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A MODEL TO FACILITATE OPTIMAL AQUACULTURE PRODUCTION
BY QUANTITATIVELY RELATING FISH GROWTH TO FEED
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A MODEL TO FACILITATE OPTIMAL AQUACULTURE PRODUCTION BY QUANTITATIVELY RELATING FISH GROWTH TO FEED AND OTHER ENVIRONMENTAL RESOURCES

By

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ABSTRACT

A MODEL TO FACILITATE OPTIMAL AQUACULTURE PRODUCTION BY QUANTITATIVELY RELATING FISH GROWTH TO FEED AND OTHER ENVIRONMENTAL RESOURCES

By

Charles S. Annett

Aquaculture, the propagation and rearing of an aquatic organism, entails the provision of all requirements for growth. The long-term process of natural selection has resulted in each type of organism having specific environmental requirements. As a result, optimal production requires the ability to relate the limits of growth of the organism to the availability of particular environmental variables in a quantitative manner. The intent of this research is to determine the usefulness of a model which simplifies the complex biochemical, biophysical elements of growth, yet also provides the ability to quantitatively relate growth to environmental variables.

A review of existing models of growth revealed that the "logistic" curve was important for initiating a consideration of rate of growth, and the "enzyme-kinetic" model important for relating growth rate to substrate concentration. These concepts, combined with a consideration of the metabolic expenditures of the organism, resulted in the formulation of a threshold-corrected hyperbolic equation to

model growth. The equation provides a means to predict the specific growth rate and conversion efficiency of an organism corresponding to specific feeding levels and the feeding level required to maintain the organism at zero growth. Furthermore, the quantitative nature of the model permits comparisons of growth responses obtained under varying environmental conditions.

The threshold-corrected hyperbolic equation was demonstrated to be useful in relating the specific growth rate of *Tilapia zillii* to three different feeds. Differences in growth were attributed to the energy and protein content of the feeds. Additional experiments were performed which revealed the usefulness of the model to relate growth to fish size and water temperature. Comparison of specific growth rates of *T. zillii* and *Sarotherodon niloticus* on identical feeds revealed that the equation provided data for the comparison of different species and their abilities to harvest, digest and assimilate feeds.

The threshold-corrected hyperbolic equation has potential application for the following: 1) identify the dietary requirements of organisms, 2) quantify the effect of environmental variables on growth, 3) quantify the ability of the organism to harvest-digest-assimilate feed, 4) provide data to determine stocking levels and harvest date, and 5) provide a basis for cost benefit considerations.

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INTRODUCTION

Aquaculture may be defined as the propagation and rearing of an aquatic organism in either an artificial or natural environment. The goal of aquaculture is the cost effective production of an organism. Cost effectiveness may be expressed in either financial terms as monetary reward, or in human terms as the production of an edible product from poorer quality resources. All aquaculture programs share the common purpose of attempting to grow an organism by creating an environment conducive to its most efficient propagation and growth. The long-term process of natural selection has resulted in each type of organism having specific environmental requirements. The unique assemblage of structural, behavioral and biochemical processes characteristic of each organism enables it to achieve maximum efficiency under a particular set of environmental constraints. Alterations in one or more of the environmental constraints results in decreased efficiency and/or yield. The development of an effective aquaculture program requires the ability to relate the limits of growth of the organism in question to the availability of particular environmental resources in a quantitative manner.

The first criterion for the growth of an organism is a continued energy supply. In addition, the individual elements required in the anabolic process must be provided. Unless a sufficient supply of energy and building components are supplied to the cellular reaction sites, growth cannot occur. The effective promotion of growth requires the identification and quantification of the types of food which efficiently stimulate growth. Unfortunately, the determination of food requirements for an organism is complicated by the numerous environmental factors which influence the metabolic processes in the organism.

Brett (1979) reported on the complex, interactive influence of numerous environmental factors on growth. Some measure of simplification was achieved by classifying biotic and abiotic factors into categories based on how they affected the growth of the organism. The four categories cited by Fry (1971) and Brett (1979) which influenced growth were: 1) controlling factors, such as temperature, which govern reaction rates; 2) limiting factors which restrict reaction rates, such as ration level; 3) masking factors which modify reactions or processes, for example, salinity; and 4) directive factors which cue, or signal particular responses, such as photoperiod.

Notwithstanding these simplifications, the growth of an organism is understood to be a complex process. First the organism must be able to successfully harvest, digest and transport all the resources required for growth to the internal reaction sites. Simultaneously, these physiological processes must occur within an environment where all the factors which influence these growth processes are within a functional range.

The task of developing a procedure to quantitatively relate growth to both food and environmental factors appears formidable. For this reason there have been numerous attempts to simplify the complexity by correlating growth with individual factors. These correlations have been investigated from both a limnological and fisheries standpoint and have included both biotic and abiotic parameters.

Numerous authors have attempted to relate fish growth or yield to limnological factors. Hayes and Anthony (1964) correlated fish production capacity with the parameters of lake area, depth, and alkalinity. In examining 150 North American lakes, the authors reported that these parameters accounted for 67% of the variability in fish productivity. Ryder (1982) employed the Morphoedaphic Index (MEI) as a means of relating fish growth to environmental parameters. Ryder's original MEI is obtained for any lake by dividing the concentration of total dissolved solids by the mean depth. Thus, the index includes some measure of the chemical and physical character of a lake. The MEI was proposed as a "reasonable compromise between unmanageable complexity and ecological oversimplification" (Ryder 1982).

While these correlations may provide a first approximation for total fish yield, they are too simplistic to provide species-specific predictions or to relate production to potentially manageable resources in the environment. Because they fail to relate growth to specific dietary or environmental requirements, these correlations provide limited insight for aquaculture management. To a large degree, these attempts to utilize abiotic parameters to predict fish production are actually an indirect means of relating the primary production capacity of the water to fish production. The parameters of area, depth, nutrient concentration, etc. have limited direct impact on fish but greatly influence the levels of primary production which ultimately represent the feed supply level for the fish.

Other attempts have been made to relate fish yield to biotic factors, usually primary production. Melack (1976) related the fish yield in tropical lakes to the levels of net phytoplankton productivity. Fish yields were reported to increase logarithmically in response to arithmetic increases in primary production. This correlation was demonstrated to provide predictive ability superior to that of the Morphoedaphic Index. However, the author expressed concern over both the reliability of the primary production data and applicability to more diverse lakes. Liang et al. (1981), using a variety of curve fitting techniques, reported that the relationship between fish yield and gross photosynthesis in several Chinese lakes could be described by arithmetic, semilog or log-log regression equations depending on the environmental conditions, diversity of harvested fish and fishing method. The authors suggested that, in general, a logistic curve may describe the full range of the fish yield/gross primary productivity relationship. Edwards et al. (1981) reported that despite constant changes in the phytoplankton community, the growth of *Tilapia nilotica* (also referred to as *Sarotherodon niloticus*) was linearly related to the mean phytoplankton concentrations in fish ponds. Similar investigation by Reich (1978) indicated a direct correlation between the harvest of *Sarotherodon galilaeus* and the total biomass of algae present from the time of fish hatch until harvest.

Because the yield of fish has been correlated to primary production, there have been numerous studies investigating the effects of fertilization on fish yield. Seymour (1980) stated that the "production of fish ponds may be increased by fertilization which encourages growth of phytoplankton and in turn the amount of food available to the fish." Swingle and Smith (1939) reported that the addition of inorganic fertilizer greatly increased fish production in ponds. The findings of Dendy et al. (1968) indicated that production of tilapia increased from 63 to 199% following inorganic fertilization. Schroeder (1974) similarly reported that the harvest of carp and tilapia increased 25 to 100% in ponds fertilized with fluid cowshed manure. The increased fish productivity was attributed to increases in primary production ranging from 10-1000 fold. In consequent research, Schroeder (1978) reported that increases in fish yield occur as a result of increases in both primary production and heterotrophic production of microorganisms. Following addition to the fish pond, the manure is quickly colonized by a microbial community which digests the organic content. These colonizing bacteria and protozoa are ingested by the fish and become an important stimulant for increased fish production.

Burns and Stickney (1980) reported that poultry wastes were effective in increasing the growth rate of *Tilapia aurea* and that production paralleled the rates of organic fertilization. Stickney et al. (1979) recognized that the purpose of organic fertilization was to increase primary productivity which, in turn, would stimulate increased fish production. The authors reported that fertilization with hog manure was effective in increasing the growth rate of *Tilapia nilotica*. While these correlations have resulted in a measure of increased fish production, they cannot be used to relate the growth of specific organisms to the availability of particular environmental resources in a quantitative manner.

Another research approach, utilized by nutritionists, involves the investigation of the ability of formulated diets to promote growth. Smith (1971) proposed the use of metabolic chambers to determine assimilation efficiencies of food components. By force-feeding the fish and quantifying the separate excrements, the digestibility and nutrient balance of the feeds may be determined. Unfortunately, this method also entails stressing the fish due to force-feeding, restraint in the chamber, abrasion, handling and ammonia concentration in the chamber. While the methodology affords a measure of control to determine the digestion-assimilation efficiencies, it simultaneously results in an alteration of normal function. Application of the results to natural environments requires the questionable assumption of unaltered food processing rates in a stressful environment.

An alternative approach involving less stress was utilized by Cho et al. (1974). Digestibility of feeds are determined by adding 1% chromic oxide (Cr_2O_3) to the diet as a tracer and measuring the subsequent concentration in the feces. The relative concentrations of the chromic oxide in feed and feces are used to calculate digestibility and assimilation rates. This method provides the opportunity of studying a population of fish in an unstressed environment. However, the method is limited by the difficulties involved with collection of representative samples of fecal material and a restriction to digestible rather than metabolizable fractions. While these approaches do provide a limited means of evaluating the digestibility and assimilation potential of feeds, they cannot be used to relate the growth of the unstressed organism directly to environmental resources in a quantitative manner.

Because of the limitations involved with using correlations or empirical models to relate growth to environmental parameters, the practicality of more theoretical models will be reviewed. One well-accepted model proposed to characterize the growth of both individuals and populations is the logistic curve. The following summary of the model is based on a review by Kingsland (1982). In 1845 Pierre-Francois Verhulst proposed that during the early stages of growth, a population would increase exponentially until such time that critical resources became limiting. Upon reaching such limits, the rate of growth was retarded resulting in a symmetrical, sigmoidal curve of growth. The sigmoidal curve labeled the "logistic" can be written in differential form as:

$$\frac{dN}{dt} = rN\left(\frac{K-N}{K}\right)$$

where K = maximum carrying capacity or upper asymptote (number)
N = number of individuals (number)
r = maximum rate of growth $\left(\frac{1}{\text{time}}\right)$
t = time

Raymond Pearl and Lowell Reed independently introduced the same growth curve in 1920 to explain the growth rate of individual organisms as well as populations of bacteria and protozoans. While some researchers sought to define the logistic as a universal law of growth, others recognized it as a logical explanation or model for growth provided certain criteria were met. These conditions were: 1) both r and K are constant, 2) no time lags exist, and 3) all individuals are equal in their effect on the reduction of the growth rate at each density. When the logistic formula is rearranged to express the rate of increase per unit, or individual, the following results:

$$\frac{dN}{Ndt} = r\left(1 - \frac{N}{K}\right)$$

The $\left(1 - \frac{N}{K}\right)$ term represents the multitude of factors which contribute to the decreasing rate of increase in growth as the size of the individual or population increases. It was suggested by Lotka in 1925 that the reasons both individuals and populations follow the same curve is that both share the same basic underlying mechanisms of growth since populations consist basically of "populations" of cells. The fundamental principals underlying the growth process were poorly understood at the time of introduction of the model. Therefore, it is fairly judged to contain both theoretical and empirical elements. Modifications of the model have since been employed to explain r and K selection (MacArthur and Wilson 1967), competition (Hixon 1980), and predator-prey relationships (Chesson 1978).

The logistic curve serves the important function of incorporating a rate dependency concept into growth modeling. Verhulst proposed that growth rates will be influenced by the availability of critical, limiting resources. However, the logistic fails to quantitatively relate changing growth rates directly to particular environmental resources. Instead, the model is simply descriptive of typical population growth rates occurring with time.

Another approach to model or characterize the growth of organisms is based on the function of enzymes. In both unicellular and multicellular animals, the biochemical reactions contributing to growth are regulated by enzymes. Even the simplest manifestations of life involve many complex enzymatically controlled reactions (Hoar 1979). The enzymes, while not contributing energy to the reactions, are necessary for the activation of the process. Specific proteinaceous enzymes associate themselves with a reactant (substrate) to form an intermediate complex. The formation of this complex allows the reaction to occur at a lower activation energy than would otherwise be possible. As the intermediate complex combines with another reactant to form a product, the enzyme is released and may be reused in the reaction process. The reaction is summarized as follows:

$$E \quad + \quad S \quad \rightleftharpoons \quad ES \longrightarrow \quad P \quad + \quad E$$

(Enzyme) (Substrate) (Enzyme-substrate) (Product) (Enzyme)

The relative amount of both enzyme (E) and enzyme specific substrate (S) is critical to the reaction rate. At low substrate concentrations, the reaction rate should be proportional to the substrate concentration (S). At these substrate levels, the enzyme (E) is able to completely react with all the substrate (S) forming the enzyme-substrate complex (ES) facilitating the reaction. However, as the concentration of substrate is increased, a higher percentage of the enzyme (E) is bound in the intermediate complex (ES) and less is available to react with substrate. Eventually, if the substrate concentration is increased sufficiently, virtually all the enzyme would be bound in the intermediate complex (ES) with none in reserve to combine with substrate. As fast as the complex breaks down, the released enzyme combines with substrate to form more intermediate complex. Under these circumstances the enzyme is referred to as "saturated," and additional units of substrate yield no increase in reaction rate. The dependence of rate on substrate concentration is shown in Figure 1. This enzyme-substrate reaction rate response is described by the Michaelis-Menten equation as follows (Engel 1977):

$$V = V_{max} \left(\frac{S}{K_s + S}\right)$$

where V = reaction rate $\left(\frac{1}{\text{Time}}\right)$

V_{max} = maximum reaction rate $\left(\frac{1}{\text{Time}}\right)$

S = substrate concentration

K_s = substrate concentration at which the reaction rate is one-half the maximum

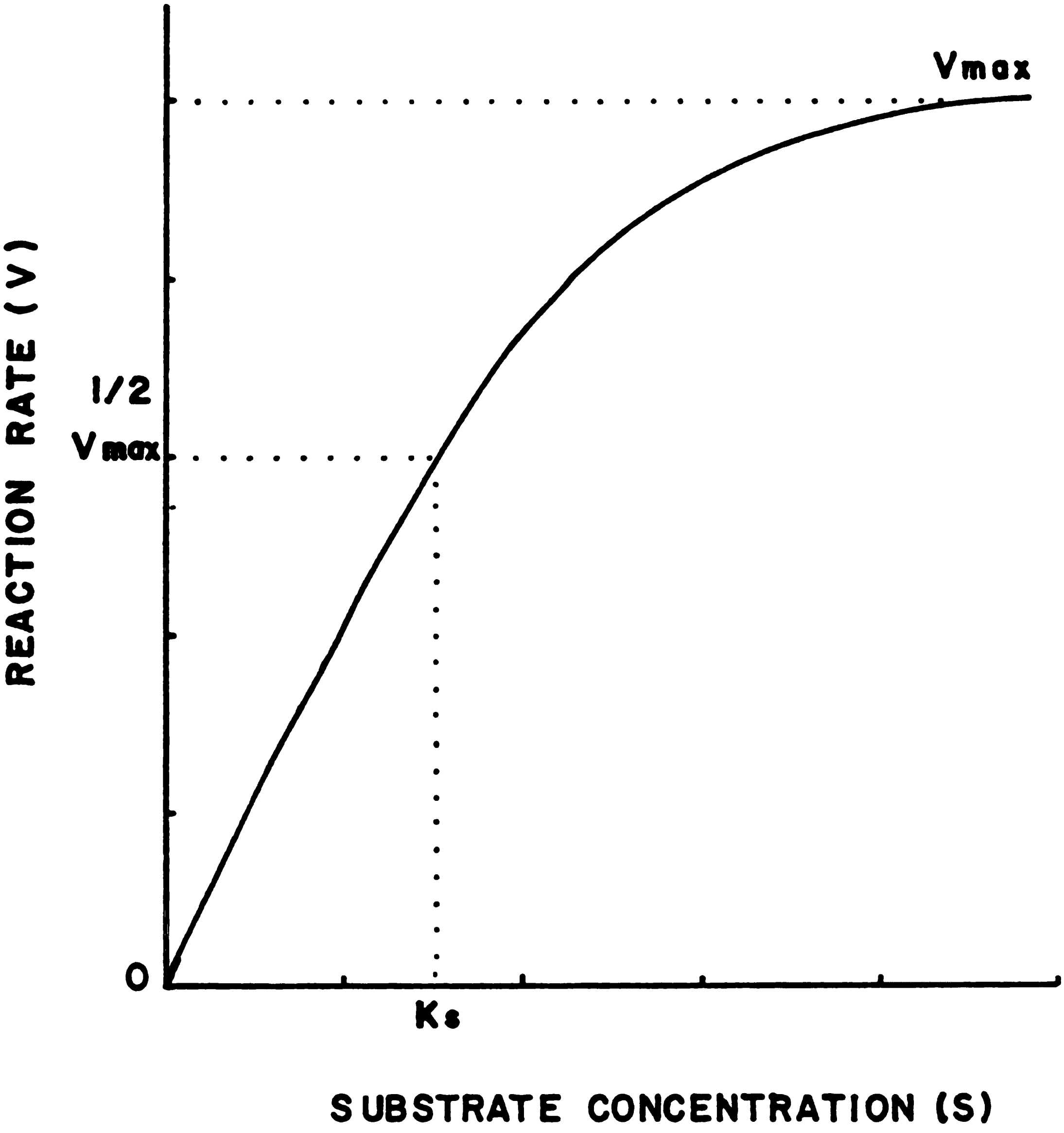

Figure 1. Dependence of rate on substrate concentration in enzyme catalyzed reaction.

In the equation, when S becomes very large compared to K_s, the reaction rate V approaches V_{max}. Conversely, when S is very low relative to K_s, the reaction rate is proportional to S.

Monod (1949), working with bacterial cultures, stated that "despite the immense complexity of the phenomena to which it testifies, growth generally obeys relatively simple laws, which make it possible to define certain quantitative characteristics of the growth cycle." These growth cycles were recognized by Monod to consist of different phases with the extent of each phase being determined by the composition of the media in which the bacteria grew. Growth was demonstrated to be restricted by limiting factors such as nutrients or adverse environmental factors. When experimental conditions were established to provide an excess of all but one limiting nutrient, and the concentration of that limiting nutrient was gradually increased, the growth of the bacteria demonstrated a rapid initial increase, then gradually lessened the rate of increase until a plateau was reached at high substrate concentrations. Monod established that the form of the relationship is similar to that determined for the enzyme-catalyzed reactions and proposed the following equation to relate growth to substrate:

$$\mu = \mu_{max} \left(\frac{S}{K_s+S}\right)$$

where μ = growth rate$\left(\frac{1}{\text{Time}}\right)$

μ_{max} = maximum growth rate$\left(\frac{1}{\text{Time}}\right)$

S = substrate concentration

K_s = substrate concentration at which the growth rate is one-half the maximum.

Clearly, this equation is the same form as the previous Michaelis-Menten equation.

The constants μ_{max} and K_s were used to compare the abilities of different nutrients to stimulate growth of the bacteria. The levels of the constants are an expression of the ability of the enzyme systems to utilize the particular limiting substrate for growth. The relation of growth to substrate is a simplification in that it does not examine the complex processes which comprise growth. Instead, the organism functions to integrate those complexities into one parameter--growth. Thus the enzyme-kinetic model of growth serves two important functions. First, it incorporates a consideration of rates of growth much like the logistic model. Secondly, the model allows the establishment of quantitative relationships between growth and specific environmental parameters.

It would appear that the enzyme-kinetic growth model fulfills the initial objective of providing the ability to relate the limits of growth of an organism to the availability of particular environmental resources in a quantitative manner. However, an examination of Figure 1 reveals that the growth curve passes through the origin allowing no provision for energy expenditures and nutrient utilization for basal metabolic activities. A review of some principals underlying these metabolic expenses will clarify their importance in relating growth to resources.

The Second Law of Thermodynamics states that no process involving an energy transformation will occur spontaneously unless there is a degradation of the energy from a concentrated form into a dispersed form. Therefore, as feed components are processed through the myriad biochemical reactions, a processing cost must be extracted from the feed to drive these transformations. It is expected that since some amount of feed must be allocated to meet these energy expenditures, the growth curve cannot pass through the origin when growth is plotted versus feeding level. Instead, at a zero feeding level, it is expected that negative growth should occur. This is necessitated because the continued functioning of the organism during starvation demonstrates that energy-consuming, life-sustaining reactions continue to occur. These processing costs, when combined with the enzyme-kinetic dynamics, would result in the hyperbolic growth curve being shifted downward to reflect the cost of energy transformations.

Organisms with extracellular digestion require that feed components diffuse through membranes prior to utilization. The diffusion processes are important in determining the amount and rate at which nutrients are transported to sites for anabolism, and waste materials carried away from catabolic sites. Transport from ingestion to reaction site often involves diffusion across several membrane barriers. The rates of passive diffusion in an aqueous media are defined by Fick's Law of Diffusion as shown below:

$$\text{Diffusion rate} = D \quad S \left(\frac{dc}{dx}\right)$$

where S = cross sectional area

D = diffusion constant

c = concentration

x = unit length perpendicular to the plane of S

$\left(\frac{dc}{dx}\right)$ = concentration gradient

At each membrane barrier, some portion of the feed, determined by the gradient, area, etc., will remain outside the membrane. Therefore, the initial level of feed is necessarily greater than the level of feed available at the internal reaction site. For this reason, the growth curve will again experience a downward shift representing a decreased growth rate due to incomplete diffusion and transport of feed components.

The growth curve proposed to represent the influence of enzyme kinetics on growth in response to limiting substrate concentration must be modified to incorporate the effects of diffusion processes and the Second Law of Thermodynamics. Both processes similarly shift the curve downward causing the appearance of a threshold feeding level (Figure 2). This feeding level, labeled S_q, represents the amount of feed required to meet the basal metabolic expenses of the organism at a zero growth rate. The equation used to describe the curve must also be modified to incorporate this shift. By subtracting the maintenance feeding level, S_q, the equation now becomes:

$$\mu = \mu_{max} \left[\frac{S-S_q}{(K_s-S_q)+(S-S_q)}\right]$$

The equation may be further simplified to:

$$\mu = \mu_{max} \left(\frac{S - S_q}{K_s+S-2S_q}\right)$$

The threshold-corrected hyperbolic equation includes the maintenance feeding level (S_q) and the maximum rate of growth (μ_{max}). These values, along with K_s, allow the prediction of growth response to any feeding level of the resource utilized. The equation appears to quantitatively describe the relationship of the specific growth rate to single limiting environmental parameters in a manner

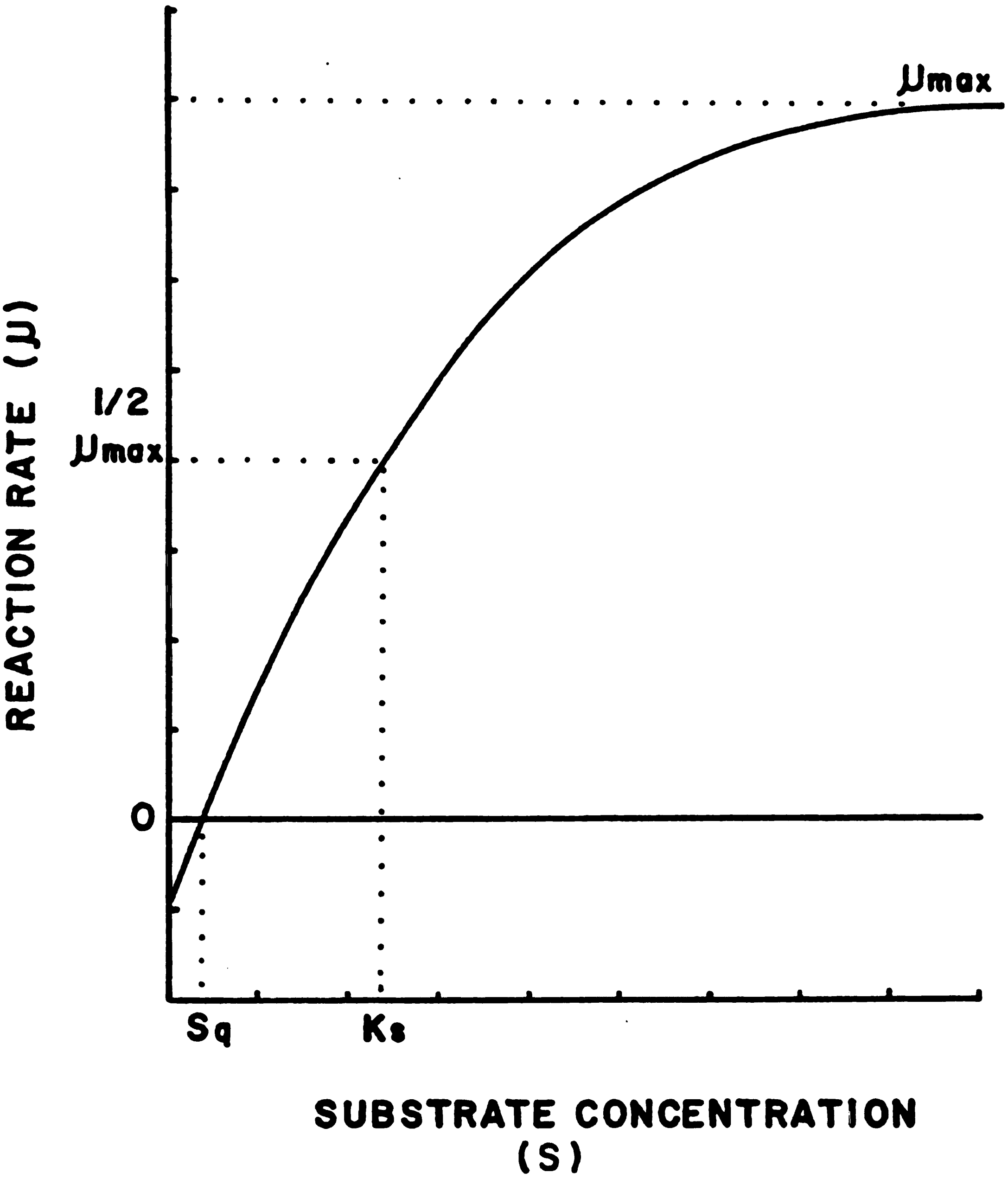

Figure 2. Threshold-corrected dependence of rate on substrate concentration.

applicable to aquaculture. The intent of this research is to determine the usefulness of this model in quantitatively relating the growth of fish to the environmental variables temperature, feed type and quality, and the type and size of fish.

MATERIALS AND METHODS

This research involves numerous laboratory experiments, each from one to five weeks in duration, designed to examine the ability of the model to quantitatively relate the growth of *Tilapia zillii* and *Sarotherodon niloticus* (also referred to as *Oreochromis niloticus*) to particular feeds under controlled conditions. The growth of *T. zillii* was evaluated as a function of the following feeds: commercial trout chow; a pelleted macrophyte (*Elodea canadensis*); and a pelleted blue-green alga (*Spirulina* sp.). The growth response of *T. zillii* to trout chow was examined at five different temperatures. Growth rates were determined for four size classes of *T. zillii* as a function of trout chow feeding levels at near optimal temperature. The growth of *S. niloticus* was examined in response to both commercial trout chow and pelleted *Spirulina*. The experimental conditions are summarized in Table 1.

The *Tilapia zillii* for the experiments were provided by Dr. Donald Garling at Michigan State University. The *Sarotherodon niloticus* were obtained from Dr. Ronald Phelps at Auburn University. Their assistance is greatly appreciated.

Each of the experimental feeds was measured on a dry-weight basis and provided in distinct size categories. The *Elodea canadensis* was harvested from a 10 acre lake located

Table 1. Summary of experimental conditions.

Feed type	Fish species	Average size (g)	Temperature °C				
			21.5°	25.0°	27.5°	30.0°	35.0°
Trout chow	*T. zillii*	0.014			X		
"	"	1.76			X		
"	"	10.0	X	X	X	X	X
"	"	38.1			X		
"	*S. niloticus*	10.0			X		
E. canadensis	*T. zillii*	10.0			X		
Spirulina sp.	*T. zillii*	10.0			X		
"	*S. niloticus*	10.0			X		

at the Michigan State University, Inland Lakes Research Study Center. The plants were blended, extruded into pellets, dried at 90°C for 24 hours, ground with a mortar and pestle, and sieved to a 1 to 2 mm size range using a U.S. Standard Sieve Series. The pelleted *Spirulina* sp. was obtained as a powder from Microalgae International Sales Corp. The powdered algae was mixed with 1% carboxy methyl cellulose binder and sufficient water to form a paste. The paste was then dried at 90°C for 48 hours, ground with a mortar and pestle, and sieved to a 1 to 2 mm size range. The Purina Trout Chow was utilized in several size categories depending on the size of the fish. In each instance, the feed was ground with a mortar and pestle, then screened to the following size classes: 0.297 to 0.420 mm; 0.420 to 1.0 mm; 1.0 to 2.0 mm; and 2.0 to 4.0 mm. All feeds were stored in sealed containers to prevent spoilage.

APPARATUS AND PROCEDURE

A series of 30 gallon aquaria each equipped with air stone aerators, heaters, and external tank filters made up the basic system for most experiments. The aquaria were located side by side under a four bulb, 34 watt, fluorescent light fixture which was regulated to a light period from 730 hrs to 1930 hrs each day. All experiments utilized cold tap water from the Michigan State University water supply system which had originated in deep groundwater sources. The aquaria were filled at least three days prior to an experiment to allow for aeration, chlorine removal, temperature adjustment and iron precipitation. Each outside tank filter contained synthetic fiber to remove particulate material and clinoptilite resin to reduce ammonia levels. Temperature control was provided by 150 watt Penn Plax aquarium heaters, except for the 35°C experiment which required 200 watt heaters. Plastic netting was placed over the aquaria to limit the renowned jumping ability of the fish.

Approximately three weeks prior to each study, all fish were transferred to aquaria adjusted to the forthcoming experimental temperature and fed the appropriate feed at approximate maintenance levels. Initially, for each experiment, individual fish were captured in a nylon net, measured for length and weight, and from zero to three of the dorsal fin spines clipped. This allowed the individual fish to be identified in succeeding weighing operations through the one to five week experimental period. The only exception to this rule was the weighing process for the fry utilized in the size experiments. Because these fish were so susceptible to injury, an alternative method was developed. All of the fry utilized in the 0.0140 g experiment were from one brood of about 150 fish. Ten of these were randomly netted for the weighing process. The weights of these ten fish were found to average 0.0140 g and ranged from 0.0139 - 0.0141 g. Therefore, twenty fry were captured with a nylon net and transferred directly to each aquarium, with the initial, individual weights in each case assumed to be 0.0140 g.

During the weekly measuring process, the fish were handled using surgical gloves and kept on moist paper toweling. Weight measurements for all the experiments were conducted prior to the morning feeding at 800 hrs. Weights were determined to tenths of a gram using a top-loading Mettler balance, with the pan covered by a moist paper towel. Due to the hardiness of the fish and the short handling time (less than one minute), the fish showed no adverse effects and demonstrated an affinity for feed within 30 minutes after their return to the aquaria.

With the exception of the 0.014 g _T. zillii_, each experiment was conducted with one to four fish per aquarium, and each fish spine clipped for identificaton. Each group of fish then received different amounts of dry feed based on a percentage of the total wet-weight of the fish per aquarium. The percentages of feed usually ranged from zero to five percent body weight per day (dry weight feed/wet weight fish), although in one experiment levels up to 10 percent were included.

The feed was measured on a dry-weight basis using a Mettler Balance Model H10T and was divided into two equal portions which were fed at 800 hrs and again at 1500 hrs.

The fish were fed twice per day to increase feed utilization efficiency as reported by Greenland and Gill (1979). Waste material was siphoned daily from each aquarium prior to the first feeding and the water replaced with conditioned water stored in an additional aquarium. Weights and lengths of the individual fish were measured weekly, and the required levels of feed recalculated using these data.

Water quality was monitored by periodic determinations of temperature, pH, dissolved oxygen, alkalinity and ammonia levels. After analysis at the beginning of the experiment, the determinations were performed weekly, except for temperature which was measured daily. Protein and caloric content determinations were performed on all the feeds utilized.

ANALYTICAL METHODS

Temperature

A standard laboratory mercury bulb thermometer was used to determine the temperature of each aquarium. Values were recorded at the time of the morning feeding.

Alkalinity

Titratable alkalinity was measured using methyl orange indicator and 0.02 N H_2SO_4 as described in Standard Methods (1980).

pH

Measurements of pH were made using either an Orion model 901 digital microprocessor ionanalyzer with an Orion Ross combination pH electrode or a Beckman Expand-Mate pH meter with a combination electrode. Both units were calibrated with standard buffer solutions.

Ammonia-Nitrogen

Ammonia-nitrogen (NH_4-N) was measured using the phenate method described by Solorzano (1969). The ammonia, phenol and hypochlorite react to form a blue-colored complex which absorbs at a wavelength of 680 m . Color development of samples and standards was measured with a Bausch and Lomb Spectronic 21 spectrophotometer.

Dissolved Oxygen

Dissolved oxygen levels were determined using a Y.S.I. model 54A dissolved oxygen meter. The instrument was calibrated weekly using a Winkler-Azide Modification procedure as outlined in Standard Methods (1980).

Caloric Content

The caloric content of the feed was determined using a Parr Oxygen Bomb Calorimeter Model 1341. Samples were dried at 90°C for 48 hours, then ground in a Wiley Mill with a 20 micron mesh screen. The ground samples were pelletized and the caloric content measured using benzoic acid as a standard.

Total Nitrogen-Protein

The total nitrogen content of feeds was determined using a Perkin-Elmer Model 240 Elemental Analyzer. Samples were dried at 90°C for 48 hours, then ground in a Wiley Mill with a 20 micron mesh screen. The Elemental Analyzer provides a determination of the total nitrogen content by combusting a known amount of sample at 1000°C, reducing the total nitrogen content to nitrogen gas, then measuring the thermal conductivity generated relative to that generated from standards of acetanilide. The total nitrogen concentrations were then multiplied by 6.25 to convert to protein levels.

The Elemental Analyzer provides accompanying readouts on hydrogen and carbon content of the samples. These data are included with the nitrogen-protein levels.

CALCULATIONS

Growth Rate

In analyzing the growth data from each experiment, it is necessary to perform a series of calculations. To clarify these calculations, the data from one experiment will be carried through each step of the process with sample calculations performed. The data obtained from a feeding trial using *Tilapia zillii* fed trout chow at 27.5°C are used for this purpose. The initial data consists of the beginning weight of each fish and the subsequent weight at the end of each week. The data in Table 2 provide the weight for each fish at each feeding level as well as the total weight of fish per aquarium, or feeding level.

The specific growth rate of each fish, as well as the average for each feeding level, is calculated for each week's data using the formula:

$$\mu = \frac{\ln W_t - \ln W_o}{t}$$

where μ = specific growth rate $\left(\frac{1}{\text{Time}}\right)$

W_t = weight at time t

W_o = initial weight

t = time (days)

For example, in calculating the specific growth rate for fish #0 at a 0% feeding level during the first week, the equation would appear:

$$\mu = \frac{\ln 12.6 \text{ gm} - \ln 13.5 \text{ gm}}{7 \text{ days}} \quad \text{or} \quad \mu = -0.00986/\text{day}$$

Similarly, the average specific growth rate for fish at the 0% feeding level during the first week would be as follows:

$$\mu = \frac{\ln 60.3 \text{ gm} - \ln 64.7 \text{ gm}}{7 \text{ days}} \quad \text{or} \quad \mu = -0.0101/\text{day}$$

The specific growth rates calculated for all the weight data in Table 2 are contained in Table 3.

Table 2. Weight in grams of *Tilapia zillii* fed trout chow at 27.5°C.

Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0% Feed					
0	13.5	12.6	12.2	12.0	11.4
1	14.5	13.2	12.5	12.1	11.8
2	14.7	13.9	13.7	13.6	13.5
3	12.7	12.0	11.6	11.6	11.4
4	9.3	8.6	8.1	7.6	7.4
Total	64.7	60.3	58.1	56.9	55.5
0.2% Feed					
0	8.5	8.2	8.1	7.8	7.5
1	7.1	7.1	7.1	7.1	7.0
Total	15.6	15.3	15.2	14.9	14.5
0.5% Feed					
0	9.0	8.7	8.4	8.3	8.1
1	6.1	6.6	7.1	7.4	7.6
Total	15.1	15.3	15.5	15.7	15.7
0.7% Feed					
0	7.2	7.3	8.1	8.6	9.1
1	6.8	6.9	6.8	6.6	6.6
Total	14.0	14.2	14.9	15.2	15.7

Table 2 (cont'd.).

Fish #	Initial	Week 1	Week 2	Week 3	Week 4
<u>2% Feed</u>					
0	6.7	6.3	6.3	6.3	7.3
1	7.4	9.2	11.1	12.6	12.3
2	6.9	8.6	9.3	9.6	10.8
3	9.0	9.1	10.8	12.5	14.2
Total	30.0	33.2	37.5	41.0	44.6
<u>3% Feed</u>					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	7.4	8.4	10.0	12.4	14.4
1	6.3	7.9	9.2	11.2	12.9
2	6.8	7.8	9.0	10.5	11.7
3	-	-	-	-	-
Total	20.5	24.1	28.2	34.1	39.0
<u>4% Feed</u>					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	14.2	17.0	17.1	17.3	20.7
1	16.4	20.9	25.0	27.2	31.7
2	15.6	18.7	21.3	26.4	31.2
3	-	-	-	-	-
Total	46.2	56.6	63.4	70.9	83.6

Table 3. Specific growth rates of *Tilapia zillii* fed trout chow at 27.5°C.

