Quantitative and qualitative aspects of energy acquisition of the cichlid fish *Oreochromis niloticus* L.

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ABSTRACT

A unified theory of fish growth and feed intake was proposed with the aid of a conceptual growth model. Feeding experiments were performed with individuals and groups of *Oreochromis niloticus* (L) (2-50g in weight) using diets with differed biochemical composition. The functional relationship between growth and feed intake was found to be linear, irrespective to the growth indices is used as the dependent variable. The protein and energy requirement of *O. niloticus* was quantified and maximum requirements of 2 - 12g fish was approximately 22g protein $kg^{-1}BW\ day^{-1}$ or 990 $kJ^{-1}\ kg^{-1}BW\ day^{-1}$ in terms of mammalian physiological fuel values. The requirements of fish of 18 - 50g weight range was approximately 12g protein $kg^{-1}BW\ day^{-1}$ with a corresponding energy intake of 650 $kJ^{-1}\ kg^{-1}BW\ day^{-1}$ for the maximum growth. Feeding fish above those limits resulted in a slight retardation of growth. The maintenance requirements were computed with the regression curves of growth and feed intake and found to be vary depending upon the equation used.

The digestibility coefficients of protein, lipid and carbohydrates were established to be as high as 90%, and the coefficients did not change over the weight range of the experimental fish. The optimum non-protein and protein energy ratio (NPE/PE) for *O. niloticus* was 1.72 and NPE was further analysed into lipid (LE) and carbohydrate energy (CE). It was found that the LE/CE ratio for *O. niloticus* is 2.25 in terms of mammalian physiological fuel values.

A development of the conceptual growth model for predictive growth modelling was discussed.

CHAPTER 1

INTRODUCTION

Among its many facets, there are two major aspects of fish farming. One, like many business ventures, has as its objective the profitable production of a high value commodity to satisfy the various demands of customers. The other, apparent in Asia, Africa and Latin America, is the provision of an inexpensive yet highly acceptable animal protein source as a basic food for a human populations. The former is particularly important in economically developed countries, however, both are equally valuable to the developing world as the agricultural sector has to provide both capital and labour for industrial development (Grigg, 1978). Whatever the ultimate goal, it is imperative that aquaculture production per unit area is increased as the available land per head is drastically declining due to over-population (FAO, 1981). However, one of the major constraints to increasing output per unit area is feeding costs which account for 30 - 60% of total cost depending on the species (Greenfield, 1970; Huguenin and Ansuini, 1978; Giachelli *et al.*, 1982; Griffin *et al.*, 1984). Reducing the cost of feed provides more opportunity for diminishing production costs than any other single expense (Lovell, 1983). Since a reduction in feed cost depends primarily on the efficient utilization of the nutrients by the animal, a better understanding of the nutrient/ energy requirement of fish in relation to their metabolism and growth is fundamental to successful production planning of any aquaculture enterprise.

Feeding and the search for food are probably the major activities in the life of most animals in the natural environment as food is scattered in both time and space

(Rozin, 1976). Hence, a major focus of attention for ecologists has been food resource acquisition by animals and there are a number of conceptual and predictive models dealing with the question of when and on what an animal should feed and should not feed (*eg.*: Schoener, 1971; Charnov, 1976; Pyke *et al.*, 1977). However, in any animal production system the food is supplied either by enhancing the natural production of food items or by the provision of prepared food. The questions which may be raised here are not only concerned with what and when but also how much feed should be given. Apart from the added cost of excessive feeding and/or attempts to increase natural food through fertilization, the quantity of food given and/or fertilizers added is particularly important in aquaculture systems as excess food or excess waste may seriously degrade the aquatic environment in which the animals are being grown (Cole and Boyd, 1986).

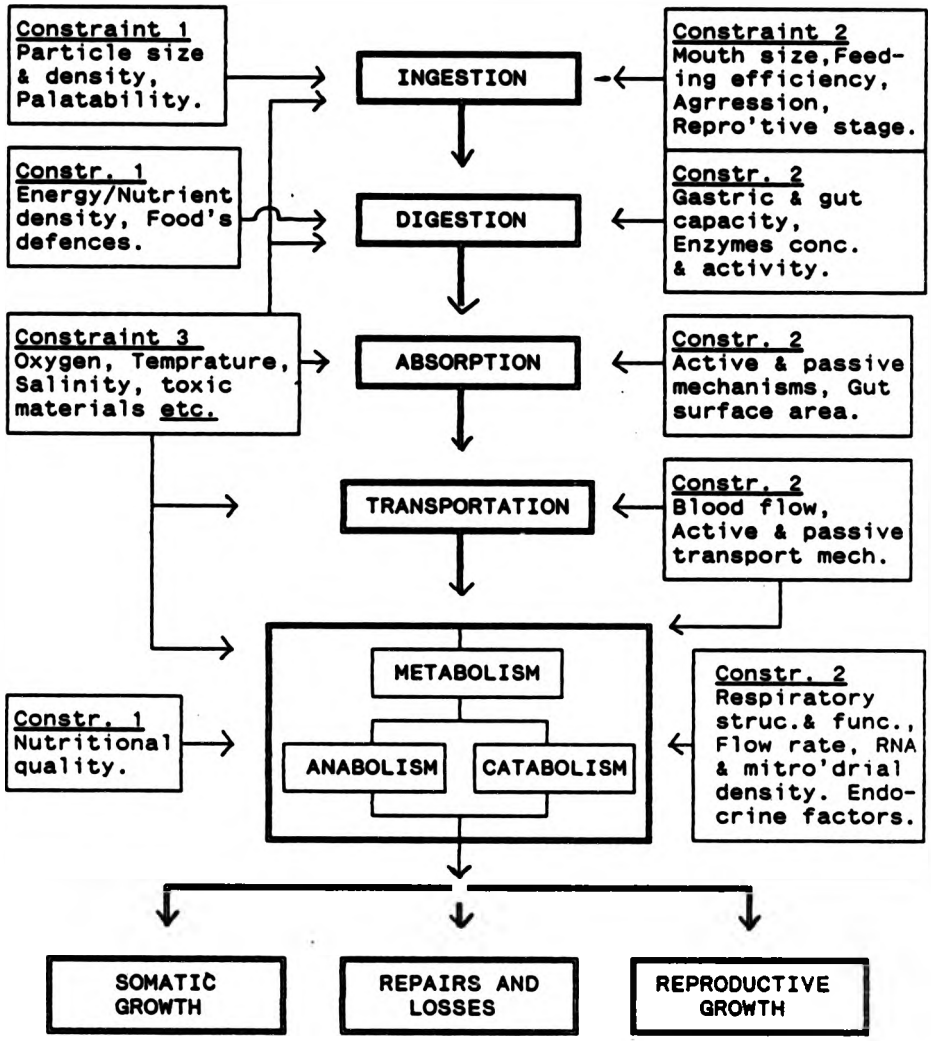
There are three principal types of constraints affecting any aquatic animal attempting to extract nutrients/energy from a food resource (Fig.1.1).

(1) Constraints imposed by food items.
i.e. Particle size and density, palatability, defence against digestion, inadequate nutrient quality *etc.*

(2) Structural, behaviourial and physiological constraints specific to the animal.
i.e. Oral and gut capacity, aggression, rate of ingestion, digestion and assimilation *etc.*

(3) Environmental constraints.
i.e. Oxygen concentration, temperature, salinity, presence of toxic materials *etc.*

Fig. 1.1 Some constraints affecting any aquatic animal attempting to extract and utilise nutrients/energy from a food resource.


Constraint 1
Particle size & density, Palatability.
Constr. 1
Energy/Nutrient density, Food's defences.
Constraint 3
Oxygen, Temprature, Salinity, toxic materials etc.
INGESTION
DIGESTION
ABSORPTION
TRANSPORTATION
Constraint 2
Mouth size, Feeding efficiency, Agrression, Repro'tive stage.
Constr. 2
Gastric & gut capacity, Enzymes conc. & activity.
Constr. 2
Active & passive mechanisms, Gut surface area.
Constr. 2
Blood flow, Active & passive transport mech.
METABOLISM
ANABOLISM
CATABOLISM
Constr. 1
Nutritional quality.
Constr. 2
Respiratory struc.& func., Flow rate, RNA & mitro'drial density. Endocrine factors.
SOMATIC GROWTH
REPAIRS AND LOSSES
REPRODUCTIVE GROWTH

The literature in the field of fish biology explaining how these constraints operate in fish is voluminous and impressive (Ivlev, 1961; review papers in Hoar and Randall, 1969, 1971, 1979 and Tytler and Calow, 1985). However, there is a clear dichotomy in both theory and practice concerning how fish growth responds to feed intake (Birkett, 1972; Brett and Groves, 1979; Brett, 1979; Ricker, 1979; Condry, 1982) and the reaction of fish to food of different energy density (Jobling, 1986). There are a considerable number of studies determining the effects of varying nutrient levels on the growth of fish (*eg.* Garling and Wilson, 1976, 1977; Jauncey, 1982) but most of the dietary formulations are based either on a percentage composition basis or on gross/metabolisable energy terms with little attempt to resolve their inter-relationships. Several detailed scientific studies, principally on temperate carnivorous and zooplanktivorous fish (Gerking, 1955, 1971; Brett *et al.*,1969; Elliott, 1975a, 1975b, 1976a, 1976b, 1976c, 1982), the omnivorous common carp, *Cyprinus carpio* (Huisman, 1974, 1976), channel catfish, *Ictalurus punctatus* (Gatlin *et al.*, 1986) and various marine fishes (*eg.* Menzel, 1960, Stirling, 1972) have focused on how food ration size is related to growth. The underlying mechanisms of energy limited growth, however, are poorly understood and maximum and optimum food rations still have no sound scientific basis. (Brett and Grove,1979). A comprehensive study would not only attempt to explain how these constraints operate in a fish, but in addition, should also help to produce a significant increase in yield and hence profit, through the optimisation of inputs.

This thesis is an attempt to explore some of those quantitative and qualitative aspects of food resource acquisition and utilization by a tropical cichlid fish,

Oreochromis niloticus (L) under confined conditions. This species is one of approximately eighty which are collectively termed tilapias and comprise the tribe Tilapiini (Trewavas,1983). Although originally of African origin, the tilapias are now widely distributed throughout the tropics. (Balarin, 1979; Philippart and Ruwet, 1982). They are primarily omnivorous, ingesting phytoplankton, zooplankton, detritus, periphyton and aquatic weeds, but are unusual in their ability to feed and digest phytoplankton, especially blue-green algae (Cyanobacteria) and bacteria, very efficiently (Moriarty *et al.*, 1973; Bowen, 1976). As a result of its plasticity in feeding habits (Bowen, 1982; Maitipe and De Silva, 1985), tolerance of adverse environmental conditions (Chervinski, 1982), resistance to disease (Roberts and Sommerville, 1982), rapid growth rate (Caulton, 1982) and its ability to convert less refined protein sources into high quality protein suitable for human consumption (Jauncey and Ross, 1982), *Oreochromis niloticus* has been hailed as the "aquatic chicken" of the future (Pullin, 1985). However, information on the qualitative and quantitative energy / nutrient requirement of this species is fragmentary or completely lacking (Jauncey and Ross, 1982). The following questions, therefore, are addressed in the present study:

(1) What are the energy/nutrient requirements of *O. niloticus* in a confined system, and are those requirements comparable with other fish species and homiothermic terrestrial animals?

(2) How does the fish respond to limited and unlimited energy/food supply ?

(3) How great a range in food composition can the fish utilize without significant reduction in growth?

In the process of exploring these aspects of fish biology an *a priori* approach, aided by conceptual models, is used. The difference between an *a priori* and an *a posteriori* approach is that the former searches for the possible causal factors involved in advance, whilst with the latter explanations are formulated after observing correlations but are taken no further (Sibly and Calow, 1986). The hypotheses generated by the *a priori* approach should be subjected to empirical validation. However, it is impossible to study all the variables involved in energy acquisition by an animal in a single short term study. Therefore, the data for synthesis was derived from a limited number of experiments and supplemented with data extracted from an extensive literature survey on tilapias and other fish species.

Studies on resource acquisition follow two general approaches (Hainsworth and Wolf, 1979). Ecological studies focus on how external parameters such as "prey" size and its density and behaviourial factors affect ingestion (Ivlev, 1961), whilst physiological studies are concerned with how internal feedback systems control feeding through chemical and electrical co-ordination [*eg.*thermostatic, glucostatic, lipostatic, hepatostatic and cholecystokinin (CCK) theories; for recent reviews see Novin *et al.*, 1976]. The present thesis is also an attempt to integrate the two approaches into a single explanatory model with various sub-models, and to formulate some basic guidelines which would be applicable to other cultured or wild fish species. This task, however, cannot be achieved without investigating some theoretical aspects of fish growth and without simplifying assumptions on fish biology.

All models are a substantial simplification of reality. They do not reveal a panoramic view of every detail of a complex process, as even the simplest event has so many variables which cannot be observed (Calow, 1976). Building conceptual models is not an end in itself but a useful exercise which may show the areas and pathways which are worthy of investigation (Phillipson, 1975). Therefore, the utility and the validity of the hypotheses presented here may be judged in relation to their contribution to an understanding of fish growth and feeding relationships, and may be enhanced by future refinements.

CHAPTER 2

2.1 Introduction.

The success of well planned terrestrial animal production has depended primarily upon the integration of energetic principles into the production process (Brody, 1945; Kleiber, 1961). Realizing the economic importance of fish and the need for better management of fish production processes, Ivlev (1939, 1945) introduced energetic concepts to aquatic animal production. Later Winberg's (1956) investigations of the biology of aquatic organisms, especially metabolic studies of fish, established the scientific foundation. This approach, perhaps due to its logical nature, has since attracted many biologists and there are a considerable number of review articles and models of fish energetics (*eg.* Ivlev, 1945; Ricker, 1946; Fry, 1947,1971; Winberg, 1956; Parker and Larkin, 1959; Windell, 1966; Paloheimo and Dickie, 1966; Ursin, 1967; Warren and Davis, 1967; Warren,1971; Beamish *et al.*, 1975; Kapoor *et al.*, 1975; Jones, 1976; Elliot, 1979; review papers in Hoar and Randal, 1979; Jobling, 1981, 1986, 1988; Cho *et al.*, 1982; review papers in Tytler and Calow, 1985; Machiels and Henken, 1986; Pandian, 1987; Weatherly and Gill, 1987).

The present study, however, is not an empirical exercise in energy budgeting; rather, it attempts to answer an apparently simple question; 'If fish ingest X quantity of food of Y quality, why do they grow in the way that they do ?' The remaining content of this chapter, therefore, is aimed at a critical analysis of the models delineating the relationship amongst food, feeding and growth (Section 2.2) and at

generating new models based on existing concepts (Section 2.3) in order to evaluate their answers to the above question

2.2 Literature review.

Growth implies the accumulation of material (energy) from the environment. Initially, therefore, a brief look at the general concepts of what fish ingest with emphasis on their requirements is essential.

2.2.1 The energy requirement of fish.

According to optimal foraging theory an animal should minimise the cost and maximise energy intake (*eg.* Pyke *et al.*, 1977). Summarising Love's (1980) data Pandian and Vivekanandan (1985) show that of 600 fish species 85% are carnivorous, 6% are herbivores, 4% omnivores, 3% detritivores and 2% scavengers. They conclude that carnivory is more profitable than any other form of feeding habit because;

(a) all other forms of feeding habits, except carnivory, demand "biting, nibbling, scraping, crushing hard structures, filtering enormous volume of water to ingest little amount of algae".

(b) Carnivores have an advantage over others as their prey size is comparatively larger. They become satiated in a relatively shorter time (Ishiwata, 1968) whereas detritivores and filter feeders have to spend a considerable time feeding (Odum, 1973, Moriarty and Moriarty, 1973).

(c) analyses of protein requirement data show that fish need more protein than terrestrial animals.

Propositions (a) and (b).

Palatability is a major factor that has been considered for fish being mainly carnivorous in feeding habit. However, herbivorous and omnivorous fish constitute 31% of tropical and 55% of sub-tropical coral reef fish communities respectively (Pandian, 1987). As the coral reef environment is rich in algae and detritus, this suggests that the problem lies not in palatability or 'prey' size but in the availability of feed in the surrounding environment. For example, mullets (*Mugil cephalus*) can extract sufficient nutrients from detritus containing less than 0.3% carbon (Moriarity, 1976). The tilapia (*O.mossambicus*) can derive adequate nutrients from the detritus containing 4.5% total amino acids (Bowen, 1980) whilst the blue tilapia (*Tilapia zilli*) can extract 75% of the protein from an aquatic weed, *Naja guadalupensis*, which contains 28% cellulose (Buddington, 1979). The grass carp, *Ctenopharyngorden idella* can also thrive on algae, *Spyrogyra maxima* (Hannifa and Venkatachalum, 1980) containing 36% mineral content whilst common carp (*Cyprinus carpio*) grow well on a diet of algae *Mougeotia gotlandica* containing 42.5% minerals (Singh and Bhanot, 1970). The diet of *Scarus oviceps* is dependent on faeces from *Zebrasoma scopas* which contains 56% ash (Bailey and Robertson, 1982). Laboratory experiments have shown channel catfish (*Ictalurus punctatus*) suffer no growth retardation fed on diets containing 30% cellulose (Gatlin *et al.*,1986). The recommended dietary fibre level for rainbow trout (*Salmo gairdneri*) is less than 10 % of the diet (Hilton *et al.*, 1983). However, Davies (1982) showed that there was no growth depression at inclusion levels of up to 16% dietary fibre and Bromley and Adkins (1984) have demonstrated that rainbow trout can tolerate 30% cellulose in the diets.

