AN ABSTRACT OF THE THESIS OF

HARI SINGH SETHI (Name) for the DOCTOR OF PHILOSOPHY (Degree)

in FISHERIES (Major) presented on 9/15/69 (Date)

Title: TEMPERATURE AND THE BIOENERGETICS OF CICHLASOMA BIMACULATUM

Abstract approved: Redacted for Privacy
Charles E. Warren

One means of evaluating the temperature requirements of an animal is to determine changes temperature causes in the uses and losses of energy and materials in the food the animal consumes. To develop energy budgets for cichlids (Cichlasoma bimaculatum) at different temperatures (20, 24, 28, 32 and 36 C) data were obtained on food intake, growth (total, fat, and protein), fecal losses, maintenance requirements, and other uses and losses of energy and materials including specific dynamic action, activity, and nitrogen excretion.

Starvation experiments were conducted to provide information on the metabolic cost of existence and on adjustments in body composition in the absence of food. Energy expenditure during starvation, estimated from heat of combustion data, was found to increase linearly with temperature over 4, 13 and 20 days of starvation.

The caloric cost of existence per fish per day was greatest during the first four days at all temperatures and decreased as

starvation was prolonged to 20 days. Caloric losses and dry weight losses showed essentially the same thing. Protein catabolism increased regularly with temperature and decreased slightly with duration of starvation. Fat catabolism did not vary significantly with temperature but decreased markedly as starvation was prolonged.

Food intake, growth, and food conversion efficiency varied greatly with changes in temperature. Cichlids were fed ad libitum on small aquatic worms of the genus Tubifex. For food consumption as well as growth, the optimum temperature dropped from 32 to 28 to 24 C as the experimental period was extended from 5 to 12 to 19 days. Optimum growth in dry weight and calories occurred at the same temperatures. Growth of cichlids through time at 20 and 28 C was nearly linear; at 24 C the curve was exponential. At 32 and 36 C, the growth curves rose and then declined. When the fish were held separately during the experiment, food conversion efficiency of cichlids was highest at 20 C and decreased with increasing temperature. Efficiencies decreased with duration of the experiment. When fish were held in groups during the experiment, efficiency was low at both high and low temperatures.

The guts of the cichlids were found to contain food up to 30 hours after an ad libitum meal at all temperatures. They were reasonably empty after 36 hours. Thus, no significant correlation was found between temperature and the length of time necessary for the

guts to empty. Food material not absorbed was measured by wet combustion. Percentages of absorption of unrestricted rations were found to be 83, 84, 88, 85 and 69 at 20, 24, 28, 32 and 36 C. Percentages of food absorption were much higher on the restricted ration than on the unrestricted ration. Absorption appears to have been optimal at 28 C.

Specific dynamic action (SDA) was estimated at different temperatures by taking the difference between oxygen consumption of unfed and fed cichlids at the same levels of activity. SDA represented 22, 20, 19, 33 and 33 percent of the food consumed at 20, 24, 28, 32 and 36 C, when the fish were swimming at 4 cm/second. SDA was generally complete within 36 hours after feeding.

Graphs showing energy and material intake, utilization, loss, and scope for growth at each temperature during different experimental periods were developed both in terms of calories per fish per day and calories per gram of fish per day. The evidence suggests that the optimum temperature for growth of this cichlid is near 24 C.

Temperature and the Bioenergetics of *Cichlasoma bimaculatum*

by

Hari Singh Sethi

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1970

APPROVED:

Redacted for Privacy

Professor of Fisheries
in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 9/15/69

Typed by Donna L. Olson for Hari Singh Sethi

ACKNOWLEDGEMENT

Most sincere appreciation is extended to Dr. Charles E. Warren and Dr. Hugo Krueger for their advice and guidance throughout the course of this study.

The helpful assistance of the personnel of the Fisheries Research Laboratories, Oregon State University is gratefully acknowledged.

Special thanks are due my wife, Renu, whose patience, encouragement and assistance made this study possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TEMPERATURE AND THE BIOENERGETICS OF CICHLASOMA BIMACULATUM

INTRODUCTION

Animals are dynamic systems in a state of continuous exchange of energy and materials with their environment. The energy exchanges are governed by the same thermodynamic laws that describe physical energy transfers and transformations. Because temperature affects chemical reaction rates so profoundly, and because most biological responses involve chemical transactions, temperature is a major parameter in virtually all biological energy exchanges.

Bioenergetics is the term used to denote the study of energy transformations in living organisms (Lehninger, 1965). The bioenergetic approach to biological problems was highly developed by Brody and his co-workers from 1930 onward and was summarized in 1945 in his classic Bioenergetics and Growth (1945). Fry (1947) made some bioenergetic studies with fish and developed the bioenergetic concept of scope for activity. Recently the Oregon State University Pacific Cooperative Water Pollution Laboratories have been involved in extensive bioenergetic studies of fish as related to their environment (Warren and Davis, 1967). The studies reported here on the bioenergetics of the cichlid fish Cichlasoma bimaculatum were

the beginning of this broader program of investigation.

Basic Concepts and Definitions

In fish, the role of temperature is multiple. Temperature and temperature changes set lethal limits and require adaptation of an animal if it is to live at temperatures that would otherwise be intolerable. Temperature influences the rate of development and growth; it is a controlling factor for metabolic rate; and, as such, it determines the limits within which the animal is free to perform. Some of the pertinent physiological parameters through which temperature exerts its influence will be considered.

Metabolism, as defined in Dorland's Illustrated Medical Dictionary (1965), is the sum of all the physical and chemical processes by which living organized substance is produced and maintained and also the transformations by which energy is made available for the needs of the organism. Metabolism may be measured by direct calorimetry in which the amount of heat liberated is determined. This is not easy, particularly for fish. Fish have a low metabolic rate and water, in which they live, is remarkable for its temperature stability.

Rubner (cited by Brody, 1945) showed that heat production could be calculated from oxygen consumption and introduced the method of indirect calorimetry. However, indirect calorimetry is

not an exact measure of the energy lost as heat to the animal, because different food and body constituents may be oxidized, and the extent and proportion of these determines the energy equivalents of oxygen. To assess the expended energy, the relative utilization of carbohydrate, fat or protein must be known, in addition to their caloric equivalents. Adequate caloric equivalents have not been determined for fish protein. Although the exact value of the coefficient will depend on the relative amounts of fat, carbohydrate and protein being metabolized, for mammals usually it is $3.42 \pm .05$ calories per milligram (4.89 cal per ml) of oxygen consumed (Winberg, 1956; Kleiber, 1961; Brody, 1945).

Both basal and resting metabolism have been used to denote the minimum metabolic rate reflecting the energy costs of bare existence. These are the costs of minimum function of circulation, respiration, excretion and muscle tone, costs which never cease, even under absolute rest. The costs also include energy losses associated with activities of enzyme systems, colloidal structures, semipermeable membranes and the maintenance of cell structure. Basal metabolism does not include the energy expended for growth or morphogenesis, the transformation of food intake or the conversion of simple precursors into more complex states (Brody, 1945).

In practice, basal metabolism is defined as the minimum energy cost when the animal is at rest in a thermoneutral (or

temperature acclimated) environment in the post-absorptive condition (Brett, 1962). The possibility of obtaining absolute rest has frequently been questioned for undomesticated animals or animals subject to excitement from handling or experimental confinement. This has led to the use of the term standard metabolism which is employed in fish metabolism. The term standard oxygen consumption has ordinarily been rather loosely applied in the literature as many workers have made no specific effort to insure that the rate of oxygen consumption is representative of that for motionless fish (Beamish and Mookherjii, 1964). Standard metabolic rate is taken to be the metabolic rate of an unfed fish whose activity is zero. This is determined by projecting to the zero level on a graph of the relationship between its metabolic rate and activity (Beamish, 1964; Brett, 1964). Thus standard metabolic rate is essentially equivalent to basal metabolic rate. It is usually measured after 24 hours of fasting, during the slump of any diurnal metabolic cycle. Active metabolic rate is the maximum rate consistent with the highest continued level of activity (Fry, 1957). Here the question of duration of maximum activity is frequently left in doubt.

Routine metabolic rate has been used to denote oxygen consumption per unit of time of fish at spontaneous levels of activity (e.g. Job, 1955; Wolschlag, 1960) or oxygen consumption recorded continuously during free activity (Brett, 1962). Routine metabolic

rate is not defined as to the plane of nutrition or the level of activity of the animal. Variation in the behavior of fish under different conditions makes it difficult to associate routine metabolic rate with any particular level of activity.

Scope for activity is defined by Fry (1947) as the difference between active and standard metabolic rates under specified conditions. An extension of Fry's (1947) concept of scope for activity was suggested by Warren and Davis (1967) in their definition of the concept scope for growth:

> Growth can be considered an activity, so perhaps the difference between the energy of the food an animal consumes and all other energy utilizations and losses can be defined as the scope for growth under particular environmental circumstances. Scope for growth, as defined, is not strictly equivalent to scope for activity. Food consumption rate is not active metabolic rate, but will be influenced by and will influence metabolic rate. Other metabolic utilizations and losses are not equivalent to standard metabolism (p. 184).

The daily intake of calories is the upper limit of the daily energy lost or expended by an animal without deterioration. All energy within this limit is not available for activities of the animal. Some of the energy is never absorbed and some is excreted unused in compounds with a measureable heat of combustion. Food intake calories reduced by non-absorbed and by excreted calories provide a measure of the daily energy available for all activities including maintenance. The calories remaining after fecal and excretory losses are variously

partitioned by an animal to provide for the cost of maintenance, the costs of movement, specific dynamic action (heat increment of feeding), and the energy impounded as an increase in body fat, carbohydrate and protein. The fractional distribution varies from moment to moment, but the food intake calories remaining after subtracting fecal and excretory losses can be variously partitioned to show the scope of several activities, or the average distribution of calories over a specified period of time. Fry (1947) envisioned the difference between the minimum oxygen consumption rate consistent with continued existence of an animal and the maximum rate of oxygen consumption which would permit the highest continued level of muscular activity as a measure or index of the muscular activity limit, range or scope available to an animal. An animal usually makes use of only part of this range or scope. The scopes for various activities usually have magnitudes far beyond the energy used in these activities. Diagrams showing the available energies for activities can be called scope diagrams. Diagrams showing the factual distribution of energies among activities may be spoken of as energy accounts.

The increase in oxygen consumption following ingestion of food was termed by Rubner (1902) the specific dynamic effect of food. Measurements of oxygen consumption taken in a fasting and resting state, as initially performed by Lavoisier, represent essentially basal metabolism. In a series of experiments in which his assistant

Seguin was the subject, Lavoisier showed that oxygen consumption, and by inference heat production, was increased above the basal state by a decrease in environmental temperature, by the ingestion of food, and by physical exercise. When food stuffs were fed individually, the increase in heat production following ingestion of protein was observed to be considerably greater than that due to carbohydrate and fat (Pike and Brown, 1967).

Determination of the basal metabolic rate requires that the subject be in the postabsorptive state, that is, without food for at least 12 hours prior to the test. The reason for withholding food is that following ingestion of food, heat production increases above the resting state. Rubner called this increased heat production the specific dynamic effect of food. In an ordinary mixed diet consisting of all food stuffs, the calorigenic effect amounts to about six percent of the energy value of the dietary intake (Benedict and Carpenter, 1918). In cattle, SDA ranges from three percent of the gross energy at 0.5 maintenance to about 20 percent at full feed; or it ranges from about eight percent of the metabolizable energy at 0.5 maintenance to 38 percent of the metabolizable energy at full feed (Brody, 1945, p. 98).

When nutrient substances were injected (thus by-passing the intestinal tract), the calorigenic effect could still be observed. The calorigenic effect, then, was assumed to be related to the fate of

food stuffs after absorption. Voit (cited in Pike and Brown, 1967) proposed that when foods are ingested and transported to the cells, the cellular mechanism is temporarily overwhelmed by a flood of metabolizable nutrients which, in some indefinable way, speed up the metabolic rate. The greater calorigenic effect observed for protein was attributed by Lusk (1931) to the more complicated series of reactions required for metabolism of amino acids, in particular the reactions involved in deamination.

Terroine and Bonnet (1926) attributed the high heat increment of protein to the quantity of amino nitrogen derived and, more specifically, to the amount of amino nitrogen deaminated. Thus the heat increment of protein would be proportional to urea nitrogen in the urine. Wilhelmj et al. (1928) expressed the relationship between the calorigenic effect and ingested protein in terms of calories for millimole of deaminated amino acid (calculated from urea nitrogen), and Borsook and Winegarden (1931) reported a positive correlation between metabolic rate and urinary nitrogen.

Although these studies are significant in that they related the calorigenic effect of food to a specific biochemical measurement, urinary nitrogen, the cellular mechanism contributing to extra heat production following the ingestion of food stuffs has remained obscure. Krebs (1964) recently proposed a theory relating calorigenic effect to the energy requirement for synthesis of ATP. Doubtless, the

problem of calorigenic effect of food will continue to be debated, and whether experimental evidence can be obtained that will precisely explain its metabolic basis is a moot question (Pike and Brown, 1967).

Brody (1945) defines growth as a biologic synthesis and production of new biochemical units. He adds that it is the aspect of development concerned with increase in living substance or protoplasm and includes three processes: cell multiplication; cell enlargement; and incorporation of material taken from the environment. The inclusion in an organism of non-protoplasmic substances, such as fat, blood plasm, and cartilage is an increase by incorporation of material from the environment, involving neither cell multiplication nor cell enlargement. According to Brody (1945) such increase is not regarded as true growth. Yet operationally, from the standpoint of quantitative measurement of growth of the organism as a whole, he believed we must consider these non-protoplasmic inclusions--whether or not they are irreversible--as parts of the growth process.

Thermal Acclimation in Fish

Animals and plants can survive many changes in the environment. If the environmental change is great, the survival usually involves some change in the animal or plant. This generalization holds

also for fish. The changes in the environment are met by changes in the animal which tend to reduce the intensity of the alterations induced by the environment. Sometimes this reduction can occur and sometimes it cannot. Mammals, for example, can induce circulatory responses in the skin and muscular alterations in respiratory movement which allow a maintenance of constant body temperature over wide variations in environmental temperatures. In fish however, particularly small fish, the physical relationship with the environment requires that temperature maintenance above or below the environmental temperatures could occur only with great expenditures of energy. Fish usually do not expend this energy, nor have they a mechanism to provide body temperatures below the environmental temperatures. Thus small fish take a temperature very close to and slightly above the environmental temperature. This automatically results in changes in metabolism, because rates of enzymatic reactions, other things being equal, depend on temperature. The fish cannot regulate its temperature, but can maintain bodily processes reasonably constant over a moderate range of temperatures if acclimation can occur.

Adaptation is a general term referring to any alteration or response of an organism which favors survival in a changed environment. Physiological adaptation refers to conformity and regulation of internal states as well as compensation by long term acclimation or

acclimatization (Prosser and Brown, 1961).

Recognition of temperature acclimation in fish dates back to the latter part of the 19th century. Since that time it has been the subject of many investigations. Thermal acclimation has been found to be widespread among fish and enables them to maintain some degree of stability of their physical and metabolic activities in spite of temperature vicissitudes. The extensive literature which describes the responses of fish to altered environmental temperatures has been adequately reviewed (Brett, 1956; Bullock, 1955; Fry, 1957; Precht, et al., 1955 and Prosser, 1962). One general response involves stabilization of oxygen consumption rates across a range of temperatures so long as the animals are acclimated to each temperature. This has survival value at moderate to high temperatures since temperature acclimation is rapid here. The significance of this response is that it allows, in the absence of a successful ability to regulate body temperature, a degree of physiological independence of environmental temperature.

The varying ability among fishes to extend their temperature tolerance through acclimation has been amply demonstrated. The incipient lethal level of the bullhead can be increased through stepwise acclimation by 10 C. For the speckled trout, the upper limit of temperature can be increased only by less than 5 C (Brett, 1956). It has been shown by Doudoroff (1942) and Brett (1944) that the

increase in ability to tolerate higher temperatures among fish is relatively rapid, requiring less than 24 hours at temperatures above 20 C. Conversely, the loss of tolerance for higher temperatures and a gain in resistance to low temperatures, is an inherently slower process requiring up to 20 days in some species to approach completion (Brett, 1956). Brett (1956) pointed out that the rates of acclimation appear to be governed by the rate of metabolism, which, if depressed by a low environmental temperature, automatically reduces the rate of acclimation. Whatever the process, Brett (1944) discovered that acclimation to a higher temperature in bullhead was inhibited under conditions of low oxygen concentration.

Allanson and Noble (1964) estimated the rate of acclimation for Tilapia mossambica as measured by the change in median time of survival in waters of different temperatures. They suggested for this fish an average acclimation rate of 1 C per 150 minutes when the fish was transferred directly from 25 C to 30 C, whereas it was observed that fish transferred from 25 to 15 C were not fully acclimated even after 20 days. Spaas (1959) similarly estimated the rate of acclimation to increased temperature for T. melanopleura and T. macrochir to be around 1 C per day or more. This extremely rapid rate of acclimation must relate to its environment and constitutes an adaptation to rapid insolation in the shallow, often isolated waters of tropical river basins in Africa, where water temperatures of up to 35 C have

been recorded during the summer rainy season (Spaas, 1959).

According to Stroganov (1956), the organism does not react passively to changes in temperature of the medium, but actively adjusts to them and to any disturbances which they may bring about in its normal physiological process. He also maintained (on the basis of differences in RQ and N/O values at different temperatures) that changes induced by temperature in the organism are not purely quantitative but qualitative as well; and involve changes in the concentration of enzymes and the enzyme control of the type and magnitude of chemical reactions (oxidation, reduction, hydrolysis, hydration and deamination). When speaking of metabolism, he had in view the large group of exchange processes of the organism with the medium. Stroganov (1956) pointed out that sometimes some aspects of metabolism may be disordered under a change in environmental temperature--e.g. water, salt, fat, carbohydrate metabolism--but most often protein metabolism is disturbed to some extent. Disturbances in protein metabolism affect energy production, growth and reproductive ability. These disorders will then be reflected in other links of the chain of metabolic processes and the chemical processes will be different from those of a normal state.

Within lethal limits, acclimation results in changes that are often conspicuous and which would appear to be intimately related to survival. Some of the variables which have been found to be altered

during acclimation are those associated with food intake, growth, circulation, and oxygen consumption. Precht et al. (1955) and Bullock (1955) give recent extensive compilations of acclimation effects. Among the different measurements of acclimation effects, oxygen consumption offers some singular advantages for comparative studies. It would appear that aside from daily caloric loss and rate of growth, oxygen consumption is the most easily measured single index of extent of overall response of an animal to its environment. There are several procedural precautions to be taken in measuring oxygen consumption of fishes; they are discussed in detail by Fry (1957, p. 30-35).

Respiratory Metabolism in Fish

Since the publication of Winkler's method for the determination of dissolved oxygen in water, much attention has been devoted to the oxygen consumption of aquatic animals. Many investigators have observed a relationship between oxygen consumption and activity (Krogh, 1914; Spencer, 1939; Spoor, 1946; Higginbotham, 1947; Graham, 1949; Prosser et al., 1957 and Basu, 1959). Three levels of oxygen consumption have been defined for fish, each in relation to activity. These are standard, routine and active (Fry, 1947).

The first measurement of oxygen consumption in relation to spontaneous activity appears to have been made by Spoor (1946). He

recorded the number of deflections of an aluminum paddle activated by water currents set in motion by the activity of a fish. Oxygen consumption plotted on the ordinates against paddle movements on abscissa indicated a linear relationship. The standard rate was established by plotting oxygen consumption against activity and extrapolating the linear relation to zero activity. The same procedure was later on followed by Fry (1957) and Brett (1964). Other types of activity recording devices have since been produced (Beamish, 1963).

On examining the literature, one is impressed with the enormity of information available on fish metabolism and growth with respect to temperature. However, the variation in energy disbursements in fish subjected to different temperatures has not been adequately covered. The aim of the present study was to investigate the relationships that may exist between the energy consumed in food and the energy retained as growth as well as the various ramifications of energy losses in cichlids at different temperatures.

MATERIALS AND METHODS

It was the general aim of the research here reported to investigate the metabolic alterations in Cichlasoma bimaculatum when exposed to a variety of temperatures. The data collected provide an estimation of the cost in energy to the fish, and perhaps an estimation of the net damage done by the temperature alterations. Studies were conducted on both starved and fed cichlids at each of the five temperatures (20, 24, 28, 32 and 36 C). Starvation and growth experiments were supplemented by studies on assimilation and SDA.

In the exposition presented here, the experimental animals and their management and the sequence of events in starvation, growth, assimilation and SDA experiments will be described.

The experimental animals used in this study were cichlid fish, Cichlasoma bimaculatum (Linnaeus), a tropical fish of the family Cichlidae. Mature cichlids for reproduction were kept in 50 gallon glass aquaria at 20 C. Fry up to a length of ten millimeters were fed newly hatched brine shrimp. Cichlids over ten millimeters in length were fed tubificid worms, Daphnia sp., and dry fish food. When the fry reached 15 mm, they were usually separated from their parents. The young fish were transferred to a 50-gallon aquarium and kept in water having a small exchange flow at 20 C. They were fed live and dry food until used in experiments.

During experiments, the cichlids were fed small aquatic worms of the genus Tubifex. An analysis of the tubificids gave: water 81 percent of the wet weight; fat 3.04 percent; and protein 15.96 percent. On combustion, one gram of wet and dry tubificids yielded approximately 1028 and 5557 calories, respectively.

The incoming water was passed through a heat exchange unit and then was piped into a series of five wooden head tanks. Each head tank was equipped with a thermostatically controlled stainless steel heater. Head tanks at high temperatures were supplied with oxygen so that a dissolved oxygen concentration between 7 to 9 mg/l was maintained in all tanks. Each head tank supplied water at a desired temperature to three test chambers. Each set of three chambers was maintained at a temperature of 20, 24, 28, 32 or 36 C. Temperature was maintained within $\pm$ 0.5 C. All experiments were conducted in a 20 C constant temperature room which was continuously lighted by cool white fluorescent tubes. Uniformity of conditions at different temperatures were maintained as far as possible. Keeping the fish in similar test chambers, at the same water flow, and presenting the same diet in sufficient quantity to ensure abundance, were the first precautions. The variations in response of fish can then be considered as attributable mainly to temperature.

Three experiments in which fish were starved, and three experiments in which fish were fed, were conducted. Experiments were

redesigned on the basis of previous experience. Thus, some seemingly minor differences in technique developed between the experiments. One important difference was with regard to control of variability in initial weights. Variability was better controlled in the later experiments. Another difference was holding the fish individually or in groups during experiments.

Starvation Experiments

About 200 four-month-old cichlids were fed a mixed diet (tubificid worms and dry fish food) in the stock tank for two weeks. Forty-eight hours before the start of the experiment, all food was removed. Each fish was blotted with a damp cotton towel, weighed and branded with pens dipped in a mixture of dry ice and acetone. The wet weight of each individual fish was determined to the nearest 0.01 gram.