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0% Feed					
0	-0.0099	-0.0046	-0.0024	-0.0073	-0.0060
1	-0.0134	-0.0078	-0.0046	-0.0036	-0.0073
2	-0.0080	-0.0021	-0.0010	-0.0011	-0.0030
3	-0.0081	-0.0048	0.0000	-0.0025	-0.0039
4	-0.0112	-0.0086	-0.0091	-0.0038	-0.0082
Average	-0.0101	-0.0056	-0.0034	-0.0037	-0.0057
0.2% Feed					
0	-0.0051	-0.0018	-0.0054	-0.0056	-0.0045
1	0.0000	0.0000	0.0000	-0.0020	-0.0005
Average	-0.0026	-0.0009	-0.0028	-0.0039	-0.0026
0.5% Feed					
0	-0.0048	-0.0050	-0.0017	-0.0035	-0.0038
1	0.0112	0.0104	0.0059	0.0038	0.0079
Average	0.0019	0.0019	0.0018	0.0000	0.0014
0.7% Feed					
0	-0.0020	0.0149	0.0086	0.0081	0.0084
1	0.0021	-0.0021	-0.0043	0.0000	-0.0011
Average	0.0021	0.0069	0.0029	0.0046	0.0041

Table 3 (cont'd.).

2.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0088	0.0000	0.0000	0.0210	0.0031
1	0.0311	0.0268	0.0181	-0.0034	0.0181
2	0.0315	0.0112	0.0045	0.0168	0.0160
3	0.0016	0.0245	0.0209	0.0182	0.0163
Average	0.0145	0.0156	0.0127	0.0120	0.0137

3.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0181	0.0249	0.0307	0.0214	0.0246
1	0.0323	0.0218	0.0281	0.0202	0.0274
2	0.0196	0.0204	0.0220	0.0154	0.0207
3	-	-	-	-	-
Average	0.0231	0.0224	0.0271	(0.0191)	0.0242

4.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0257	0.0008	0.0017	0.0256	0.0257
1	0.0346	0.0256	0.0120	0.0219	0.0346
2	0.0259	0.0186	0.0307	0.0239	0.0259
3	-	-	-	-	-
Average	0.0290	(0.0162)	(0.0160)	(0.0235)	0.0290

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

The use of the specific growth rate formula provides advantages over both the incremental and relative growth rate formulas. The incremental growth formula

$$\text{Incremental Growth} = \frac{\text{Weight}_{\text{final}} - \text{Weight}_{\text{initial}}}{\text{time}}$$

has the deficiency of not relating weight gain to initial weight. For example, using the incremental growth formula, a 100 g weight gain by a 1.0 g fish is equal to the growth rate of a 1000 g fish which gains 100 g. The relative growth formula, shown below, is a linear approximation to the logistic formula.

$$\begin{matrix}\text{Relative}\\ \text{Growth}\end{matrix} = \left(\frac{\text{Weight}_{\text{final}} - \text{Weight}_{\text{initial}}}{\text{time}}\right)\left(\frac{1}{\text{Weight}_{\text{initial}}}\right)$$

This method has the advantage of providing a measure of growth per unit weight of the organism. Using this formula to calculate the specific growth rate of the fish #0 during the first week at the 0% feeding level results in a figure of -0.01020/day, slightly lower than the -0.00986/day obtained with the specific growth rate equation. While the relative growth rate formula provides a close approximation of the logistic growth rate, especially over short time periods, the more accurate specific growth rate equation will be employed in this study. Utilization of either the specific growth rate, or its approximation, has two additional advantages. First, the specific growth rate formula is easily rearranged to the exponential equation $W_f = W_i e^{\mu t}$ as follows:

$$\mu = \frac{(\ln W_f - \ln W_i)}{t}$$

$$\mu t = (\ln W_f - \ln W_i)$$

$$e^{\mu t} = \frac{W_f}{W_i}$$

$$W_f = W_i e^{\mu t}$$

The rearranged formula is useful in determining the weight of the organism or population at any point in time. A second advantage is the opportunity to utilize the data in relating growth to feed concentrations under defined conditions of limiting resources with the base equation

$$\mu = \mu_{max}\left(\frac{S-S_q}{K_s+S-2S_q}\right)$$

Determination of S_q, μ_{max} and K_s Constants

Linear Regression. The next stage in the definition of the growth curve is a determination of the maintenance feeding level (S_q). This constant is essential in utilizing the threshold-corrected hyperbolic equation and must be determined prior to its usage. Values for S_q are obtained by performing a regression of specific growth rate values against the natural log (ln) of the corresponding percent feeding levels. In order to include the values at the 0% feeding level in the regression, a value of one was added to each level of percent feed. The equation for the regression then becomes: $\mu = a + b \ln (1 + \text{percent feed})$. The constants a and b were determined by regression. The specific growth rate (μ) was then set at zero and the equation solved for percent feed at a specifc growth rate of zero. The example data in Table 3 yield an S_q value of 0.37 percent feed.

Following calculation of the S_q value, the data were used to determine the other constants, K_s and μ_{max}, in the threshold-corrected hyperbolic curve formula. Values of these constants are calculated using a linear transformation of the original formula of Dowd and Riggs (1965) which for the threshold-corrected model becomes as follows:

$$\frac{S - S_q}{\mu} = \frac{K_s - S_q}{\mu_{max}} + \frac{1}{\mu_{max}} (S - S_q)$$

When values for $\frac{(S - S_q)}{\mu}$ are linearly regressed against values for $(S - S_q)$, the reciprocal of the slope equals μ_{max}, and this value times the intercept of the line equals $K_s - S_q$. Using the results of the sample experiment, summarized in Table 4, μ_{max} was calculated to be 0.0641 and K_s was calculated to be 5.04.

Table 4. Regression data and growth constants calculated for *T. zillii* fed trout chow at 27.5°C.

S	S_q	$S - S_q$	μ	$\frac{S - S_q}{\mu}$
0	0.37	-0.37	-0.0057	64.91
0.2		-0.17	-0.0026	65.38
0.5		0.13	0.0018	72.22
0.7		0.33	0.0041	80.49
2		1.63	0.0142	114.79
3		2.63	0.0242	108.68
4		3.63	0.0290	125.17
$\mu_{max} = 0.0641$		$K_s = 5.04$	$S_q = 0.37$	

The data fit the regression line of the transformed equation with an r^2 of 0.91.

This series of calculations has resulted in the determination of values for the three constants of the threshold-corrected hyperbolic curve: K_s, S_q and μ_{max}. These values are then incorporated into the original equation. Using the data from the example, the equation would appear:

$$\mu = 0.0641 \left(\frac{S - 0.37}{S + 4.30}\right)$$

The equation now is useful for obtaining the rate of growth resulting from any given ingestion level of the trout chow under similar environmental conditions. The calculation of the three constants has resulted in a quantification of the complete growth range by identifying the maximum rate of growth (μ_{max}), the half saturation value (K_s), and the intercept at zero growth, or maintenance ration (S_q).

Two important assumptions are made when utilizing the data to define the growth curves. First, when more than one fish are in one aquarium, each fish cannot be expected to consume equal feed, or the same percentage of its body weight per day. Physical and behavioral differences should cause some individuals to consume a greater and some a lesser share of the total feed. As a result, only the growth in the total weight of the fish per tank is utilized in defining the growth curves. This procedure insures that the weight and specific growth rate of the fish corresponds to the specified rate of feeding. At least this is certain if all the feed was ingested, which leads to the second assumption. In order for growth data to be incorporated in the defining of the curves, all feed must have been consumed. Whenever feed was observed to remain overnight in an aquarium, that week's data were excluded from further calculations since the observed growth of the fish cannot be related to the intended percentage body weight ingested. Occasions of incomplete feeding are indicated on the tables of growth rates for each experiment by enclosing the figures in parentheses. These data are not included in the averages listed in the far right column of the table.

Non-linear regression. It has been demonstrated that a linear transformation of the threshold-corrected hyperbolic curve is useful in determining values of μ_{max} and K_s. However, when the equation is transformed, the possibility exists that some areas of the curve may obtain more power in determining the slope of the line than others, thus disproportionately affecting the calculation of μ_{max} and K_s, and the definition of the curve. Therefore, another procedure, non-linear regression was utilized in analyzing the data.

Non-linear regression analysis was performed using a BMDP-PAR computer program (Dixon, 1983). In this analysis, the threshold-corrected hyperbolic formula is specified by FORTRAN in a function statement with μ_{max}, K_s and S_q as unknown constants. The experimental data from an individual trial are then entered with the average μ values being the dependent variable. The values of μ_{max}, K_s and S_q calculated by the linear regression technique are then entered as initial estimates for these parameters, along with upper and lower constraints to contain the search if something should fail to converge. The program then examines the data by an iterative "examine and modify" series of steps. Through a series of iterative-least squares analysis, the program provides an estimate for each of the constants. In addition, standard deviations of the estimates, correlations between the parameters, predicted values, and observed values of the variables are listed.

Efficiency

An additional calculation provides a means of examining growth relative to the individual units of feed supplied. This is accomplished by dividing the calculated values of μ

by the corresponding substrate level (S) and plotting this value (μ/s) versus S. These plots establish "efficiency" curves which demonstrate the ability of each unit of feed to stimulate growth. The data from the sample experiment are shown below:

Table 5. Growth efficiency data calculated for *T. zillii* fed trout chow at 27.5°C.

Calculated μ	S	μ/S
-0.0055	0	-
-0.0024	0.2	-0.012
0.0017	0.5	0.0034
0.0042	0.7	0.0060
0.0076	1	0.0076
0.0166	2	0.0083
0.0231	3	0.0077
0.0280	4	0.0070
0.0319	5	0.0064
0.0350	6	0.0058
0.0376	7	0.0054

The calculated growth curve and "efficiency" curve, as well as the observed data points are plotted in Figure 3. This calculation of efficiency is similar to the more traditional calculation of feed conversion efficiency, where the change in the fishes weight is divided by the amount of feed ingested. Direct comparisons could be made if the growth rate calculation used in the efficiency determination utilized the relative rather than the specific growth rate equation.

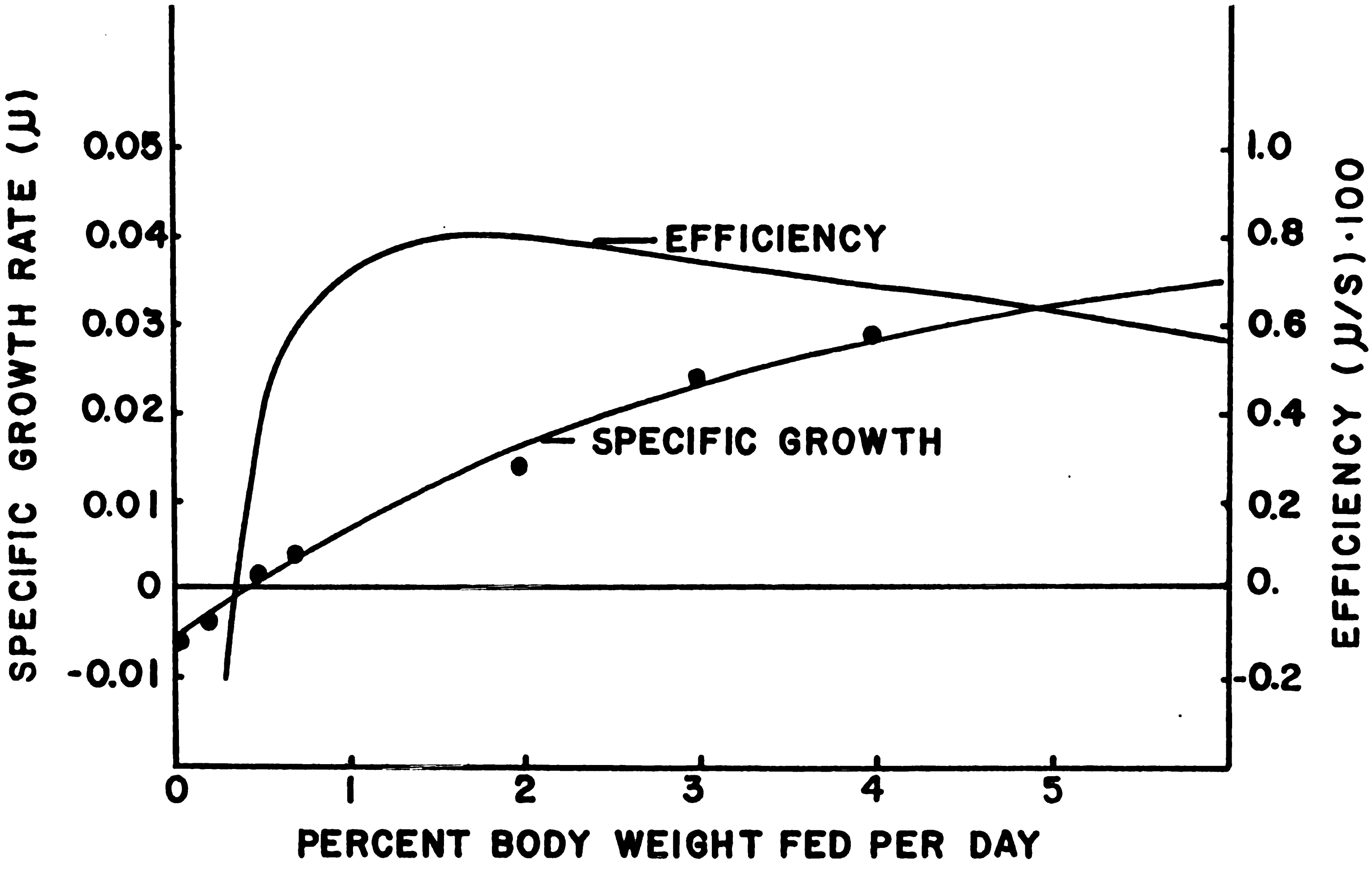

Figure 3. Observed growth data and the predicted specific growth rate and efficiency curves for *T. zillii* fed trout chow at 27.5°C.

RESULTS

The basic data obtained during the various experiments are presented in appendices. Appendix A contains the weekly length and weight measurements for individual fish and the average fish weight in each aquarium. Appendix B consists of the specific growth rates calculated from the weight data and also indicates which data are excluded from consideration due to incomplete feeding. Appendix C contains the results of water quality determinations performed during the experiments. Appendix D contains the results of protein and energy analyses performed on all the feeds utilized in the experiments as well as manufacturers' analyses of the feeds. Because these data are provided in appendices, this chapter will be a presentation of the calculated constants and equations which describe the growth curves for each experiment. These data are grouped into four sections: temperature, size, feed type, and species comparison.

Temperature - Growth

The first experiment involved the determination of the growth of *Tilapia zillii* fed trout chow at 27.5°C. Using the procedures described in the Materials and Methods chapter, the average specific growth rate at each feeding level was calculated. These data were then used to calculate values for the constants μ_{max}, K_s and S_q, using both the linear and non-linear regression techniques. Table 8 contains the original observed growth data, the constants calculated by both techniques, the predicted growth rates using each data analysis technique, and the efficiencies calculated for each feeding level using the linear regression constants. In addition, the table contains the coefficient of determination (r^2) for the linear regression and the standard deviations for the predicted data points calculated by the non-linear regression analysis.

Following this experiment, identical feeding trials were performed with *Tilapia zillii* fed trout chow, except that water temperatures were maintained as follows: 21.5°C, 25°C, 27.5°C, 30°C, 35°C. Data for each of these experiments were similarly calculated and are presented in Tables 6-11. A summary of the constants calculated by both regression techniques, for each temperature is contained in Table 12. Inspection of Table 12 reveals that the maximum growth rate occurred at 27.5°C. Therefore, this temperature was used in subsequent experiments.

The constants, μ_{max}, K_s and S_q, calculated by linear regression, were utilized to construct growth curves representing the predicted growth throughout the feeding range. The growth curves for each of the five temperatures are included in Figure 4.

Table 6. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 3.3 g) fed trout chow at 21.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0459	0.77	3.23	0.99
non-linear regression	0.0416	0.80	2.77	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
1	0.0038	0.0039	0.0038	0.00033	0.390
2	0.0159	0.0153	0.0158	0.00029	0.765
3	0.0217	0.0218	0.0219	0.00020	0.727
4	0.0259	0.0261	0.0258	0.00030	0.653

Table 7. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 4.0 g) fed trout chow at 25.0°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0519	0.38	4.30	0.97
non-linear regression	0.0542	0.41	4.57	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s) \cdot 100$
0	-0.0060	-0.0056	-0.0060	0.00115	-
1	0.0069	0.0071	0.0067	0.00079	0.710
2	0.0139	0.0152	0.0150	0.00075	0.760
3	0.0220	0.0208	0.0208	0.00066	0.693
5	0.0281	0.0281	0.0285	0.00109	0.623

Table 8. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 10.3 g) fed trout chow at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0641	0.37	5.04	0.91
non-linear regression	0.1403	0.46	13.7	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency (μ/s)•100
0	-0.0057	-0.0055	-0.0050	0.00126	-
0.2	-0.0026	-0.0024	-0.0028	0.00095	-1.20
0.5	0.0018	0.0017	0.0005	0.00075	0.340
0.7	0.0041	0.0042	0.0025	0.00078	0.600
2	0.0142	0.0166	0.0146	0.0011	0.830
3	0.0242	0.0231	0.0227	0.00099	0.770
4	0.0290	0.0280	0.0297	0.00158	0.700

Table 9. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 6.8 g) fed trout chow at 30°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0489	0.42	5.20	0.88
non-linear regression	0.0471	0.46	4.76	-

% body weight fed/day	observed specific growth rate	predicted growth		std. deviation non-linear regression	predicted efficiency (μ/s)·100
		linear regression constants	non-linear regression constants		
0	-0.0053	-0.0047	-0.0056	0.00178	-
1	0.0046	0.0053	0.0053	0.00122	0.530
2	0.0115	0.0121	0.0124	0.00117	0.605
3	0.0196	0.0171	0.0175	0.00102	0.570
5	0.0233	0.0239	0.0241	0.00170	0.523

Table 10. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 7.3 g) fed trout chow at 30°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0483	0.47	4.52	0.91
non-linear regression	0.0468	0.50	4.17	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
0	-0.0070	-0.0063	-0.0073	0.00205	-
1	0.0049	0.0056	0.0056	0.00145	-
2	0.0126	0.0132	0.0136	0.00132	-
3	0.0214	0.0186	0.0189	0.00116	-
5	0.0247	0.0255	0.0257	0.00192	-

Table 11. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 7.3 g) fed trout chow at 35°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0341	0.50	4.57	0.95
non-linear regression	0.0385	0.55	5.44	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
0	-0.0051	-0.0048	-0.0049	0.000737	-
1	0.0040	0.0037	0.0032	0.000498	0.370
2	0.0081	0.0092	0.0088	0.000488	0.458
3	0.0128	0.0129	0.0128	0.000429	0.430
5	0.0185	0.0179	0.0183	0.000708	0.358

Table 12. Summary of growth constants calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 6.3 g) fed trout chow at five temperatures.

	μ_{max}		S_q		K_s	
°C	linear	non-linear	linear	non-linear	linear	non-linear
21.5	0.0459	0.0416	0.77	0.80	3.23	2.77
25.0	0.0519	0.0542	0.38	0.41	4.30	4.57
27.5	0.0641	0.1403	0.37	0.46	5.04	13.70
30.0	0.0489	0.0471	0.42	0.46	5.20	4.76
30.0	0.0483	0.0468	0.47	0.50	4.52	4.17
35.0	0.0341	0.0385	0.50	0.55	4.57	5.44

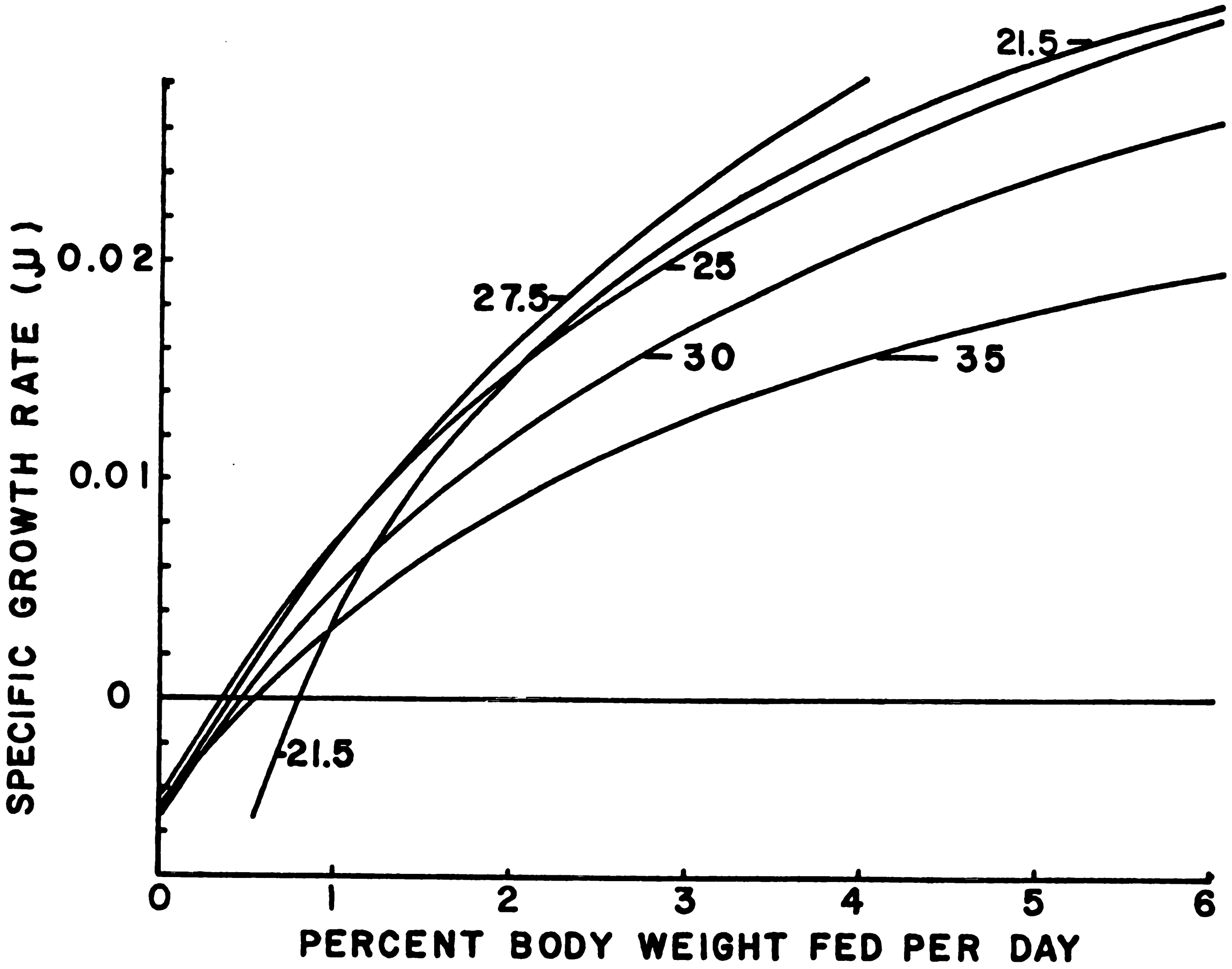

Figure 4. Predicted specific growth rate responses for *T. zillii* (average weight 6.3 g) fed trout chow at five temperatures.

Size - Growth

The next series of experiments was designed to investigate the effects of fish size (weight) on growth. Four feeding trials were conducted, each utilizing different average weight *Tilapia zillii* fed trout chow at 27.5°C. The previous feeding trial using *Tilapia zillii* at 27.5°C utilized fish averaging 10.0 g, and this was included as one of the four experiments. The smallest size group averaged 0.0140 g. Special weighing procedures for this size class were noted previously in the Materials and Methods chapter. The two remaining size classes had average weights of 1.76 and 38.1 g. Trout chow was provided with the particle size ranges adjusted to the fish's weight as listed in Table 13.

Table 13. Trout chow particle size corresponding to each size class of *T. zillii*.

Average fish weight (grams)	Trout Chow Size Range (mm)
0.0140	0.297 - 0.420
1.76	0.420 - 1.0
10.0	1.0 - 2.0
38.1	2.0 - 4.0

The three constants defining the growth curves (μ_{max}, S_q, and K_s) were again calculated by the two regression techniques. These data, observed and predicted growths and efficiencies, are contained in Tables 14-16. The values for the constants used to determine predicted specific growth rates are shown in Table 17. The constants determined by linear regression were utilized to construct the predicted growth curves for each size class shown in Figure 5.

Table 14. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for 0.0140 g (average weight) *T. zillii* fed trout chow at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.1052	1.55	3.27	0.99
non-linear regression	0.1075	1.49	3.29	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
1	-0.0401	-0.0505	-0.0404	0.0058	-5.05
2	0.0233	0.0217	0.0238	0.0044	1.09
3	0.0459	0.0481	0.0491	0.0036	1.61
5	0.0782	0.0703	0.0711	0.0032	1.41
10	0.0857	0.0875	0.0888	0.0051	0.87

Table 15. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for 1.76 g (average weight) _T. zillii_ fed trout chow at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0797	0.89	3.89	0.71
non-linear regression	0.2638	0.34	23.52	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
1	0.0085	0.0028	0.0073	0.00369	0.280
2	0.0170	0.0215	0.0176	0.00232	1.075
3	0.0239	0.0329	0.0271	0.00276	1.097
5	0.0483	0.0461	0.0441	0.00270	0.922
7	0.0572	0.0534	0.0588	0.00384	0.763

Table 16. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for 38.1 g (average weight) _T. zillii_ fed trout chow at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0295	0.48	3.54	0.97
non-linear regression	0.0356	0.51	4.50	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
0	-0.0053	-0.0055	-0.0052	0.00061	-
1	0.0042	0.0043	0.0039	0.00053	0.428
2	0.0092	0.0098	0.0097	0.00039	0.489
3	0.0139	0.0133	0.0136	0.00057	0.443

Table 17. Summary of growth constants calculated by both linear and non-linear regression techniques for four size classes of *T. zillii* fed trout chow at 27.5°C.

	μ_{max}		S_q		K_s	
	linear	non-linear	linear	non-linear	linear	non-linear
0.014 g	0.1052	0.1075	1.55	1.49	3.27	3.29
1.76 g	0.0797	0.2638	0.89	0.34	3.89	23.52
10.0 g	0.0641	0.1403	0.37	0.46	5.04	13.7
38.1 g	0.0295	0.0356	0.48	0.51	3.54	4.50

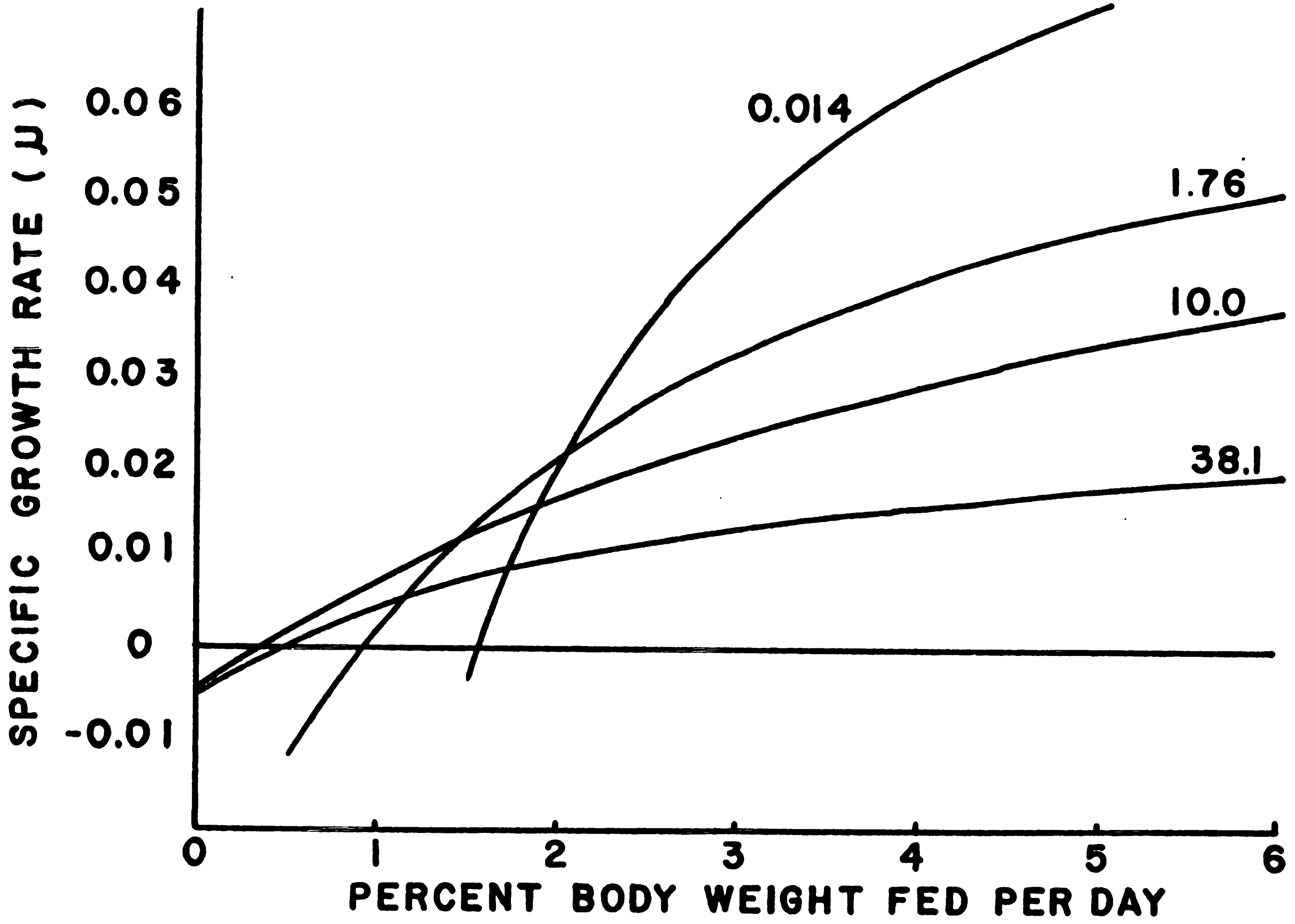

Figure 5. Predicted specific growth rate response for four size classes of T. zillii (average weights 0.014 g, 1.76 g, 10.0 g and 38.1 g) fed trout chow at 27.5°C.

Feed Type - Growth

In order to study the effect of feed type on growth, two additional feeding trials were performed with *Tilapia zillii* at 27.5°C. Pelleted *Elodea canadensis* and pelleted *Spirulina* were utilized for comparison against the trout chow study already performed. As previously described, specific growth rates were calculated, regression analyses performed and constants determined. The data from these trials are listed in Tables 18-19 and summarized in Table 20. The constants calculated by linear regression were utilized to construct the predicted growth curves for each feed shown in Figure 6.

All the feeds were analyzed for energy content (calories/g), total nitrogen, total carbon, and total hydrogen. These data are contained in Appendix D. The total nitrogen content was multiplied by 6.25 to convert to protein (Goodhard and Shils, 1978). In addition to the three feeds utilized in this feed comparison, analyses also were performed on live, suspended *Spirulina* sp. algae. These data are also in Appendix D.

Table 18. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 2.1 g) fed *Spirulina* algae at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0413	0.55	7.21	0.53
non-linear regression	0.1731	0.69	30.0	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
0	-0.0043	-0.0037	-0.0042	0.00031	-
1	0.0023	0.0026	0.0018	0.00022	0.260
2	0.0071	0.0074	0.0074	0.00018	0.369
3	0.0123	0.0111	0.0126	0.00021	0.370
5	0.0224	0.0165	0.0221	0.00034	0.331

Table 19. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *T. zillii* (average weight 13.8 g) fed pelleted *Elodea canadensis* at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.0115	0.76	3.20	0.96
non-linear regression	0.0110	0.65	2.91	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s) \cdot 100$
0	-0.0043	-0.0052	-0.0044	0.00056	-
1	0.0010	0.0010	0.0014	0.00044	0.100
2	0.0047	0.0039	0.0041	0.00033	0.194
3	0.0056	0.0051	0.0056	0.00031	0.184
5	0.0070	0.0073	0.0072	0.00049	0.146

Table 20. Summary of growth constants calculated by both linear and non-linear regression techniques for *T. zillii* fed three feeds at 27.5°C.

	μ_{max}		S_q		K_s	
	linear	non-linear	linear	non-linear	linear	non-linear
Trout Chow	0.0641	0.1403	0.37	0.46	5.04	13.7
Elodea canadensis	0.0115	0.0110	0.76	0.65	3.20	2.91
Spirulina sp.	0.0413	0.1731	0.55	0.69	7.21	30.0

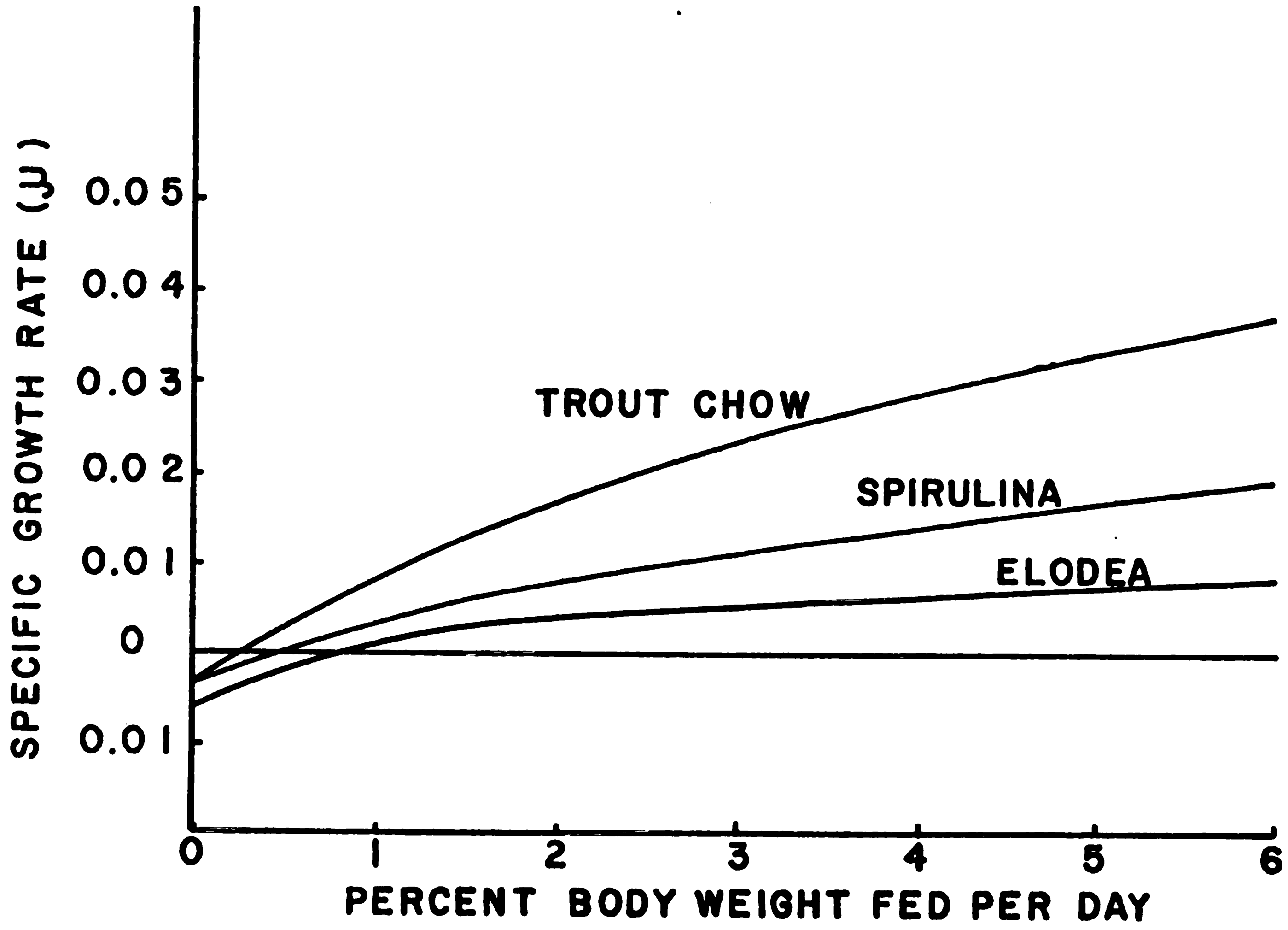

Figure 6. Predicted specific growth rate response for T. zillii fed trout chow, pelleted *Spirulina* and pelleted *E. canadensis* at 27.5°C.

Species Comparison

After examining the ability of the model to quantify the effects of temperature, size and feed type on the growth of *Tilapia zillii*, experiments were designed to identify differences in growth experienced by different species under identical environmental conditions. Therefore, feeding trials were performed using *Sarotherodon niloticus* fed trout chow and pelleted *Spirulina* at 27.5°C. The average size of the fish was 4.1 g for the trout chow trial and 3.9 g for the pelleted *Spirulina* trial. As previously described, the specific growth rates were calculated, regression analyses and other calculations performed. The findings from the trials are provided in Tables 21-22. Comparisons of the data calculated for both species when fed both trout chow and *Spirulina* are shown in Table 23. Growth curves constructed from constants calculated by linear regression are shown in Figure 7.