Proposition (c).

The physiological basis for carnivory among fish is that; (I) as fish are poikilothermic they do not spend energy in regulating body temperature (Nijkamp *et al.*, 1974); (II) fish derive more metabolisable energy from protein than mammals as they do not have to spend energy on urea production (Rumsey, 1978).

Irrespective of their feeding habit at the adult stage, however, most species are carnivorous during their larval stage (Dabrowski, 1984) and only switch to an adult diet during the juvenile stage. This may be due to the higher protein requirements of the larval fish. A similar situation has been demonstrated for humans where an infant should derive 37% protein requirement from essential amino acid (EAA) and a child at 10 yrs, 33% from EAA, whereas an adult needs only 15% of their protein requirment from EAA (Young *et al*, 1981; Millward, 1985).

Fish nutritionists traditionally present their data on nutritional requirements of fish on a percentage basis of the diet and generally agree that fish require more protein than mammals and birds (Pandian, 1987). The ranges reported are between 25- 75% protein for all fishes and 25-50% for tilapias. There are two important reasons for re-evaluating the situation.

First, the majority of these studies have been on newborn fish and it is known for fish that protein requirements change with body size, and hence age (Jauncey and Ross, 1982). Secondly, is it correct to use a percentage to quantify the nutritional requirement? If a percentage is to be used, the index has a meaning only if it is based on a percentage of body weight, as the denominator should relate to the fish not to the diet. The food requirement expressed as a percentage

the diet would provide a recipe for the feed manufacturer but would not indicate the quantitative requirement of a fish. For example protein requirement should be expressed as mg protein/100g fish and not as g protein/100g diet. The former reveals the protein requirement of fish whilst the latter provides the recipe for preparing the diet [While this study was in progress, Bowen (1987) forwarded a similar argument after analysing available data in the literature].

The primary source of energy for metabolic work in fishes is considered to be protein and lipid rather than carbohydrate as in mammals and birds. Fish lack an insulin regulatory system and are therefore similar to diabetic mammals and cannot utilize carbohydrate efficiently (Nagai and Ikeda, 1973; Jauncey and Ross, 1982; Hepher, 1988). Discrepancies are again evident in the literature. The digestibility of carbohydrate is assumed to be 60% for tilapias by Jauncey and Ross (1982) yet Wang *et al.* (1985) reported a digestibility coefficient as high as 96% of crude starch for *O. niloticus*. Kutty (1972) reported that *O. mossambicus* starved for 36h and then exercised generate all aerobic energy from catabolising protein. However, Moorthy *et al.*, (1980) noted considerable depletion of glycogen when the same species was starved for 30 days. Murat and Serfaty (1975) concluded that the common carp was unable to derive energy from glycogen. Davison and Goldspink (1984) found, however, that goldfish *Carassius auratus* (a cyprinid similar to common carp in feeding habits) derived energy from glycogen for metabolism whereas the carnivorous *Salmo trutta* relied on lipid breakdown at all swimming speeds.

The growth of plaice, *Pluronectes platessa*, is markedly increased by the addition of carbohydrate to the diets (Cowey *et al.*, 1975) and reductions in dietary carbohydrate levels have been shown to result in growth inhibition in carp (Yone,

1979). Rainbow trout have been fed diets containinig 41% cooked wheat or 38% wheat meal by Edwards *et al.* (1977) and 32% wheat meal or 21% wheat meal+13% glucose without significant reductions in growth. Yet a diet with 30% glucose resulted in significantly lower weight gain in the same species with higher heat generation (Beamish *et al.*, 1986). Jauncey (1982) showed that carbohydrate up to an inclusion level of 25% in the diet positively contributes to the growth of tilapias. Teshima (1985) claims that maximum growth was obtained by 40% dextrin in the diet of *O. niloticus* and Wee and Ng (1986) added 60% cassava root meal to the nile tilapia's diet to obtain maximum growth.

Although the picture appears confused with respect to energy requirements of fish, when the optimum protein energy to total energy ratio (based on gross energy) of diets is studied a close similarity among fishes irrespective of feeding habits is apparent. For example, the optimum total energy to protein energy ratio for rainbow trout is 1:2 (Lee and Putnam, 1973), for *O. mossambicus*, 1:1.85 (Jauncey, 1982) and for *O. niloticus* 1:2.33 (Wee and Ng, 1986). This similarity may be due to fishes being ectothermic in nature.

Once acquired, material (energy) has to be used for various purposes which means work has to be done. Hence the total energy requirement would be a resultant of the net energy required for work and for the 'desired' growth rate. Thus one has to consider how fish partition acquired energy in order to understand their energy requirements. As mentioned earlier, there is a voluminous quantity of literature on these aspects. No attempt, therefore, has been made to present a complete review of fish energetics in this thesis. The following section, however attempts to clarify some energetic concepts which have particular relevant to feeding and growth.

2.2.2 Energy partitioning in fish.

There are three major type of models used to describe energy partitioning of fish.

(a) Ivlevs' mass balance equation.

(b) Winberg's equation.

(c) Von Bertalanffy equation.

The basis of all three models is that growth is the net result of the difference between acquired energy and dissipated energy. The theoretical foundation of (a) and (b) is the same and whilst (c) embraces the idea of synthesis and degradation it has only a superficial resemblance to the former models.

2.2.2.1 Ivlev's mass balance equation.

There are two fundamental concepts in bioenergetics, the law of energy conservation (the first law of thermodynamics) and the law of additivity of reaction heats (Hess's law of constant energy summation) (Wiegret, 1968; NRC, 1981).

The first law of thermodynamics states that the internal energy change between two states of a closed system is equal to the difference between heat added to the system from the surroundings and the work done by the system on the environment.

$$En = Q - W \qquad [2.1]$$

where,

En = internal energy change,

Q = heat added to the system,

W = work done by the system.

Hess's law of constant heat summation claims that when a reaction can be expressed as the algebraic sum of a sequence of two or more component reactions, the heat of the reaction

is the algebraic sum of these components.

$$H_{TOTAL} = H_1 + H_2 + \ldots\ldots + H_n \qquad [2.2]$$

where,

H_{TOTAL} = overall heat change,

H_1 = heat change in first step

H_2 = heat change in second step,

H_n = heat change in n^{th} step.

Ivlev (1939, 1945) considered organisms to be open thermodynamic systems which allow both matter and energy to enter and leave and he proposed that energy transactions in aquatic organisms could be treated in a similar fashion to physical systems. Another assumption is that the work done by the fish on its environment, and *vice versa*, is negligible (Wiegert, 1968).

The model proposed by Ivlev (1939) assumes linearity (additivity) of energy transactions of an animal over a defined time period. The original version has been subjected to various modifications by Winberg (1956) and Warren and Davis (1967). The fullest and most recent version is given by Brett and Groves (1979):

$$I = G + M + E \qquad [2.3]$$

Expanding the terms:

$$I = (G_s + G_r) + (M_s + aM_{R-S} + bM_{F-S} + cM_{A-S}) + (F + U + Mu)* \qquad [2.4]$$

where, I = amount of food consumed, G = growth, M = total metabolic work, E = excretion, G_s = somatic growth, G_r = reproductive growth, M_s = standard (basal or resting) metabolism, M_{R-S} = routine metabolism, M_{F-S} = feeding metabolism, M_{A-S} = active metabolism, F = faecal losses, U = urinary losses, Mu = losses due to mucus secretion, and a,b,c = constants denoting the fraction of time each type of

metabolism is used [* all values are in energy units (cal. or J.)].

This mass balance equation partitions the ingested energy into three major components, growth, metabolism and excretion. There have been many attempts to quantify the above terms. Most of the variables, however, have been measured in the laboratory under controlled environmental conditions (Brett , 1964) after acclimatising fish for a period of time.

The term growth (G) represents the somatic growth (G_s) plus reproductive growth (G_r) and can be quantified on a wet or dry weight basis.

Quantification of ingestion (I) is relatively easy under laboratory conditions when feeding a known amount of food of pre-determined composition. Field estimations of food consumption have been done on the basis of gastric evacuation rate (eg. Bajakov, 1935; Daan,1973; Elliot and Persson, 1978).

Excretion refers to the non-utilized part of ingested food, and it is divided into non-digested (faecal), non-assimilated (*eg.* urinary) and to various other losses such as mucus. Faecal and excretory losses (F and E) have been estimated in aquaria (Elliot, 1976d) or in metabolic chambers holding individual fish (Smith, 1971) or groups of fish (Knights, 1985) fed either voluntarily or by tube (Smith,1980). The inert marker method has been widely used to estimate faecal waste (when total faecal collection is not possible) with different techniques such as collection of settleable materials (Cho *et al.*, 1982), suction techniques to collect faeces from the lower intestine (Windell et al., 1978), filtration techniques and centrifugation (Smith,1980). These methods may be criticised on several grounds such as stress induced in fish in metabolic chambers and in tube

feeding experiments (NRC, 1981), rapid leaching of nutrients within the first hour of faecal production (Windell et al., 1978; Smith, 1980), differential rate of passage of the inert marker in the alimentary tract (Bowen, 1978, Jorgensen and Jobling, 1988) and incomplete digestion of food in faeces stripped from the lower intestine (NRC,1981).

The energy content of faeces is measured by bomb calorimetry. Excretory losses in the form of ammonia and urea can be converted into energy equivalents employing conversion factors of 24.85 kJ mg^{-1} for ammonia nitrogen and 23.05 $kJmg^{-1}$ for urea nitrogen assuming that protein contains 16% nitrogen (Elliot and Davison, 1975; Elliot, 1976d). No attempts have been made to directly quantify mucus losses (Mu), although, Elliot (1976d) after employing a wet combustion technique on aquarium water holding starved fish concluded that losses were negligible.

Metabolism is the energy expenditure of fish through a series of chemical reactions within the body (Beamish and Dickie, 1967). Metabolic rate (M) is measured by indirect calorimetry in laboratory-designed chambers and oxy-caloric coefficients of energy- yielding materials (*i.e.* carbohydrate, 14.77 kJ mg^{-1} O_2 consumed; lipid, 13.72 kJ mg^{-1}; protein, 13.39-13.60 kJ. mg^{-1}, Elliot and Davison, 1975) have been used to convert oxygen consumed to energy units. Calculations of a general value are conventionally performed by taking the respiratory quotient into consideration (Brody, 1945; Kleiber, 1961). Elliot and Davison (1975) proposed a mean value of 14.14 kJ mg^{-1} O_2 consumed but stressed that the value can range between 13.77-14.31 kJ mg^{-1} depending on the feeding habits and food composition.

Four arbitrary levels of metabolism have been identified and this is one of the major drawbacks in this model. Total

metabolism is divided into; (a) standard metabolism which is the energy used to maintain the life processes during the fasting and resting stages; (b) routine metabolism, which is defined as a "recording of a routinely active animal" (Calow, 1985); (c) feeding metabolism, the energy expenditure associated with feeding; and (d) active metabolism, the expense of energy for a fish undergoing sustained activity (*i.e.* swimming).

Basal metabolism (M_B) cannot be measured accurately as spontaneous activities cannot be eliminated (Beamish and Mookherjii, 1964). It is termed, therefore, standard metabolism and is sometime defined as the metabolic rate of fasting fish whose activity level is projected to zero with the aid of a regression curve (Beamish, 1964; Brett, 1964). Variability in measurement can be expected as some species are known to exhibit circadian rhythms in oxygen consumption *eg. Huro salmoides, Ameiurus meals*, Clausen, 1933, 1936 as cited by Winberg,1956; *Cyprinus carpio*, Oya and Kimata, 1938; *O.mossambicus*, Nagarajan and Gopal, 1983; *O.niloticus*, De silva *et al.*, 1986; Ross and McKinney, 1988).

Routine metabolism (M_{R-B}) is an arbitrary division according to experimental conditions and the value ranges from standard metabolic rate to half of the active metabolic rate (Fry, 1971).

Feeding metabolism (M_{F-B}) refers to the increase in metabolic rate following ingestion and has been conventionally called specific dynamic action (SDA), "a term created by Rubner in order not to commit himself to a definite explanation" (Kleiber,1961). This has subsquently been explained as the energy cost of digestion, absorption and also amino acid deamination (Beamish *et al.*, 1975). Recent evidence suggests that SDA might represent at least part of the cost

of synthesis (Jobling, 1981, 1983; Parry, 1983; Vahl, 1984; Calow, 1985; Flatt, 1985) but the term still has no exact definition.

The maximum sustainable activity of fish swimming steadily has been defined as the active metabolic rate (M_{A-S}) and the difference between the standard and active metabolic rate has been termed as the metabolic scope of fish (Fry, 1957).

Metabolic rate is empirically expressed as an allometric (*i.e.* dy/dx=k.y/x) relationship with two parameters (*i.e.* a and b):

$$M = aW^b \qquad [2.5]$$

where, M = O_2 consumed or energy expended (O_2 mg/hr or kJ/hr), W = body weight in grams, a = mass co-efficient representing the level of metabolism and b = mass exponent denoting instantaneous rate of change.

Dividing by body weight:

$$M/W = aW^{b-1} \qquad [2.6]$$

where M/W = metabolic turnover rate which indicates the fraction of chemical energy given off as heat (Kleiber,1975). Using Rubner's famous surface rule (as cited by Von Bertalanffy in his paper) Von Bertalanffy (1957) categorised fish as metabolic type 1, which consisted of animals whose basal metabolic rate was proportional to the 2/3 power of the weight. Winberg (1956), based on his own experiments and available data from the literature, showed $M = 0.297\ W^{0.81}$ O_2 ml/hr ($M \approx 0.3W^{0.8}$, standard or resting metabolic rate at 20°C) for all freshwater fishes. The temperature correction was done by using Krogh's normal curve.

This type of energy balance equation was recommended by the IBP handbook (IBP, 1975) and is widely used for empirical studies on constructing energy budgets and predictive growth

modelling. (*eg.* Knights, 1985; Ross *et al.*, 1988). The model provides a valuable insight on partitioning of ingested energy by a fish under confined conditions. For example, Brett and Grove (1979) reported the following partitioning for carnivorous and herbivorous fish respectively:

100I = 29G + 44M + 7U + 20F

100I = 20G + 37M + 2U + 41F

Does this approach help to answer the original question which is that if fish ingest X quantity of food of Y quality, why do they grow in the way that they do ?

The major advantage of this approach is its sound theoretical foundation. However, the above model suffers severely from the lack of clear definitions with regard to energy partitioning of fish (*eg.* SDA, routine metabolic rate). The other major problem with energy budgets may be best explained by quoting Winberg (1956). In his pioneering work on the "Rate of metabolism and food requirement of fish" Winberg raised the question in the prologue: "What is the metabolic rate of fish ? We refer, ofcourse not to a particular measurement made under experimental conditions, but values which describe the metabolic rate of fish in nature". The answer has yet to be given. Researchers, to date, have only speculated on the possible values of metabolic rate of fish in the natural environment based on laboratory results. However, with recent advances in technology, such as ECG techniques, it may be possible to estimate the metabolic rate of fish in nature in the near future (Jobling, 1985).

However, there are two fundamental problems associated with this approach:

(a) As with the first law of thermodynamics, the balance equation gives a recipe for finding the change in energy between two states.(*i.e.* initial and final energy values).

However, it does not say how to find these values and why those values change as they do. What it basically says is that if an energy store is decreased in one place in the body, the reduction should be replaced by another component (*i.e.* food).

(b) The mass balance equation considers the total energy as the common currency in metabolic activities and it does not specify the type of energy involved (*i.e.* protein energy, lipid energy *etc.*) Hence, it will give the recipe for retained energy but does not state the origin of retained energy in relation to food quality.

There are many practical difficulties with aquatic animals involved in measuring those values accurately but it should not devalue the underlying theoretical foundation (NRC,1981). In order to overcome these difficulties, Winberg (1956) simplified the formula as follows.

2.2.2.2 Winbergs' equation.

Winberg (1956) provided a tentative procedure for computing the food requirements (in calories) of fish. The logic is that the metabolisable energy intake of fish should at least satisfy its energy expenditure for growth and metabolism. The basic energy equation, therefore, may be expressed as:

$$\Omega IE = G + M \qquad [2.7]$$

where Ω = 0.8 (physiologically useful fraction of energy) and M = maximum possible average metabolic rate (assumed to be equal 2 X 'routine metabolic rate'). In this equation Winberg (1956) assumes that there will be only 20% energy losses associated with all types of food that fish eat.

This equation has been mis-quoted as the basic energy equation (*eg.* Ursin,1967; Kerr, 1971, 1982), as the balanced energy equation (*eg.* Ware, 1975; Jobling, 1985) and also has been used as a starting point for constructing predictive growth models (*eg.* Paloheimo and Dickie, 1965; Zaika, 1973). With some frustration, Winberg (1984) wrote "...the difference in [*ΩIE-M*] [*if M is used to denote the metabolic rate*] will denote not growth rate, but rate of production of the specimen. .....Nowhere and never did I use it as a basis for the study of growth. Traditional interpretation of growth as a balance of assimilation [*ΩIE*] and metabolic expenditure [*M*] cannot be the basis of practical study of growth, due to unresolved difficulties in determining the magnitude of [ΩIE]". In other words, Winberg (1984) has stressed that his model cannot be used to answer the question raised in this thesis. There are, nevertheless, advantages to this model: food ration can be crudely approximated (*eg.* Kerr, 1982) and theoretical explanations of growth processes can be investigated (Jobling, 1985).