Fifteen groups of seven fish, each fish identified with a brand, were then transferred to the test chambers (5 temperatures X 3 chambers per temperature X 7 fish per chamber). Initially, the water temperature in each chamber was 20 C. Over a two hour period the temperature was changed to 20, 24, 28, 32 or 36 C. Samples of fish were taken on days 0, 4, 13 and 20. (The day zero sample was taken from the stock tank on the day of starting the experiment and there was no acclimation period.) Wet weight of each

fish to the nearest 0.01 gram was taken and then the fish were placed separately in a 70 C oven for drying. After five days, the dry material was weighed on a Mettler H-15 balance to the nearest 0.1 mg, and then ground with a motar and pestle.

Heat of combustion, fat, and ash values were determined from aliquots of the dried sample. Calorimetry with Parr semi-micro oxygen bomb calorimeter No. 1411 was employed to determine the caloric values of the dried aliquots. The procedures described in the Parr Instrument Company Manuals 128 and 130 and in Supplement No. 1 were followed. Fat was extracted in a Goldfisch continuous extraction apparatus, with reflux distillation of the solvent for a period of five hours. The ash content was determined by burning an aliquot of each sample in a muffle furnace at a temperature of 600 C for five hours. The amount of the protein containing fraction in each sample was determined by subtracting the weight of fat and of ash from the total dry weight of the sample.

Growth Experiments

Cichlids from a single brood reared at 20 C were used. After a starvation period of 48 hours, these fish were weighed on a Mettler top-pan balance to the nearest 0.01 gram. Fifteen groups of four fish each were then transferred, for individual treatment, to plastic jars in the 15 test chambers where the temperature had been

brought down to 20 C.

The four fish were held individually in four one-gallon plastic jars which were tied together and placed in each test chamber. The walls of these jars were perforated about two inches above the bottom. An air stone was placed in the center of each set of four perforated jars and the bubbling air kept the water circulating through the perforations without disturbing the food at the bottom of the jars. With this arrangement four fish could be fed independently in the same tank and their individual food consumption and growth determined (Appendix Figures 1 and 2).

Feeding was resumed immediately after placement in the jars. The temperature of the chambers was raised gradually to the desired level within two hours. The cichlids were fed ad libitum and tubificid worms left over were quantitatively recovered to determine the food consumption. The worms were blotted on paper towels and weighed to the nearest tenth of a gram before and after feeding. Since at 36 C the worms could not live longer than six hours, they were replaced with a fresh supply after every four hours. In order to maintain a uniformity of food availability at all temperatures, the clumps of worms forming at lower temperatures were dispersed by hand every four hours.

There was no acclimation period and the initial sample (day zero sample) was taken on the day the experiment was started.

Samples were taken after 5, 12, and 19 days of feeding. The fat, water, and ash contents along with heats of combustion of the cichlids were determined for the individually fed fish and for controls.

Assimilation Experiments

A large portion of the food ingested is never absorbed into the body proper and is lost in the feces. Some energy is lost in the urinary excretions. Additional losses may occur from skin and gills. All these may be summed as excretory losses. But the methods used in this study yielded estimates of what were primarily fecal wastes.

One study was conducted to estimate the time required for digestion of a meal. Three to four gram fish were divided randomly into five groups of 20 fish each and then transferred to the test chambers in which water temperatures had been brought down to 20 C. The desired temperatures of 20, 24, 28, 32 and 36 C were gradually attained within two hours. An acclimation period of eight days was allowed during which the fish were fed for five days and then starved for three days. Since starving fish had been found picking on each other during starvation study, the fish to be used in the assimilation experiment were starved in isolation. After this starvation period, the fish were fed tubificid worms and allowed to eat for one hour. Fish were sacrificed at intervals of 18, 24, 30, 36,

42 and 48 hours after feeding and their intestinal tracts examined under the microscope. It was found that at all five temperatures tested the intestines were reasonably empty by the 36th hour after feeding.

Two sets of experiments were conducted to estimate excretory losses. In one set, after starving the individual fish for five days, weighed amounts of food were provided in the test chambers. Invariably fish swallowed their food within five minutes and nothing was left behind. In the other set of experiments, the fish after starvation were fed ad libitum for one hour.

At each temperature, one fish was fed at a time and was then transferred to a 100 ml beaker containing 30 ml of water taken directly from the head tanks. Each beaker was then covered with a piece of fish net fastened by a rubber band and was inserted through a 4 X 4 X 1 inch styrofoam float which kept the beaker floating in the test chamber for 36 hours. After this period, each fish was taken out gently from the beaker and dissected to expose the digestive tract. The undigested food material remaining in the intestine was pressed into 15 ml of water taken from the head tank. This 15 ml of water was kept separately from the 30 ml of water in the beaker and refrigerated. Estimations of organic matter were made later the same day by the iodate wet combustion method (Davis and Warren, 1965).

The amount of oxygen required for combustion of the organic materials in the water was thus determined. Similar determinations were made on tubificids in order to obtain standard values from which to calculate the total amounts of food fed in terms of milligrams of oxygen necessary for combustion. By dividing the milligrams of oxygen required to oxidize the fecal and other organic material in the samples by the milligrams of oxygen required to oxidize one milligram of food fed, an approximate value for the percent availability was derived. This value was used to determine the energy percentages lost in fecal wastes.

Oxygen Consumption of Unfed and Fed Fish

To study the effect of temperature on the oxygen requirements for activity and for SDA, ten cichlids weighing three to four grams were acclimatized for three weeks at temperatures 20, 24, 28, 32, and 36 C. During this acclimation period, these fish were fed just sufficient tubificids to maintain a constant weight. Each fish was starved for 48 hours and weighed before being introduced into the swimming tunnel (Appendix Figure 3) which was designed to accommodate one fish at a time. A period of 24 hours was allowed for training of the fish in the tunnel, during which it was subjected to the lowest water velocity of 4 cm/sec. The Tygon tubes 12 and 13 were left open during this conditioning period so that a fresh supply

of water was available to the fish.

Before starting the measurement of the oxygen consumed, all the air bubbles were removed from the system and tubes 12 and 13 were kept closed for one hour. The water in the closed system was allowed to recirculate for two minutes to insure uniformity of oxygen concentration in all parts of the system and then the initial sample was taken for the determination of oxygen concentration. A terminal sample was taken one hour later. The difference in oxygen concentration between the initial and final sample multiplied by the volume of water circulating in the closed system gave the oxygen consumption of the fish in one hour. Dissolved oxygen concentrations were measured by the Alsterberg azide modification of the Winkler method.

The determination of oxygen consumption was repeated after feeding, and the extra oxygen consumed after the administration of food was determined. The oxygen determinations of the fed fish were made within a period of two to three hours after taking food. The fish swimming at the five temperatures were periodically checked to insure their uninterrupted performance. Individual fish were used several times.

Two experiments were conducted at each of four high water velocities (6, 8, 10 and 12 cm/second) and four experiments at the lowest water velocity of 4 cm/sec. One experiment at a water

velocity of 4 cm/sec was conducted at each temperature for a period of 36 hours during which oxygen determinations of the fed fish were made at three to four hour intervals.

To determine the mass of food eaten, the water velocity was reduced to a minimum and a known weight of tubificids was placed at the bottom of the large chamber. Then the gate screen of the tunnel was lowered and with an L-shaped wire the fish was directed to the chamber containing the food. The fish was allowed a period of one to two hours for eating and during this period the system was left open so that it could be flushed. After feeding was over, the gate screen was lowered, the fish directed into the tunnel and the screen was put back again. The left over worms were syphoned out and weighed and the amount of food consumed was determined. In some experiments fish were fed limited but equal amounts of ration whereas in others they were fed ad libitum.

At each temperature a series of oxygen losses were estimated by using the closed system without the fish in the tunnel. The mean of these values was later subtracted from the oxygen consumption of the fish. At the end of each experiment 50 ml of Chlorox bleach was added to the water circulating in the closed systems. After two hours, the system was flushed with fresh water for 24 hours.

Oxygen consumption was determined by multiplying the change in concentrations by the volume of circulating water (3.5

liters). The oxygen cost of taking food at a specific time after a meal was obtained by subtracting the oxygen consumption for unfed cichlids from the oxygen consumption of fed cichlids. In the 36 hour experiment, the oxygen consumptions per hour of the fed fish were plotted on the ordinates against time on the abscissa and the area under the curve evaluated for milligrams of oxygen during the 36-hour period; from this was subtracted 36 times the oxygen consumed per hour by the unfed fish. The difference represented the cost of taking food (SDA) at a given temperature when a cichlid was forced to swim at a given velocity. The oxygen consumptions in milligrams were converted into calories by multiplying by 3.36 (Brody, 1945).

RESULTS AND INTERPRETATION

Starvation

Information useful in developing an energy budget of an animal includes the metabolic cost of bare existence, the amount of oxygen consumed or the amount of carbon dioxide released under different conditions; the amount and composition of food consumed; body weight and composition changes; and amounts and kinds of waste products. Starvation experiments were performed to provide information on the minimum energy requirement or metabolic cost of existence and on adjustments in body composition in the absence of food. The metabolic cost of existence was estimated from heat of combustion data of control fish on day zero and at different intervals after withdrawal of food.

In considering the results of the starvation experiments, caloric data will be considered first, next wet and dry weight changes, and then protein, fat and ash changes. Comparisons will first be made of the data obtained when starvation was prolonged over 4, 13 or 20 days at different temperatures. Then data will be compared for the daily intervals of 0-4, 5-13 and 14-20. Some discussion of the ratios of metabolic rates at different temperatures (Q_{10} and Q_1) will be introduced and comment will be made on the possibility of the development and presence of metabolic homeostasis over a range of

temperature. Most of the data presented are averages derived from observations on seven cichlids; in a few instances these averages represent only six fish.

Behavior and Fatalities

Behavior can be considered as the consequence as well as the cause of physiological changes in an animal. Survival and success of an animal depends on its behavior. This was not a study of cichlid behavior. Nevertheless, some note of the behavior of the fish is necessary, as changes in behavior in part explain the results obtained. The cichlids were generally active at the intermediate temperatures (24, 28, and 32 C) and quiet at the extreme temperatures (20 and 36 C). The stress of starvation may be increased or decreased, depending on the level of activity of the fish. The activity of the fish very much depends on whether they were tested individually or in groups. Because of the lack of social interaction, fish tested individually were generally less active.

Deaths were frequent among cichlids at high temperatures, particularly at 36 C, where four fish died on the second day of starvation. Cichlids, in the early stages of starvation did not eat dead cichlids. However, the cichlids tended to become cannibalistic after prolonged starvation. Early removal of dead cichlids prevented this from becoming a problem; but in one experiment, the remains of a

cichlid were recovered.

Energy Expenditure at Different Temperatures

Energy expenditure during starvation, estimated from heat of combustion data, was found to increase quite linearly with temperature over 4, 13 and 20 days of starvation (Table 1; Figure 1). Linearity was clear over the 4-day and the 20-day period of starvation; but over the 13-day period a simple straight line relationship between calories lost and temperature did not fit the data properly, there being a plateau between 24 and 28 C. The caloric cost of existence per fish per day decreased as starvation was prolonged to 20 days.

The data in Table 1 and most of the other tables presented are given on a per fish basis. This was done because the data were collected on a per fish basis, and direct presentation of the data obtained is without the bias introduced by complex calculations. For comparative purposes, data on a per gram basis are often more useful and such data have been provided in energy budgets described in the Discussion. Absolute increase in weight of feeding fish or decrease in weight of starving fish is a function of body size. Some of the differences in data presented in this thesis are undoubtedly due to this. But differences in the size of fish between treatments were usually not great enough to alter general conclusions. Nevertheless,

Table 1. Daily energy expenditures during starvation in calories per cichlid.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	43	75	100	118	161
Day 0-13	42	60	62	72	78
Day 0-20	36	47	59	71	82
Day 0-4	43	75	100	118	161
Day 5-13	41	54	44	52	41
Day 14-20	25	24	53	68	88
From oxygen consumption data for fish swimming at 4 cm/sec, day 20	53	60	78	86	80

Note: The first three lines represent the average caloric loss per cichlid over 4, 13 or 20 day periods of starvation. The average initial weight on day zero was 1.013 grams (4552 calories) per cichlid. The next three lines represent average caloric loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Table 2. Experimental periods, temperatures, and average wet weights of starved cichlids in grams.

	Temperature (C)				
	20	24	28	32	36
Day 0	4.45	4.45	4.45	4.44	4.57
Day 4	4.29	4.29	4.16	4.24	4.16
Day 13	4.22	4.08	4.04	3.84	3.74
Day 20	4.12	3.98	3.85	3.69	3.60
Day 0-4	4.37	4.37	4.30	4.34	4.36
Day 5-13	4.25	4.18	4.10	4.04	3.95
Day 14-20	4.17	4.03	3.94	3.76	3.67
Day 0-13	4.35	4.25	4.24	4.15	4.07
Day 0-20	4.28	4.45	4.48	4.06	4.47

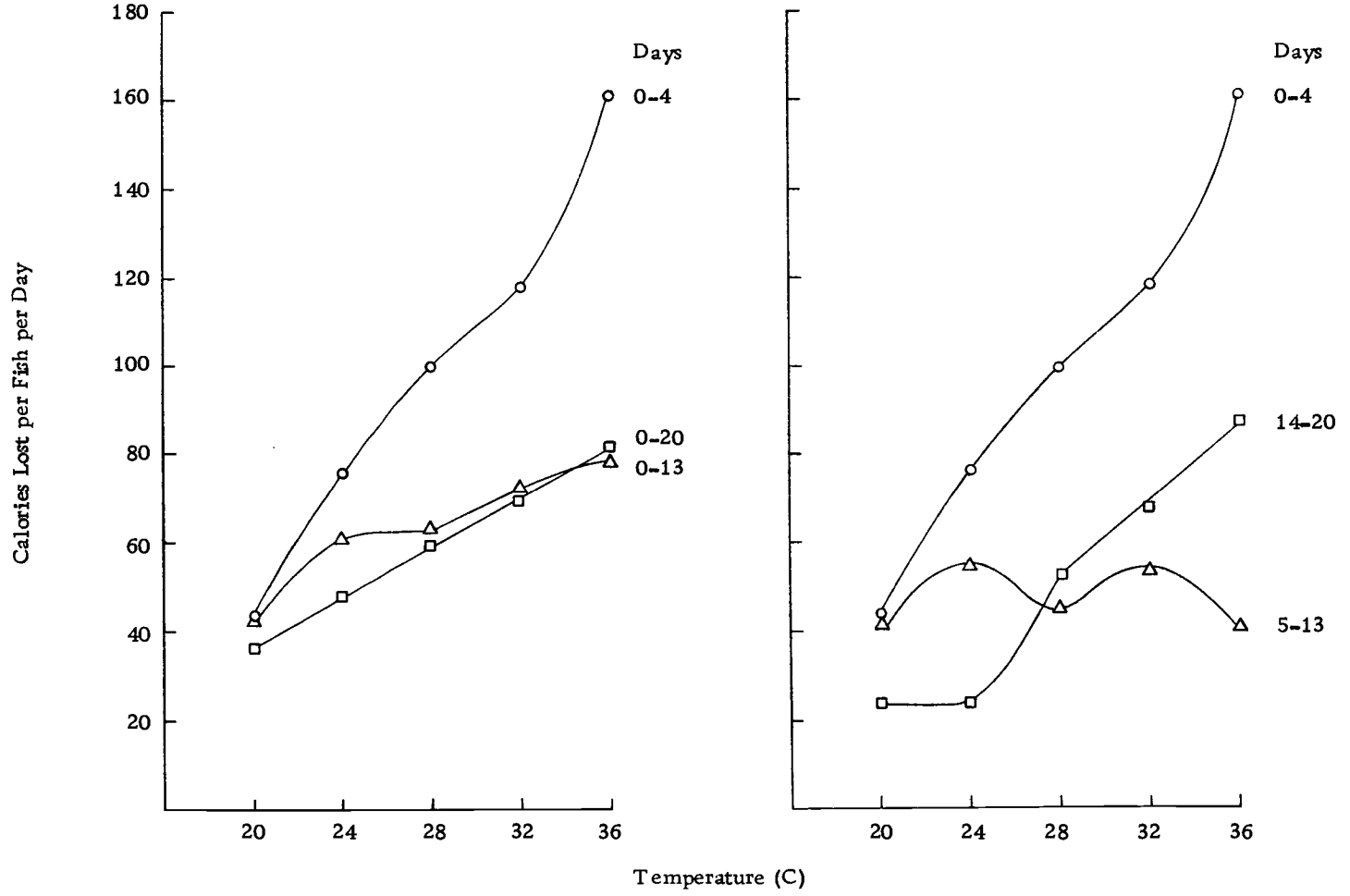

Figure 1. Average daily loss of energy per cichlid during starvation at different temperatures over indicated time periods.

presentation of mean weights of starving fish (Table 2) and growing fish (Table 13) will make it possible for the reader to assess the effects of any differences in the size of the fish.

Loss of body materials per fish per day, in calories, at all temperatures was greatest during the first four days and decreased for the next nine days at all temperatures tested (Table 1, Figure 1). The decrease in daily energy dissipation from the initial four-day period to the second nine-day period was negligible at 20 C; but the difference increased to 21, 56, 66, and 120 calories per fish per day at four degree intervals from 20 to 36 C. Thus energy losses per day were much higher in the first four day period than in the period from the 5th to the 13th day. Presumably the first period includes the costs of reactions to the new temperature, the costs of adjustment, as well as the cost of existence.

The caloric cost of existence, varied irregularly with temperature during the period 5-13 days, the daily losses being 41, 54, 44, 52 and 41 calories at four degree intervals between 20 and 36 C. Obviously, the energy dissipation by cichlids during this period of fasting was not much different at the various temperatures. This could suggest some metabolic homeostasis in the face of temperature change; this was suggested by Stroganov (1956) and Roberts (1967) on the basis of their experiments on oxygen consumption at different temperatures. This possible metabolic homeostasis of short

duration will be considered further below.

Although there may have been a brief period of metabolic homeostasis from the 5th to the 13th day, from the 14th to the 20th day, the daily energy dissipation increased linearly with temperature from 28 to 36 C. Energy dissipation at 20 and 24 C did not change much.

Metabolic Rate Ratios

The temperature coefficient of a process is the ratio of its speed or velocity at a given temperature to its velocity at a temperature 10 C lower. Thus the Q_{10} for a process of speed K_t at temperature t and K_{t+10} at a temperature ten degrees higher is:

$$Q_{10} = \frac{K_{t+10}}{K_t} \qquad (1)$$

If we wish to assume that the rate of caloric loss of a starving animal is proportional to its metabolic rate, we may extend equation 1 to:

$$Q_{10} = \frac{\text{Rate of caloric loss at } t+10}{\text{Rate of caloric loss at } t} \qquad (2)$$

If we know the rate of caloric loss at any two temperatures (H_1 at temperature t_1 and H_2 at t_2) we may use the formula

$$\text{Log } Q_{10} = \frac{10(\text{Log } H_1 - \text{Log } H_2)}{t_1 - t_2} \qquad (3)$$

if we assume that the rate of reaction increases continuously as temperature is increased and that there is a constant rate of increase per degree change in temperature. With the aid of equation (3), numerical values of Q_{10} for caloric losses during starvation were estimated for the four temperature intervals studied. These equations represent ratios of rates of processes. Ordinarily metabolic rates are calculated per gram or per kilocalorie of body mass. The rates computed here are on a per fish basis. This should cause no change in the estimated ratios so long as the fish are of nearly the same size. Both methods of computation were tested and yielded ratios no more than a few percent different.

The values for Q_{10} change with different ranges of temperature and with different periods of starvation (Table 3). Calculated over a 20-day period, the numerical values of Q_{10} tended to decrease slightly with increasing ranges of temperature. Although the Q_{10} values over a 20-day period may seem to vary simply with temperature, there appear to be some differences in the Q_{10} values during different periods of starvation. These differences may be more the result of experimental error than real.

The concept of Q_{10} is important in general or industrial

Table 3. Q_{10} and Q_1 ratios for rates of caloric losses during starvation of cichlids.

Temperature range	Period of days covered			
	0-4	5-13	14-20	0-20
Q_{10}				
20 to 24 C	4.00	1.96	.903	1.92
24 to 28 C	2.04	.598	7.17	1.74
28 to 32 C	1.51	1.51	1.85	1.58
32 to 36 C	2.15	.551	1.88	1.41
20 to 36 C	2.26	1.00	2.17	1.66
Q_1				
20 to 24 C	1.15	1.07	.99	1.07
24 to 28 C	1.07	.95	1.22	1.06
28 to 32 C	1.04	1.04	1.06	1.05
32 to 36 C	1.08	.94	1.06	1.04
20 to 36 C	1.08	1.00	1.08	1.05

Table 4. Wet weight loss per fish per day in milligrams, at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	40.5	39.5	72.5	51.0	101.5
Day 0-13	19.6	26.4	35.2	48.0	50.0
Day 0-20	16.0	23.5	31.7	36.5	43.3
Day 0-4	40.5	39.5	72.5	51.0	101.5
Day 5-13	10.3	20.6	22.0	46.6	28.0
Day 14-20	9.3	18.0	21.0	15.2	29.7

Note: The first three lines represent the average wet weight loss per cichlid over 4, 13 or 20 day periods of starvation. The average initial wet weight on day zero was 4.44 grams per cichlid. The last three lines represent average wet weight loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

chemistry but its introduction into biochemistry and physiology seems unfortunate. Very few biological phenomena can be studied over several 10 C intervals. A more logical concept for biochemistry and physiology is Q_1; the ratio of a rate at T + 1 to the rate at T. Q_1 values have been calculated and presented along with Q_{10} values (Table 3). A value of 1.15 for Q_1 means that there is a 15 percent increase in the rate of a process per degree increase in temperature; a value of 1.0 means that temperature had no effect; and a value below one means that the rate decreased as temperature increased. Q_1 values in Table 3 ranged from 0.94 to 1.22. As with Q_{10} the Q_1 ratios varied irregularly with time and temperature.

Wet Weight Losses at Different Temperatures

Daily wet weight loss per fish during the first four days of starvation varied irregularly with the temperature; essentially the same daily loss occurred at 20 and 24 C and higher losses occurred at 28 and 36 C; a seemingly anomalous intermediate weight loss occurred at 32 C (Table 4). The daily wet weight losses clearly increased with temperature over 13 and 20 day periods of starvation. The average daily rate of wet weight loss was found to be greatest during the first four days and then decreased during successive time intervals as starvation was continued.

Dry Weight Losses at Different Temperatures

Dry weight relationships are likely to be more consistent than wet weight relationships, because the experimental error of determination is less and body water percentages may change with temperature. For example, the dry weight data give no evidence of the anomalous result for the first four days at 32 C which was indicated by the wet weight data (Table 5). The average daily dry weight loss during the first four days of starvation increased with temperature from 20 to 36 C. The average daily dry weight losses over 13 days of starvation increased from 20 to 32 C, but it was the same at 32 and 36 C. Over 20 days of starvation, the average daily loss increased with temperature from 20 to 36 C. The daily rate of dry weight loss was greatest during the first four days and then decreased as the starvation was extended to 13 and 20 days except for two minor deviations. Hence, generally, daily losses in dry weight became less as starvation was prolonged but increased as temperature was increased.