Table 21. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *S. niloticus* (average weight 4.1 g) fed trout chow at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.2552	0.34	22.7	0.51
non-linear regression	0.2192	0.35	18.9	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency (μ/s)•100
0	-0.0041	-0.0040	-0.0042	0.00161	-
1	0.0066	0.0073	0.0074	0.00099	0.731
2	0.0197	0.0176	0.0179	0.00112	0.882
3	0.0260	0.0271	0.0273	0.00110	0.905
5	0.0441	0.0440	0.0439	0.00167	0.881

Table 22. Observed specific growth rates and predicted specific growth rate data calculated by both linear and non-linear regression techniques for *S. niloticus* (average weight 3.9 g) fed pelleted *Spirulina* at 27.5°C.

	μ_{max}	S_q	K_s	linear regression r^2 fit
linear regression	0.1602	0.43	14.9	0.71
non-linear regression	0.2744	0.50	26.8	-

		predicted growth			
% body weight fed/day	observed specific growth rate	linear regression constants	non-linear regression constants	std. deviation non-linear regression	predicted efficiency $(\mu/s)\cdot 100$
0	-0.0055	-0.0049	-0.0053	0.00068	-
1	0.0058	0.0060	0.0051	0.00042	0.600
2	0.0141	0.0156	0.0148	0.00048	0.782
3	0.0241	0.0241	0.0238	0.00048	0.804
5	0.0401	0.0384	0.0401	0.00072	0.791

Table 23. Summary of growth constants calculated by both linear and non-linear regression techniques for *T. zillii* and *S. niloticus* fed similar feeds at 27.5°C.

	μ_{max}		S_q		K_s	
	linear	non-linear	linear	non-linear	linear	non-linear
Tilapia zillii						
Trout Chow	0.0641	0.1403	0.37	0.46	5.04	13.7
Spirulina sp.	0.0413	0.1731	0.55	0.69	7.21	30.0
Sarotherodon niloticus						
Trout Chow	0.2552	0.2192	0.34	0.35	22.7	18.9
Spirulina sp.	0.1602	0.2744	0.43	0.50	14.9	26.8

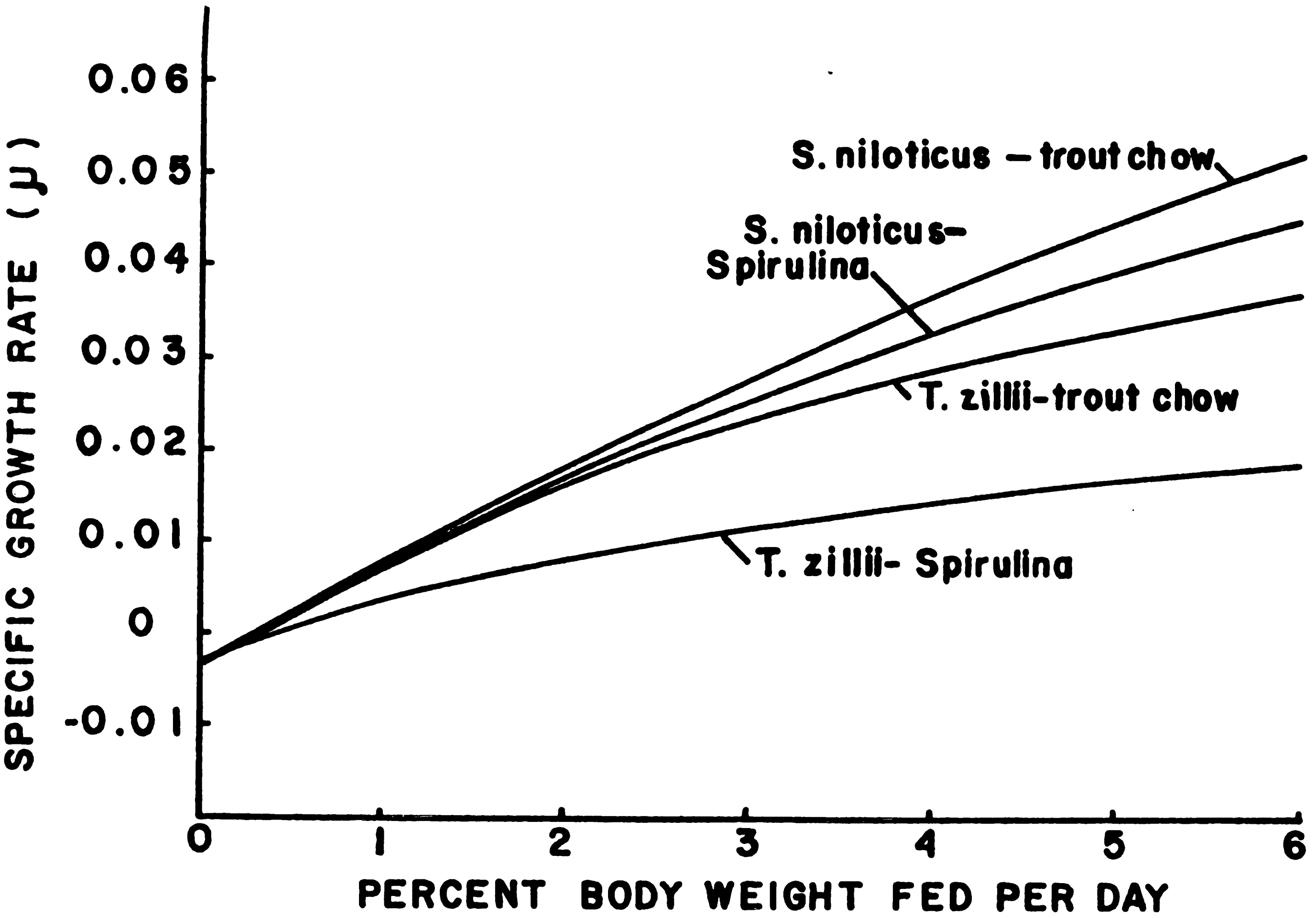

Figure 7. Predicted specific growth rate response for *T. zillii* and *S. niloticus* fed trout chow and pelleted *Spirulina* at 27.5°C.

DISCUSSION

Discussion concerning the usefulness of the proposed model to quantitatively relate fish growth to environmental resources will be divided into the following four areas: 1) variability, 2) growth-feed relationships, 3) growth-size relationships and 4) growth-temperature relationships.

VARIABILITY

An examination of the data indicates that great variability is present in the growth rates among fish at the same feeding level. Variability is present both among fish in the same aquarium and for the individual fish from week to week. While the variability is demonstrated in all the feeding trials, the experiment with *Tilapia zillii* fed trout chow at 27.5°C was chosen as an example. More specifically, an examination of the specific growth rates at the 2% feeding level (Appendix B-3) reveals a range from -0.0088/day to 0.0315/day during the first week for the individual fish. Variability also was present for each individual fish from week to week. Fish #2 experienced a range in specific growth rate from 0.0311/day the first week to -0.0034/day the fourth week. Therefore, growth variability, both for individuals with time and between individuals, is an important consideration.

This variability is the result of several complex factors which interact to determine the ingestion rate, the processing efficiency, assimilation rate and eventually the growth rate. Included in these factors are the genetic makeup of the fish, environmental factors, behavior patterns and sex of the individual. All of these factors interact to determine the rates and efficiencies of the processes which determine the growth rate. Because of the complexities of these interactions, a detailed study of the variables has not been attempted. Rather, the variability itself has been examined to determine how to process and interpret the data. Some general observations on the sources of variability are included.

One factor with a large potential of affecting variability is the ingestion rate. This also provides a good opportunity to demonstrate the attempt to examine the variability itself without attributing a causative mechanism to it. The best example of the variability of ingestion rate occurred during the feeding trial of _Sarotherodon_ _niloticus_ on _Spirulina_. Over the course of the five-week study, fish #0 at 1% feed and fish #2 at 3% feed each were observed to ingest little, if any, feed. At the end of the five weeks, the fish had lost 22.6 and 10.7% of their initial body weight respectively, yet refused to ingest the pelleted _Spirulina_ algae which promoted excellent growth in the other fish sharing the aquarium. After the study was completed, these fish were isolated and fed trout chow. Each fish immediately packed its mouth full to the point where its jaw could not close, and even then attempted to pick up additional pieces which could not fit. The explanation of such behavior is left to other researchers.

A less bizzare example is encountered in the aforementioned feeding trial of _Tilapia zillii_ fed 2% trout chow at 27.5°C. Judging only from the resultant specific growth rates, it appears that the individual fish experienced differential ingestion rates from week to week. If fish #2 had ingested the same percentage feed from week to week, its growth rate would not have so dramatically decreased each week as was the case. Fish #1 experienced the opposite growth pattern, having the lowest specific growth rate initially (-0.0088/day) and the highest the fourth week (0.0210/day). While it is recognized that other variables have the potential of effecting differential growth rates from week to week, I assume that changing ingestion rates is a major factor.

In order to clarify the growth variability associated with differential ingestion, an experiment was designed having three aquaria all containing four _Sarotherodon niloticus_ under similar conditions of feed (3% trout chow), temperature (27.5°C) and size (8.8-14.0 g). The results of the two-week study are shown in Table 24.

Table 24. Specific growth rates of *Sarotherodon niloticus* fed 3% trout chow per day -- four fish per tank.

	Tank 1	Tank 2	Tank 3
Week 1			
Fish 1	0.0296	0.0079	0.0186
Fish 2	0.0164	0.0297	0.0351
Fish 3	0.0307	0.0236	0.0184
Fish 4	0.0319	0.0331	0.0208
Average	0.0273	0.0243	0.0241
all 12 data points	0.0247±0.0043		
average of each tank	0.0252±0.0030		
Week 2			
Fish 1	0.0159	0.0132	0.0135
Fish 2	0.0225	0.0237	0.0201
Fish 3	0.0165	0.0203	0.0194
Fish 4	0.0121	0.0118	0.0209
Average	0.0165	0.0181	0.0186
all 12 data points	0.0175±0.0022		
average of each tank	0.0177±0.0018		
Two-Week Averages			
Fish 1	0.0223	0.0105	0.0161
Fish 2	0.0195	0.0267	0.0276
Fish 3	0.0236	0.0219	0.0189
Fish 4	0.0220	0.0225	0.0209
Average	0.0219	0.0212	0.0214
all 24 data points	0.0215±0.0006		
average of each week	0.0211±0.0038		
two-week average	0.0211±0.0009		

Over the two-week period, there were several reversals in the relative magnitude of growth within each aquarium. In the first two aquaria, the fish with the highest specific growth rate the first week experienced the lowest specific growth rate the second week. In aquarium one, the fish having the lowest first week specific growth rate had the highest second week specific growth rate. Overall, the specific growth rate ranged from 0.0079/day to 0.0351/day.

The data in Table 24 were used in calculating means and 95% confidence intervals for the means. During the first week, if all 12 data points are used in calculating the mean, an average specific growth rate of 0.0247±0.0043/day is obtained. If only the averages for each aquarium are used, the mean specific growth rate is 0.0252±0.0030/day. For the second week, if calculations are based on all 12 data points or just the aquaria averages, the mean specific growth rates are 0.0175±0.0022/day and 0.0177±0.0018/day respectively. Over the two-week period, if all data points are utilized, the mean specific growth rate is 0.0215± 0.0006/day; if the six weekly aquaria averages are used, the mean specific growth rate is calculated as 0.0211± 0.0038/day; and if only the three two-week averages for each aquarium are utilized, the calculated mean is 0.0211± 0.0009/day. It appears that essentially identical means are calculated if either the individual data are utilized or if aquaria averages are used in the calculations. The variability, expressed as standard deviation or confidence interval, is affected by the calculation technique, but the mean is not. Therefore, throughout this study, the mean was chosen as the most representative expression of the data.

The reproducibility between the three aquaria also was monitored in the study. Throughout the two-week study, aquaria two and three contained fish experiencing specific growth rates within 3% of each other. While aquarium one had a slightly higher average specific growth rate the first week, it was slightly lower the second week. The two-week averages demonstrate excellent reproducibility, being within 3.2% of each other.

Another study was conducted to investigate growth variability by placing one, two, three, and four fish in separate aquaria, each fed 3% trout chow under identical conditions. The results of the two-week study are shown in Table 25. Once again in this experiment, variability in individual specific growth rates was significant, ranging from 0.0149/day to 0.0339/day. However, whether the overall growth means were calculated from individual or from aquaria average values, the same two-week values were obtained.

One trend in the mean specific growth rate data, although not different at a 75% significance level (Student's t test), might deserve further attention. As the number of fish per aquarium increased, the growth rate steadily decreased. These differences might reflect an

Table 25. Observed specific growth rates, averages, and 95% confidence intervals (two-tailed t test) for variability study.

	Tank 1	Tank 2	Tank 3	Tank 4
Week 1				
Fish 1	0.0271	0.0339	0.0257	0.0261
Fish 2	-	0.0215	0.0206	0.0272
Fish 3	-	-	0.0310	0.0223
Fish 4	-	-	-	0.0195
Average	0.0271	0.0277 ±0.0391	0.0258 ±0.0088	0.0238 ±0.0042
Week 2				
Fish 1	0.0228	0.0171	0.0149	0.0151
Fish 2	-	0.0161	0.0222	0.0151
Fish 3	-	-	0.0149	0.0163
Fish 4	-	-	-	0.0237
Average	0.0228	0.0166 ±0.00316	0.0173 ±0.0071	0.0175 ±0.00487
Two-Week Averages				
Fish 1	0.0249	0.0255	0.0203	0.0206
Fish 2	-	0.0188	0.0214	0.0211
Fish 3	-	-	0.0230	0.0193
Fish 4	-	-	-	0.0216
Average	0.0249 ±0.0063	0.0222 ±0.0087	0.0215 ±0.0050	0.0207 ±0.0032
Mean calculated from individuals				
	0.0249	0.0222	0.0215	0.0207
Mean calculated from weekly average				
	0.0249 ±0.0017	0.0222 ±0.0045	0.0215 ±0.0035	0.0207 ±0.0026

increased energy expenditure caused by increased activity and aggression as the fish become more crowded.

A trend which was clearly expressed in the two variability feeding trials (Tables 24-25) is that the growth rate tends to decrease during the second week of the experiment. I hypothesize that the growth rate may decrease slightly when fish are experiencing rapid growth. However, the magnitude of decrease seen in all seven variability study aquaria and in 70% of the other feeding trials is too large to attribute to such a mechanism.

A possible explanation of the trend may involve the conditioning of the fish prior to experimentation. Before each feeding trial, the fish were held at the experimental temperature they were to experience, and they were given the same feed for a period of at least three weeks. In each case, the amount of feed was approximated to be between maintenance ration and 1% body weight/day. Bilton and Robins (1973) conducted feeding trials investigating the effects of starvation and subsequent feeding on sockeye salmon fry. They reported that following a one to four week starvation period, fish fed ad libitum experienced accelerated growth. The authors suggested the fish were able to utilize feed more efficiently following starvation. Although the fish used in these studies were not starved prior to the feeding trials, a majority (and in the case of the variability studies, all) of the fish were exposed to higher feeding levels during the experiment. Perhaps the temporarily increased efficiency may be attributed to body stores of important growth components which are lacking other depleted, essential reactants. When the missing reactants are supplied during increased feeding levels, the growth is accelerated until the stores are depleted. Because of this important consideration, the maximum possible data were involved in the calculation of growth constants. Only data associated with incomplete feed utilization were excluded.

The final study investigating variability in growth involved the rearing of three broods of _Sarotherodon niloticus_. Each brood was fed an excess of trout chow from the time the fry were first observed free-swimming until 30 days from hatch. (The first brood was weighed 27 days from hatch.) At this time, each fish was individually weighed and the specific growth rate calculated assuming a uniform initial weight at hatch of 0.002 g. The following table lists the growth data for each brood.

Table 26. Ranges in weight and specific growth rate obtained from three broods of _S_. _niloticus_.

Brood	# fish	weight range (g)	specific growth rate range	Average specific growth rate (S.D)
I	98	0.0617-0.2731	0.1270-0.1821	0.1532±0.0107
II	60	0.0606-0.4805	0.1137-0.1827	0.1513±0.0112
III	105	0.0404-0.7333	0.1002-0.1968	0.1505±0.0260

Once again, these data reflect the same pattern of tremendous variability amongst individuals, yet consistency of the mean. When the broods are compared on the basis of frequency for different size ranges (Figure 8), broods I and II have many similarities. Despite the distinctly different frequency distribution of brood III, the mean specific growth rate is still essentially unchanged from that recorded for broods I and II.

The linear and non-linear regression techniques for determining the constants of μ_{max}, K_s and S_q provided similar results on some feeding trials and very divergent results on others. For the temperature study, the two sets of constants were very similar except for the 27.5°C trial (Table 12). For this experiment, all the non-linear regression constants were extremely high and lacked biological reality. An examination of the data calculated for the size experiments (Table 17) reveals good agreement between the regression techniques for the 0.014 g fish and 38.1 g fish, but not the 1.76 g size. Once again, the non-linear technique produced unrealistically high values for μ_{max} and K_s. Concerning both the feed type and species comparison experiments (Tables 20 and 23), very poor agreement was obtained for all the pelleted Spirulina studies. In both cases, the non-linear regression values for μ_{max} and K_s were comparatively high.

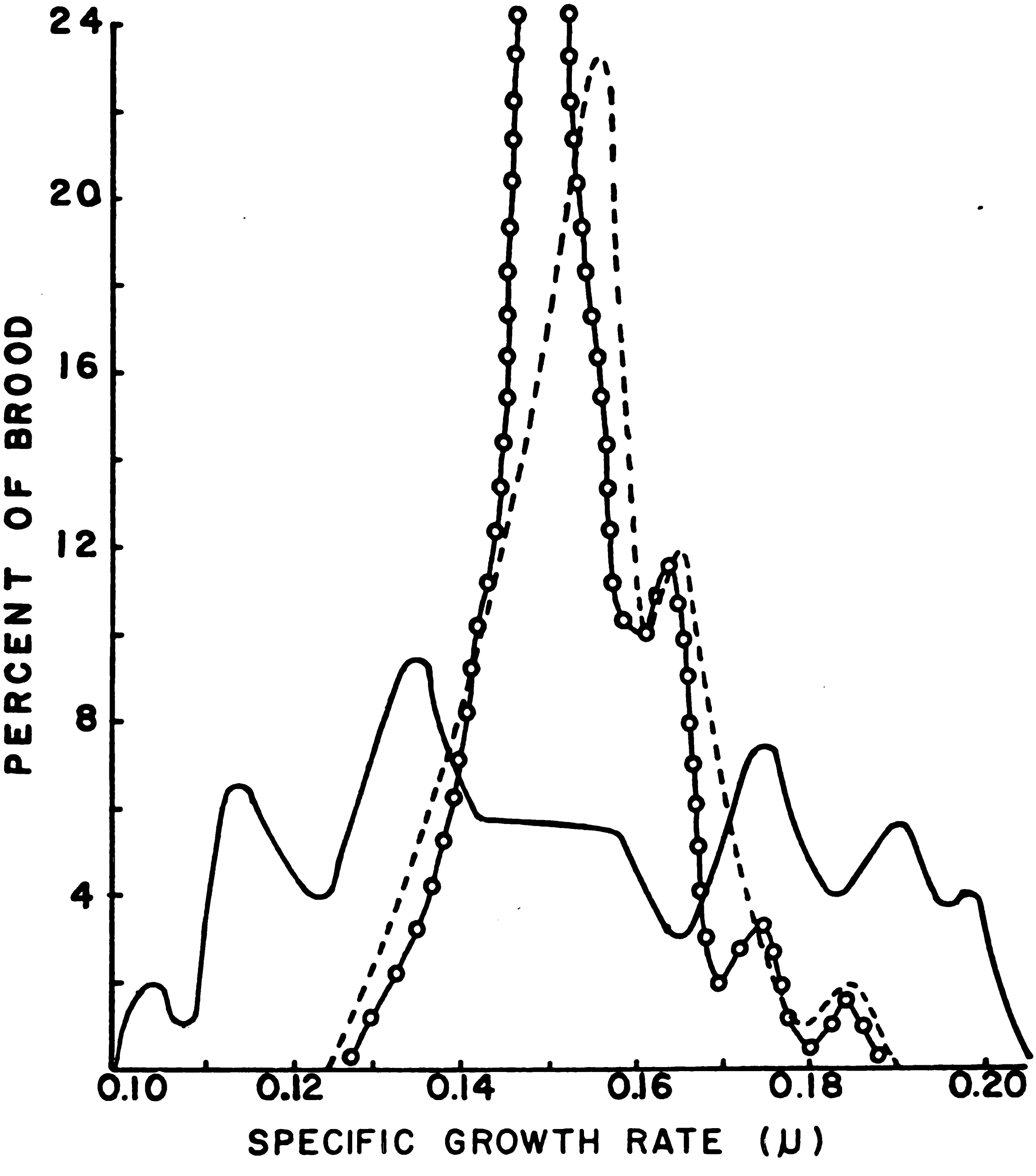

Figure 8. Frequency distribution for the weights of individuals from three broods of S. *niloticus* thirty days from hatch.

An examination of the observed and predicted specific growth rate data reveals that both the linear and non-linear regression techniques provided accurate predictions of growth rates within the experimental feeding range. Despite this similar predictive capacity, some growth constants obtained by non-linear regression were biologically unrealistic. This paradox may be explained by examining the growth curves representing these problematic experiments (Figure 9). Each instance of divergent non-linear regression constants corresponded to a growth curve exhibiting minimal curvature and relatively high specific growth rates. Apparently, when the computer program used to calculate the non-linear regression constants attempted to fit these data to the threshold-corrected hyperbolic equation, the lack of curvature caused the determination of unrealistically high values for μ_{max} and K_s. When closer constraints were placed on the initial estimates for the constants, the program would simply run up to the imposed limits.

While these comparatively high constant values are able to predict specific growth rates within the limited experimental feeding range, they are unable to continue realistic predictions corresponding to higher feeding levels. This is demonstrated in Figure 10 where the predicted growth curves from both linear and non-linear regression constants are plotted for 1.76 g _T. zillii_ fed trout chow at 27.5°C.

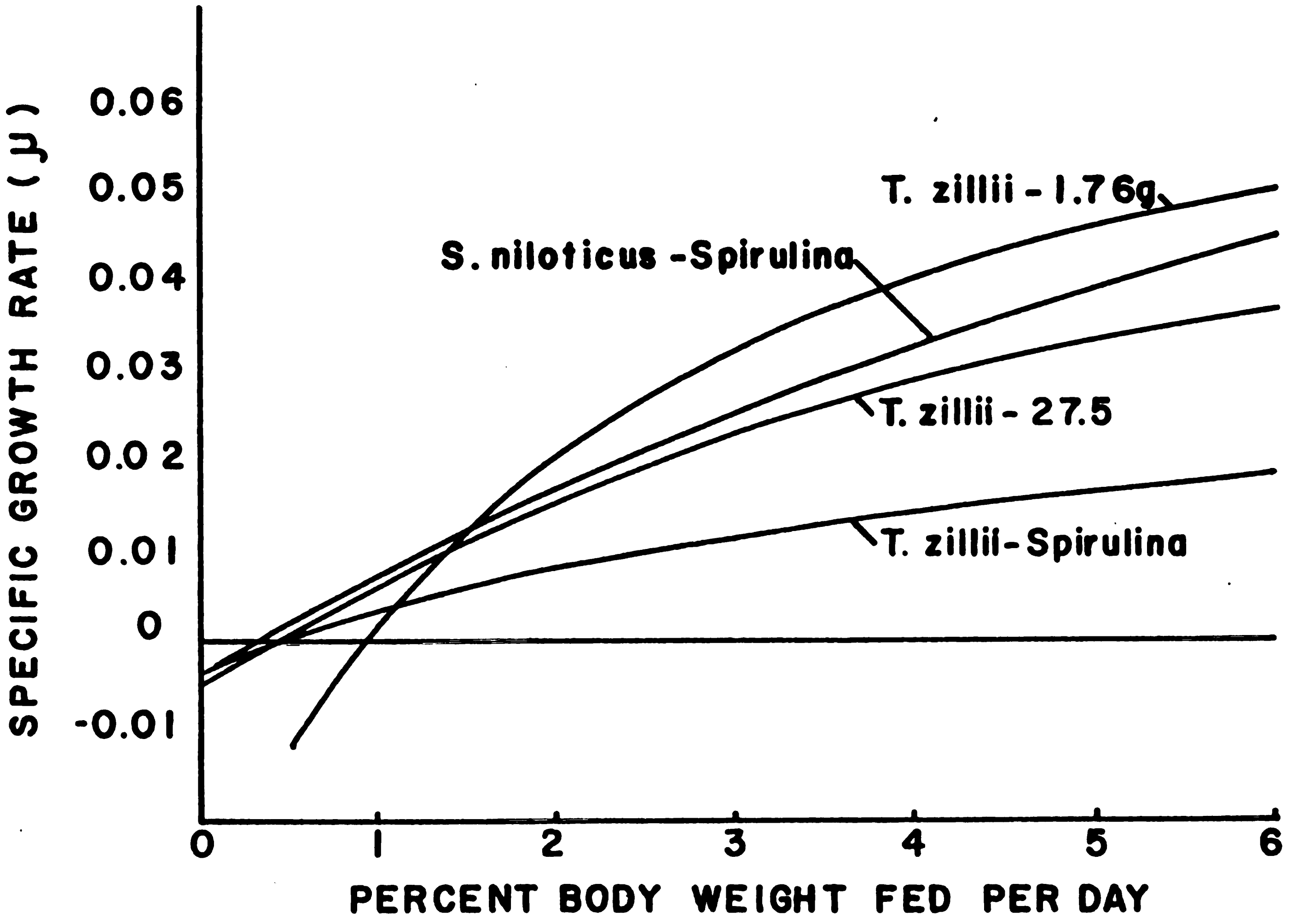

Figure 9. Predicted specific growth rate response for four experiments restricting usage of non-linear regresssion analyses.

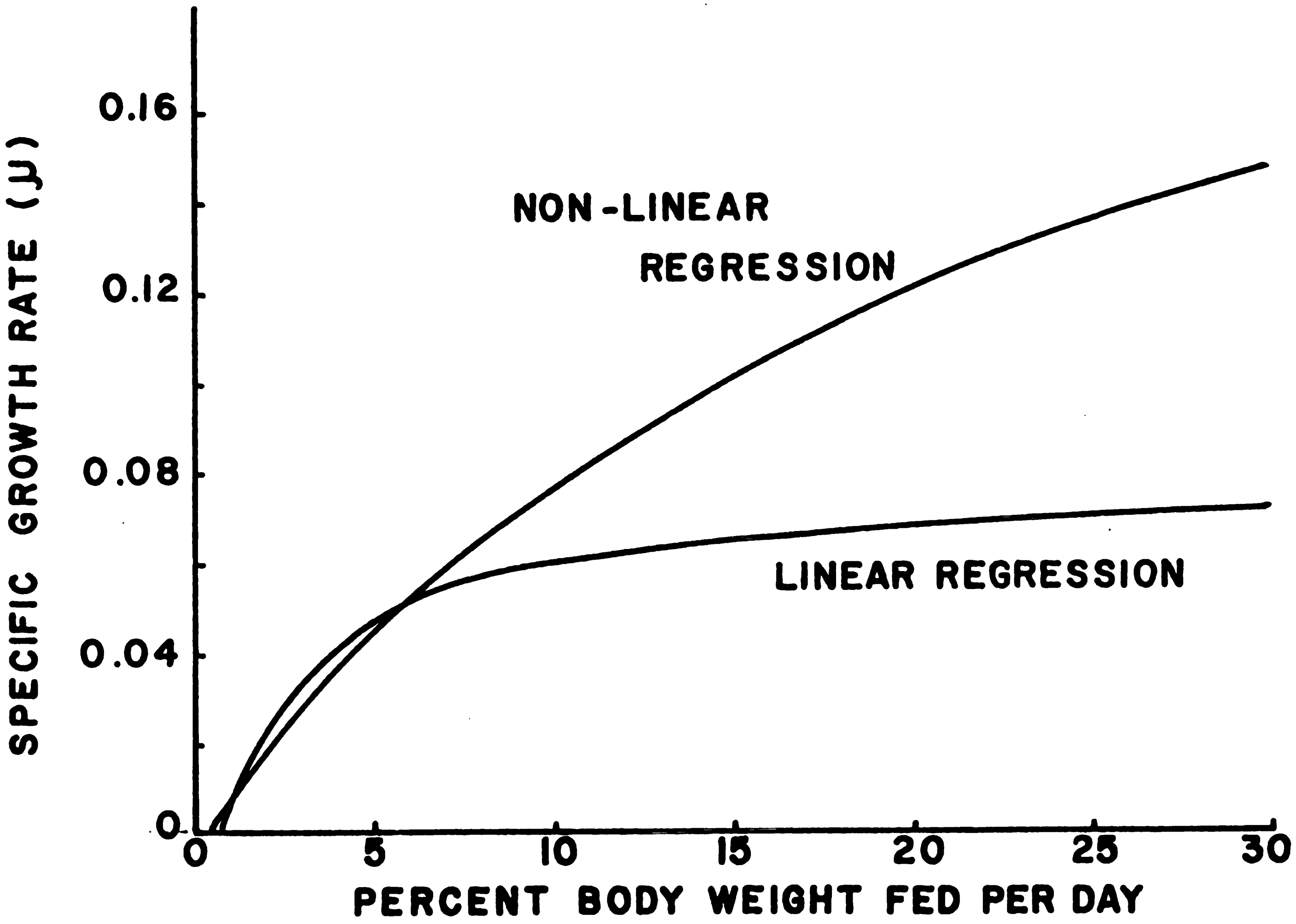

Figure 10. Comparison of the growth response predicted by both linear and non-linear regression analysis for 1.76 g *T. zillii* fed trout chow at 27.5°C.

Because the predicted growth rates are extended over a broad feeding range, the divergence between the regression techniques becomes apparent.

Since the non-linear regression analysis technique was effective in determining constants when sufficient curvature was present, it is hypothesized that if higher feeding levels were utilized, more curvature in the observed data would be evident and the program successfully used. Due to these difficulties, only the constants determined by linear regression are utilized in the discussion of the data.

GROWTH-FEED RELATIONSHIP

The capacity of a feed to stimulate growth of an organism is dependent on its ability to provide all the components necessary for growth to the appropriate reaction sites within the organism. In judging the quality of a feed, there are three basic considerations: 1) the feed must contain the necessary components in sufficient concentration; 2) the organism must be capable of capturing and digesting the feed and transporting the components to the internal reaction sites; and 3) toxins which would hinder growth must not be present.

Feed Quality (Protein-Energy)

While numerous vitamins, minerals and nutrients have been recognized as essential to the growth of fish, this discussion will be limited to examining the relationship between protein, energy and growth. Proteins are large, complex molecules composed of up to 20 amino acids, 10 of which have been shown to be essential to fish (NRC 1983). Ingested amino acids may be assimilated into new protein or used in repair throughout the body. Being a major component of structural units, protein may represent 65-75% of the total body weight on a dry-weight basis. Both quantity and quality are important considerations for protein supply. If insufficient dietary protein is provided, organisms are capable of withdrawing protein from existing tissue to maintain vital functions.

Sufficient protein levels must be supplied to provide for maintenance and activity, and to provide an excess for growth. However, if more protein is supplied than can be incorporated into new tissue, the excess will be used for energy, representing an expensive energy supply. In addition, the proper balance, or quality of amino acids, must be provided. If any essential amino acids are lacking in the diet, tissue production and growth will be limited. The minimum amount of protein (percentage of feed) needed to supply adequate amino acids and produce maximum growth has been determined for several species of fish (NRC 1983). These levels range from 31-38% for the common carp to 56% for *Tilapia aurea* fry.

Several authors have investigated the growth promoting ability of different protein levels in iso-caloric diets.

Davis and Stickney (1978) reported that the growth of Tilapia aurea increased consistently as the percentage of protein was increased in series from 15 to 36%. Mazid et al. (1979) reported similar findings using Tilapia zillii fingerlings fed variable protein levels. As protein levels were increased, up to 35% corresponding increases of growth occurred. However, when protein levels exceeded 35%, gradual growth retardation occurred. Dupree and Sneed (1966) also concluded that increases in protein level up to 40% promote increased growth, but that above 40%, gradual decreases occur. The authors attributed these decreases to the increased metabolic cost of processing the excess protein. Boyd and Goodyear (1971) summarized the importance of protein content by defining it as the most important aspect of the nutritional value of food.

Energy requirements for protein synthesis in fish are reported to be lower than those required by warm-blooded animals due to: 1) avoidance of energy expenditures to maintain a constant body temperature, 2) requiring less energy to maintain their position in an aqueous media, and 3) excretion of nitrogen waste more efficiently as ammonia rather than urea or uric acid (NRC 1983). Nevertheless, energy is still required for maintenance, activity and growth.

Although warmwater fish can utilize proteins, carbohydrates and lipids as energy sources (Garling and Wilson

1976), they are not comparable in either cost or effectiveness. Lipids are an important source for energy because they are less expensive than protein, have a high energy content, and are essential in hormone function, membrane structure and vitamin absorption (NRC 1983).

Sufficient non-protein energy must be supplied to "spare" the expensive protein for growth, yet an energy excess must be avoided to prevent the organism from decreasing protein intake as a result of having satisfied all energy requirements. Because of these considerations, dietary energy requirements are often expressed as a function of dietary protein level. Optimal ratios of kcal digestible energy to grams of protein were reported to range from 7.8 to 9.7 for various fish species (NRC 1983).

The three pelleted feeds fed to Tilapia zillii in this study had a wide range in both protein and energy levels (Appendix D-4). The commercial trout chow was considered a high quality feed providing 40% protein, an 11.4 energy: protein ratio, and all the vitamins and minerals necessary for growth. (Energy units for all pelleted feeds were expressed in units of kcal/g total energy.) The pelleted Spirulina contained an abundance of protein at 60.5%, but a lower energy:protein ratio of 7.3. The protein content of the trout chow supplies approximately 47% of the total feed energy, while the protein content of the Spirulina represents over 73% of the total feed energy. According to the content analysis of Spirulina provided by Microalgae International Sales Corp., lipids supply 13% of the total energy and carbohydrate 14%. The pelleted Elodea canadensis contains a much lower protein content at 21% and a lower energy: protein ratio at 3.8.

The data obtained from the various feeding trials are listed in Tables 18-20. The data fit the linear transformation of the threshold-corrected hyperbolic equation with a coefficient of determination (r^2) of 0.91 ($n = 7$), 0.96 ($n = 5$) and 0.53 ($n = 5$) for trout chow, Elodea, and Spirulina respectively. The range in values for the constants, especially μ_{max} and S_q, indicate that growth stimulation has differed greatly for the three feeds as shown in Figure 6. To facilitate comparison of the effects of the divergent protein concentrations on the growth of the fish, the same growth values are plotted versus protein in Figure 11. Both sets of growth curves clearly demonstrate that the trout chow with an optimal blend of protein, energy and essential components, stimulates superior growth. The pelleted Elodea is only capable of producing a maximal growth rate 18% that of trout chow. In addition, the S_q values indicate that twice as much Elodea is needed to maintain constant body weight. These data from the growth curves clearly reflect the protein and energy deficiencies present in the Elodea. The 20% protein content, the reduced total energy content, and the 3.8 energy:protein ratio of the Elodea would be

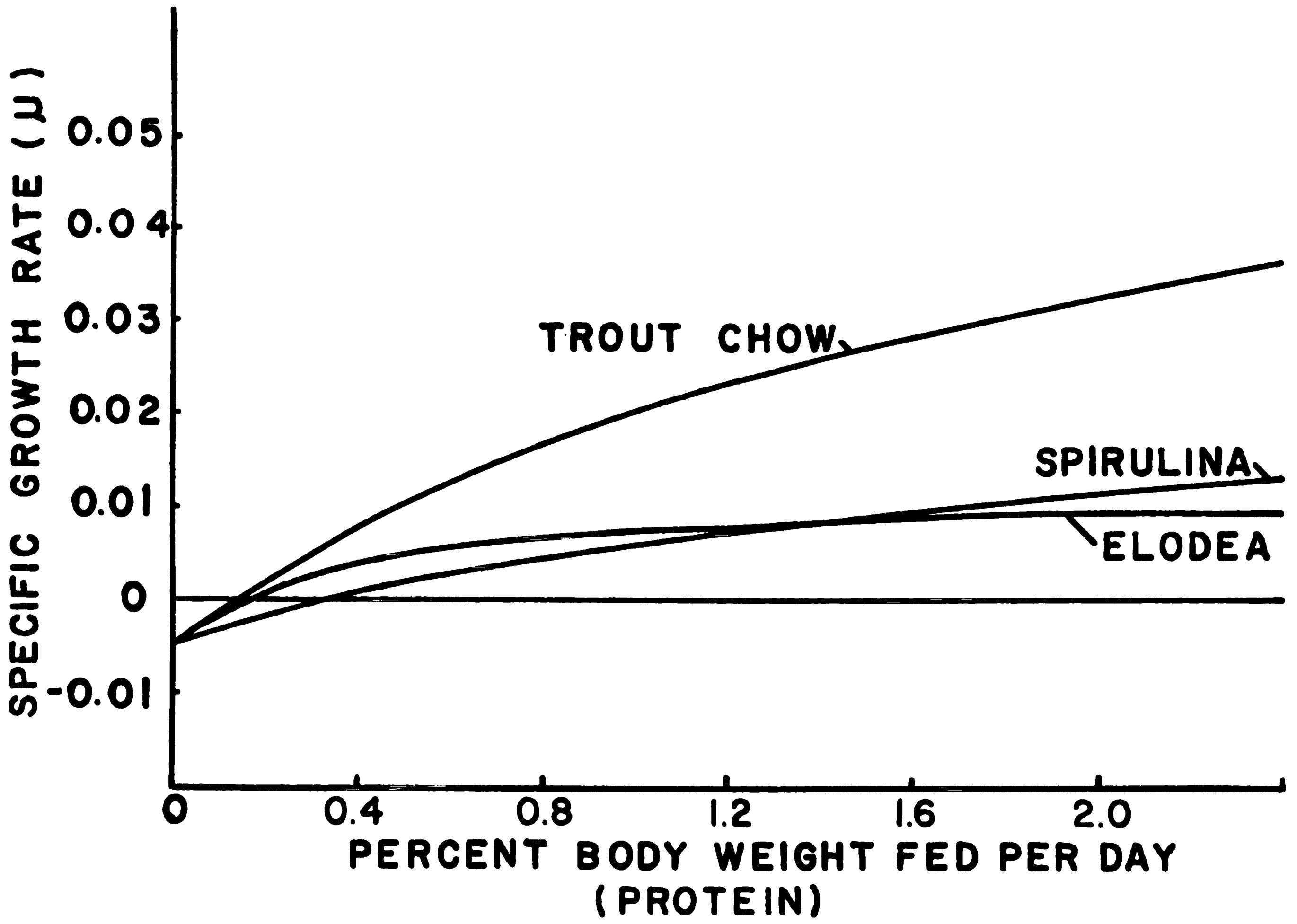

Figure 11. Predicted specific growth rate response of T. zillii fed trout chow, pelleted Spirulina and pelleted E. canadensis at 27.5°C and compared on a protein-fed basis.

expected to cause energy and especially protein shortages, which would limit growth.