Although there is no direct link, many authors use the Von Bertalanffy equation as a balance process similar to the above equation (*Eg.* Zaika, 1973) and the Von bertalanffy equation is the most widely used formula in fisheries management. In the following section, therefore, the Von Bertalanffy equation is briefly reviewed.

2.2.2.3 Von Bertalanffy equation.

This was one of the first attempts to define organismic growth in mathematical terms and PÜtter (1920) has been credited as the founder of the equation by Von Bertalanffy (1938). The energy balance equations do not contain a time

dimension but results are integrated by the user. The characteristic feature of the Von Bertalanffy equation is that it has a time dimension and the equation (in integrated form) belongs to a family of curves with a single rate constant which is s-shape or sigmoid in shape over the time domain.

According to the Von Bertalanffy equation growth (dW/dt) can be expressed as the net result of two counteracting process, assimilation (S: synthesis) and dissimilation (C: catabolism or degradation):

Growth = Synthesis - Degradation

$dW/dt = hS - kC$

where, t = time; W = weight and h, k = constants for synthesis and degradation respectively.

Based on the assumption that synthesis is proportional to a surface area for absorption and that degradation is directly proportional to body weight, the growth equation take the form of:

$dW/dt = hW^{2/3} - kW$

or a more general form:

$$dW/dt = hW^{n} - kW^{m} \qquad [2.8]$$

where, n = instantaneous rate of synthesis,

m = instantaneous rate of degradation.

Upon integration, when n=2/3 and m=1:

$W_t = [W^{1/n}_{a} - (W^{1/n}_{a} - W^{1/n}_{0})\ e^{-k(t-t0)}]^{n}$ Von Bertalanffy (1938),

where,

W_a = asymptotic size (mature weight, *cf.* Brody, 1945),

W_0 = initial weight at time t_0,

e = natural base for exponential and logarithmic function,

n = 3,

or as integrated by Beverton and Holt (1957),

$W_t = W_a\ [1 - e^{-k(t-t0)}]^{n}$

or as integrated by Pauly (1979),

$W_t = W_\infty [1 - e^{-KD(t-t0)}]^{1/D}$ where K = k/3 and D = 3(1-n).

A physiological basis for this equation has been sought by many authors. Ursin (1967) employing a number of pragmatic assumptions, re-defined the catabolic term as a function of metabolic rate and proposed the exponent m of equation 2.8 = 0.8. He assumed that n was equivalent to the exponent of the body weight to gut surface power function, but failed to find empirical evidence. When n=2/3 and m = 0.8, the equation cannot be integrated easily, hence, methods of numerical integration have been suggested for solving the equation.

Pauly (1979, 1981) attempted to justify this equation in physiological terms by proposing that synthesis is proportional to O_2 supply (as indicated by Winberg, 1956), hence to metabolic rate. Hughes (1972), after measuring the gill surface area of many different fishes found that body weight and gill surface area have an allometric relationship with an exponent value close to 0.8. He proposed that active metabolic rate may be a function of gill surface area. Combining those ideas Pauly (1979) suggested that the parameter n should be equivalent to the exponent of metabolic rate or to the gill surface vs body weight relationship. Like Von Bertalanffy, he also assumed that m = 1.

Ziaka (1973) combined Winberg's equation 2.7 and the Von-Bertalanffy equation. Rearranging equation 2.7 ($G = \Omega IE + M$) so that it is written as $dW/dt = \Omega IE - M$, he argued that ΩIE is proportional to food intake and can be represented as a function of body weight. The term M is equivalent to degradation (C), and therefore, can be expressed as an allometric function of body weight as proposed by Ursin (1967). In this sense, Winberg's equation and the Von Bertalanffy equation are assumed to be similar. As mentioned

earlier, Winberg (1984) was critical of those using his equation for 'practical growth studies' as the term M does not represent all of the losses which occur during the fishes life-span (if interpreted as the metabolic rate) and because of problems associated with determining QIE.

Sibly and Calow (1986) in an elegant work in theoretical ecology, very briefly explained the Von Bertalanffy equation in terms of ingestion and catabolism. In this interpretation, ingestion $I = hW^n$ and, as with Von Bertalanffy, catabolism $C = kW^m$. If the functional relationship between body size and feeding rates are known and if the relative values of parameters m and n are known, growth pattern and rate can be predicted. This definition coupled with the concept of energetic efficiency provides a clue on the metabolic fate of ingested energy. Before any attempt is made to take this further, it is necessary to clarify the concept of energetic efficiency of fish production.

2.2.3 Energetic efficiency of fish production.

If two variables are correlated, the ratio between the dependent variable (*eg.* growth) and the independent variable (*eg.* food intake) is termed as 'coefficient' or simply 'efficiency' (*eg.* coefficient of growth or growth efficiency). Since the magnitude of the dependent variable is a function of the magnitude of the independent variable the relationship is called a 'functional response' or 'functional relationship'. If there exists a direct proportionality in the change in magnitude of variables then the adjective 'linear' is prefaced before the word 'relationship'. It is said to be 'positive curvi-linear', if the change in the former increases with a decreasing rate relative to change in the latter (*i.e.*

the law of diminishing returns). If the opposite is true, it is termed 'negative curvi-linear'.

2.2.3.1 Growth coefficients.

Although the most important aspect of growth for aquaculture is its efficiency, the literature on fish energetics provides an extremely confused picture on the relationship between food intake and growth, and hence growth efficiency. A linear relationship has been found by several authors (*eg.* Gerking, 1971; Birkett,1972; Stirling,1972; Tyler and Dunn,1976; Flowrdew and Grove,1980; Allen and Wooten, 1982; Condry, 1982) and a curvi-linear relationship has been justified by others (*eg.* Paloheimo and Dickie, 1965; Brett *et al.*, 1969; Kerr, 1971; Elliot, 1975b; Huismann, 1974; Smith and Thrope,1976, Brett and Groves, 1979, Hogendoorn, 1983). It may be useful at the outset, therefore, to illustrate just how confused the situation and concepts in the literature are with respect to the relationship between feed intake and growth.

In addition to the mass balance equation, Ivlev (1939) defined three coefficients of energy utilization for growth. The first order energy or growth co-efficient (K_1) reveals the fraction of gross energy retained in the body and the second order growth co-efficient (K_2) is the fraction of metabolisable energy reserved in the body.

First order energy/growth co-efficient (K_1) = G/IE [2.9]
where, G = energy increase in animal product, I = gross food intake in g. and E = energy density of food.

Second order energy co-efficient (K_2) = G/ΩIE [2.10]
where, Ω = fraction of used energy (*i.e.* ΩIE = metabolisable energy).

Metabolisable energy is defined as the difference between the ingested energy and the excreted energy (NRC, 1981).

$$ME = IE-(FE+UE+GLE) \quad [2.11]$$

where ME = metabolisable energy, IE = ingested energy, FE = faecal energy, UE = urinary energy, GLE = energy excreted via gills.

The absorbed energy (AE) is defined as:

$$AE = IE - FE.$$

Therefore:

$$(K_2) = G/[IE-(FE+UE+GLE)] = G/[AE -(UE+GLE)] \quad [2.12]$$

Hence, from equations [2.9], [2.10] and [2.12];

$$K_1 = \Omega K_2 \quad [2.13]$$

$$K_1 [IE] = K_2 [IE -(FE+UE+GLE)] = K_2 [ME] \quad [2.14]$$

Ivlev's second order energy co-efficient is equivalent to gross efficiency (Brody, 1945) and also to Kleiber's (1961) total efficiency. Winberg (1956) objected to Ivlev's third order co-efficient (K_3) as it contained a term 'primary heat' which Ivlev defined as a product of exothermic heat or inevitable loss. As this is difficult to measure and as at least part of exothermic heat would account for feeding metabolism, it has not been used in any practical situation.

2.2.3.2 A negative exponential relationship.

Paloheimo and Dickie (1966) analysing published data for several species of fish showed that K_1 is a negative exponential function as it is expressed on the basis of cumulative food intake for the experimental period.

$$K_1 = G/IE. \quad t = e^{-a-bI}$$

where t = time and a, b = constants. This implies that:

$$\log K_1 = a - bI$$

and hence, the co-efficient of growth begins with a maximum value and then declines by a constant factor of e^{-b} exponentially with increasing food intake (ration). Establishing this empirical relationship they combine their equation with Winberg's to construct a growth model. Kerr (1971) further modified Paloheimo and Dickie's model but no data has subsequently been found to support this empirical model.

2.2.3.3 A positive curvi-linear relationship.

Brett *et al.* (1969) fitted a curve by eye, even though their data showed a near linear relationship between feed intake and growth (at optimal temperature) in order to include their experimental group of fish fed an excess of feed without quantification of feed intake. Brett *et al.* (1969), however, mentioned that "in the case of excess ration [excess fed A group] the uneaten particles were washed out of the tank within 3-8 min. from the time of introduction" yet included the data point in order to demonstrate a curvi-linearity. Later Huisman (1974) for common carp, Elliot (1975b) for brown trout fed on *Gammurus pulex* and Hogendoorn (1983) for African catfish demonstrated the same relationship. Stauffer (1973), as cited by Ricker (1979) fitted a sine curve to the functional relationship between growth and feed intake but could not "explain the growth phenomenon".

If growth and feed intake are curvi-linearly related, the efficiency is a parabolic function of the latter. Kleiber (1961) argued against this as follows. "The law of diminishing returns does not properly fit the conditions of animal feeding, because from the metabolisable energy of ingested food, an animal must produce either an animal product or

heat". Analysing the above concepts of growth in relation to food intake, Ricker (1979) concluded that they have "not produced any relationship for everyday use based on physiologically meaningful concepts. It now seems safe to conclude that no such simple relationship exists".

2.2.3.4 A linear relationship in growth efficiency.

Other than k_1 and K_2 co-efficients, the partial efficiency of growth, [e_p], (Kleiber, 1961) has been widely used in fish energetics studies. This term is similar to net efficiency of Brody (1945).

Partial efficiency [e_p] = $G/(IE - M_n) = K_3$ [2.15]

where; M_n = maintenance requirement.

In energetics studies on aquatic organisms all of the food energy categories (*i.e.* gross, digestible or metabolisable energy) have been employed (Warren and Davis, 1967) but in terrestrial animals metabolisable energy value is principally used as the denominator *eg.* Kleiber (1961).

Partial efficiency (e_p) has been termed the third order co-efficient of growth (K_3) by many authors who have worked with aquatic animals (*eg.* Stirling, 1972; Flowerdew and Grove, 1983) and this is also used in this thesis in order to maintain consistency amongst symbols. It should be emphasised, however, the K_3 in this sense is different from Ivlev's K_3 co-efficient.

Re-arranging terms of equation 2.15:

$$G = K_3IE - K_3M_n \qquad [2.16]$$

Equation 2.16 says that there exists a linear relationship between food intake and growth where K_3 is independent of the rate of ingestion. A linear relationship between growth and ingestion implies that; Y=mX-c, or Y/X=m- b/X, hence when X

—> a, Y/X—> m. Coefficient K_3, therefore, is a constant and coefficients K_1 and K_2 should be a monotone increasing function over the domain of food intake (*eg.* Gerking, 1971; Birkett, 1972; Stirling, 1972). Analysing data in the literature with the aid of a regression method termed the piecewise regression technique, Condry (1982) argued that much of the available data agrees with the linearity concept.

From equation 2.16, the maintenance requirement can be calculated from the intercept as follows:

Intercept $c = K_3M_n$; therefore $M_n = c/K_3$

2.2.3.5 Growth efficiency and fish size.

It is in the interest of fisheries scientists to understand the relationships among body size, feed intake, growth and its efficiency. Jones (1976) showed that K_3 [a_2 according to symbols used by Jones (1976)] and body weight have a negative curvilinear relationship for cod, *Gadus morhua*.

$$K_3 = rW^{-s}. \qquad [2.17]$$

This equation indicates the fraction of surplus intake (above maintenance requirement) retained for growth as a function of body weight.

Pauly (1979, 1981) analysing Menzel's (1960) data for Bermuda reef fish, *Epinephelus gluttatus* demonstrated that K_1 can be also represented as an allometric function of body weight.

$$K_1 = pW^{-q} \qquad [2.18]$$

This equation explains the fraction of ingested energy deposited as a function of body weight.

In another attempt to perfect the curve fitting exercise, Pauly (1986) proposed a new model in the form of:

$$K_1 = 1 - (W/W_\infty)^\beta \qquad [2.19]$$

where, W = body weight at particular time in growth,

W_∞= asymptotic body weight.

With this form of the model, it is presumed that fish have an asymptotic body size. Pauly (1986) presented equation 2.19 in an empirical form without mentioning how he developed the formula.

Silvert and Pauly (1987) multiplied equation 2.19 by the term ingestion $I = hW^n$ and argued that the equation justified the Von Bertalanffy equation.

$dW/dt = [1 - (W/W_\infty)^\beta].hW^n$ and hence:

$dW/dt = hW^n - kW^\beta$

where, $m = (n + \beta)$ and $k = h/W_\infty^\beta$

This means that the physiological interpretation of the Von Bertalanffy equation Sibly and Calow (1986) has been substantiated by Silvert and Pauly (1987) with their empirical model. Silvert and Pauly (1987) further claimed that this equation can be integrated to find a general solution implying that m = 1 as for the original Von Bertalanffy equation.

However, when the exponent n is equal to 1, a similar equation can be derived in the following way.

From equation 2.8, when m=1

$$dW/dt = hW^n - kW \qquad [2.20]$$

Let $\mu = 1-n$. Substituting into equation 2.20;

$dW/dt = hW^{1-\mu} - kW$

When $W \longrightarrow W_\infty$, $dW/dt \longrightarrow 0$

Therefore $hW_\infty^{1-\mu} = kW_\infty \Longrightarrow k = hW_\infty^{-\mu}$ [2.21]

Substituting equation 2.21 into equation 2.20 and rearranging terms:

$$dW/dt = hW^{1-\mu}[1- (W/W\alpha)^\mu] \qquad [2.22]$$

Allowing $\mu = \beta$, results in $n = (1 - \beta)$. The empirical data provided by Pauly (1986) does not justify their claim. For example, analysing Pandian's (1967) data for snakehead, *Ophiocephalus straitus* Pauly (1986) showed that $\beta = 0.07$ which

implies $(1 - \beta) = 0.93$. Analysing the same data in the present study, it was found that feed intake is proportional to $W^{0.75}$ implying $n = 0.75$. It is reasonable to conclude, therefore, that Silvert and Pauly's (1987) claim that their equation is equal to Von Bertalanffy's when $m = 1$ is based on false premises.

It may be evident now how confused the concepts of feed intake and growth relationship are in fish biology literature. In the following section, therefore, it is attempted to analyse these concepts by generating new models.

2.3 Modelling feed intake and growth.

Although the empirical relationship shown by Jones (1976) is quite stimulating and deserves further analysis, Pauly (1986) set three conditions among which the most serious according to Pauly (1986) is that "the parameters (p and q; r and s) have no biological meaning and they do not provide information which can be interpreted via another model". Although the biological meaning of the parametric values of the empirical relationship of metabolic rate vs body weight is still subject to argument (Heusner, 1982; Feldman and McMahon, 1983), the allometric equation is widely used in theoretical biology. Hence, whether the parameters have a meaning or not is not a problem in model building as long as there exists a statistically proven empirical relationship among variables. Despite this warning, therefore, this thesis intends to analyse the above empirical models in order to investigate their implications for the relationship between body weight and growth efficiency and to analyse how much light they can shed on an understanding of the relationship between growth and feed intake in fish.

2.3.1 Relationship of K values and body weight and its implications.

If the functional relationships between K values vs body weight and metabolic rate vs body weight are known, it is possible to predict the patterns of resource (energy) allocation between metabolism and growth. The equations 2.17 and 2.18 have been shown to fit the growth efficiency data for bluegill sunfish (*Lepomis macrochirus*) and cod (*Gadus morhua*) by Jones (1976) and for Bermuda reef fish, *Epinephalus guttatus*, snakehead, *Ophiocephalus striatus* and anglefish, *Holocanthus bermudensis* by Pauly (1981,1986) and have yielded correlation co-efficients (r) above 0.8. It is assumed, therefore, that these empirical relationships are not mathematical artifacts (*i.e.* nonsensical correlations) but a true phenomenon related to the real world.

2.3.1.1 Model 1A: Patterns of energy allocation in relation to K_1.

A mass balance equation for growth can be written in the following form:

Energy retained + energy loss as heat + energy loss as matter =IE.

The energy retained can be calculated from the equation 2.18 and the energy loss as heat from the body can be calculated from equation 2.5. Since the mass balance equation has no time dimension, by integrating time and subject to a number of assumptions, it can expressed in the form of body weight.

Assumption 1

Loss of mucus (MU), scales (SE) *etc.* is negligible (Elliot, 1976d) over a short period and in the long term they are assumed to be a part of growth.

Assumption 2

The reproductive output over long time period is considered as a part of somatic growth.