The values of Q_1 for the rates of dry weight loss were estimated for different periods (Table 6). The numerical values of Q_1 for dry weight losses did not show much change either with period of starvation or temperature range. The consistent results for dry weight data suggest much of the variation in Q_{10} and Q_1 values

Table 5. Dry weight loss per fish per day in milligrams, at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	5.5	13.5	14.2	16.7	20.0
Day 0-13	6.7	10.0	11.8	13.0	13.0
Day 0-20	5.5	7.8	9.3	10.5	12.5
Day 0-4	5.5	13.5	14.2	16.7	20.0
Day 5-13	7.3	8.4	10.8	11.3	10.0
Day 14-20	3.3	3.8	4.7	6.0	11.4

Note: The first three lines represent the average dry weight loss per cichlid over 4, 13 or 20 day periods of starvation. The average initial dry weight on day zero was 1.013 grams per cichlid. The last three lines represent average dry weight loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Table 6. Q_1 values for rates of dry weight losses during starvation of cichlids.

	Period of days covered			
Temperature range	0-4	5-13	14-20	0-20
20 to 24 C	1.25	1.03	1.04	1.09
24 to 28 C	1.01	1.06	1.05	1.04
28 to 32 C	1.04	1.00	1.06	1.03
32 to 36 C	1.04	.97	1.17	1.04
20 to 36 C	1.08	1.01	1.08	1.05

based on caloric loss (Table 3) were due to errors in caloric determinations.

Since dry weight losses during the last week of starvation indicated a linear relationship with temperature, wet weight ambiguities were presumably due to the variable water losses and variable percentages of body water at different temperatures. It will be noted that daily water losses vary irregularly with time and with temperature (Table 7) during the first, second, and third periods of starvation; yet they give consistent losses over either a 13 or a 20 day period.

Table 7. Net water loss per fish per day in milligrams at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	35	26	58	34	81
Day 0-13	13	16	22	35	37
Day 0-20	10	15	22	26	31
Day 0-4	35	26	58	34	81
Day 5-13	3	12	6	35	18
Day 14-20	6	14	22	9	18

Note: The first three lines represent the average water loss per cichlid over 4, 13 or 20 day periods of starvation. The average initial wet weight on day zero was 4.44 grams per cichlid. The last three lines represent average water loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Generally we should expect caloric losses to follow fairly

closely dry weight losses, and the data bear out this expectation, though there are differences (Figures 1 and 2). Some of these differences appear to be due to errors in caloric determinations. But a lesser relative loss in calories than in dry weight could suggest greater protein metabolism, whereas the reverse could suggest greater fat metabolism. Thus some of the discrepancies between the rates of caloric loss and rates of dry weight loss during the last two periods may be attributed to the proportion of protein and fat metabolized by the fasting cichlids.

Protein Loss at Different Temperatures

The separation of protein and fat losses incurred by cichlids during the different periods of starvation permits assessment of the relative importance of these energy sources at each temperature (Figures 3 and 4). Protein losses were calculated by subtracting fat and ash losses from dry weight losses (Table 8). Protein catabolism increased with temperature and decreased with the duration of starvation. The protein loss at 36 C was about 2.6 times as great as that at 20 C over a period of 20 days.

The Q_1 values for protein loss varied little with time and with temperature (Table 9). Of greatest interest were the higher values at temperature extremes during the first four days of starvation.

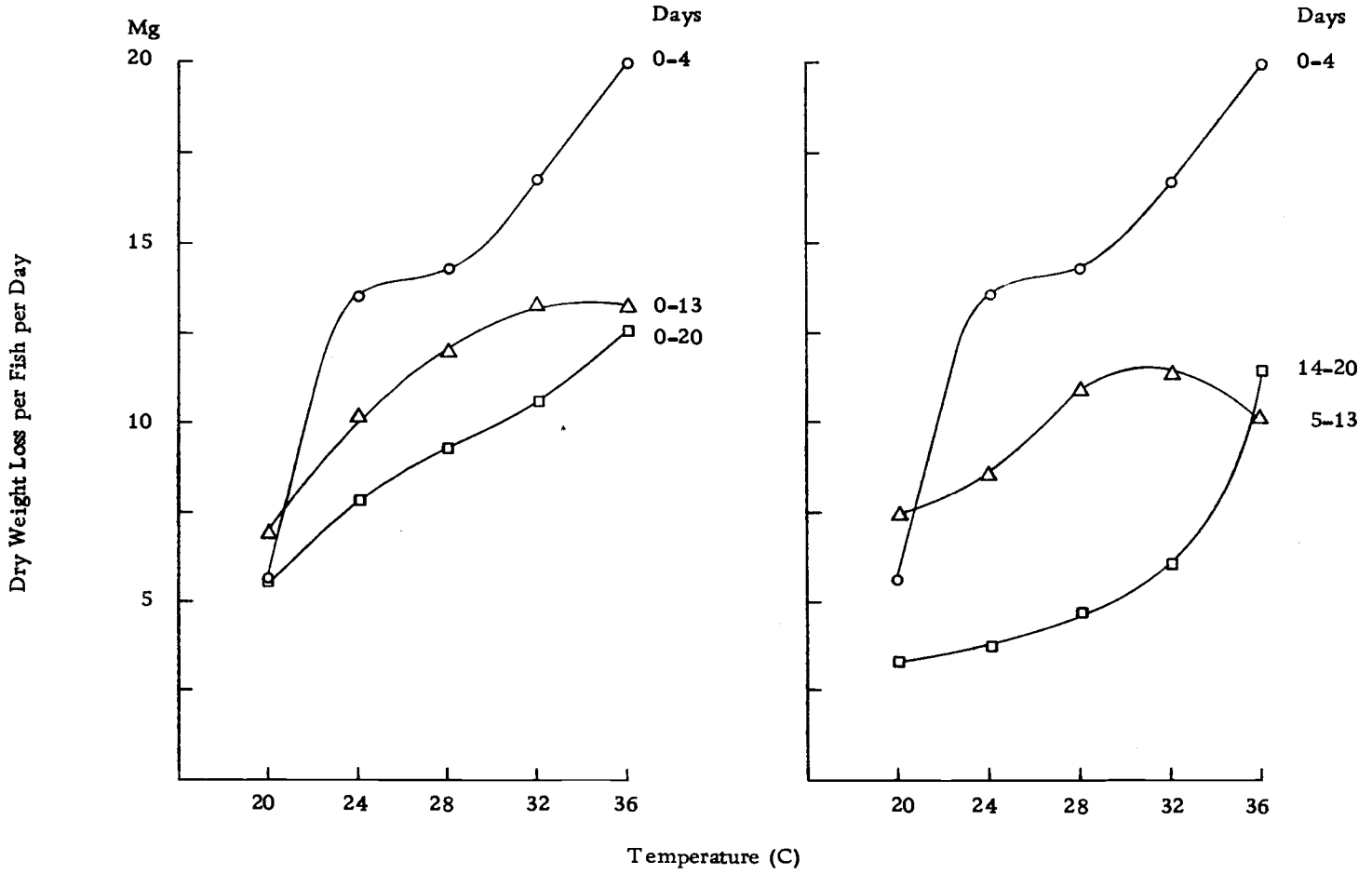

Figure 2. Average daily loss of body substance (energy reserves) of starving cichlids, expressed as milligrams per fish per day during starvation while exposed to different temperatures over indicated time periods.

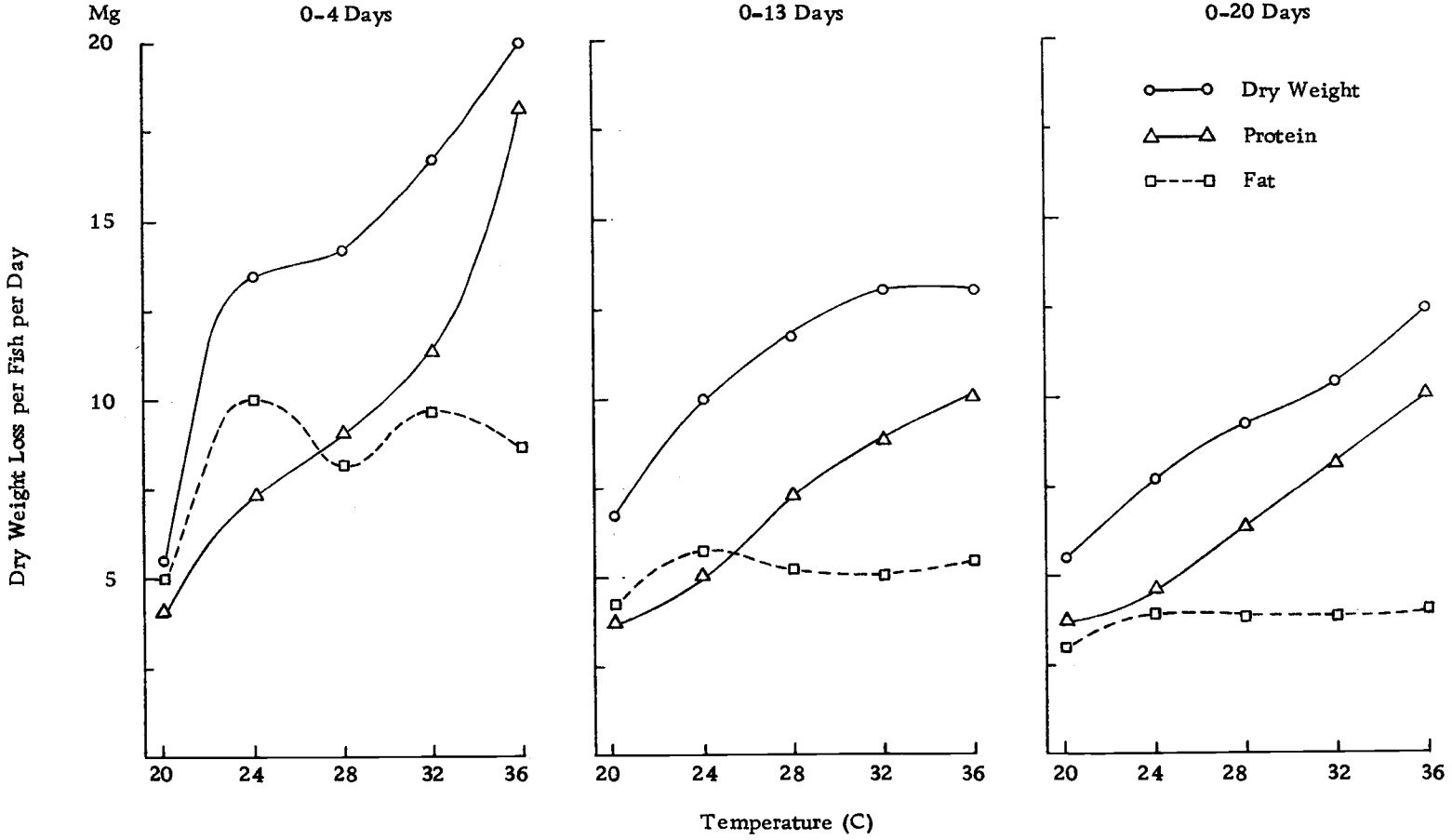

Figure 3. Weight decrements during starvation, expressed as milligrams per fish per day, of dry tissue, protein and fat in cichlids exposed to different temperatures over indicated time periods.

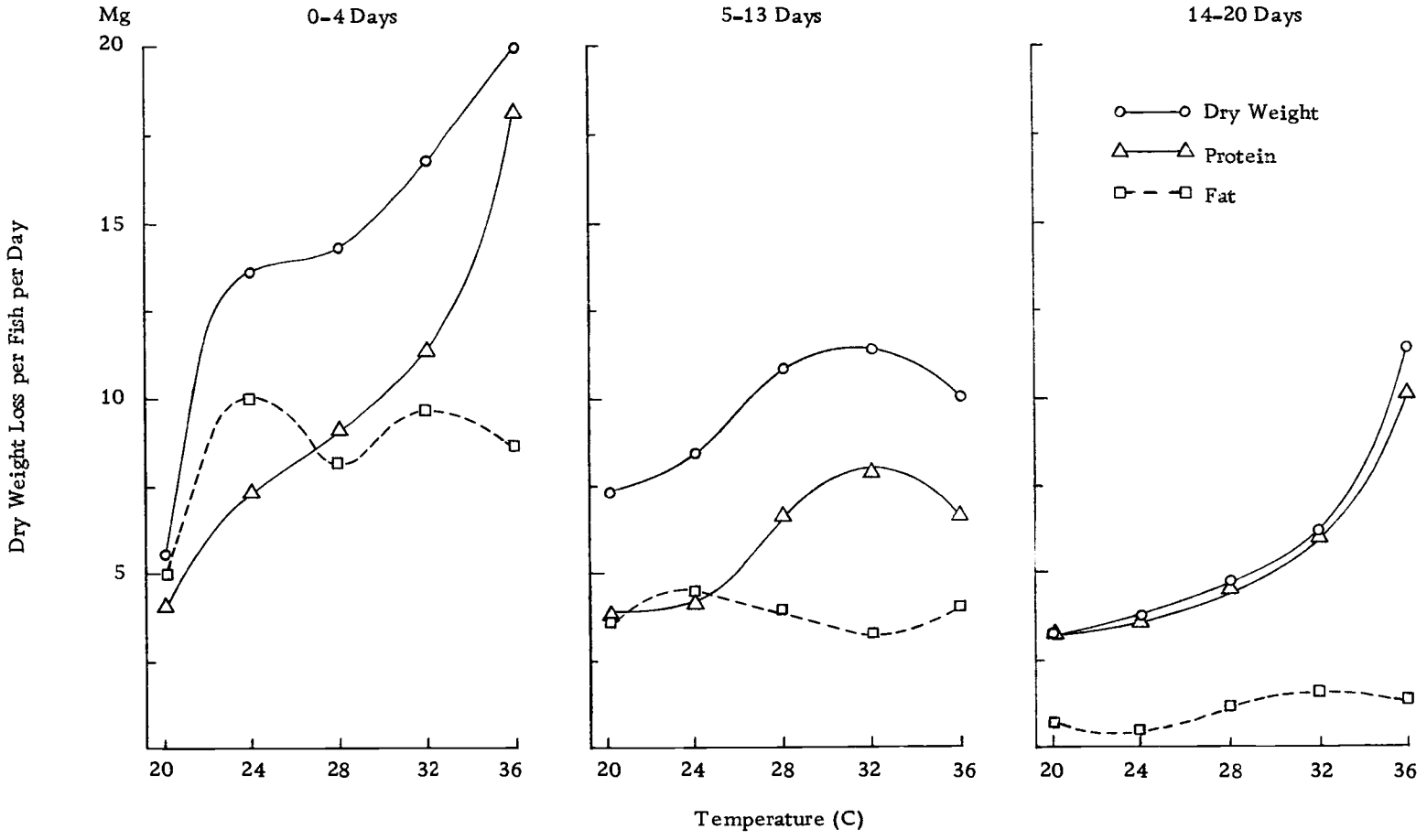

Figure 4. Weight decrements during starvation, expressed as milligrams per fish per day, of dry tissue, protein and fat in cichlids exposed to different temperatures over indicated time periods.

Table 8. Protein loss per fish per day in milligrams at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	4.0	7.2	9.0	11.2	18.0
Day 0-13	3.8	5.0	7.3	8.8	10.0
Day 0-20	3.7	4.5	6.3	8.0	10.0
Day 0-4	4.0	7.2	9.0	11.2	18.0
Day 5-13	3.8	4.0	6.5	7.8	6.6
Day 14-20	3.6	3.7	4.5	6.6	10.1

Note: The first three lines represent the average protein loss per cichlid over 4, 13 or 20 day periods of starvation. The average initial dry weight on day zero was 1.013 grams per cichlid. The last three lines represent average protein loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Table 9. Q_1 values for rates of protein losses during starvation of cichlids.

	Period of days covered			
Temperature range	0-4	5-13	14-20	0-20
20 to 24 C	1.15	1.01	1.00	1.05
24 to 28 C	1.05	1.12	1.04	1.08
28 to 32 C	1.05	1.04	1.09	1.06
32 to 36 C	1.12	.96	1.11	1.05
20 to 36 C	1.09	1.03	1.06	1.06

Fat Loss at Different Temperatures

Fat was measured as crude fat from ether extract. The average daily rates of fat loss were somewhat similar at all temperatures during 13 and 20 days of starvation (Table 10 and Figures 3 and 4). Average daily losses during the first four days of starvation varied irregularly with temperature. Daily fat losses during the first four days were much higher than from the 5th to 13th day; a further decrease occurred from the 14th to 20th days. Thus losses decreased with time. The decrease in fat utilization at 20 C was the only significant change with temperature.

Table 10. Fat loss per fish per day in milligrams at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	5.0	10.0	8.2	9.7	8.7
Day 0-13	4.2	5.8	5.3	5.0	5.4
Day 0-20	3.0	3.9	3.8	3.8	4.0
Day 0-4	5.0	10.0	8.2	9.7	8.7
Day 5-13	3.8	4.4	4.0	3.3	4.0
Day 14-20	.71	.43	1.1	1.6	1.4

Note: The first three lines represent the average fat losses per cichlid over 4, 13 or 20 day periods of starvation. The average initial dry weight on day zero was 1.013 grams per cichlid. The last three lines represent average fat loss per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Comparatively, then, protein catabolism was found to be slightly greater than fat catabolism on a weight basis. Protein catabolism increased regularly with temperature and decreased slightly with duration of starvation, whereas fat catabolism did not vary significantly with temperature but was decreased markedly as starvation was prolonged. Q_1 values for fat catabolism varied greatly and make little sense (Table 11).

Table 11. Q_1 values for rates of fat losses during starvation of cichlids.

Temperature Range	Period of days covered			
	0-4	5-13	14-20	0-20
20 to 24 C	1.19	1.03	.88	1.06
24 to 28 C	.95	.97	1.26	.99
28 to 32 C	1.04	.95	1.09	1.00
32 to 36 C	.97	1.05	.96	1.01
20 to 36 C	1.02	1.00	1.04	1.01

Stimpson (1965) reported that the goldfish acclimated to 24 C and 37 C, unlike the rat, does not utilize tissue glycogen or triglycerides during the early stages of fasting but metabolizes mainly protein. Furthermore, the results of experiments on the eel (Lovern, 1940), on salmon during migration (Chang and Idler, 1960), and on the lungfish during aestivation (Janssens, 1964) suggest that these animals may utilize protein in preference to carbohydrates, which, to begin with, are a meager energy reserve. The results of the present

study demonstrate that during the early period (0-4 days) of starvation nonacclimated cichlids at all temperatures except 36 C used body proteins in weight amounts not much different from those of body fat. However, the greater metabolism of protein by weight as compared to fat in the later periods of starvation is in agreement with the findings from studies reported above. But on a caloric basis, body fat, even though consumed in smaller amounts, contributed almost as much or even more than did body protein during different periods of starvation. Thus mere mass measurements without consideration of caloric content lead to inadequate bioenergetic inferences.

Body Ash Content at Different Temperatures

Body fat and protein were catabolized in the starving cichlids and lost. The amount of ash content per fish generally increased during the course of starvation (Table 12). The average daily increase in ash at all temperatures was found to be maximum during the first four days and was reduced as the starvation was prolonged. The increase in ash varied irregularly with temperature. Of the three measured body components (protein, fat and ash), ash was found to be the most variable on a percentage basis.

Table 12. Ash increase per fish per day in milligrams at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-4	3.5	3.8	3.0	4.2	6.7
Day 0-13	1.3	.80	.80	.90	2.4
Day 0-20	1.2	.60	.80	1.3	1.6
Day 0-4	3.5	3.8	3.0	4.2	6.7
Day 5-13	.33	-.44	-.22	-.55	.55
Day 14-20	1.0	.30	1.0	2.1	.14

Note: The first three lines represent the average ash increase per cichlid over 4, 13 or 20 day periods of starvation. The average initial dry weight on day zero was 1.013 grams per cichlid. The last three lines represent average ash increase per cichlid per day for consecutive periods of 4, 9 (5th to 13th day) and 7 (14th to 20th day) days of starvation.

Stresses of Temperature and Starvation

Extremes of temperature, pressure, low levels of oxygen, pollutants, fatigue or disease are stresses. In many instances starvation can be considered a stress. Brett (1958) writes of loading and inhibiting stresses. Loading stress is any environmental influence that places undue burden on an organism. High temperatures such as 32 and 36 C may act as loading stresses by inducing disorders in water, salt, fat, carbohydrate and protein metabolism in some fish. These disorders are then reflected in increased standard and perhaps total metabolic rates. This may provide the energy necessary for the animal to survive the stress by partially returning the animal to

its pre-stress composition and function.

Inhibiting stress, on the other hand, are factors that reduce the ability of the organism to carry out normal functions. The labile metabolic processes are suppressed and the possible utilization of energy is reduced. Temperatures even as high as 20 C may restrict the metabolic rates of tropical fish sufficiently to be considered inhibiting stresses. At extremes of temperature, the stress of starvation may be either suppressed or exaggerated, depending on the needs of the animal. The maintenance cost under the restriction of the inhibiting stress is probably usually reduced. Under loading stress the maintenance will always be greater. Within the optimum temperature range the cost of maintenance is higher than at low temperatures but the stress of starvation is operating under thermal conditions which are neither loading nor inhibiting. During the course of this study, cichlids at 20 and 36 C exhibited little activity most of the time, a fact which indicates that thermal stress at 36 C, acting as loading factor, led to reductions of spontaneous activity, as much as did the inhibiting temperature of 20 C.

During starvation, cichlids at all temperatures exhibited the highest metabolic rate during the first four (0-4) days. This rate then dropped during the next nine (5-13) days. During the last week of starvation (14-20 days) metabolic rate increased slightly at 28 to 36 C, but it remained low at 20 and 24 C. This can be attributed

primarily to costs of temperature acclimation and to lag in the adjustment of fish to starvation. Starvation itself leads to energy conserving metabolic and behavioral adjustments. Perhaps also during the initial period of temperature adaptation, changes in body temperature of fish may lead to transient reactions of excitation or inhibition in the cells and tissues (Stroganov, 1956). Other conditions being equal, the ability of the fish to increase metabolic rate during acclimation provides the energy necessary for this and gives the organism greater assurance for survival.

The initial stimulative phase induced by a temperature increase was followed by a relatively quiescent phase (5-13 days) when adjustment had been made to the thermal stimulation and the fish had reduced their metabolic rate in response to starvation. The cost of existence in terms of calories, during 5-13 days of starvation, was not as affected by temperature as it was during the periods 0-4 and 14-20 days. Reduction in muscular activity to conserve energy in the absence of food would be more likely to occur under laboratory conditions than in nature. Fish starved in the homogenous environment provided by a laboratory tank, all of which is within their visual and olfactory range have little stimulus for investigative or food seeking behavior. During the last week of starvation (14-20 days), nipping of other fish, perhaps motivated by increased hunger led to greater activity and increased metabolic rate, particularly at the

higher temperatures. Here, of course, the stress of starvation was greatest.