Further examination of the two figures reveals that the pelleted Spirulina was capable of producing a μ_{max} only 64% of that for trout chow. This occurred despite a higher protein content at 60.5%. A significantly higher feeding level was also required for maintenance feeding with Spirulina. One possible explanation for this reduced growth stimulation is that the high protein represents an excess. The work of Dupree and Sneed (1966), referred to earlier, indicated that a protein excess may incur additional processing costs and lower growth as the excess nitrogen is eliminated. If this were a major factor, it would not be expected to become evident until relatively high feeding levels are reached. Instead, the Spirulina is shown to be a dramatically inferior feed at even 1% body weight per day (bw/d) where protein would not be in excess.

Another possible explanation lies in the 7.3 energy: protein ratio of Spirulina. This low ratio, in conjunction with protein providing 73% of the total feed energy, indicates that protein will be required to provide a portion of the energy costs of the fish and be unavailable for growth. Therefore, an unknown percentage of protein, probably well below the 60.5%, will actually be available to stimulate growth. This limitation of the protein quality introduces additional considerations. The amino acid content of

Spirulina is listed in Appendix D-2. In comparing these values with the requirements listed by the National Research Council (1983), it is possible that lysine, methionine and phenylalanine all could be growth limiting in *Spirulina*. The combined action of limiting amino acids and protein utilization for energy supply could represent factors which would diminish the ability of the *Spirulina* protein to stimulate growth, despite the high quantity present.

A comparison of the growth curves in Figures 6 and 11 also reveals that while the trout chow consistently stimulated superior growth, the relative positions of the *Elodea* and *Spirulina* growth curves are reversed in the two figures. For equal quantities of protein, the *Elodea canadensis* stimulated superior growth up to a feeding level of approximately 1.5% bw/d-protein. Above this feeding level, the *Spirulina* stimulated superior growth. However, the 1.5% bw/d-protein feeding level is equivalent to about 7.5% bw/d-*Elodea* and only 2.3% bw/d-*Spirulina*. At these feeding levels, there would be dramatic differences in the retention time of feed in the gut, with the *Spirulina* having increased time for digestion and assimilation. The ability of the pelleted *Elodea* to promote better growth than the pelleted *Spirulina* on an equal protein basis again suggests that the quality of the *Spirulina* protein is lacking. The efficiency curves for each feed, shown in Figure 12, reinforce such interpretation. A comparison of the feeding levels at which

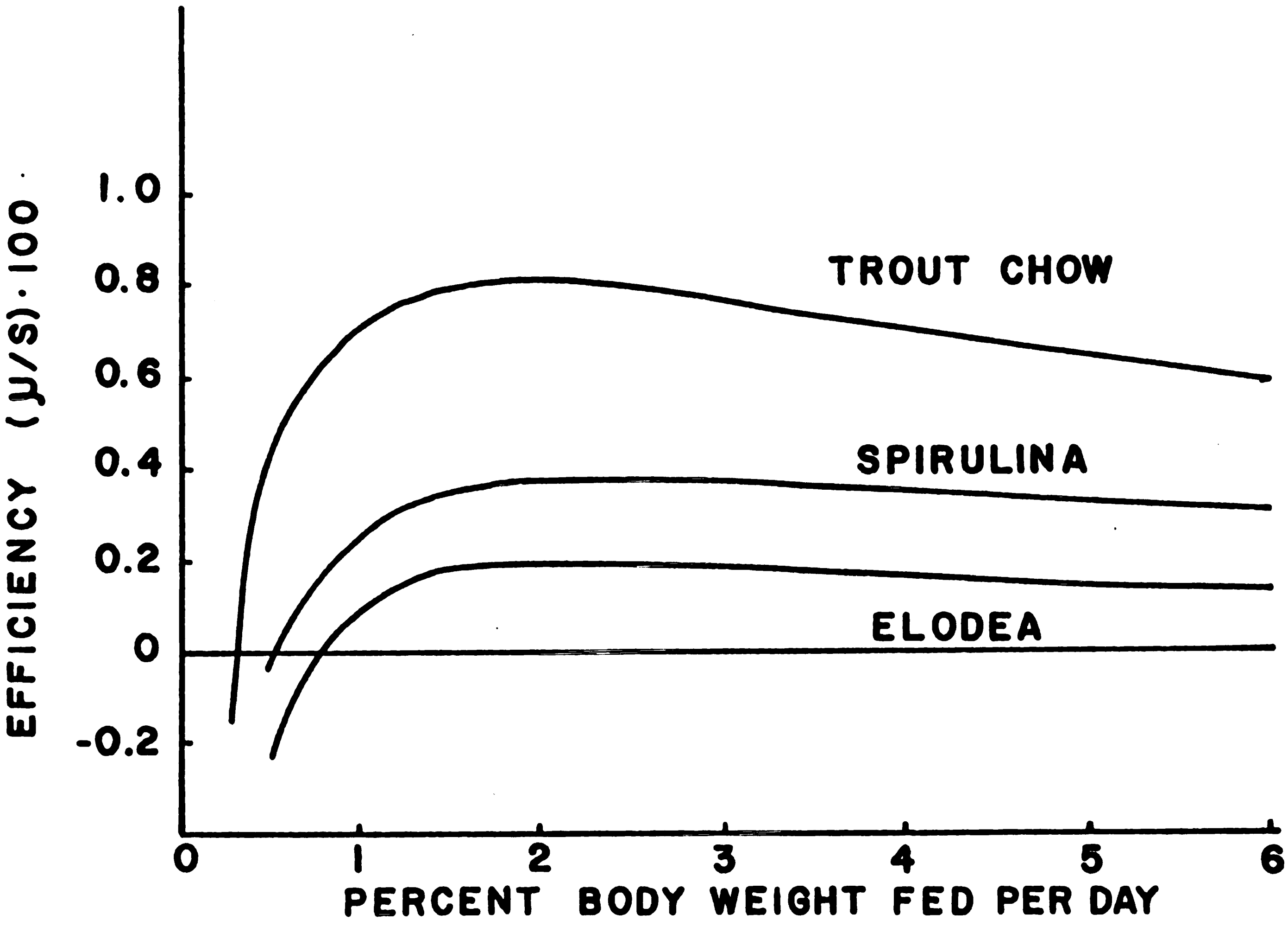

Figure 12. Efficiency growth curves for _T. zillii_ fed trout chow, pelleted _Spirulina_ and pelleted _E. canadensis_ at 27.5°C.

maximum efficiency occurs reveals levels of 1.8%, 2.1% and 2.5% (bw/d) for trout chow, Elodea and Spirulina respectively. The comparatively high value for Spirulina suggests a deficiency in feed quality which necessitates higher feeding levels for high promotion of growth.

Although the consideration to this point has centered on energy and protein, it must be recognized that the fish are integrating the total feed quality into one parameter--growth. The discussion of feed quality was limited to these parameters because they are perhaps the two major criteria when considering feed quality. The enzyme-kinetic growth model was demonstrated to be capable of producing growth curves and constants which could be used to compare and identify the relative potential of the different feeds to stimulate growth based on protein and energy content. Additional experiments, using other feed components as variables, could similarly quantify the relative importance of these parameters in promoting growth. Eventually, such experimentation could identify optimal feed compositions for specific species in unstressed environments.

Harvest and Digestibility

Two other considerations were judged critical in determining the ability of feed to stimulate growth: 1) the presence of toxins, and 2) the capability of the organism to capture and digest the feed and transport the components to the internal reaction sites. While toxins are potentially an important factor causing decreased growth, I do not believe them to have been a factor in these trials. The trout chow is a commercial feed formulation, the powdered *Spirulina* blue-green algae have been approved for human consumption, and the pelleted *Elodea canadensis* have been successfully utilized in feeding trials involving ruminants. However, the other factors of capture, digestion and assimilation efficiency are believed to have considerable import. Feed capture and processing are necessarily related and have been simultaneously influenced by selection pressures. Because of these considerations, the morphological structures and mechanisms associated with feed capture and processing for various cichlids are discussed.

The cichlids, in general, are characterized by unique jaw structures which distinguish them from other families in the order Perciformes. These characteristic structures were described in detail by Liem (1974). The distinctive features of the jaws are as follows: 1) the joint between the skull base and the upper pharyngeal jaw is synovial, offering controlled mobility; 2) the lower pharyngeal jaws are united; and 3) muscles which in other percoids are attached to the dorsal gill arch and upper pharyngeal are instead attached to the lower pharyngeal. These features accomplish three important functions. First, the united, lower pharyngeal jaw provides for increased control and degree of jaw movement. Second, the different muscle attachment allows the lower pharyngeal to exert strong biting action against the upper pharyngeal. Third, the free floating, synovial, upper pharyngeal, with its predominant forward-backward movement, working with the other features provides for efficient food mastication (Liem 1974).

This unique function of the pharyngeal jaws as an efficient food processor removes the responsibility from the premaxillary and mandibular jaw, which usually function as both collectors and processors of food. As a consequence, the cichlid family is characterized by a high degree of specialization of both the pharyngeal jaws and the mandible and premaxillaries. For example, cichlid pharyngeal dentition was shown in scanning electron micrographs to vary from large, blunt teeth in mollusc-eating fish, to pointed, serrated, blade-like teeth in piscivors (Liem 1974).

Bowen (1982) described the pharyngeal teeth as ranging from fine, thin hooked structures on plankton eaters to coarse, robust structures on macrophyte consumers. This specialization and versatility has probably been an important factor which enabled the cichlids to successfully populate numerous, diverse niches (Greenwood 1964; Greenwood 1973; Liem 1974). Several authors (Greenwood 1973; Liem 1974; Brooks 1950; Fryer and Iles 1972) have described the high level of endemism or "explosive speciation" exhibited by cichlids in the Rift Valley Lakes. This evidence of selection has been attributed to the continuous spawning of the cichlids, the long history of tectonic activity in the area, and the specialized mouth structures. In summary, the cichlids as a family are characterized by specialized structures which have enabled different species to compete efficiently for a variety of feed resources.

As a result, it is reasonable to expect that the *T. zillii* and *S. niloticus* used in this study possess specialized structures which would enable them to efficiently utilize particular resources. This is especially true since both species originated in a wide, common range across central Africa, including the Rift Lakes where speciation and specialization is so prevalent (Philippart and Ruwet 1982). In concert with the feeding patterns evident during the early development stages of most fishes, young *Tilapia* (up to two inches) feed on zooplankton (LeRoux 1956; Drenner et al. 1982). Upon maturing, the various species tend to specialize on particular feed resources, although many maintain a varied diet. When confronted with scarcity of their preferential feed, many species readily utilize other resources (LeRoux 1956). *T. zillii* are predominantly macrophyte and filamentous algae feeders (Philippart and Ruwet 1982; Bowen 1982; Buddington 1979; Fryer and Iles 1972).

Microscopic examination of the oral dentition reveals that both jaws contain three rows of teeth with the anterior being bicuspidate, chisel-shaped, and the posterior being tricuspidate. When a leaf is seized, it is gripped firmly by the inner rows and severed by the outer row of blade-like teeth (Fryer and Iles 1972). Because the front teeth are located at the tip of the snout, they have also been observed to function as scrapers of periphyton.

Personal observation showed the gill rakers to be short, rigid, sharply pointed and slightly curved inward. The open spacing and short length suggest a relatively inefficient filtration mechanism. The upper pharyngeal consists of duplicate pads of dense dentition. Individual teeth are stocky, yet narrow with serrated edges facing posteriorly. The lower pharyngeal is a triangularly-shaped, dense pad of dentition. The anterior portion consists of thin, sharp rods, curved and pointed posteriorly. From anterior to posterior, the teeth gradually become broader and more upright until the last few rows closely resemble the upper pharyngeal teeth, with the notable exception that the serration faces anteriorly. The combined assemblage of jaws, gill rakers and pharyngeals appears to provide an efficient mechanism for biting, tearing and shredding material before passage to the stomach.

Adult S. niloticus are reported to effectively feed on phytoplankton (Edwards et al. 1981; Harbott 1975; Moriarty et al. 1973). Moriarty and Moriarty (1973) demonstrated that S. niloticus can assimilate 70-80% of the carbon ingested as blue-green algae. Assuming that this species is able to efficiently assimilate phytoplankton, it is expected that mouth structures, as a result of selection pressures, would be shaped and arranged such that phytoplankton could be separated from the water and transported to the esophagus.

Greenwood (1953), in an examination of the phytophagus _T. esculenta_, described an efficient system for harvesting algae through the combined action of mucus secretion, gill rakers and pharyngeal teeth. When suspended phytoplankton is drawn into the buccal cavity, it becomes mixed with the copious mucus which is secreted from the buccal and pharyngeal epithelium and gill arches. The mucus-algae aggregates are carried posteriorly to the gill rakers which, while not fine enough to filter individual cells, are capable of retaining the aggregates and transporting them to the pharynx. Here the mucus-algae aggregate comes in contact with the anterior pharyngeal teeth. Both the paired upper and fused lower pharyngeal are covered with numerous fine teeth. Those on the anterior, lower pharyngeal are recurved while the posterior teeth are cuspidate and erect. The upper pharyngeals possess a hook-like tip directed toward the esophagus. As the aggregates contact this dentition, the upper pharyngeal is moved backward with the hooked tips passing between the lower pharyngeal teeth, raking the aggregates toward the esophagus.

Moriarty (1973) suggested that the size and shape of the phytoplankton particles affects the effectiveness of filtration of the mucus-algae aggregates with the larger and filamentous forms more efficiently retained. Furthermore, Fryer and Iles (1972) described the filtering action of micro-branchial spines located on the gill arches of *Tilapia*. These numerous, fine projections, each with lateral spines, may act as an efficient sieve trapping very small particles. The particles would be removed from this sieve if the final closure of the opercula were delayed until after the mouth began to open, causing a momentary reverse in current which would blow the entrapped particles into the mucus-covered buccal cavity.

Drenner et al. (1984) suggested that the size selective grazing of *Tilapia aurea* on phytoplankton may be due to the efficient retention of particles by microbranchial spines. This species was stated to size-selectively feed on phytoplankton larger than 25 μm, and escape-selectively feed on zooplankton. No apparent relationship of fish size to the size of particle selectively ingested was seen over a range of 4.3 - 18.7 cm standard length fish.

A microscopic examination of the mouth parts of *S. niloticus* used in this study revealed distinctive contrasts to the *T. zillii* and confirmed the observations of the previously mentioned authors. The *S. niloticus* possessed four irregularly aligned rows of tricuspidate teeth on the upper and lower jaw. The teeth resembled the smaller dentition found in the posterior rows of the *T. zillii* jaw. The gill rakers were fleshy, closely packed projections covered with copious mucous. The upper pharyngeal consisted of paired pads of densely packed teeth. Individual teeth were tall and thin, aligned on a posterior-anterior axis. Each tooth possessed a hook-shaped tip which faced posteriorly. The lower pharyngeal was a triangular pad with the anterior portion bearing thin, sharp rods curved and pointed posteriorly. From anterior to posterior, the teeth become broader and more upright until the last few rows closely resemble the upper pharyngeal teeth, with the notable exception that the hook-shaped tip faces anteriorly.

The most distinctive difference between the two species is the presence of the microbranchial spines on the gill arch of the *S. niloticus*. A single row of projections were observed to exist on the anterior side of the second and third gill arches. Each projection was observed under 400 x magnification to extend lateral spines to each side with spacing approximated at two microns. These spines appeared fully developed when sampled from a 50 g, 15 cm fish with eight to ten lateral spines present on each side of the projection. However, when viewed on a 0.6 g, 3.2 cm fish, the projections were barely visible and only two lateral spines, at most, were present. A 2.2 g, 4.7 cm fish had a maximum of three lateral spines on each side. Once again, when examining the combined assemblage of jaws, gill rakers, microbranchial spines, and pharyngeals, it appears that S. niloticus is capable of processing a variety of feeds, but is especially suited as an adult for processing algae. Until the microbranchial spines develop to provide an efficient filtering mechanism to harvest suspended algae, it would be dependent on larger food particles.

The physiological features involved in the collection, processing and transport of the feed to the esophagus are easily discerned and indicate that each species possesses distinctive features which allow it to efficiently harvest a particular feed type. However, the efficient utilization of the feed involves the capability of digestion and assimilation into body tissues.

Just as each species evidences particular oral structures which enable it to harvest and process particular feeds, so the digestive physiology should evidence features which would facilitate utilization of the feeds. For S. niloticus to successfully utilize phytoplankton, a mechanism must be present to lyse the cell walls, thereby permitting assimilation in the intestine. A biochemical breakdown of the phytoplankton cell walls would require cellulose enzymes, and the mucilagenous sheath of blue-green algae would require hemi-cellulase or RNAase enzymes (Payne 1978). Stickney and Shumway (1974) reported that, based on their investigations into the occurrance of cellulase activity in

62 species, fish lack the ability to form cellulase and must obtain such activity either from their food or through the establishment of a cellulase producing bacterial flora in the alimentary tract. Prys and Blaszczyk (1977) further reported that fish are unable to maintain a specialized cellulolytic gut flora. In the absence of a biochemical capability, a physical or chemical mechanism must be relied upon. Payne (1978) determined that several species of mullet which feed on phytoplankton possess a muscular gizzard containing sand particles. Through the grinding action of this gizzard, the fish is able to physically lyse the cells and render them susceptible to further digestion. However, S. niloticus lacks a gizzard, and the mouth parts described are incapable of lysing small algal cells, so physical disruption of the cell walls seems of little import.

Moriarty and Moriarty (1973) and Moriarty et al. (1973) provided an excellent description of the chemical mechanism S. niloticus employs to lyse phytoplankton cell walls. They reported that acid secretion in the sac-like stomach of the fish follows a diurnal cycle associated with feeding activity. At dawn, before feeding has commenced, the stomach is empty and the pH is 7.0. As feeding begins, the stomach expands and acid secretion is triggered, dropping the pH to 2.0-3.0. As feeding continues, a large portion of the algae is reportedly not subject to sufficiently low pH, and the digestion and assimilation are inefficient. Higher pH values are generally apparent in the upper stomach while only in the lower stomach, where the pH drops to 1.4, is cell lysis effective. After feeding ceases, the acid secretion continues for some time and the accumulated algae are all subjected to pH values well below 2.0. When this occurs, the algal cells were observed to be completely lysed and the subsequent digestion and assimilation almost complete. Harbott (1975) reported similar observations on stomach pH values for _S_. _niloticus_ and also determined that cells of the blue-green algae _Microcystis_ were lysed in vitro by acid conditions below pH 1.65. The efficiency of digestion and assimilation of phytoplankton was thus demonstrated to be dependent on sufficient exposure to acid conditions below pH 2.0 which would lyse the cell walls.

While studies are lacking identifying the particular digestive mechanisms in _T_. _zillii_, they have been shown capable of efficient digestion of macrophytes. Buddington (1979) reported that when _T_. _zillii_ were fed _Najas_ _guadalupensis_, they were capable of assimilating 75.1% of the protein, 75.9% of the fat, and 45.4% of the caloric content of the plant.

It was previously expressed that an evaluation of feed quality must involve joint considerations of harvestability, composition and digestibility. This seems especially appropriate in light of the evidence that selective pressures have resulted in simultaneous physiological refinements in the ability of particular species to harvest and digest particular feeds. If this understanding is correct, then a comparison of the growth of different species on different feed types should reflect not only the quality of feed composition, but also the relative pre-digestion processing abilities and the digestion-assimilation efficiencies. It was for this reason that I designed an experiment to determine if the proposed threshold-corrected hyperbolic equation was effective in quantitatively evaluating the digestive-assimilation efficiencies of different species utilizing different feeds.

Species Comparison

In this study, both *S. niloticus* and *T. zillii* were each fed both pelleted trout chow and the blue-green algae *Spirulina*. The results are summarized in Table 23 and Figure 7. In addition, the efficiencies for each of the feeding trials are shown in Figure 12. It is apparent from the constants and the growth curves that each species consistently demonstrated superior growth when fed trout chow. The trout chow is formulated to promote excellent growth and may have stimulated growth close to each species' genetic capability. This represents another important capacity of the growth model. Based on this assumption, the comparison of the growths of the two species on *Spirulina* leads to significant conclusions.

When the efficiencies of growth (from 1-7% bw/day) for each species on Spirulina are calculated as a percentage of their efficiency of growth on trout chow, the T. zillii ranged from 36.3% to 51.2%, while the S. niloticus ranged from 52.8% to 91.5%. While each species was unable to fully utilize the high protein content of the Spirulina to promote growth, the S. niloticus was clearly more efficient than T. zillii.

This relative difference in ability to utilize pelleted Spirulina to stimulate growth may be attributed to either different abilities to digest the feed, or different abilities to assimilate the components, or both. The ability of fish to digest Spirulina, or any alga, has been noted to be dependent on an ability to break down the cell walls. The digestive system of S. niloticus has been documented as being capable of lysing algal cell walls, and this study confirms its ability to grow well with Spirulina as the sole food source. The inferior growth response exhibited by both species when fed Spirulina, as compared with trout chow, may again be attributed to either limiting amino acids or protein utilization for energy rather than growth.

The literature has documented no physiological adaptations which would enable T. zillii to lyse algal cell walls. Instead, this species has been consistently described as a macrophyte feeder. The digestive conditions necessary to process macrophytes would probably not provide the biochemical, chemical or physical mechanisms necessary to lyse algal cell walls. Yet the _T. zillii_ was able to achieve a measure of growth on the pelleted _Spirulina_. This could be, at least partially, attributed to previous lysing of algal cell walls during the harvesting-drying-pelleting process. Microscopic examination of the powdered algae revealed that many of the cell walls were ruptured. If only a portion of the algal cell walls were lysed prior to ingestion by the fish, and only the _S. niloticus_ were capable of lysing the remaining percentage, then the relative difference in ability to utilize the _Spirulina_ could occur.

It is also possible that the relative efficiencies of utilization of pelleted _Spirulina_ by the two species is due to differential assimilations of equally digested materials. If selective pressures have resulted in one species possessing physical mechanisms which enable it to effectively utilize a particular feed, those same pressures may have resulted in changes in assimilation processes which facilitate the effective incorporation of feed into tissue by the same species. Quite possibly, an interaction of both differential digestion and assimilation produce the differential efficiencies demonstrated by the growth curves.

The threshold-corrected hyperbolic equation is thus shown capable of quantitatively identifying the ability of different species to utilize different feeds. Some of the general sources of the differential efficiencies may be identified by comparisons of the growth curves. However, it should be understood that when growth is related to substrate, the growth parameter represents the integration of all the complicated mechanisms, processes and reactions associated with feed harvest, digestion and assimilation. Thus far, the model has been effective in relating the quality of different feeds to one species and also comparing species on different feeds. These studies, because of their structure, have been limited to examining the growth response due to composition of feed and the ability to digest and assimilate. The remaining variable in the consideration of feed and growth was harvestability.

Although both _T. zillii_ and _S. niloticus_ were shown capable of growing on pelleted _Spirulina_, this has little relevance to real-world situations. The mechanical harvesting of algae is usually inefficient and energy intensive. To attain practical relevance as a feed source, the fish must demonstrate an ability to harvest the algae directly from the water and effectively assimilate it.

Numerous authors (Melack 1976; Almazon and Boyd 1978; Edwards et al. 1981) have reported correlations between _Tilapia_ growth and levels of primary production or plankton abundance. While these studies are useful in determining general relationships, they have not related fish growth to particular feeds or identified the source or means of harvest, whether scraping of periphyton, size selective capture of zooplankton, indiscriminate filtration, etc.

A brief experiment was conducted to determine the ability of both fish species to harvest live suspended Spirulina from the water. Two 20 l aquaria, each equipped with 150 watt heaters and 15 cm air bars, received 15 l of conditioned water which was maintained at 27.5°C. Approximately 200 ml of a dense, live Spirulina culture were added to each aquarium. After extensive aeration and mixing, a 10 ml aliquot was removed, diluted, and the algal cell concentration determined by microscopic examination. These concentrations were then utilized to calculate the total number of algal cells per aquaria. Following this initial cell count, a 22 g S. niloticus was added to one aquarium while the other remained a control without fish. Subsequent sampling for cell count analysis followed the schedule listed in Table 27. Following completion of this experiment, an identical trial was performed using the same S. niloticus in one aquarium and a 47 g T. zillii in the other. The results of cell count analysis in all four trials are contained in Table 27.

Over the period of 32 hours, the T. zillii was able to harvest $2.71 \cdot 10^9$ cells. These cells were estimated to weigh 0.271 g (avg. cell weight was $1.0 \cdot 10^{-7}$mg-dry weight) on a dry-weight basis or 4.44 g on a wet-weight basis (93.9% moisture). Therefore, this fish was able to harvest 9.4% of

Table 27. Concentration of _Spirulina_ sp. cells in 15.2 l volume.

Time from spike of algae (hours)	Control $\cdot 10^9$cells	_Tilapia zillii_ $\cdot 10^9$cells	_Sarotherodon niloticus_ Trial I $\cdot 10^9$cells	_Sarotherodon niloticus_ Trial II $\cdot 10^9$ cells
0	13.5	9.50	10.01	12.01
2	-	9.59	10.66	7.74
4	-	10.88	10.28	4.97
6	-	10.29	9.19	4.00
8	-	8.91	6.66	3.32
10	-	9.46	4.81	2.76
12.5	-	9.26	2.70	1.36
24	-	8.49	2.85	0.45
28	-	8.39	1.32	-
32	13.0	7.67	1.07	0.17

its body weight over the study period or 14% of the total algal cells present. The small decrease in cell number in the control aquarium indicates that some adsorption and settling of algal cells did occur. These aggregations of cells could provide opportunities for capture apart from filtration of suspended algal cells.

The *S. niloticus*, during the first trial, was able to harvest $8.44 \cdot 10^9$ cells which represented 84.3% of the total cells present. The filtration efficiency of the fish, which had previously received only pelleted feeds, improved during the second trial when it filtered out $11.3 \cdot 10^9$ cells or 94.4% of the total algal cells present. Therefore, on the average, the fish was able to harvest 73.6% of its body weight over the study period. Visual observations clearly indicated that, based on either the improved clarity of the water, or the abundant green feces present, the *S. niloticus* could effectively filter suspended *Spirulina* cells from the water. Microscopic examination of the greenish feces revealed that many filaments of *Spirulina* were still intact and that limited cell disruption had occurred. Nitrogen analysis of these feces indicated that almost 79% of the original concentration remained. However, the transformation of suspended algae to fecal material does not represent conversion to an unavailable form for *S. niloticus*. I have observed that *S. niloticus* in holding aquaria receiving limited feed are effective in ingesting and recycling fecal material.

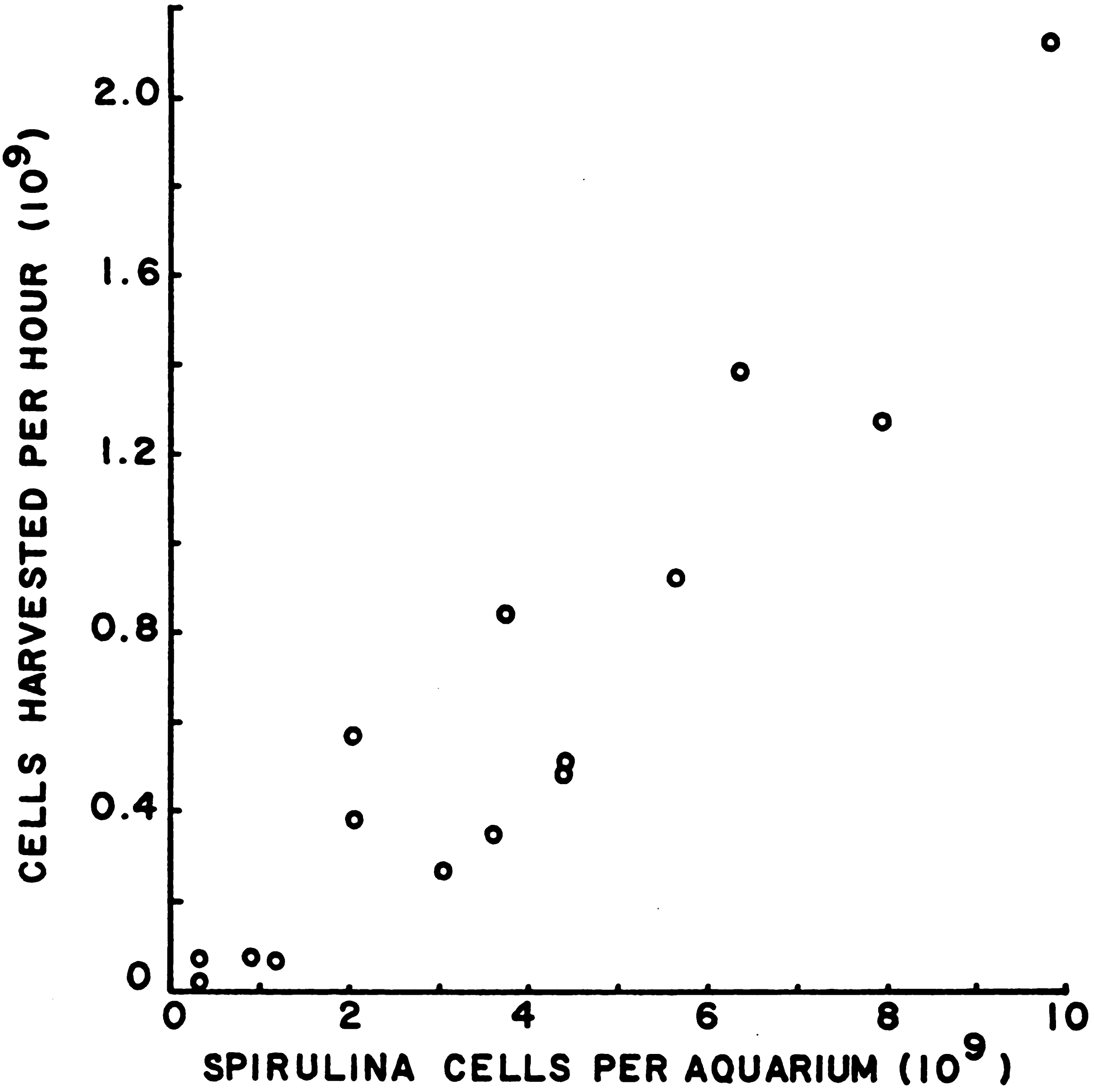

Figure 13. Relationship between the ability of *S. niloticus* to harvest suspended *Spirulina* cells and the concentration of suspended cells.

Following a six hour acclimation period, the removal rate of Spirulina cells by S. niloticus decreased dramatically with time in each trial. The rate of filtering of S. niloticus was examined by plotting the cells harvested/hour against the total cell concentration. These data are plotted in Figure 13 (with the exception of the initial six hours of the first trial). The data indicate that harvest rate is directly related to cell concentration up to an average aquarium concentration of almost $1 \cdot 10^{10}$ cells or $6.58 \cdot 10^{5}$ cells/ml. It was expected that the microbranchial spine-mucous harvest mechanism would become saturated at a high cell density and the harvest rate level off. This saturation was not evident at a cell concentration representing an extremely dense algal bloom.

Although there was no question concerning the ability of S. niloticus to harvest suspended Spirulina cells from the water, the tremendous passage rate through the gut left some doubt concerning its ability to effectively grow under these conditions. Therefore, the same fish was used on a longer term, four-day experiment, during which the aquarium received daily additions of the Spirulina culture to maintain initial concentrations of $1.62 \cdot 10^{10}$ cells/aquarium. Over the four-day period, the fish harvested $5.36 \cdot 10^{10}$ suspended Spirulina cells, or 5.38 g dry-weight (87.9 g wet-weight). Obviously, this represents a tremendous ingestion rate--almost 100% bw/day-wet weight. Over the four-day period, the fish grew from 22.0 g to 23.6 g, a specific growth rate of 0.0176/day. Despite the rapid gut passage rate, the fish was able to withdraw sufficient nutrients to sustain growth. The actual growth of the *S. niloticus* may be of lesser practical significance than the removal of the suspended algae from the water column. The conversion of the suspended algae into fecal material accomplishes two additional important functions. First, it serves to greatly clarify the water which would have profound consequences for the water chemistry and the biota. Second, it could serve to transform the previously unavailable suspended algae into a practical, high quality food source. The fecal material is easily ingested and potentially of high protein content. These factors are of obvious practical usefulness for aquaculture and especially polyculture.

GROWTH-SIZE RELATIONSHIP

Body size has long been recognized as a factor affecting the growth of organisms. Brett and Shelbourn (1975) stated that the fundamental laws of growth for all animals attest to a continuous decrease in growth rate with increasing size subject to some variation in the rate of decrease according to developmental stages. Bertalanffy (1957) identified the following individual parameters as being affected by body size: total metabolism, heart rate, respiratory rate, excretion, chemical composition, and enzyme content. Despite the understanding of these relationships, the exact causative mechanism relating size and growth are poorly understood.

Bertalanffy (1957), in his study of metabolism, indirectly related body size to growth. Initially, a relationship between metabolic rate and body surface area was examined. The surface area of a body was shown to be directly related to the metabolic rate as shown in Rubner's famous surface rule. Unfortunately, there are considerable difficulties involved in measuring surface area. The surface area was, in turn, shown to be directly related to weight. The formula of Meeh, $S = bw^{2/3}$, expresses that surface area (S) is obtained by multiplying the 2/3 power of the weight (W) by an appropriate constant (b). By combining these considerations, the surface rule of metabolism accordingly states that the metabolic rate (M) is proportional to the fractional power of the weight, or $M = bW^{\delta}$. Therefore, as the body weight increases, the relative metabolic rate decreases.

Bertalanffy then continued by defining three basic types of metabolism. The first type, characteristic of fish, has a metabolic rate which was related to surface area by the 2/3 power of weight. Other types of metabolism were either directly related to weight or by a power intermediate between 2/3 and one. Several purported bases for these relationships were then reviewed and found unsatisfactory. These included: thermoregulation, anatomy and physiology of the circulatory system, types of respiratory apparatus, anatomical or chemical changes related to body size, respiration changes at the cellular level, and changes in enzymatic systems. While each factor may be involved in establishing the size-growth relationship, none were singularly capable of complete explanation. Bertalanffy (1957) postulated the presence of unidentified factors in the intact organism which regulated the respiration of tissues and, therefore, metabolism.

Following his relating of metabolism to size, Bertalanffy developed a direct relationship between growth and metabolism, and also thereby, a relationship between size and growth. The basic consideration was that growth was the result of a counteraction of synthesis and destruction. This interaction was explained by the general formula:

$$\frac{dw}{dt} = nW^m - kW^n$$

Here the change in body weight with time ($\frac{dw}{dt}$) is equal to the difference between anabolism (nW^m) and catabolism (kW^n). The exponents m and n indicate that both processes are proportional to some power of the body weight (W). This important assumption was justified by the consideration that all physiological processes hitherto investigated can be expressed in such a power formula. Based on various physiological facts, the catabolism exponent is approximated to be one. The exponent of the anabolism (metabolism) was previously shown to be 2/3 for fish. Therefore, the general formula becomes:

$$\frac{dw}{dt} = nW^{2/3} - kW$$

This theoretical formula for growth, based on a weight-metabolic rate relationship, represents a sigmoid growth curve.

Paloheimo and Dickie (1965), while appreciating the value of growth curves for comparison purposes, stressed the importance of the biological interpretation of the parameters describing the statistical fitting. As a result, the authors questioned the general physiological interpretation offered by Bertalanffy and suggested an alternative consideration by relating growth to energy. Rather than the abstract, nonmeasurable concepts of anabolism and catabolism, growth was related to quantifiable energy parameters as shown in the basic energy formula:

$$\frac{dw}{dt} = pR - T$$

Here the growth is equal to the metabolizable energy content of the ration (pR) minus the total metabolic energy expenditure (T). The metabolic energy cost is again related to weight as $T = \alpha W^{\delta}$, where α is a constant and δ defines the rate of change in metabolism with body weight. Therefore, the general formula becomes:

$$\frac{dw}{dt} = pR - \alpha W^{\delta}$$

The authors then continued with a lengthy examination of ration energy and feeding efficiency, concluding that growth efficiency decreases for each unit increase in ration. Therefore, as either weight or ration increases, the growth efficiency is expected to decrease.