Assumption 3

Assimilation remains at an average level independent of body weight as shown by Pandian (1967) for snakehead, and metabolisable energy (physiologically useful energy) is a constant fraction of ingested energy (Winberg, 1956; Elliot, 1976d).

Defining the following:

From equation 2.18 $K_1 = G/IE = pW^{-q}$

Therefore $G = IE \cdot pW^{-q}$

From equation 2.5 $M = aW^b$ and assuming total metabolism can be expressed as c times standard metabolism:

total heat cost = $c[aW^b]$

Hence a mass balance equation can take the form of;

Energy retained + energy loss as heat + energy loss as matter =IE.

$$IE.pW^{-q} + c[aW^b] + (FE+UE+GLE) = IE \quad \text{energy/unit time} \quad [2.23]$$

Re-arranging terms in equation 2.23:

$$IE.pW^{-q} + c[aW^b] = IE - (FE+UE+GLE)$$

and substituting equation 2.11 and allowing c.a=d;

$$IE.pW^{-q} + dW^b = ME \quad [2.24]$$

Feed intake (IE) increases as a positive power function of body weight as has been demonstrated for many animals including fish (*eg.* Ziaka, 1973; Peters, 1983; Calder, 1984; Sibly and Calow, 1986; Jobling, 1988).

$$IE = uW^{v} \qquad [2.25]$$

Substituting equation 2.25 into equation 2.24:

$$uW^{v}.pW^{-q} + dW^{b} = ME$$

and dividing by body weight (W) and allowing u.p=g:

$$gW^{v-(1+q)} + dW^{b-1} = ME\ W^{-1}Time^{-1} \qquad [2.26]$$

Empirical evidence suggests that b<1 and v<1 for most animals including fish (Peters,1983; Jobling, 1988).

Subject to conditions that (1+q)>>v and b<<1, equation 2.26 can be written as:

$$gW^{-k} + dW^{-j} = ME\ W^{-1}Time^{-1} \qquad [2.27]$$

where, $-k = v-(1+q)$ and $-j = b-1$.

Equation 2.27 shows that the fraction of metabolisable energy dissipated as heat or recovered for growth (somatic + reproductive) per unit body weight is a function of body weight.

There are three possible relationships with above parameters in exponents.

(a) $|k| < |j|$

(b) $|k| = |j|$

(c) $|k| > |j|$

Each of those can be coupled with the same possibilities of parameters $|g|<|d|$, $|g|=|d|$ and $|g|>|d|$ resulting in nine possible patterns of energy allocation between growth and metabolism.

Fig. 2 is a plot of $ME\ W^{-1}$ vs body weight and graphically shows the possible patterns of energy allocation between growth and metabolism. The nonsensical regions given in Fig.2.1 indicate the region where the model has no meaning as there is a zero energy allocation to metabolic work and metabolic function would cease (death?). W_{∞} is the asymptotic weight.

There are three major patterns of energy allocation in Fig.2.1. They are:

(a) Divergence allocations (Fig.2.1 b, c, g, h),

(b) Convergence and divergence allocations (Fig.2.1a,i),

(c) Parallel allocation (Fig.2.1 d, e, f).

The growth patterns can be further divided into;

Pattern a: Divergence allocations,

Type a.1 Energy allocation for metabolism > for growth. This would result in asymptotic growth (Fig.2.1 g and h)

Type a.2 Energy allocation for growth > for metabolism. This type of allocation will result in continuous growth and this shown in Fig.2.1 b and c.

Pattern b: Convergence and divergence allocations,

Type b.1 At the initial stage of life, the energy for metabolism > for growth; at later stages the opposite is true; this will result in a pattern of continuous growth. W_1 in Fig. 2.1a can take any value from W_0 to maximum possible size.

Type b.2 An opposite process to type b1 and there will be asymptotic growth (Fig.2.1i).

Pattern c: Parallel allocations,

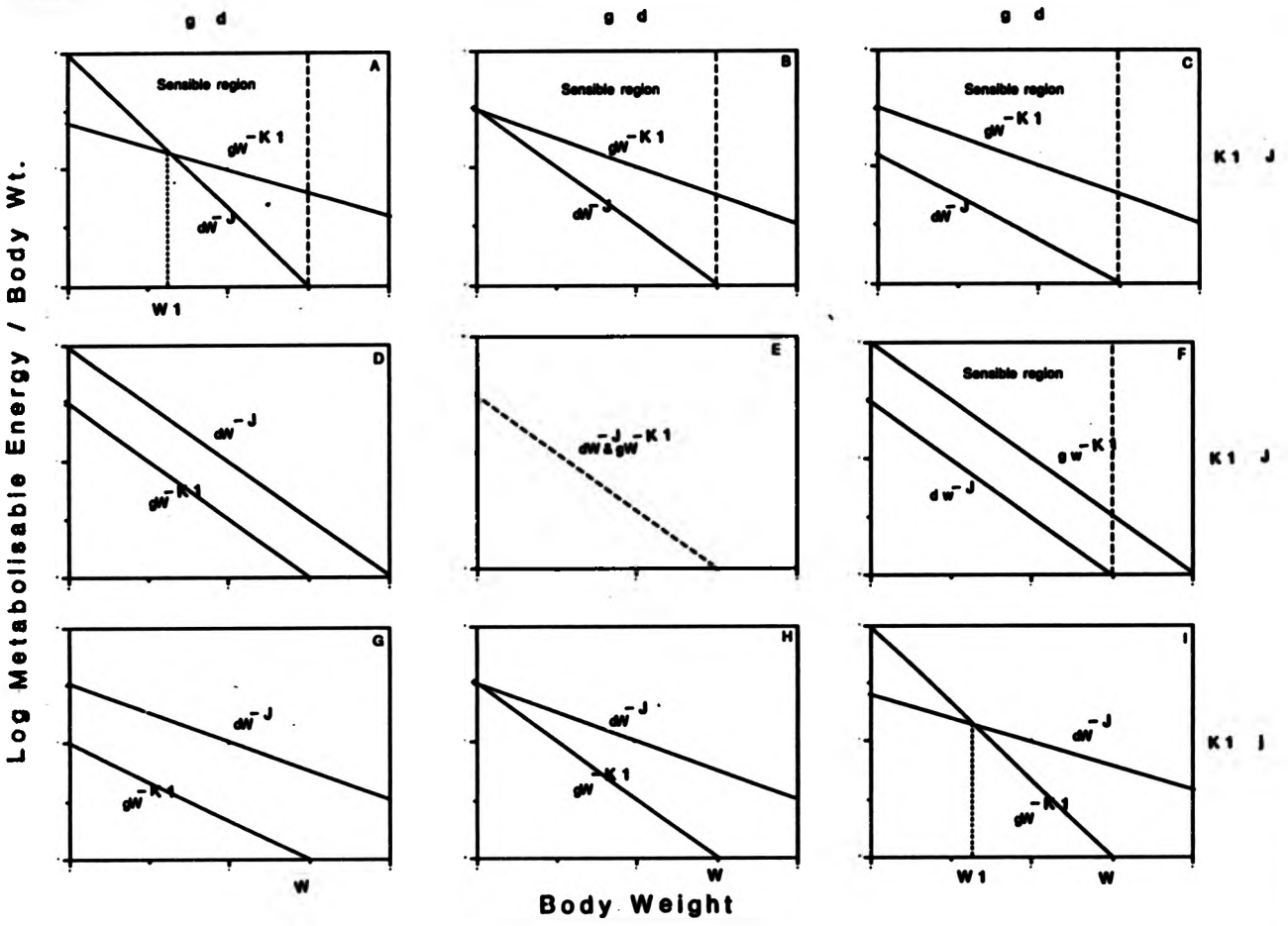
Type c.1 Energy allocation to metabolism > for growth; this will result in asymptotic growth (Fig.2.1d).

Type c.2 Energy allocation to growth > for metabolism; this will lead to continuous growth (Fig.2.1f).

Type c.3 Equal allocations (50:50) for growth and metabolism will result in continuous growth (Fig.2.1e).

According to Brett and Grove (1979), the energy allocation for metabolism is higher than for the growth of most fishes. If this was the case, only types a1 and c1 are

Fig. 2.1 Patterns of energy allocation between growth and metabolism as a function of body weight according to Model 1.


g d
A
Sensible region
W 1
B
C
K 1 J
D
E
F
G
H
I
K 1 I
W
Log Metabolisable Energy / Body Wt.
Body Weight

possible (Fig. 2 d, g and h).

2.3.1.2 Model 1B: Patterns of energy allocation in relation to K_g.

K_g in equation 2.17 also can be treated in a similar fashion.

Since K_g is the fraction of food energy retained after maintenance losses, a mass balance equation for surplus energy intake can be presented in the following form.

Energy retained from surplus intake +
energy losses as heat from surplus intake +
material losses from surplus intake $= IE_{pr}$

where,

IE_{pr} = total energy available for production.

The maintenance requirement can also be given as an allometric function of body weight (Jones, 1976; Jobling, 1988).

$$M_n = u_s W^{vs}$$

Therefore, surplus intake = $IE - M_n = uW^v - u_s W^{vs}$

Defining the following;

heat cost of maintenance = $e(aW^b)$,

Therefore, the losses as heat from surplus intake

$$= [c(aW^b) - e(aW^b)] = yW^b,$$

losses of materials = f(FE+UE+GLE),

where, f=fraction of losses above maintenance.

With the help of equation 2.17 and the functions defined above, the mass balance equation can be written in the following form for energy utilisation for production .

$$(uW^v - u_s W^{vs})\, rW^{-s} + yW^b + f(FE+UE+GLE) = IE_{pr} \qquad [2.28]$$

Defining a balance equation of metabolisable energy transactions for production from equation 11:

$$ME_{pr} = IE_{pr} - f(FE+UE+GLE). \qquad [2.29]$$

Let $uW^{v} - u_{s}W^{vs} = uW^{k}$

Re-arranging terms in equation 2.28 and substituting equation 2.29 with the assumptions 1, 2, 3 and the definition of maintenance requirement, it can be shown that:

$$uW^{k} \cdot rW^{-s} + yW^{b} = ME_{pr} \qquad [2.30]$$

and dividing equation 2.30 by body weight (W) and allowing u.r=h:

$$hW^{k-(1+s)} + yW^{b-1} = ME_{pr}\, W^{-1} Time^{-1} \qquad [2.31]$$

Subjected to conditions that $(1+s)>>k$ and $b<<1$, equation 2.31 take the form of:

$$hW^{-k_1} + yW^{-j} = ME_{pr}\, W^{-1} Time^{-1} \qquad [2.32]$$

where, $-k_1 = k-(1-s)$ and $-j = b-1$.

Equation 2.32 demonstrate that the partitioning of metabolisable energy available for production also follows the same patten as gross energy intake.

2.4 Concluding remarks.

Models 1a and 1b demonstrate the close link between growth, metabolism and body weight of fish. These models, however, are based on an empirical observation of K values and the body weight relationship. The legitimacy of these models, therefore, depends upon the validity of the basic empirical model provided by Jones (1976).

The advantage of these models is that they show that if the functional relationships between feed intake and growth efficiency in relation to body weight are known, growth pattern and rate can be predicted in a simple but physiologically meaningful way. This may be achieved by small modifications to model 1 and it provides an advantage over all other models in its inability to give an over-estimation of production, as is the case with laboratory based energy

budgets (Ross *et al.*, 1988) and the calculations may be easily accomplished with a pocket calculator rather than a microcomputer.

This goal, however, can only be achieved by resolving the controversy on the relationships between food, feeding and growth. It may be evident from preceding discussion that this problem cannot be solved completly with empirical work. It was decided, therefore, to investigate some theoretical aspects of animal biology basing the models generated on a 'hypothetical' fish.

CHAPTER 3

"Forming hypotheses is one of the most precious faculties of the human mind and is necessary for the development of science. Sometimes, however, hypotheses grow like weeds and lead to confusion instead of clarification. Concepts should relate as directly as possible to observations and measurements, and be distorted as little as possible by explanatory elements. .....so that the operational concepts can grow and function"

Max Kleiber (1961) on Specific Dynamic Action.

MODELLING THE FISH GROWTH VERSUS FEEDING RELATIONSHIP: A UNIFIED THEORY

3.1 Introduction.

Probably no branch of biology other than fisheries and aquaculture has extensively used growth models. The prologue of any scientific paper dealing with growth modelling of fish lies in a few simple, though very ambitious, statements. Prediction of growth of fishes in the natural/aquaculture environment is important as it can be used as a management tool in decision making. Since growth is the difference between what fish ingest and the waste it produces, a model can be constructed using energetic principles and a few mathematical equations. A recent addendum is that computation can be easily accomplished with the help of a cheaply available microcomputer.

Fifty years after Ivlev (1939), this approach has failed to offer a thorough understanding of the underlying mechanisms of fish growth and its relationship with feed intake, probably because underneath the physiological justifications in most models there lies an unguided empirical inter-relationship. Ursin (1967) questioned his own model pondering "whether the model has anything to do with the real world". Fryer (1987) expressed deep concern about the mathematical invasion of biology's charm. Yet models proliferate.

As aquaculture and fisheries are in the realm of applied biology, one can argue that the need here is to provide tools for management and not to deal with theoretical aspects of fish growth. It is known, however, that optimality is the desired situation in any production process. In physical sciences, this would be achieved by investigating the relationship between physical inputs and outputs. The

processing unit can be designed and modified according to requirements. In aquaculture, however, the processing unit is biological in nature which allows very limited modifications (*eg.* genetic engineering). Thus, the profit to the entrepreneur is entirely dependent upon how the processing unit itself optimizes the inputs. It is necessary, therefore, to understand how the processing unit deals with inputs in order to know how to optimize production costs. If the underlying principles are well understood, biologists will be able to provide 'real tools' for management rather than a model based on partial knowledge of a complex process.

As an alternative to the above approach, there are growth models in theoretical biology based on the assumption that an organism is programmed to follow a pre-defined life strategy. The growth process, therefore, is under active internal regulation. There are constraints and trade-offs within the system and hence growth is optimized (*eg.* Weiss and Kavanau, 1957; Kavanau, 1961; Hubbell, 1971; Sibly and Calow, 1986). Although, the exact control mechanisms have not being defined (Calow, 1977; Jobling, 1985) this approach contributes more to an understanding of growth rather than predicting it precisely. Attempts are made in the present study, therefore, to construct a simple conceptual model of fish growth which has a potential to serve as a basic guide for a more precise model in the future.

In the process of model building the following procedures were followed;

(a) to develop a hypothetical growth model based on energetics principles,

(b) to use mathematical tools only if necessary and to make the model as explanatory as possible,

(c) to check the validity of the hypotheses against data that are available in the fish biology literature,

(d) to design experiments on aspects of the growth which are immediately important to the species of interest in the present study.

3.2 A hypothesis of growth in relation to feed intake.

The term growth in general implies a change in magnitude of some variable such as size, number *etc.* and it can be positive or negative. Negative growth is sometimes termed 'degrowth' and the term growth in this thesis represents positive growth.

Growth is a result of digesting raw materials and absorbing nutrients, polymer formation minus the cost of the work performed in these processes. The term nutrient refers only to those substances taken into the body and utilised as:

- the building blocks (BB) for the formation of structural components,
- a substrate (DSE) for energy metabolism (*i.e.* to supply energy for maintenance and growth),
- a catalyst in at least one step of these processes.

The building blocks comprise both non-dispensable building blocks (NDB) [*i.e.* building blocks that cannot be synthesised in the body at a rate sufficient to meet their needs or are not synthesised at all, *eg.* essential amino acids (EAA), essential fatty acids (EFA), minerals *etc.*] and dispensable building blocks (DBB) (*i.e.* building blocks that can be synthesised in the body by using non-specific sources).

The acquired nutrients are then used for maintenance and organismic growth. The latter can be sub-divided into: (a) the building up of 'hard' structures (*eg.* bone formation), (b) the building up of 'soft' structures (*eg.* muscle formation), and (c) storage of energy (*eg.* lipid, glycogen *etc.*).

The conceptual model presented in the remaining portion

of this chapter investigates the fate of absorbed energy in the synthesis of structural materials (sections 3.3 & 3.3.1). It then examines the relationship between energy cost and material formation (section 3.3.2). Section 3.3.3 investigates the possible factors controlling feed intake. The digestive process is explored in section 3.4. Finally, in section 3.5 the possible functional relationship between growth and feed intake is analysed.

3.3 The relationship between absorbed energy and synthesis.

As a starting point, let us assume that a fish absorbs 'A' quantity of food material which contains 'B' fraction of non-dispensable building blocks. In the process of structural material formation, the body will maintain a constant ratio (n) of DBB to NDB.

Let us assume the following:

- all remaininig NDB (B') after maintenance are used for the synthesis,
- there is sufficient DBB in the diet to assist NDB to build up polymers,
- there is adequate energy (*i.e.* substrate for energy generation) in the diet (DSE, dietary substrate energy),
- formation of each unit of polymer from monomers cost a fixed amount of energy.

Under these simplified conditions, growth (G), therefore, can be expressed as:

$$G = (B' + nB')A + EXE \qquad [3.1]$$

$$n = DBB/NDB$$

$$EXE = DSE - (M_n + H_pE) + EXDBB$$

where, EXE = surplus energy after costs of maintenance (M_n) and product formation (H_pE) have been subtracted plus the storage energy from excess DBB (EXDBB) present in the food.