Roberts (1967) who studied Lepomis gibbosus at temperatures of 10, 15, 20 and 25 C suggested that secondary control mechanisms (extra cellular as against intra cellular), such as systemic integration by nervous, endocrine, circulatory mechanisms might be less active at low temperatures. They may be means of energy conservation over ranges in temperatures that require rapid energy turnover.

Q_{10} and Metabolic Homeostasis

Roberts (1967) reported a temperature zone of metabolic homeostasis (standard metabolic rate did not vary with temperature); the values of Q_{10} for acclimated Lepomis gibbosus were one in the acclimated temperature range of 10-17 C. For starving cichlids in the present study, an equivalence or homeostasis of total metabolism over a temperature range was noted during the period between the 5th and 13th day of starvation. The total caloric losses included not only standard metabolism but also energy loss due to spontaneous activity and to nitrogen excretion. If we regard differences in spontaneous activity at various temperatures to be negligible during the 5-13 day period of starvation, then some homeostasis with respect to standard metabolism may be suggested over the temperature range from 20-36 C.

Since the energy expenditure for spontaneous activity is related to body weight, reduction in energy loss is a natural consequence of decreasing mass of cichlids during starvation. Beamish (1964) suggested that a gradual decrease in metabolic rate of starving fish was due to subsiding spontaneous activity. The present study however, indicates that cichlids at 28, 32 and 36 C expended more energy during the last week (14-20 days) of starvation than they did during 5-13 days. The spontaneous activity (visual observation) of seven cichlids starving together actually increased during the last week of fasting. Studies by Smith (1935a, 1935b), Phillips and Brockway (1954) and Beamish (1964) reporting a decrease in metabolic rate with starvation time were based on oxygen consumption of individual fish. The gradual subsidence of spontaneous activity with time of starvation suggested by Beamish (1964) may be due to the fish being held separately. When starved as a group, cichlids were observed to increase their activity between 13 and 20 days of starvation.

Food Consumption and Growth

Growth is defined as elaboration of protoplasm. Hence, changes in protein content of the body may be considered as an appropriate measurement of growth (Gerking, 1955). From a bioenergetic viewpoint, growth can be defined as an increase in energy

content of the organism. In terms of calories, growth obviously depends on food intake and upon the fraction of food intake that can be converted into and conserved as cell contents or cell products.

After completion of the first growth experiment, some ambiguities in the data were evident. Since the fish in this growth experiment had been fed ad libitum during the acclimation period of six days, the weight differences between various temperatures were quite pronounced before the experiment was begun. To avoid this weight discrepancy no acclimation period was provided before the beginning of the other growth experiments and the starvation studies. Differences over the first five and four days of growth and starvation studies can be considered as changes over an acclimation period; further changes over longer periods of time can be interpreted as changes of adapted or acclimated animals.

Three studies on the effect of temperature on food intake and growth were carried out, each experiment redesigned on the basis of previous experience. Thus, some minor differences in technique developed within these three experiments. In spite of minor differences in procedure in the three experiments, the effects of time and temperature on food consumption and growth were similar between experiments. Food consumption was highest initially at 32 C and as time progressed the highest food consumption occurred at lower temperatures. However, the magnitude of shift in the temperature

at which the high food consumption occurred was not the same in all experiments. There were also significant differences in efficiency of conversion of food to tissue. The third of these experiments was the best in design and technique. It is mainly this experiment which will be considered here. Consideration of the results of the other experiments will be given mainly when important differences were found. In the experiment presented, cichlids were fed continuously for 5, 12 or 19 days from a common day zero. Cichlids were transferred from holding tanks at 20 C and placed in individual containers at temperatures of 20, 24, 28, 32 and 36 C. On days 0, 5, 12 and 19 data were collected from individual cichlids on wet weight, dry weight, ash, fat, and heat of combustion. Most of the data presented in this section represent averages derived from data on four cichlids at each temperature. Nevertheless (as in the starvation experiments), mean weights of growing fish are presented in Table 13 to make it possible for the reader to assess the effects of any differences in the size of the fish.

Table 13. Experimental periods, temperatures, and average wet weights of fed cichlids in grams.

	Temperature (C)				
	20	24	28	32	36
Day 0	4.45	4.50	4.36	4.32	4.37
Day 5	4.90	5.21	5.40	5.44	4.64
Day 12	6.33	6.40	6.76	6.33	4.83
Day 19	7.65	9.78	8.43	5.75	4.43
Day 0-5	4.67	4.85	4.88	4.88	4.58
Day 6-12	5.61	5.80	6.08	5.88	4.73
Day 13-19	7.00	8.09	7.60	6.04	4.63
Day 0-12	5.42	5.36	5.58	5.39	4.59
Day 0-19	5.99	7.06	6.40	5.07	4.39

Mortality and Behavior of Feeding Cichlids at Different Temperatures

The cichlids were generally more active at the intermediate temperatures (24, 28, 32 C) than at the extreme temperatures (20 and 36 C). Hierarchy among cichlids was observed at all temperatures but it was not so obvious at 36 C. Under extreme thermal stress (36 C), cichlids were found struggling for existence (rapid opercular movement, little activity and little food intake) rather than exercising their hierarchical rights. Furthermore, dispersion rather than clumping of tubificid worms used for food was greater at the highest temperature. This elminated the possibility of food being guarded by a single dominant fish. In general, the cichlids at 36 C looked emaciated and were nervous; they would dash about on

slight disturbance. At 36 C, one fish died on the second day of feeding.

The behavior of feeding cichlids was different than that of starving cichlids. Dominant fish keeping watch over clumps of tubificid worms reduced the exploratory activity of other cichlids as well as their own. During starvation, this was reversed when dominant cichlids chased other fish. Since fish were held individually in the third experiment, social interaction did not occur.

In assimilation studies, most of the fish fed to repletion died at 32 and 36 C when left in 30 ml of water for 36 hours. Mortality among fish that were fed limited rations was lower at these temperatures. Poor water quality as well as high temperature may have been involved in mortalities of fish fed ad libitum.

Food Consumption at Different Temperatures

Food intake, growth, and food conversion by cichlids varied greatly with changes in temperature. During the first five days, food consumption increased with increasing temperature 20 to 32 C; during the second period (6th to 12th days) food consumption increased over the range from 20 to 28 C; and during the last period (13th to 19th days) it increased only between 20 and 24 C. Obviously food intake was not related to temperature in a simple manner (Table 14, Figure 5).

Table 14. Food consumption of cichlids (milligrams dry weight per fish per day) at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	63	107	170	191	57
Day 0-12	117	136	173	157	51
Day 0-19	131	199	168	86	48
Day 0-5	63	107	170	191	57
Day 6-12	156	158	176	132	47
Day 13-19	155	307	158	-34*	43

*Please see text for explanation of negative value.

Note: The first three lines represent the average daily food consumption per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams per cichlid. The last three lines represent average food consumption per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding. Cichlids were fed tubificid worms.

During the first five days of ad libitum food intake, the average daily dry weight of food taken per fish increased from 63 mg at 20 C to 191 mg at 32 C, and then dropped to 57 mg at 36 C. Intake varied between fish; variability was similar at 20, 24 and 28 C, was very high at 32 C, but at 36 C was similar to that at the lower temperatures (Figure 5a).

Over 12 days of ad libitum feeding, food consumption per fish increased from 117 mg at 20 C to 173 mg at 28 C; but it dropped to 156 mg at 32 C and to 51 mg at 36 C. Variability of food intake at all temperatures studied was much greater over the 12-day period

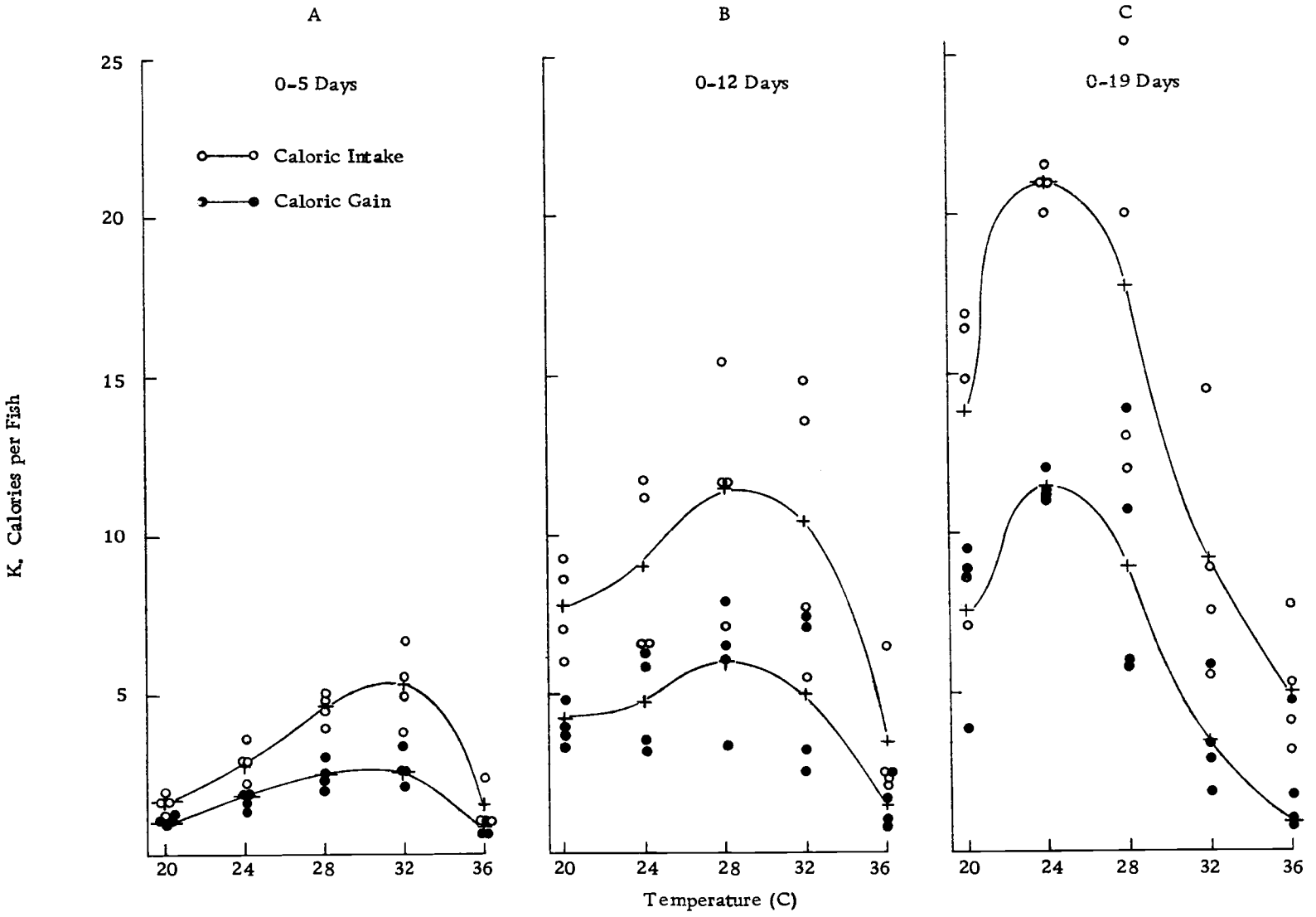

Figure 5. Relationship between caloric intake, caloric gain and temperature for cichlids during three periods of ad libitum feeding. Open circles indicate the total caloric intake of a single cichlid over the indicated time periods. Solid circles indicate the total caloric gain over the indicated time periods. Averages are indicated by +.

than over the five-day period (Figure 5b). During 19 days of ad libitum feeding, daily dry food intake per fish increased from 131 mg at 20 C to 199 mg at 24 C but dropped to 167 mg at 28 C and still further to 86 and to 48 mg at 32 and 36 C. At 20 C, intake for one fish was 65 mg and for the other three was near 160 mg (Figure 5c). All cichlids at 24 C showed an almost identical daily food intake near 200 mg. Over 19 days, the variability of food intake at 28 C was greater than that at other temperatures. The highest food consumption per cichlid per day, about 260 mg was shown by a cichlid at 28 C, and the smallest consumption at 28 C was slightly above 100 mg. Variability at 36 C was low. In general, variability was low at 20 C over 5, 12 or 19 days. Variability increased with time at 28 C. At 24, 32 and 36 C variability was high over 12 days but was reduced over the 19-day period, especially at 24 and 36 C. In spite of the changes in variability with temperature, definite trends were clear. Over a 5-day period the greatest food consumption was found at 32 C; over a 12-day period at 28 C and over a 19-day period at 24 C. From the 6th to 12th day food consumption was greatest at 28 C and from 13 to 19 days was greatest at 24 C.

At 20 C the daily food (dry) consumption per fish increased from 63 mg during the first five days to 156 mg over the 6-12 day period and did not appreciably change subsequently (Table 14). Daily food consumption at 24 C increased in successive periods

throughout the experiment. In striking contrast, there was little change in food intake throughout the experiment at 28 C. The daily food intake at 32 C decreased from 191 mg during the first five days to 132 mg over 6-12 days and finally to near zero from the 13th to 19th days. Thus food consumption at 32 C decreased with time whereas at 24 C food consumption increased with time. At 36 C, there was little change in food consumption with time, food consumption here being little more than a maintenance ration (Table 14).

The negative value for average food intake during the period 13 to 19 days at 32 C (Table 14) is a result of the method of determining intake for this period. Differences between the 0-12 day group and the 0-19 day group from which this value was estimated were responsible. In the 0-12 day period two of the four cichlids at 32 C had low intakes; and three of the four in the 0-19 day period had low intakes. A higher incidence of low intake cichlids in the group from 32 C sacrificed on the 19th day would account for the negative figure of -34.

Since dry weight measurements without caloric information on the materials consumed are not adequate for bioenergetic studies, caloric intakes of cichlids at different temperatures and during different experimental periods are given in Table 15.

Table 15. Caloric intake of cichlids per fish per day at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	350	595	945	1062	326
Day 0-12	652	760	964	870	285
Day 0-19	730	1108	932	481	267
Day 0-5	350	595	945	1062	326
Day 6-12	870	876	980	733	255
Day 13-19	860	1705	878	-185*	237

*Please see text for explanation of negative value.

Note: The data of Table 14, indicating dry weights of tubificid worms consumed, have been converted to caloric equivalents by multiplying dry weights by 5.557. The average initial dry weight per cichlid on day zero was 0.987 grams and the average caloric content was 3967 calories.

Growth in Dry Weight at Different Temperatures

As might be expected, food consumption and growth were closely parallel at all temperatures. Except for the first five-day period, one might easily think graphs of the relationships between food consumption and temperature and growth and temperature differed only in scale. For growth as well as food consumption, the optimum temperature dropped from 32 to 28 to 24 C as the experimental period extended from 5 to 12 to 19 days (Figure 5). Thus, for short periods of time, high temperatures increased food intake and dry weight gain; over longer periods, even with food always present, food intake and weight gains had maxima at lower

temperatures (Table 16).

Table 16. Dry weight gain (milligrams per fish per day), of cichlids on ad libitum intake of tubificids at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	34	56	80	88	14
Day 0-12	54	60	75	60	20
Day 0-19	62	93	74	28	8
Day 0-5	34	56	80	88	14
Day 6-12	67	61	71	40	23
Day 13-19	77	150	71	-27*	-10*

*Please see text for explanation of negative values.

Note: The first three lines represent the average dry weight gain per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams per cichlid. The last three lines represent average dry weight gains per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding.

At 20 C, growth was essentially a linear function of time. At 24 C, growth was much the same as at 20 C over the first 12 days but was greater over the next seven days. At 28 C growth was essentially constant through time. It declined at 32 C and was variable at 36 C (Table 16).

Growth in Calories at Different Temperatures

Optimum growth in calories, as did optimum growth in dry weight, occurred at 32 C during the first five days but at 28 C and

then at 24 C during the second and third periods of the experiment (Table 17). At each temperature, growth in calories differed somewhat from growth in dry weight as the experiment progressed through time. The daily gain in dry weight at 20 C increased from 67 mg during the 6-12 day period to 77 mg over a period of 13-19 days, whereas the daily caloric gain per fish decreased during the 13-19 day period. This may have been due to protein elaboration being relatively greater than fat elaboration during the last period of the experiment. At 24 C, both daily dry weight and caloric gain per fish increased as feeding was prolonged. At 28 C, even though the daily increase in dry weight remained the same (71 mg), the daily caloric gain per fish declined over the last two periods. This suggests relatively greater protein production during the last period.

Table 17. Caloric gain of cichlids on ad libitum intake of tubificids per fish per day at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	250	370	517	537	162
Day 0-12	364	397	500	423	137
Day 0-19	403	603	473	181	50
Day 0-5	250	370	517	537	162
Day 6-12	452	395	484	355	78
Day 13-19	424	960	430	-238*	-101*

*Please see text for explanation of negative values.

Note: The first three lines represent the average caloric gain per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams (3967 calories) per cichlid. The last three lines represent average caloric gain per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding.

Increases in Protein, Fat, and Ash at Different Temperatures

The gains in protein, fat and ash per fish at different temperatures and over the three experimental periods, when the cichlids were fed tubificid worms ad libitum, are presented in Figure 6 and in Tables 18, 19 and 20. Over the first five days, the daily protein gain per fish increased with temperature to 32 C and then decreased markedly at 36 C. In last two successive periods of the experiment, the maximum protein gain shifted downward first to 28 C and then to 24 C.

Except at 20 and 36 C, the daily protein gain per fish dropped

during the second experimental period (Table 18). At 20, 24, and 28 C, protein gain was as high or higher during the last period than in earlier periods.

Table 18. Protein gain (milligrams dry weight per fish per day) of cichlids on ad libitum intake of tubificids at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	25	39	54	55	6
Day 0-12	25	29	38	34	9
Day 0-19	32	51	44	15	4
Day 0-5	25	39	54	55	6
Day 6-12	25	21	26	18	12
Day 13-19	42	88	54	-17*	- 6*

*Please see text for explanation of negative values.

Note: The first three lines represent the average protein gain per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams per cichlid. The last three lines represent average protein gains per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding.

Over the first five-day period, the daily fat gain per fish increased with temperature to 32 C and then decreased at 36 C (Table 19). Again, the optimum temperature decreased first to 28 C and then to 24 C during the last two periods of the experiment. The daily gain in crude fat per cichlid on the tubificid diet at 20 C increased and then decreased as the experiment progressed through time (Table 19). At 24 C, the daily fat gain increased through time.

The daily fat gain at 28 C increased and then decreased.

Table 19. Fat gain (milligrams dry weight per fish per day) of cichlids on ad libitum intake of tubificids at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	7	16	22	25	4
Day 0-12	24	26	29	23	7
Day 0-19	25	35	23	10	2
Day 0-5	7	16	22	25	4
Day 6-12	36	33	34	22	10
Day 13-19	26	50	14	-11*	- 7*

*Please see text for explanation of negative values.

Note: The first three lines represent the average fat gain per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams per cichlid. The last three lines represent average fat gains per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding.

In general, at temperatures 20 to 32 C the daily protein gain per fish either remained the same (at 20 C) or decreased over the second period. In direct contrast to this trend, the daily fat gain per fish at temperatures studied except 32 C, increased during the same period (6-12 days). Over 13-19 days, daily protein gain per fish increased markedly at 20 and 28 C whereas the daily fat gain per fish increased only at 24 C and declined at 20 C. At 20 C, increased protein production with decreased fat production during the 13th to 19th days may account for an increase in dry weight even though the food

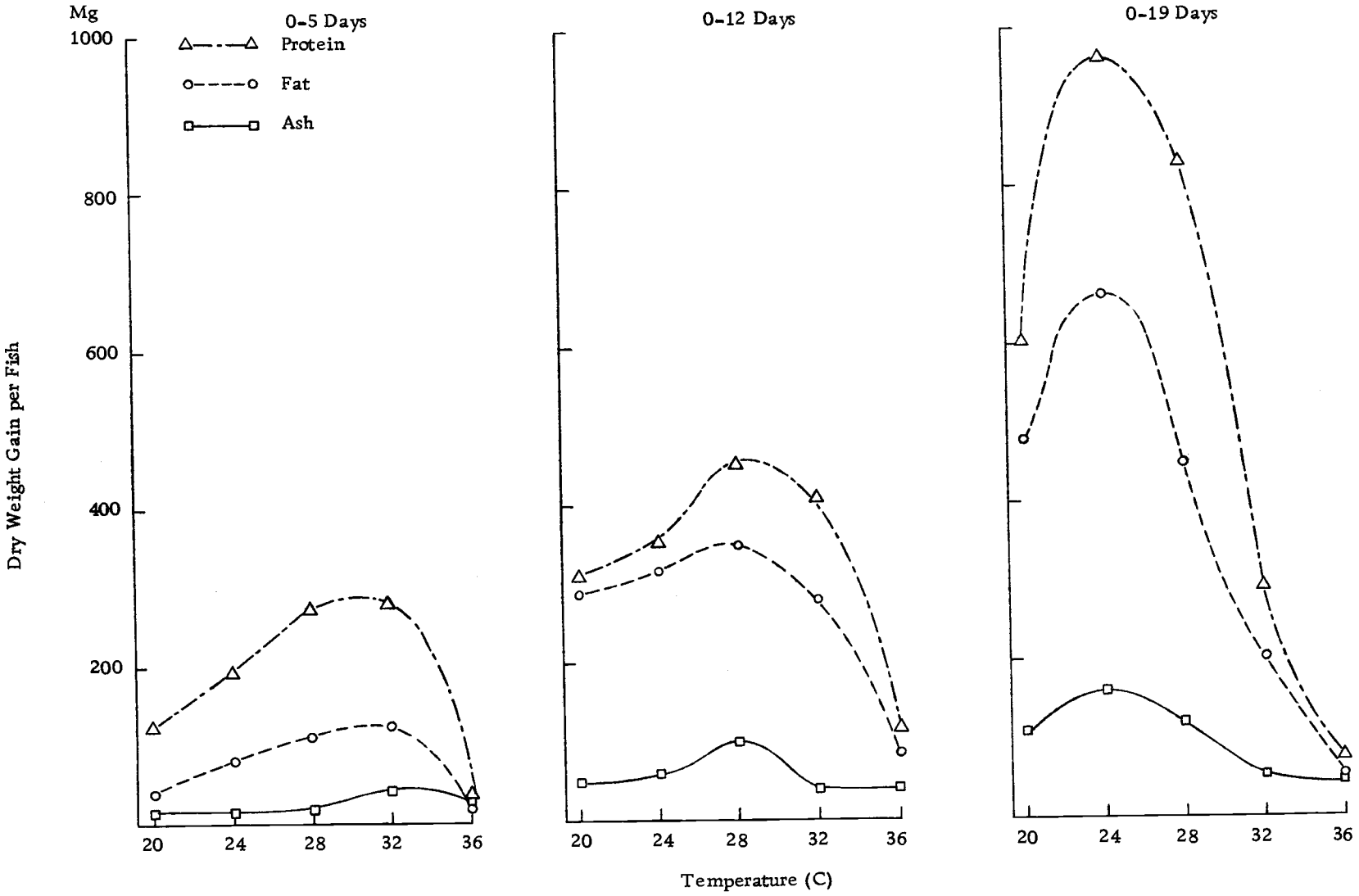

Figure 6. Average total dry weight increments per fish of protein, fat and ash in cichlids exposed to different temperatures over indicated time periods. Cichlids were fed ad libitum on tubificid worms.

intake remained the same during the last two periods (6th to 19th days). Over the 13 to 19 days of feeding a greater protein production may also be attributed to smaller but frequent food intakes. Food intake in bulk may lead to greater SDA and ultimately to a smaller protein production and to a greater fat deposition. Cichlids when fed ad libitum after having been on maintenance ration are likely to indulge in over eating at least in the early periods of feeding. This may account for the greater fat production during the second period (6-12 days). Over the last period (13-19 days) cichlids might have become accustomed to abundant food supply and thus more discreet in their diet.