Elliott (1976), in his consideration of the energetics of feeding brown trout, developed formulas which related both the energy of metabolism and food intake to the weight of the fish. Both formulas were identical as shown below:

$$\text{Energy intake (or metabolism)} = aW^{b_1} e^{b_2 T}$$

Here W represented weight, a, b_1 and b_2 were constants determined empirically, and T was temperature. Because b_1 and b_2 are fractional constants, both energy intake and/or energy expenditure per unit would decrease with increased weight. The change in rate of growth would be a function of the relative rate of decrease in both energy intake and metabolism expressed by the empirical constants a, b_1 and b_2.

A comparison of these theoretical considerations reveals important common factors. Although based on different assumptions and underlying principles, each analysis concluded that the metabolic rate (catabolism) would decrease proportionately to a fractional power of weight of the organism. Bertalanffy (1957) supported this hypothesis even though he expressed catabolism in the form kW^n with $n = 1$. The setting of n to 1 was a means of mathematical simplification justified by the belief that the deviation from unity was small and the basic equation rather insensitive to the change. A second common principle was that the energy intake rate or anabolism would also decrease proportionately to a fractional power of weight of the organism. Bertalanffy (1957) expressed anabolism as proportional to the 2/3 power of weight for fish, while Elliott (1976) and Paloheimo and Dickie (1965) expressed the energy intake as proportional to empirically determined fractional powers of the weight. All three authors similarly conclude that growth rate is related to weight as a function of these interacting processes, both of which decrease with increasing size.

Empirical research tends to confirm many of these theoretical expectations. Niimi and Beamish (1974) reported that the amount of ration representing satiation decreases with increasing size in largemouth bass. Furthermore, the maintenance costs for zero growth (S_q) was also shown to decrease with increased size. Brett (1979) reported that increased size had a greater restricting effect on growth than on metabolic rate, though both were affected; and also suggested that age, independent of size, may have some effect on growth rates. In reviewing growth curves reported for three species of fish, Brett reported that increasing size resulted in a decreasing maximal growth rate and decreased maintenance levels per unit weight for all species. Gerking (1971), studying the effect of body weight on protein metabolism of bluegill sunfish, determined that the efficiency of protein utilization steadily decreased as the weight of the fish increased. The author suggested that the decreased efficiency may, in part, be attributed to a decreased rate of feeding with increased size but offered no further explanation.

Both theoretical expectations and empirical studies agree that growth rate and maintenance costs decrease with increased size. This phenomenon has been generally related to numerous physiological processes and feeding energetics. Unfortunately, no definitive explanation has been found which precisely explains the controlling mechanisms. The relationship may be the cumulative expression of several of the aforementioned variables interacting with age and genetic composition. I do not intend to propose that the enzyme-kinetic growth model defines a single controlling factor producing the weight-growth relationship. Instead, I propose the model as a tool to quantitatively monitor the weight-growth relationship by taking a holistic look at the system. Whether the actual mechanisms producing the response are controlled at the cellular or organismic level, whether it's more genetically or environmentally influenced, or whether it's any of a number of interacting, complex physiological processes, the model proposes to utilize the fish as an integrator of all the components.

Because each of the size-related biochemical processes is governed by enzyme-catalyzed reactions, it is proposed that the integrated response will follow the same pattern which typifies the singular enzyme-substrate reaction. Although the rate of reaction may change with size, the basic nature of the reaction remains unchanged. Therefore, whether at the singular reaction level or the complex, integrated organismic level, the response pattern is similarly characterized by the threshold-corrected hyperbolic growth model.

If it were true that the size of an organism affected, by whatever means, the rate of delivery of substrates or the balance between anabolism and catabolism, these changes should be reflected in the integrated growth response to substrate level. Thus, the model is developed with consideration of the organism's interaction with the environment and how that interaction changes with the increasing size of the organism.

The results of the experiments dealing with size and growth are contained in Tables 14-17 and Figure 5. In addition, the efficiency curve for each size class fish is plotted in Figure 14. It is apparent from Figure 5 that the growth curve for each size class of T. zillii closely

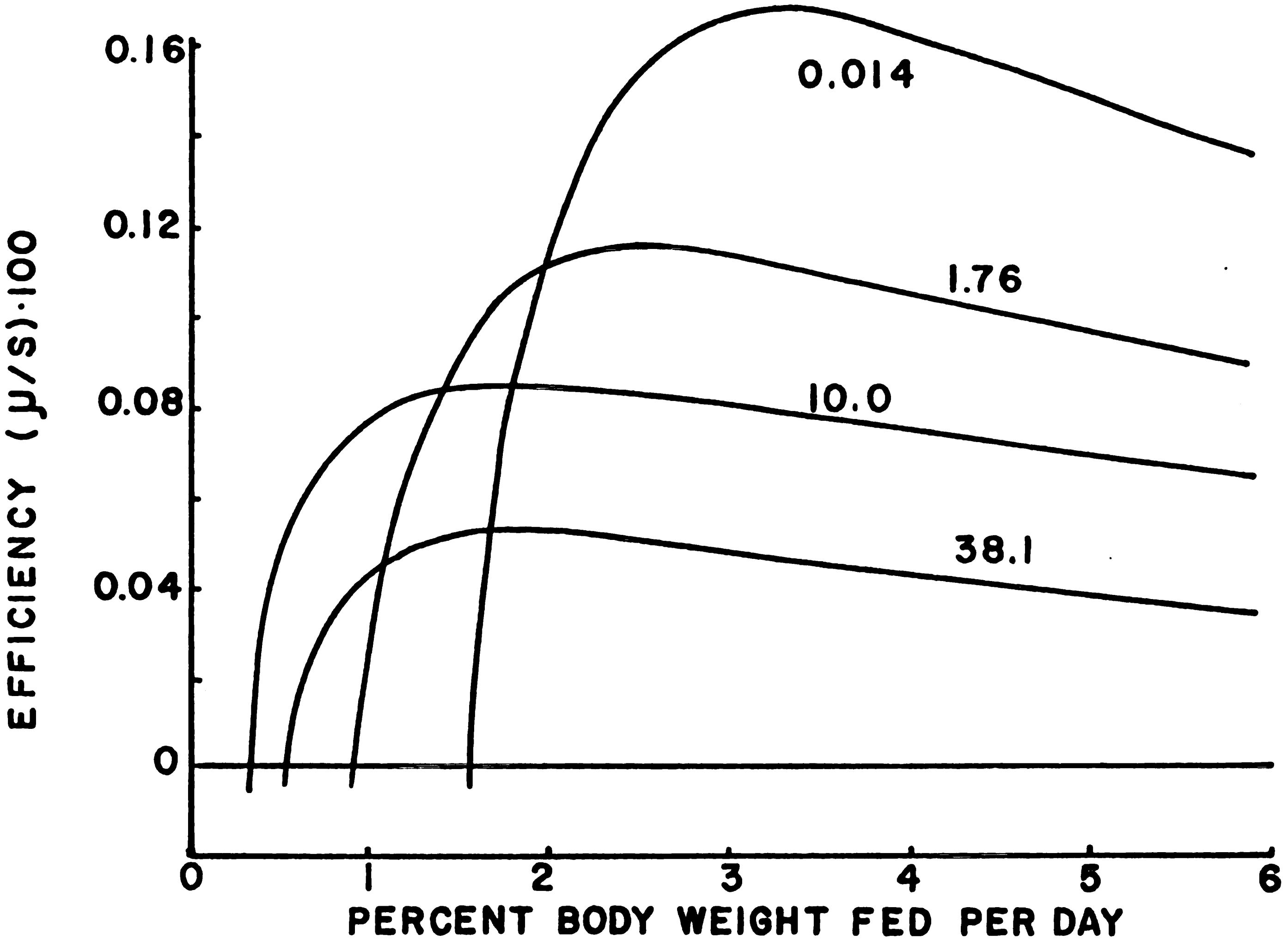

Figure 14. Efficiency growth curves for four size classes of _T. zillii_ (average weights 0.014 g, 1.76 g, 10.0 g and 38.1 g) fed trout chow at 27.5°C.

follows the threshold-corrected hyperbolic curve model. A regression of the observed growth data, utilizing the transformed linear equation, results in coefficients of determination (r^2) of 0.99 ($n = 5$), 0.71 ($n = 5$), 0.91 ($n = 7$) and 0.97 ($n = 4$) for the 0.014, 1.76, 10.0, and 38.1 g size classes respectively. The data demonstrate that the model is capable of quantitatively expressing the integrated growth response as a function of varying substrate concentrations for the various sizes of fish. Although the general shape of each curve adheres to the model, the individual constants of each curve reflect distinct differences which, by comparison, provides insight into the growth dynamics of the fish.

Overall, the most apparent contrast between the curves is that at high feeding levels (above 3%), there is a dramatic decrease in growth and efficiency with increasing size. These findings correspond with both the theoretical expectations and previous research reports on fish growth. This decrease in growth and efficiency is especially significant since the maximum growth rate (μ_{max}) has been reduced by 2/3 when the fish has attained only a 38 g size. If the fish were to reach their potential of about 1 kg, the great majority of growth would occur at a specific growth rate below 0.03.

The calculated values of μ_{max} for the different size classes of fish are plotted in Figure 15, along with the

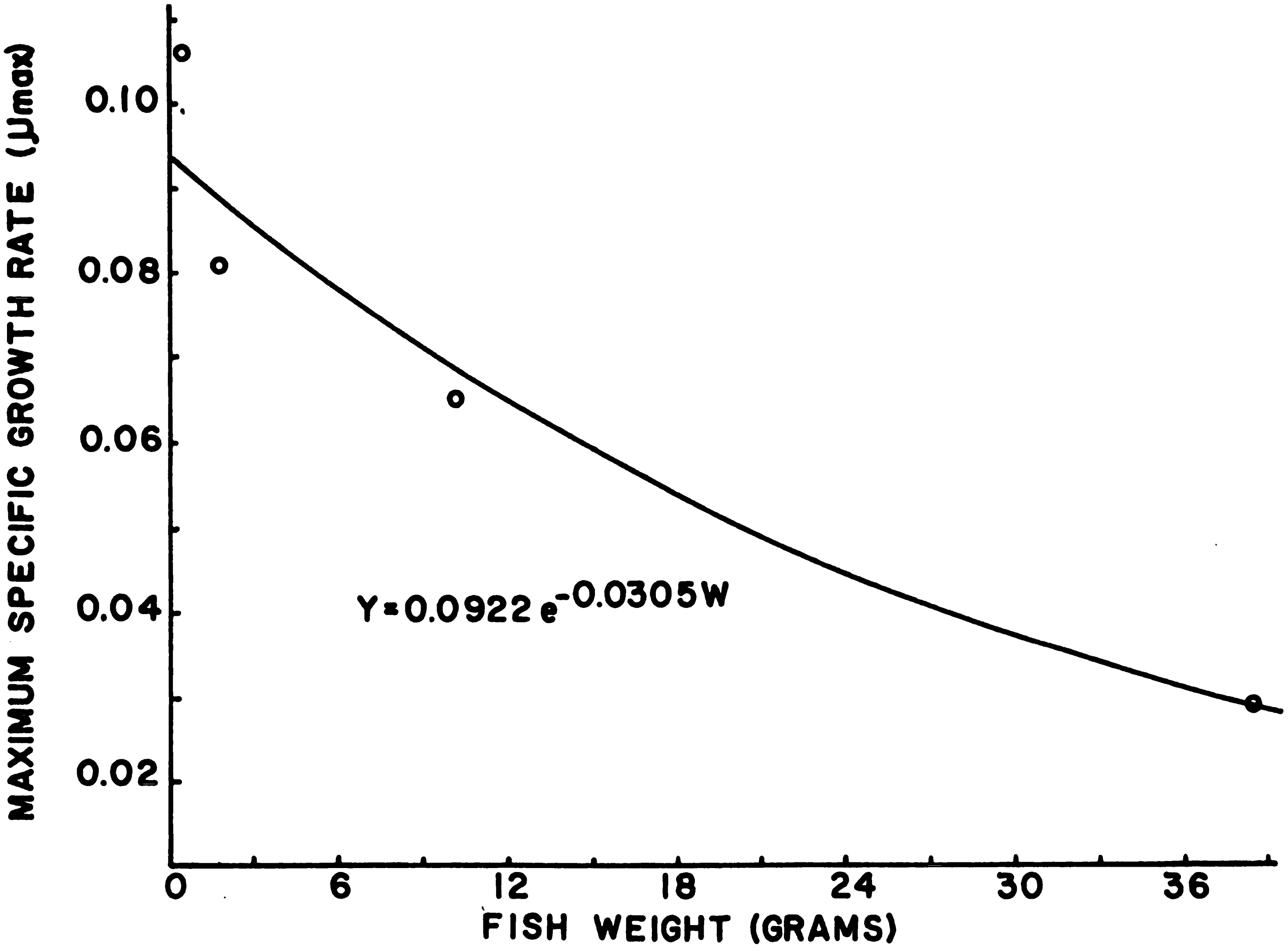

Figure 15. Calculated μ_{max} values for four size classes of <u>T. zillii</u> fed trout chow at 27.5°C and the exponential curve fit to the data ($r^2 = 0.97$; $n = 4$).

curve described by the exponential equation μ_{max} = $0.0922e^{-0.0305W}$. A regression of the calculated μ_{max} data, utilizing the exponential equation, results in a coefficient of determination (r^2) of 0.97 (n = 4).

Whatever the controlling mechanism for the growth process, tremendous emphasis is placed on early, rapid growth. As the fish quickly accumulate size, perhaps enough to escape predation or to facilitate utilization of different feed resources, the growth rate rapidly diminishes.

And yet, these considerations have only involved growth at high feeding levels above 3%. Below this level, quite different growth dynamics are evidenced. At low percentage feeding levels, numerous crossover points occur between the growth curves for the different sized fish (Figure 5). In examining the growth curve of the 0.0140 g fish, it is noted that the line intersects the other growth curves at feeding levels between 1.5 and 2% bw/d. At feeding levels of 1.5%, the relative positions have entirely reversed, and the smallest size fish experience the lowest growth rate. These contrasts and intersections are the result of widely divergent maintenance requirements for the various size classes. While the smallest fry demonstrate superior growth at high feeding levels, they simultaneously expend high levels of energy to maintain that level of performance.

The 0.014 g fish are shown to require a ration level of 1.55% bw/d for maintenance. At this same percentage of feed, the 1.76 g fish would be growing at a specific growth rate of 0.015. These dynamics are also demonstrated in the efficiency curves. Above a 2% bw/d feeding level, the 0.014 g fish are superior to larger fish in their ability to process feed and convert it to fish tissue. However, this relative advantage in efficiency changes drastically when feeding levels drop below 2% bw/d. This size class is most competitive at high percentage feed levels with their feed processing geared for fast growth at high cost. When the high maintenance cost has been paid, the potential for growth is tremendous. The feeding level for maximum efficiency of this size fish is approximately 3.2% bw/d. Above this level, a gradual reduction occurs due to decreased diffusion-assimilation efficiencies and the saturation of enzyme reaction rates. At ration levels above 3.2% bw/d, each additional unit of feed added contributes to an already nearly saturated system, and the efficiency of processing each unit of feed decreases. Below the maximum efficiency feeding level, the growth per unit feed decreases rapidly. This may be attributed to the high maintenance costs requiring an increasing percentage of the total feed, leaving little for growth.

The 1.76 g fish demonstrates different priorities of operation. At higher percentage levels of feed, this size is clearly less efficient than the smaller fish. However, at approximately 2% bw/d feed for each, the two experience equal growth rates. At lower, equal percentage body weight feeding levels, the larger fish, with a 43% reduction in maintenance costs, becomes the more efficient processor.

This trend continues with the 10 g fish whose maintenance costs are 76% lower than for the smallest fish. The maintenance feeding levels (S_q) are plotted versus size in Figure 16. Also shown in the figure is the curve represented by the equation: $S_q = 0.906 - (0.149 \ln W)$. A regression of the maintenance feeding level data, utilizing the log equation, results in a coefficient of determination (r^2) of 0.94 ($n = 4$).

Reduced maintenance costs with increased size also are reflected in the steadily decreasing feeding levels for maximum growth efficiency. Feeding levels for maximum efficiency were 3.2%, 2.5%, and 1.7% bw/d for the 0.014 g, 1.76 g and 10 g fish respectively.

At high percentage feeding levels, the 38.1 g fish exhibited relatively low specific growth rates and levels of efficiency indicating a limited ability to process high feed levels. Because of its lower maintenance requirements, the 38.1 g fish are able to experience specific growth rates higher than the 0.014 g fish at equal percentage feed levels below 1.7% bw/d. Similarly, the 38.1 g fish are able to grow faster than the 1.76 g fish when feed levels are below 1.12 bw/d. However, the 38.1 g fish is never able to process equal percentages of feed more efficiently than the 10 g

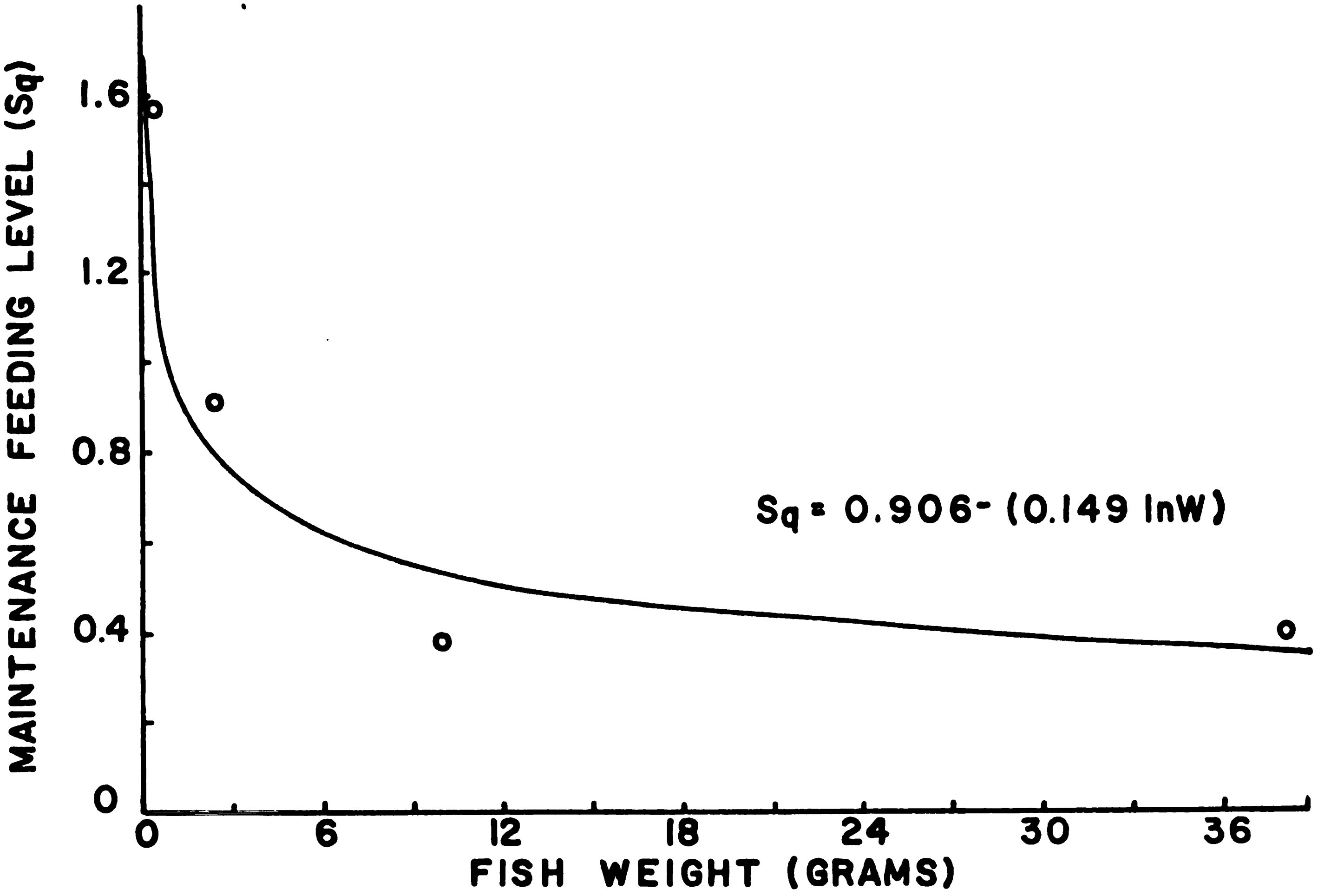

Figure 16. Calculated S_q values for four size classes of *T. zillii* fed trout chow at 27.5°C and the log equation fit to the data ($r^2 = 0.94$; $n = 4$).

fish, due to higher maintenance costs. While the small difference in maintenance costs (0.11% bw/d) may not be statistically significant, it may represent an important trend in the anabolism-catabolism balance indicating the increased costs necessary to maintain an aging fish. It would be interesting to define the growth curve of a relatively large fish (200-250 g) to compare maintenance costs on this size class.

It is important to realize that these comparisons of growth have been made on the basis of feeding levels expressed in percentages of body weight. Therefore, these comparisons reflect only the abilities of each size class fish to utilize similar percentages of feed -- not equal amounts of feed. When a 10 g fish and a 0.014 g fish are both fed 1.5% bw/d trout chow, the 10 g fish will experience a higher specific growth rate and demonstrate a higher efficiency of feed utilization. However, the 1.5% bw/d for the 10 g fish equals 0.15 g of trout chow per day, which corresponds to over 1,000% bw/d for the 0.014 g fish. Therefore, the smaller size classes are more efficient processors of equal weights of feed.

In summary, the enzyme-kinetic growth model was demonstrated to be useful for quantifying the differential growth response of various sizes of Tilapia zillii. The growth curves indicated that at high feeding levels, both the growth rate and efficiency decrease steadily with increasing size. However, at lower feeding levels, the relative growth rates and efficiencies are greatly affected by maintenance requirements. The data also suggest that although maintenance costs decrease rapidly during the fry-fingerling transition, eventually, as adults, the maintenance costs stabilize or may even increase slightly.

These changes in μ_{max} and S_q with size may be incorporated into the original growth formula and utilized to predict the growth of fish at any size for the given conditions. The maximum growth rate (μ_{max}) was effectively related to weight with the exponential formula:

$$\mu_{max} = 0.0922e^{-0.0305W}$$

This value for μ_{max} may be substituted into the original threshold-corrected hyperbolic curve which then would appear:

$$\mu = (0.0922e^{-0.0305W})\left(\frac{S - S_q}{K_s + S - 2S_q}\right)$$

The maintenance feeding level was related to weight by the log formula $S_q = 0.906 - (0.149\ln W)$. This value also may be incorporated into the original formula as follows:

$$\mu = (0.0922e^{-0.0305W})\left[\frac{S - (0.906 - 0.149\ln W)}{K_s + S - 2(0.906 - 0.149\ln W)}\right]$$

Using this formula, the growth of any size *T. zillii* at any given feeding level of trout chow for the given conditions could be calculated.

The formula could be further refined by incorporating a mathematical expression which related K_s to weight. The data obtained for K_s may be related to weight by a quadratic equation with a coefficient of determination (r^2) of 0.98 ($n = 4$). However, this quadratic equation projects an unrealistic cessation of growth at a 55 g size level. Obviously, this is unrealistic and again points out the desirability of feeding trials involving larger sized fish.

GROWTH-TEMPERATURE RELATIONSHIP

Temperature is recognized as an important variable affecting the well-being of organisms (Hill 1976). Because fish are poikilothermic, temperature has been reported as the primary environmental factor influencing their physiology (Caulton 1983). However, the well-being or growth of an organism may also be viewed as the integration of the efficiencies at which each of the physiological processes are occurring within the organism. It is at this level, the biochemical reactions and biophysical mechanisms, that temperature has its direct effect.

An important principal underlying all reactions is that as temperature increases, the movement of particles increase. The increased movement of particles causes both an increase in the kinetic energy and an increase in the frequency of collision between molecules. More importantly, the number or percentage of collisions having sufficient energy to participate in a reaction are greatly increased (Solomons 1976). This principle and a consideration of activation energies of reactions led Arrhenius to relate temperature to the metabolic rate of organisms as expressed in the equation: metabolic rate $= a10^{bT}$, where a and b are constants and T is temperature (Hill 1976). In summary, biochemical reactions have been shown to be temperature dependent.

Biophysical mechanisms such as diffusion and adsorption are also important factors in physiological processes. For organisms with extracellular digestion, the diffusion processes are important in determining the rate at which nutrients are transported to sites for anabolism, and waste materials carried away from catabolism sites. Transport from ingestion to reaction site involves diffusion across several membrane barriers. Passive diffusion usually occurs in an aqueous medium, wherein rates are predicted according to Fick's Law of Diffusion. The Fick's Law diffusion constant is determined by the amount of random motion of the particles in the aqueous solution. Therefore, as the temperature increases, the kinetic energy of the particles increase and the diffusion rate increases (Florey 1969).

Because both the rate of supply of reactants and the kinetic energy of the molecules increase with higher temperature, it would seem that the rates of the physiological processes should also increase. However, this relationship is clearly limited in biological systems where organisms experience both upper and lower temperature limits for their efficient operation and existence. Other factors must be involved affecting the basic relationship between temperature and the physiological processes.

In both unicellular and multicellular animals, the biochemical reactions are regulated by enzymes. Even the simplest manifestations of life involve many complex enzymatically controlled reactions (Hoar 1979). The enzymes, while not contributing energy to the reactions, are necessary for the activation of the process.

Enzyme reactions, like other reactions, are dependent on the amount of molecular motion and kinetic energy. As the temperature increases, the reaction velocity of the enzyme with the substrate increases (Hill 1976). However, because enzymes are proteins, they are susceptible to structural disruption and denaturation at higher temperatures (Atlas and Bartha 1981). Without the proper functioning enzyme, the biochemical reactions are stifled at high temperatures. A consideration of enzymes has added new dimension to the influence of temperature on organisms. Rather than a system of reactions whose rates increase progressively with increasing temperature, instead, an integrated complex of enzymatically controlled reactions are occurring within specific temperature bounds.

Indeed, the system is further complicated by a consideration of isozymes, different chemical forms of the same enzymes which function effectively at two different temperature levels. Organisms have been shown capable of interconverting from the production of one isozyme to another, thereby maintaining reaction rates over a broader temperature range (Hill 1976). Thus, rather than a dramatic change in reaction rates with changing temperature, a gradual change might be experienced as a series of isozymes operate over a temperature range.

Temperature boundaries are also affected by the solidification of lipids at low temperatures. Lipids are important components of membranes, and their solidification can seriously disrupt diffusional processes. The extent of solidification is affected by the degree of saturation of the lipids. The higher the degree of saturation, the higher the solidification temperature. Organisms adapted for low temperatures are usually characterized by lipids of low saturation (Hill 1976). As temperatures decline, the degree of solidification will affect the diffusion rates across membranes. This, in turn, will affect the rates of the enzymatically controlled reactions. These effects of low temperature on rates of reactions contributes to causing a decreased protein synthesis rate in bacteria (Atlas and Bartha 1981).

Temperature is thus shown to have a general effect on the physiological processes occurring in organisms. The factors of activation energy levels, enzyme function and denaturation, diffusion and lipid solidification all interact to delineate a temperature range within which the organism can survive. Rather than abrupt boundaries to these ranges, it is expected that isozymes would function to dampen the negative repercussion of high or low temperature extremes.

In addition to the general influence of temperature on reactions, it is possible to identify specific effects of temperature on individual physiological processes. For example, Smit (1967) reported that the digestive rate in fish is determined by three processes whose intensities are temperature dependent: 1) enzymatic action, 2) gastric juice secretion, and 3) stomachal motility. It was determined that both the quantity and composition of gastric secretion in brown bullheads is affected by temperature. Gastric juice output was shown to be maximal at 25°C with decreasing volume at both higher and lower temperatures.

Food intake rate is another variable affecting the digestive efficiency of fish. The rate of food intake has been shown to be dependent on temperature for several fish species. Peters and Boyd (1972) reported that the maximum feeding rate of the hogchoker depended primarily on temperature, and that the relationship was characterized by a quadratic equation. Kinne (1960), working with desert pupfish, similarly found that ingestion increased as the temperature was raised from 15°C to 30°C, but decreased when the temperature was further elevated. Caulton (1977) reported that the ingestion rate of *Tilapia rendalli* was optimal at about 27°C and decreased significantly above or below that temperature.

The efficiency of digestion is also affected by temperature. Jobling and Davies (1983) reported that increased temperature decreased the gut detention time and also increased the rates of digestion and evacuation. Caulton (1983) similarly reported that increased temperature caused an increase in assimilation efficiency due to more complete feed trituration and improved preassimilatory processing of feed. Because the changing temperature affected various aspects of feed processing differentially, the author suggested that the overall effect of temperature on the ingestion-digestion-assimilation process must be considered.

Brett (1967) reported on the effects of temperature on the swimming performances of young sockeye salmon. Those studies demonstrated that the capacity of the fish to perform was adversely affected by temperatures above and below an optimal temperature of 15°C. Brett theorized that an internal control was operating as a limiting factor, possibly in the capacity to deliver oxygen, remove metabolic products, provide adequate substrate or activate enzymatic processes. Kutty and Sukumaron (1975) reported similar findings that swimming performance varied in response to temperatures with high and low extremes causing deterioration and failure. The authors suggested that temperature acts through the central nervous system to control the rates of processes.

Many studies have examined the relationship between temperature and metabolic rate. Denzer (1968) reported that the respiratory rate for both rainbow trout and *Sarotherodon niloticus* varied with temperature. Caulton (1978) reported data obtained using a continuous flow respirometer which indicated that increases in temperature from 16°C to 37°C resulted in increases in oxygen uptake and routine metabolic energy expenditures by *Sarotherodon mossambicus*. Elliott (1976) demonstrated that respiration of brown trout increased with increasing temperature until a maximum was reached at 17.8°C, followed by decreases at two higher temperatures. The same pattern of a maximum energy flow occurring at an intermediate temperature with decreased energy at lower or higher temperatures was reported for the processes of food intake, waste excretion, specific dynamic action and respiration attributed to physical activity. Only the standard metabolism was found to continually increase with increased temperature. Caulton (1983), in summarizing the effect of temperature on feeding and metabolism, reported that an increasing temperature leads to increased assimilation but also to increasing costs due to elevated specific dynamic action. Therefore, in order to properly gauge the effect of temperature on an organism, the overall effect must be considered.

Fish species are commonly reported to exist within discrete temperature ranges. Broad ranges are reported in reference to the ability to survive and much narrower ranges for optimal growth and reproduction. A variance in temperature ranges for different fish species has resulted in the usage of terms such as thermophilic (loving high temperatures), eurythermal (having a broad temperature range), and stenothermal (having a narrow temperature range) (Philippart and Ruwet 1982). For example, numerous authors have documented the relatively high temperature range of _Tilapia_. Denzer (1968) reported that _Tilapia nilotica_ could survive within a range of 11°C to 42°C. Chervinski and Lahav (1976) confirmed the lower limit of _Sarotherodon niloticus_ by noting that most fingerling died upon a two-day exposure to 11°C water. Philippart and Ruwet (1982), experimenting with _S. niloticus_, determined a lower temperature limit of 10-13°C and an upper limit of 34-36°C. The same authors, working with _T. zillii_, reported a lower range of 6-10°C and an upper limit of 36-37°C. Brett et al. (1969) determined that sockeye salmon evidenced a dramatically lower range extending from 1-25°C.

It thus appears that the physiological processes of organisms are bounded by temperature limits and that within these limits the reaction rates vary with temperature. The temperature dependent rates of some physiological reactions and processes may be quantitatively defined. However, when these processes are examined in the context of the complex, interdependent matrix of systems within the organism, the complexity of the network defies complete quantitative definition.

One means of simplifying the diverse effects of temperature on the various components of the system is to quantify growth and utilize this parameter as an integrator of the complex interactions. It is expected that the growth-temperature relationship, since it is a cumulative expression of the processes described earlier, will evidence two trends similar to those experienced by the contributing factors. First, because all the reactions involved in the growth process are enzymatically controlled, it is anticipated that at any given temperature the growth response to substrate will also follow the threshold-corrected hyperbolic curve characteristic of enzyme-substrate reactions. Secondly, if factors such as diffusion, ingestion, digestion, respiration, etc. exhibit distinct similar responses over a range of temperatures, it is anticipated that growth will exhibit a similar response over the same temperature range. This anticipated response is again based on enzyme kinetics. As temperature changes, the rates of the enzyme-controlled reactions will change. These rate changes should cause a shift in the growth response curve but should not change the basic characteristic of the response.

With the exception of the standard metabolic rate, the processes contributing to growth have been reported to exhibit an optimal reaction rate at an intermediate temperature and decreasing rates with either decreasing or increasing temperature. Therefore, it is hypothesized that growth, the integrator of these components, will express similar trends.

Kinne (1960) determined that desert pupfish experienced a maximal growth rate at 30°C with decreasing growth at both increased and decreased temperature. Growth was reported to decrease in the following order: 30°; 25°, 35°, 20°, and 15°C. Dwyer et al. (1983) reported similar responses in studying the effect of temperature on brook trout growth. The highest rate of growth occurred at 16°C and decreased in the following series of temperatures: 13°, 19°, 10°, 7°, 4°C. Platt and Hauser (1978), studying the growth rate of *T. zillii* at various temperatures, determined an optimal maximum growth rate occurred between 28.8°C and 31.4°C. When the temperature was elevated or lowered, growth was reported to decrease. When the growth data of Platt and Hauser (1978) are plotted against temperature, the data are fit by a quadratic curve with an r^2 of 0.79. This quadratic curve had roots at 21.2°C and 37.6°C and optimal growth at 29.4°C. Brett et al. (1969) reported a similar type response for sockeye salmon in a much lower temperature range. Optimal growth was reported at 15°C with progressive decreases at 10°, 20°, 5°, 1° and 24°C.

The observed and calculated data resulting from my feeding studies where T. zillii were fed trout chow at five different temperatures are listed in Tables 6-11 and summarized in Table 12. The growth curves constructed from the calculated constants μ_{max}, K_s and S_q are shown in Figure 4. The regression of the specific growth rate data against the linear transformation of the threshold-corrected hyperbolic equation results in coefficient of determination values (r^2) of 0.99 ($n = 4$), 0.97 ($n = 5$), 0.91 ($n = 7$), 0.88 ($n = 5$), 0.91 ($n = 5$) and 0.95 ($n = 5$) for temperatures of 21.5°, 25.0°, 27.5°, 30.0°, 30.0° and 35.0°C respectively.

The high degree of correlation of the data to the threshold-corrected hyperbolic curve indicates that the enzyme-kinetic model is useful in quantitatively monitoring growth-substrate responses over a range of temperatures. The close comparison between the observed and predicted data throughout the 0-5% bw/d feeding range also emphasizes the accuracy of the model. This is especially significant since the feeding levels encountered in natural environments would usually fall within this range. These data from the model confirm the first of the two expected trends: growth at each temperature is quantitatively related to substrate by the threshold-corrected hyperbolic curve.

Despite the similarity in shape of the growth curves attributed to a common mechanism, there are still significant differences between actual specific growth rates found at different temperatures. Although both feed and temperature were variables in these experiments, a similar sequence of feeding levels was employed for each series of temperature experiments. Therefore, when growth response patterns are compared between each series of temperature experiments, the differences may be attributed to temperature. The introductory discussion of temperature leads to the expectation that changes in temperature should affect both the supply of reactants and rates of the enzyme-catalyzed reactions. The enzyme-kinetic base of the model, in turn, leads to the expectation that changes in temperature should also affect the growth constants. The model has the advantage of facilitating comparison between growth curves by providing the three constants which define the curve and the ability to calculate growth corresponding to any feeding level.

A comparison of the constants for the feeding trials reveals significant trends. The calculated values for μ_{max} indicate that at high feeding levels, the greatest growth occurred at 27.5°C and tapered off sharply at either higher or lower temperatures. Calculated μ_{max} values are plotted as a function of feeding level in Figure 17, along with a curve described by the quadratic equation $\mu_{max}=-0.1863+0.0181T-3.37\cdot10^{-4}T^2$. The μ_{max} data fit the quadratic equation with an r^2 of 0.77 (n = 5). From the equation, optimal μ_{max} is predicted to occur at 26.8°C and zero growth at both 14 and 40°C. These data follow the same general trend for *T. zillii* reported by Platt and Hauser (1978).

These calculations also were performed using the data of Brett et al. (1969) on sockeye salmon. The authors hand fit growth curves to data obtained at 1°, 5°, 10°, 15° and 20°C. Maximum specific growth rates were then determined from the asymptotes to each curve. These data were fit with a quadratic curve ($\mu_{max}=6.87\cdot10^{-3}+1.96\cdot10^{-1}T-6.73\cdot10^{-3}T^2$) with an r^2 of 0.99 (n = 5). The optimal μ_{max} for sockeye salmon determined from the equation was predicted at about 15°C with zero growth occurring at both 0°C and 29°C.