This implies that there is a simple linear relationship between absorbed materials/energy and growth for a particular quality of food. If this simple model is subjected to perturbation for various situations such as degradation of building blocks, cost of polymer formation *etc.*, this will indicate whether there is any possibility of deviation from linearity. The following sections are aimed at analysing this simple model.

3.3.1 Synthesis of the structural materials and degradation of building blocks.

A portion of the available building blocks may be used as a substrate for energy generation. This occurs when there is insufficient substrate for energy generation or when some building blocks are necessary for energy generation processes.

If the former condition has been fulfilled by the dietary source, the latter condition implies a somewhat bizarre situation as it suggests that some essential nutrients, such as EAAs, may be used for energy generation. However, this may be clarified by investigating biochemical efficiency (k_b) of absorbed materials. The k_b) is defined here as:

k_b = (ATP output /substrate input) X 100

The units can be either joules or ATP molecules per unit of substrate (Table 3.1). ATP output can be calculated from an investigation of the biochemical pathways of intermediate metabolism. The details of these reactions can be found in standard biochemistry texts (*eg.* Lehninger,1970; Dagley and Nicholson, 1970 and Stryer 1975). Using these pathways ATP output per unit of substrate can be calculated (Schulz, 1978; Machiels and Henken, 1986). The ATP values presented in Table 3.1 are based on the mechanisms for aerobic respiration and the assumption that 90% ammonia and 10% urea would be produced

Substrate	Mol.Wt.	ATP/mol	ATP/100g
Glucose	180	36	20.0
Tri-oleylglycerol	884	452	51.1
EAA			
Argenine	174	37.0	20.9
Histidine	155	29.3	18.9
Isoleucine	131	42.8	32.6
Leucine	131	41.8	31.9
Lysine	146	37.0	25.3
Methionine	149	21.0	14.1
Phenylalanine	165	39.8	24.1
Threonine	119	22.8	19.1
Tryptophan	204	46.5	22.8
Valine	117	32.0	27.4
NAA			
Alanine	89	17.8	19.9
Aspartic acid	133	17.8	13.3
Asparagine	132	17.8	17.8
Cysteine	121	14.8	12.2
Glutamic acid	147	26.8	18.2
Glutamine	146	26.8	26.8
Glycine	75	8.8	11.7
Hydroxyproline	131	28.8	22.0
Proline	115	31.8	27.6
Serine	105	14.8	14.1
Tyrosine	181	43.8	24.2

Table 3.1. Biochemical efficiency (K_b) of energy-yeilding processes. K_b is presented as the moles of ATP/ 100g of substrate.

by amino acid catabolism.

The mechanisms of amino acid degradation in omnivores and herbivores operate according to the Michaelis constant (K_s) (Krebs, 1972). This may play a central role in amino acid catabolism in fish (Walton and Cowey, 1982; Walton, 1986) irrespective of feeding habit. It is also known that branched chain amino acids (*i.e.* iso-leucine, leucine and valine) especially leucine which is a ketogenic amino acid, are degraded in the muscles whereas most others are catabolised in the liver (Smith and Elia, 1983). Young *et al.* (1985) summarising data available for mammalian species, stated that the K_s for leucine amino-acyl synthetase (ligase) is about 6 µmol, which is 30 times higher than the K_s for other synthetases (usually <0.2 µmol). However, tissue concentrations of essential amino acid are 0.5-1.5 mM and the K_s values for branched chain amino acid transferases are about 4mM. Young *et al.*, (1985) showed that irreversible oxidation, however, increases when intake increases. They argued that the requirement for indispensable amino acids, therefore, might be a function of the mechanisms and the extent of oxidation, rather than the level required for protein anabolism.

It is evident from Table 3.1 that the highest efficiency per unit of input is derived from lipids. However, the k_s values for most essential amino acids are greater than the value for glucose. In the light of the evidence presented and taking k_s values into account, it is reasonable to assume that a part of the NDBs degrades in the body. The DBBs, therefore, are subjected to inevitable degradation as the synthesis process cannot be continued without NDB. On the other hand, if the degradation is controlled by the K_s of enzymes, it is reasonable to assume that synthesis and degradation are directly propotional to intake. In fact, Houlihan *et al.*, 1988 showed that in cod, *Gadus morhua*, protein synthesis and

degradation are linearly related.

From these observations and with limited available data, it is possible to construct hypothetical response curves for whole body tissue synthesis and degradation in relation to feed intake at a particular temperature (Fig. 3.1). The following assumptions are made in constructing Fig.3.1.

(1) Synthesis and degradation obey the rules of enzyme kinetics.

(2) The rate of synthesis (S) and degradation (D) are controlled by the K_s values of enzymes. The rate is, therefore, directly proportional to absorbed building materials (B_i) ($S \propto B_i$; $D \propto B_i$).

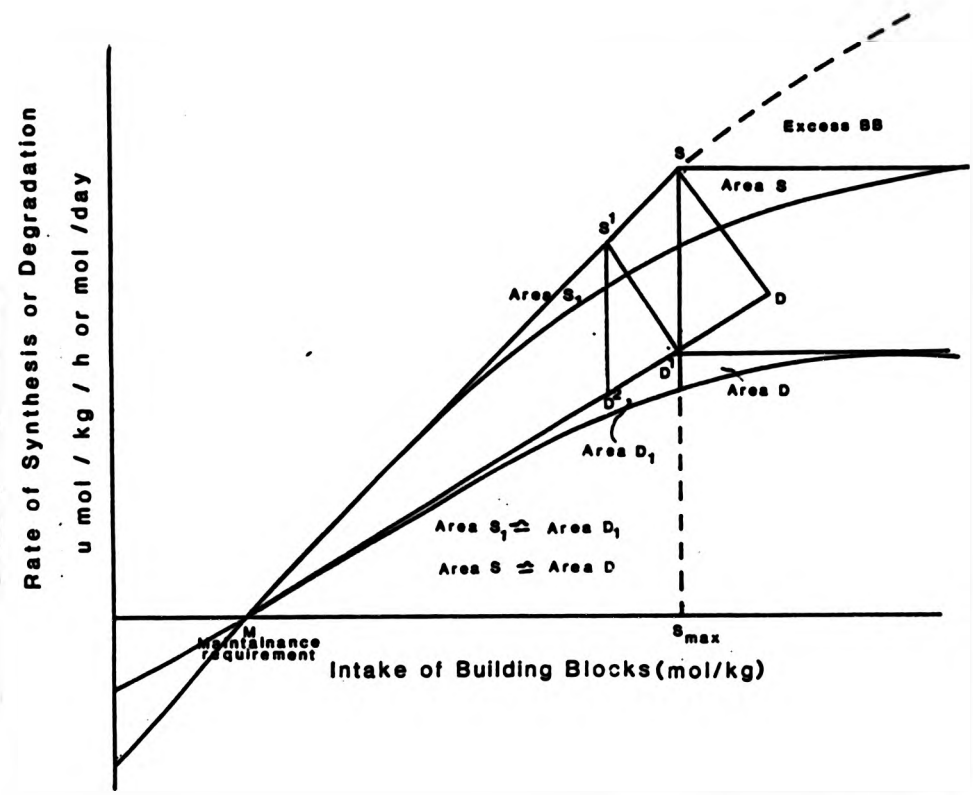
(3) Synthesis approaches asymptotically an upper bound due to the limitations set by enzyme kinetics and reaction surfaces (*eg.* mitrochondrial membranes). For example, if the synthesis is assumed to have an upper limit, the rate, therefore: $dS/dB_i = k_1(S_{max} - S)$ and $dD/dB_i = k_2(D_{max} - D)$ where, S_{max} = maximum synthesis capacity per unit of time, k_1 and $k2_1$ = rate constants for synthesis and degradation. Upon integration: $S = S_{max}(1 - e^{-k1B_i})$ and $D = D_{max}(1 - e^{-k2B_i})$. The net synthesis of structural materials, therefore, is the area occupied between the synthesis and degradation curves.

(4) The maximum capacity can be delineated by drawing lines from maintenance requirement and from upper asymptote. It was assumed that the areas $S_1 \approx D_1$ and $S = D$ in Fig 3.1. The sharpness of the breakpoint, S, shown in Fig.3.1 will depend upon the reaction rate which in turn may depend upon environmental temperature.

There is ample evidence for terrestrial homeotherms that there is a clear breakpoint in the protein synthesis curve as shown for a number of species (*eg.* Young *et al.*, 1985).

S_{max} in Fig.3.1 indicates the saturation point or plateau

Fig. 3.1 Curves depicting the expected response rates of structural materials synthesis in relation to varying feed intake.
[See text for explanation of letters.]


Rate of Synthesis or Degradation
u mol / kg / h or mol /day
Excess BB
S
Area S
S¹
Area S₁
D
D¹
D²
Area D
Area D₁
Area S₁ ≙ Area D₁
Area S ≙ Area D
M
Maintainance requirement
S max
Intake of Building Blocks (mol/kg)

value for synthesis. If intake exceeds S_{MAX}, the remaining building blocks will be converted to energy storage compounds. In the case of amino acids, they are deaminated and converted into glycogen or lipid otherwise they may be toxic to the fish. This implies that when intake level exceeds S_{MAX}, degradation must continue. The breakpoint shown on the degradation curve (which is similar to the synthesis curve) is for conceptual clarity. Since net synthesis is the difference between the rates of synthesis and degradation, the breakpoint shown in Fig 3.1 indicates that total synthesis of structural materials reaches a plateau value after S_{MAX}. Excess degradation after S_{MAX}, therefore, is shown in the upper part of synthesis curve. A similar hypothetical synthesis curve has been presented by Millward (1985) for humans, but the degradation concepts and curves in the present study differ from his presentation.

In the triangle MSD in Fig. 3.1: $SD \| S'D'$ and $SD' \| S'D^2$. Therefore, triangles SDD' and $S'D'D^2$ are congruent. Hence the ratio $SD'/S'D^2 = g$, is a constant. This implies that synthesis of structural materials is linearly related to absorbed materials until S_{MAX} and thereafter is independent of the rate of food acquisition. This, however, may depend upon the environmental temperature.

This phenomenon obeys Blackman's kinetics in which there is a linear relationship between growth and substrate concentration until some other factor limits any further increase in growth (Blackman, 1905; Condrey, 1982). Hence equation 3.1 still holds true for optimal temperature up to S_{MAX}; however, B' in equation 3.1 should be replaced by b and the expression thus changed to:

$$G = (b + nb)A + EXE = gA - EXE \qquad [3.2]$$

where b = net fraction of essential building blocks remaining after degradation.

Predictions.

Up to S_{max} within the optimal temperature range:

(1) Net synthesis is a linear function of absorbed energy.

Prediction 1 implies that:

Degradation of amino acids is a function of absorbed energy. Excretion of ammonia and urea is an approximately linear function of absorbed energy. If fish are offered an 'unbalanced' diet, the rate of excretion will be higher than for a 'balanced' diet due to unfavourable NDB/DBB ratio. The same is true for a diet containing insufficient non-protein energy.

3.3.2 Energy utilisation for synthesis.

The energy cost for product formation has been investigated theoretically by applying stoichiometry for biochemical reactions (*eg.* Buttery and Boorman, 1976). For example, the theoretical cost of protein deposition can be calculated as follows:

Cost of activation of amino acid:

Amino acid + enzyme + ATP ——> [Enz-amino acyl-AMP] +P P

[Enz-amino acyl-AMP] + tRNA ——> Enzyme + AMP + amino acyl-tRNA

AMP + ATP ——> 2ADP

P P ——> 2Pi

Amino acid + 2ATP ——> amino acyl-tRNA + 2ADP + 2Pi

Cost of polymerisation: 2 GTP ——> is equal to 2 ATP

assuming the cost of transportation of amino acid through cellular membranes = 1ATP $mol.mol^{-1}$ amino acid

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Total energy cost for peptide bond synthesis, therefore
= 5ATP mol.mol^{-1} amino acid.

Assuming the energy is to be generated from glucose catabolism:

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$ G = 2870 kJ

Assuming 1 mol of glucose produces 36 ATP,

Energy cost = (5/36) X 2870 = 398.6 kJ mol^{-1} amino acid.

Average molecular weight of amino acid = 118

Therefore, the theoretical cost of protein deposition = 3.4 kJ g^{-1}

Energy content of the deposited protein = 23.6 kJ g^{-1}

Therefore, total cost of deposition = 27 kJ g^{-1} protein.

Details of the reaction pathways can be found in biochemistry text books (*eg.* Stryer, 1975). Assumptions made are similar to Buttery and Boorman (1976) and Reeds *et al.* (1985).

The above example shows that it is reasonable to assume that the energy cost of product formation is a linear function of absorbed energy and hence growth to S_{max}.

Let us assume that a fish is offered a diet with an energy density greater than required for maintenance, synthesis and waste product formation. Extra energy will be accumulated in the body. Then:

Remaining energy =

$$EXE = [NDE - (M + H_pE + Excretion)] + \text{energy from EXBB}$$

It is logical to predict (Prediction 2), therefore, that energy accumulation up to S_{max} is a linear function of absorbed energy.

Until now the model dealt with energy absorbed up to S_{max}. The following section investigates the consequences of energy intake above S_{max}.

3.3.3 Do fish ingest beyond S_{MAX} ?

The model predicts that the synthesis of structural materials remains steady after S_{MAX}, hence, all extra energy has to be converted to lipid or glycogen.This situation is shown in Fig. 3.2.b which is essentially another way of presenting Fig. 3.2.ba.

Suppose a fish is offered a formulated diet of high nutrient density. If the capacity of the gastrointestinal tract is high and the food is easily digestible, the fish may ingest and digest more food than necessary in a day.

In theory, there are two possible ways in which energy can be absorbed beyond S_{MAX}.

(a) Over-consumption;

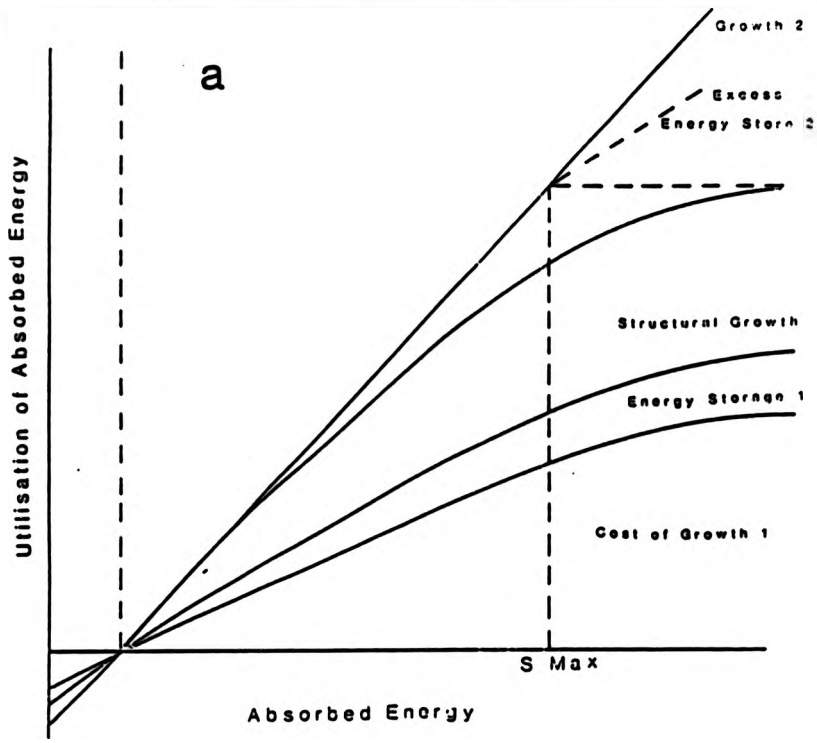
i.e. ingestion of more food than fish can use for structural material synthesis. This is possible if ingestion is controlled, at least partially, by stretch receptors in the gastrointestinal tract but the evacuation (and hence digestion) is controlled by osmo-receptors in the duodenum wall as described by Hunt (1980).

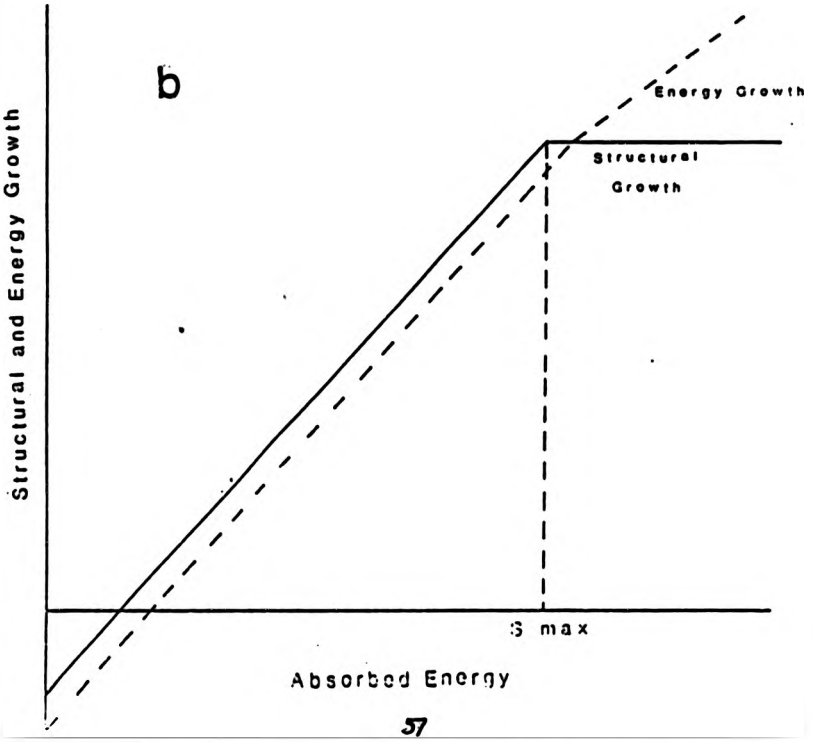
(b) Rapid digestion and absorption.

