

AN ABSTRACT OF THE THESIS OF

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Title: SOME EFFECTS OF DISSOLVED OXYGEN CONCENTRATION  
ON FEEDING, GROWTH AND BIOENERGETICS OF

JUVENILE COHO SALMON

Abstract approved: \_\_\_\_\_

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Charles E. Warren

The effects of three levels of dissolved oxygen (8, 5 and 3 mg/l) upon the feeding, growth and bioenergetics of juvenile coho salmon, Oncorhynchus kisutch (Walbaum), were determined in laboratory studies. Experiments with individual fish were conducted during the summer, fall and spring to measure rates of food consumption, standard metabolism, waste production, activity-specific dynamic action, and growth. House fly larvae were fed to the young salmon and the temperature was kept constant year-round at 15 C.

The results indicated that differences due to the dissolved oxygen concentration were not great. Food consumption rate and the slope of growth rate curves were reduced only at 3 mg/l dissolved oxygen and only at near maximum food consumption rates which appear to be higher than juvenile coho salmon generally encounter in nature.

However, normal competition for food and space in stream life (not present in these experiments) would be expected to curtail feeding in naturally occurring populations of juvenile cohos exposed to dissolved oxygen levels near 3 mg/l.

Energy budgets were constructed from caloric determinations of the various uses made of the energy in the consumed food. These revealed that the reduced slope of the growth curve exhibited by 3 mg/l fish at near maximum feeding levels was due to sharp increases in energy requirements for activity-specific dynamic action. These energy budgets also offered the explanation that the greater loss of weight by the starved juvenile cohos kept at 8 mg/l dissolved oxygen (as compared to those kept at 3 and 5 mg/l) was due to their greater activity.

Juvenile cohos kept at 5 mg/l dissolved oxygen in these studies grew at least as well as those at 8 mg/l. Again, however, this was considered to be an artifact of this experimental procedure. Fish reported upon here were not required to expend energy in competition for food and space as are those living in nature.

Some Effects of Dissolved Oxygen Concentration  
on Feeding, Growth and Bioenergetics of  
Juvenile Coho Salmon

by

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SOME EFFECTS OF DISSOLVED OXYGEN CONCENTRATION  
ON FEEDING, GROWTH AND BIOENERGETICS OF  
JUVENILE COHO SALMON

INTRODUCTION

The degree of success in fulfilling the potential for growth which juvenile coho salmon, Oncorhynchus kisutch (Walbaum), or any organism for that matter, attain depends in large part both on the availability of useful energy and materials in its food and on limitations imposed by the environment. The dissolved oxygen concentration of the water is just one of the influencing conditions of the environment. It has, however, long been recognized as one of the most important. The staff and students of the Pacific Cooperative Water Pollution and Fisheries Research Laboratory at Oregon State University (more commonly known as the Oak Creek Laboratory) for a number of years have been studying the effects of various levels of oxygen dissolved in water on freshwater fishes in order to help establish water quality criteria required for the well being of fish. The study reported herein is part of an overall program directed to determining the influence dissolved oxygen exerts on the feeding, growth, and bioenergetics of juvenile coho salmon. The overall investigation has involved studies in bottles, aquaria, respirometers, and swimming channels, and other experiments in the more natural environments provided by laboratory streams and outdoor observation

channels. This report covers aquarium and respirometer studies with young coho salmon conducted in the carefully controlled environment of a laboratory system, which would yield data from which energy budgets describing the fate of food energy, as affected by the dissolved oxygen content of the water, could be developed.

### The Bioenergetic Approach

The bioenergetic approach is concerned with the sources and fates of the energy and material resources that nourish an organism. This is not a new approach to animal husbandry, but its practical application to fishes has been made only in recent years through a bioenergetic equation developed by Warren and Davis (1967).

Much of the foundation for the bioenergetic analysis of the effects of environmental factors on animals has been laid down by the studies of a few investigators, especially Brody (1945), Ivlev (1945), Fry (1947), and Winberg (1956). Brody (1945), in his extensive discussion in Bioenergetics and Growth, showed how the various food and nutrient categories are related to the first and second laws of thermodynamics. Several of the five energy categories (gross energy, digestible energy, metabolizable energy, net energy, and specific dynamic action) discussed by Brody are incorporated into the diagram that Warren and Davis (1967) used to explain the derivation of their energy budget equation (Fig. 1).

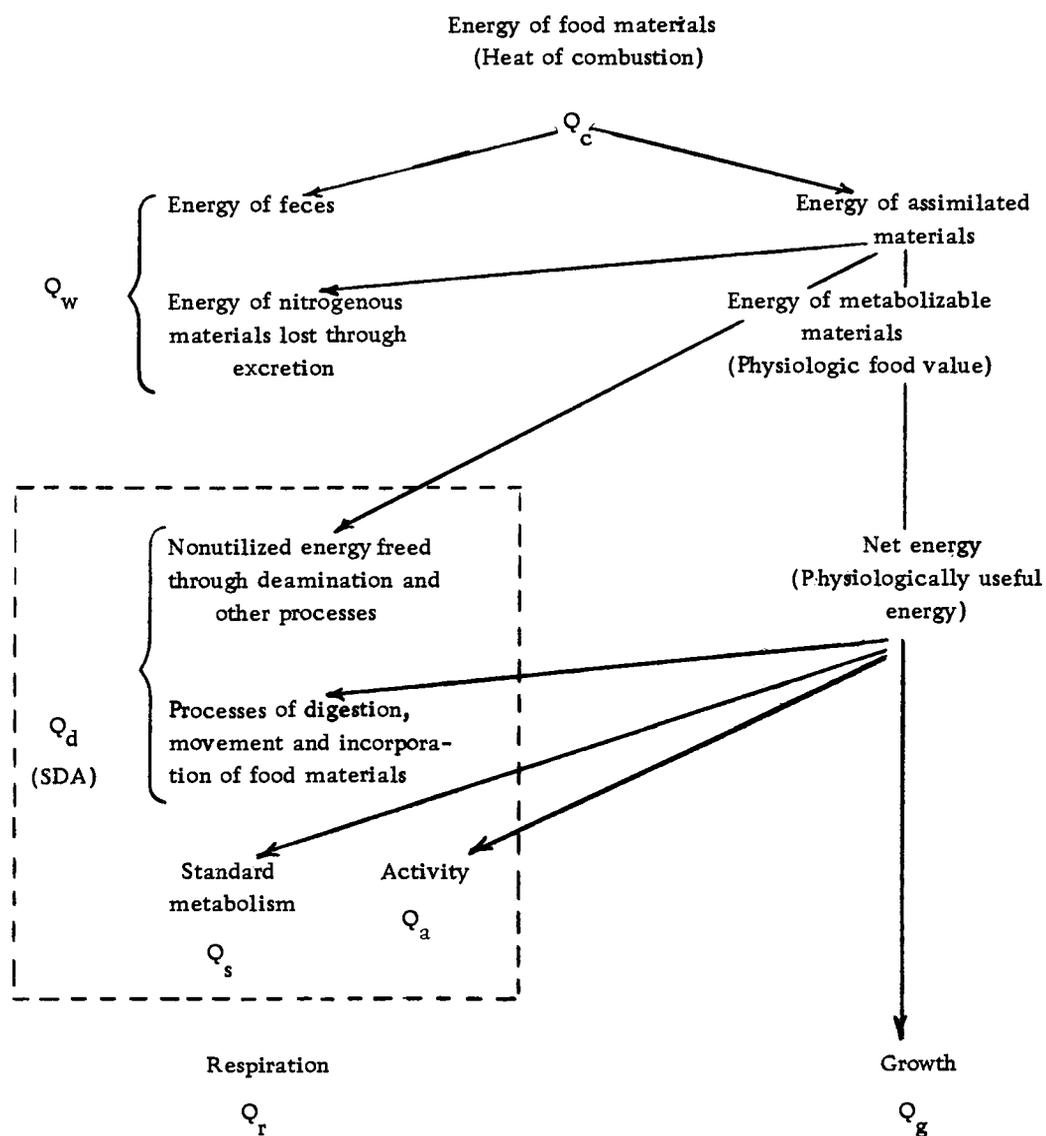


Figure 1. The fates or categories of uses of the energy and materials in the food an animal consumes. (from Warren and Davis, 1967)

Ivlev (1945) was one of the first to apply a bioenergetic equation to aquatic organisms. Building on work of previous investigators who diagrammed pathways for energy transformations within living organisms, Ivlev described six energy categories and three energy coefficients of growth (or growth efficiencies). The categories he described as food energy ( $Q$ ), energy for growth ( $Q'$ ), and energy of the unutilized parts of the food--that which is eliminated as waste ( $Q_r$ )--all seem self-explanatory and easily measurable. But the other three categories--primary heat ( $Q_t$ ), external work ( $Q_v$ ), and internal work ( $Q_w$ )--seem difficult to define and/or measure precisely or easily. These do form a framework, however, that no doubt aided Warren and Davis (1967) in developing an equation with fairly distinct energy categories.

Fry (1947), after years of studying fish metabolism, presented his conceptualization of how the environment affects the activity of animals through its action on their metabolism. He discussed several categories of environmental factors and the manner in which "scope for activity" (the metabolic capacity to perform all activities over and above those accounted for in standard metabolism) is affected by these factors. Growth is one type of activity, and after considering Fry's concepts Warren and Davis (1967) defined "scope for growth" as the difference between the energy value of an animal's food and the energy value of all uses and losses of food other than growth, under a particular set of environmental conditions.

Winberg (1956) reviewed much of the literature, including his own contributions, in Rate of Metabolism and Food Requirements of Fishes, an excellent reference volume, which has been translated into English. In this volume, he discusses much of the fish metabolic work done in Russia and in the rest of the world, thus providing a very usable text on such studies. He also includes much material on energy budgets and various energy use categories.

Warren and Davis (1967), in presenting their bioenergetic equation, used a diagram to show the fates of the energy resources taken into an animal's body (Fig. 1). Of the energy consumed ( $Q_c$ ), part is eventually absorbed through the walls of the alimentary tract, i.e., assimilated, and the remainder passes through and out of the animal's body as fecal waste (part of  $Q_w$ ). Not all the substances assimilated are utilized by the animal for work or growth, and those not metabolized are excreted by the kidneys or gills (remainder of  $Q_w$ ) as ammonia, urea, creatine, creatinine, perhaps trimethylamine oxide (TMAO), and other nitrogenous products. The remaining energy and material resources in metabolizable assimilated substances either supply the energy for the various activities of the animal ( $Q_s$ ,  $Q_a$ , and  $Q_g$ ) or appear as specific dynamic action ( $Q_d$ ). This latter category results from the deamination of proteins (and similar processes in the metabolism of carbohydrates and fats) or from digestion and handling of materials leading to new tissue elaboration.

These same categories (Q) from the diagram of Warren and Davis are used in their energy budget (see Methods and Materials). This energy budget was selected for the studies with young coho salmon because it is the most usable one available and also because working at the same laboratory with these investigators offered the obvious advantage of immediate consultation.

### The Effects of Dissolved Oxygen

Many studies have been made observing the effects of low dissolved oxygen on such parameters as survival, development, food consumption, growth, swimming ability, standard (near minimum) metabolism, and active (maximum) metabolism of both warm and cold water fishes. Most of the earlier studies were reviewed by Winberg (1956), as mentioned previously. Several investigations of special pertinence have been reported in the literature since that time.

Beamish (1964a) reported that the standard metabolic rate for speckled (brook) trout observed at dissolved oxygen levels considerably below 50 percent saturation first increased with decreasing oxygen concentration (as the fish worked harder to get the required oxygen from the water), then decreased (as the lower lethal level was reached). He also observed that in the range of independent respiration (higher levels where the respiration rate was independent of the dissolved oxygen level) the standard metabolic rate remained

constant, but that acclimation to lower dissolved oxygen resulted in lowering of this rate.

In another study, Beamish (1964b) observed that when speckled trout were starved, their standard metabolic rates did not reach minimum levels until after three days. For other species, he noted that other investigators had reported this time to vary from 24 hours up to long periods of time in which respiration continues to decline. Beamish kept records of "spontaneous activity" in his studies and believed that the time required to reach minimum standard oxygen consumption rate reflected the period during which the previous meal was still exerting an oxygen demand.

Herrmann (1958), working with young coho salmon, found the lethal level to be between 2.3 and 3.3 mg/l dissolved oxygen and observed that food consumption and growth declined slightly as the oxygen level was reduced from 8.3 to 6.0 or 5.0 mg/l and that the reduction was much more marked at lower dissolved oxygen levels.

Davison (1954) observed that cohos in 9.0 mg/l dissolved oxygen fed more actively than at 3.0 or lower levels, and that rate of food consumption, growth, and efficiency of conversion decreased markedly at concentrations less than 6.0 mg/l. He also noted a marked effect of temperature on lethal levels of dissolved oxygen at temperatures above 22 C and no effect below 16 C.

Fisher (1963), in studies on young cohos, observed that growth rates were markedly reduced at 2.5 and 3.0 mg/l as compared to controls. He also observed higher food conversion ratios for fish on restricted rations versus those on unrestricted (therefore higher) rations at all dissolved oxygen levels above 3.0 mg/l. The ratio was low at 3.0 mg/l for a restricted ration, which indicates an effect of low dissolved oxygen levels on food conversion ability.

If further justification for such a study as this were needed, this passage by Warren (1971, p. 135) seems to be very satisfactory:

Every successful organism must, during some period of its life, grow. And, when not growing, it must replace structural components destroyed by age or harm as long as it lives. The availability of energy and materials for these and other purposes is one of the essential conditions of the environment. But other conditions of the environment will determine an organism's ability to obtain and utilize energy and materials. Here, then, is a most general, a most important, a most difficult, and, perhaps for these reasons, a most fascinating biological problem.

## METHODS AND MATERIALS

### Experimental Animals and Acclimation

The fish used in these experiments were young coho salmon collected by seine periodically throughout the year from the Yaquina River just upstream from Nashville, Oregon. In each season of study, fish of similar size were used. Those used in the different seasons ranged in size from fry that had recently emerged from the gravel to fingerlings of the same year-class, when they had become about one year old but had not yet become smolts.

Fish were transported to the Oak Creek Laboratory in groups of about 200 on each collection date, and here they were transferred to a 50-gallon aquarium. They were then acclimated for a period of two to three weeks to the laboratory water, which was maintained at 15 C and near air-saturation levels of dissolved oxygen. During this period, the fish were fed slightly more than a maintenance ration of housefly larvae (the same food used later in experiments), which were obtained in large numbers from the Entomology Department culture facilities at Oregon State University. During this general acclimation period, the coho salmon were exposed daily to 12 hours of "cool white" fluorescent lighting and 12 hours of darkness, the same as during the experiments.

Following this period of initial acclimation to laboratory conditions, the fish were transferred to "styrofoam" aquaria divided by transparent "plexiglas" partitions into eight compartments, so that each compartment contained one fish in about 1.5 liters of water. Twelve of these aquaria, holding a total of 96 fish, were used to acclimate the cohos to conditions like those used later during experiments. During these test-like conditions of acclimation, four aquaria were maintained at each of three levels of dissolved oxygen--8.0, 5.0, and 3.0 mg/l  $\pm$  0.15 mg/l. The fish in two aquaria at each dissolved oxygen concentration were later used for growth studies and those in the other two aquaria were used in assimilation, standard metabolism, and specific dynamic action experiments.