The daily ash gain per fish during the first five days was maximum at 32 C. The maximum occurred at successively lower temperatures as the experiment progressed through its three periods. Ash gain at the lower two temperatures increased as the experiment progressed. At higher temperatures it tended to decrease (Table 20).

There is a definite pattern in the daily increase of the three body components: protein, fat and ash, for it was highest at 32 C over the first five days, at 28 C over 12 days and at 24 C over 19 days.

Most animals grow and live well with little or no dietary fat, but since fat is a favorable storage substance it can be interconverted to and from carbohydrates and formed from protein. Fat may be

deposited after high caloric intake even when no fat is fed. Fat being inert chemically and rich in energy, it makes an ideal storehouse of potential energy.

Table 20. Ash gain (milligrams per fish per day) of cichlids on ad libitum intake of tubificids at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Day 0-5	2.6	2.2	4.4	9.2	5.2
Day 0-12	4.0	5.0	9.0	3.0	3.0
Day 0-19	6.0	8.0	6.0	3.0	2.5
Day 0-5	2.6	2.2	4.4	9.2	5.2
Day 6-12	5.4	7.0	11.7	-1.1*	1.7
Day 13-19	8.5	14.0	2.7	2.7	1.2

*Please see text for explanation of negative values.

Note: The first three lines represent the average ash gain per cichlid over 5, 12 or 19 day periods of ad libitum feeding. The average initial dry weight on day zero was .987 grams per cichlid. The last three lines represent average ash gains per cichlid per day for consecutive periods of 5, 7 (6th to 12th day) and 7 (13th to 19th day) days of ad libitum feeding.

Data in Table 21 indicate that, during the first five days of feeding, fat intake generally was slightly higher than fat gain, except at 36 C where it was considerably higher. During the second period (6-12 days) fat gain was greater than fat intake at all temperatures. During the last period (13-19 days) fat gain was slightly greater than fat intake at 20 C and 24 C but lower at higher temperatures. Obviously, all gains greater than intakes imply neogensis of fat; all

intakes greater than gain imply net catabolism of fat.

Table 21. Total fat intake and total gain in body fat (milligrams per cichlid) over indicated period of time on the tubificid diet.

	Temperature (C)				
	20	24	28	32	36
0-5 Days					
Intake in fat	44	75	120	133	40
Gain in fat	38	80	110	125	18
6-12 Days					
Intake in fat	153	154	172	130	46
Gain in fat	254	235	242	156	68
13-19 Days					
Intake in fat	151	300	155	- 32	42
Gain in fat	188	350	98	- 81	- 47

Protein intake throughout the experiment remained considerably greater than gain in body protein of cichlids at all temperatures (Table 22). As protein intake was always greater than protein gain and as fat gain was often greater than fat intake, part of the protein fraction was probably converted into fat by cichlids fed ad libitum on tubificid worms.

Table 22. Total protein intake and total gain in body protein (milligrams per cichlid) over indicated period of time on the tubificid diet.

	Temperature (C)				
	20	24	28	32	36
0-5 Days					
Intake in protein	251	428	680	764	230
Gain in protein	126	196	273	277	28
6-12 Days					
Intake in protein	876	883	984	738	262
Gain in protein	180	152	181	130	88
13-19 Days					
Intake in protein	867	1718	884	-186	239
Gain in protein	294	616	380	-120	- 45

Body Weight - Time Relationships

The curves of growth of cichlids over a 19-day period are different at various temperatures (Figure 7). Cichlids at 20 and 28 C had growth curves nearly linear, as compared to the exponential curve of growth at 24 C. At 32 and 36 C, the growth curves rose and then declined. It is apparent from the growth curves that prolonged exposure to unusual temperatures has biological effects which differ from short exposure. On the basis of very short-term experiments with cichlids one might conclude erroneously that a temperature of 32 C was favorable.

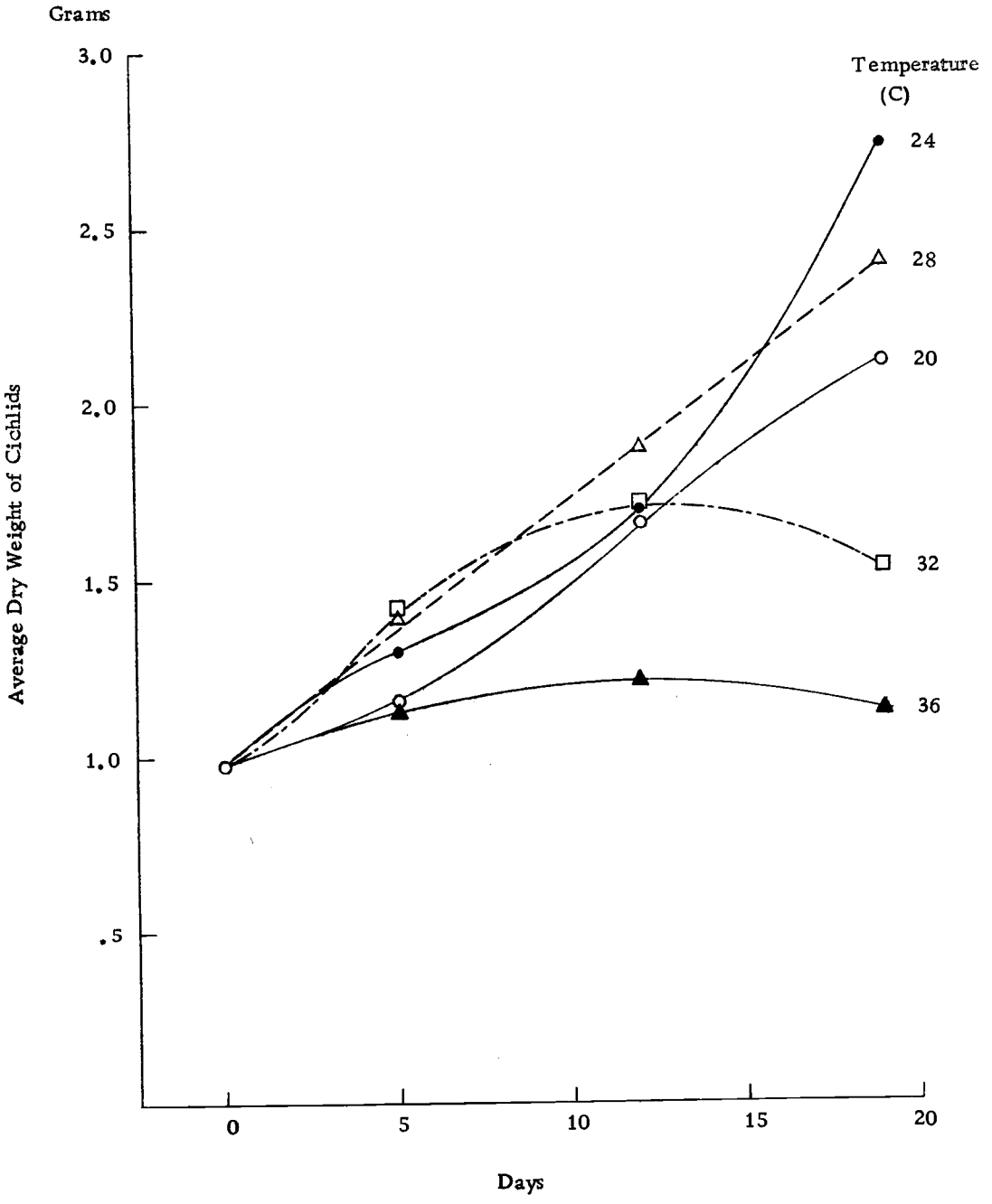

Figure 7. Relationship between dry weight, temperature and feeding time for cichlids. Average dry weights are plotted on the ordinates and duration of feeding on the abscissa. Curves are plotted of dry weight against time for five different temperatures.

Efficiency of Food Conversion

In fish held and fed individually, food conversion efficiency was highest at 20 C and decreased nearly regularly with increasing temperature during 5, 12, and 19 day periods of exposure (Figure 8a). The principal reason for this was undoubtedly the increase in maintenance metabolism of the relatively quiet fish held separately when temperature was increased. Efficiencies decreased with duration of the experiment. The initially high food consumption rates led to higher efficiencies. Decline in food consumption through time decreased food conversion efficiencies, since a greater proportion of the food went to maintenance.

The story was entirely different when fish were held in groups as in the first and second experiments. Here efficiency was low at both high and low temperatures (Figure 8b). Fish did not consume much food at low temperatures. This need not lead to low efficiency here if the fish are quiet, for their maintenance metabolism is low. But in groups the fish were not quiet and this combined with low consumption could lead to low conversion efficiencies.

Nonassimilation and Excretion

Food can be converted by fish into different amounts of body material and useful energy, depending on temperature, quality of

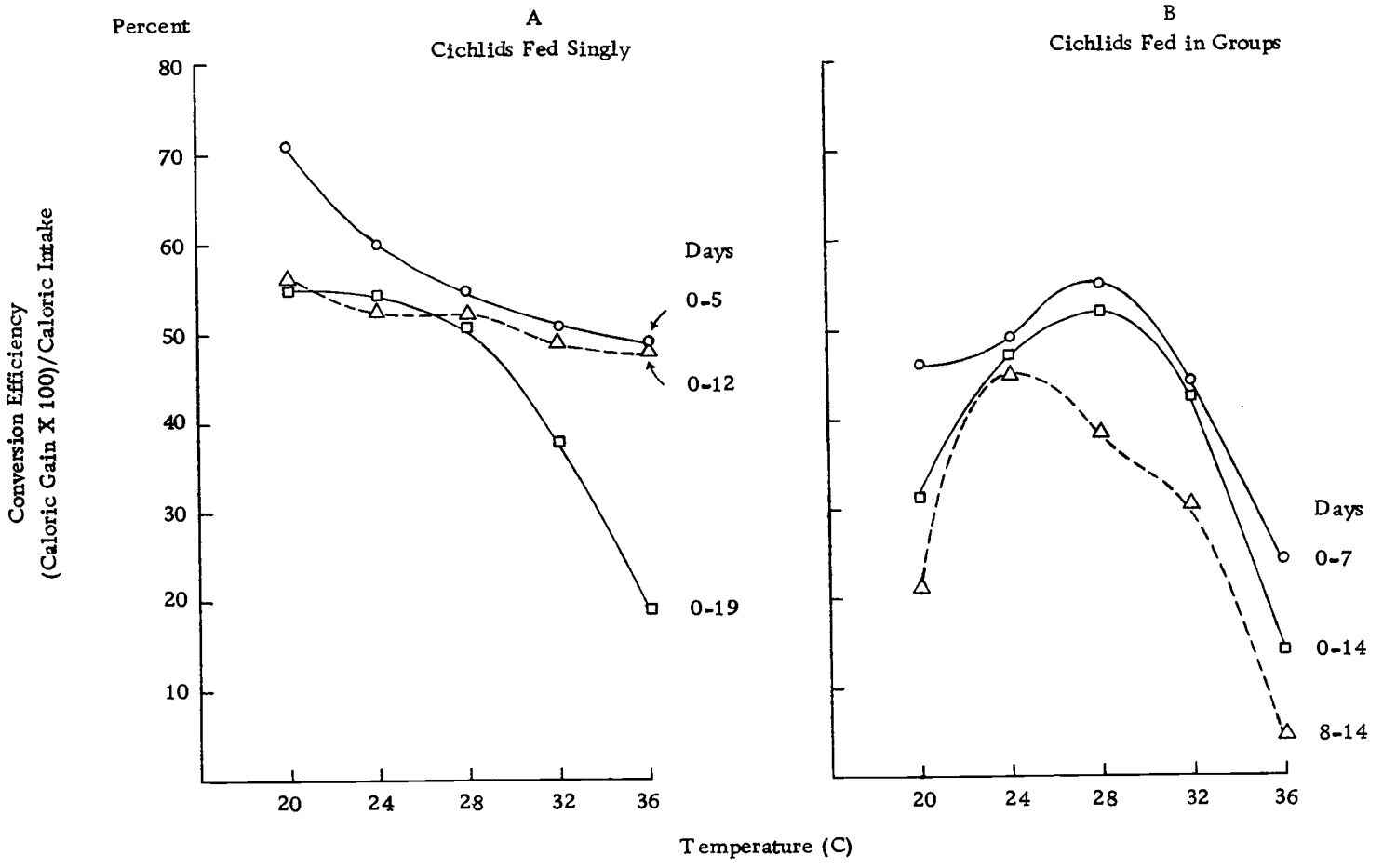

Figure 8. Relationship between conversion efficiency and temperature of cichlids fed singly and in groups over indicated time periods.

food, amount of food available, and energy needs of the organism. One effect of temperature is to alter the extent of digestion and absorption of food. Less efficiently digested food will lead to the production of feces with higher caloric value, which can be estimated by wet combustion. In the wet combustion determination of caloric equivalents, the milligrams of oxygen required for oxidation of an organic mass at 190 C under pressure in a potassium iodate sulphuric acid solution were obtained and multiplied by 3.36 to provide approximate caloric equivalents.

In determining the caloric content of feces, it is important to minimize bacterial decomposition. In order to keep the decomposition of excreta from cichlids at a minimum, a time interval had to be determined which was long enough for digestion and absorption of food and elimination of feces but not long enough for much bacterial decomposition to occur. The guts of the cichlids were found to contain food up to 30 hours after an ad libitum meal at temperatures ranging from 20 to 36 C. They were reasonably empty after 36 hours. No significant correlation was found between temperature and the length of time necessary for the guts to empty.

Although digestion may be considered as essentially completed at 36 hours, the hind gut of the cichlid was not completely empty even after five days of starvation. Cichlids were found to defecate within five minutes after they were fed a discrete meal. The

defecation of cichlids immediately after a meal was adopted as a means of emptying their hind gut almost completely. The fragment of undigested food from the previous meal was immediately removed from the medium in which a fish was to be kept for the next 36 hours to collect its excreta. At the termination of an experiment, the fish were dissected to recover the last fragment of food from the hind guts for wet combustion.

In order to determine the quantity or percentage of a meal digested or available to a cichlid, the cichlids were fed restricted amounts of food in some experiments and all the food they could consume in an hour in other experiments. The restricted ration was near 20 mg of tubificids (wet weight) or 20.5 calories. The caloric intakes for the cichlids fed ad libitum are given in Table 23. The cichlids were fed singly in styrofoam tanks and then placed singly in beakers containing 30 ml of water. Tubificids remaining in the styrofoam tanks were weighed. Three cichlids were studied at each temperature on the restricted diet and one on the one hour ad libitum intake.

Thirty-six hours after feeding the cichlids were removed from the beakers with 30 ml of water. The 30 ml with soluble and insoluble contents were divided into two 15 ml aliquots and the iodate oxygen required for oxidation at 190 C under pressure was determined and converted into caloric equivalents. The fragment of food,

recovered from the hind gut of the cichlid at the termination of the experiment, was similarly oxidized to obtain its caloric equivalent. The materials oxidized in the above three samples came mainly from the digestive tract, some from mucus and very little from nitrogen excretion at gills and kidneys, since this is mainly as ammonia. The caloric equivalents obtained are given in Table 23 as calories in feces.

Table 23. Caloric content of feces of cichlids at different temperatures.

	Temperature (C)				
	20	24	28	32	36
Ad libitum intake					
Caloric intake	55	112	135	112	98
Calories lost in feces	9	18	16	16	30
Calories absorbed	46	94	119	96	68
Percentage absorbed	83	84	89	86	70
Percentage in feces	17	16	11	14	30
Restricted ration					
Caloric intake	19	23	23	22	21
Calories lost in feces	1	1	1	2	2
Calories absorbed	18	22	22	20	19
Percentage absorbed	93	94	95	93	91
Percentage in feces	7	6	5	7	9

Note: Cichlids had been acclimated for 20 days or more at the specified temperature, and had been kept on maintenance diet. Cichlids were then deprived of food for three days and then given a single meal. Figures represent average values of three experiments. The difference in the caloric intake on the restricted diet were experimental and did not depend on the cichlids at all.

The percentage of food consumed occurring in the feces was much lower on the restricted ration than on the unrestricted ration

(Table 23, Figure 9). The data on unrestricted rations are most applicable to the present study, of course, as ad libitum feeding was used. On unrestricted rations the fish assimilated their food very poorly at 36 C. Assimilation appears to have been optimal at 28 C.

Specific Dynamic Action

Specific dynamic action (SDA: The heat increment of feeding or the calorigenic effect of food) represents the extra heat incident to the utilization of food. SDA was estimated at different temperatures by taking the difference between oxygen consumptions of unfed and fed (ad libitum for one hour) cichlids at the same levels of activity (Figure 10). SDA plus activity was also estimated by subtracting from food intake starvation metabolism, calories in feces, and growth calories, as will be considered in Discussion. From the extra oxygen consumed after a meal, the heat increment incident to the process of handling food was expressed in caloric equivalents. Two SDA experiments at each of five temperatures and each of the four swimming speeds (approximately 6, 8, 10 and 12 cm/sec) and four experiments at a swimming speed of 4 cm/second were conducted. In each experiment, oxygen consumption was determined for a period of one hour, two to three hours after the meal was eaten. In one experiment at a swimming speed of 4 cm/second, oxygen consumption measurements were made at short intervals for a period of 36 hours at each of five

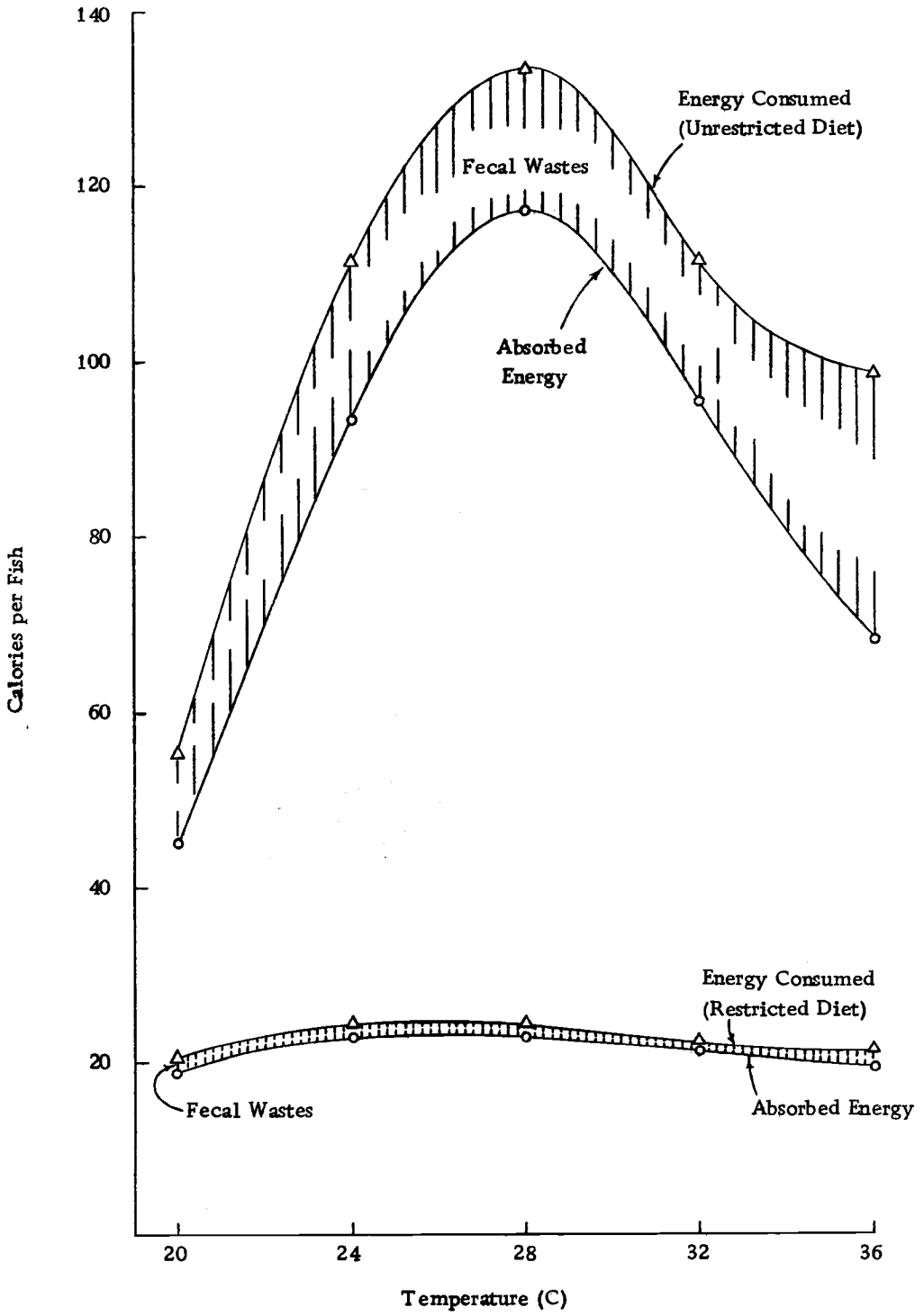

Figure 9. Relationship between restricted and unrestricted food intake, absorbed energy and temperature for cichlids fed on tubificid worms. About twenty calories per fish were provided on the restricted intake and for the ad libitum intake one hour was allowed. Energy from fecal wastes was subtracted from intake to give absorbed energy (see text).