A similar concept has been proposed by Jobling (1986, 1988) termed gastrointestinal overloading where rapid digestion and absorption result in a temporary shift of absorbed energy beyond S_{MAX} after each meal. Hence, the situation (b) will be similar to situation (a) if fish cannot store building blocks for a short period of time.

There are two possible reasons for postulating that a fish will be unable to over-eat or continue to digest rapidly for long periods of time. One is that the fish will face an energy storage problem and the other is that fish will confront a power rating problem. These two problems, however, may not be mutually exclusive.

Fig. 3.2 Curves delineating expected responses of structural and energy growth in relation to varying feeding rates.


a
Growth 2
Excess
Energy Store 2
Structural Growth
Energy Storage 1
Cost of Growth 1
S Max
Utilisation of Absorbed Energy
Absorbed Energy


b
Energy Growth
Structural
Growth
S max
Structural and Energy Growth
Absorbed Energy

3.3.3.1 Energy storage problem.

In fish, there are no specialised tissues for energy storage (Pond, 1981). Major sites for storage are in the muscles as in tilapias (Tan, 1971) and salmonids (Pond, 1981), in the liver as in sharks (Pond, 1981) and cod (Jobling, 1988) or as fat bodies in the abdominal cavity (Pond, 1981; *eg.* tilapias, pers. obs.). The lipid content of liver in sharks (elasmobranchs) is specialised as a buoyancy regulator and is totally independent of diet or nutritional status, and is, therefore, ignored in the present study. In most other fish, nutritional status may effect lipid storage. If fish absorb food beyond S_{max} this will alter their body composition implying allometric growth between structural and storage materials. It is necessary, therefore, to look at the scaling factors of body composition.

Skeleton to body mass relationship.

There are two basic functions of the skeleton (Calder, 1984): one is to support the body against gravity and the other is to provide a rigid framework for muscular attachment. Unlike terrestrial animals, it can be assumed that the buoyancy of water neutralises the weight of most fish. The skeletal mass, therefore, may increase isometrically with total body weight. Indeed, Reynolds and Karlotski (1977) provided empirical evidence to support this claim for teleost fishes (five species) such that:

$$W_s = 0.033 + 0.016\ W_b^{1.03}$$

where W_s = dry skeleton mass; W_b = wet body mass.

Since the exponent is not significantly different from 1, the above equation can be simplified to:

$$W_s = 0.02 \quad 0.05\ W_b$$

or as a percentage $[(W_s / W_b) \times 100]$ dry skeletal mass accounts for 2-5% of wet body mass independent of body size. Assuming

that fish contain 75% water the dry skeletal mass will account for 8 - 20% on a dry weight basis depending on the species.

Skeletal muscle mass (B_s) to body mass relationship.

Isometric growth of the skeleton implies that the muscles attached to them may also follow the same pattern. Calder (1984), showed that the skeletal muscle for most animals occupies a major fraction of the body independent of the size of the animal *eg.* tunas, (*Katsuwonus pelamis*) 68%, salmonids,(*Salmo irideus*) 55-67% and for goldfish,(*Carassius auratus*) 33-45% skeletal muscle mass to total body mass).

Lipid accumulation and storage.

Let us assume that energy storage in the muscles is in extracellular fluid (ECF) or attached to cylindrical muscle fibres or bones. Hence the space available for quantity of lipid (Lip) accumulation would be intuitively clear, so that:

Lip = total volume - $[(W_s/g_1) + (W_y/g_2)]$ + LFECF)

where g_1 and g_2 is specific gravity of skeletal muscles and bones and LFECF = volume of lipid free extracellular fluid.

This implies that the amount of lipid storage in muscles is subjected to an upper limit depending upon the size of fat free extracellular fluid.

The volume of the abdominal cavity should be directly proportional to skeletal and muscle growth as growth in linear dimensions is a necessity for the increase in the volume of the abdominal cavity. Hence the accumulation of lipid in the abdominal cavity (in liver or in viscera) is also constrained by the growth of the skeleton and muscle mass. This implies that the total lipid storage capacity is controlled by the growth of structural materials.

The argument may be summarised as follows. Teleost fish do not possess specialised organs for lipid storage. Hence for total lipid storage:

Stored Lipid α Body Volume

Body volume α $(W_s + W_g)$

Hence accumulation of lipid in a normal fish:

Lipid accumulation α $(W_s + W_g)$

Total body lipid is conventionally given as (assuming glycogen fraction is relatively negligible):

Lipid = Body weight - (Protein + Ash + Water).

Let us assume that protein and ash represent (approximately) total body muscles and skeleton. If food materials are absorbed beyond S_{max} protein and carbohydrate have to be converted to stored lipid and excess minerals have to be excreted. The synthesised fractions of protein and ash are a constant beyond S_{max}. The upper limit for lipid storage beyond S_{max}, therefore, will be mainly determined by the minimum amount of water which is necessary to maintain the metabolic function of the body. The moisture content of different tissues is varied. There is evidence from the literature that the total body moisture content rarely falls below 2/3 of wet body weight of fish. The water fraction in terrestrial animals is a constant, with 1% or less deviation on a fat free basis (Pitts and Bullard, 1968). It is difficult to find an exact value for water content of fish. Water content will, however, be related to the metabolic needs of the animal, and therefore there must exist a minimum level.

Under the above circumstances, if fish ingest and absorb energy beyond S_{max} it cannot be continued for a long time (depending upon previous nutritional history) due to their inability to store excess energy. The alternatives remaining are either to reduce digestibility of ingested food or to reduce ingestion.

Predictions.

(3) It is impossible for a fish to absorb energy beyond S_{max} for long period due to differential rate of structural growth and energy growth. With *ad libitum* feeding,

therefore, a wide fluctuation in the rate of ingestion (hence in growth) will take place. The corollary is that with *ad libitum* feeding, total food consumption over a defined period (and hence growth) will mainly be determined by fishes' ability to expend excess energy. An exercised fish which has been fed *ad libitum*, therefore, may attain a marginally higher growth rate than non-exercised fish.

(4) If growth in energy and structural materials is allometric due to over-consumption, but not a result of rapid digestion and absorption, a marginally higher rate of growth and food conversion will be achieved by ingesting (and hence absorbing) energy at a rate below S_{max}.

(5) If the rate of ingestion is constant, a rhythmic digestibility in a defined time period will result.

The lipogenic theory which explains that the long-term energy balance is controlled by 'energy memory' was initially proposed by Kennedy (1961) for terrestrial animals. In fish which accumulate lipid in muscles, the best indicator for fattiness will be the condition factor [$(W/L^n)*100$ where W = weight and L = length]. Birkett (1972) showed that growth efficiency and condition factor are linearly related in plaice, *Pleuronectes platessa*. Stirling (1972, 1977) demonstrated that the condition factor of European bass, *Dicentrarchus labrax* increased asymptotically due to lipid accumulation with *ad libitum* feeding and that there was a direct relationship between food intake and condition factor. Jobling (1988) reviewed the growth data for cod, *Gadus morhua* and showed that hepatosomatic index [HSI = (liver weight/body weight)*100] and specific growth rate were inversely related.

The mechanism controlling feed intake may occur via the

hypothalamus and endocrine factors. An advance mathematical treatment can be undertaken assuming that there are templates which induce growth and antitemplates which inhibit growth as was assumed by Weiss and Kavanau (1957) in constructing their growth model. As the exact form of the regulatory mechanism, however, requires clarification, this has not been investigated in the present study. A highly theoretical treatment of allometric growth associated with mortality risk can be found in Sibly and Calow (1987).

3.3.3.2 Power rating problem.

In theory, there are two important aspects of metabolism in relation to growth. One is the metabolic scope, which is the difference between active and standard metabolic rate and the other is the scope for growth, which is explained as the difference between energy ingested for maximum growth and energy dissipiated in an animal (Warren and Davis, 1967).

Let us assume that fish ingest and absorb food over S_{max}. The work done on food processing under this condition most probably covers their total metabolic scope. Beamish (1974) found that in largemouth bass, *Micropterus salmoides*, metabolic rate following a meal increased up to 100% and Soofiani and Hawking (1982) showed that SDA itself can take up all the metabolic scope of young cod. This implies that fish tend to ingest energy above their capacity to process.

Fish, however, will not be able to continue to ingest excess energy for long, as this condition will result in an O_2 budgeting problem. Fish will suffer from physiological hypoxia and it may yield required energy for metabolism from anoxic sources (Gnaiger, 1983). On the other hand, a life strategy which sustains a very high metabolic rate will not be able to continue as the higher metabolic rate will result

in high costs of wear and tear to systems for example, mitochondria and enzymes and hence increase the risk of mortality. It is known that the reliability (and also durability) of a machine is higher when the system operates well below its maximum power rating and this may also be true for animals (Priede, 1985). Hence the alternative is to reduce ingestion suggesting fluctuation in feed intake (and hence growth) with *ad libitum* feeding. The corollary to prediction 3, which stated that an exercised fish which has been fed *ad libitum*, may attain a marginally higher growth rate than non-exercised fish is, however, not true under this condition.

The model so far deals with absorbed energy and not ingested energy. Construction of a plausible model for optimal digestion in fish will be investigated in the following section.

3.4 An hypothesis of optimal digestion for fish.

In theory, there are two important aspects of digestion of acquired food.

(a) The rate of passage of ingested food through a series of chambers in the gastro-intestinal tract.

(b) The rate of digestion (*i.e.* digestive or absorption efficiency which is the amount of ingested material extracted per unit time).

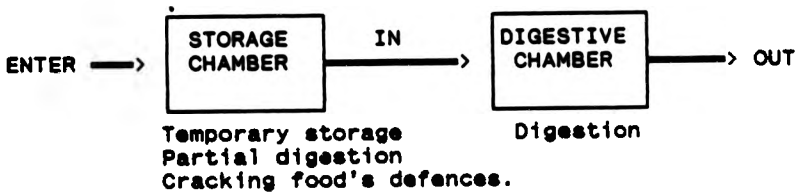
The rate of food passage through the gut has been subjected to thorough investigation in fish (*eg.* Tyler, 1970; Elliot, 1972; Fänge and Grove, 1979; Jobling, 1981) as it is an important facet in estimating food consumption, especially in nature. Digestive efficiency, however, is the most important aspect of resource acquisition but little work has been performed on fish species. An attempt is made in this thesis, therefore, to generate a model of digestive efficiency

of fish in relation to food passage through the gut.

Sibly (1981) devoloped a model to explain how food should be processed by a herbivore which possessed a continuous flow digestive system. Sibly's model, with slight modifications, can also be used to explain batch processing of food as well as continuous processing by a fish with a simple stomach. It was decided, therefore, to use the above model as the prototype for the explanations presented in this thesis. The emphasis in the present study, however, is not to make predictions about fish morphology in an evolutionary sense but to check the usefulness of this theory in understanding how food is processed by a fish and its practical value for aquaculture.

The terms used to explain digestion are confusing. The rate of passage through the gastro-intestinal tract is variously termed digestion, emptying or evacuation. The term 'evacuation' is used in this thesis. The rate of digestion is also known as digestibility, digestion co-efficient, digestive or digestion efficiency, absorption efficiency, assimilation efficiency or coefficient and extraction efficiency. The terms 'digestive' or 'absorption efficiency' are preferred in the present study.

Suppose the ingestive and digestive activities can be simplified into two hypothetical chambers as indicated below.



Within this simplification, digestion and absorption can be considered as a continuous flow digestive process between compartments. If the food particles are small and

homogeneously mixed, the time lag is considered to be negligible (Jobling, 1981).

The simplest way to model digestive activity is to considered it as a first order chemical reaction.

$$En + I \longleftrightarrow [EnI] \longleftrightarrow En + P$$

where En = enzyme, I = substrate and P = product concentrations respectively. Since the rate-limiting step is the product formation from the enzyme-substrate complex:

$$I \longrightarrow P \quad \text{for a first order reaction.}$$

The rate, therefore:

$$-dI/dt = k_d I = dP/dt = k_d(I_0 - P)$$

where, I = food concentration, P = product concentration, t = time and k_d = rate constant.

Upon integration:

$$I = I_0 e^{-kdt}$$

$$P = I_0 (1 - e^{-kdt})$$

Multiplying both sides by the energy content (E) of the food:

$$IE = I_0 E e^{-kdt} \qquad [3.3]$$

$$PE = I_0 E (1 - e^{-kdt}). \qquad [3.4]$$

As fish are poikilotherms, the rate of digestion is affected by environmental temperature. If the effect of temperature (T) on the rate of change of reaction is assumed to be exponential (Krogh, 1914; Elliot, 1972), $k_d = ae^{bT}$, k_d in the above equation can be replaced by ae^{bT} .

A similar equation has been developed by Sibly and Calow (1986) for a cylindrically-shaped intestine:

$$N_t = N_0 e^{-4dt/cM},$$

$$N_p = N_0 (1 - e^{-4dt/cM}),$$

where, N_t = number of unprocessed particles at time t, N_0 = number of unprocessed particles at time t = 0, N_p = number of processed particles at time t, d = particle diameter, c = gut diameter and M = frequency of muscular action. Details can be found in Box 2.3 in Sibly and Calow (1986). In this model

4d/cM is similar to k_d.

A two-dimensional representation of the above model is given in Fig. 3.3. Cracking the defences of food in Fig. 3.3 can be considered negligible with formulated food. Fish, therefore, would start digestion immediately after consumption. The above model predicts that when ingestion is limited, the retention time, t, has to be maximised in order to obtain maximum profit from ingested energy (*cf.* Sibly, 1981).

3.4.1 Work performed by the digestive chamber for short period T_1.

Suppose a fish is offered a single meal to satiation. If it is assumed that a food item containing E kJ g^{-1} energy enters the storage chamber at a rate of I g s^{-1}, then the total energy content in the storage chamber is IE kJ.

It is assumed that the stomach works in pulse action and releases food into the intestine in batches (*eg.* Jobling, 1986). Suppose food enters the digestive chamber (the upper intestine) at a rate of $I_{in}E$. Let the energy digested and absorbed after an infinitesimally small time interval, dt, be $I_{in}E \int f(t)\,dt$.

Therefore:

Energy in digestive chamber $= I_{in}E.T_1$

Concentration of formed product after time t

$$= f(t) = I_0E\,(1 - e^{-kdt})$$

Energy remaining in digestive chamber

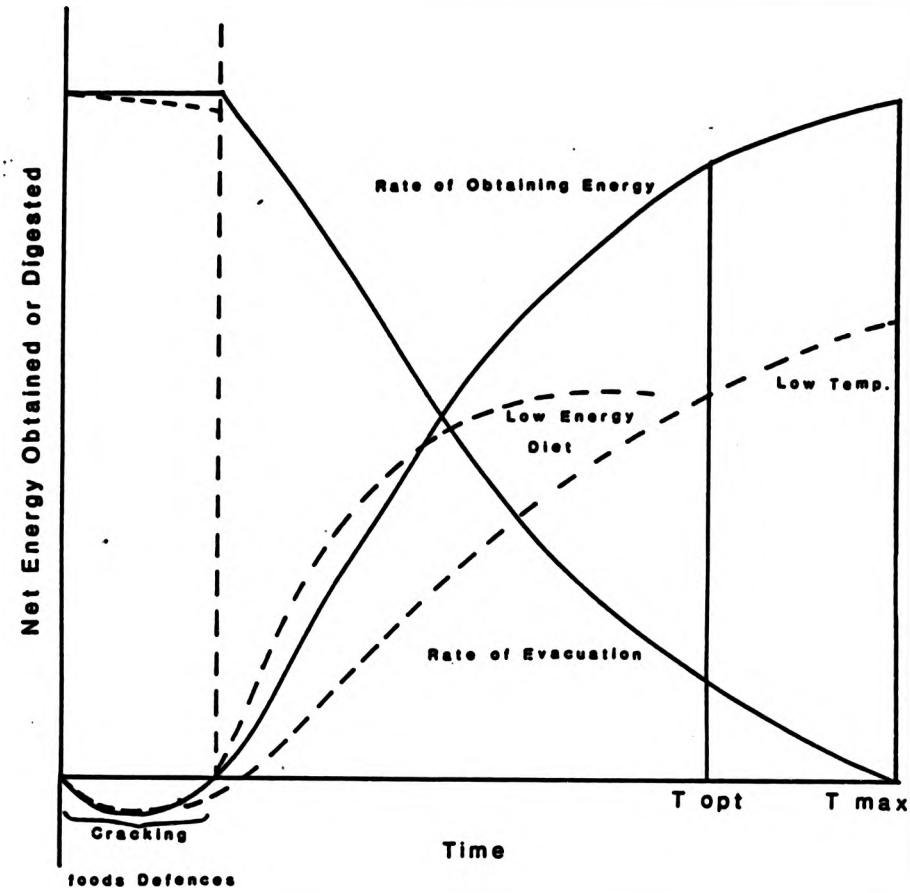
$$= I_{in}E.T_1 - I_{in}E \int f(t)dt$$

Energy remaining $= I_{in}E\,[T_1 - f(t)\int dt]$

I_{in} = Energy remains / $E\,[T_1 - f(t)\int dt]$. [3.5]

Equation 3.5 states that the rate of throughput of energy from the storage chamber to the digestive chamber, therefore,

Fig 3.3 Hypothetical digestion curves for a fish (modified from Sibly, 1981).