This latter acclimation period was 17 days in duration. The fish were fed slightly more than a maintenance ration during the first eight days, and from the 9th to the 14th day they were fed the same ration as planned for the test. Then they were deprived of food during the last three days before an experiment was begun. This test-condition acclimation occurred in the partitioned "styrofoam" aquaria, with the exception that fish which were going to be tested in the swimming tube respirometers were placed in the respirometer on the ninth day and remained there until the experiment began.

The fish were weighed on the 16th day of the acclimation period, replaced in the proper test chambers, and allowed 24 hours to "calm

down" before the selected test was begun. The fish used in the food assimilation efficiency tests were transferred from the "styrofoam" chambers to one-liter "plexiglas" chambers, after being weighed on the 16th day.

### Apparatus and Water Supply

Several types of test chambers were used in these experiments. All were constructed of materials previously tested and considered to be non-toxic. These included "plexiglas," "styrofoam" coated with "hatchery white" enamel paint, "Buna-N" rubber (water pump parts), "aged" black rubber stoppers, stainless steel, "tygon" and polyethylene tubing, and aluminum coated with polyester resin.

Swimming tube respirometers (Fig. 2) were used for standard metabolism and cost of food handling experiments. The fish holding portion of this chamber was a cylindrical section of "plexiglas" tube 6.4 cm in diameter and 25.0 cm long with walls 0.5 cm thick. On each end there was a removable 0.5 cm mesh stainless steel screen, which kept the fish restricted to this area. A 10 cm piece of black polyethylene sheeting was wrapped around the central portion of this tube to provide the fish a sheltered place decreasing outside disturbances.

The water flowed through the swimming tube section into a 10 cm cuboidal chamber, through which the fish were also introduced into

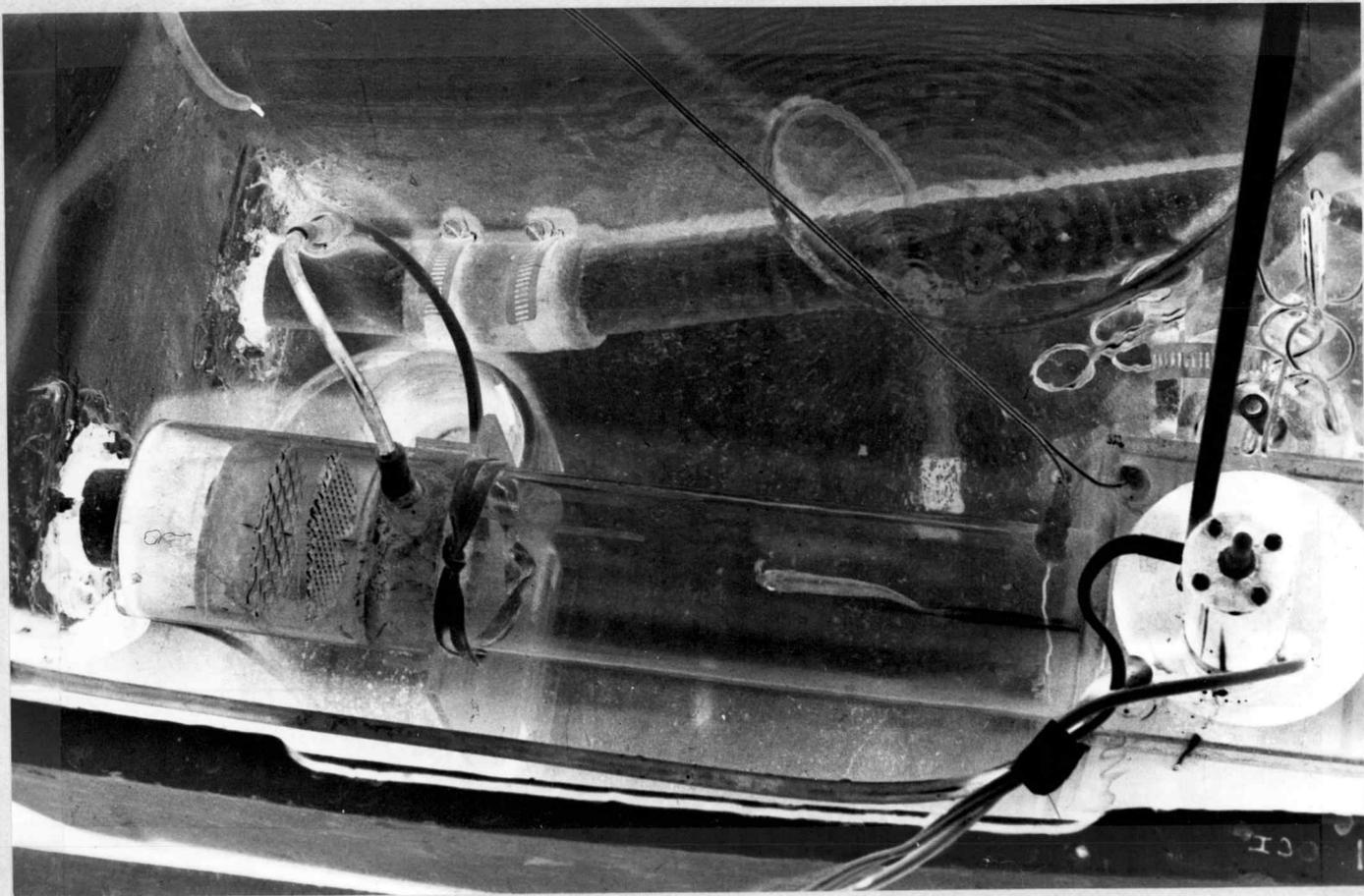


Figure 2. Top view of one swimming tube respirometer used in studies of juvenile coho salmon.

the tube. The water then traveled through "plexiglas" and polyester coated aluminum tubing back to the pump which circulated it through the system. Perforated "plexiglas" baffles between the pump and swimming portion of the tube helped to evenly distribute the flow of water. The velocity could be varied from 0.3 to 0.7 foot per second by a simple in-service adjustment on a lever-operated, variable-diameter pulley, placed between the motor and the water pump. Each respirometer contained 3.5 liters of water and had its own 0.5 Hp 3500 rpm motor, variable speed pulley, water pump, and water supply.

The main part of the respirometer was contained in a "styro-foam" water bath, which maintained the temperature within the respirometer at  $15\text{ C} \pm 0.2$ . The bath water was completely isolated from the test water and was continually circulated through a head box with submerged stainless steel heating and cooling coils. Oxygen measurements were made by inserting the motorized probe of the oxygen meter into a temporarily unstoppered hole in the top of the respirometer.

The enamel-coated "styrofoam" aquaria mentioned previously were tropical-fish shipping containers measuring 40 cm x 40 cm x 18 cm (Fig. 3). The water volume in each of these was maintained at 12.0 liters by a constant level standpipe drain. Perforated transparent "plexiglas" partitions, which allowed circulation of water but

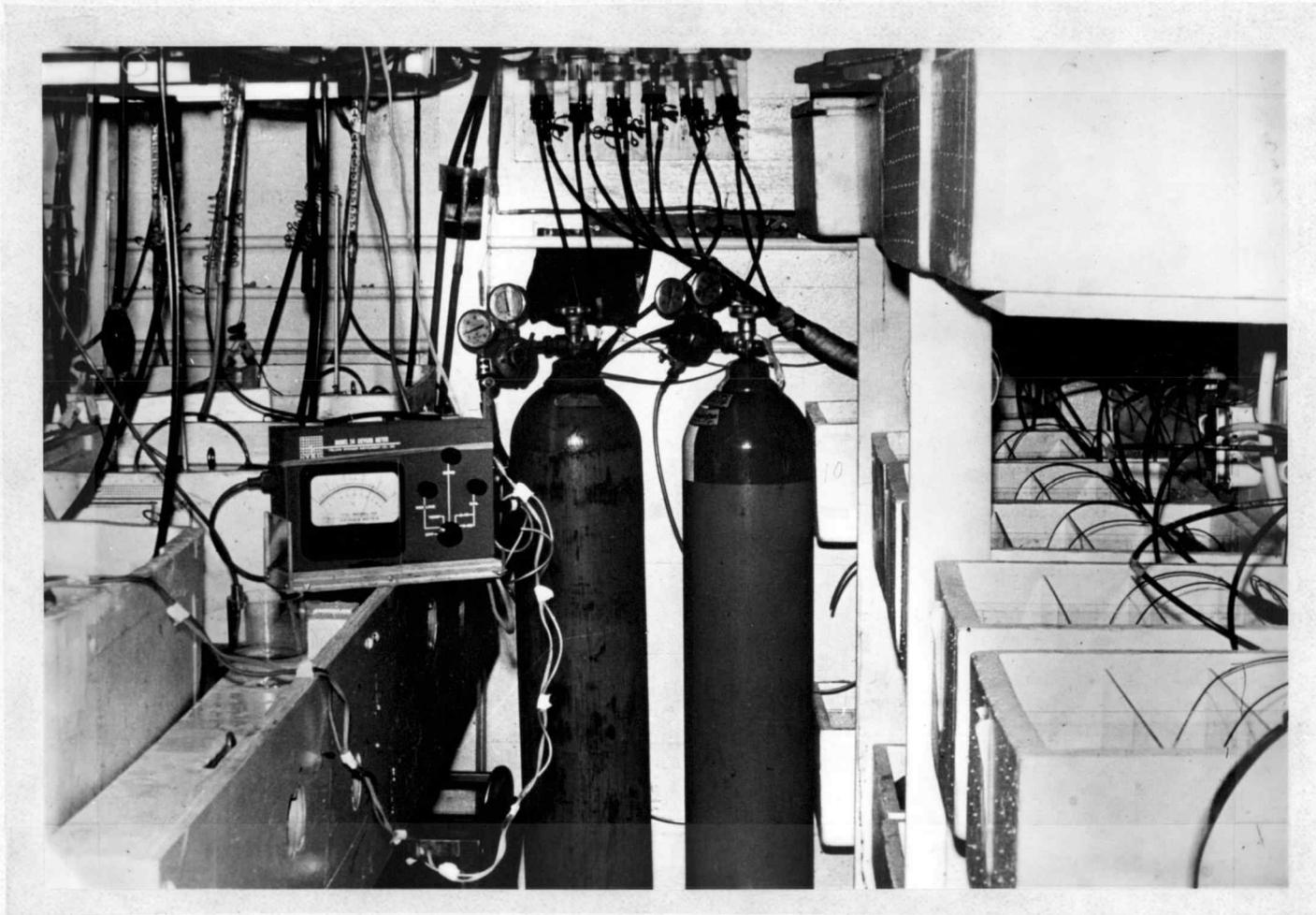


Figure 3. The experimental area used for the bioenergetic studies with juvenile coho salmon. Swimming tube respirometers on the left and growth study aquaria on the right.

not passage of food organisms, divided each aquarium into eight compartments, each containing 1.5 liters of water. A manifold system consisting of four pieces of flexible, 1 mm polyethylene "spaghetti" tubing distributed the inflowing water to different points in each aquarium at about 200 ml/min/aquarium. Each aquarium was covered with a transparent polyethylene lid to prevent the fish from jumping out and to keep insects and other potential food organisms from falling in. The fish were not forced to swim in these containers, and so their activity was random--generally greatest when stimulated by feeding activities of neighboring fish, or by food introduced into their own chambers. The head-box, temperature-control units maintained the temperature at 15 C in these chambers.

Six "plexiglas" containers consisting of two covered compartments (one liter capacity each) were used in experiments on the efficiency of food assimilation at the three dissolved oxygen levels being studied and at several levels of food consumption. The water in each of these compartments was isolated from the others and was not exchanged as was the water during the other experiments. These chambers were placed into the previously described "styrofoam" aquaria, which served as water baths to maintain the 15 C test temperature.

The water used in these experiments was from a spring-fed stream from which water is diverted through polyethylene and

aluminum pipe to a 1,000 gallon holding tank, a sand filter, and then polyethylene tubing leading to the various experimental areas of the Oak Creek Laboratory.

The temperature of the test water was maintained at 15 C for both the acclimation period and the experiments. Constant temperature was desired because size and age of fish, and dissolved oxygen were selected variables, and varying temperature would make more difficult the task of relating causes and effects. A temperature of 15 C is near optimal for juvenile coho salmon except during the winter months.

The lighting scheme, 12 hours of light and 12 hours of darkness, was maintained throughout the year to eliminate another variable that could have effects on the feeding, growth, and bioenergetics of the salmon.

The three dissolved oxygen concentrations (8.0, 5.0, and 3.0 mg/l) were selected to give a rather high one where no impairment of the selected physiological parameters was expected, an intermediate one where there might be some effects, and a low one where effects were expected. The 3.0 mg/l concentration was selected as being near the lowest level juvenile coho salmon could be expected to endure during these relatively short term tests. Herrmann (1958) and Fisher (1963) both encountered mortality among young cohos exposed to dissolved oxygen levels slightly below 3.0 mg/l. The 5.0 mg/l level

was selected because this concentration has been considered as suitable for cold water fish species, on the basis of the study by Ellis (1937). These levels were maintained in the continuously renewed water (which supplied the studies on growth, standard metabolism and specific dynamic action) by use of nitrogen stripping columns. The oxygen concentrations in the chambers for the food assimilation efficiency tests were maintained by bubbling a small amount of a mixture of air (75 percent) and nitrogen (25 percent) from a compressed gas cylinder through the water in the chambers.

#### The Bioenergetic Equation

In a study such as this one, there is a definite need to have all measurements expressed in the same terms. Since all of these measurements were made directly as calories or could be easily converted to calories, this unit was considered most appropriate. In order to compare and interrelate the various uses of the food resources available to the juvenile coho salmon, it was necessary to use a balanced equation including all the important known energy utilization categories. The bioenergetic equation developed by Warren and Davis (1967) is the most usable one available at present and was selected for use in these experiments. With this equation, the effects an environmental factor (e. g. , dissolved oxygen) may have on an animal's acquisition and use of its energy resources can be studied.

This equation is generally of the form  $Q_c = Q_g + Q_w + Q_r$  where:

$Q_c$  = energy value of the food consumed,

$Q_g$  = total change in energy value of materials of the body  
(measured as positive or negative growth),

$Q_w$  = energy value of all fecal and nitrogenous waste products  
eliminated from the body, and

$Q_r$  = value of all energy metabolically utilized or degraded  
in all ways and for all purposes (equivalent to total  
oxygen consumption measurements).

This latter category can be further reduced to energy use compartments of a more specific nature by letting  $Q_r = Q_s + Q_d + Q_a$  where:

$Q_s$  = energy value equivalent to that degraded in the metabolism  
of an unfed, inactive fish (measured as standard  
metabolism),

$Q_d$  = energy degraded in the course of digestion, assimilation,  
and storage of materials consumed (measured as  
specific dynamic action), and

$Q_a$  = energy degraded in the course of muscular activity  
over and above that of the standard metabolism of the  
animal.

Then substituting these into the first equation gives:

$$Q_c = Q_w + Q_g + Q_s + Q_d + Q_a, \text{ or}$$

$$Q_c - Q_w = Q_s + Q_d + Q_a + Q_g.$$

The equation in these terms deals with total energy consumption and utilization over a given test period. To facilitate some of the most useful interpretations, the terms need to be converted to rates per

unit of body mass per unit of time. One way to do this is to let

$$A_i = Q_i / W^x t \text{ where:}$$

i = c, w, g, s, d or a,

$W^x$  = the energy value in kcal of fish body material to some mean power x,

t = time in days.

The equation then becomes  $A_c - A_w = A_s + A_d + A_a + A_g$ .