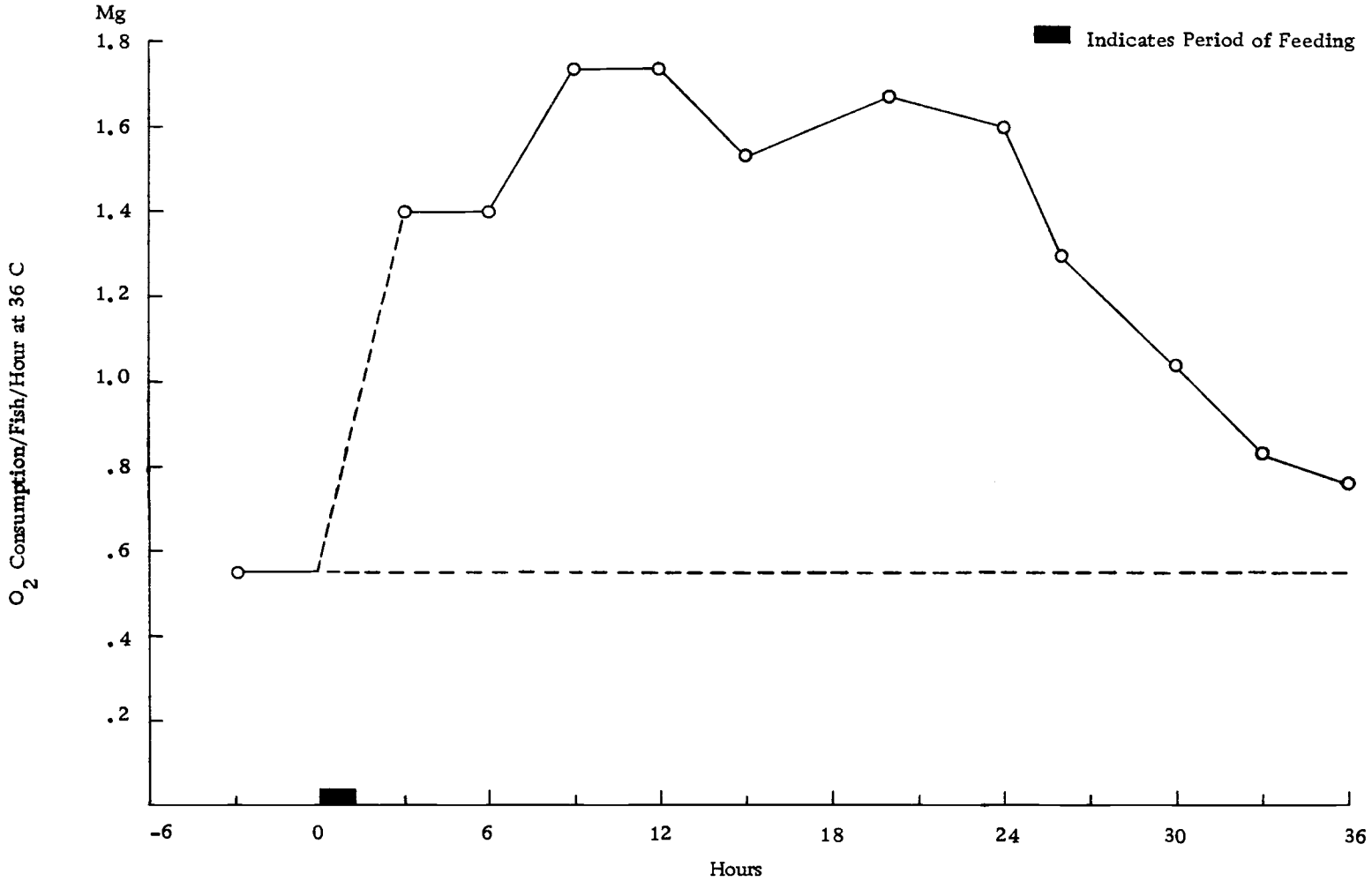

Figure 10. Oxygen consumption of a cichlid due to specific dynamic action. Upper solid line represents oxygen consumption of fed fish, lower solid and dotted line indicates oxygen consumption of same fish starved for three days. The area between the dotted and solid curves gives the extra oxygen consumption due to SDA. One such graph was prepared for each temperature.

temperatures. Cichlids had been maintained for 20-40 days on a maintenance ration.

Considerable effort was spent in trying to determine the magnitude of SDA from studies of oxygen consumption. Over 400 measurements of oxygen consumptions were made on some 13 cichlids over five different temperatures and five different velocities of water. The effort was not sufficient in the light of the variables encountered. The weight of individual cichlids varied from 2.1 to 4.4 grams. The oxygen consumption of unfed fish did not increase regularly at any temperature with the velocity of water. There was a general increase in oxygen consumption with temperature at velocities of 8, 10 and 12 cm/sec of water flow; at no velocity was the relationship between oxygen consumption and temperature linear or regular and the relationship at the three high velocities could not be said to be parallel. Data on the extra oxygen consumption after a meal and on food intake were also irregular.

The cichlids at 20, 24, 28, 32, and 36 C consumed 11.0, 16.0, 17.4, 27.7, and 29.5 milligrams of extra oxygen respectively in 36 hours to handle .168, .254, .293, .272, and .292 grams of food. The cichlids lost 37, 53, 58, 93, and 99 calories from a total of 172, 261, 301, 279, and 300 calories consumed. Therefore, SDA represented 22, 20, 19, 33, and 33 percent of the food consumed at 20, 24, 28, 32, and 36 C, when the fish were swimming at 4 cm/second.

The extra oxygen consumption associated with SDA reached its peak within the first three to six hours at temperatures where fish ate less food. At temperatures where fish ate more, SDA reached its peak much later at nine to twelve hours after feeding. In addition to this primary peak of SDA, there was a secondary peak between 20 to 26 hours at each temperature (Figure 10).

The time differential in primary peaks of SDA may be attributed to digestive processes requiring more time to handle a large amount of food. This would delay the full impact of the assimilated food on metabolism. The secondary peaks of SDA observed at all temperatures may owe their occurrence to food components that defy immediate assimilation and do not raise the metabolic rate until they are finally digested.

DISCUSSION

An over-all picture of the utilization and loss of the energy and materials in the food the cichlids consumed at different temperatures during different periods of the experiment can be given by a graphical presentation of energy and material budgets at the temperatures tested. Food intake and total growth were measured directly, as was the increment in fat. The protein increment of growth was determined by difference. Ancillary experiments permitted a reasonable approximation of the percentage of the food consumed at each temperature that was lost as fecal wastes. What is here called maintenance metabolism was estimated through starvation experiments at each temperature. Because some of the caloric loss of starving fish is in the form of reduced nitrogen compounds, primarily ammonia, a correction was necessary to estimate the metabolic rate of the starving fish. To do this, 14 percent of the caloric value of the protein loss of the starving fish was subtracted from their total caloric loss, the difference being assumed to approximate their metabolic rate.

Because the fish were generally quiet during starvation, maintenance metabolism perhaps approximates standard metabolic rate. It is, however, higher than standard metabolic rate by the caloric cost of any activity. Over the entire experiment, maintenance metabolism tended to increase with temperature, as standard metabolic

rate would be expected to do. Nevertheless, during the earlier period of the experiment, it tended to be highest near temperatures at which food consumption was greatest, this suggesting the activity of the fish also to be greatest at these temperatures.

The difference obtained when total growth, maintenance metabolism and fecal wastes are subtracted from food consumption includes specific dynamic action, any activity above that of the starving fish, nitrogenous wastes, and any errors in measurement. Specific dynamic action and nitrogenous wastes are undoubtedly the major components of this difference term.

Graphs showing energy and material intake, utilization, loss, and scope for growth at each temperature during different experimental periods will be presented both in terms of calories per fish per day and calories per gram of fish per day. Food intake and growth are functions of the size of the fish when food is unlimited, as it was during this experiment. Fish which consume more food and grow faster at one temperature than other fish at another temperature soon begin to eat more food not only because the temperature is favorable but also because they are bigger. This tends to exaggerate the apparent effect of temperature on food consumption and growth unless the data are presented in rate terms, here calories per gram of fish per day. In the graphs in rate terms, all energy utilizations and losses have been calculated on the basis of the mean wet weight of the

fish (one half of initial plus final values) during each experimental period. Actually, in this experiment, no great difference between the graphs on a per fish basis and the graphs on a per gram basis is apparent. This is because the duration of the experiment was not sufficiently long for the fish at different temperatures to become greatly different in size. Nevertheless, the maxima on the rate curves are not as exaggerated as those on the curves plotted on a per fish basis. The flattening of the rate curves becomes more apparent over longer periods of the experiment.

During the first five days of the experiment, food consumption of the cichlids was greatest at 32 C (Figures 11 and 12). Nevertheless, growth at 32 C was little greater than it was at 28 C, because fecal losses, maintenance metabolism, and other uses and losses of energy and materials were greater at 32 C than at 28 C. Here, then, was already a suggestion that optimal conditions for cichlids would be at temperatures lower than 32 C. Food consumption and growth were much less at the temperature extremes of 20 and 36 C (Figures 11 and 12). Since the fish had no prior acclimation to the test temperatures, the first five days of the experiment can be considered a period of acclimation with any associated costs, a period of adjustment of food consumption and growth rates.

Over the first 12 days of the experiment, maximum food consumption and maximum growth clearly occurred at 28 C (Figures 13

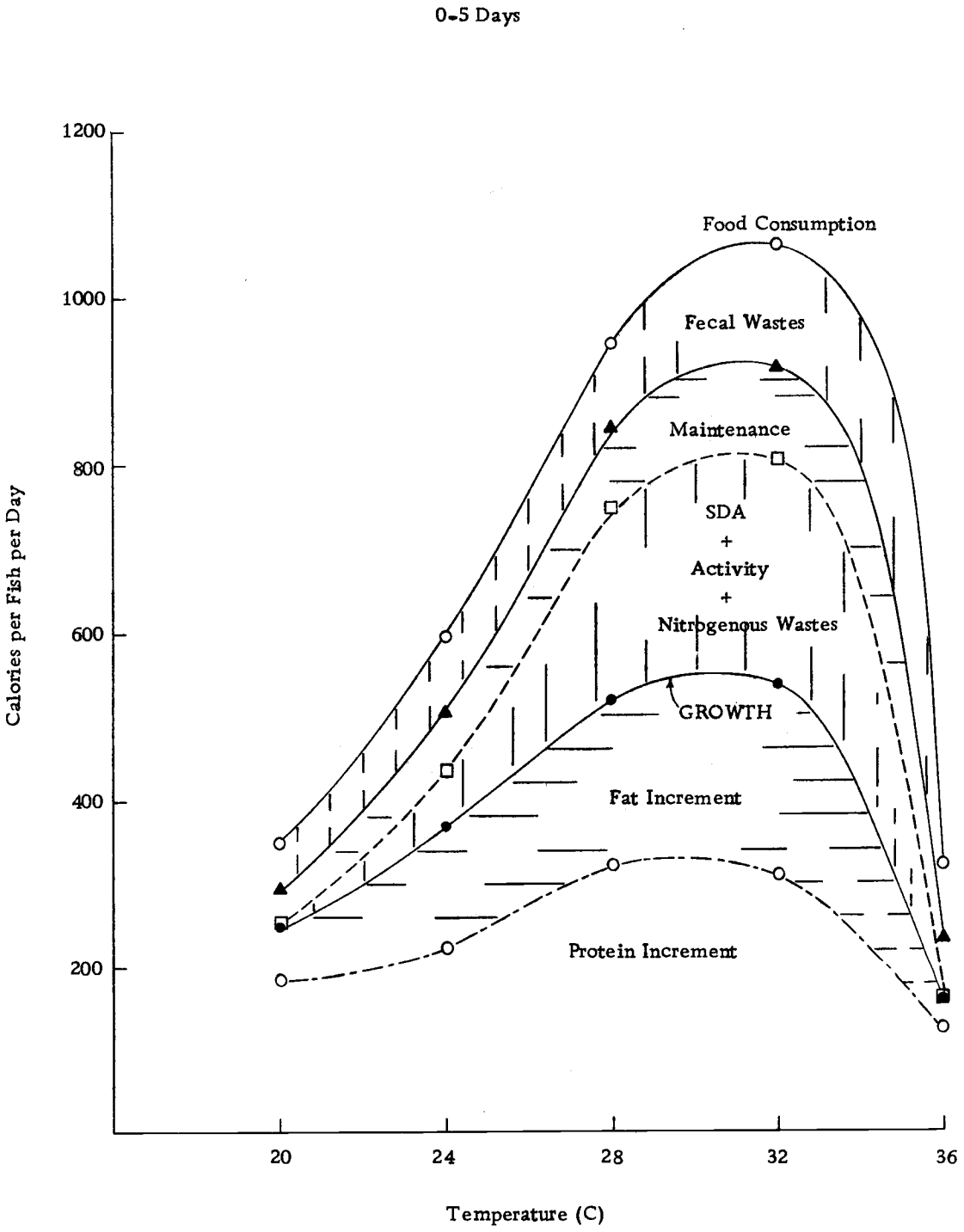

Figure 11. Budget or scope diagram for cichlids. Influence of temperature on food intake, growth, excretory losses, and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of five days and data are shown as calories per fish per day.

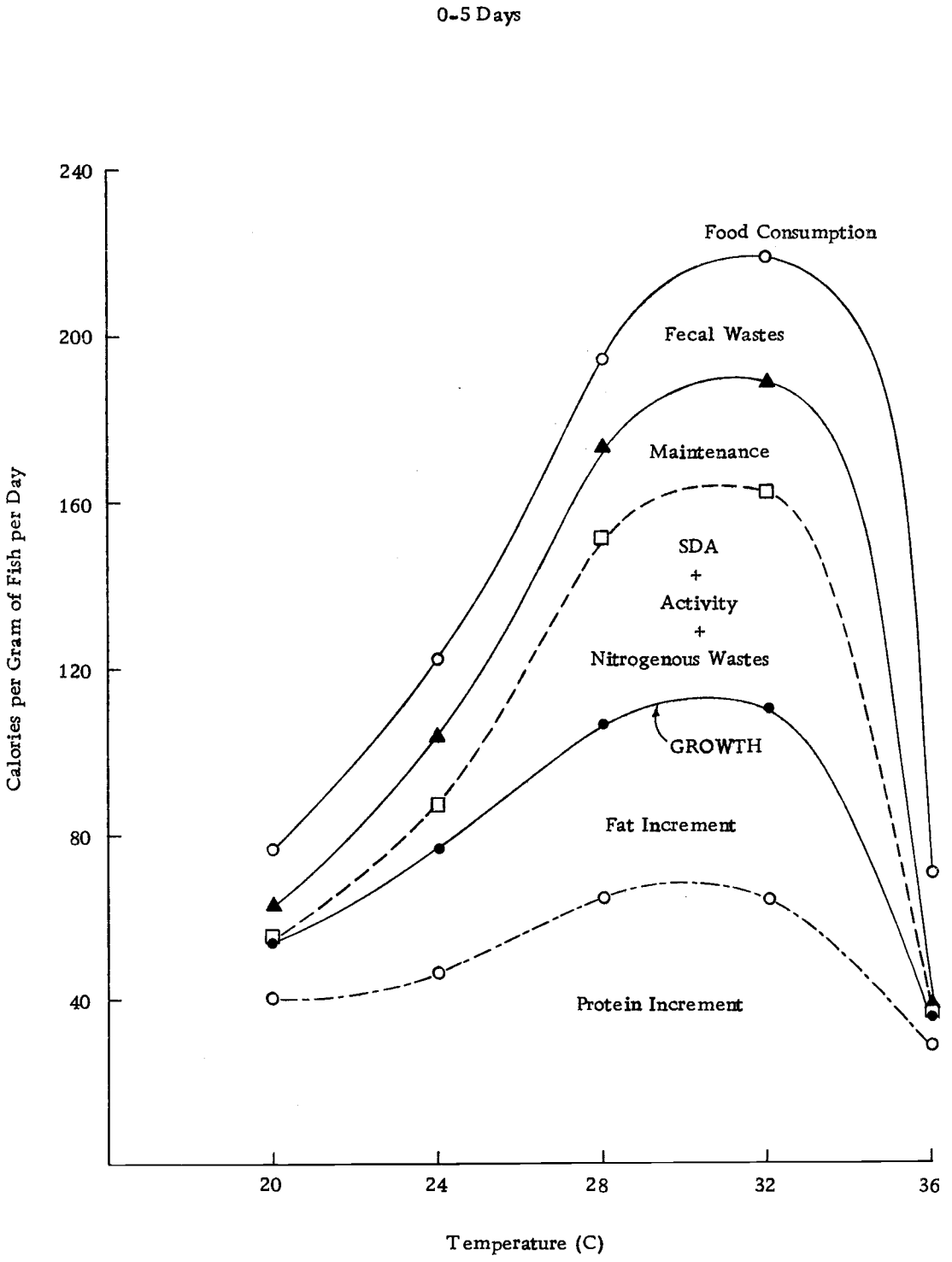

Figure 12. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of five days and data are shown as calories per gram of fish per day.

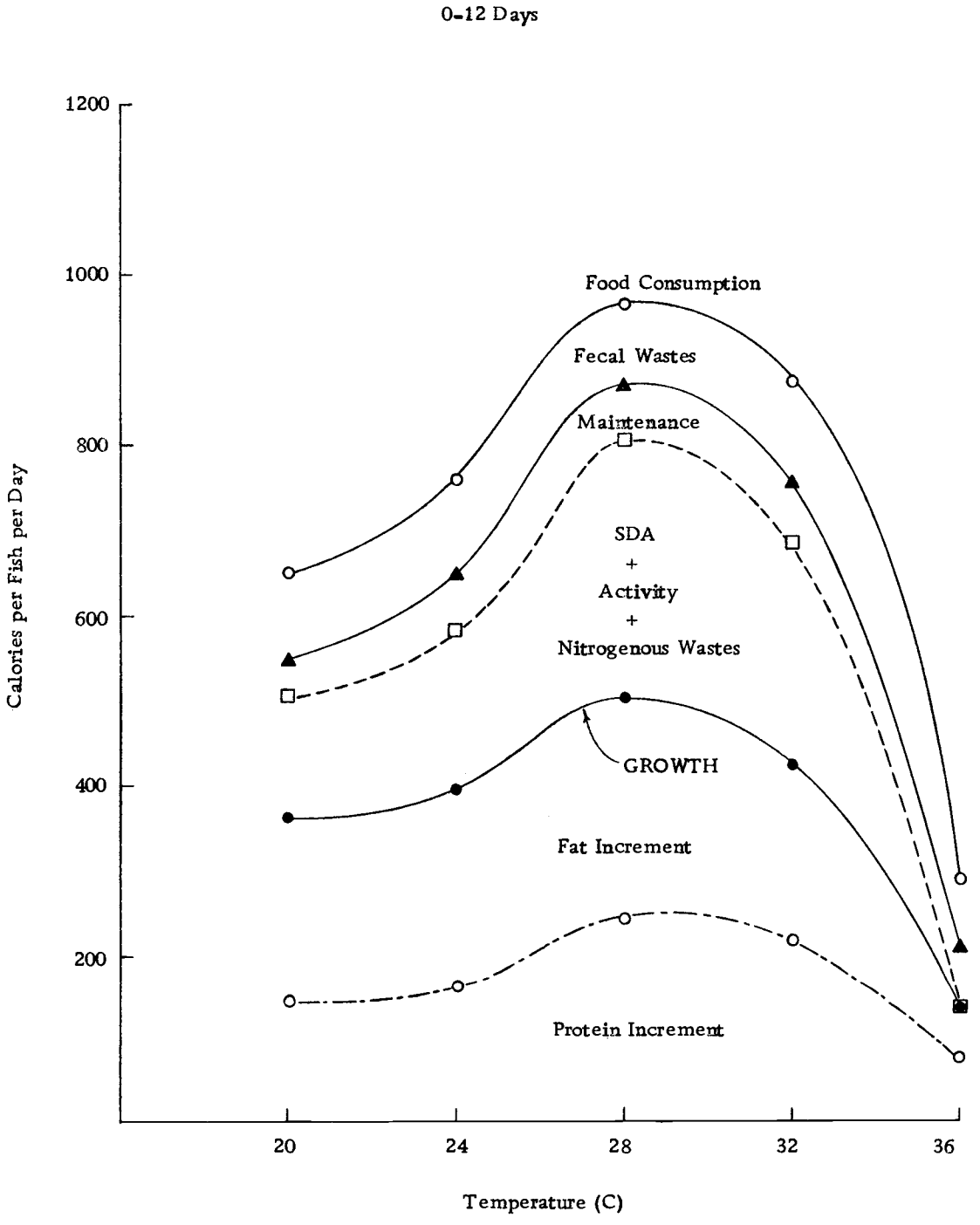

Figure 13. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of 12 days and data are shown as calories per fish per day.

and 14). But their effectiveness of food utilization for growth can be seen to have been better at still lower temperatures, for here losses of energy and materials were much less.

The evidence that the fish might be getting along best at temperatures lower than 28 C is confirmed when the energy budgets for the entire 19 days of the experiment are considered (Figures 15 and 16). Then we see that maximum food consumption and growth occurred at 24 C. And though food consumption was considerably higher at 28 C than at 20 C, growth was little better. At 20 C, the fish were much more effective in utilizing for growth the food they consumed than were fish at any of the higher temperatures. The energy budgets permit us to see some of the important reasons for this. Maintenance and other losses and uses of energy and materials were reduced at the lowest temperature. Had rations been restricted rather than unlimited, there is every reason to believe that maximum growth would have occurred at a temperature lower than 24 C. Averett (1969) showed that restricting the ration of juvenile coho salmon (Oncorhynchus kisutch) reduced the optimal temperature for growth.

Examination of the energy budgets of the cichlids during the last two periods of the experiment throws some light on the periodic shifts in energy and material utilization that lead to the downward shift in optimal temperature as the experiment progressed through

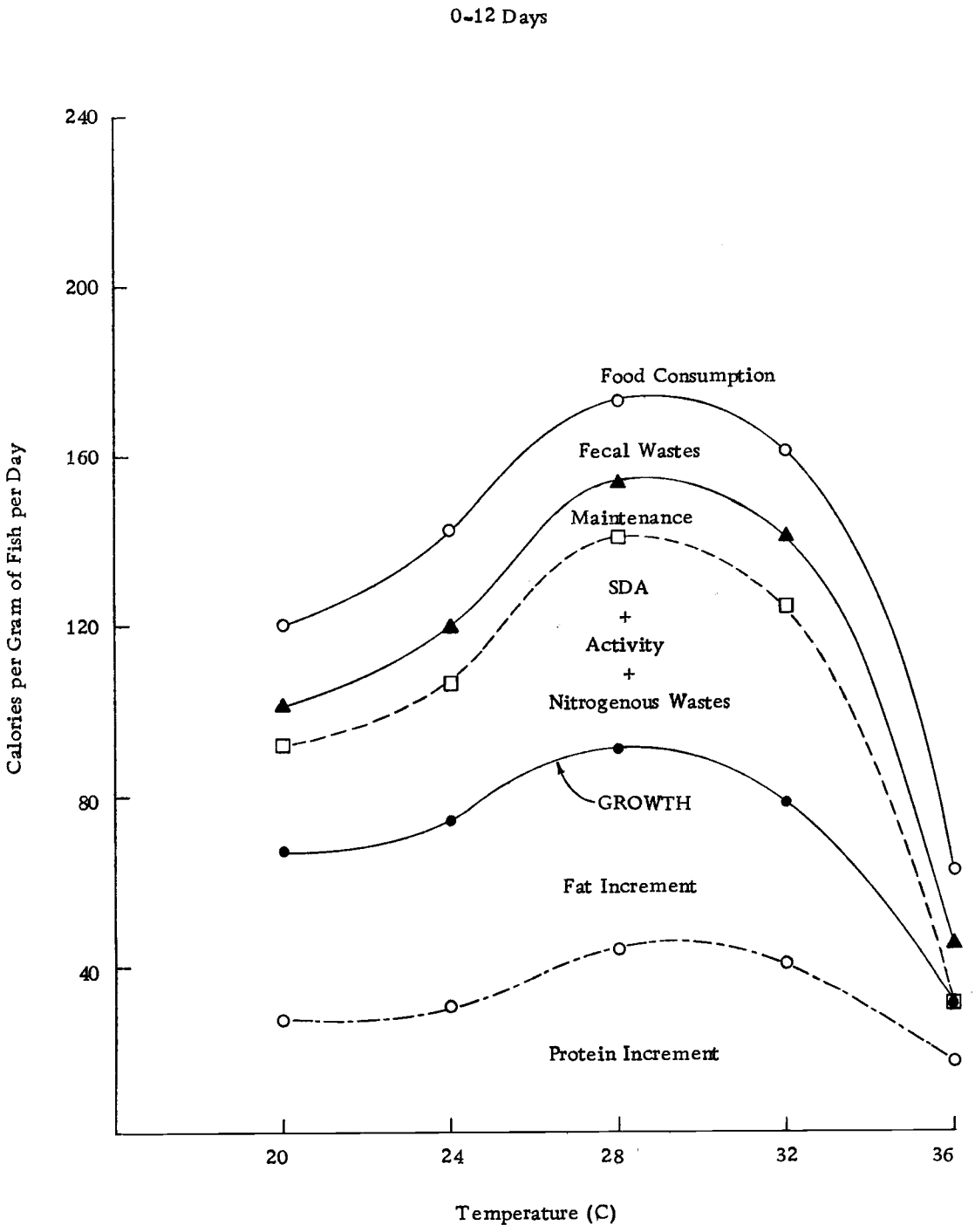

Figure 14. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of 12 days and data are shown as calories per gram of fish per day.

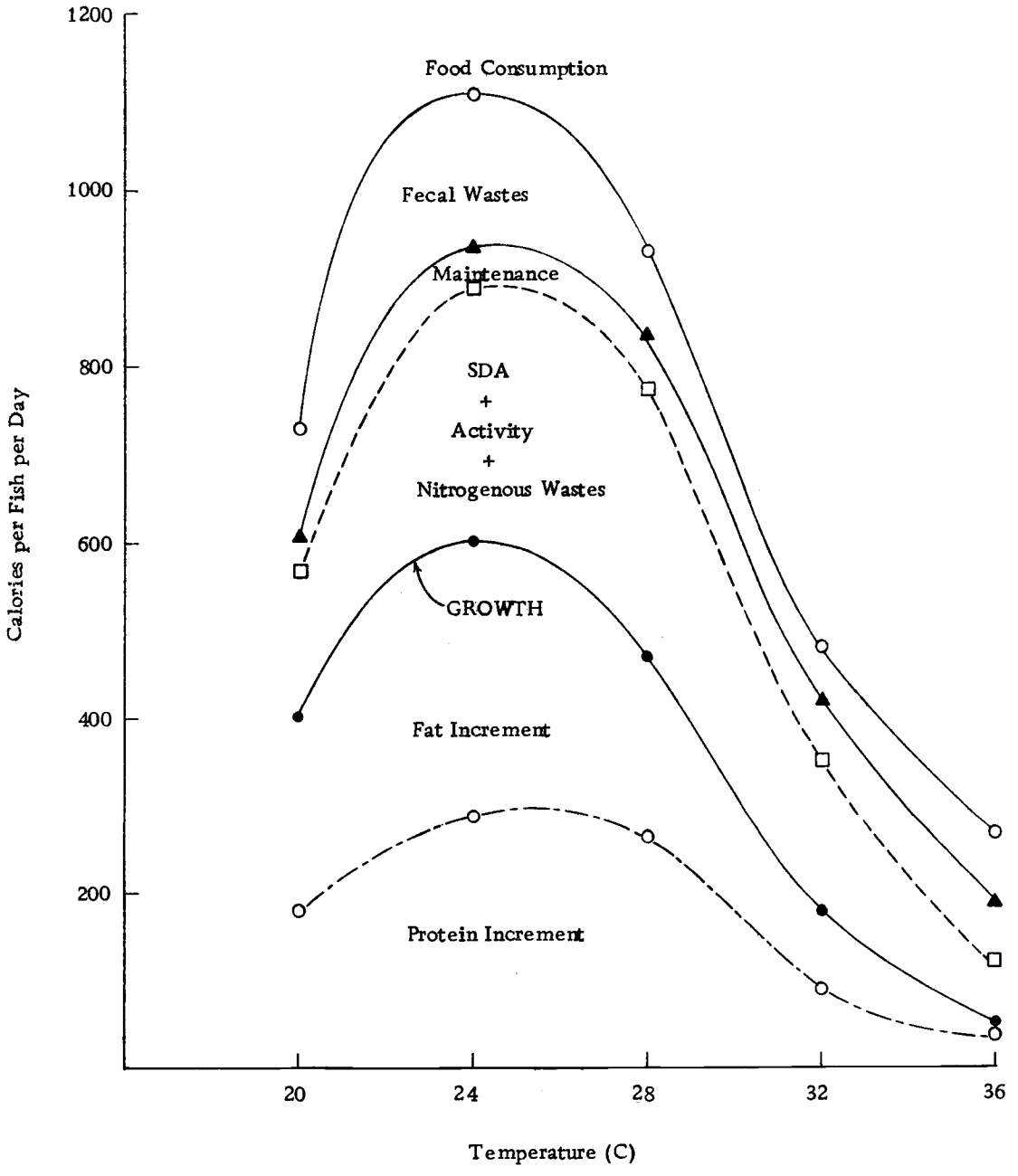

Figure 15. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of 19 days and data are shown as calories per fish per day.

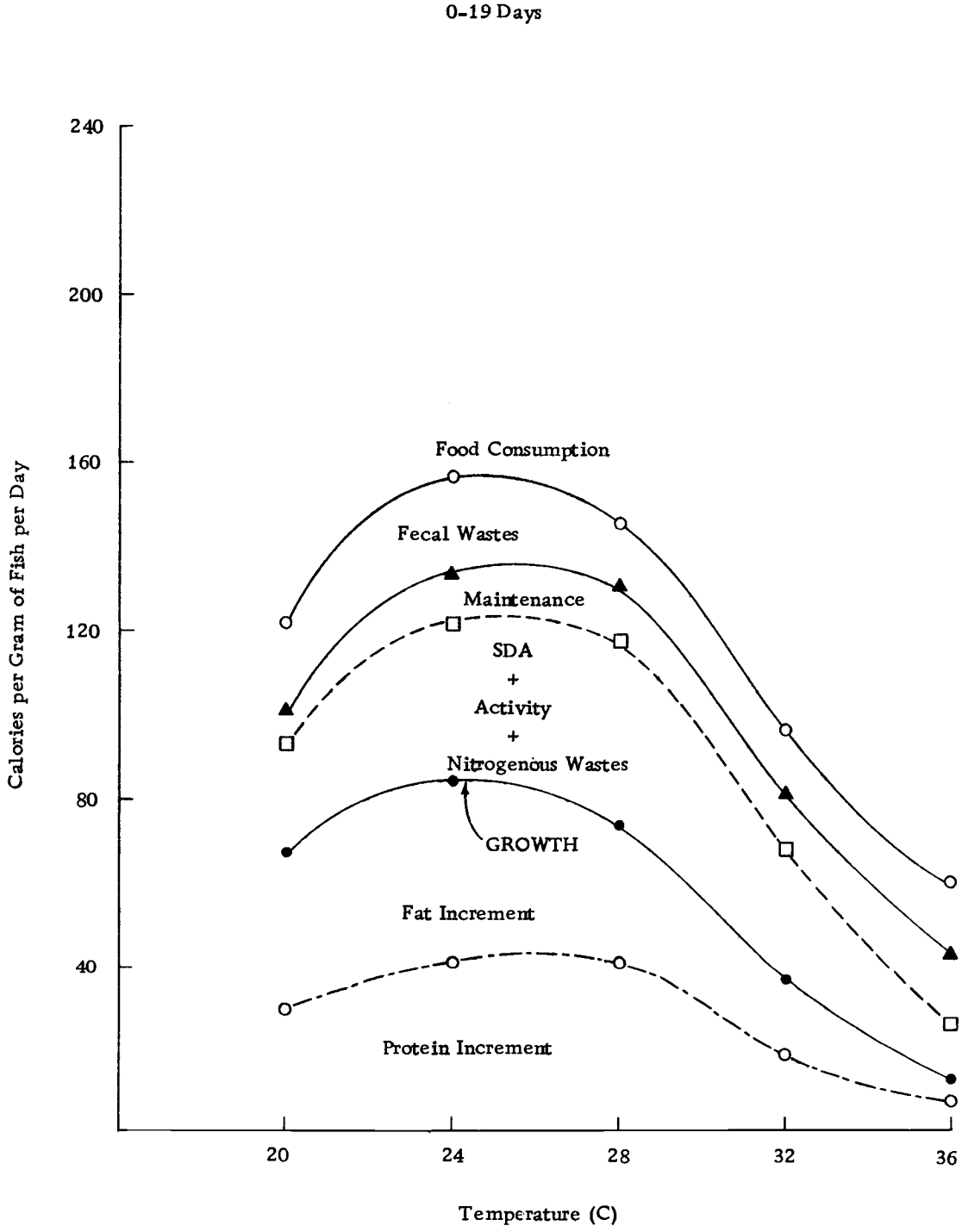

Figure 16. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum for a period of 19 days and data are shown as calories per gram of fish per day.

its entire 19 days. From the 6th to the 12th day of the experiment, food consumption and growth were higher at temperatures lower than 28 C than at higher temperatures (Figures 17 and 18). Growth at 28 C was little higher than growth at 20 C. In rate terms, growth was the same and food consumption was nearly the same at 20 and 28 C (Figure 18).

This was true also during the last period of the experiment, 13 to 19 days (Figures 19 and 20). But in this period, the fish consumed much more food at 24 C; and, since this food was used reasonably effectively for growth, growth was clearly best at this temperature. After passing through various periods of adjustment, the fish apparently found 24 C to be very much the optimum for growth.

The concept of food intake requires positive values and the curve of food intake is so plotted in Figures 19 and 20. However, in the subtraction of the data for the 12-day period from the data for 19-day period to give the data for the period from the 13th to 19th days, negative values of food intake were obtained. On the basis of the number of times the cichlids were fed over 12 days and over 19 days, it was decided that 8/9 of the food intake of the 19 day period occurred during the first 12 days and 1/9 between the 13th and 19th days. Hence food intake for Figures 19 and 20 was taken as one ninth of the food intake over the 19 day period.

Cichlasoma bimaculatum is a tropical fish. Its lower lethal

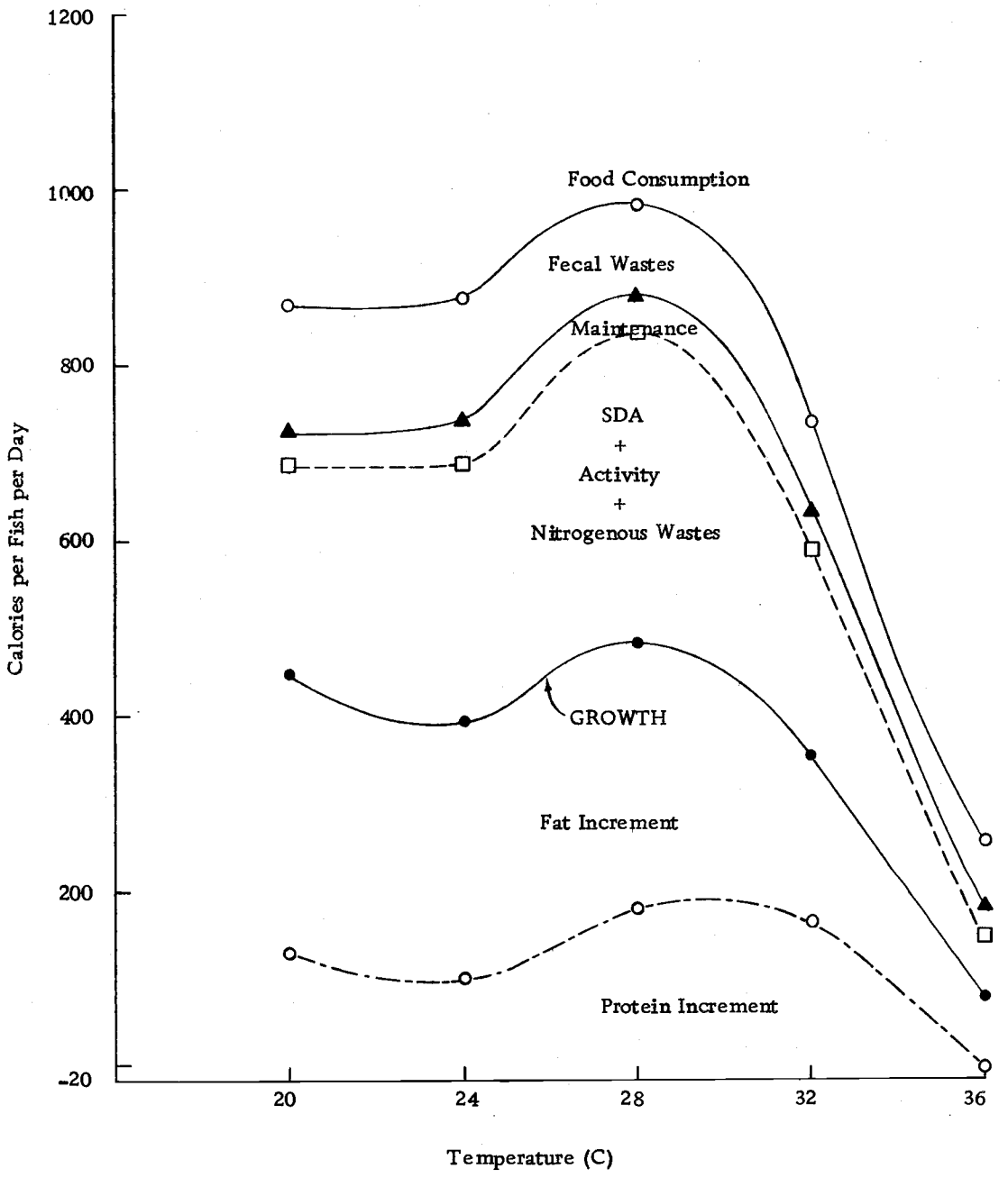

Figure 17. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum over the 6th to 12th days and data are shown as calories per fish per day.

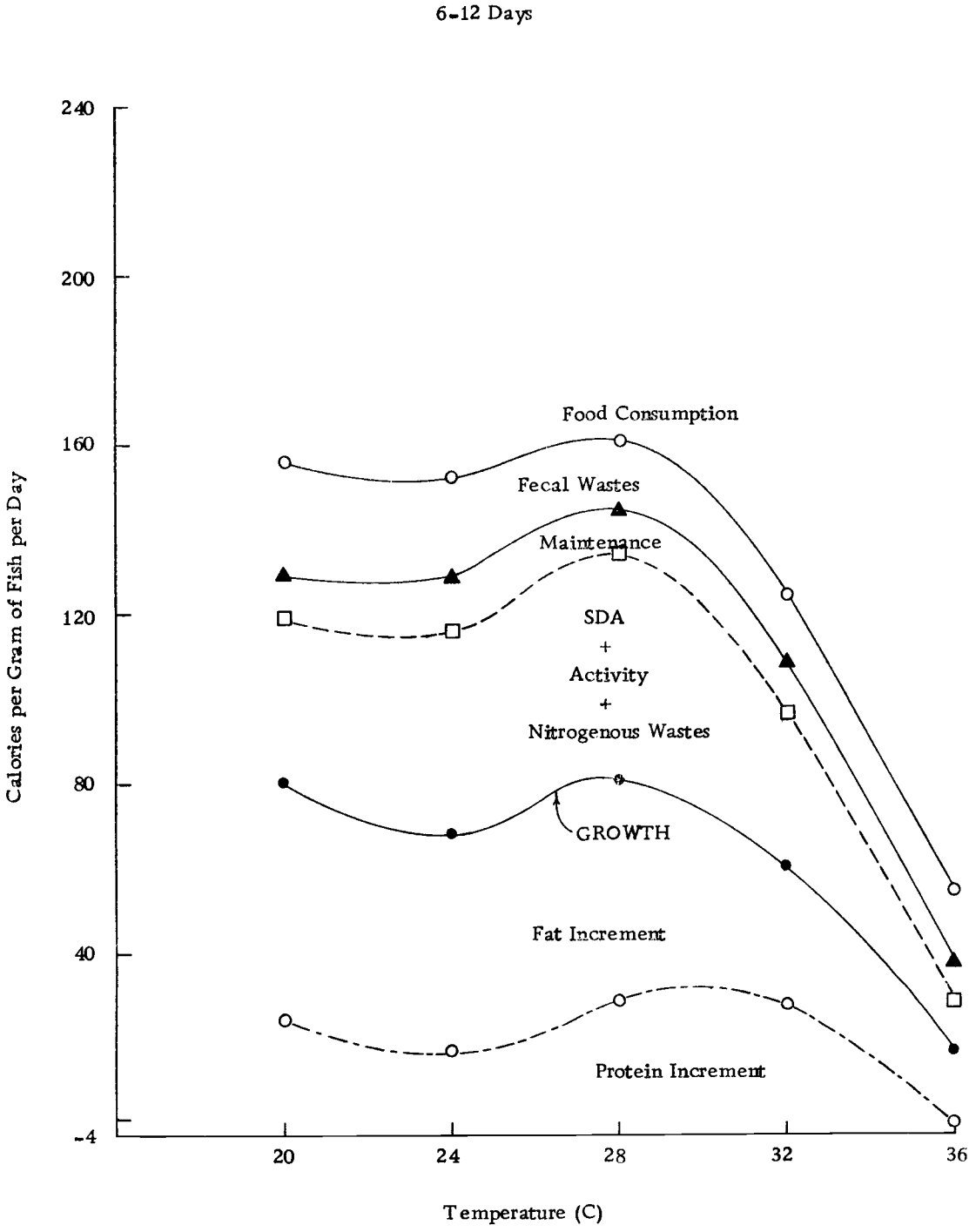

Figure 18. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum over the 6th to 12th days and data are shown as calories per gram of fish per day.

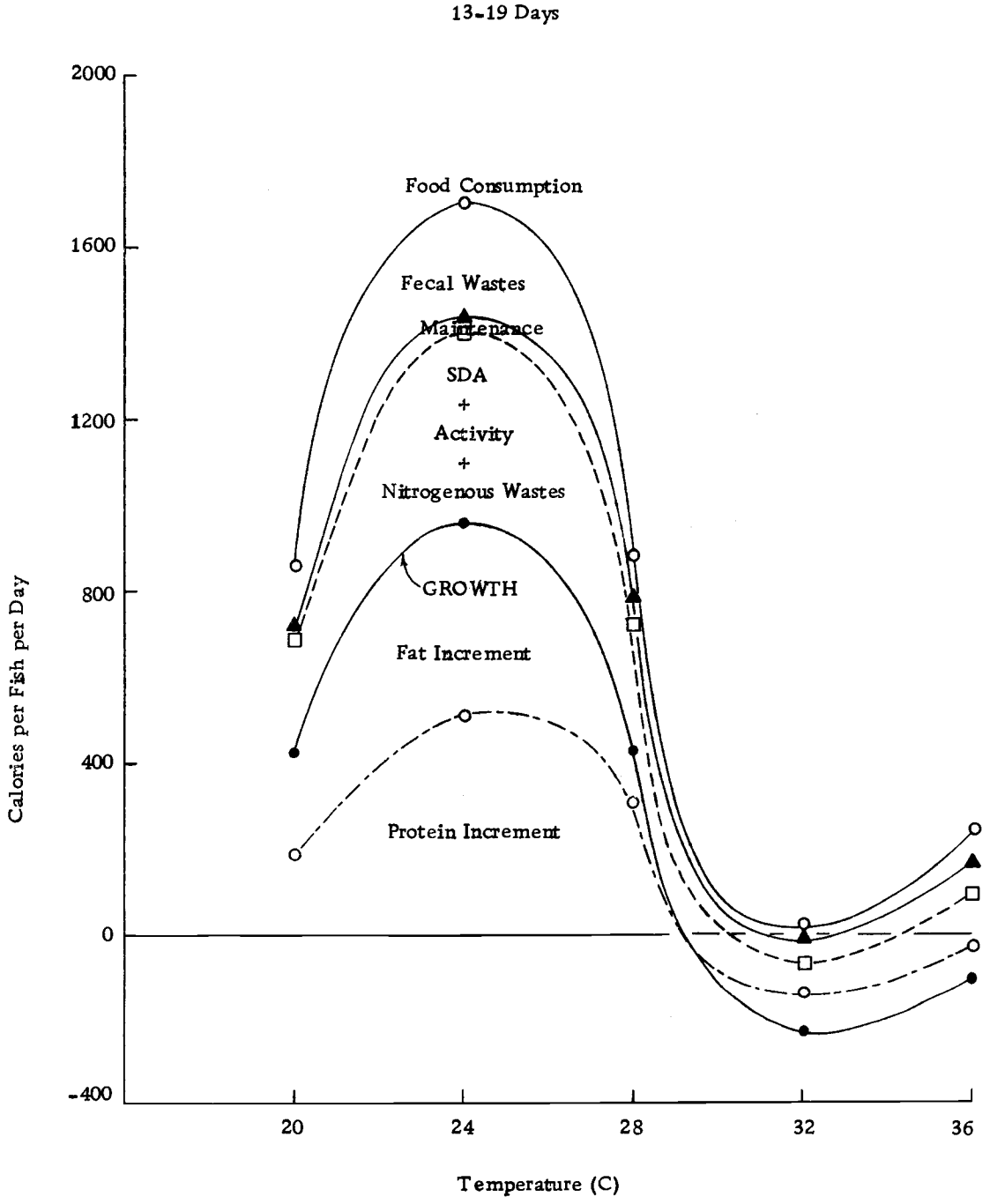

Figure 19. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum over the 13th to 19th days and data are shown as calories per fish per day.