Net Energy Obtained or Digested
Rate of Obtaining Energy
Low Temp.
Low Energy
Diet
Rate of Evacuation
T opt
T max
Cracking
foods Defences
Time

will be determined by the energy density of the food (*cf.* Jobling, 1981) and the instantaneous rate of digestion in the digestive chamber. Hence it can be predicted (prediction 6) that if the PE:TE ratio (*i.e.* protein energy to total energy) in a diet is low, fish will be unable to obtain enough proteinaceous building blocks for structural material synthesis. Fish, however, may attempt to obtain enough building blocks at a maximum rate of food processing, resulting in higher energy accumulation. A diet with a high PE:TE ratio will exceed S_{max} and also result in a lipid accumulation and power rating problems. The expected dose response curve for a short time will be one that reaches a plateau, and for a long period of time is a curvilinear function of the independent variable (PE:TE ratio). The time (*i.e.* weeks or months) here will be dependent upon the previous nutritional history of the fish.

It is known that digestion takes place in the upper intestine. According to equation 3.5, therefore, the rate of intestinal digestion may follow the rate of gastric evacuation with a small time difference. If one neglects the time difference, the relative shape and retention time of the digestion curve can be approximated from gastric evacuation studies.

There are a number of gastric evacuation models in fish biology and the best model describing the relationship is still under debate (*eg.* Jobling, 1986; Persson, 1986).

The most widely used models are:

$I = k_s I_0$ GER α linear (*eg.* Hunt, 1960),

$dI/dt = k_s I$ GER α exponential (*eg.* Elliot,1972),

$dI/dt = k_s I^{0.67}$ GER α surface area of food bowls (*eg.* Fänge and Grove, 1979),

$dI/dt = k_s I^{0.5}$ GER α square root of ingested energy (*eg.* Jobling, 1981).

where, I = weight/energy of ingested food, t = time, k_s = rate constant and GER = gastric evacuation rate.

The exponential model (Hunt and Spurrell, 1951) for gastric evacuation predicts that the rate of passage through the gut during the initial stages is faster than during the later stages. The opposite is true for the square root model which was originally proposed by Hopkins (1966) for humans. The surface area model (Tyler, 1970; Fänge and Grove, 1979) provides a compromise between the former and the latter (*i.e.* the curve passes in between the former and the latter curves). The major objective of gastric evacuation models is to calculate the food ration of fish in the natural environment. Linear, surface area and square root models have been developed due to dissatisfaction with the exponential model to describe the evacuation rate on an 'as fed' basis. However, if the evacuation rate is expressed in terms of total digestible organic matter (hence energy), the exponential model produces an excellent fit to empirical data of fish (Elliot, 1972; Jobling, 1986). Therefore, it is reasonable to assume that the exponential model is adequate to describe the rate of gastric evacuation in terms of energy or digestible organic matter.

Hence, for the digestion of a single meal from equation 3.3:

$$IE = IE_0 \, e^{-kdt}.$$

It is assumed that the above equation is approximately equal to:

$$IE = IE_0 \, e^{-kst}$$

which is an empirical model from gastric evacuation studies of fish (Elliot, 1972).

Now the relative value for T in Fig. 3.3 can be estimated approximately from gastric evacuation studies. The time taken for complete evacuation may be considered as the maximum

retention time, T_{max}.

3.4.2 Work performed by the digestive chamber over a long time period T [*i.e.* day]

If the gut has a limited capacity and if the energy requirement of a fish is higher than the amount of food that can be stored in the storage chamber at a time, a fish may ingest a number of meals depending upon the energy density of the food and the environmental temperature. A plausible model for digestion of multiple meals can be given as:

$$IE = IE_0 \left(e^{-kd1t1} + e^{-kd2t2} + \ldots\ldots + e^{-kd(n-1)t(n-1)} e^{-kdntn}\right) \qquad [3.6]$$

Assuming that the rate of gastric evacuation is approximately equal to the rate of digestion:

$$IE = IE_0 \left(e^{-ks1t1} + e^{-ks2t2} + \ldots\ldots + e^{-ks(n-1)t(n-1)} e^{-ksntn}\right)$$

where, 1, 2 ...(n-1) and n denotes the number of meals a day. The model is similar to the multiple meal evacuation model provided by Elliot (1972). If a second meal enters the storage chamber before complete digestion of the first one (or assuming that the digestion of multiple meals can be considered to be equivalent to a large fish digesting a single meal), Fig. 3.3 will be the net result of the summation of equation 3.6. The relative shape and value of T can be predicted from gastric evacuation studies provided that k_d is approximately equals to k_s.

Prediction 7.

Within the above multiple meal model, there are two possible strategies of digestion depending upon the behavior of the |k| values (rate constants).

Digestive strategy 1:

$k_{s1} \simeq k_{d1} = k_{s2} \simeq k_{d2} \ldots = k_{d(n-1)} \simeq k_{s(n-1)} \simeq k_{sn} \simeq k_{dn}$

Here the gastric evacuation rate is assumed to be

approximately equivalent to digestion rate and both rates are independent of meal frequency. Digestive efficiency may not significantly deviate from its maximum value. The faecal production and the net energy obtained, therefore, are linearly related to the rate of ingestion.

Digestive strategy 2:

Strategy 2 results from two possible relationships between k_s and k_d values.

(a) $k_{s1} < k_{s2} \ldots < k_{s(n-1)} < k_{sn}$ and k_d values follow the opposite due to rapid rate of evacuation.

(b) $k_{s1} = k_{s2} = k_{s(n-1)} = k_{sn}$ but $k_{d1} > k_{d2} \ldots > k_{s(n-1)} > k_{dn}$ because digestive efficiency decreases due to a reduction in enzyme activity and concentration with time. In this situation, k_d and k_s are not related to each other and the curve in Fig. 3.3 cannot be approximated from gastric evacuation studies.

For both cases (*i.e.* a and b) of digestive strategy 2 the relationship would yield an exponential increase in production of faecal energy and an exponential decrease in the net rate of obtaining energy per unit time. In other words T_{max} in Fig. 3.3 would shift to T_{opt} gradually with higher feeding rates.

A plausible explanation for this strategy of digestion has been provided for a continuous flow digestive system by Sibly (1981) as follows. Defining the following:

Rate of energy entering the digestive chamber = $I_{in}E$

Disregarding absorption (for details see Sibly, 1981),

Energy in the digestive chamber (EnD) = $I_{in}E.T$

Therefore, $I_{in}E = EnD/T$

Net rate of obtaining energy $= I_{in}E.f(T)$

$= EnD \; . \; [f(T)/T]$

The optimum strategy for the fish would thus be to obtain the highest possible cumulative energy per day by maximising $f(T)/T$. This situation may be very advantageous for fishes in

temperate water as both k_s and k_d decrease at lower temperatures.

A third digestive pattern under strategy 2 (sub-strategy 2) would result from particular experimental conditions with restricted feeding. The digestive efficiency would be at T_{max} until the amount fed was approximately equivalent to the size of single meal and then decrease exponentially with increasing meal size. An idealised graphical representation of production of faecal waste under the above strategies is given in Fig.3.4.

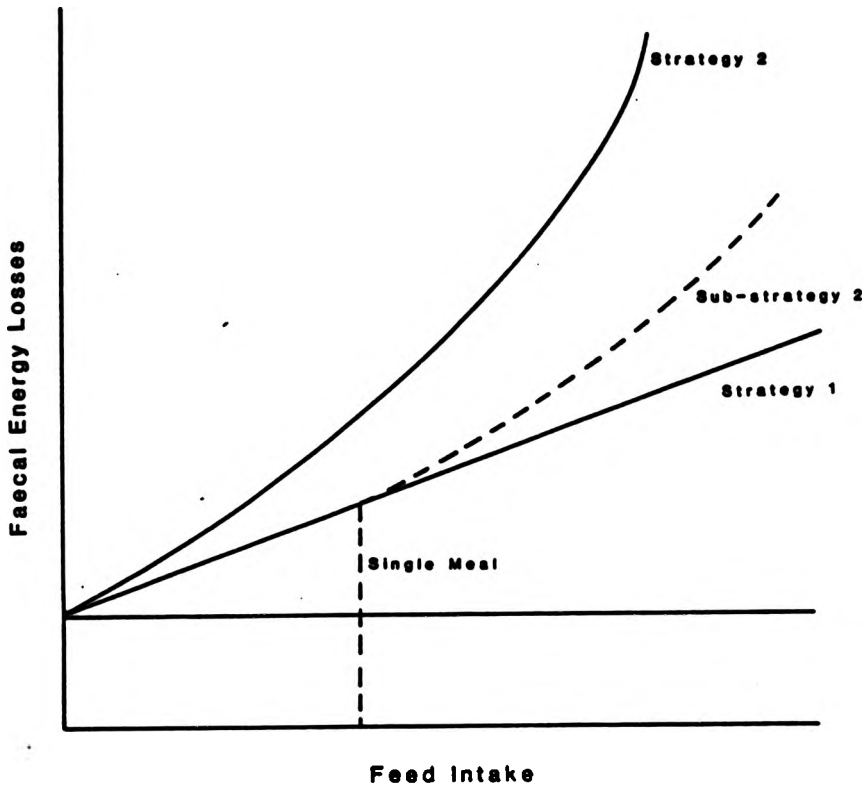
3.5 The relationship between growth and ingested energy.

The growth pattern and rate associated with ingested energy will mainly be determined by the energy allocation pattern (*i.e.* costs of food acquisition, mechanical action food passage through the gut, digestion, absorption and product formation) and the resources acquisition pattern (*i.e.* digestive strategy).

3.5.1 Growth pattern 1

Digestive strategy 1 will result in a linear increase in faecal waste. If food is artificially supplied (not by enhancing natural production of food), the cost of food acquistion may be neglegible. The costs associated with digestion and absorption should also be a linear function of ingestion rate. The absorbed energy is a linear function of the ingested energy (Fig.3.4). It has been shown previously that the cost of product formation will be a linear function of energy. Growth, therefore, is a linear function of ingested energy (Fig.3.5). Indices of growth efficiency; K_1 and K_2 are monotone increasing functions of ingested energy and K_3 is a constant (Fig.3.6).

Fig 3.4 Hypothetical curves delineating faecal production under two digestion strategies.


Strategy 2
Sub-strategy 2
Strategy 1
Single Meal
Faecal Energy Losses
Feed Intake

Fish will not be able to ingest beyond S_{max} for long and there will be a wide fluctuation in feed intake. Growth with *ad libitum* feeding, therefore, will be marginally less than fish fed near S_{max}. Thus, if the X and Y axes represent food offered and growth respectively in a graphical presentation of functional relatinoship between food intake and growth, a linear growth phase followed by a decline in growth will be the result (area ex in Fig.3.5) .

3.5.2 Growth pattern 2.

Growth pattern 2 will result from digestive strategy 2. Under this strategy there is an exponential increase in faecal energy. The net energy obtained, therefore, follows the law of diminishing returns. The cost associated with digestion, absorption and product formation also follows the same pattern with ingested energy. Growth, therefore, will be a positive curvilinear function of the rate of ingestion (Fig.3.5). The growth efficiencies K_1 and K_2 increase to a plateau value soon after maintenance requirement is exceeded, and then decreases. Partial efficiency also decreases as shown in Fig. 3.6.

Under artificial experimental conditions, sub-strategy 2 rather than strategy 2 itself will be dominant. Hence absorption, (and hence growth) will be linearly related to ingestion during the first part of the curve, the curvei-linearity only appearing during the latter part of the curve. This is the reason for the faliure to fit a suitable mathematical function with biological meaning. However, if one neglects the possible non-linear cost associated with digestion and absorption under digestive strategy 2 (or upper part of the sub-strategy 2), there will be an approximately linear relationship between absorbed energy and growth as the cost after absoption is lineraly related to absorbed energy.

Fig. 3.5 Expected growth responses *versus* feed intake in relation to different digestive strategies.

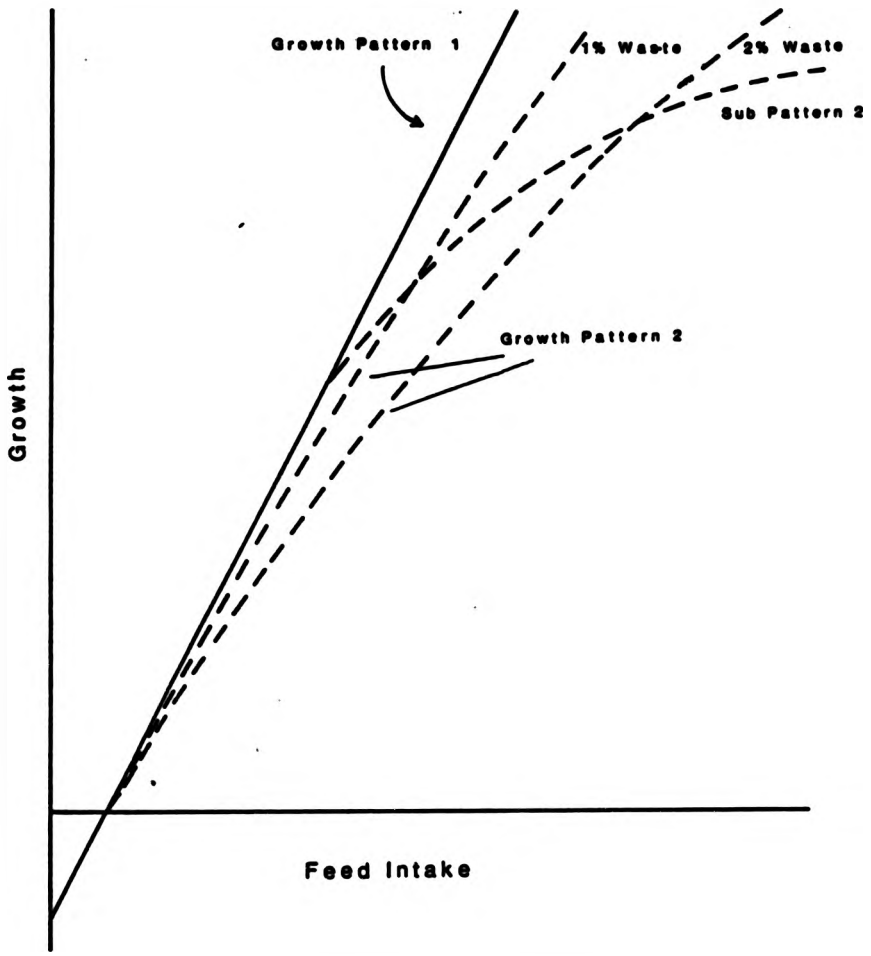
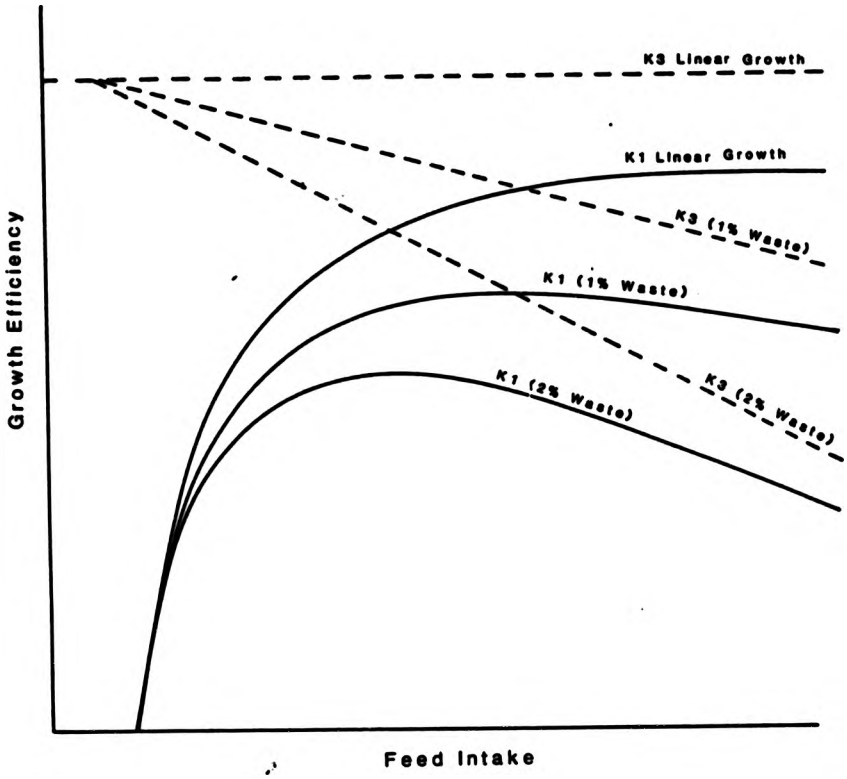

Growth Pattern 1
1% Waste
2% Waste
Sub Pattern 2
Growth Pattern 2
Growth
Feed Intake

Fig 3.6 Expected functional relationship between growth efficiency indicies and feed intake in relation to different digestive strategies.