Since growth apparently occurs only after all the other energy costs have been satisfied, the quantity of growth depends upon the magnitude of all the other categories in these equations. In fact, if consumption ( $A_c$ ) is below a certain level (the maintenance ration), energy will come from the materials of the body previously laid down as growth, and the " $A_g$ " for this period of food consumption will be negative. In such cases, it might be functionally more useful to transpose the " $-A_g$ " term to the left side of the equation, which in effect adds this negative growth to the food consumption, " $A_c$ " as an energy source.

### Experimental Procedures

Four kinds of experiments were performed with the young coho salmon periodically throughout the study period from the summer of 1968 through the spring of 1969. These four kinds of tests were performed during each of three periods of the year on the same year

class of salmon as they aged through the year. The experiments conducted in June and July will be referred to as summer studies; those of October and November will be considered fall studies; and those of February and March will be called spring studies. The details of the various experimental procedures are described next.

### Growth Experiments (A<sub>g</sub>)

After test-condition acclimation in the "styrofoam" aquaria, the fish were deprived of food for 48 hours and then weighed individually to determine their initial wet weights. The fish were not anesthetized and were blotted on damp cheesecloth before they were weighed in weighed amounts of water. An initial sample of ten fish randomly selected from the acclimated salmon was wet weighed, dried for four days at 70 C and weighed again (dry weight) and then subsamples were oxidized in a bomb calorimeter. After wet weighing, the test fish were replaced in the aquaria where they were still individually kept in semi-isolation, as they had been during the acclimation period. After approximately 24 hours, the fish were offered their first-day food ration. The fly larvae were offered while alive, after having been kept under refrigeration until time to weigh them just prior to feeding the fish. Daily samples of the food were analyzed for wet-dry weight relationships and caloric values.

There were 16 salmon (contained in two aquaria) at each dissolved oxygen level, and in each aquarium one fish was starved and others received 1, 2, 3, 4 or 5 percent of their wet body weights in food. The two remaining fish were fed as much as they would consume at one offering and were considered to be fed an "intermittent satiation" ration (Doudoroff and Shumway, 1970). The larvae were offered one at a time, until two were refused consecutively. Therefore, there were two fish at each combination of dissolved oxygen concentration and ration level, except for the four on intermittent satiation rations at each dissolved oxygen concentration.

After 14 consecutive days of feeding, the salmon were deprived of food for 48 hours and weighed and caloric values determined in the calorimeter in the same manner as at the beginning of the test. Temperature and dissolved oxygen concentrations were recorded daily and feces were removed as they accumulated.

The efficiency with which food is converted to growth is a very important facet of any animal growth study. I calculated two types of efficiencies from the data obtained in the study, gross growth efficiency and net growth efficiency. Gross growth efficiency is simply  $G/C$  where:

$G$  = the growth rate in cal/kcal/day,

$C$  = the food consumption rate in cal/kcal/day.

Net growth efficiency is represented by  $G/C - M$  where:

M = the maintenance ration, the food consumption rate at the point where the growth rate is just zero (the point where neither negative nor positive growth occurs).

#### Food Assimilation Efficiency Tests ( $A_w$ )

Food assimilation experiments were performed on six fish at each of the three dissolved oxygen concentrations studied. Two fish were tested at each of three ration levels, starvation, 3 percent of wet body weight per day, and intermittent satiation. The fish were acclimated as previously described and were placed in the "plexi-glas" containers, one fish to a container, after their initial wet weights were measured. Twenty-four hours later, they were fed the preselected ration and then held for an additional four days to allow the consumed meal to pass through the alimentary canal. The liter of water containing the waste products was acidified with 1 ml of  $H_2SO_4$  and refrigerated until energy content measurements could be made, usually within two weeks. Partial caloric value of the mixture of test water and excreted materials was determined by a wet combustion method having potassium dichromate as the oxidizing agent (American Public Health Association, 1965, p. 510). This method does not measure any of the nitrogenous waste materials (Maciolek, 1962; and personal observations). Further analyses were made to determine the proportion of the nitrogen waste measured as

ammonia, and colorimetric determinations were made of the ammonia present in the samples. A factor of 5.3 was used to convert milligrams of ammonia to calories of ammonia (Lange, 1967), so this could be added to the non-nitrogenous waste component to obtain " $A_w$ ."

#### Specific Dynamic Action Tests ( $A_d$ )

These studies were performed in the swimming-tube respirometers, and the salmon were required to swim at about 0.3 ft per second. This was done to help to eliminate variations in oxygen consumption that would result from random activity of the fish.

After a five-day acclimation period in the respirometers, during which time the fish were offered the same ration level as was to be used in the test, the fish were deprived of food for 24 hours. During the next 24-hour period, a base line oxygen consumption rate was established by means of oxygen measurements made at the beginning and end of a series of consecutive one-hour periods. The fish were then fed the test ration, and another series of one-hour oxygen consumption measurements was made to measure the specific dynamic action (cost of handling the food). After each one-hour oxygen consumption period, the respirometers were flushed with the test water until the oxygen levels were restored to the original test concentrations (requiring approximately 15 minutes) and another hour-long test

was begun. This was terminated in 24 to 36 hours, after the oxygen consumption had dropped back to the previously determined base level.

After these tests, the fish were removed and weighed and several "blank" oxygen consumption determinations were made on the respirometers without the fish. After subtracting the blank values from the total rates of oxygen utilization, the oxygen consumption values for the fish were converted to calories by using an oxygen-calorific coefficient of 3.42 calories per milligram of oxygen consumed (Warren, 1971). As will be explained later, the area between the oxygen-consumption base line and the time track of oxygen consumption after feeding the fish was taken to represent oxygen utilized in food handling.

#### Standard Metabolism Tests ( $A_s$ )

Standard metabolism experiments were also performed in the swimming-tube respirometers. During the last five days of the acclimation period (which occurred in the respirometer), the fish were fed slightly more than a maintenance ration, approximately 3 percent of their body weight. The fish were then starved for a period of 24 hours, removed from the tubes, weighed, and then returned to the tubes. Twenty-four hours after being weighed, they were then subjected to a series of one-hour long oxygen consumption measurement periods, during which they were forced to swim at a series of

water velocities (0.3 up to 0.7 ft/sec). Each fish experienced two or three one-hour periods at each velocity up to the maximum velocity it could maintain. Sufficient time was allowed between hour-long tests for any oxygen debt that may have been incurred to be paid off. After a series of velocities had been completed, oxygen consumption rates were plotted against swimming velocities and a line was drawn through the lowest points and extrapolated back to the point of zero activity. The oxygen consumption at that zero activity point was considered to be the standard metabolic rate (Beamish, 1964a, b; Brett, 1964).

A previous study (Thatcher, unpublished data) had shown that this method consistently gave lower (and therefore presumably more accurate) standard metabolism values than either a black box method, or another method measuring oxygen consumption of anesthetized fish. The oxygen consumption values determined by these tests were converted to caloric values by using the oxy-calorific coefficient of 3.42.

#### Activity ( $A_a$ )

No actual experiments were conducted to measure the costs of activity over and above costs incurred by resting, unfed fish. Activity costs were calculated as the remainder obtained by subtracting the total energy use rates (cal/kcal salmon/day) of the three measured

categories " $A_g$ ", " $A_w$ " and " $A_s$ " from the food consumption rates (cal/kcal salmon/day). For reasons discussed later, this " $A_a$ " category was combined with the SDA ( $A_d$ ) as one energy use category, for purposes of interpretation of results.

## RESULTS AND INTERPRETATION

### Food Consumption and Growth

Relationships between food consumption rates and growth rates based on data from the experiments with juvenile coho salmon during the three seasons of study (summer 1968, fall 1968, spring 1969) and at the three dissolved oxygen concentrations tested (8.0 mg/l, 5.0 mg/l, 3.0 mg/l) are presented in Figures 4, 5, and 6. Relationships between gross efficiency and net efficiency of growth and food consumption rate, based on the smoothed growth curves, are also shown in these figures. The growth curves for the three seasons and the three levels of dissolved oxygen were not greatly different. Nevertheless, some apparently important differences require consideration.

The form of growth curves tended to be curvilinear for the fish at 5.0 and 3.0 mg/l dissolved oxygen, but it was more nearly linear at 8.0 mg/l (Figs. 4, 5, 6). Curvilinear growth curves define gross efficiency of growth curves that reach a maximum and then decline and net efficiency of growth curves that tend to decline throughout the range of food consumption levels. Linear growth curves define gross efficiency of growth curves that approach an asymptote and net efficiencies that tend not to change with changes in consumption rate (Warren, 1971). These relationships are apparent in the figures.

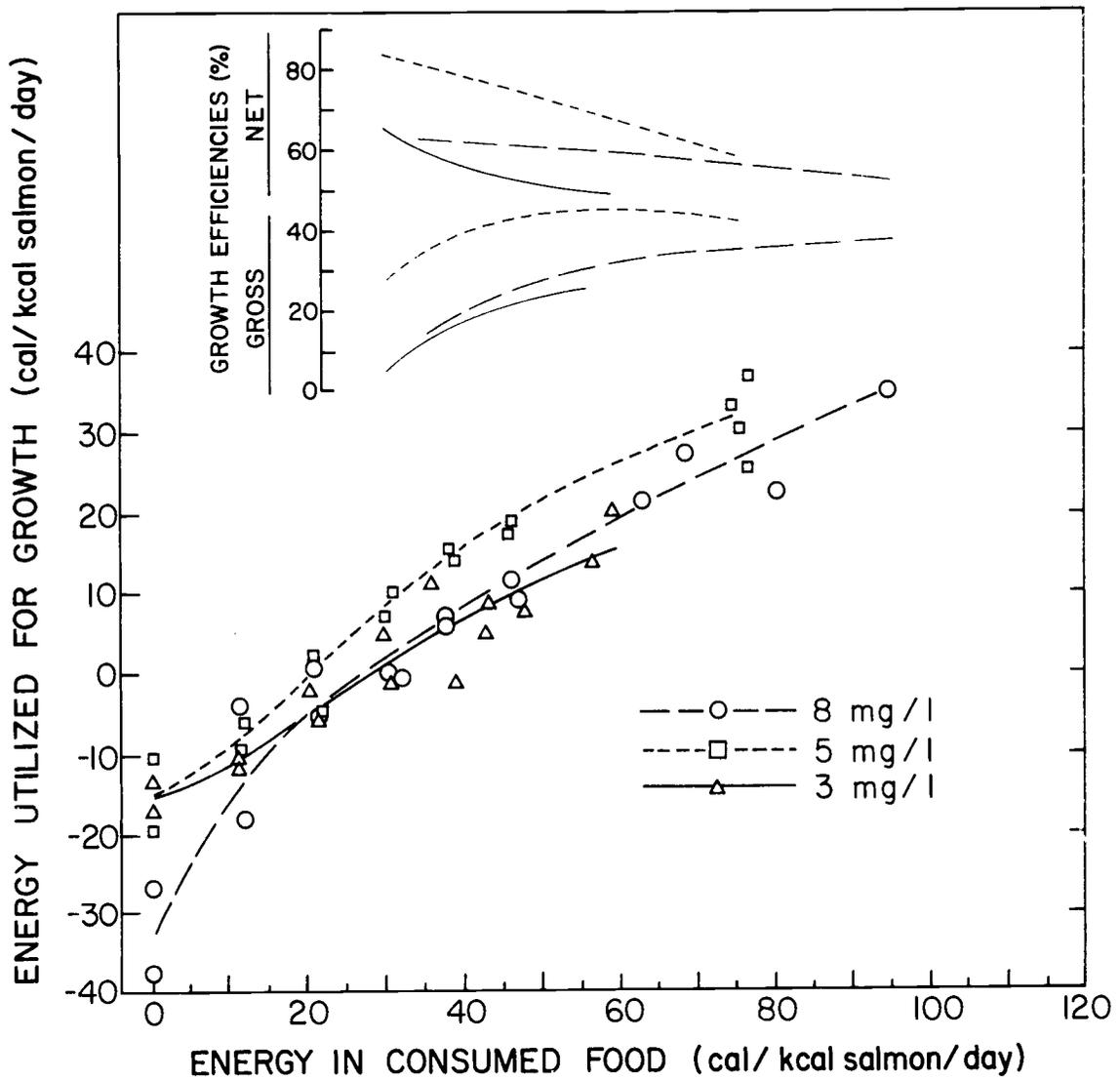


Figure 4. Relationships between food consumption rate, growth rate, efficiencies of growth, and dissolved oxygen concentration for juvenile coho salmon in summer (June and July) experiments conducted at 15 C. The curves are fitted to mean values. The maximum consumption limits were determined by the fish; the lower consumption rates were set by the investigator.

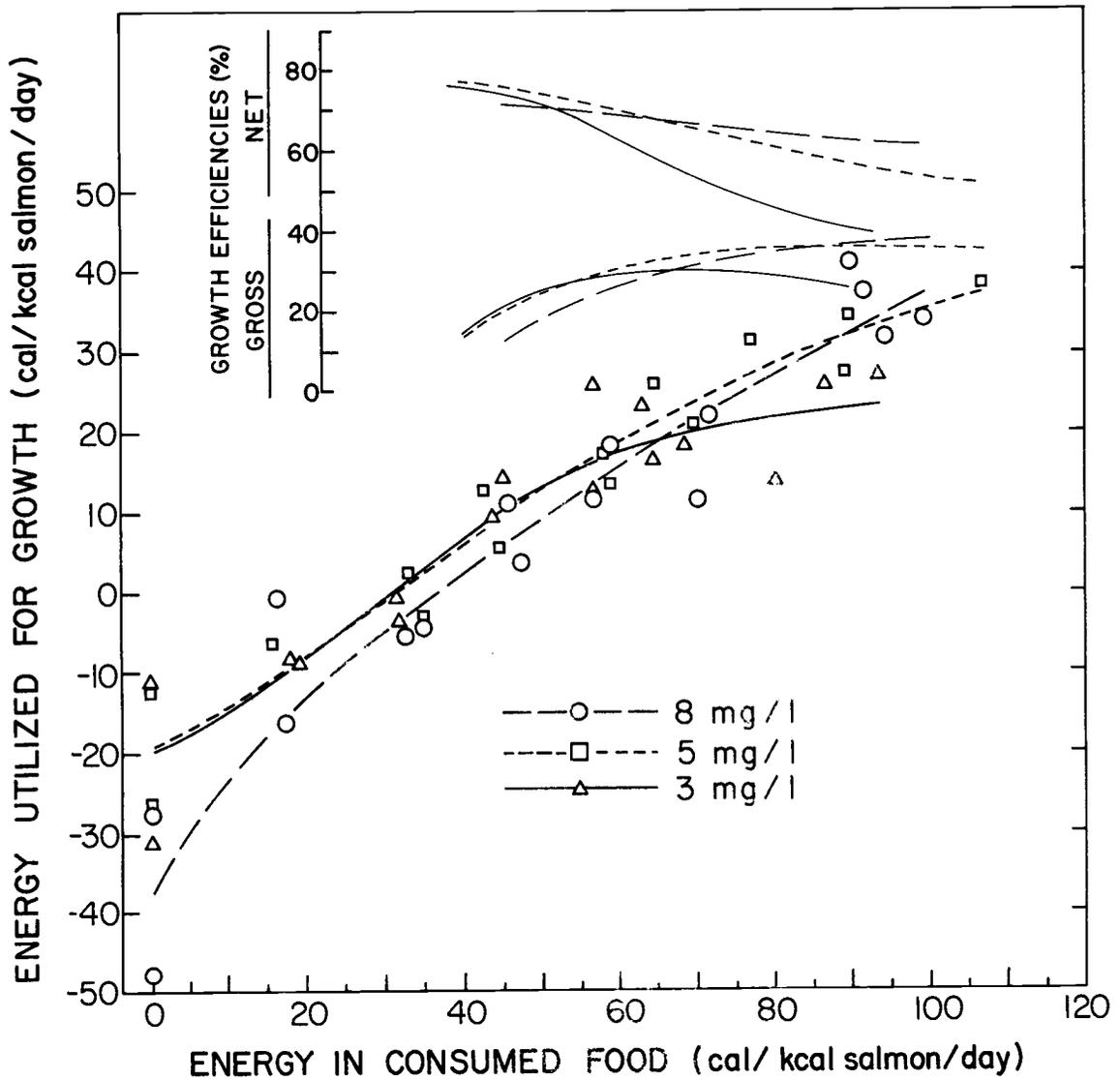


Figure 5. Relationships between food consumption rate, growth rate, efficiencies of growth, and dissolved oxygen concentration for juvenile coho salmon in fall (October and November) experiments conducted at 15 C. The curves are fitted to mean values. The maximum consumption limits were determined by the fish; the lower consumption rates were set by the investigator.