Despite the excellent fit to the data, there is reason for caution in interpretation. Both the lower threshold and optimal temperatures correspond well with previous research findings. However, the predicted upper temperature for growth threshold appears very high for sockeye salmon. Indeed, the authors had conducted an additional experiment at 24°C in which half the fish died and negative growth was reported. Therefore, caution should be taken in extending apparent trends beyond the experimental temperature range. Apparently the sockeye salmon are well adapted for cold

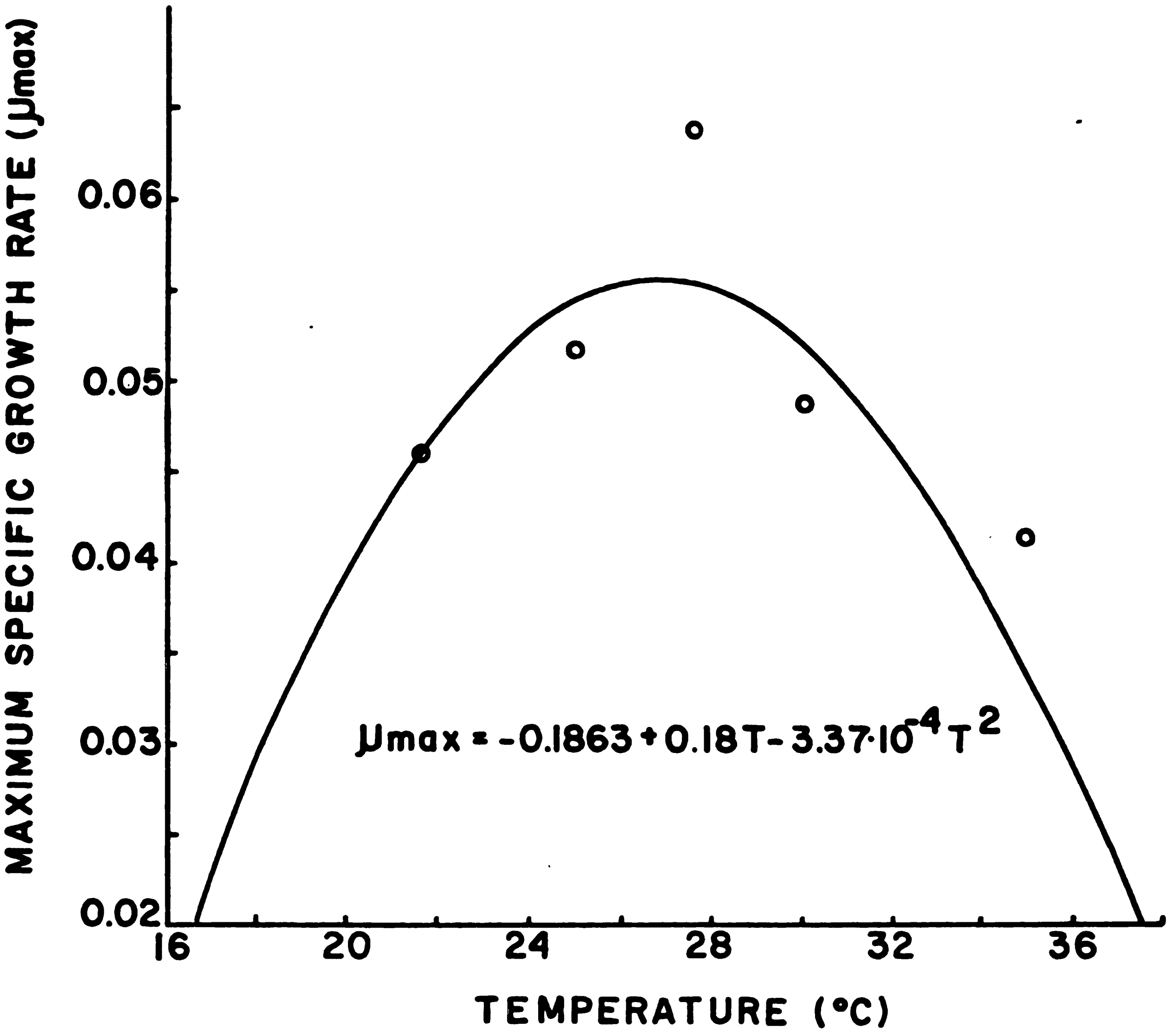

Figure 17. Calculated μ_{max} values for *T. zillii* (average weight 6.3 g) fed trout chow at 21.5, 25, 27.5, 30 and 35°C and the quadratic curve fit to the data ($r^2 = 0.77$; $n = 5$).

water temperatures with their enzyme-catalyzed reactions capable of performing down to 0°C. However, at higher temperatures, somewhere between 20°C and 24°C, they exhibit a drastic loss of ability to process feed.

Therefore, the literature data and the data from this study confirm the second expected trend, that growth acting as an integrator of the complex physiological processes would exhibit optimal rates at intermediate temperatures with decreased rates at both extremes. As temperature affects the supply rates of reactants, molecular activity, and enzyme efficiencies, both the rates of individual reactions and the integrated growth response (specific growth rate) shift similarly. The model provides not only the ability to quantitatively compare the μ_{max} at each experimental temperature level but also to use the quadratic fit to predict μ_{max} for any temperature. Furthermore, the quadratic equation may be substituted for μ_{max} in the original growth equation as shown below:

$$\mu = (-0.186+0.181T-3.37\cdot 10^{-4}T^2)\left(\frac{S-S_q}{S+K_s-2S_q}\right)$$

Thus the model permits the determination of <u>T</u>. <u>zillii</u> growth at any given feeding level of trout chow, under the controlled conditions, partially corrected for any temperature within the experimental range, for that size class of fish.

Despite the importance associated with maximum growth rates, there are other factors involved in evaluating feeds and growth. The natural environment rarely provides sufficient feed levels to support maximum growth rates of fish. Therefore, such factors as maintenance costs and growth efficiency at low feeding levels are important criteria for assessing feed quality and fish growth. The threshold-corrected hyperbolic curve provides quantitative mechanisms for comparison at these low feeding levels.

The maintenance feeding level of an organism, S_q, is useful because it identifies the amount of feed required to maintain the organism with zero growth. The S_q levels calculated for each temperature are summarized in Table 12 and plotted against temperature in Figure 18. The data points in Figure 18 are fit by the quadratic equation $S_q = 4.906 - 0.306T + 5.18 \cdot 10^{-3}T^2$ with an r^2 of 0.76 ($n = 5$). Minimum maintenance costs are predicted to occur at a temperature of 29°C. As the temperature decreases from this point, it lowers the level of molecular activity, enzyme efficiency and diffusion rate. Therefore, the amount of substrate required to provide the maintenance level of reactants would increase. As temperature increased from the optimal, it would be expected to increase molecular activity but decrease enzyme efficiency and possibly cause structural disruption and denaturation. As a result, the amount of substrate required to supply a given unit of reactant would increase.

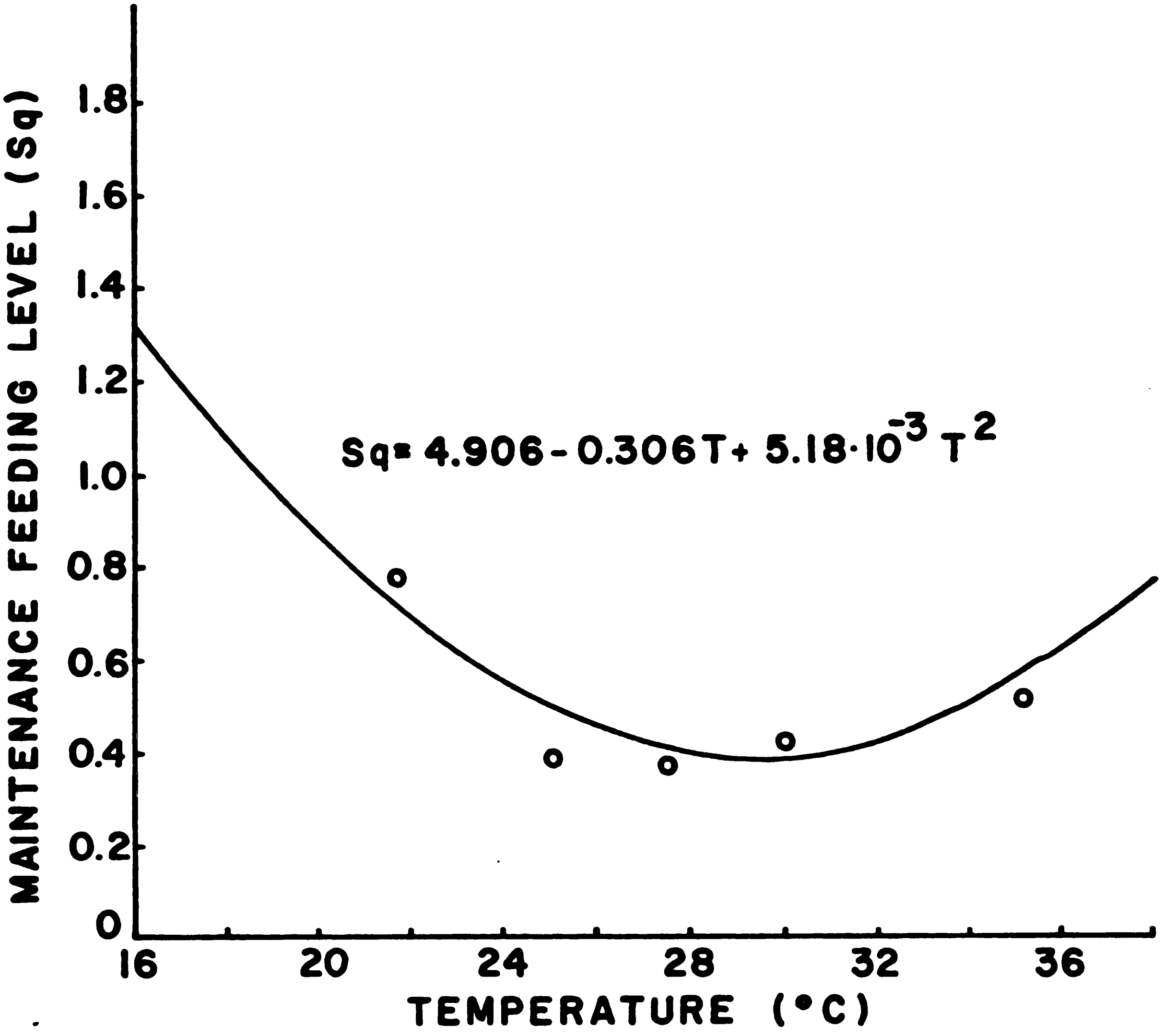

Figure 18. Calculated S_q values for T. zillii (average weight 6.3 g) fed trout chow at 21.5, 25, 27.5, 30 and 35°C and the quadratic curve fit to the data (r^2 = 0.76; n = 5).

The importance of the S_q values is demonstrated in examining the growth curves in Figure 4. At a 3% feeding level, the fish at 21.5°C have the second highest growth rate. However, at a 0.9% feeding level, the same temperature stimulates the lowest growth rate. The relatively rapid decrease in growth for fish at 21.5°C at low feed levels is caused by the high S_q of 0.77% bw/d. The elevated S_q at low temperatures suggest that *T. zillii* is poorly adapted to a cold water environment, especially at low feeding levels.

Because the S_q data may be fitted by a quadratic function, the data may again be incorporated into the growth equation already temperature-corrected for μ_{max} as shown below:

$$\mu = (-0.186+0.0181T-3.337\cdot 10^{-4}T^2)\left(\frac{S-(4.906-0.306\ T+5.18\cdot 10^{-3}T^2)}{S+K_s-2(4.906-0.306\ T+5.18\cdot 10^{-3}T^2)}\right)$$

The model now permits the determination of *T. zillii* growth at any given level of trout chow, under the controlled conditions more fully corrected for any temperature.

Brett et al. (1969) also reported on the maintenance feeding requirements of sockeye salmon over a temperature range of 1°-20°C. The S_q data of Brett et al. (1969) can be related to temperature by the exponential equation: $S_q = 0.321\cdot 10e^{(0.105\cdot 10T)}$ with an r^2 of 1.00. Unlike the quadratic nature of the *T. zillii* S_q data, these cold water fish demonstrate maintenance costs which continue to increase with rising temperature. At 20°C the fish have an S_q of approximately 2.6% body weight/day. The enzyme-controlled reactions involved with maintenance and growth are well-adapted to cold water situations and rapidly lose efficiency with rising temperature.

The remaining constant used to define the growth curve is K_s. This constant is important in defining the slope of the curve, and the data collected in this study are summarized in Table 12. When the K_s values are plotted against temperature, the data fit the quadratic equation $K_s = -15.066+1.318T-0.0216T^2$, with an r^2 of 0.87 ($n = 5$). This equation may now be fitted into the threshold-corrected hyperbolic equation to obtain the rather imposing equation shown below:

$$\mu = (-0.186+0.0181T-3.37\cdot 10^{-4}T^2)\left[\frac{S-(4.906-0.306T+5.18\cdot 10^{-3}T^2)}{S+(-15.066+1.318T-0.0216T^2)-2(4.906-0.306T+5.18\cdot 10^{-3}T^2)}\right]$$

Thus, the model now is useful for predicting the specific growth rate of <u>T</u>. <u>zillii</u> for any level of trout chow, and at any temperature under the conditions of this study in the manner shown in Figure 19.

To insure against adding to the complexity of the equation, the discussion will quickly shift to another important consideration which justifiably receives much attention--feed conversion efficiency. Feed costs are often a major

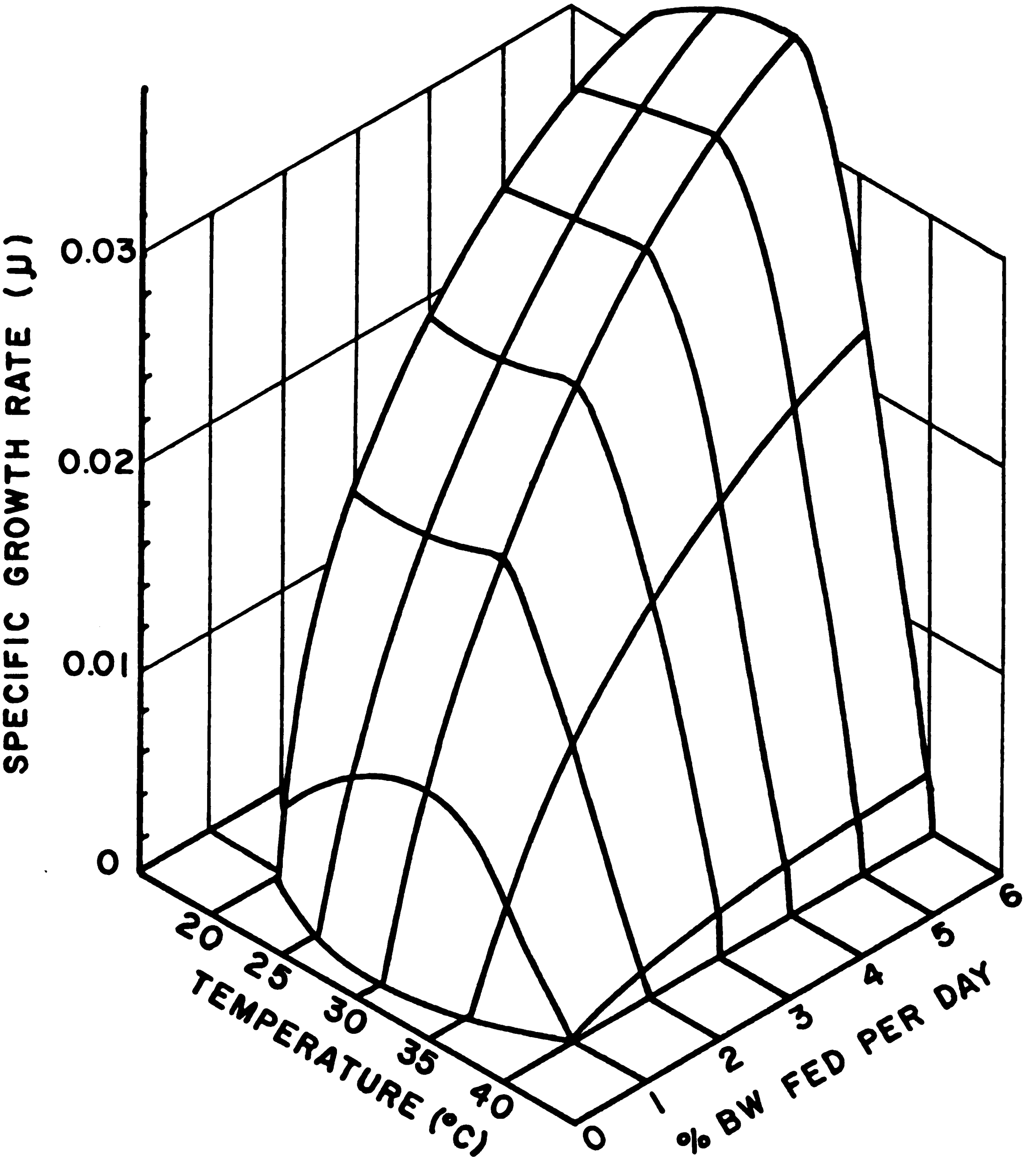

Figure 19. The three-dimensional relationship between the specific growth rate response and both temperature and feeding level of trout chow for *T. zillii* (average weight 6.3 g).

financial consideration in aquaculture. Therefore, it is important to understand the efficiency with which feed is being converted into edible tissue. Frequently this process is quantified by a simple conversion factor calculated by dividing the final weight of the organism by the total weight of feed utilized. This conversion factor may be transformed to percent conversion efficiency by multiplying by 100.

Conversion efficiency is determined both by the quality of the feed and the ability of the organism to assimilate the feed. Hopefully the considerations to this point have made it clear that measures of feed conversion are actually measures of the efficiency at which the enzyme-catalyzed reactions involved with growth process the feed. Therefore, it is anticipated that the threshold-corrected hyperbolic equation should be useful for providing a quantitative basis for determining feed conversion efficiency at any feeding level. Furthermore, it should be understood that before growth can occur, the maintenance costs of the organism (S_q) must be met. Thereafter, growth or feed conversion efficiency is a measure of the relative amount of feed required for maintenance and that which is left as an excess available for growth. As a result, the feed conversion efficiency should fluctuate dependent on the feeding level, as a function of μ_{max}, S_q, and K_s which characterize the shape of the curve. Any variable factor which causes a change in either μ_{max}, S_q or K_s will also cause a shift in conversion efficiency.

Despite the emphasis placed on rapid growth, the high feeding rates necessary to produce such growth may not represent optimal efficiency rates. It was previously reported that Kinne (1960) discovered that desert pupfish experienced superior growth at 30°C. However, when these same data are expressed in terms of weight gain per weight of feed, the fish reared at 20°C experienced the greatest conversion efficiency. The higher temperature stimulated higher ingestion and growth rates, but the digestion-assimilation process was reduced in efficiency. The conversion efficiencies were related to temperature in a pattern similar to growth since the efficiencies were reported to decrease with the following order of temperatures: 20°, 15°, 25°, 30°, 36°C. Intermediate temperatures stimulated the maximum feed conversion efficiency and decreased with both lower and higher temperatures. The author suggested that conversion efficiency is affected by age, heredity, environmental history of the fish and metabolic rate.

Brett et al. (1969) also determined the maximum efficiency feeding levels for sockeye salmon reared at different temperatures. These values were obtained by hand fitting a curve through growth data plotted against feeding level and drawing a tangent from the origin to the curve. The feeding level corresponding to the tangent junction with the curve represented the maximum efficiency feeding rate. When these feeding levels of optimal efficiency were plotted against temperature, the data points were fitted by the straight line equation: efficiency = $0.542+0.308T$ with an r^2 of 0.99.

The method of calculation of efficiencies for this study was explained in the Materials and Methods section. The determination simply involves dividing the specific growth rate (μ) by the feeding level (%S). If μ is expressed as $\frac{1}{\text{Time}}$ and %S is expressed as $\frac{\text{Feed}}{\text{Fish}}$, then the efficiency units become $\frac{\frac{\text{Fish}}{\text{Feed}}}{\text{Time}}$, thus giving a measure of fish growth relative to both feed and time. Efficiency curves from each of the temperature experiments are plotted in Figure 20. Each of the curves has a similar shape with an abruptly ascending limb tapering to a maximum point followed by a gradual descending slope. The enzyme-kinetic consideration suggests that the initial sharp increase is due to the fact that at low feeding levels all the feed components will be efficiently diffused and processed through the enzyme-catalyzed reactions.

Efficiencies will be negative until sufficient feed is provided at S_q to meet maintenance costs. As feeding levels continue to increase, the additional units are efficiently processed until competition begins at diffusion sites and the recycling enzymes cannot process the increasing supply as effectively. At yet higher feed levels,

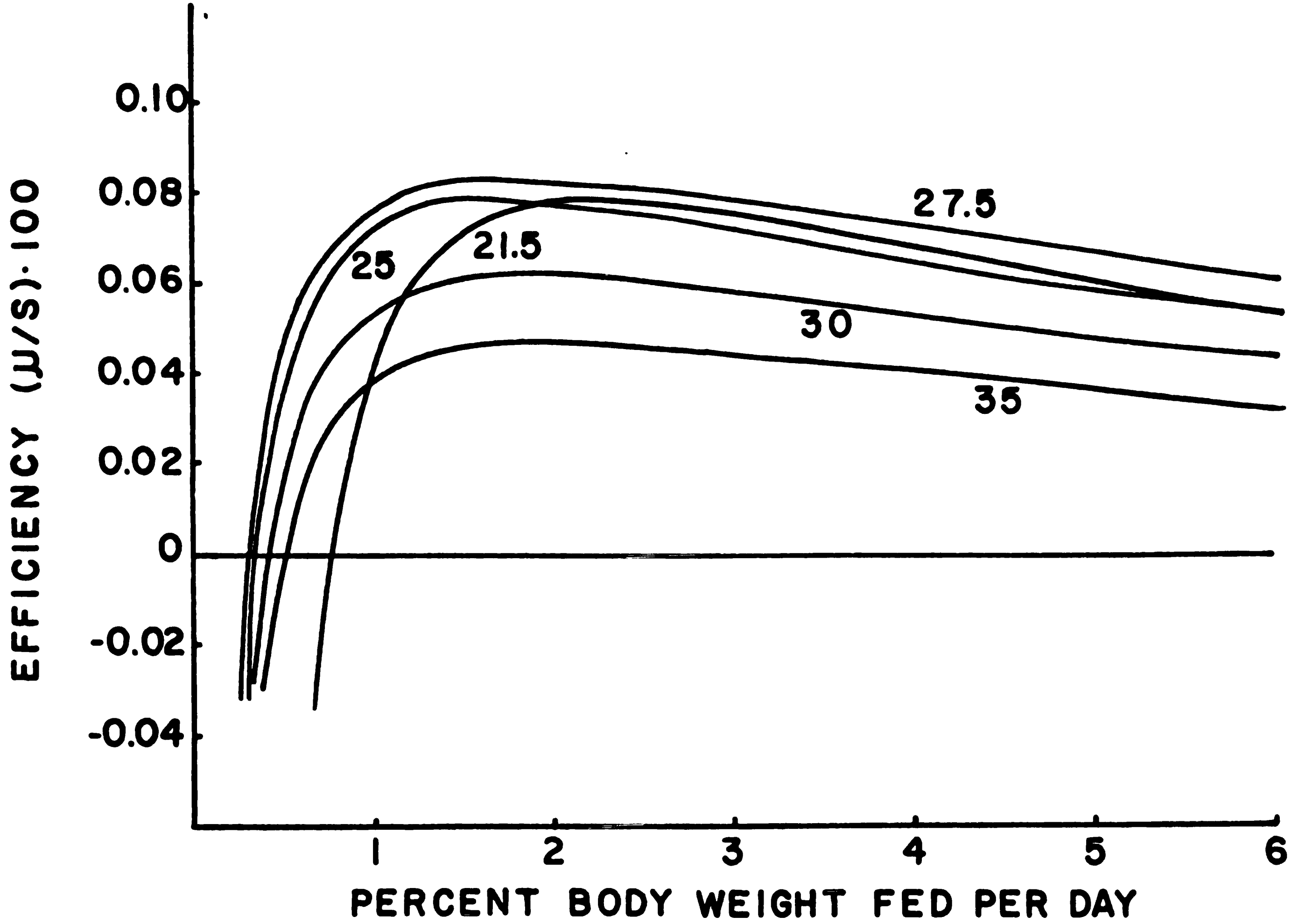

Figure 20. Efficiency growth curves for T. zillii (average weight 6.3 g) fed trout chow at 21.5, 25, 27.5, 30, 35°C.

growth is still increasing at this point, but because some feed components escape diffusion through the membranes, and the enzyme processing rate cannot increase as fast as the supply, the growth per unit feed increases more slowly. Eventually the trade-off between increased supply and less complete diffusion and diminished increase in reaction rate will result in a peak in efficiency. Thereafter, the enzyme-catalyzed reactions approach saturation and cannot respond as well to further increased supply, causing the reaction rate per unit feed to decrease.

A comparison of the curves in Figure 20 and the three-dimensional Figure 21 reveals that temperature has a profound effect on efficiency. The efficiencies demonstrated at 27.5°C are superior to all other feeding trials throughout the feeding range, and at times are almost twice that at 35°C. The changing S_q levels associated with different temperatures also have a dramatic effect. This relationship is most evident when examining the 21.5°C efficiency curve (Figure 20). At feeding levels above 2% bw/d, this feeding trial demonstrated the second highest efficiency levels. Yet because of the high maintenance costs of 0.77% bw/d at feeding levels below 1% bw/d, this trial experiences the lowest efficiency.

The feeding levels stimulating the maximum conversion efficiency for all five temperatures lie within 1.5-2.1% bw/d. As feeding levels increase beyond this range, they

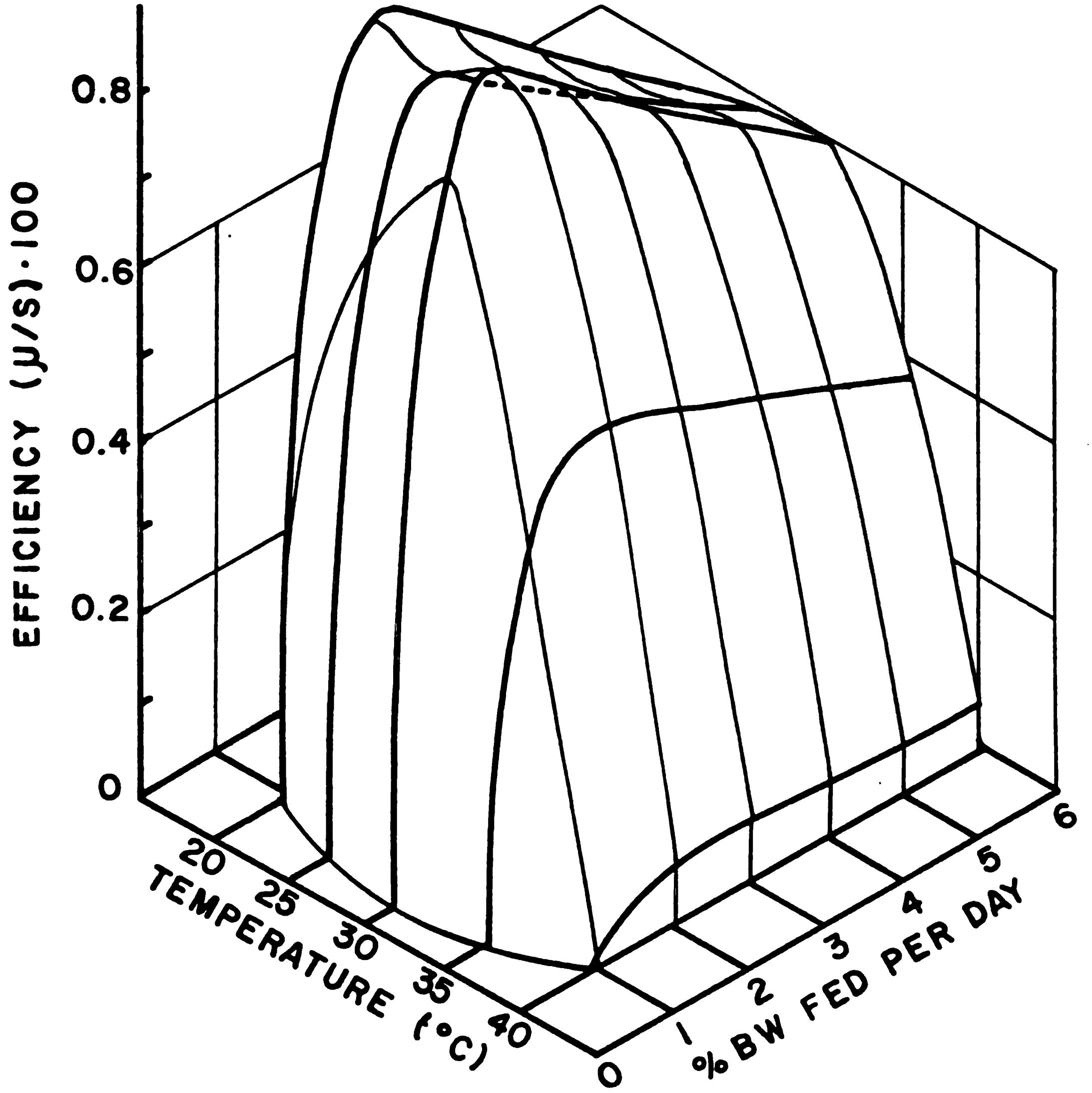

Figure 21. The three-dimensional relationship between feed conversion efficiency and both temperature and feeding level of trout chow for *T. zillii* (average weight 6.3 g).

continue to stimulate faster growth but at lessened efficiency due to diminished diffusion-enzyme processing rates. The 27.5°C temperature for optimal processing seems to represent a compromise between the optimal temperatures for the three growth constants which are 26.8°C for μ_{max}, 29.0°C for S_q and 30°C for K_s.

SUMMARY

The effective rearing of an organism requires the provision of all feed components and environmental conditions necessary for growth. Whether the rearing involves an extensive, natural feed situation or an intensive, artificial feeding program, the basic consideration is the same--to produce an organism by providing all the components necessary to stimulate growth in a cost-effective manner. Effective management thus requires the following information: 1) the environmental requirements of the organism, 2) dietary requirements of the organism, and 3) identification of the ability of the organism to harvest, digest and assimilate the form of feed. In addition, there is usually a consideration of whether these conditions can be provided in a cost-effective manner.

In seeking to effectively rear an organism, the overwhelming complexity necessitates some form of simplification to avoid confusion concerning the interactions and feedbacks in the system. And yet, to manage effectively requires some level of understanding concerning these processes and the relationship of the organism to the environment.

Historically, a rather pragmatic approach has been taken in seeking to increase aquaculture productivity. The complexity of the natural system has resulted in heavy reliance on empiricism wherein fish productivity is related to a single environmental parameter such as fertilization. These studies align closely with the "empirical ecology approach" proposed by Rigler (1982). Rigler proposed the empirical approach as a preferable, common ground for both theoretically-based limnologists and more practically-based fisheries managers. Rigler stated that when patterns in the behavior of the natural system are described by correlations, they are useful in establishing management practices. The literature supported the usefulness of correlations to increase fish productivity. For instance, correlations between fertilization and fish productivity have resulted in increased fish production. Unfortunately, such correlations have not led to the identification of the mechanisms and rates which stimulated these increases.

It is ironic that Rigler, in attempting to unite the sciences of limnology and fisheries management under the banner of empiricism, found it necessary to discredit both. All too often different investigative techniques are approached with an either/or attitude. Seldom are different methodologies discussed in an attempt to explore, combine and utilize the applicable elements of each. Rigler (1982), in summarizing the usefulness of the empirical approach, states that "a really useful and interesting correlation inevitably stimulates some of us to move on to the next phase of science--that of replacing the empirical theory with an explanatory theory." Thus, while degrading the attempts of the theoretical ecologists to develop models to explain and predict the workings of the natural system, Rigler ignores that the ultimate goal of each is the same--a desire to understand the natural system. The distinction between the approaches is not the goal but rather the means to a common goal. Admittedly, correlations by themselves are inadequate to quantitatively relate growth to particular environmental resources or to explain the basis of the observed patterns.

Following consideration of the strengths and weaknesses of the empirical approach, it is advantageous to question the necessity, or possibility, of identifying the mechanisms and rates by which growth is related to environmental parameters. If these relationships could be quantified, it would unquestionably enhance the understanding of the natural system, which was expressed as a common goal of all approaches. Therefore, the issue becomes a singular question of whether or not it is possible to quantitatively define the relationships.

The practicality of relating growth to environmental resources was explored by reviewing previous theoretical approaches to modeling growth. This review revealed that the "logistic" model had initiated a consideration of rate of growth during different phases and that the "enzyme-kinetic" model had incorporated the capacity to quantitatively relate specific growth rate to individual environmental parameters. The enzyme-kinetic model was subsequently altered to incorporate an allowance for energy-feed component expenditures associated with basal metabolism.

The threshold-corrected hyperbolic equation is intended as a research tool which includes aspects of both theoretical models and correlations to achieve the goal of understanding and prediction of the natural system. In addition to providing insight into the rates and mechanism of growth, it is anticipated that this research methodology will provide opportunity to recognize the commonalities inherent in the disciplines of limnology and fisheries management.

Kitaka (1972) expressed the belief that the fields of geology, chemistry, physics and biology (including limnology and fisheries management) are all interdependent. The author stated that "limnological factors have also been shown to influence feeding, growth rates and survival of fish." As the growth rate of fish is shown to be dependent on environmental resources, the interdependence of limnology and fisheries management is made evident. Because the model has both empirical and theoretical elements, the appearance of a mutual exclusiveness between these modeling approaches is also avoided.

The proposed growth model, provides a research methodology which, on the one hand simplifies the system, yet also facilitates the quantitative relating of growth to feed, environmental variables and digestive abilities of the organism. Simplification is achieved by allowing the organism to become an integrator of its internal biochemical-biophysical complexity. While the individual components are not quantitatively examined by the model, the integrated response to feed and environmental variables is monitored and related directly to these factors.

The threshold-corrected hyperbolic equation was first examined to determine its usefulness in quantifying the effects of the amount and type of different feeds on growth. Controlled experiments were conducted to quantitatively relate the growth rate of _T_. _zillii_ to three feeds: commercial trout chow, pelleted _Elodea_ _canadensis_, and pelleted _Spirulina_. It was demonstrated that the threshold-corrected hyperbolic equation and the constants which define it (μ_{max}, K_s and S_q) could be used to effectively predict the fish growth response to each feed. These quantitative relationships were also shown effective in relating fish growth to the protein and energy content of each feed.

The growth response of _S_. _niloticus_ to both trout chow and pelleted _Spirulina_ also was quantitatively defined by the model. The determination of growth constants for each organism on similar feeds permits comparisons between the species and the relating of differences to the unique morphology of each species.

The effectiveness of the model was next investigated concerning its ability to quantitatively define the relationship between growth-substrate and size. Controlled experiments were conducted using various sizes of _T_. _zillii_. It was determined that for each size class of fish, the threshold-corrected hyperbolic curve accurately described the growth-substrate relationship. As the size of the fish changed, the functions of the growth equation (μ_{max}, K_s and S_q) also changed, and these functions were related to size by either logarithmic, quadratic or exponential equations. When these relationships (equations) were incorporated into the growth equation, it was useful for predicting the growth response of fish at any size and at any feeding level under the controlled conditions.

The model also was examined relative to its usefulness in quantifying the effects of temperature on growth. Based on controlled experiments conducted on _Tilapia_ _zillii_ with temperature as a variable, it was demonstrated that growth could be quantitatively related to substrate at each temperature. Furthermore, each component of the growth curve (μ_{max}, K_s, S_q) was shown to change as a variable relative to temperature. These relationships could be described by either a quadratic or a power function formula. When these temperature dependent factors were incorporated together in the growth formula, the equation became useful for predicting the effect of temperature on the growth of _T_. _zillii_ at any feeding level throughout a broad temperature range. The practicality of the model was thus demonstrated in accurately quantifying a growth-substrate-temperature relationship in agreement with theoretical expectations and previous research findings.

These findings demonstrate that use of the threshold-corrected hyperbolic equation is effective in quantifying the effect of environmental and feed variables on growth. The experiments involving both feed types and species relate to the ability to identify dietary requirements of an organism and the ability to utilize the feed. Growth curves constructed using the model were shown to accurately reflect the growth response of _T. zillii_ to different feeds. The constants defining the curves could then be compared and related to protein and energy content of the feeds. Other components (protein, essential amino acids, vitamins, etc.) could similarly be used as the variable in such experiments and the optimal concentration of each determined from the growth response. Optimal species-specific feed formulations could thus be developed based on the integrated ability of the fish to digest and assimilate the feed under non-stressed conditions. Furthermore, similar studies could be conducted on various forms of "natural" feeds. By comparing the growth curves produced when feed is presented to the organism in different forms, conclusions may be drawn concerning the organism's abilty to harvest, digest and assimilate the feed.

The temperature experiments demonstrated the model to be useful in quantitatively defining the effect of an environmental variable on growth. Similar controlled studies using other variable factors such as ammonia concentrations, dissolved oxygen levels, pH, concentrations of specific toxins, etc. could be conducted to similarly identify the environmental range for individual species concerning these factors. Through a series of such experiments, the optimal environmental conditions for any species could be identified.

The quantitative nature of the model also facilitates the determination of an additional critical dimension of growth--conversion efficiency. Because the model permitted the calculation of growth rates corresponding to any feeding level, the growth per unit of feed also could be calculated for the entire feeding range. These data demonstrate the efficiency with which each unit of feed is converted into fish tissue. Because the profit motive underlies most aquaculture operations, these conversion efficiencies are of critical importance. Profit margins of any animal husbandry operation are basically a function of sale price and cost of production with feed being a major production expense.