K3 Linear Growth
K1 Linear Growth
K3 (1% Waste)
K1 (1% Waste)
K3 (2% Waste)
K1 (2% Waste)
Growth Efficiency
Feed Intake

3.5.3 Does temperature affects the growth pattern ?

There is a possibility of a third situation due to differential rates of digestion, absorption and synthesis which may result from undesirable environmental temperatures. It may be clear from the above reasoning that for the normal growth:

Digestion α absorption α synthesis α degradation.

Digestion, however, is an extracellular process and in a strict zoological sense takes place outside the body (if one is connected to the surrounding environment from mouth to anus). Hence, after enzyme secretion, the fish has only a limited control over the digestion process. The major part of the absorption process may also be via passive mechanisms. Synthesis, however, is an intracellular process and this will be metabolically regulated.

Let us assume that a fish is offered a diet which is easily digestible at a temperature other than optimal. Such a fish would digest and absorb ingested energy at a rate higher than it can synthesise structural materials and excess materials may be subjected to inevitable degradation. The net result will be a curvi-linear relationship between growth and ingested energy. A detailed study of growth in relation to environment temperature, however, is out with the scope of the present study.

3.6 Concluding remarks.

There are two ways of making hypotheses and predictions about the real world with an *a priori* approach. One is by making predictions through simple hypotheses. When the prediction is not obvious, probably as a result of unresolved mechanisms, mathematical manipulation has been employed. The model

presented here is a compromise between the two approaches and is in its infancy. Before attempting any complex mathematical treatment it is necessary to look at the experimental evidence for the *priori* expectations from the model presented for feed intake and growth for fish.

At the initial planning stage, the present study was divided into two major parts: studies on the growth efficiency of fish for formulated diets and the efficiency of growth of natural food such as green and blue-green (Cyanobacteria) algae. However, as time became a major constraint during the latter stages of the study, only growth efficiency in relation to formulated food is presented in the thesis.

CHAPTER 4

MATERIALS AND METHODS

4.1 Introduction

The objective of this chapter is to provide a detailed description of the materials and methods commonly used in the present study, so that the following chapters can deal largely with data presentation and discussion. Specific methods related to particular experiments are detailed in the relevant sections of Chapter 5.

4.2 Replication

The most fundamental principle in any experimental design is that the treatments should be replicated. However, the number of replicates (sample size) in an experiment is constrained by two major factors (Bros and Cowell, 1987), namely, the power of statistical analysis (*i.e.* smaller sample sizes would result in less poweerful statistical analysis) and cost (*i.e.* larger samples would generate more powerful statistics but the cost in terms of money, time, equipment, *etc.* may be prohibitive). These constraints are especially true for biological investigations, where variability among subjects is notorious. However, if one posseses *a priori* information on the variability among treatments, optimum sample size can be computed using statistical techniques given by, for instance, Sokol and Rohlf (1981), Zar (1984) and Bros and Cowell (1987).

The cost in terms of total experimental time, number of animals, space and the number of tasks that can be performed daily are the major constraints in the present study. Attempts were made, therefore, to select sample sizes which were commensurate with available time without having a major effect

on any statistical analysis. Most of the studies were conducted with individual animals, but when available time and materials were a critical factor, several fish were pooled and used for biochemical analysis. As the resolving power of the statistical tests concerned with this study was mainly determined by the standard error of the means (SEM, s/ n), this procedure would have resulted in less powerful statistical analysis.

Biochemical analysis based on pooled samples does not provide an exact description of change in body composition of a fish population. However, it was adequate to reveal gross differences in composition among different treatments. As the samples were homogeneously mixed, the expected mean from an analysis based on individual animals should not be highly different from the value obtained from pooled analysis. On the other hand, there is evidence in the literature suggesting that a larger sample does not necessarily yield additional information on proximate composition of fish (Brett *et al*, 1969; Stirling, 1972).

4.3 Experimental animals

4.3.1 Source

The fish *O. niloticus* used in the experiments were obtained from a genetically pure strain (McAndrew and Majumdar,1983) maintained in the tropical fish facility at the Institute of Aquaculture, Stirling. Prior to the experiments, fish were reared on a commercial trout pellet of appropriate size (Ewos Aquaculture International, U.K).

4.3.2 Size

Attempts were made to select fish for each experiment from a single brood in order to avoid genetic differences becoming

a prominent variable amongst treatments. However, if the number of fish from one brood was not sufficient, two broods of the same age were mixed and reared together until the beginning of the experiments. Prior to each experiment, all fish in the holding tank were removed, anaesthetized in a closed system containing benzocaine (Ethyl-4-amino benzoate; Ross and Geddes, 1979) and graded according to size (fish with average weight of 2g were grouped into 0.1g size classes, fish between 10-20g in weight into 1g size classes and fish more than 50g in weight into 2g size classes). Assuming that the fish weight was normally distributed, the mode of the frequency distribution curve was selected as the desired initial weight. The size range used in each experiment was determined by the number of fish required for that particular study. There was a large variance in weight amongst fish of the same age weighing more than 10g. It was necessary, therefore, to use fish with a wider range in initial weights than desired and because of this, differences became further exaggerated during the experiments.

Selected fish were transferred to the experimental systems two weeks before each experiment and acclimatized to the diets and the environmental conditions within the systems.

4.3.3 Density

O. niloticus is known to be a very aggressive species (Mishrigi and Kubo, 1978), and the fish used in all trials were extremely aggressive, irrespective of size. The experiments conducted with groups of fish had to be abandoned after 3-4 weeks due to an insufficient number of fish remaining in some treatments. Therefore, most of the experimental results reported in the present study were from individual fish. However, a compromise solution regarding sample size was formulated, taking into account the available

space, tanks, numbers of fish per treatment and maximum possible work load per day.

Considering the size-related space requirements of fish, it was decided that the stocking density should not exceed 8-10 g per litre of aquarium water. The standard oxygen requirement for a given body weight was computed using the allometric equation relating body weight and oxygen consumption [*i.e.* M O_2/kg/hr= aW^b (W: weight in grams, a & b: constants) Ross and Ross, 1984]. The flow rate was adjusted to provide five times more oxygen to the system (above critical concentration) than the calculated basal metabolic demand. It was found that the adjusted flow rate maintained the dissolved oxygen concentration above 80% saturation. A comparison of the data obtained with that available in the literature indicated no evidence that the stocking densities employed were limiting growth or survival.

4.4 Experimental systems

All experiments were conducted in three warm water recirculating systems in the tropical facility of the Institute of Aquaculture, Stirling, where air temperature was maintained above 20°C in a closed system and photoperiod was automatically regulated providing a 12:12 hours light to dark regime (8.00-20.00 hours light period, U.K. time).

4.4.1 System 1

This recirculating system consisted of forty-eight 20l square plastic tanks, each of which was partitioned into four chambers of equal size using 1 mm nylon mesh attached to plastic tubing (Figs.4.1 a - c). Tanks were covered with black polythene in order to reduce stress on the fish from the

surrounding environment. Individual fish were randomly assigned to each chamber, making a total of 4 fish per tank depending on the number of fish used per treatment. The aquaria were provided with continuous recirculated fresh water, at a velocity of 11 min^{-1} from a header tank of 230l capacity attached to an electric pump (PV 100, capacity 150 l min^{-1}, Breresford, England) placed in a sump tank. The delivery water was well aerated to maintain a dissolved oxygen level above 80% saturation. The aquaria drained into a 230l waste settling tank and water then passed through five 230l biofilter tanks, containing plastic filter medium (Mass Transfer ltd., Hobons Lane, Cumbria) connected in series to a 230 l sump tank. Overflow water from the header tank is returned through an overflow pipe to the sump tank via five filter trays containing filter wool, gravel and cockle shells designed to trap small suspended solid waste particles and to maintain the pH and conductivity at desired levels.

The temperature was maintained at 28.0 ± 0.5°C by a 3 kw thermostatically controlled immersion heater installed in the header tank. Water losses due to evaporation were made up by a continuous fresh water input of approximately 100 ml/min.

4.4.2 System 2

In principle, this system was similar to System 1, though smaller in terms of the size of the rearing chambers, sump and header tanks, filters, pumps *etc.* Six independent rearing units were constructed, of which four contained two rows of six 2l compartments (Fig.4.2 a-b) and two contained four rows of six 4l compartments. Each chamber held a single fish. The water was recirculated using a submersible 'Otter' pump (Beresford, England) providing approximately 750 ml/min to each compartment. The temperature was maintained at 28.0 ± 0.5°C

Fig. 4.1a Diagram of the layout of System 1, Tropical Facility, Institute of Aquaculture, showing arrangement of waste settlement tanks, fish tank tables and drainage system.

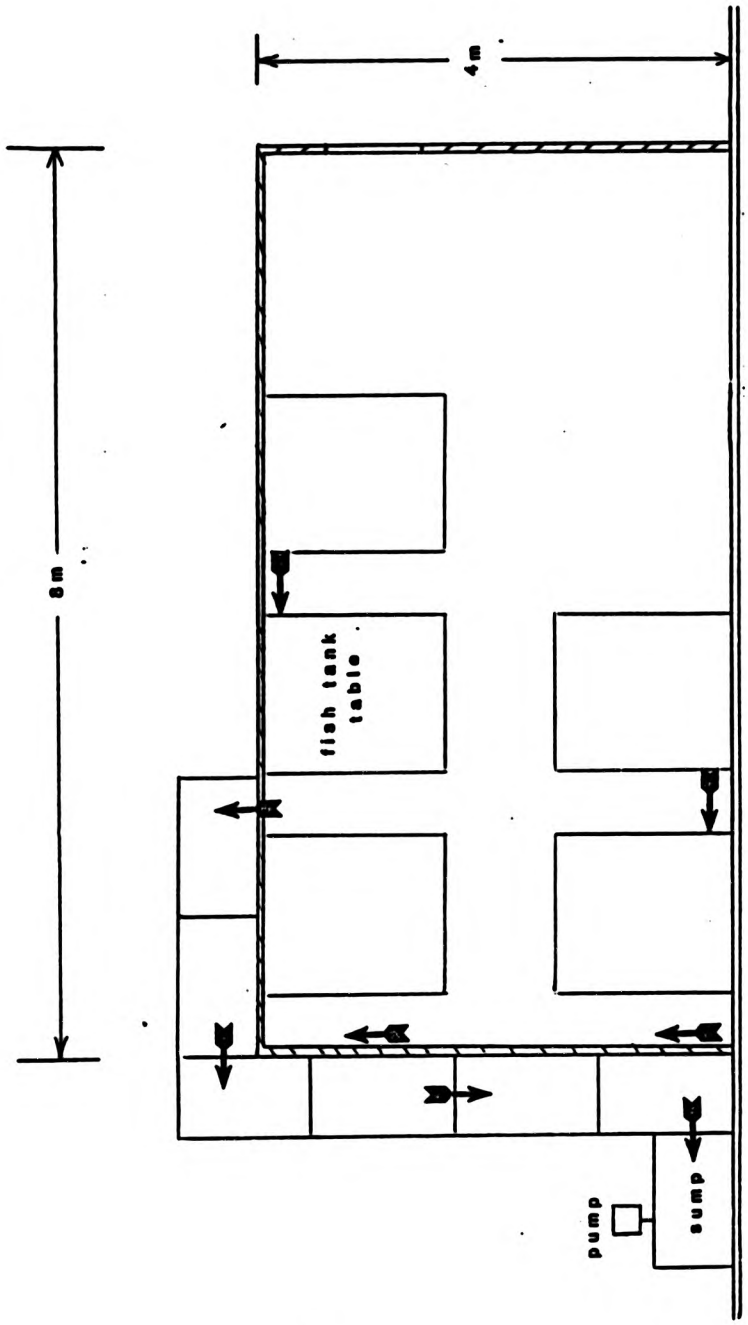

4m
8m
fish tank
table
pump
sump

Fig. 4.1b Diagram of heater tanks, overflow and filter tray system.

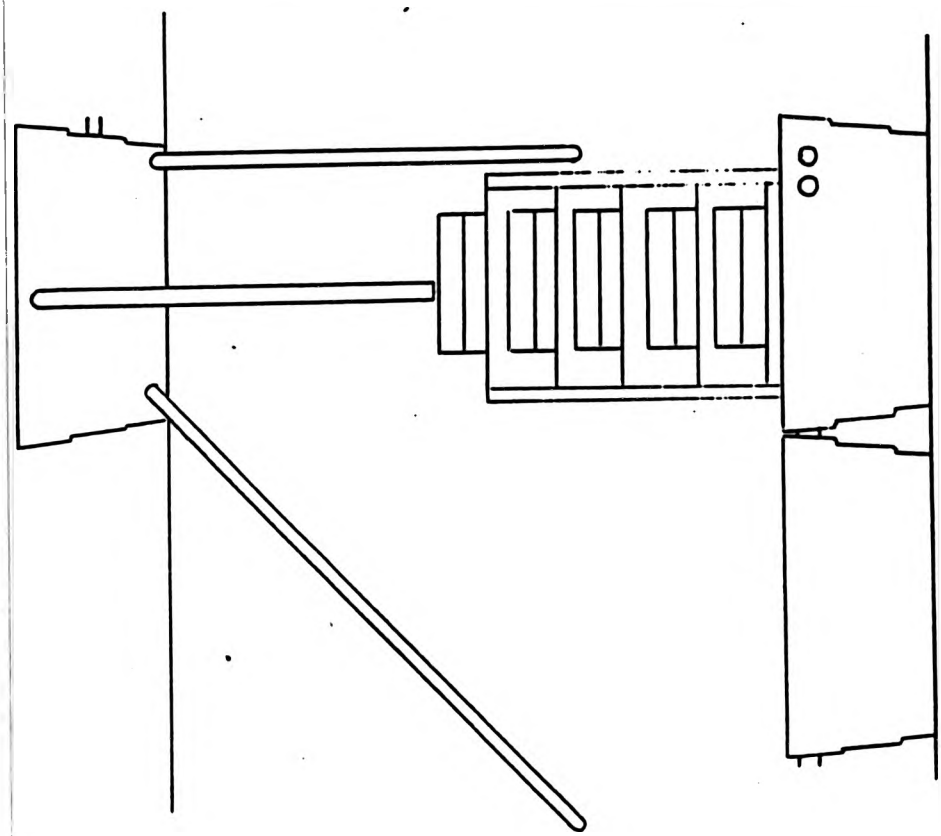
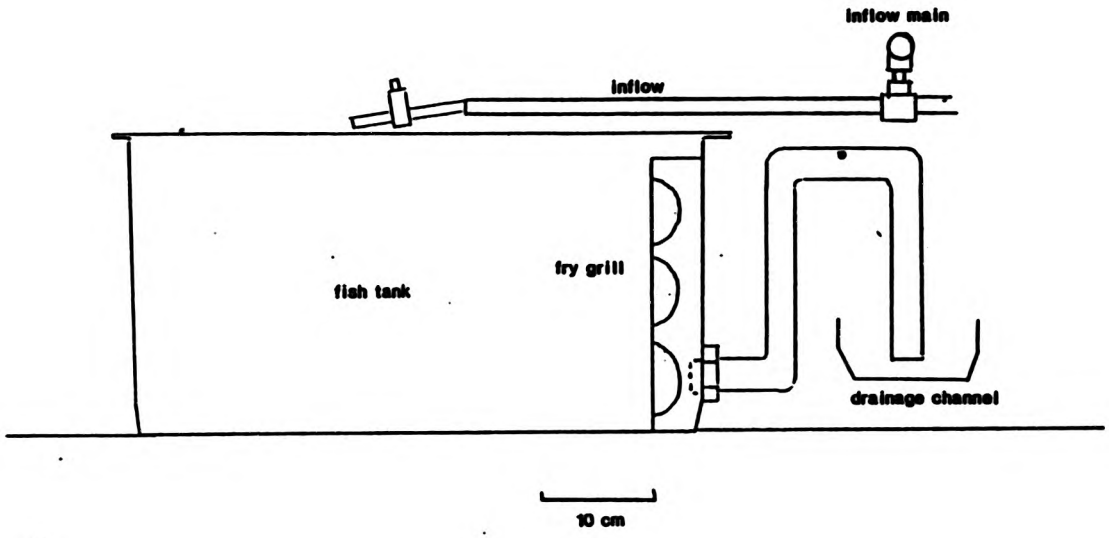


Fig 4.1c Side elevation of perspex tank and waterflow pipes in System 1 (*N.B.* The tank is divided into 4 equal-sized chambers using 1mm mesh netting).


inflow main
inflow
fry grill
fish tank
drainage channel
10 cm

by six 300 Watt thermostatically controlled 'Atlantis' glass heaters (Thomas's Ltd., Halifex, England), submerged in the header tank. Approximately 10% of the total water volume was replaced daily with pre-heated water to avoid build up of soluble wastes.

4.4.3 System 3

System 3 comprised two adjacent warm water rearing systems of thirty two and sixteen circular fish tanks (9 l in volume, self cleaning) respectively. Three serially connected settling tanks (containing plastic filter materials), a sump and header tank (each 125 l in volume) and two filters (containing gravel, cockle shells and filter wool) completed each system (Fig.4.3). The water was recirculated with a pump (PV 22, capacity 40 l/min) providing 1.5 l/min to each rearing tank. Water temperature was maintained at 28.0 ± 0.5 °C by eight 300 watt 'Atlantis' thermostatically controlled submersible heaters. