on Energy Transduction. February 7-9, 1973. Quoted from Biomedical News IV(4), Washington D. C., p. 1, col. 3. April 1973.

Niimi, A. J. 1972. Changes in the proximate body composition of largemouth bass (Micropteras salmoides) with starvation. Canadian Journal of Zoology 50:815-819.

Parr Instrument Company. 1958. Instructions for the No. 1411 combustion calorimeter. Manual No. 128. Moline, Illinois. p. 22.

_____. 1960. Oxygen bomb calorimetry and combustion methods. Manual No. 130. Moline, Illinois. p. 56.

Saddler, James B. 1967. Fatty acids of exercised and non-exercised salmon. Ph.D. thesis. Corvallis, Oregon State University. 155 numb. leaves.

Shaw, W. N., H. S. Tubiash, and A. M. Backer. 1967. Freeze-drying for determining total solids in shellfish. Journal of Fisheries Research Board of Canada 24(6):1413-1417.

Simatos, Denise and L. Rey. 1965. Advances in freeze drying. Federation Proceedings, Supplement No. 15. 24(2):213-215.

Sperry, Warren. 1955. Lipid Analysis. In: Methods of Biochemical Analysis, Vol. II. ed. by David Glick, New York, Interscience Pub. Inc.

Starling, E. H. 1930. Principles of human physiology. "Rev. ed." by C. L. Evans. Philadelphia, Lea & Febiger. p. 1039.

Stevens, E. E. and E. C. Black. 1966. The effect of intermittent exercise on carbohydrate metabolism in Rainbow Trout (Salmo gairdnerii). Journal of the Fisheries Research Board of Canada 23(4):471-485.

Tinsley, I. J., H. M. Krueger and J. B. Saddler. 1973. Fatty acid content of coho salmon, Oncorhynchus kisutch (Walbaum): A statistical approach to changes produced by diet. Journal of the Fisheries Research Board of Canada 30:1661-1666.

Vinogradov, A. P. 1953. The elementary chemical composition of marine organisms. (Translators: J. Efron and J. K. Setlow). New Haven, Sears Foundation. 646 p.

Warren, C. E. and G. E. Davis. 1967. Laboratory studies on the feeding, bioenergetics, and growth of fish. In: The Biological Basis for Freshwater Fish Production (S. D. Gerking, ed.). Oxford, Blackwell Scientific Publications. p. 174-214.

Washburn, Edward W. (ed.) 1929. International Critical Tables of Numerical Data, Physics, Chemistry and Technology. National Research Council of U. S. A., Vol. V. New York, McGraw-Hill. p. 162-171.

Winberg, G. G. 1960. New information on metabolic rates in fishes. Translation Series No. 194. Fisheries Research Board of Canada. 202 p.

_____. 1961. New information on metabolic rates in fishes. Translation Series No. 362. Fisheries Research Board of Canada. 11 p.