Initially, when examining the growth curves, it might appear that since growth continues to increase with higher feeding levels, the fish should always receive a high feeding level which would stimulate near maximal growth. However, when examining any of the efficiency curves, it is evident that at elevated feeding levels, the ability of each unit of feed to stimulate growth is decreased. Optimal efficiency feeding levels for both species under all conditions examined ranged from 1.6 to 3.2% bw/d. At these feeding rates the maintenance costs of the organism are provided and additional feed remains to stimulate growth. This additional feed is efficiently processed by the organism and converted to tissue. When feed levels are increased beyond this maximal efficiency point, additional feed is available for growth but each unit of feed is less efficiently diffused and processed through the system of enzyme-catalyzed reactions. As a result, the economically optimal feeding rate becomes a complex consideration of balancing the market price of the organism against the feeding costs at different efficiencies.

The threshold-corrected hyperbolic equation also has useful application to other considerations important in aquaculture. The model was demonstrated to facilitate the prediction of specific growth rates of organisms of various sizes, at different feeding rates of specific feeds. These data permit the prediction of weight of the organism with time. Therefore, the time to reach marketable size at various feeding levels, and different efficiencies could be calculated. A decrease in feeding level might increase the efficiency of feed utilization but would increase the time to harvest due to a decreased growth rate. These calculations, along with considerations of feed cost and market value, would permit the determination of cost-effective feeding levels.

Another factor, indirectly involved in all considerations to this point, is the stocking level of fish. The level of stocking, along with the amount of feed added, determines the percent body weight of feed available to the fish. The proposed model would permit the calculation of stocking levels which would result in optimal, cost-effective, feeding levels and allow for changes in the size of the fish.

Extensive empirical research has been useful in establishing effective feeding tables for hatching operations. For example, Leitritz and Lewis (1980) compiled feeding tables for rainbow trout based on the recognition that the feeding ration for optimal growth is affected by both size and temperature. The threshold-corrected hyperbolic equation has a two-fold advantage over the empirical process. First, the model provides insight into the underlying mechanisms of growth and how it is influenced by numerous variables. Secondly, through inexpensive, short-term experimental procedures which avoid stress to the fish, the model facilitates the relating of specific growth rate and conversion efficiency to a broad range of feeding levels and provides the maintenance ration level.

The potential usefulness of the model also applies to natural feeding situations. Here the growth of the fish cannot be related to known increments of external feed. Instead, growth must somehow be correlated with the amount of natural feeds produced in the environment. The productivity of natural waters has frequently been estimated through correlations to biotic and abiotic variables. Unfortunately, these correlations only provide rough estimates of total productivity and are not capable of quantifying the production of specific feed types. The growth model has demonstrated the ability to accurately predict fish growth relative to particular levels of various feed types. If the availability of specific natural feeds could be determined, then the growth of the fish could not only be predicted but also predetermined by controlling the number and size of fish in the environment.

This predictive capacity for fish growth requires the identification of both standing crop and growth rate of the natural feed. This information could be obtained by utilizing the growth model to relate the growth of natural feeds to environmental parameters. The quantification of growth of natural feeds becomes the basis for predicting the growth of the fish. In each case, growth is based on the dietary requirements of the organism, the existing environmental conditions, and the ability of the organism to utilize the particular substrate for growth.

In summary, the threshold-corrected hyperbolic equation has the following potential application: 1) identify the dietary requirements of organisms, 2) quantify the effect of environmental variables on growth, 3) quantify the ability of the organism to harvest-digest-assimilate feeds, 4) provide a basis for cost-benefit considerations by identifying feed conversion efficiencies, and 5) provide growth data which relates to stocking rate and harvest time.

These applications are also possible in diverse situations due to the inexpensive, uncomplicated nature of the analytical technique. The basic data acquisition requires only a series of aquaria, an accurate balance to weigh the fish and feed, and routine laboratory equipment. More sophisticated analyses of protein content, vitamins, amino acids, etc. are desirable to enhance data interpretation. However, the basic procedure of quantitatively relating the specific growth response of fish to substrate concentrations under controlled conditions provides important data identifying optimal growth conditions. As a result, this research technique has practical application for enhancing aquaculture in remote areas and lesser developed countries.

While this research has been limited to aquaculture applications, the potential scope is much broader. The growth model is based on principals of diffusion mechanisms, the first and second laws of thermodynamics and enzyme-catalyzed reactions. These underlying principles are believed to function similarly in most organisms. Therefore, this model may provide a basis for quantitatively defining growth processes in other natural systems.

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APPENDIX A

Table A-1. Weights and lengths of *Tilapia zillii* fed trout chow at 21.5°C.

1% Feed

	Initial		Week 1		Week 2	
Fish #	cm	gm	cm	gm	cm	gm
0	5.7	3.2	5.8	3.2	5.9	3.3
1	5.5	2.6	5.5	2.4	5.6	2.6
2	6.0	3.4	6.0	3.5	6.1	3.7
3	5.9	3.5	5.9	3.6	6.2	3.8
Total Weight		12.7		12.7		13.4

2% Feed

	Initial		Week 1		Week 2	
Fish #	cm	gm	cm	gm	cm	gm
0	6.3	4.1	6.3	4.3	6.5	5.0
1	5.6	2.7	5.6	2.9	5.8	3.2
2	6.5	4.6	6.9	5.5	7.0	6.3
3	5.6	3.0	5.9	3.4	6.0	3.5
Total Weight		14.4		16.1		18.0

3% Feed

	Initial		Week 1		Week 2	
Fish #	cm	gm	cm	gm	cm	gm
0	6.0	3.5	6.1	4.0	6.3	4.5
1	5.6	3.0	5.8	3.5	6.0	4.0
2	5.6	2.8	5.8	3.2	5.9	3.6
3	5.6	2.9	5.7	3.5	6.0	3.9
Total Weight		12.2		14.2		16.0

4% Feed

	Initial		Week 1		Week 2	
Fish #	cm	gm	cm	gm	cm	gm
0	5.3	2.7	5.6	3.4	Discontinued	
1	6.2	3.8	6.4	4.8		
2	6.2	4.3	6.4	4.7		
3	5.6	2.8	5.9	3.4		
Total Weight		13.6		16.3		

Table A-2. Weights and lengths of *Tilapia zillii* fed trout chow at 25°C.

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.0	2.1	5.0	2.0	5.0	1.9	5.0	1.9	5.0	1.8	5.0	1.8
1	6.0	3.5	6.0	3.3	6.0	3.2	6.0	3.0	6.0	2.8	6.0	2.8
2	5.9	3.5	5.9	3.3	5.9	3.1	6.0	3.0	6.0	2.9	6.0	2.8
3	6.6	5.1	6.7	4.6	6.7	4.5	6.7	4.2	6.7	4.1	6.7	4.1
Total Weight		14.2		13.2		12.7		12.1		11.6		11.5

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.4	2.7	5.7	3.1	5.8	3.5	6.1	3.7	6.4	3.8	6.4	3.8
1	6.4	4.3	6.6	4.3	6.6	4.5	6.8	5.0	7.1	5.7	7.2	5.9
2	5.9	3.4	6.0	3.8	6.2	3.9	6.3	3.9	6.3	4.0	6.3	2.8
3	6.6	5.6	7.4	5.8	7.1	5.9	7.1	6.0	7.2	5.8	7.7	4.1
Total Weight		16.0		17.0		17.8		18.6		19.3		20.4

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.0	3.6	6.3	4.4	6.6	4.9	6.8	5.3	7.0	5.7	7.0	6.2
1	5.8	3.3	6.0	3.5	6.1	3.7	6.2	4.2	6.6	4.7	6.7	5.1
2	5.9	3.6	6.3	4.3	6.6	5.1	6.9	5.4	7.0	6.0	7.1	6.3
3	7.3	6.9	7.5	7.7	7.9	8.1	8.1	8.6	8.2	9.3	8.3	9.5
Total Weight		17.4		19.9		21.8		23.5		25.7		27.1

Table A-2 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.4	2.7	5.9	3.3	6.2	3.9	6.5	4.2	6.5	4.5	6.6	4.9
1	6.0	3.4	6.3	4.3	6.7	5.0	7.0	5.3	7.1	6.1	7.3	6.3
2	5.7	3.0	5.7	3.2	6.1	3.6	6.3	4.0	6.5	4.3	6.6	4.8
3	6.7	5.3	7.1	6.3	7.5	7.1	7.7	7.7	8.0	8.8	8.2	9.7
Total Weight		14.4		17.1		19.6		21.2		23.7		25.7

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.2	3.9	6.4	5.1	6.9	5.9	7.1	6.1	7.3	6.2	7.3	7.0
1	6.2	4.1	6.7	5.3	7.1	6.5	7.4	7.9	7.9	9.4	8.0	9.8
2	6.1	3.8	6.3	4.2	6.4	3.7	6.5	4.2	6.6	4.5	6.8	5.5
3	6.7	5.7	7.0	6.7	7.5	7.3	7.5	7.3	7.6	7.3	7.6	7.5
Total Weight		17.5		21.3		23.4		25.5		27.4		29.8

Table A-3. Weight in grams of *Tilapia zillii* fed trout chow at 27.5°C.

0% Feed					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	13.5	12.6	12.2	12.0	11.4
1	14.5	13.2	12.5	12.1	11.8
2	14.7	13.9	13.7	13.6	13.5
3	12.7	12.0	11.6	11.6	11.4
4	9.3	8.6	8.1	7.6	7.4
Total	64.7	60.3	58.1	56.9	55.5
0.2% Feed					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	8.5	8.2	8.1	7.8	7.5
1	7.1	7.1	7.1	7.1	7.0
Total	15.6	15.3	15.2	14.9	14.5
0.5% Feed					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	9.0	8.7	8.4	8.3	8.1
1	6.1	6.6	7.1	7.4	7.6
Total	15.1	15.3	15.5	15.7	15.7
0.7% Feed					
Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	7.2	7.3	8.1	8.6	9.1
1	6.8	6.9	6.8	6.6	6.6
Total	14.0	14.2	14.9	15.2	15.7

Table A-3 (cont'd.).

2% Feed

Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	6.7	6.3	6.3	6.3	7.3
1	7.4	9.2	11.1	12.6	12.3
2	6.9	8.6	9.3	9.6	10.8
3	9.0	9.1	10.8	12.5	14.2
Total	30.0	33.2	37.5	41.0	44.6

3% Feed

Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	7.4	8.4	10.0	12.4	14.4
1	6.3	7.9	9.2	11.2	12.9
2	6.8	7.8	9.0	10.5	11.7
3	-	-	-	-	-
Total	20.5	24.1	28.2	34.1	39.0

4% Feed

Fish #	Initial	Week 1	Week 2	Week 3	Week 4
0	14.2	17.0	17.1	17.3	20.7
1	16.4	20.9	25.0	27.2	31.7
2	15.6	18.7	21.3	26.4	31.2
3	-	-	-	-	-
Total	46.2	56.6	63.4	70.9	83.6

Table A-4. Weights and lengths of *Tilapia zillii* fed trout chow at 30°C.

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.4	6.0	7.4	5.6	7.4	5.4	7.5	5.3	7.5	5.0	7.5	4.9
1	7.9	6.9	7.9	6.4	7.9	6.2	7.8	6.0	7.8	5.4	7.8	5.3
2	7.6	7.1	7.6	6.8	7.6	6.6	7.6	6.6	7.6	6.4	7.6	6.2
3	9.0	11.4	9.0	10.7	9.0	10.4	9.0	10.1	9.0	10.0	9.0	9.7
Total Weight		31.4		29.5		28.6		28.0		26.8		26.1

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.5	5.6	7.5	5.0	7.5	4.9	7.5	5.5	7.6	5.9	7.7	6.7
1	8.1	6.1	8.1	8.2	8.5	9.0	8.6	9.3	8.7	9.3	8.8	10.0
2	7.3	5.9	7.6	6.1	7.6	6.2	7.6	5.9	7.7	6.3	7.7	5.6
3	8.5	8.2	8.5	7.6	8.5	7.8	8.7	7.9	8.6	8.0	8.7	8.0
Total Weight		25.8		26.9		27.9		28.6		29.5		30.3

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.7	3.9	6.7	3.8	6.7	3.7	6.7	3.7	6.7	3.6	6.7	3.4
1	7.2	5.7	7.5	6.5	7.8	7.1	8.0	7.4	7.9	7.5	8.0	7.5
2	8.2	8.8	8.6	10.6	9.0	12.0	9.2	13.3	9.6	15.2	9.8	16.3
3	8.7	8.9	8.7	9.3	8.8	9.7	9.1	10.2	9.1	11.1	9.6	13.6
Total Weight		27.3		30.2		32.5		34.6		37.4		40.8

Table A-4 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.7	4.6	7.3	6.0	7.8	7.4	8.1	8.4	8.6	10.6	8.6	10.7
1	6.5	4.3	7.3	6.1	7.6	6.6	7.8	7.2	7.8	7.5	8.0	8.5
2	7.6	7.4	8.3	8.5	8.6	10.1	9.0	12.4	9.2	12.6	9.2	13.4
3	8.1	8.7	8.5	10.0	8.9	11.6	9.2	12.5	9.7	14.7	10.1	17.0
Total Weight		25.0		30.6		35.7		40.5		45.4		49.6

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	8.2	8.8	9.1	14.0	9.8	17.6	10.3	18.6	10.6	21.3	11.0	24.3
1	7.1	5.3	7.2	5.3	7.5	6.5	7.8	7.5	7.9	8.0	8.0	7.7
2	7.5	6.3	7.8	7.3	7.9	7.7	8.1	9.3	8.4	10.0	8.4	11.3
3	7.2	6.3	7.9	8.2	8.5	10.0	8.7	11.0	8.7	11.9	8.8	12.2
Total Weight		26.7		34.8		41.8		46.4		51.2		55.5

Table A-5. Weights and lengths of *Tilapia zillii* fed trout chow at 35°C.

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.8	7.6	7.8	7.0	7.8	6.7	7.8	6.7	7.8	6.6	7.8	6.5
1	9.0	10.5	9.0	9.7	9.0	9.5	9.0	9.5	8.9	9.3	8.9	9.0
2	8.0	7.5	8.0	7.3	8.0	7.3	8.0	7.0	8.0	6.8	9.0	6.6
3	9.2	11.6	9.0	11.0	9.0	10.8	8.9	10.0	9.0	9.3	9.0	9.0
Total Weight		37.2		35.0		34.3		33.2		32.0		31.1

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.3	5.7	7.3	5.9	7.3	6.0	7.3	6.1	7.5	6.2	7.5	6.2
1	7.6	5.3	7.6	6.3	7.7	6.1	7.5	5.9	-	-	-	-
2	7.7	6.5	7.6	6.4	7.8	7.0	8.0	7.6	8.3	8.0	8.3	7.9
3	8.1	7.6	8.2	7.7	8.3	7.7	8.3	7.7	8.3	7.9	8.3	8.7
Total Weight		25.1		26.3		26.8		27.3		22.1		22.8

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	8.1	8.2	8.4	9.4	8.5	9.4	8.6	9.2	8.7	9.5	8.8	9.7
1	7.7	7.0	7.9	7.6	7.9	7.9	8.2	8.5	8.4	8.9	8.4	8.9
2	7.4	6.6	7.7	7.3	7.8	7.2	7.9	7.8	7.9	7.2	8.0	7.9
3	8.8	10.0	8.8	9.7	8.9	10.7	9.2	11.9	9.6	13.8	10.0	15.7
Total Weight		31.8		34.0		35.2		37.4		39.4		42.2

Table A-5 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	-	-	-	-	-	-	-	-	-	-	-	-
1	7.2	5.7	7.5	5.8	7.5	6.0	7.6	6.3	7.7	6.1	7.7	6.5
2	7.7	6.0	8.0	7.3	8.0	8.5	8.3	9.3	8.4	10.1	8.6	10.5
3	8.0	7.5	8.0	7.9	7.8	6.1	-	-	-	-	-	-
Total Weight		19.2		21.0		20.6		15.6		16.2		17.0

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.3	6.0	7.9	7.3	7.9	7.2	8.0	7.6	8.2	8.3	8.2	9.3
1	7.2	6.0	7.7	6.4	7.6	6.3	7.7	6.6	8.0	8.1	8.1	8.7
2	7.2	5.3	7.5	6.0	7.6	6.4	7.8	7.0	8.0	7.4	8.2	8.6
3	-	-	-	-	-	-	-	-	-	-	-	-
Total Weight		17.3		19.7		19.9		21.2		23.8		26.6

Table A-6. Weights of *Tilapia zillii* fry fed trout chow at 27.5°C.

	Initial weight (gm)	Final weight (gm)
1% Feed	0.0140	0.01057
2% Feed	0.0140	0.01648
3% Feed	0.0140	0.0193
5% Feed	0.0140	0.0242
10% Feed	0.0140	0.0255

Table A-7. Weights of *Tilapia zillii* fry (1.75 g average initial weight) fed trout chow at 27.5°C.

1% Feed

Fish #	Initial gm	Week 1 gm
0	0.8	1.0
1	2.4	2.5
2	1.4	1.6
3	1.9	1.8
Total weight	6.5	6.9

2% Feed

Fish #	Initial gm	Week 1 gm
0	1.8	2.0
1	2.9	3.2
2	1.9	2.1
3	1.3	1.6
Total weight	7.9	8.9

3% Feed

Fish #	Initial gm	Week 1 gm
0	1.5	1.8
1	2.3	2.4
2	1.3	1.8
3	1.5	1.8
Total weight	6.6	7.8

5% Feed

Fish #	Initial gm	Week 1 gm
0	1.6	2.5
1	2.5	3.3
2	1.5	1.9
3	2.1	3.1
Total weight	7.7	10.8

Table A-7 (cont'd.).

7% Feed		
Fish #	Initial gm	Week 1 gm
0	1.9	2.8
1	1.7	2.6
2	1.4	1.9
3	1.5	2.4
Total weight	6.5	9.7

Table A-8. Weights and lengths of 38.1 g average weight *Tilapia zillii* fed trout chow at 27.5°C.

	Initial		Week 1		Week 2	
	cm	gm	cm	gm	cm	gm
0% Feed	1.40	47.7	1.39	44.8	1.39	44.3
1% Feed	1.27	33.1	1.28	34.1	1.30	35.1
2% Feed	1.25	35.0	1.28	37.5	1.32	39.6
3% Feed	1.30	38.3	1.33	42.2	1.39	44.1

Table A-9. Weights of *Tilapia zillii* fed pelleted *Elodea canadensis* at 27.5°C.

0% Feed

Fish #	Initial gm	Week 1 gm	Week 2 gm	Week 3 gm	Week 4 gm	Week 5 gm
0	9.3	8.9	8.9	8.7	8.6	8.5
1	7.5	7.4	7.1	6.9	6.6	6.5
2	16.7	16.7	16.7	16.5	16.5	16.3
3	17.4	17.4	17.2	16.8	16.2	15.9
Total Weight	50.9	50.4	49.9	48.9	47.9	47.2

1% Feed

Fish #	Initial gm	Week 1 gm	Week 2 gm	Week 3 gm	Week 4 gm	Week 5 gm
0	-	-	-	-	-	-
1	11.8	12.6	12.6	12.5	12.4	12.6
2	15.6	16.2	15.8	15.5	15.7	16.2
3	18.4	19.4	19.4	19.1	18.9	18.6
Total Weight	45.8	48.2	47.8	47.1	47.0	47.4

2% Feed

Fish #	Initial gm	Week 1 gm	Week 2 gm	Week 3 gm	Week 4 gm	Week 5 gm
0	10.3	11.3	12.2	12.6	12.8	12.9
1	15.2	16.5	16.5	16.5	16.5	16.4
2	17.5	18.4	18.7	18.5	19.5	19.6
3	17.4	18.5	19.4	20.7	20.7	22.2
Total Weight	60.4	64.7	66.8	68.3	69.5	71.1

3% Feed

Fish #	Initial gm	Week 1 gm	Week 2 gm	Week 3 gm	Week 4 gm	Week 5 gm
0	10.6	11.8	12.3	12.4	12.9	13.0
1	9.9	11.2	11.5	12.0	12.0	12.4
2	14.2	15.4	16.0	16.5	17.3	17.4
3	15.0	16.0	16.2	16.6	17.2	17.6
Total Weight	49.7	54.4	56.0	57.5	59.4	60.4

Table A-9 (cont'd.).

5% Feed Fish #	Initial gm	Week 1 gm	Week 2 gm	Week 3 gm	Week 4 gm	Week 5 gm
0	11.6	13.0	14.0	14.8	15.0	16.3
1	7.8	8.4	8.8	9.3	9.9	10.2
2	10.1	11.2	11.8	12.3	12.9	13.0
3	16.0	17.6	18.1	18.3	18.4	18.7
Total Weight	45.5	50.2	52.7	54.6	56.2	58.2

Table A-10. Weights and lengths of _Tilapia_ _zillii_ fed pelleted _Spirulina_ sp at 27.5°C.

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.1	2.5	5.2	2.6	5.3	2.4	5.2	2.3	5.4	2.3	5.4	2.5
1	5.7	2.7	5.7	2.7	5.7	2.7	5.8	2.7	5.8	2.7	5.7	2.7
2	5.0	2.2	5.1	2.2	5.1	2.1	5.1	2.2	5.1	2.2	5.1	2.0
3	5.2	2.1	5.1	2.0	5.1	1.9	5.2	2.1	5.1	2.2	5.1	2.1
Total Weight		9.5		9.5		9.1		9.3		9.4		9.3

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.3	2.4	5.5	2.6	5.5	2.6	5.5	2.8	5.6	2.7	5.6	2.8
1	5.7	2.8	5.7	2.8	5.7	2.9	5.7	2.7	5.6	2.6	5.6	2.5
2	5.1	2.5	5.2	2.6	5.4	2.4	5.3	2.1	5.3	2.0	5.4	2.4
3	5.2	2.1	5.1	2.3	5.1	2.4	5.1	2.3	5.1	2.3	5.1	2.1
Total Weight		9.8		10.3		10.3		9.9		9.6		9.8

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.3	2.8	5.5	2.9	5.7	2.6	5.4	2.5	5.5	2.7	5.5	2.8
1	5.9	3.4	6.0	4.1	6.0	4.4	6.4	4.9	6.7	5.5	6.8	5.6
2	5.9	3.2	6.0	3.4	6.0	4.1	6.4	4.9	6.6	5.2	6.7	5.2
3	4.7	1.7	4.8	1.7	4.7	1.6	4.7	1.6	4.7	1.6	4.7	1.5
Total Weight		11.1		12.1		13.0		13.9		15.0		15.1

Table A-10 (cont'd.).

5% Feed	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.9	3.2	6.0	3.5	6.0	3.2	6.1	3.4	6.2	4.0	6.4	4.3
1	5.2	2.4	5.5	3.2	5.3	2.8	5.3	3.1	5.3	3.2	5.3	3.5
2	5.8	3.2	5.8	3.6	5.8	3.2	5.8	3.4	6.1	4.1	6.3	4.1
3	6.1	3.6	6.3	4.2	6.6	4.9	6.9	6.1	7.1	7.3	7.0	7.1
Total Weight		12.4		14.5		14.1		16.0		18.6		19.0

Table A-11. Weights and lengths of *Sarotherodon* *niloticus* fed trout chow at 27.5°C.

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.5	3.8	6.5	3.6	6.2	3.6	6.2	3.6	6.2	3.5	6.2	3.4
1	6.3	3.6	6.3	3.4	6.2	3.3	6.1	3.3	6.1	3.2	6.1	3.1
2	6.4	3.8	6.4	3.5	6.2	3.4	6.2	3.4	6.2	3.3	6.2	3.3
3	6.3	3.7	6.3	3.5	6.3	3.5	6.3	3.5	6.1	3.3	6.1	3.1
Total Weight		14.9		14.0		13.8		13.8		13.3		12.9

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.2	3.3	6.2	3.3	6.2	3.5	6.2	3.5	6.3	3.5	6.3	3.6
1	6.1	3.2	6.2	3.3	6.4	3.8	6.6	4.0	6.6	4.4	6.7	4.7
2	6.2	3.6	6.4	3.7	6.5	3.9	6.5	3.9	6.7	4.0	6.7	4.2
3	6.8	4.6	7.0	4.8	7.1	5.3	7.1	5.4	7.3	5.6	7.4	6.0
Total Weight		14.7		15.1		16.5		16.8		17.5		18.5

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	7.0	5.0	7.3	5.9	7.7	7.1	8.0	8.0	8.3	9.3	8.8	10.3
1	6.8	4.8	7.2	5.4	7.5	6.2	8.0	7.2	8.2	8.1	8.5	8.5
2	6.9	4.8	7.2	5.6	7.6	6.6	8.0	7.5	8.3	8.8	8.7	10.1
3	7.0	5.1	7.2	5.7	7.6	6.8	8.0	7.6	8.3	8.8	8.8	10.3
Total Weight		19.7		22.6		26.7		30.3		35.0		39.2

Table A-11 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.6	4.2	7.0	5.2	7.6	6.8	8.0	8.2	8.5	10.2	9.0	12.0
1	6.8	4.8	7.4	6.4	7.9	7.6	8.5	9.0	9.0	11.6	9.6	13.8
2	6.6	4.3	6.9	5.1	7.2	5.6	7.6	6.6	7.9	7.2	8.0	8.1
3	6.8	4.9	7.0	5.5	7.5	6.6	7.9	7.7	8.2	9.2	8.7	11.3
Total Weight		18.2		22.2		26.6		31.5		38.2		45.2

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.2	3.6	6.7	4.9	7.4	6.5	8.2	8.6	8.7	11.0	9.2	14.0
1	6.2	3.5	6.9	5.2	7.5	6.6	8.1	9.0	9.3	12.7	9.6	16.6
2	6.4	3.9	7.0	5.6	7.7	7.4	8.5	9.7	9.0	13.5	10.1	18.0
3	6.5	3.9	7.1	6.1	8.0	8.5	8.9	11.6	9.7	15.6	10.5	21.1
Total Weight		14.9		21.8		29.0		38.9		52.8		69.7

Table A-12. Weights and lengths of *Sarotherodon niloticus* fed pelleted *Spirulina* sp at 27.5°C.

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.6	2.4	5.5	2.3	5.5	2.1	5.5	2.0	5.5	2.0	-	-
1	6.2	3.8	6.2	3.5	6.2	3.2	6.2	3.1	6.1	3.1	6.1	3.1
2	6.3	3.6	6.2	3.4	6.3	3.4	6.3	3.3	6.2	3.2	-	-
3	6.1	3.3	6.1	3.0	6.0	3.0	6.0	3.0	6.0	2.9	6.0	2.7
Total Weight		13.1		12.2		11.7		11.4		11.2		5.8

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.1	3.1	6.0	2.8	6.0	2.8	5.9	2.6	5.9	2.5	5.9	2.4
1	6.5	4.1	6.7	4.4	6.9	5.0	7.0	5.3	7.1	5.7	7.2	6.1
2	6.5	4.2	6.5	4.2	6.5	4.3	6.6	4.5	6.8	4.7	6.8	5.0
3	6.7	4.5	6.8	4.4	6.8	4.5	6.9	4.9	6.9	5.0	6.9	5.1
Total Weight		15.9		15.8		16.6		17.3		17.9		18.6

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.8	5.3	7.1	5.9	7.4	6.5	7.7	7.4	8.1	8.1	8.2	8.7
1	6.8	4.9	7.0	5.1	7.0	5.4	7.3	5.8	7.2	6.1	7.5	6.4
2	6.9	5.1	7.2	5.7	7.4	6.5	7.7	7.2	8.0	8.0	8.1	8.7
3	6.7	4.7	7.0	5.6	7.5	6.5	7.9	7.4	8.1	8.1	8.4	9.0
Total Weight		20.0		22.3		24.9		27.8		30.3		32.8

Table A-12 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.2	3.4	6.2	3.1	6.2	3.3	6.2	3.3	6.2	3.3	6.2	3.4
1	6.3	3.6	6.7	4.6	7.2	6.3	8.0	8.3	8.5	10.5	9.1	13.3
2	6.0	2.8	5.9	2.7	5.9	2.6	5.9	2.6	5.8	2.5	5.7	2.5
3	6.1	3.5	6.6	4.7	7.2	6.1	7.9	7.6	8.1	9.5	9.0	11.7
Total Weight		13.5		15.1		18.3		21.8		25.8		30.9

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.6	4.3	7.0	5.5	7.8	8.0	8.7	11.6	9.6	15.6	10.5	21.0
1	6.0	3.2	6.5	4.0	7.0	5.0	7.2	6.0	7.8	7.7	8.5	10.3
2	6.2	3.4	6.7	4.6	7.0	5.3	7.5	7.2	8.3	9.4	9.0	12.2
3	7.1	5.5	7.8	8.1	8.6	11.1	9.5	14.3	10.2	18.8	11.0	23.2
Total Weight		16.4		22.2		29.4		39.1		51.5		66.7

Table A-13. Weights and lengths of *Tilapia zillii* fed trout chow at 30°C. (duplicate trial)

0% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	6.4	4.6	6.5	4.1	6.5	3.9	6.5	3.8	6.5	3.6	6.5	3.4
1	5.8	2.6	5.8	2.4	5.5	2.3	5.5	2.3	5.4	2.1	5.4	2.1
2	5.8	3.5	6.0	3.1	6.0	3.1	6.0	2.8	6.0	2.8	6.0	2.8
3	5.8	3.6	6.0	3.2	6.0	3.1	6.0	3.0	6.0	2.9	6.0	2.9
Total Weight		14.3		12.8		12.4		11.9		11.4		11.2

1% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	4.9	1.9	5.0	1.8	5.0	1.8	5.0	1.8	5.0	1.7	5.0	1.6
1	5.7	3.2	5.8	2.7	5.9	2.7	5.8	2.6	5.8	2.7	5.9	2.8
2	6.0	3.8	6.0	3.3	6.1	3.5	6.2	3.6	6.2	3.7	6.2	3.7
3	5.4	2.8	5.7	3.1	5.9	3.6	6.2	4.0	6.2	4.2	6.5	4.4
Total Weight		11.7		10.9		11.6		12.0		12.3		12.5

2% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	4.7	1.6	5.1	2.1	5.2	2.2	5.3	2.2	5.3	2.3	5.5	2.6
1	5.5	3.0	6.0	3.5	6.3	4.0	6.1	4.4	6.6	4.8	6.9	5.4
2	5.2	2.4	5.1	2.1	5.5	2.6	5.8	3.0	6.1	3.6	6.4	4.1
3	7.2	6.9	7.4	7.0	7.7	7.5	7.8	8.1	8.1	9.1	8.4	9.5
Total Weight		13.9		14.7		16.3		17.7		19.8		21.6

Table A-13 (cont'd.).

3% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.0	2.0	5.3	2.5	5.6	3.3	6.2	4.3	6.8	5.1	7.2	6.1
1	6.1	4.0	6.7	4.5	6.7	5.2	7.2	5.9	7.5	6.9	8.1	8.8
2	6.0	3.8	6.5	4.5	6.5	4.5	6.7	4.9	7.0	5.6	7.4	6.6
3	5.5	2.9	6.0	3.3	6.1	3.6	6.4	4.2	6.5	5.0	6.8	5.4
Total Weight		12.7		14.8		16.6		19.3		22.6		26.9

5% Feed

	Initial		Week 1		Week 2		Week 3		Week 4		Week 5	
Fish #	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0	5.3	2.3	5.5	2.6	5.5	2.4	5.5	2.2	5.6	2.6	6.0	3.7
1	6.2	4.1	6.5	4.3	6.7	4.3	6.7	4.3	6.9	5.0	7.3	5.8
2	5.7	3.2	6.4	4.7	7.0	6.1	7.5	7.2	7.8	8.1	8.0	8.7
3	6.4	4.7	6.9	5.4	7.0	6.3	7.5	7.4	7.8	8.0	8.0	8.8
Total Weight		14.3		17.0		19.1		21.1		23.7		27.0

APPENDIX B

Table B-1. Specific growth rates of *Tilapia zillii* fed trout chow at 21.5°C.

1% Feed

Fish #	Week 1	Week 2	Average
0	0.0	0.0044	0.0022
1	-0.0114	0.0114	0.0
2	0.0041	0.0079	0.0060
3	0.0040	0.0077	0.0059
Average	0.0	0.0076	0.0038

2% Feed

Fish #	Week 1	Week 2	Average
0	0.0068	0.0215	0.0142
1	0.0102	0.0141	0.0121
2	0.0255	0.0194	0.0225
3	0.0179	0.0041	0.0110
Average	0.0159	0.0159	0.0159

3% Feed

Fish #	Week 1	Week 2	Average
0	0.0191	0.0168	0.0191
1	0.0220	0.0191	0.0220
2	0.0191	0.0168	0.0191
3	0.0269	0.0155	0.0269
Average	0.0217	(0.0171)	0.0217

4% Feed

Fish #	Week 1	Week 2	Average
0	0.0329	Ceased Feeding Discontinued	0.0329
1	0.0334		0.0334
2	0.0127		0.0127
3	0.0277		0.0277
Average	0.0259		0.0259

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-2. Specific growth rates of *Tilapia zillii* fed trout chow at 25°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.007	-0.007	0.00	-0.0077	0.00	-0.0044
1	-0.008	-0.004	-0.0092	-0.0099	0.00	-0.0064
2	-0.008	-0.009	-0.0047	-0.0048	-0.0050	-0.0064
3	-0.015	-0.003	-0.0099	-0.0034	0.00	-0.0062
Average	-0.0104	-0.0055	-0.0069	-0.0060	-0.0012	-0.0060

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.020	0.017	0.0079	0.0038	0.00	0.0098
1	0.00	0.006	0.0151	0.0187	0.0049	0.0090
2	0.016	0.004	0.00	0.0036	0.0070	0.0060
3	0.005	0.002	0.0024	-0.0048	0.0163	0.0043
Average	0.0087	0.0066	0.0063	0.0053	0.0079	0.0069

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.029	0.015	0.0112	0.0104	0.0120	0.0164
1	0.008	0.008	0.0181	0.0161	0.0117	0.0126
2	0.025	0.024	0.0082	0.0151	0.0070	0.0182
3	0.016	0.007	0.0086	0.0112	0.0030	0.0107
Average	0.0192	0.0130	0.0107	0.0128	(0.0076)	0.0139

Table B-2 (cont'd.).

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.029	0.024	0.0106	0.0099	0.0122	0.0263
1	0.034	0.022	0.0083	0.0201	0.0046	0.0275
2	0.009	0.017	0.0151	0.0103	0.0157	0.0130
3	0.025	0.017	0.0116	0.0191	0.0139	0.0209
Average	0.0245	0.0195	(0.0112)	(0.0159)	(0.0116)	0.0220

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.038	0.021	0.0048	0.0023	0.0173	0.038
1	0.037	0.029	0.0279	0.0248	0.0060	0.037
2	0.014	-0.018	0.0181	0.0099	0.0287	0.014
3	0.023	0.012	0.00	0.00	0.0039	0.023
Average	0.0281	(0.0134)	(0.0123)	(0.0103)	(0.0120)	0.0281

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-3. Specific growth rates of *Tilapia zillii* fed trout chow at 27.5°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0099	-0.0046	-0.0024	-0.0073	-0.0060
1	-0.0134	-0.0078	-0.0046	-0.0036	-0.0073
2	-0.0080	-0.0021	-0.0010	-0.0011	-0.0030
3	-0.0081	-0.0048	0.0000	-0.0025	-0.0039
4	-0.0112	-0.0086	-0.0091	-0.0038	-0.0082
Average	-0.0101	-0.0056	-0.0034	-0.0037	-0.0057

0.2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0051	-0.0018	-0.0054	-0.0056	-0.0045
1	0.0000	0.0000	0.0000	-0.0020	-0.0005
Average	-0.0026	-0.0009	-0.0028	-0.0039	-0.0026

0.5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0048	-0.0050	-0.0017	-0.0035	-0.0038
1	0.0112	0.0104	0.0059	0.0038	0.0079
Average	0.0019	0.0019	0.0018	0.0000	0.0014

0.7% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0020	0.0149	0.0086	0.0081	0.0084
1	0.0021	-0.0021	-0.0043	0.0000	-0.0011
Average	0.0021	0.0069	0.0029	0.0046	0.0041

Table B-3 (cont'd.).

2.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	-0.0088	0.0000	0.0000	0.0210	0.0031
1	0.0311	0.0268	0.0181	-0.0034	0.0181
2	0.0315	0.0112	0.0045	0.0168	0.0160
3	0.0016	0.0245	0.0209	0.0182	0.0163
Average	0.0145	0.0156	0.0127	0.0120	0.0137

3.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0181	0.0249	0.0307	0.0214	0.0246
1	0.0323	0.0218	0.0281	0.0202	0.0274
2	0.0196	0.0204	0.0220	0.0154	0.0207
3	-	-	-	-	-
Average	0.0231	0.0224	0.0271	(0.0191)	0.0242

4.0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0257	0.0008	0.0017	0.0256	0.0257
1	0.0346	0.0256	0.0120	0.0219	0.0346
2	0.0259	0.0186	0.0307	0.0239	0.0259
3	-	-	-	-	-
Average	0.0290	(0.0162)	(0.0160)	(0.0235)	0.0290

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-4. Specific growth rates of *Tilapia zillii* fed trout chow at 30°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0099	-0.0052	-0.0027	-0.0083	-0.0029	-0.0058
1	-0.0107	-0.0045	-0.0047	-0.0151	-0.0027	-0.0075
2	-0.0062	-0.0043	0.0	-0.0044	-0.0045	-0.0039
3	-0.0091	-0.0041	-0.0042	-0.0014	-0.0044	-0.0046
Average	-0.0089	-0.0044	-0.0030	-0.0062	-0.0038	-0.0053

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0162	-0.0029	0.0165	0.0100	0.0182	0.0051
1	0.0423	0.0133	0.0047	0.0	0.0104	0.0141
2	0.0048	0.0023	-0.0071	0.0094	-0.0168	-0.0015
3	-0.0109	0.0037	0.0018	0.0018	0.0	-0.0007
Average	0.0060	0.0052	0.0035	0.0044	0.0038	0.0046

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0037	-0.0038	0.0	-0.0039	-0.0082	-0.0039
1	0.0188	0.0126	0.0059	0.0019	0.0	0.0078
2	0.0266	0.0177	0.0147	0.0191	0.0100	0.0176
3	0.0063	0.0060	0.0072	0.0121	0.0290	0.0121
Average	0.0144	0.0105	0.0089	0.0111	0.0124	0.0115

Table B-4 (cont'd.).