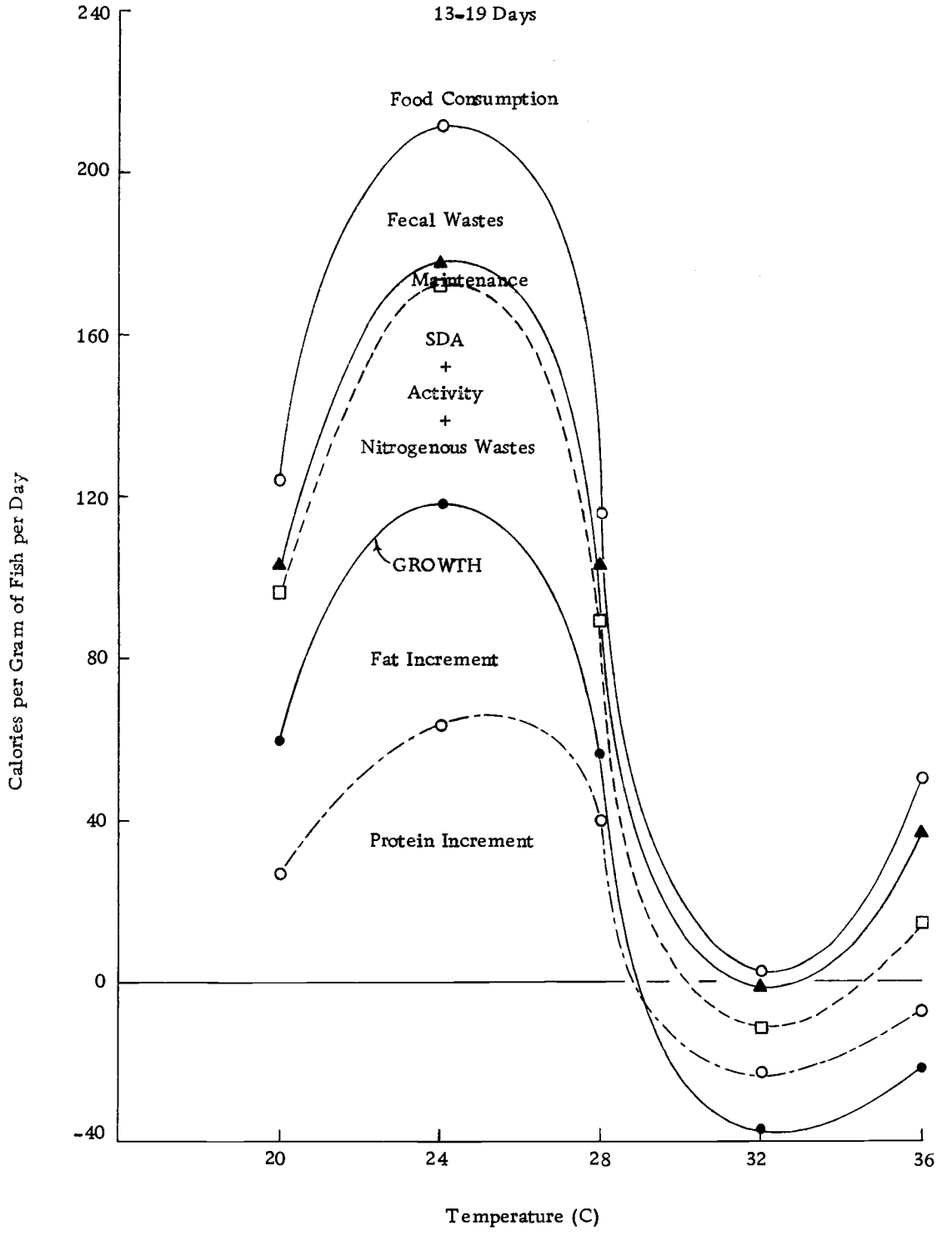

Figure 20. Budget or scope diagram for cichlids. Influence of temperature on the food intake, growth, excretory losses and uses of energy of food intake. Cichlasoma bimaculatum was fed ad libitum over the 13th to 19th days and data are shown as calories per gram of fish per day.

temperature is not much below 20 C. It is hard to believe that it would thrive in nature at temperatures near this. The temperature here found to be optimum for growth over a 19 day period, the temperature of 24 C, may be a reasonable estimate of the temperature optimal for this animal in nature.

BIBLIOGRAPHY

Allanson, B. R. and R. G. Noble. 1964. The tolerance of Tilapia mossambica (Peters) to high temperature. Transactions of the American Fisheries Society 93: 323-332.

Averett, R. C. 1969. Influence of temperature on energy and material utilization by juvenile coho salmon, Oncorhynchus kisutch. Ph. D. thesis. Corvallis, Oregon State University. 74 numb. leaves.

Basu, S. P. 1959. Active respiration of fish in relation to ambient concentration of oxygen and carbon dioxide. Journal of the Fisheries Research Board of Canada 16: 175-212.

Beamish, F. W. H. 1964. Influence of starvation on standard and routine oxygen consumption. Transactions of the American Fisheries Society 93: 103-107.

Beamish, F. W. H. and P. S. Mookherjii. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, Carassius auratus L. Canadian Journal of Zoology 42: 161-175.

Benedict, F. G. and T. M. Carpenter. 1918. Food ingestion and energy transformations, with special reference to the stimulating effect of nutrients. Washington, D. C. 335p. (Carnegie Institute of Washington. Publication no. 261)

Borsook, H. and H. M. Winegarden. 1931. The energy cost of the excretion of urine. Proceedings of the National Academy of Science 17: 3-12.

Brett, J. R. 1944. Some lethal temperature relations of Algonquin Park fishes. Toronto, 49p. (University of Toronto Studies. Biological Series no. 52. Publications of the Ontario Fisheries Research Laboratory no. 63)

Brett, J. R. 1956. Some principles in the thermal requirements of fishes. Quarterly Review of Biology 31(2): 75-87.

Brett, J. R. 1958. Implications and assessments of environmental stress. In: The investigation of fish-power problems, ed. by

P. A. Larkin. Vancouver, University of British Columbia. p. 69-83.

Brett, J. R. 1962. Some considerations in the study of respiratory metabolism in fish, particularly salmon. Journal of the Fisheries Research Board of Canada 19(6): 1025-1038.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21: 1183-1226.

Brody, S. 1945. Bioenergetics and growth with special reference to the efficiency complex in domestic animals. New York, Reinhold. 1023p.

Bullock, T. H. 1955. Compensations for temperature in the metabolism and activity of poikilotherms. Biological Review 30: 311-342.

Chang, V. M. and D. R. Idler. 1960. Biochemical studies on sockeye salmon during spawning migration. Canadian Journal of Biochemistry and Physiology 38: 553-558.

Davis, G. E. and C. E. Warren. 1965. Trophic relations of a sculpin in laboratory stream communities. Journal of Wildlife Management 29: 846-871.

Dorland's illustrated medical dictionary. 1966. 24th ed. Philadelphia, Saunders. 1724p.

Duodoroff, P. 1942. The resistance and acclimation of marine fishes to temperature changes. I. Experiments with Girella nigricans (Ayres). Biological Bulletin 83: 219-244.

Fry, F. E. J. 1947. Effects of the environment on animal activity. Toronto. 62p. (University of Toronto Studies. Biological Series no. 55. Publications of the Ontario Fisheries Research Laboratory no. 68)

Fry, F. E. J. 1957. The aquatic respiration of fish. In: The physiology of fishes, ed. by M. E. Brown. Vol. 1. p. 1-63.

Gerking, S. D. 1955. Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. Physiological Zoology 28: 267-282.

Graham, J. M. 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, Salvelinus fontinalis. Canadian Journal of Research, sec. D, 27:270-288.

Higginbotham, A. C. 1947. Notes on the oxygen consumption and activity of catfish. Ecology 28: 462-464.

Janssens, P. A. 1964. The metabolism of aestivating African lungfish. Comparative Biochemistry and Physiology 11: 105-117.

Kleiber, M. 1961. The fire of life. New York, Wiley. 454p.

Krebs, H. A. 1964. The metabolic fate of amino acids. In: Mammalian protein metabolism, ed. by H. N. Munro and J. B. Allison. Vol. 1. New York, Academic. p. 125-176.

Krogh, A. 1914. The quantitative relation between temperature and standard metabolism in animals. Internationale Zeitschrift fur Physikalischemische Biologie 1: 491-508.

Lehninger, A. H. 1965. Bioenergetics. New York, Benjamin. 258p.

Lovern, J. A. 1940. Fat metabolism in fishes. 14. The utilization of ethyl esters of fatty acids by the eel and their effect on depot fat consumption. Biochemical Journal 34: 704-708.

Lusk, G. 1931. The specific dynamic action. Editor's review. Journal of Nutrition 3: 519-530.

Phillips, A. M., Jr. and D. R. Brockway. 1954. Effect of starvation, water temperature and sodium amytal on the metabolic rate of brook trout. Progressive Fish Culturist 16: 65-68.

Pike, R. L. and M. L. Brown. 1967. Nutrition: An integrated approach. New York, Wiley. 542p.

Precht, H. et al. 1955. Temperatur und Leben. Berlin, Springer. 514p.

Prosser, C. L. et al. 1957. Acclimation of goldfish to low concentrations of oxygen. Physiological Zoology 30: 137-141.

Prosser, C. L. 1962. Acclimation of poikilothermal vertebrates to low temperatures. In: Comparative physiology of temperature regulation, ed. by J. P. Hannon and E. Viereck. Fort Wainwright, Alaska, Artic Aeromedical Laboratory. p. 1-44.

Prosser, C. L. and F. A. Brown. 1961. Comparative animal physiology. Philadelphia, Saunders. 688p.

Roberts, J. L. 1967. Metabolic compensations for temperature in sunfish. In: Molecular mechanisms of temperature adaptations; a symposium ed. by C. Ladd Prosser. Washington, D. C., American Association for the Advancement of Science. p. 245-262.

Rubner, M. 1902. Die Gesetze des Energieverbrauchs bei der Ernährung. Leipzig, Deutiche.

Smith, H. W. 1935a. The metabolism of lungfish. I. General considerations of the fasting metabolism of the active fish. Journal of Cellular and Comparative Physiology 6: 43-67.

Smith, H. W. 1935b. The metabolism of the lungfish. II. Effect of feeding meat on the metabolic rate. Journal of Cellular and Comparative Physiology 6: 335-349.

Spaas, J. T. 1959. Contribution to the biology of some cultivated Cichlidae: temperature, acclimation, lethal limits and resistance in three Cichlidae. Biologische Jahrbuch. Dononaea 27: 21-38.

Spencer, W. P. 1939. Diurnal activity rhythms in freshwater fishes. Ohio Journal of Science 39: 119-132.

Spoor, W. A. 1946. A quantitative study of the relationship between the activity and oxygen consumption of goldfish, and its application to the measurement of respiratory metabolism in fishes. Biological Bulletin 91: 312-325.

Stimpson, J. H. 1965. Comparative aspects of the control of glycogen utilization in vertebrate liver. Comparative Biochemistry and Physiology 15: 187-197.

Stroganov, N. S. 1956. Physiological adaptability of fish to the temperature of the surrounding medium. Jerusalem, Israel Program for Scientific Translations, 1962. 108p.

Terroine, E. F. and R. Bonnet. 1926. Le mechanisme de l'action dynamique specifique. Annales de Physiologie et de Physico-chimie Biologique 2:488-508.

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, ed. by S. D. Gerking. Oxford, England, Blackwell. p. 175-214.

Wilhelmj, C. M., J. L. Bollman and G. C. Mann. 1928. Studies on the physiology of the liver. XVII. The effect of the removal of the liver on the specific dynamic action of amino acids administered intravenously. American Journal of Physiology 87:497-509.

Winberg, G. G. 1956. Role of metabolism and food requirements of fishes. Montreal. 239p. (Fisheries Research Board of Canada. Translation Series no. 194. Translated from Nauchnye Trudy Belorusskova Gosudarstvenno Universiteta Imeni. Vol. I. 253p.)

Wohlschlag, D. E. 1960. Metabolism of an Antarctic fish and the phenomenon of cold adaptation. Ecology 41:287-292.

APPENDIX

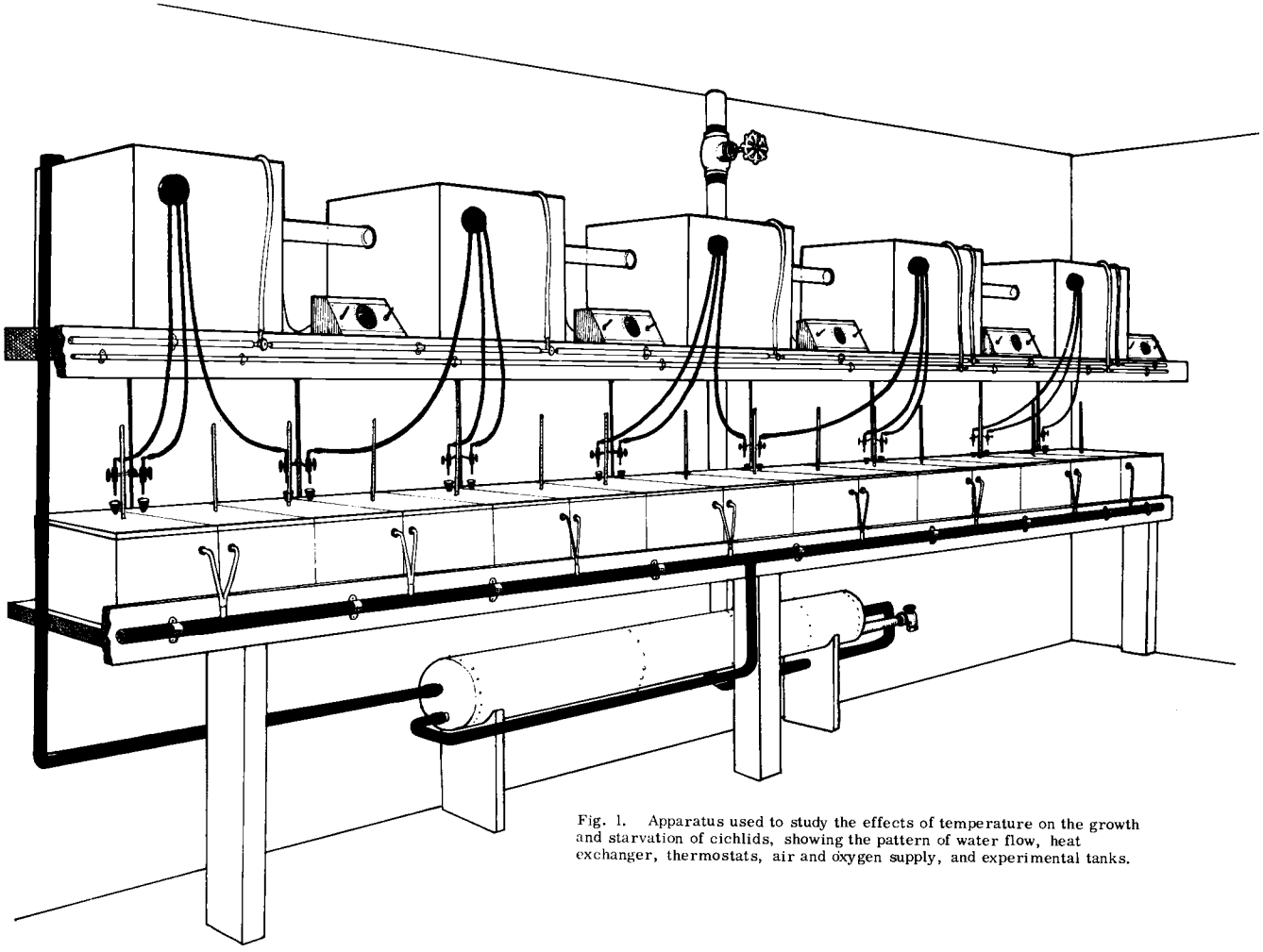

Fig. 1. Apparatus used to study the effects of temperature on the growth and starvation of cichlids, showing the pattern of water flow, heat exchanger, thermostats, air and oxygen supply, and experimental tanks.

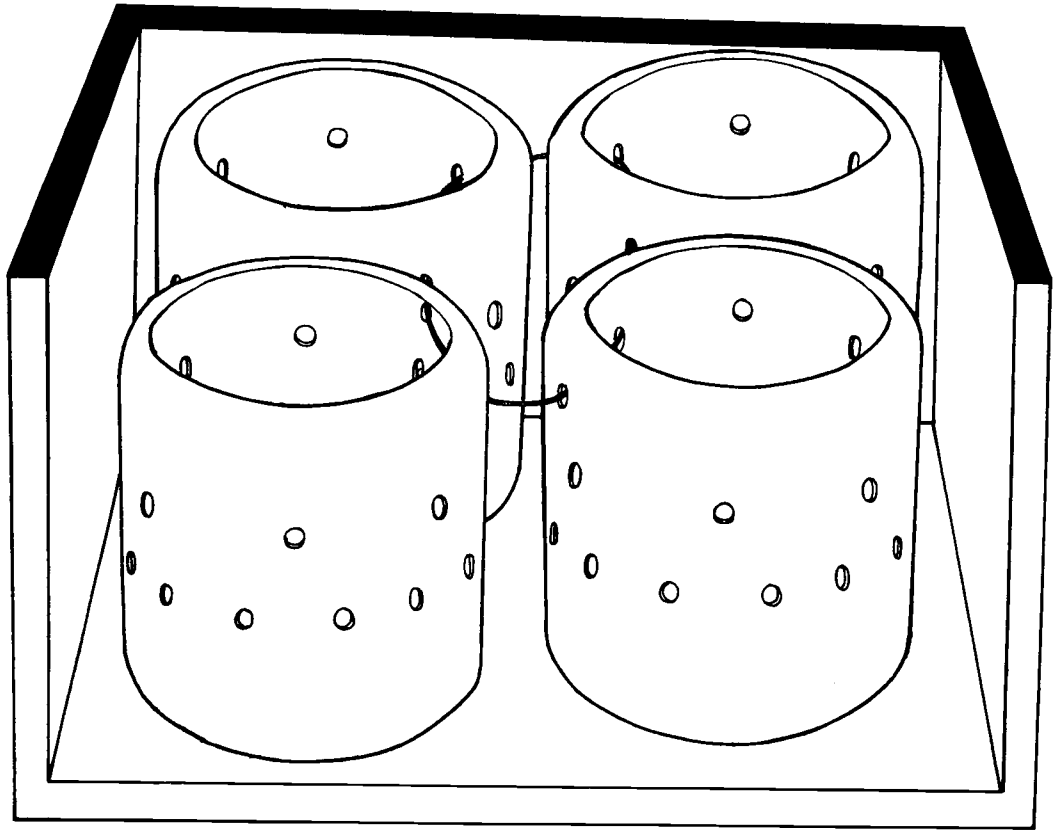

Fig. 2. Plastic jars with perforated walls used to hold four cichlids separately in each experimental tank for feeding and growth experiment.

Respirometer

The respirometer (see numerals on the diagram in Figure 3), was designed as a water tunnel (152 mm long X 51 mm inside diameter) connected with a 124 X 94 X 147 mm plexiglass chamber on one end and with a 124 X 51 X 147 mm chamber on the other. A removable stainless steel screen (2) was placed at each end of the tunnel. Terminals (3) to electrify the downstream screen were inserted through the tunnel and the rubber stopper (15). The tunnel was fitted with a thermometer (7) to measure the temperature of water circulating from the large to the small chamber. A forward cover area (4) of the tunnel, darkened by a wide ring of black plastic was provided and was frequently used by the fish.

Each chamber was provided with an entrance at the top which could be sealed with a rubber stopper (6). The wide access port (5) of the large chamber was used for loading and unloading the tunnel with fish. The inlet (13) for fresh supply of water into the large chamber and the outlet (12) to flush the system from the small chamber could be opened or shut off as the necessity demanded. Water was recirculated through the respirometer system (1) by a centrifugal pump (8) and the velocity could be regulated by a gate valve (10) and was read from the position of a float in the flow meter tube (9) connected to the large chamber through Tygon tubing (16).

A part of the recirculating water could be temporarily diverted from the main stream (Tygon tubing 11 connecting the small chamber to the pump) to pass through two sample bottles (14). Total volume of the recirculating water was 3.5 liters.

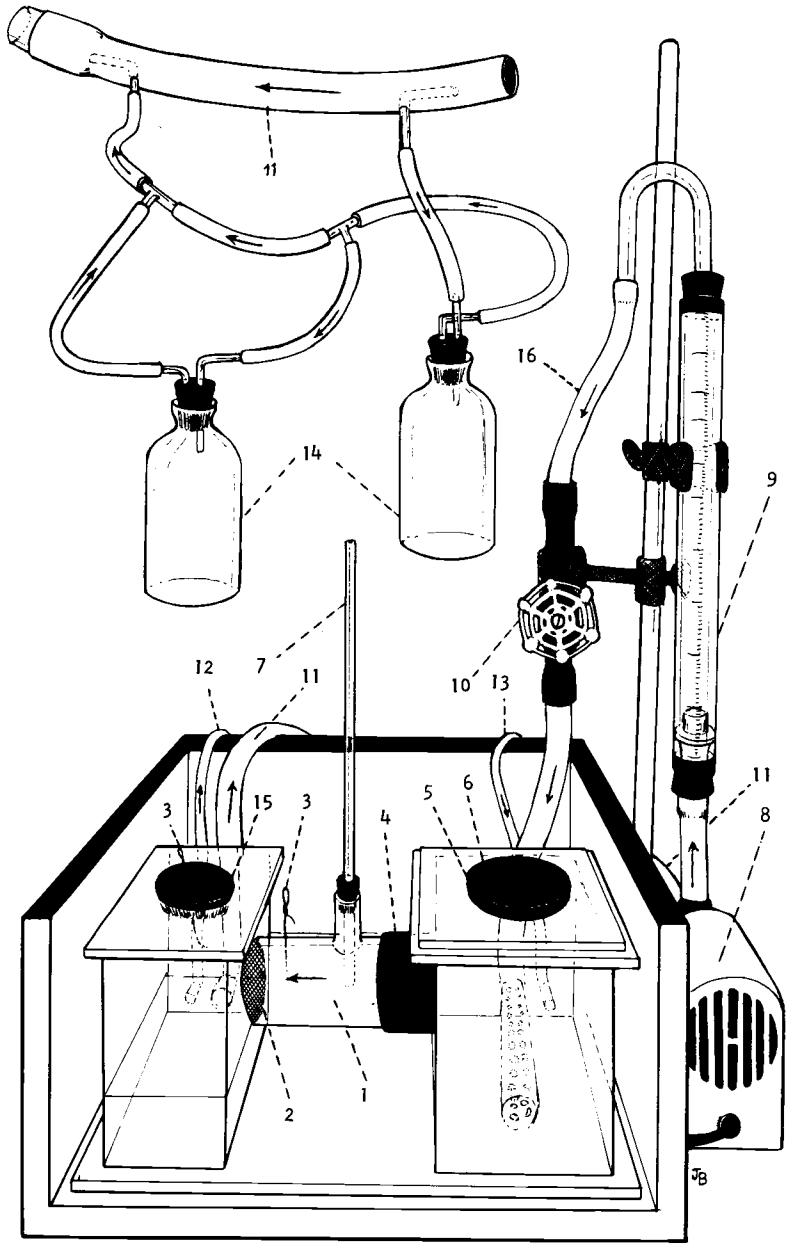

Fig. 3. Tunnel respirometer used for determining the oxygen consumption of unfed and fed fish.