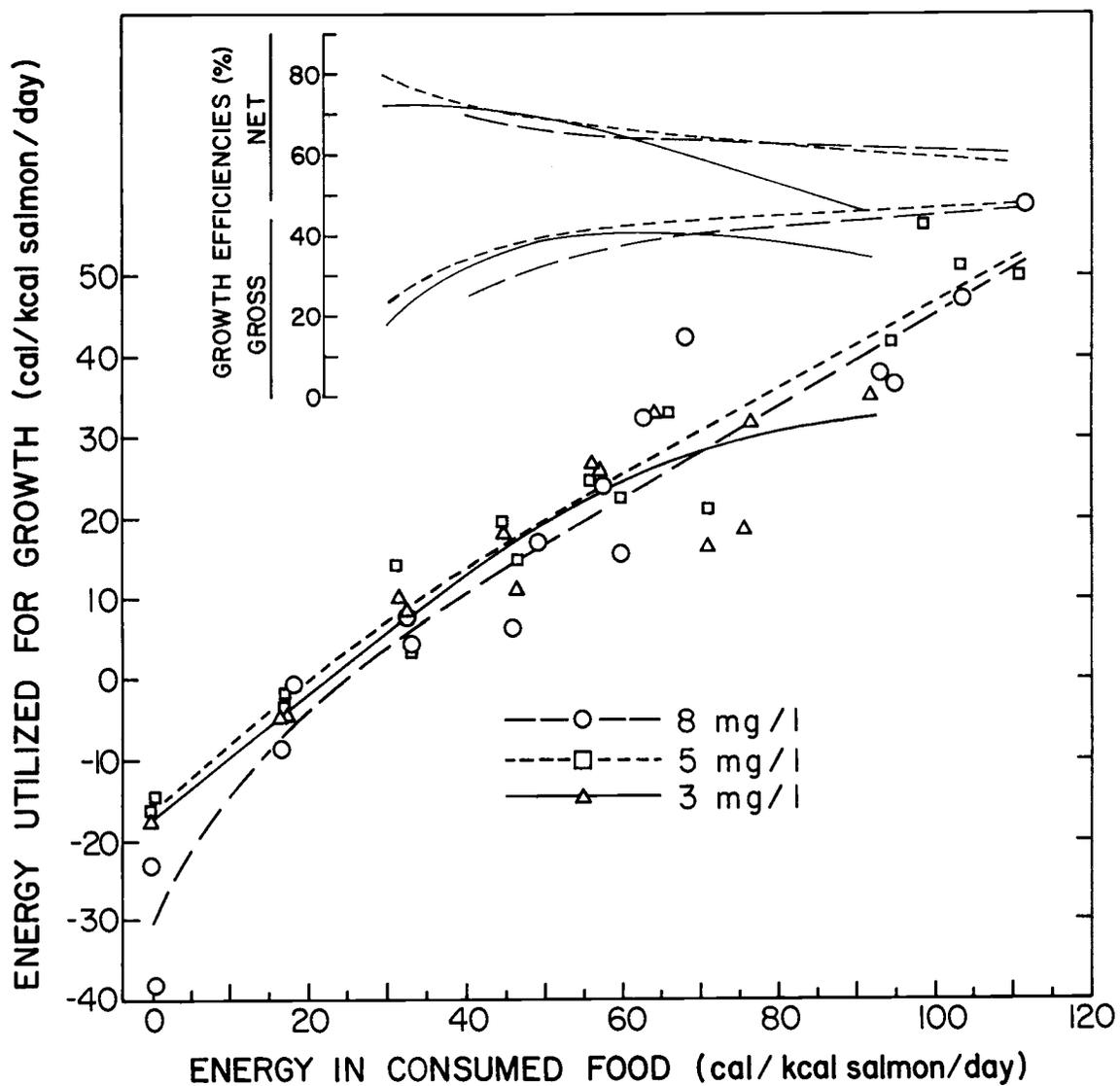


Figure 6. Relationships between food consumption rate, growth rate, efficiencies of growth, and dissolved oxygen concentration for juvenile coho salmon in spring (February and March) conducted at 15 C. The curves are fitted to mean values. The maximum consumption limits were determined by the fish; the lower consumption rates were set by the investigator.

Food consumption rates at the "intermittent satiation" (as much as a fish would consume at one unlimited food offering) level of feeding tended to be much lower for fish held at dissolved oxygen concentrations of 3.0 mg/l than for those at 5.0 mg/l and 8.0 mg/l (Figs. 4, 5, 6). This effect was obvious in all three seasons of study.

The rate of loss of body tissue at zero food consumption was greater for the cohos kept at 8.0 mg/l dissolved oxygen than for those at 5.0 or 3.0 mg/l, in all three seasons (Figs. 4, 5, 6). This will be elaborated upon in the section on energy budgets, where apparent correlations between this greater loss of body tissue and the fate of the energy involved at 8.0 mg/l will be considered.

At high food consumption levels, the rate of increase in growth rates per unit increase of food consumed tended to decrease more noticeably in the fish kept at 3.0 mg/l than in those at 8.0 or 5.0 mg/l during the fall and spring experiments. This was not apparent during the summer study, perhaps because maximum food consumption rate at 3.0 mg/l was noticeably lower (and apparently within the limits where the fish can convert food into growth fairly efficiently) during that season than during the other seasons. The effects noted in the fall and spring seasons will be interpreted in the section on energy budgets, where fates of energy and material can be taken into account.

One of the most striking features of the curves illustrating gross efficiency of growth was the much higher value reached by the

fish at 5.0 mg/l dissolved oxygen than those reached by the fish at 8.0 or 3.0 mg/l, during the summer experiment (Fig. 4). The gross efficiency curves for the fish at 3.0 mg/l in the fall and spring were noticeably different from those for the fish at 8.0 or 5.0 mg/l. The curves for the fish at 3.0 mg/l reached a maximum at intermediate feeding levels and then declined with further increases in consumption, while in general the curves for the fish at 8.0 and 5.0 mg/l continued to increase as the food consumption increased.

The net growth efficiency curves of the fish at the three oxygen concentrations were quite different for the summer experiment (Fig. 4), the 8.0 mg/l curve declining little with increasing consumption, in contrast to the 5.0 and 3.0 mg/l curves. The net efficiency of growth curves based on the fall and spring experiments had much more negative slopes in the case of fish kept at 3.0 mg/l dissolved oxygen than in the cases of those kept at 8.0 or 5.0 mg/l.

In summary of the food consumption and growth data, during the summer study the fish kept at the dissolved oxygen concentration of 5.0 mg/l exhibited greater growth than those at 8.0 or 3.0 mg/l at most food consumption levels near and above the maintenance level (Fig. 4). Fish kept at 3.0 mg/l consumed less food than at 8.0 or 5.0 mg/l. The coho salmon at 8.0 and 3.0 mg/l appeared to grow at about the same rates (though lower than at 5.0 mg/l) at comparable food consumption levels above the maintenance level. At zero food

consumption, those at 8.0 mg/l lost more energy and body materials. Gross growth efficiencies were noticeably higher at 5.0 mg/l, while the slope of the net growth efficiency curve at 8.0 mg/l declined much less than at 5.0 or 3.0 mg/l. In the fall, the most noticeable characteristic of the growth curves was a decrease in slope of the food consumption-growth curve at higher rations in the case of the fish held at the 3.0 mg/l oxygen concentration (Fig. 5). Fish at 3.0 mg/l again consumed less food than at 8.0 or 5.0 mg/l. The loss of energy and materials at zero food consumption by the fish at 8.0 mg/l again was greater than that of those at 5.0 or 3.0 mg/l. The gross growth efficiency curve dropped off sharply at 3.0 mg/l and the net efficiency curve exhibited a much greater slope than at 8.0 or 5.0 mg/l. In the spring experiments, there was again a marked decrease in slope of the growth curve at high ration levels for the fish held at 3.0 mg/l (Fig. 6). Also, fish at 3.0 mg/l consumed less food than at 8.0 or 5.0 mg/l. And again at zero food consumption, the fish at 8.0 mg/l exhibited a greater loss of body materials than did those at 5.0 or 3.0 mg/l. Then too, the gross efficiency curve dropped off sharply at 3.0 mg/l and the net efficiency curve had a much greater slope at 3.0 mg/l than at 8.0 or 5.0 mg/l.

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3.0 mg/l, during the summer experiment (Fig. 4). The gross efficiency curves for the fish at 3.0 mg/l in the fall and spring were noticeably different from those for the fish at 8.0 or 5.0 mg/l. The curves for the fish at 3.0 mg/l reached a maximum at intermediate feeding levels and then declined with further increases in consumption, while in general the curves for the fish at 8.0 and 5.0 mg/l continued to increase as the food consumption increased.

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#### Nonabsorption (Fecal) and Nitrogenous Wastes

The relationships between dissolved oxygen concentration, food consumption rate, and the two categories of waste energy measured (nonabsorbed materials and nitrogenous wastes) are shown in Figures 7, 8, and 9. Nonabsorbed energy was essentially fecal wastes, which were measured as chemical oxygen demand by the liquid combustion method already described. Nitrogenous wastes were assumed to be principally ammonia and were measured as such.

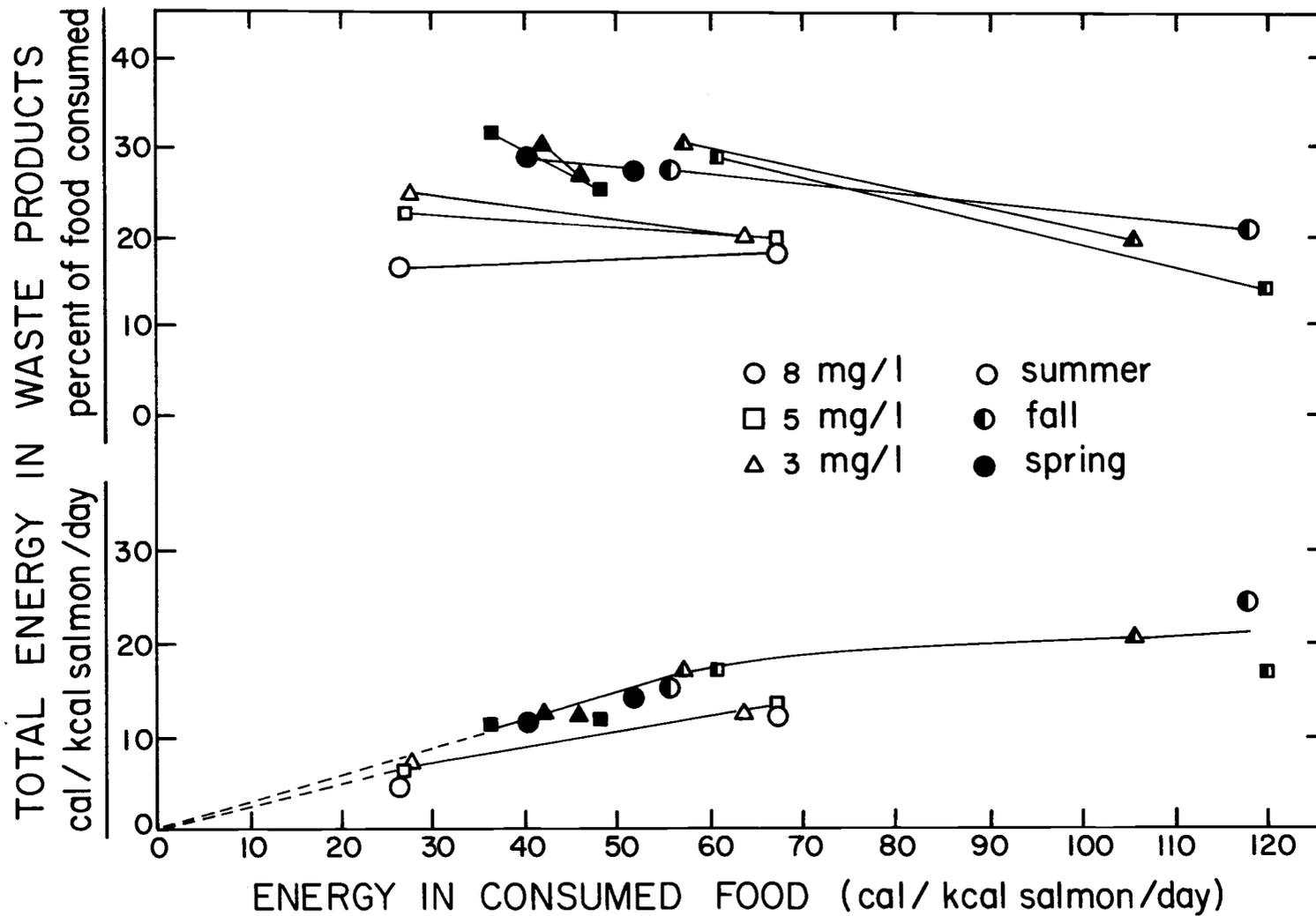


Figure 7. Relationships between total energy in the waste products, food consumption rate, season, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies conducted at 15 C. Each data point represents the mean of two fish.

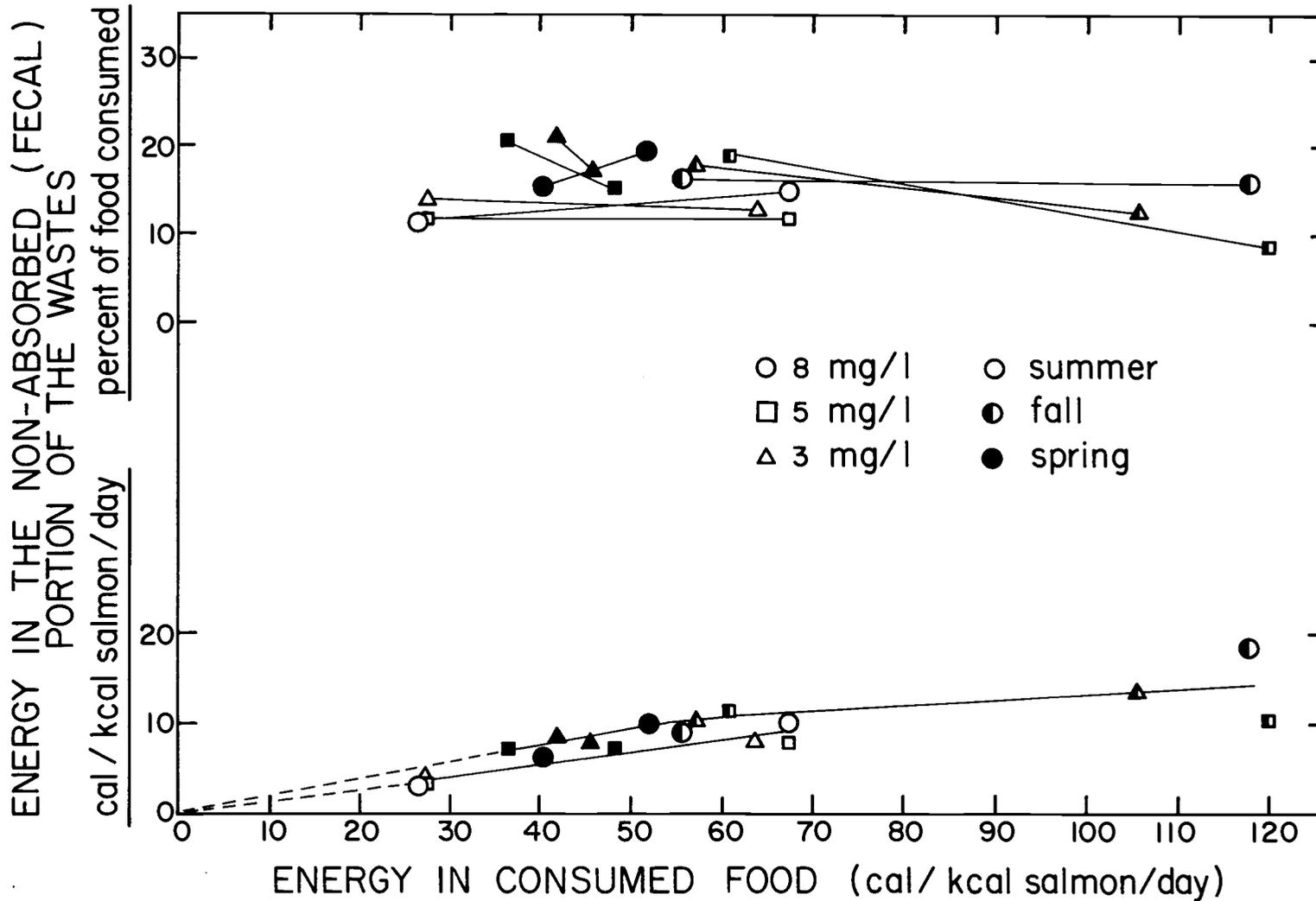


Figure 8. Relationships between energy in the non-absorbed (fecal) portion of the wastes, food consumption rate, season, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies conducted at 15 C. Each data point represents the mean of two fish.

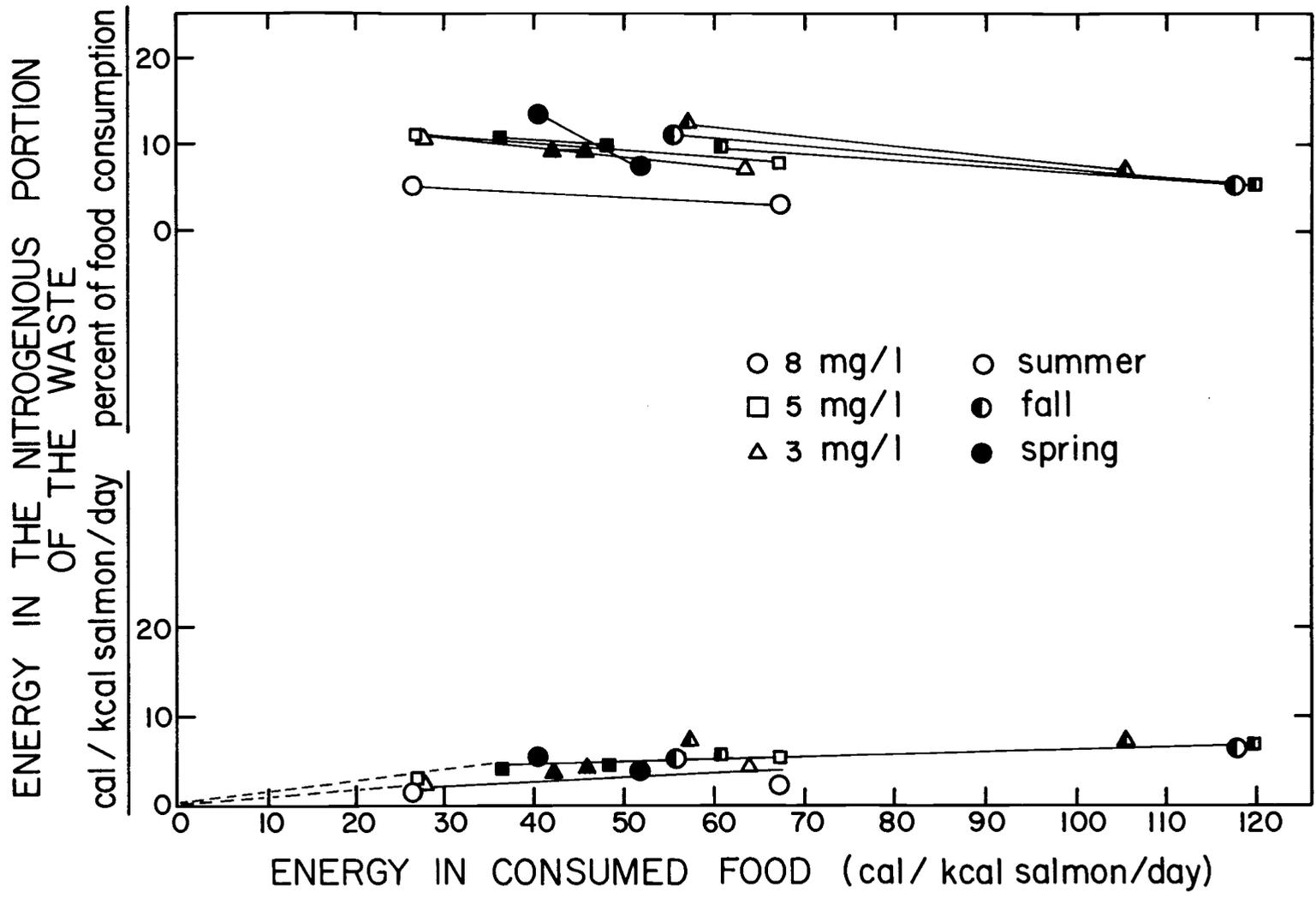


Figure 9. Relationships between energy in the nitrogenous portion of the wastes, season, food consumption rate, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies conducted at 15 C. Each data point represents the mean of two fish.

Figure 7 shows the total waste produced in relation to food consumption rate on a percent of food consumed basis and on the rate basis of calories per unit of fish weight per day. Figures 8 and 9 show nonabsorbed energy and nitrogenous wastes on similar bases. At similar food consumption levels, the energy of total waste products (on a rate basis) was slightly lower during the summer study period than during the fall or spring periods. Correspondingly, the percentage of food consumed that appeared as waste products was lowest in the summer.

Generally, in each season at the levels of food consumption studied, the total rate of energy loss through waste products increased slightly with increasing food consumption. The percentage of the food consumed that appeared as total waste products, however, generally decreased as food consumption increased. Over all seasons and consumption levels, the total waste energy represented about 20 to 30 percent of the consumed food energy.

Dissolved oxygen concentration generally appeared to have little effect on the total wastes produced. There is, however, an interesting indication that at 8.0 mg/l the percentage of food consumed appearing as waste products did not decline so much with increasing food consumption as it did at 5.0 and 3.0 mg/l.

In the nonabsorbed energy category (fecal material) there appeared to be slightly less waste, per unit food consumed or per unit

fish biomass, by the fish in the summer than in the fall or spring. As food consumption rate increased, there was a tendency for nonabsorbed waste products to increase when these were expressed as cal/kcal salmon/day. Fecal waste products, expressed as percentage of food consumed, did not change much with increasing food consumption, if all seasons and groups are considered together. There may have been some tendency for the percentage of food appearing as nonabsorbed waste to increase with increasing consumption in the group held at 8.0 mg/l. Other than those, no relationship between oxygen concentration and the nonabsorbed waste category was apparent.

Energy values of the nitrogenous waste category, calculated from the measured ammonia levels in the waste, are shown in Figure 9. With the possible exception at 8.0 mg/l in the summer, there were no apparent seasonal differences, or differences owing to dissolved oxygen concentration. There was very little if any increase in the rate of loss of energy in the nitrogenous waste category with rather large increases in the food consumption rate. Thus, as food consumption levels increased, there was a trend for the percentages of the food consumed which appeared as nitrogenous waste to decrease.

#### Activity and Specific Dynamic Action

Specific dynamic action (SDA) was estimated on the basis of increase in the metabolic rate of fish after feeding, when an attempt

was made to keep the fish at a constant but curtailed level of activity (see Methods). Figure 10 shows graphical representations of data from (a) a successful experiment, and (b) an unsuccessful experiment. Estimates of SDA based on reasonably successful experiments are given in Appendix Table 1. The relationships between dissolved oxygen concentration, food consumption rate, and the collective requirements for activity and SDA, as determined with energy equations by difference, are illustrated in Figures 11, 12, and 13.

Two major problems were encountered in attempting to measure the SDA of the juvenile coho salmon in these experiments. Even though held in a current of constant velocity, these fish did not swim in one position or at one speed for very long at a time, but rather were quite erratic in their swimming behavior. Consequently, a flat base line of oxygen consumption could hardly be expected to occur; although some experiments yielded reasonably good results (Figure 10a), often the results were too erratic (Figure 10b).

Since quantifying the SDA depended on measuring the area between the oxygen consumption baseline and the line defining oxygen consumption after ingestion of food (the shaded areas in Fig. 10a and b), determining the end point of the SDA expenditure was also important. It was nearly impossible to determine this end point in test runs that yielded results like those in Figure 10b. Sometimes, even in relatively good experiments, the SDA dependent oxygen consumption

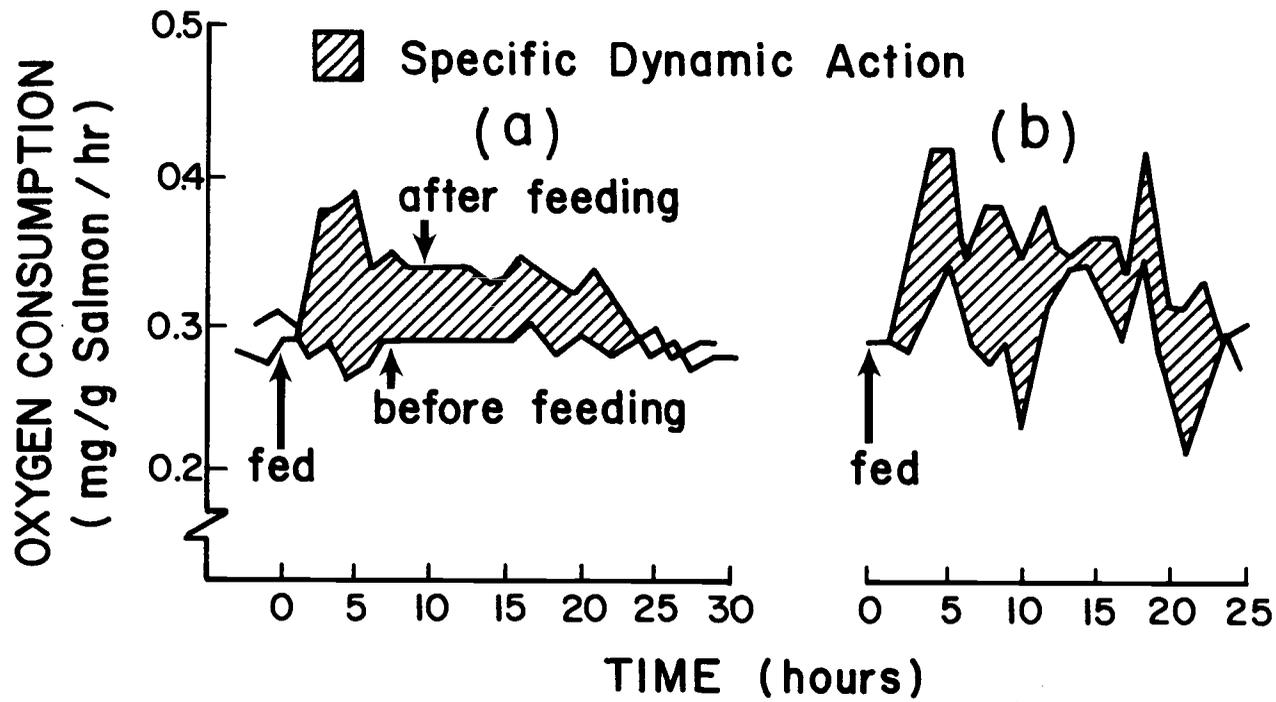


Figure 10. Specific dynamic action plot for a swimming juvenile coho salmon in laboratory experiments conducted at 15 C. (a) A relatively good experiment; (b) a very poor experiment.

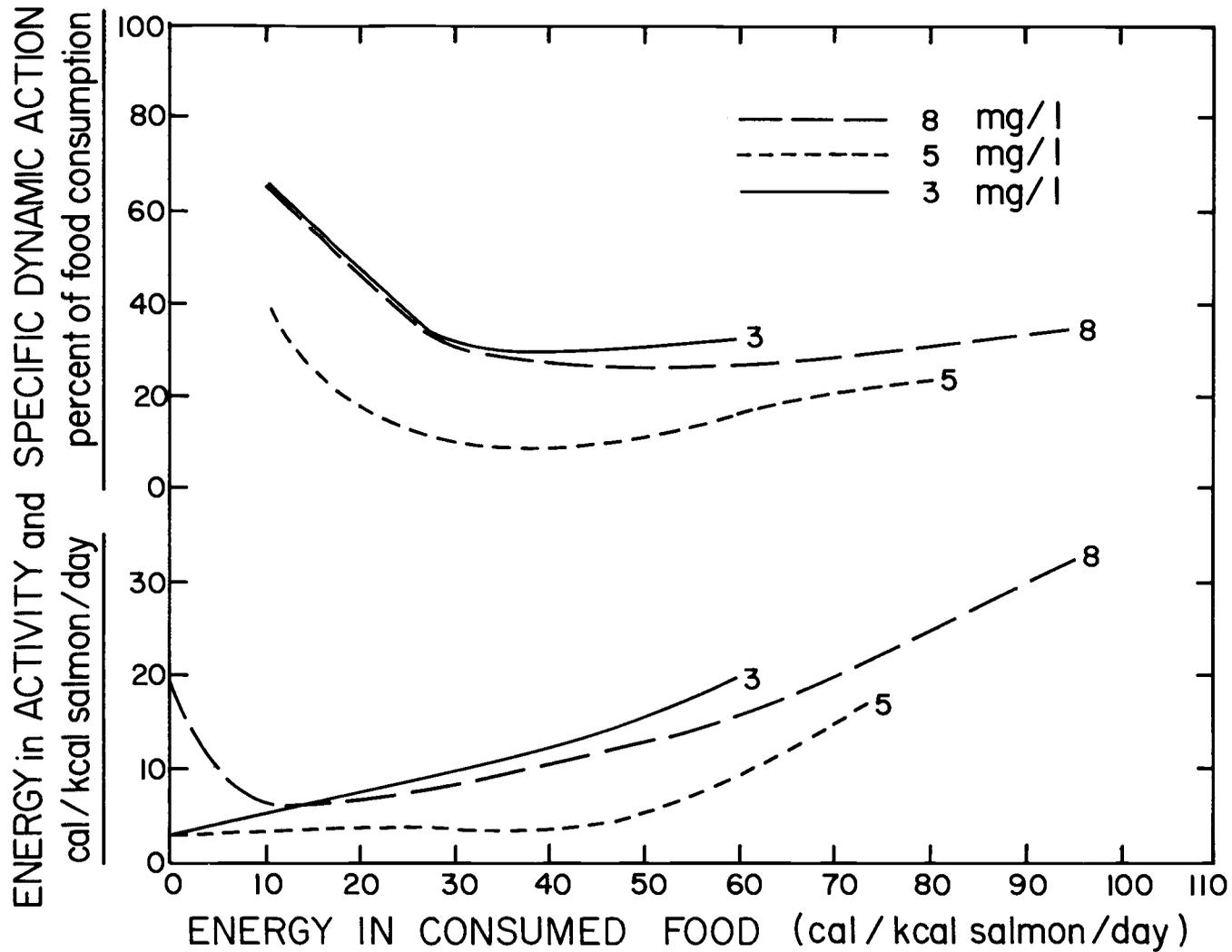


Figure 11. Relationships between food consumption rate, activity and specific dynamic action, and dissolved oxygen concentration in the summer (June and July) for juvenile coho salmon in laboratory studies at 15 C. The curves are fitted to mean values derived by difference from the other data ( $A_c$ ,  $A_w$ ,  $A_s$  and  $A_g$ ).

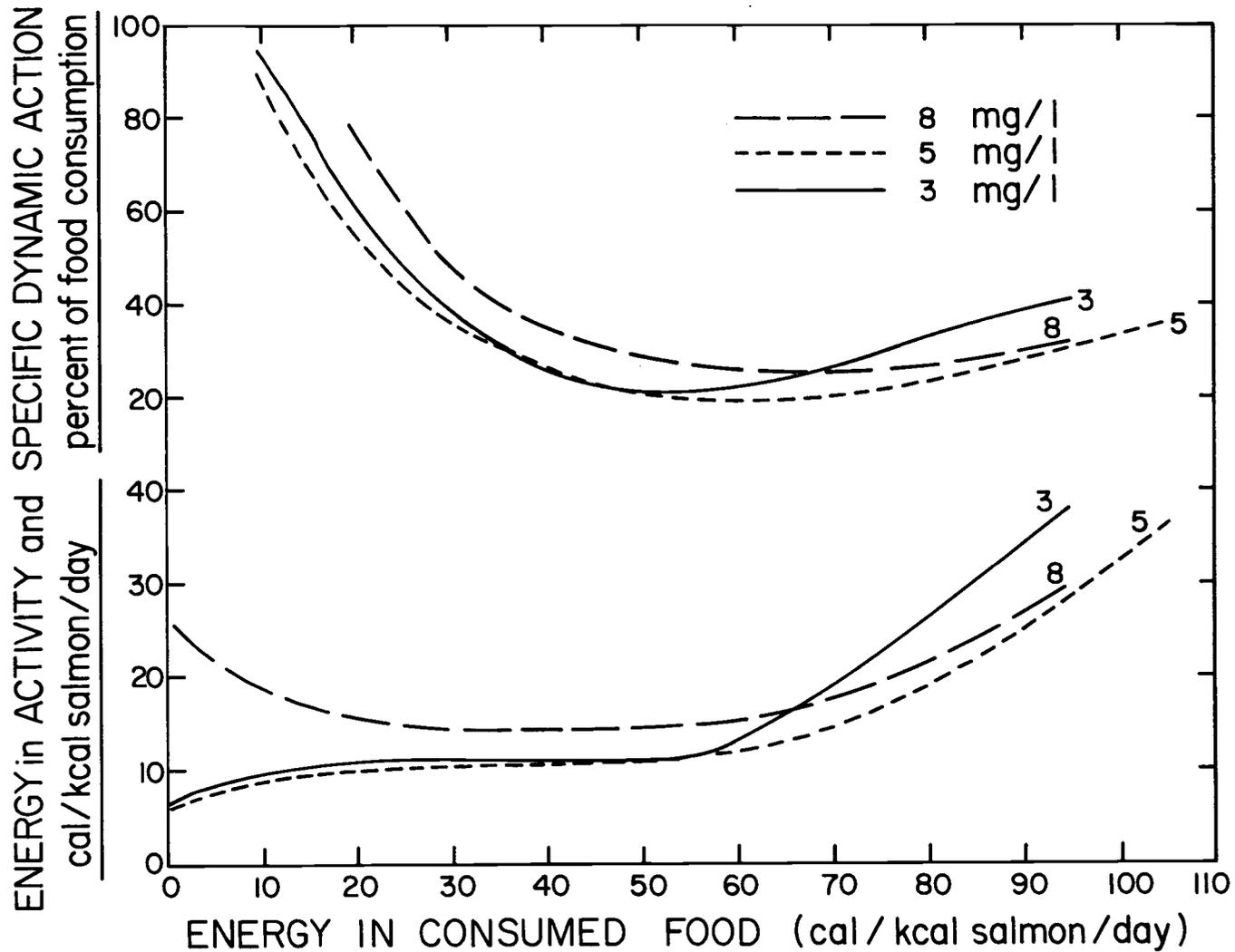


Figure 12. Relationships between food consumption rate, activity and specific dynamic action, and dissolved oxygen concentration in the fall (October and November) for juvenile coho salmon in laboratory studies at 15 C. The curves are fitted to mean values derived by difference from the other data ( $A_c$ ,  $A_w$ ,  $A_s$  and  $A_g$ ).

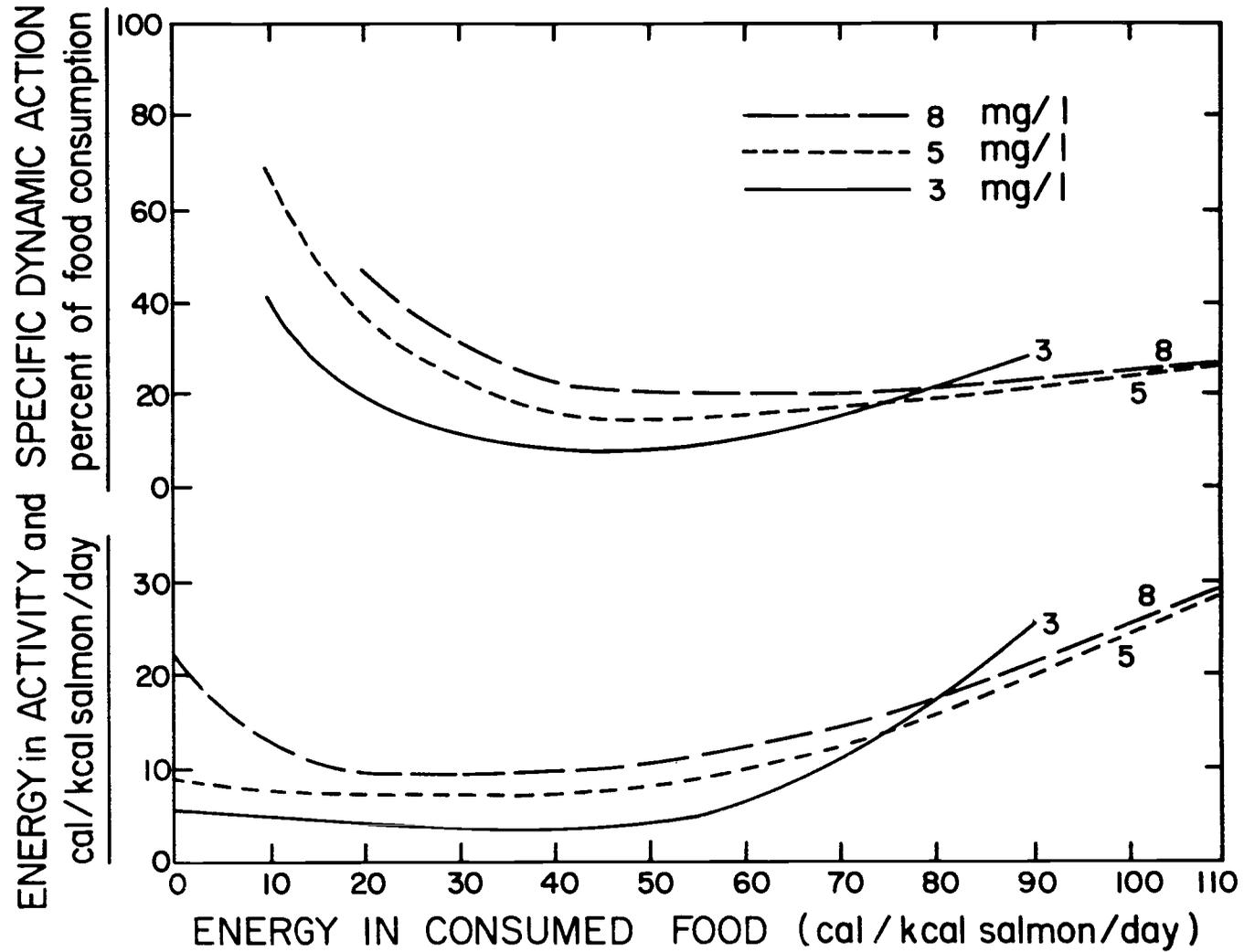


Figure 13. Relationships between food consumption rate, activity and specific dynamic action, and dissolved oxygen concentration in the spring (February and March) for juvenile coho salmon in laboratory studies at 15 C. The curves are fitted to mean values derived by difference from the other data ( $A_c$ ,  $A_w$ ,  $A_s$  and  $A_g$ ).

line would drop to or below the baseline, but then rise above it again for several hours. It was difficult to decide in these cases whether the latter increase in oxygen consumption was due to SDA or activity.

The values derived from the graphic representations of the SDA data (similar to Fig. 10a and b) are given in Appendix Table 1. For reasons explained in the next paragraph, no SDA experiments were conducted during the spring study period. The values for the summer and fall seasons are not greatly different, but there did seem to be a tendency for SDA values to be a little higher in the summer than the fall. Whether this is a true picture of SDA costs varying between the two seasons cannot be determined with certainty from these data.

The utility of SDA derived from fish kept in forced activity chambers can be questioned on another basis. Recently, review of the methods used in this present study and in a previous study by Averett (1969) indicated that estimates of SDA based on fish at one forced activity level could not reliably be used in developing energy budgets in the growth studies reported here, which involved fish not forced to swim. Warren (1971) pointed out that energy that would appear as SDA in a rather inactive fish apparently can be used for muscular work by an active fish. As a result, the SDA of the active fish would be reduced. Therefore, the two energy use categories " $A_d$ " and " $A_a$ " of the Warren and Davis (1967) energy budget have been combined in further analyses of my data.

The relationships between dissolved oxygen concentration, food consumption rate, and the combined costs of activity and SDA, as determined from energy equations by difference (budgets), for the summer season are shown in Figure 11. At the zero food consumption level, the energy expenditure for activity and SDA in cal/kcal salmon/day was much higher at 8.0 mg/l dissolved oxygen than at 5.0 or 3.0 mg/l. At food consumption rates near and above the maintenance level (20-28 cal/kcal salmon/day) the cost of this category of energy use was very similar at both 8.0 and 3.0 mg/l. The fish at 5.0 mg/l exhibited lower values for activity and SDA over the entire range of food consumption rates in this same season.

In the summer, the energy utilization in cal/kcal salmon/day for activity and SDA increased at a fairly steady rate with increasing food consumption for the fish studied at 8.0 and 3.0 mg/l dissolved oxygen. At 5.0 mg/l, such energy utilization remained constant up to intermediate food consumption levels and then increased quite sharply with further increase in food consumption.

The relationships between dissolved oxygen concentration, food consumption rate, and combined costs of activity and SDA, as determined by difference (budgets), for juvenile coho salmon in the fall study period are shown in Figure 12. The expenditure of energy for activity and SDA in rate terms was greater from starvation levels up to intermediate food consumption levels for the fish kept at 8.0 mg/l

dissolved oxygen than for those kept at 5.0 or 3.0 mg/l. At higher food consumption levels, energy use in this combined category increased sharply for cohos at 3.0 mg/l, this group of fish exhibiting the greatest requirements for activity and SDA at high feeding levels. The fish at 8.0 and 5.0 mg/l exhibited a similar but not so marked increase at high food consumption levels. Thus, for the fish at all oxygen exposure levels in the fall, except those at 8.0 mg/l receiving starvation-level rations, the energetic costs of activity and SDA remained fairly constant up through intermediate food consumption levels, but at still higher feeding rates these costs increased quite markedly.

Relationships between dissolved oxygen concentration, food consumption rate, and the combined costs of activity and SDA, as determined by difference (budgets), for fish during the spring season are shown in Figure 13. Once again at zero food consumption the expenditures of energy in cal/kcal salmon/day for activity and SDA by fish studied at 8.0 mg/l were much higher than for those kept at 5.0 or 3.0 mg/l. There was little or no difference in the costs of activity and SDA at 8.0 or 5.0 mg/l dissolved oxygen as food consumption increased from near maintenance levels up to maximum values. Energy utilization in this category appeared to be lower for the fish at 3.0 mg/l at all food rations up to about 70-80 cal/kcal salmon/day. Above that level, these energy costs became greatest at 3.0 mg/l.

During the spring, with the exception of the cohos kept at 8.0 mg/l dissolved oxygen and starvation rations, the expenditures of energy for activity and SDA were fairly constant up to the intermediate food consumption rates. Above that level of consumption, the expenditures of energy in this category increased at all oxygen exposure levels. The increase was quite sharp at 3.0 mg/l, less so at 8.0 and 5.0 mg/l.

There were two interesting relationships that appeared to some degree in all seasons. First, there was a tendency for energy expenditures for activity and SDA to remain on a plateau at the intermediate food consumption levels, which may be similar to feeding rates in nature. There also was a tendency for the costs of activity and SDA at 8.0 mg/l to be higher than at 5.0 or 3.0 mg/l in the intermediate to low food consumption range.

#### Standard Metabolism

Relationships between swimming velocity and energy expended while swimming from which standard metabolic rates for coho salmon at different oxygen concentrations and seasons of the year were estimated (by extrapolation back to "0" swimming velocity) are presented in Figure 14. The energetic costs of standard metabolism were very similar at all tested dissolved oxygen levels during both the summer and fall periods of study, these being between 12 and 13 cal/kcal

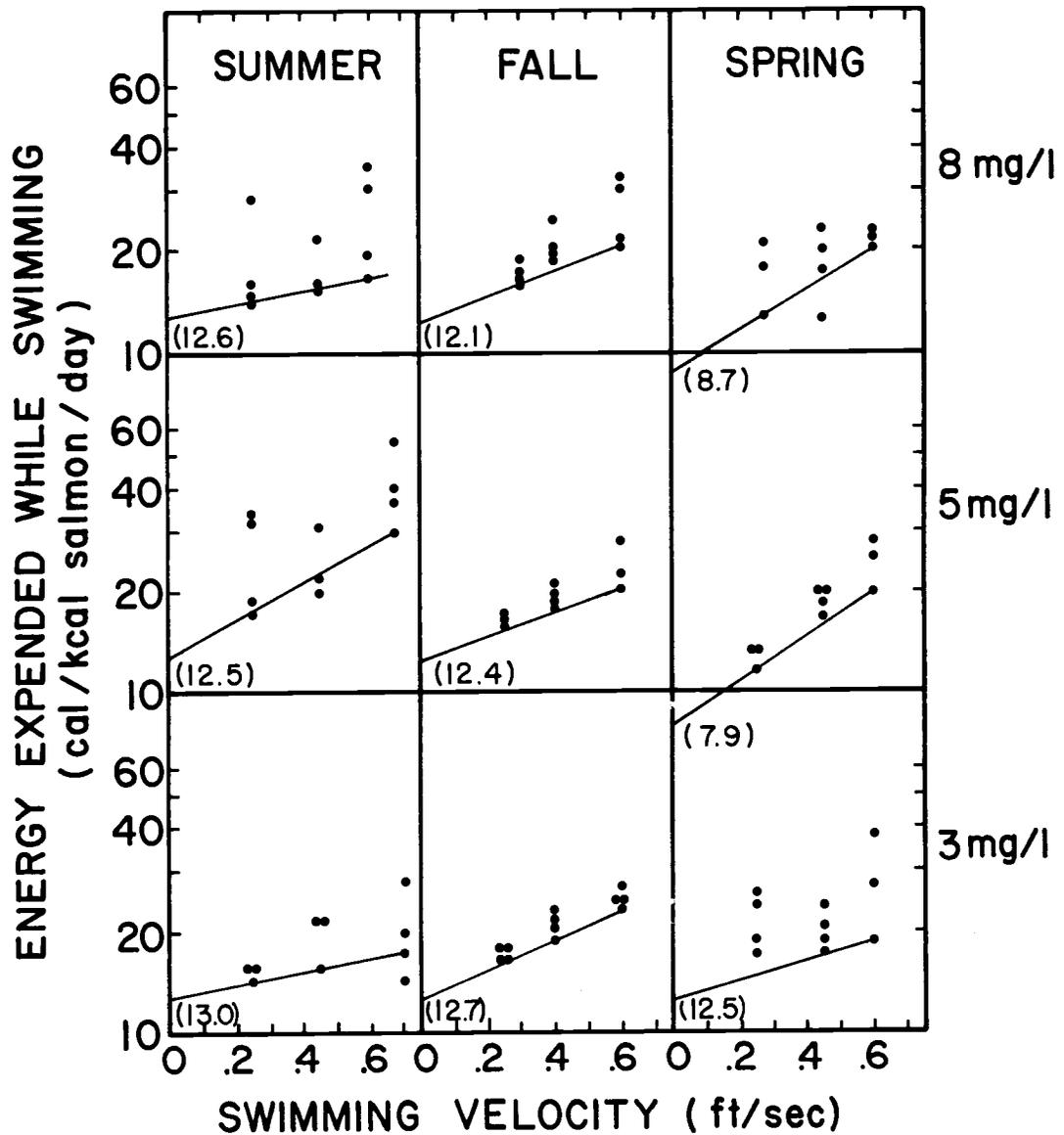


Figure 14. Relationships between swimming velocity, energy expended while swimming, season, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies at 15 C. The values in parentheses are the extrapolated estimates (at "zero" swimming) in cal/kcal salmon/day.

salmon/day. During the spring season, the expenditures for standard metabolism were apparently lower than during the previous seasons for the fish kept at 8.0 and 5.0 mg/l dissolved oxygen. The fish kept at 3.0 mg/l apparently did not experience this reduced requirement for standard metabolism.

### Energy Budgets

The data from preceding sections on food consumption, growth, waste production, activity and SDA, and standard metabolism have been combined in the graphic presentations of energy budgets in Figures 15, 16, and 17. For construction of these budgets, values were taken from the smoothed curves fitted to the growth and total waste product data (Figures 4, 5, 6, and 7), and from the data on standard metabolic rates; costs of activity and SDA were determined by difference. These values for the budgets were taken from the curves at food consumption rate intervals of 10 cal/kcal salmon/day, beginning at zero food consumption and extending up to the maximum food consumption rates encountered. All energy in the consumed food was accounted for in the vertical bars. Also negative growth (loss of body tissues) at less than maintenance feeding levels was considered as energy that should be accounted for in the vertical bars, and therefore the bars at those food consumption levels extend into the negative region of the vertical axis.

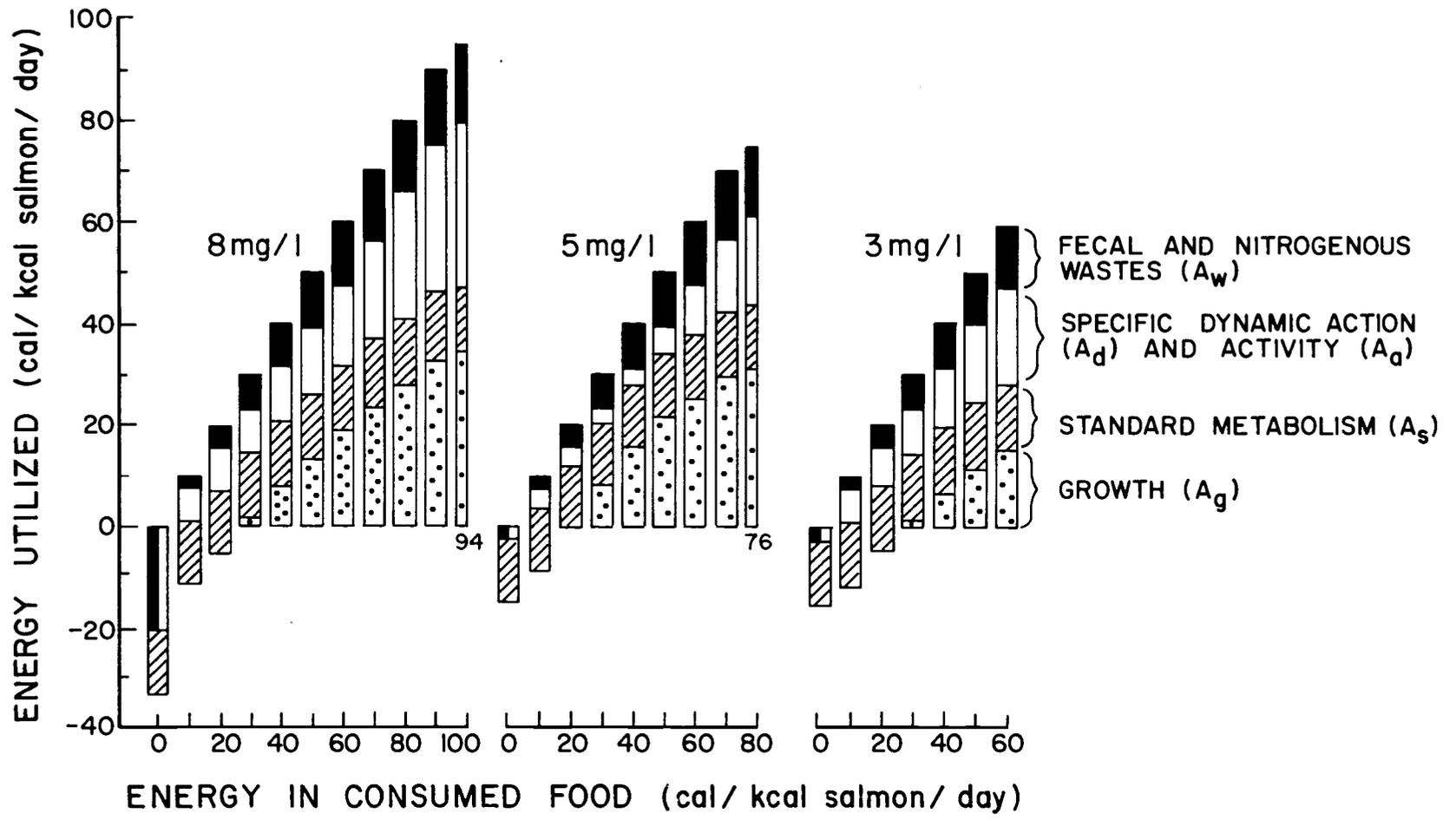


Figure 15. Energy budgets showing relationships between food consumption rate, energy and material uses and losses, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies at 15 C in the summer (June and July).

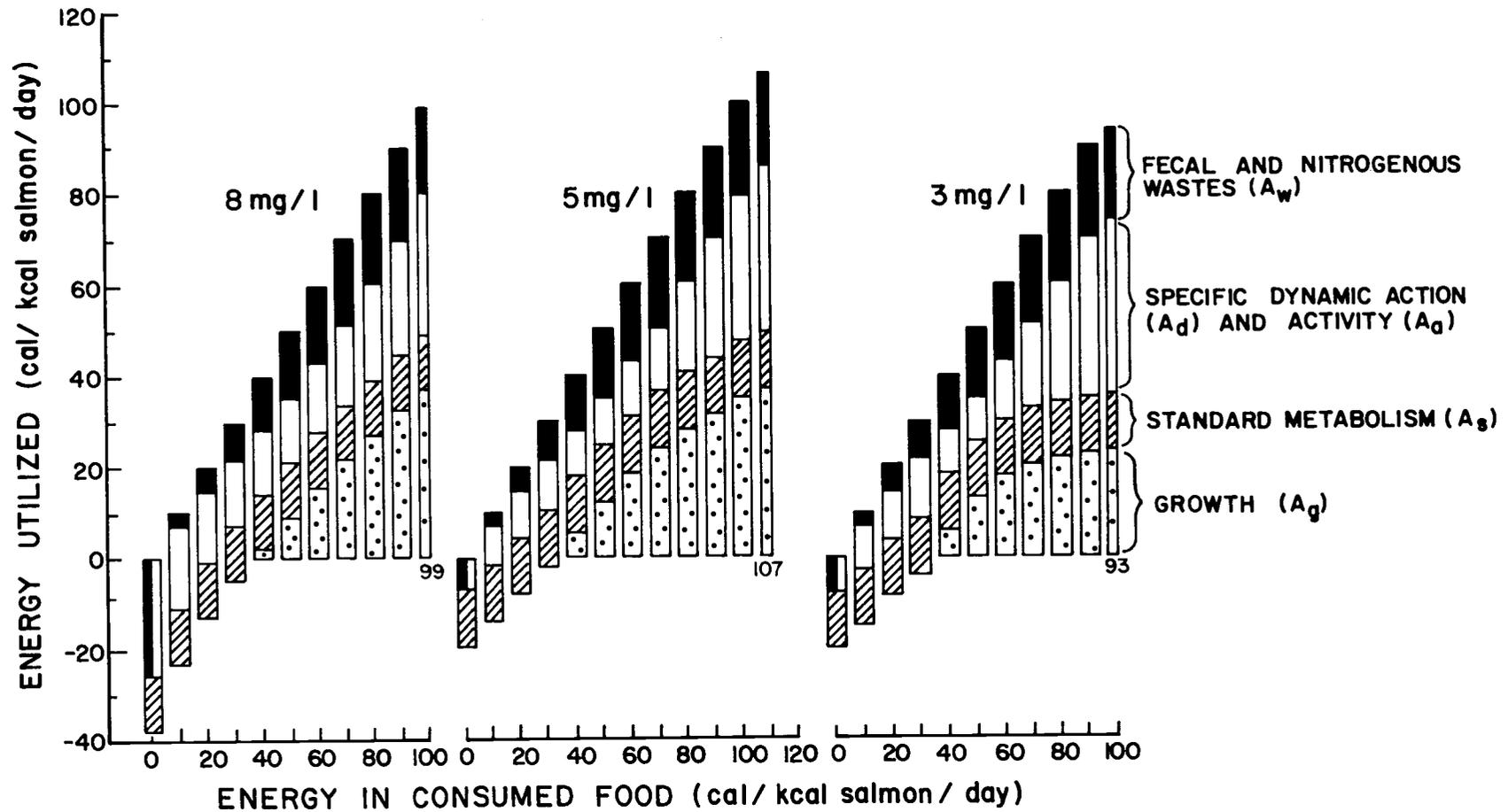


Figure 16. Energy budgets showing relationships between food consumption rate, energy and material uses and losses, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies at 15 C in the fall (October and November).

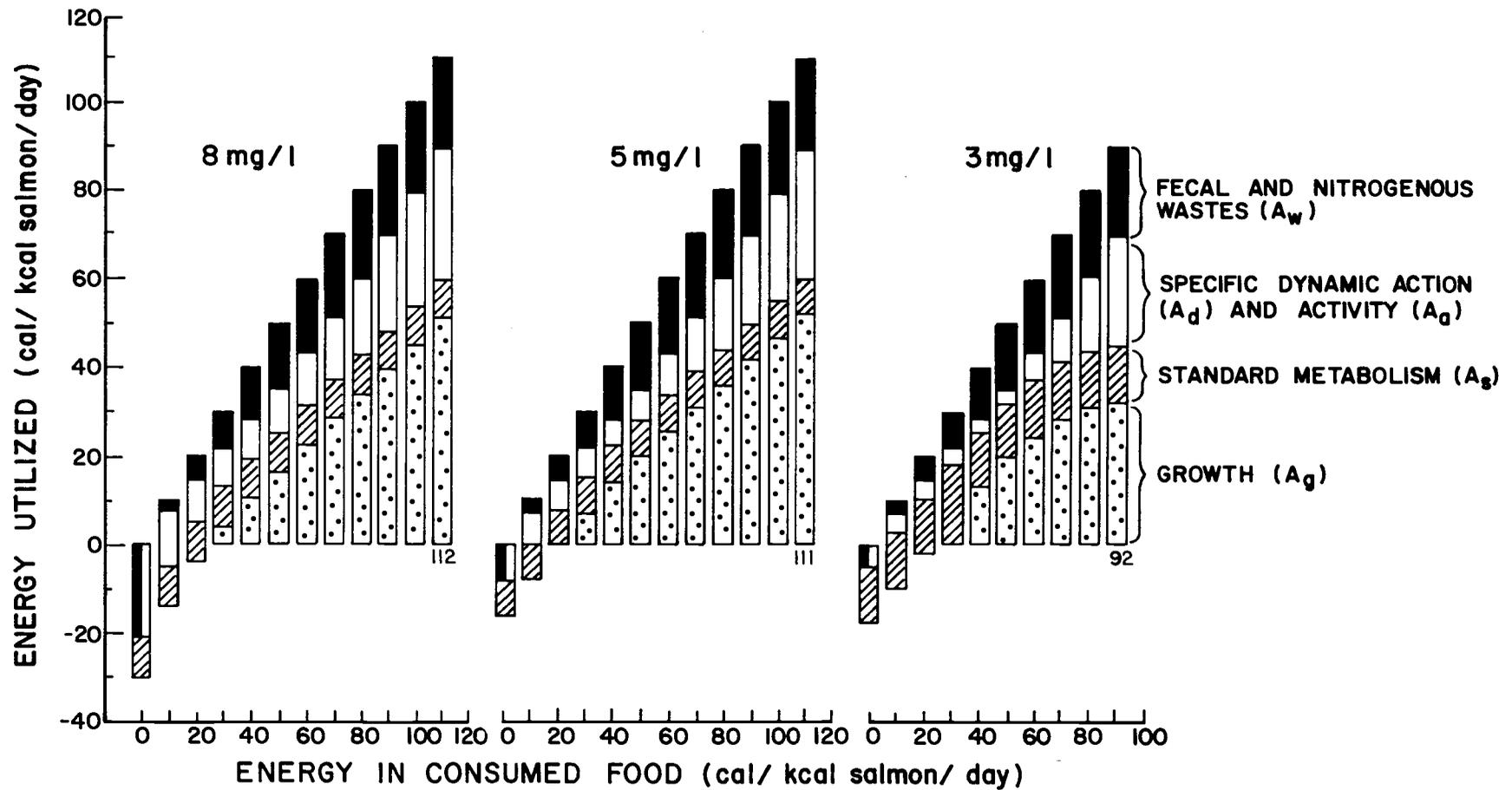


Figure 17. Energy budgets showing relationships between food consumption rate, energy and material uses and losses, and dissolved oxygen concentration for juvenile coho salmon in laboratory studies at 15 C in the spring (February and March).

## Summer

In addition to the obvious effect of reduced dissolved oxygen concentration restricting the maximum food consumption during the summer, there was also a deleterious effect on growth (Fig. 15). At given levels of food consumption, growth rates tended to be greater at 5.0 mg/l than at 8.0 or 3.0 mg/l dissolved oxygen. The rates of energy use for standard metabolism and the rates of total waste energy production were similar at all levels of dissolved oxygen exposure and food consumption rates, but these represented decreasing proportions of the food energy consumed with increases in consumption rates (see also Fig. 7). This, of course, was partly responsible for the general relationship of greater growth at higher rates of food consumption. There were, however, important differences in the energy required for the combined category of swimming activity and SDA at the various dissolved oxygen concentrations and feeding levels. These differences were the principal factor accounting for the variations in growth rates observed at different dissolved oxygen levels. Over much of the range of food consumption rates, energy expenditures for activity and SDA by the fish kept at 5.0 mg/l dissolved oxygen were only about half the amounts used by the fish at 8.0 mg/l and were only one-third to one-half the amounts used by the fish at 3.0 mg/l. Thus, there was an apparent inverse relationship between growth rate and rate of energy use for activity and SDA,

which accounted for the poorer growth at 8.0 and 3.0 mg/l than at 5.0 mg/l at all food consumption rates. Also, the large rate of energy expenditure for activity and SDA by the unfed cohos at 8.0 mg/l dissolved oxygen accounted for the much larger loss of body mass at this oxygen level than at 5.0 or 3.0 mg/l.

### Fall

During the fall study period, as is shown in Figure 16, the anticipated relationship of reduced dissolved oxygen concentration inhibiting the food consumption rate of the cohos at 3.0 mg/l, which was observed during the previous season, was repeated. The maximum food consumption rate of the fish in the fall was slightly greater at 5.0 mg/l dissolved oxygen than at either 8.0 or 3.0 mg/l, for the fish offered an intermittent satiation ration. Up to intermediate food consumption rates, growth rates were similar at all three oxygen exposure levels, but at higher feeding rates the growth rates of the fish at 8.0 and 5.0 mg/l continued to increase markedly, in notable contrast to those of the fish at 3.0 mg/l. Increases in consumption rate beyond about 60 cal/kcal salmon/day resulted in very little increase in energy utilization for growth in the case of the fish kept at 3.0 mg/l dissolved oxygen.

The energetic cost of standard metabolism and the energy in the total wastes were similar at all oxygen concentrations at given

food consumption rates. But, as in the summer season, there was an inverse relationship between energy use for activity and SDA, and for growth rate. At 3.0 mg/l, energy use in the activity and SDA segment increased markedly with increasing food consumption at higher feeding levels, and consequently the increase in growth rate was less. Also, the marked loss of body mass at 8.0 mg/l under starvation conditions appears in large part to have been owing to large energy requirements for activity and/or SDA (Fig. 16).

### Spring

During the spring experiment, the maximum food consumption rate of the cohos offered intermittent satiation rations was again lowest in the fish kept at 3.0 mg/l dissolved oxygen (Fig. 17). Growth of fish at 5.0 and 8.0 mg/l continued to increase steadily with increases in food consumption up to maximum values of about 110 cal/kcal salmon/day. Conversely, growth rates of the fish at 3 mg/l did not increase much with increases in food consumption beyond about 65 cal/kcal salmon/day. Below that level of consumption, there was little difference in growth rates between fish at the three different oxygen levels.

As in the previous seasons, the total waste components of the energy budgets were similar at all three oxygen levels at given food consumption rates. Standard metabolic rate may have been somewhat

higher at 3.0 mg/l than at either 5.0 or 8.0 mg/l dissolved oxygen concentration, which could account for some of the difference in growth observed. The energy cost of the activity and SDA category was quite low at 3.0 mg/l dissolved oxygen as compared to costs at 8.0 or 5.0 mg/l, up to food consumption rates of about 65 cal/kcal salmon/day. Above that level of food consumption, however, this cost increased greatly at 3.0 mg/l; this was apparently the major factor reducing growth rate at that level of dissolved oxygen exposure at the higher feeding rates. As in previous experiments, the greater loss of body mass of the starved cohos kept at 8.0 mg/l dissolved oxygen, as compared with those at 5.0 and 3.0 mg/l, was accounted for by the increased energy expenditure for activity and/or SDA.

## DISCUSSION

The primary objective of this research project was to determine the effects of reduced dissolved oxygen concentrations upon the growth of juvenile coho salmon. To accomplish this, two of the most rewarding areas of investigation appeared to be the food consumption and the bioenergetics of the use of this food by these fish. Figures 15, 16 and 17 provide a means of examination of possible causal relations involved in differences in food consumption, bioenergetics, and growth pointed out in the previous section (Results and Interpretation). In addition, an important part of any such study is the consideration of how the data relate to fish in nature, as well as to the findings of other investigators.

### Food Consumption

The most obvious effect of lowering the dissolved oxygen concentration in these experiments was reduction of the maximum food consumption rates of the fish offered the daily intermittent satiation rations (allowed to consume as much as they would of one unlimited food offering). This reduction in food consumption occurred during each of the three seasons but only at the 3.0 mg/l dissolved oxygen level. There were no appreciable differences between the maximum feeding rates of the fish at 5.0 and 8.0 mg/l.

Such limiting of food consumption has been reported many times previously for juvenile cohos subjected to the stress of reduced oxygen levels. Sometimes inhibition of feeding has been found to occur at any reduction in oxygen concentration below air saturation values (Fisher, 1963; Trent, 1972), but it has been observed by other investigators to occur only when the dissolved oxygen level was reduced to 5.0 mg/l or lower (Davison, 1954; Herrmann, 1958; Hutchins, 1972).

Since the particular effect of reduced feeding was observed in this present study only at the maximum feeding levels (above 60 cal/kcal salmon/day in the summer and above 80 in the fall and spring), the question arose as to whether these experimental food consumption maxima were or were not higher than feeding rates in nature.

Feeding rates of juvenile cohos in nature are understandably quite variable, depending on a number of factors. Included in these are the availability of appropriate food organisms, competition for food, energy requirement necessary to capture live prey, and various water quality parameters. The overall effect of this combination of influences is such that cohos in nature would seldom be expected to consume food at rates similar to the maximum rates observed in these laboratory experiments.

Food consumption rates have apparently never been directly measured for fish in nature. In one recent study, Averett (1969),

using a combination laboratory-field technique, estimated that young cohos in Tobe Creek (Oregon) were able to obtain natural food organisms at only 50 cal/kcal salmon/day in April and May. The fish in this creek were living under typical small stream conditions for the species, and this figure is considered to be representative of a natural consumption rate, at least for those two months. This value (50 cal/kcal salmon/day) has also been discussed and tentatively accepted by Warren (1971).

Making the assumption that 50 cal/kcal salmon/day is a near maximum food ingestion rate for young cohos in streams, and also assuming that the total effect of other influencing factors was similar in my aquarium fish and those in nature, then generally in streams no limitation of feeding rates would be anticipated due to reductions in dissolved oxygen down to 3.0 mg/l. I believe, however, that the latter assumptions are unrealistic. In the wild, young cohos must search for their food and in most cases they must also compete with other fish for the available food and space. Under reduced oxygen conditions it is probable that the increased energy utilization necessitated by food capturing and competition, combined with the increased energy expenditure required to extract oxygen from the water, would result in a reduction in the former activities and therefore would curtail feeding. High growth rates, resulting from the high food consumption rates, by the cohos at reduced oxygen levels in the experiments

reported herein were due to obvious reductions in swimming activity. The combination of these events would be most unlikely in a real world situation.

### Growth

In addition to the observed reduction in food consumption (which would obviously limit the maximum potential for growth) discussed in the previous section, two other important effects of the dissolved oxygen level upon the growth of young coho salmon are apparent in the energy budgets (Figures 15, 16 and 17). There was a decrease in the slope of the growth curve of the fish kept at 3.0 mg/l dissolved oxygen at higher food consumption levels, and a much greater negative growth rate (greater loss of weight) by the starved fish kept at 8.0 mg/l.

Decreases in the slope of growth curves were observed only in the fall and spring in the juvenile cohos kept at 3.0 mg/l dissolved oxygen and having food consumption rates greater than about 65 cal/kcal salmon/day. Fish at other oxygen levels and food consumption rates as high as 110 cal/kcal salmon/day did not exhibit this.

The energy budgets (Figures 16 and 17) reveal that activity and specific dynamic action (SDA) was the only energy use category that increased sharply at the food consumption levels where growth curves

of the fish at 3.0 mg/l dissolved oxygen began to level off. Direct observations of these fish revealed no increase in swimming activity compared to similarly fed fish at higher oxygen levels. As previously discussed, the actual measurements of SDA in experimental apparatus were not reliable enough to determine whether there was an effect due to the oxygen level. Nevertheless, it seems that a well fed fish would have a greater oxygen demand imposed upon it by the high energy costs of deaminating excess amino acids, the major component of SDA (Warren, 1971). And, subjecting this well fed fish to greatly reduced oxygen levels (e. g., 3.0 mg/l) would demand a greater expenditure of energy for the extraction of oxygen from the water necessary to satisfy the requirement imposed by the increased SDA. As a result, there would be an increase in the energy use category identified here as activity and SDA. This could well explain the reduction in the growth curve observed in the 3.0 mg/l fish in the fall and spring.

It would follow then that the explanation for the absence of a sharply declining growth curve at 3.0 mg/l in the summer is that the maximum food consumption rates (which were much lower than in fall or spring) occasioned less of an excess of amino acids and were still well within the limits of the ability of the fish to extract sufficient oxygen from the water at low energy cost.

Caution must again be taken in attempting to apply these data to the real situations of juvenile cohos in streams. If natural feeding rates are below 65 cal/kcal salmon/day, and if energy utilization required by competition for food and space were as low as in these experiments, then these data suggest there would be no effect on the growth rate directly due to dissolved oxygen levels down to 3.0 mg/l. As discussed previously, seldom in nature would required activity be as low as observed in this study. And, since both growth rate and activity rate are the major components of a fish's scope for activity (Fry, 1947), as one increases the other must decrease. At times of low food availability, the energy expended in feeding activity would increase at an increasing rate with higher feeding levels and one would observe a reduced slope in the growth curve at food consumption levels lower than those reported here.

The greater loss of weight by the starved fish kept at 8.0 mg/l oxygen, as compared to those kept at 3.0 and 5.0 mg/l, was obviously due to their greater activity. The budgets (Figures 15, 16 and 17) show that the increased energy costs were due to large expenditures in the activity and SDA category, and personal observations revealed that increased swimming activity was a prime factor. The starved fish at 8.0 mg/l typically exhibited a frenzy of swimming for up to 30 minutes during the time nearby fish were consuming food. Fish at other rations or dissolved oxygen concentrations often became

quite active at feeding time, but their increased activity levels persisted for a maximum of only four or five minutes.

Other investigators have also reported higher activity levels for cohos kept at higher dissolved oxygen levels. Herrmann, Warren and Doudoroff (1962) found that fish at high oxygen concentrations were active throughout the daytime and exhibited aggression or defense of territory which was not observed at low oxygen levels. Davison (1954) reported that cohos were more active while feeding at high oxygen levels than at 3.0 or 2.0 mg/l.

One other observation concerning the growth of these experimental cohos deserves some consideration. This was the finding that the fish kept at 5.0 mg/l dissolved oxygen grew at least as well as, and at times apparently better than, those at 8.0 mg/l in all three seasons. The energy budgets indicate that there were generally no differences in the energy categories for standard metabolism or waste products at these two oxygen concentrations. Also, for the most part, the energy requirements in the activity and SDA category were similar at both oxygen levels; there were, however, occasions when the fish at 5.0 mg/l apparently exhibited a lower requirement for energy in this category. On these occasions, there was an indication that these fish grew better (i. e., had higher gross efficiencies) than those at 8.0 mg/l.

Similar relationships between growth and moderately reduced dissolved oxygen levels have been reported by other researchers. Hutchins (1972) has observed that juvenile cohos held individually in forced-swimming channels generally exhibited reduced growth only at oxygen levels below 5.0 mg/l. Herrmann, Warren and Doudoroff (1962) reported no change in the food conversion ratio of cohos until the dissolved oxygen concentration was reduced to 4.5 mg/l. Fisher's (1963) studies included one experiment in which growth at 5.0-6.0 mg/l was little reduced from that at higher levels. In some recent coho studies (Warren, Doudoroff and Shumway, 1973), the investigators reported that in six out of eight experiments there was little or no reduction in growth rate above about 5.0 mg/l dissolved oxygen.

Whether there is any relationship between the growth patterns of juvenile coho salmon in these laboratory studies (all those cited above) and fish in nature cannot be determined from the available data. However, there is a good possibility that the similarities in growth observed at near saturation levels of oxygen down to about 5.0 mg/l may be an artifact due to the artificial experimental environment. This has recently been partially verified in laboratory stream studies with juvenile chinook salmon. Hutchins (reported in Warren, Doudoroff and Shumway, 1973) observed that at high food availability (as in my experiments) growth rate of these salmon was dependent

on dissolved oxygen concentration at all oxygen levels; the higher the oxygen concentration, the higher the growth rate.

In my own studies, the fish at 5.0 mg/l were obviously expending less energy in swimming activity than those at 8.0 mg/l. The former were frequently observed to be resting while the latter, in nearby aquaria, were observed to be cruising around their individual compartments searching for food or nipping at the transparent dividers separating them from fish in adjacent compartments. In natural conditions where successful fish must expend energy competing for food, territory, and other required activities, a dissolved oxygen concentration of 5.0 mg/l may restrict these necessary activities. In those situations, cohos living at 5.0 mg/l dissolved oxygen may not grow nearly as well as fish at higher oxygen levels.

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Appendix Table 1. Relationships between dissolved oxygen concentration, food ration size, percent of food ration appearing as specific dynamic action (SDA), and length of time over which the SDA was exerted.

Season	Dissolved Oxygen Concentration (mg /l)	Ration Size (% of body weight)	Specific Dynamic Action (% of ration)
Summer	3.0	3.0	8.8
		3.0	10.0
		4.0	7.5
		6.5	5.5
Fall	3.0	2.5	2.2
		3.0	8.2
		5.0	5.9
		6.0	4.1
Summer	5.0	3.0	7.7
		4.0	10.4
		6.0	10.5
		11.0	6.5
Fall	5.0	3.0	3.8
		3.0	5.8
		6.0	4.0
		6.0	8.1
Summer	8.0	3.0	15.2
		3.5	15.1
		6.0	6.5
		12.5	7.0
Fall	8.0	2.7	8.0
		3.0	12.0
		5.0	5.7
		6.0	3.3