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0380	0.0300	0.0181	0.0332	0.0013	0.0241
1	0.0499	0.0113	0.0124	0.0058	0.0179	0.0195
2	0.0198	0.0246	0.0293	0.0023	0.0088	0.0170
3	0.0199	0.0212	0.0107	0.0232	0.0208	0.0191
Average	0.0289	0.0220	0.0180	0.0163	0.0126	0.0196

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0663	0.0327	0.0079	0.0194	0.0188	0.0316
1	0.0	0.0291	0.0204	0.0092	-0.0055	0.0147
2	0.0210	0.0076	0.0270	0.0104	0.0175	0.0165
3	0.0377	0.0284	0.0136	0.0112	0.0036	0.0227
Average	0.0379	0.0262	0.0149	0.0141	(0.0115)	0.0233

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-5. Specific growth rates of *Tilapia zillii* fed trout chow at 35°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0117	-0.0062	0.0	-0.0021	-0.0022	-0.0045
1	-0.0113	-0.0030	0.0	-0.0030	-0.0047	-0.0044
2	-0.0039	0.0	-0.0060	-0.0041	-0.0043	-0.0037
3	-0.0076	-0.0026	-0.0110	-0.0104	-0.0047	-0.0072
Average	-0.0087	-0.0029	-0.0047	-0.0052	-0.0041	-0.0051

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0049	0.0024	0.0024	0.0023	0.0	0.0024
1	0.0247	-0.0046	-0.0228	died	-	-
2	-0.0022	0.0128	0.0117	0.0073	-0.0018	0.0056
3	0.0019	0.0	0.0	0.0037	0.0138	0.0039
Average	0.0067	0.0027	(-0.0011)	0.0046	0.0045	0.0040

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0195	0.0	-0.0031	0.0046	0.0030	0.0048
1	0.0117	0.0055	0.0105	0.0066	0.0	0.0069
2	0.0144	-0.0020	0.0114	-0.0114	0.0133	0.0051
3	-0.0044	0.0140	0.0152	0.0212	0.0184	0.0129
Average	0.0096	0.0050	0.0087	0.0074	0.0098	0.0081

Table B-5 (cont'd.).

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	died	-	-	-	-	-
1	0.0025	0.0048	0.0070	-0.0046	0.0091	0.0025
2	0.0280	0.0217	0.0128	0.0118	0.0055	0.0280
3	0.0074	-0.0369	died	-	-	0.0074
Average	0.0128	(-0.0027)	(0.0104)	(0.0054)	(0.0069)	0.0128

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0280	-0.0020	0.0077	0.0126	0.0163	0.0280
1	0.0092	-0.0022	0.0066	0.0293	0.0102	0.0092
2	0.0177	0.0092	0.0128	0.0079	0.0215	0.0177
3	died	-	-	-	-	-
Average	0.0185	(0.0014)	(0.0090)	(0.0165)	(0.0159)	0.0185

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-6. Specific growth rates of *Tilapia zillii* fry (0.0140 g average weight) fed trout chow at 27.5°C.

Average	Specific Growth
1% Feed	-0.0401
2% Feed	0.0233
3% Feed	0.0459
5% Feed	0.0779
10% Feed	0.0854

Table B-7. Specific growth rates of *Tilapia zillii* fry (1.76 g average weight) fed trout chow at 27.5°C.

Feed	Fish #		Specific Growth
1% Feed	0		0.0319
	1		0.0058
	2		0.0191
	3		0.0077
		Average	0.0085
2% Feed	0		0.0151
	1		0.0141
	2		0.0143
	3		0.0297
		Average	0.0170
3% Feed	0		0.0261
	1		0.0061
	2		0.0465
	3		0.0261
		Average	0.0239
5% Feed	0		0.0638
	1		0.0397
	2		0.0338
	3		0.0556
		Average	0.0483
7% Feed	0		0.0554
	1		0.0607
	2		0.0436
	3		0.0671
		Average	0.0572

Table B-8. Specific growth rates of 38.1 g average weight *Tilapia zillii* fed trout chow at 27.5°C.

0% Feed	Week 1	Week 2	Average
	-0.0089	-0.0016	-0.0053
1% Feed	Week 1	Week 2	Average
	0.00425	0.00413	0.0042
2% Feed	Week 1	Week 2	Average
	0.00986	0.00778	0.0088
3% Feed	Week 1	Week 2	Average
	0.01385	(0.00629)	0.0138

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-9. Specific growth rates of *Tilapia zillii* fed pelleted *Elodea canadensis* at 27.5°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0063	0.00	-0.0032	-0.0017	-0.0017	-0.0026
1	-0.0019	-0.0059	-0.0041	-0.0063	-0.0022	-0.0041
2	0.00	0.00	-0.0017	0.00	-0.0017	-0.0035
3	0.00	-0.0017	-0.0034	-0.0052	-0.0027	-0.0026
Average	-0.0014	-0.0014	-0.0029	-0.0030	-0.0021	-0.0022

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-	-	-	-	-	-
1	0.0094	0.00	-0.0011	-0.0011	0.0023	0.0019
2	0.0054	-0.0036	-0.0027	0.0018	0.0045	0.0011
3	0.0076	-0.00	-0.0022	-0.0015	0.0023	0.0003
Average	0.0073	-0.0012	-0.0021	-0.0003	0.0012	0.0010

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0132	0.0109	0.0046	0.0022	0.0011	0.0064
1	0.0117	0.00	0.00	0.00	-0.0009	0.0022
2	0.0072	0.0023	-0.0015	0.0075	0.0007	0.0032
3	0.0088	0.0068	0.0093	0.00	0.0099	0.0070
Average	0.0098	0.0046	0.0032	0.0025	0.0033	0.0047

Table B-9 (cont'd.).

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0153	0.0059	0.0012	0.0056	0.0011	0.0058
1	0.0176	0.0038	0.0061	0.00	0.0047	0.0064
2	0.0116	0.0055	0.0044	0.0068	0.0008	0.0058
3	0.0092	0.0018	0.0035	0.0051	0.0033	0.0046
Average	0.0129	0.0041	0.0038	0.0046	0.0024	0.0056

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0163	0.0106	0.0079	0.0019	0.0119	0.0097
1	0.0106	0.0066	0.0079	0.0089	0.0043	0.0077
2	0.0148	0.0075	0.0059	0.0068	0.0011	0.0072
3	0.0136	0.0040	0.0016	0.0008	0.0023	0.0045
Average	0.0140	0.0069	0.0051	0.0041	0.0050	0.0070

Table B-10. Specific growth rates of *Tilapia zillii* fed *Spirulina* sp at 27.5°C.

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0056	-0.0114	-0.0061	0.0	-0.0030
1	0.0	0.0	0.0	0.0	0.0
2	0.0	-0.0066	0.0066	0.0	0.0033
3	-0.0070	-0.0073	0.0143	0.0066	0.0079
Average	(0.0)	(-0.0061)	0.0031	0.0015	(0.0021)

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0114	0.0	(0.0106)	(-0.0052)	0.0114
1	0.0	0.0050	(-0.0102)	(-0.0054)	0.0
2	0.0056	-0.0114	(-0.0130)	(-0.0070)	0.0056
3	0.0130	0.0061	(-0.0061)	(0.0)	0.0130
Average	0.0071	(0.0)	(-0.0014)	(-0.0044)	0.0071

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0050	-0.0156	-0.0056	0.0109	0.0050
1	0.0267	0.0101	0.0154	0.0165	0.0267
2	0.0087	0.0267	0.0255	0.0085	0.0087
3	0.0	-0.0087	0.0	0.0	0.0
Average	0.0123	(0.0069)	(0.0096)	(0.0109)	0.0123

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Average
0	0.0128	-0.0128	0.0087	0.0232	0.0128
1	0.0411	-0.0191	0.0145	0.0045	0.0411
2	0.0168	-0.0168	0.0087	0.0267	0.0168
3	0.0220	0.0220	0.0313	0.0256	0.0220
Average	0.0224	(-0.0040)	(0.0181)	(0.0215)	0.0224

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-11. Specific growth rates of *Sarotherodon niloticus* fed trout chow at 27.5°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0077	0.0	0.0	-0.0040	-0.0041	-0.0032
1	-0.0082	-0.0043	0.0	-0.0044	-0.0045	-0.0043
2	-0.0117	-0.0041	0.0	-0.0043	0.0	-0.0040
3	-0.0079	0.0	0.0	-0.0084	-0.0089	-0.0051
Average	-0.0089	-0.0021	0.0	-0.0053	-0.0044	-0.0041

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0	0.0084	0.0	0.0	0.0040	0.0025
1	0.0044	0.0201	0.0073	0.0136	0.0094	0.0110
2	0.0039	0.0075	0.0	0.0036	0.0070	0.0044
3	0.0061	0.0142	0.0027	0.0052	0.0098	0.0076
Average	0.0038	0.0127	0.0026	0.0058	0.0079	0.0066

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0236	0.0264	0.0170	0.0215	0.0146	0.0206
1	0.0168	0.0197	0.0214	0.0168	0.0069	0.0163
2	0.0220	0.0235	0.0183	0.0228	0.0197	0.0213
3	0.0159	0.0252	0.0159	0.0209	0.0225	0.0201
Average	0.0196	0.0238	0.0181	0.0206	0.0162	0.0197

Table B-11 (cont'd.).

3% Feed						
Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0305	0.0383	0.0267	0.0312	0.0232	0.0299
1	0.0411	0.0246	0.0242	0.0363	0.0248	0.0302
2	0.0244	0.0134	0.0235	0.0124	0.0168	0.0181
3	0.0165	0.0260	0.0220	0.0254	0.0294	0.0454
Average	0.0284	0.0258	0.0242	0.0275	0.0240	0.0260
5% Feed						
Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0440	0.0404	0.0399	0.0352	0.0345	0.0388
1	0.0566	0.0341	0.0443	0.0492	0.0383	0.0445
2	0.0517	0.0398	0.0387	0.0472	0.0411	0.0437
3	0.0639	0.0474	0.0444	0.0423	0.0431	0.0482
Average	0.0544	0.0408	0.0420	0.0436	0.0397	0.0441

Table B-12. Specific growth rates of *Sarotherodon niloticus* fed pelleted *Spirulina* sp at 27.5°C.

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0061	-0.0130	-0.0070	0.0	died	-0.0065
1	-0.0117	-0.0128	-0.0045	0.0	0.0	-0.0058
2	-0.0082	0.0	-0.0043	-0.0044	died	-0.0042
3	-0.0136	0.0	0.0	-0.0048	-0.0102	-0.0057
Average	-0.0102	-0.0060	-0.0037	-0.0025	-0.0048	-0.0055

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0145	0.0	-0.0106	-0.0056	-0.0058	-0.0055
1	0.0101	0.0183	0.0083	0.0104	0.0097	0.0117
2	0.0	0.0034	0.0065	0.0062	0.0088	0.0062
3	-0.0032	0.0032	0.0122	0.0029	0.0028	0.0053
Average	(-0.0009)	0.0071	0.0059	0.0049	0.0055	0.0058

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0153	0.0138	0.0185	0.0129	0.0102	0.0142
1	0.0057	0.0082	0.0102	0.0072	0.0069	0.0076
2	0.0159	0.0188	0.0146	0.0151	0.0120	0.0152
3	0.0250	0.0213	0.0185	0.0129	0.0151	0.0186
Average	0.0155	0.0158	0.0157	0.0123	0.0113	0.0141

Table B-12 (cont'd.).

3% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.0132	0.0089	0.0	0.0	0.0043	0.0
1	0.0350	0.0449	0.0394	0.0336	0.0338	0.0373
2	-0.0052	-0.0052	0.0	-0.0056	0.0	-0.0032
3	0.0421	0.0372	0.0314	0.0357	0.0297	0.0345
Average	0.0180	0.0275	0.0250	0.0241	0.0258	0.0241

5% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.0352	0.0535	0.0531	0.0423	0.0425	0.0453
1	0.0319	0.0319	0.0260	0.0356	0.0416	0.0334
2	0.0432	0.0202	0.0438	0.0381	0.0372	0.0365
3	0.0553	0.0450	0.0362	0.0391	0.0300	0.0411
Average	0.0432	0.0401	0.0407	0.0394	0.0369	0.0401

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

Table B-13. Specific growth rates of *Tilapia zillii* fed trout chow at 30°C. (duplicate trial)

0% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.016	-0.007	-0.0037	-0.0077	-0.0082	-0.0070
1	-0.011	-0.006	0.00	-0.0130	0.00	-0.0061
2	-0.017	0.00	-0.0145	0.00	0.00	-0.0064
3	-0.017	-0.005	-0.0047	-0.0048	0.00	-0.0062
Average	-0.0158	-0.0045	-0.0059	-0.0061	-0.0025	-0.0070

1% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	-0.008	0.00	0.00	-0.0082	-0.0087	-0.0042
1	-0.024	0.00	-0.0054	0.0054	0.0052	0.0013
2	-0.020	0.008	0.0040	0.0039	0.0039	0.0041
3	0.015	0.021	0.0151	0.0070	0.0066	0.0125
Average	(-0.0101)	0.0089	0.0048	0.0035	0.0023	0.0049

2% Feed

Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.039	0.007	0.00	0.0064	0.0175	0.0139
1	0.022	0.019	0.0136	0.0124	0.0168	0.0168
2	-0.019	0.031	0.0204	0.0260	0.0186	0.0153
3	0.002	0.010	0.0110	0.0167	0.0061	0.0091
Average	0.0080	0.0148	0.0118	0.0160	0.0124	0.0126

Table B-13 (cont'd.).

3% Feed						
Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.032	0.040	0.0378	0.0244	0.0256	0.0319
1	0.017	0.021	0.0180	0.0224	0.0347	0.0225
2	0.024	0.00	0.0122	0.0191	0.0234	0.0158
3	0.018	0.012	0.0220	0.0249	0.0110	0.0178
Average	0.0219	0.0164	0.0215	0.0225	0.0249	0.0214

5% Feed						
Fish #	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.018	-0.011	-0.0124	0.0239	0.0504	0.018
1	0.007	0.00	0.00	0.0215	0.0212	0.007
2	0.055	0.037	0.0237	0.0168	0.0102	0.055
3	0.020	0.022	0.0230	0.0111	0.0136	0.020
Average	0.0247	(0.0167)	(0.0142)	(0.0166)	(0.0186)	0.0247

Figures in parentheses represent growth rates which occurred during weeks when all feed was observed not to be eaten. These data are not included in overall averages.

APPENDIX C

Table C-1. Results of water chemistry analyses performed while *Tilapia zillii* were fed trout chow at 21.5°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Average
1	9.0	8.9	8.9	8.93
2	8.9	8.9	9.1	8.96
3	8.9	8.8	9.0	8.90
4	9.0	8.6	8.9	8.83

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Average
1	0.01	0.43	0.43	0.29
2	0.01	0.66	0.74	0.47
3	0.01	0.81	0.89	0.57
4	0.01	0.98	0.82	0.60

pH

% Feed	Initial	Week 1	Week 2	Average
1	8.30	8.20	8.20	8.23
2	8.30	8.25	8.25	8.27
3	8.35	8.25	8.20	8.27
4	8.30	8.25	8.20	8.25

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Average
1	21.7	21.6	21.4	21.5
2	21.9	21.6	21.4	21.5
3	21.4	21.3	21.4	21.3
4	21.8	21.7	21.5	21.6

Table C-2. Results of water chemistry analyses performed while *Tilapia zillii* were fed trout chow at 25°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	7.8	7.9	8.0	7.9	7.8	7.8	7.86
1	7.9	8.0	8.0	7.7	7.9	7.7	7.90
2	7.9	8.0	7.9	7.8	7.9	7.8	7.88
3	8.0	8.0	7.8	7.8	7.9	7.8	7.88
5	8.0	8.0	7.8	7.8	8.0	7.9	7.92

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.11	-	0.17	-	0.18	0.17	0.16
1	0.10	-	0.22	-	0.27	0.26	0.21
2	0.10	-	0.31	-	0.38	0.33	0.28
3	0.09	-	0.19	-	0.38	0.39	0.26
5	0.11	-	0.30	-	0.40	0.42	0.31

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.91	8.86	8.87	-	8.83	8.79	8.85
1	8.91	8.83	8.87	-	8.80	8.79	8.83
2	8.90	8.79	8.83	-	8.81	8.80	8.83
3	8.87	8.70	8.73	-	8.71	8.72	8.75
5	8.88	8.60	8.60	-	8.58	8.60	8.65

Table C-2 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	25.0	24.9	24.9	25.0	25.0	25.0	25.0
1	24.5	24.8	25.0	25.0	24.9	25.0	24.9
2	25.5	24.9	25.0	24.9	25.0	25.0	25.0
3	26.5	25.3	25.0	25.0	25.0	25.0	25.1
5	25.0	24.9	25.0	25.0	25.0	25.0	25.0

Alkalinity

Alkalinity values exhibited little fluctuation averaging 359 mg/l $CaCO_3$ for all aquaria.

Table C-3. Results of water chemistry analyses performed while *Tilapia zillii* were fed trout chow at 27.5°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
0	8.0	7.9	-	7.9	7.8	7.70
0.2	8.1	8.1		8.1	8.4	8.17
0.5	8.1	8.2		8.1	8.4	8.20
0.7	8.1	8.1		8.2	8.4	8.20
1	7.9	7.9	-	8.0	8.0	7.95
2	8.1	8.0	-	7.8	7.6	7.88
3	8.0	7.8	-	7.7	7.9	7.85
4	8.0	7.6	-	7.7	7.8	7.78

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
0	0.04	0.08	-	0.13	0.18	0.11
0.2	0.03	0.28		0.15	0.01	0.12
0.5	0.03	0.18		0.02	0.01	0.06
0.7	0.03	0.15		0.02	0.02	0.06
1	0.03	0.17	-	0.27	0.24	0.18
2	0.03	0.31	-	0.22	0.26	0.21
3	0.01	0.47	-	0.49	0.40	0.34
4	0.01	0.58	-	0.61	0.38	0.40

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
0	8.93	8.89	-	8.89	8.86	8.89
0.2	8.25	8.50		8.55	8.45	8.44
0.5	8.30	8.50		8.60	8.50	8.47
0.7	8.35	8.50		8.60	8.50	8.49
1	8.93	8.91	-	8.80	8.78	8.86
2	8.89	8.83	-	8.67	8.56	8.74
3	8.89	8.80	-	8.66	8.62	8.74
4	8.94	8.81	-	8.59	8.50	8.71

Table C-3 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
0	26.0	27.2	27.5	27.3	27.2	27.3
0.2	27.5	27.7	27.4	26.6	28.4	27.5
0.5	27.4	27.7	27.5	27.6	27.5	27.5
0.7	27.7	27.7	27.5	27.5	27.5	27.6
1	26.0	27.0	27.6	27.5	27.3	27.4
2	26.0	27.7	27.6	27.4	26.9	27.5
3	26.5	27.0	27.5	27.5	27.3	27.3
4	27.0	27.2	27.7	27.6	27.5	27.5

Alkalinity

Alkalinity values exhibited little fluctuation averaging 338 mg/l $CaCO_3$ for all aquaria.

Table C-4. Results of water chemistry analyses performed while _Tilapia_ _zillii_ were fed trout chow at 30°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.9	8.8	-	8.7	8.6	8.7	8.74
1	8.8	8.8	-	8.6	8.6	8.5	8.66
2	8.7	8.6	-	8.4	8.5	8.5	8.54
3	8.8	8.6	-	8.4	8.2	8.4	8.48
5	8.8	8.7	-	8.5	8.3	8.1	8.48

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.01	0.08	0.04	0.01	0.01	0.01	0.03
1	0.01	0.09	0.06	0.01	0.01	0.01	0.03
2	0.01	0.16	0.05	0.03	0.01	0.04	0.05
3	0.01	0.17	0.05	0.21	0.10	0.05	0.10
5	0.01	0.01	0.05	0.17	0.27	0.16	0.11

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.86	8.88	8.88	8.89	8.73	8.52	8.79
1	8.73	8.73	8.73	8.70	8.71	8.74	8.72
2	8.76	8.69	8.65	8.63	8.63	8.61	8.66
3	8.76	8.48	8.46	8.42	8.44	8.51	8.51
5	8.64	8.36	8.40	8.43	8.42	8.51	8.46

Table C-4 (cont'd.).

<u>Temperature</u> (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	29.0	29.5	29.9	29.8	29.9	30.0	29.8
1	29.5	29.9	30.0	29.9	29.9	30.0	30.0
2	29.5	29.9	29.9	29.9	29.9	30.0	29.9
3	30.5	29.8	29.9	29.7	30.0	30.0	29.9
5	30.0	30.0	30.0	29.9	30.1	30.0	30.0

<u>Alkalinity</u>

Alkalinity values exhibited little fluctuation averaging 354 mg/l $CaCO_3$ for all aquaria.

Table C-4 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	29.0	29.5	29.9	29.8	29.9	30.0	29.8
1	29.5	29.9	30.0	29.9	29.9	30.0	30.0
2	29.5	29.9	29.9	29.9	29.9	30.0	29.9
3	30.5	29.8	29.9	29.7	30.0	30.0	29.9
5	30.0	30.0	30.0	29.9	30.1	30.0	30.0

Alkalinity

Alkalinity values exhibited little fluctuation averaging 354 mg/l $CaCO_3$ for all aquaria.

Table C-5. Results of water chemistry analyses performed while *Tilapia zillii* were fed trout chow at 35°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.7	8.7	-	8.5	8.5	8.6	8.60
1	8.9	8.6	-	8.7	8.6	8.8	8.72
2	8.8	8.8	-	8.7	8.6	8.5	8.68
3	8.8	8.8	-	8.6	8.3	8.1	8.52
5	8.7	8.8	-	8.5	7.9	7.7	8.32

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.01	0.15	0.02	0.01	0.01	0.03	0.04
1	0.01	0.18	0.04	0.01	0.01	0.02	0.04
2	0.02	0.43	0.43	0.06	0.01	0.07	0.17
3	0.02	0.57	0.53	0.11	0.04	0.08	0.22
5	0.03	0.74	0.57	0.36	0.15	0.21	0.34

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.77	8.52	8.56	8.64	8.61	8.56	8.61
1	8.87	8.73	8.68	8.61	8.61	8.60	8.68
2	8.81	8.70	8.69	8.64	8.63	8.64	8.68
3	8.88	8.66	8.66	8.69	8.68	8.67	8.71
5	8.80	8.64	8.61	8.56	8.56	8.60	8.62

Table C-5 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	34.5	33.8	34.6	34.8	34.6	34.7	34.5
1	34.0	34.8	34.8	35.2	34.3	35.3	34.9
2	32.0	34.2	35.1	35.2	35.2	34.8	34.9
3	34.0	34.6	35.1	34.9	34.9	34.5	34.8
5	34.0	34.8	35.5	35.0	34.9	35.0	35.1

Alkalinity

Alkalinity values exhibited little fluctuation averaging 329 mg/l $CaCO_3$ for all aquaria.

Table C-6. Results of water chemistry analyses performed while *Tilapia zillii* fry (0.0140 g average weight) were fed trout chow at 27.5°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Average
1	8.0	7.8	7.9
2	8.0	7.7	7.8
3	7.9	7.7	7.8
5	7.9	7.9	7.9
10	8.0	7.8	7.9

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Average
1	0.01	0.04	0.02
2	0.01	0.02	0.01
3	0.01	0.01	0.01
5	0.01	0.01	0.01
10	0.01	0.05	0.03

pH

% Feed	Initial	Week 1	Average
1	8.60	8.60	8.60
2	8.65	8.60	8.62
3	8.70	8.65	8.67
5	8.70	8.65	8.67
10	8.70	8.65	8.67

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Average
1	27.0	27.1	27.1
2	28.0	28.7	28.7
3	27.5	28.1	28.1
5	27.5	27.4	27.4
10	27.5	27.6	27.6

Table C-7. Results of water chemistry analyses performed while *Tilapia zillii* fry (1.75 g average weight) were fed trout chow at 27.5°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Average
1	7.8	7.7	7.7
2	7.9	7.8	7.8
3	7.8	7.8	7.8
5	7.8	7.8	7.8
7	7.9	7.8	7.8

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Average
1	0.01	0.07	0.04
2	0.02	0.12	0.07
3	0.01	0.14	0.07
5	0.01	0.26	0.13
7	0.02	0.37	0.19

pH

% Feed	Initial	Week 1	Average
1	8.65	8.35	8.50
2	8.60	8.15	8.37
3	8.70	8.30	8.50
5	8.70	8.15	8.42
7	8.70	8.25	8.47

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Average
1	27.0	27.4	27.4
2	28.0	27.6	27.6
3	27.5	27.5	27.5
5	26.0	27.4	27.4
7	27.5	27.5	27.5

Table C-8. Results of water chemistry analyses performed while *Tilapia zillii* (38.1 g average weight) were fed trout chow at 27.5°C.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Average
0	8.0	7.9	8.0	7.97
1	8.0	8.0	7.9	7.97
2	7.9	8.0	7.9	7.93
3	8.1	8.0	8.0	8.03

pH

% Feed	Initial	Week 1	Week 2	Average
0	8.81	8.79	8.68	8.76
1	8.73	8.70	8.76	8.73
2	8.80	8.62	8.59	8.67
3	8.75	8.63	8.51	8.63

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Average
0	27.5	27.5	27.7	27.6
1	27.5	27.5	27.8	27.6
2	27.5	27.5	27.7	27.6
3	27.5	27.5	27.7	27.6

Alkalinity

Alkalinity values exhibited little fluctuation averaging 329 mg/l $CaCO_3$ for all aquaria.

Table C-9. Results of water chemistry analyses performed while *Tilapia zillii* were fed pelleted *Elodea canadensis*.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	7.70	8.30	9.40	9.90	9.90	8.81	9.00
1	8.05	8.25	9.20	9.90	9.95	8.67	9.00
2	7.75	8.15	9.30	9.85	9.90	8.47	8.90
3	7.75	8.20	9.25	9.90	8.30	8.67	8.68
5	7.80	8.00	9.15	9.80	9.90	8.50	8.86

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.01	0.42	0.38	0.38	0.22	0.05	0.24
1	0.02	0.39	0.39	0.03	0.05	0.03	0.15
2	0.02	0.63	0.73	0.06	0.04	0.04	0.25
3	0.02	0.17	0.13	0.04	0.05	0.03	0.07
5	0.01	0.16	0.11	0.03	0.04	0.03	0.06

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.78	8.81	8.76	8.80	8.77	8.81	8.79
1	8.85	8.90	8.68	8.60	8.62	8.67	8.72
2	8.62	8.64	8.60	8.52	8.52	8.47	8.56
3	8.83	8.69	8.51	8.56	8.38	8.67	8.61
5	8.85	8.58	8.59	8.47	8.53	8.50	8.59

Table C-9 (cont'd.).

Alkalinity

Alkalinity values exhibited little fluctuation averaging 350 mg/l $CaCO_3$ for all aquaria.

Temperature

Temperatures for all aquaria remained at room temperature throughout the study period averaging 24.5°C.

Table C-10. Results of water chemistry analyses performed while *Tilapia zillii* were fed pelleted *Spirulina* sp.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
1	8.1	8.1	7.9	8.1	8.0	8.04
2	8.2	8.1	7.9	8.1	7.9	8.04
3	8.1	8.1	7.8	8.0	8.1	8.02
5	8.1	8.0	7.9	7.9	7.8	7.94

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
1	0.03	0.31	0.28	0.02	0.05	0.14
2	0.02	0.50	0.03	0.05	0.09	0.14
3	0.03	0.70	0.02	0.02	0.05	0.16
5	0.03	1.22	0.03	0.13	0.07	0.30

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
1	8.35	8.45	8.35	8.35	8.50	8.40
2	8.30	8.45	8.40	8.50	8.55	8.44
3	8.30	8.45	8.35	8.50	8.55	8.43
5	8.30	8.45	8.35	8.40	8.20	8.34

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Average
1	28.5	27.7	27.6	27.6	27.5	27.6
2	28.5	27.6	27.6	27.6	27.4	27.6
3	28.5	27.7	27.6	27.8	27.4	27.6
5	27.5	27.4	27.6	27.6	27.7	27.5

Alkalinity

Alkalinity values exhibited little fluctuation averaging 343 mg/l $CaCO_3$ for all aquaria.

Table C-11. Results of water chemistry analyses performed while *Sarotherodon niloticus* were fed trout chow.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.10	-	7.85	7.90	7.80	7.50	7.83
1	7.90	-	7.95	7.90	7.90	7.80	7.89
2	8.10	-	7.85	7.90	7.80	7.70	7.87
3	8.10	-	7.95	7.85	7.80	7.75	7.89
5	8.20	-	7.80	7.70	7.55	7.35	7.72

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.01	-	0.06	0.02	0.08	0.04	0.04
1	0.01	-	0.06	0.04	0.05	0.06	0.04
2	0.01	-	0.07	0.04	0.07	0.06	0.05
3	0.01	-	0.07	0.04	0.05	0.06	0.05
5	0.02	-	0.09	0.06	0.09	0.07	0.07

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.50	-	8.45	8.70	8.60	8.65	8.58
1	8.50	-	8.40	8.60	8.65	8.65	8.56
2	8.50	-	8.40	8.60	8.55	8.60	8.53
3	8.55	-	8.40	8.60	8.55	8.60	8.54
5	8.55	-	8.35	8.45	8.40	8.35	8.42

Table C-11 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	28.0	27.4	27.4	27.4	27.4	27.5	27.4
1	29.0	26.9	26.8	27.6	27.5	27.5	27.2
2	27.5	27.4	27.6	27.6	27.8	27.6	27.6
3	27.5	27.8	27.2	27.4	28.8	27.9	27.8
5	27.5	27.6	27.5	27.5	27.5	27.3	27.5

Alkalinity

Alkalinity values exhibited little fluctuation averaging 390 mg/1 $CaCO_3$ for all aquaria.

Table C-12. Results of water chemistry analyses performed while *Sarotherodon niloticus* were fed pelleted *Spirulina* sp.

Dissolved Oxygen (mg/l D.O.)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.20	-	8.00	7.90	8.00	7.80	7.98
1	8.20	-	7.80	7.60	7.50	7.70	7.76
2	8.20	-	7.40	7.45	7.50	7.45	7.60
3	8.10	-	7.45	7.50	7.00	7.40	7.49
5	8.10	-	7.50	7.40	7.30	7.05	7.47

Ammonia Nitrogen (mg/l-N)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	0.01	-	0.07	0.04	0.04	0.04	0.04
1	0.01	-	0.07	0.04	0.05	0.04	0.04
2	0.02	-	0.07	0.04	0.05	0.04	0.04
3	0.02	-	0.13	0.04	0.11	0.04	0.07
5	0.01	-	0.13	0.06	0.08	0.05	0.07

pH

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	8.45	-	8.40	8.50	7.95	8.20	8.30
1	8.45	-	8.30	8.45	8.25	8.55	8.40
2	8.45	-	8.25	8.35	8.20	8.40	8.33
3	8.50	-	8.35	8.40	8.15	8.40	8.36
5	8.50	-	8.30	8.25	8.20	8.25	8.30

Table C-12 (cont'd.).

Temperature (°C -- 7 day averages)

% Feed	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Average
0	27.5	27.4	27.3	27.5	27.4	27.5	27.4
1	27.5	27.4	27.4	27.6	27.1	27.5	27.4
2	27.5	27.6	27.6	27.6	27.3	27.5	27.5
3	27.5	27.6	27.4	27.4	27.4	27.7	27.5
5	28.0	28.1	27.9	27.6	27.7	27.7	27.8

Alkalinity

Alkalinity values exhibited little fluctuation averaging 373 mg/l $CaCO_3$ for all aquaria.

APPENDIX D

Table D-1. Chemical analysis of Purina Trout Chow, Grower, Size #3, (provided by manufacturer).

Guaranteed Analysis

Crude protein not less than	40.0%
Crude fat not less than	9.0%
Crude fiber not more than	5.0%
Ash not more than	10.0%
Added minerals not more than	1.5%

Ingredients

Fish meal, soybean meal, wheat middlings, ground yellow corn, dried whey, soybean oil, dried yeast, dehydrated alfalfa meal, corn gluten meal, calcium carbonate, salt, ethoxyquin (a preservative), vitamin A supplement, D activated animal sterol (source of vitamin D-3), menadione sodium bisulfite (source of vitamin K activity), vitamin E supplement, DL methionine, vitamin B-12 supplement, ascorbic acid, biotin, choline chloride, folic acid, pyridoxine hydrochloride, thiamin, niacin, calcium pantothenate, riboflavin supplement, copper sulfate, manganous oxide, ferrous carbonate, calcium iodate, cobalt carbonate, zinc sulfate.

Table D-2. Typical chemical analysis of *Spirulina* sp. algae used in pelleted algae feeding trials (analysis provided by manufacturers).

Chemical Composition	
Moisture	7.0%
Ash	9.0%
Proteins	71.0%
Crude fiber	0.9%

Essential Amino Acids	
Isoleucine	4.13%
Leucine	5.80%
Lysine	4.00%
Methionine	2.17%
Phenylalanine	3.95%
Threonine	4.17%
Tryptophan	1.13%
Valine	6.00%

Nonessential Amino Acids	
Alanine	5.82%
Arginine	5.98%
Aspartic Acid	6.43%
Cystine	0.67%
Glutamic Acid	8.94%
Glycine	3.46%
Histidine	1.08%
Proline	2.97%
Serine	4.00%
Tyrosine	4.60%

Vitamins		
Biotin (H)	average	0.4 mg/kg
Cyanocobalamin (B-12)	average	2 mg/kg
d-Ca-Pantothenate	average	11 mg/kg
Folic Acid	average	0.5 mg/kg
Inositol	average	350 mg/kg
Nicotinic Acid (PP)	average	118 mg/kg
Pyridoxine (B-6)	average	3 mg/kg
Riboflavine (B-2)	average	40 mg/kg
Thiamine (B-1)	average	55 mg/kg
Tocopherol (E)	average	190 mg/kg

Minerals	
Calcium (Ca)	1,315 mg/kg
Phosphorus (P)	8,942 mg/kg
Iron (Fe)	580 mg/kg
Sodium (Na)	412 mg/kg
Chloride (Cl)	4,400 mg/kg
Magnesium (Mg)	1,915 mg/kg
Manganese (Mn)	25 mg/kg
Zinc (Zn)	39 mg/kg
Potassium (K)	15,400 mg/kg
Others	57,000 mg/kg

Table D-3. Results of analyses performed on feeds to determine carbon, hydrogen, nitrogen, protein and caloric content.

Spirulina--Pelleted

	% Nitrogen	% Protein	% Carbon	% Hydrogen	K cal/g
	9.64	60.2	43.7	7.10	4.65
	9.71	60.7	43.9	7.11	4.62
Avg	9.67	60.5	43.8	7.10	4.63

Spirulina--Cultured

	% Nitrogen	% Protein	% Carbon	% Hydrogen	K cal/g
	10.56	66.0	49.1	7.27	-
	10.61	66.3	49.2	7.70	-
	10.65	66.6	49.3	7.64	-
Avg	10.61	66.3	49.2	7.54	-

Commercial Trout Chow

	% Nitrogen	% Protein	% Carbon	% Hydrogen	K cal/g
	6.55	40.9	42.7	7.03	4.84
	6.37	39.8	41.4	6.68	4.74
	6.41	40.1	42.4	6.80	-
Avg	6.44	40.2	42.2	6.84	4.79

Elodea canadensis--Pelleted

	% Nitrogen	% Protein	% Carbon	% Hydrogen	K cal/g
	3.40	21.2	40.7	6.1	-
	3.30	20.6	40.3	6.1	3.86
	3.30	20.6	40.1	5.8	3.84
Avg	3.33	20.8	40.4	6.0	3.85

APPENDIX E

Table E-1. Composition of *Spirulina* media.

Nutrient	Concentration
$NaHCO_3$	13.61 g/l
Na_2CO_3	4.03 g/l
K_2HPO_4	0.50 g/l
$NaNO_3$	2.50 g/l
K_2SO_4	1.00 g/l
NaCl	1.00 g/l
$MgSO_4 \cdot 7H_2O$	0.20 g/l
$CaCl_2 \cdot 2H_2O$	0.04 g/l
Metal Solution	6.0 ml/l
Micronutrient Solution	1.0 ml/l
Thiamin·HCl	0.10 mg/l
Biotin	0.5 μg/l
B_{12}	0.5 μg/l
Composition of Metal Solution	
Na_2-EDTA	750. mg/l
$Na_2MoO_4 \cdot 2H_2O$	4. mg/l
$CoCl_2 \cdot 6H_2O$	2. mg/l
$FeCl_3 \cdot 6H_2O$	97. mg/l
$MnCl_2 \cdot 4H_2O$	41. mg/l
$ZnCl_2$	5. mg/l
Composition of Micronutrient Solution	
Na_2-EDTA	50.0 mg/l
$CuSO_4 \cdot 5H_2O$	19.6 mg/l
$CoCl_2 \cdot 6H_2O$	20.0 mg/l
H_3BO_3	618.4 mg/l
$MnCl_2 \cdot 4H_2O$	36.0 mg/l
$Na_2MoO_4 \cdot 2H_2O$	12.6 mg/l
$ZnSO_4 \cdot 7H_2O$	44.0 mg/l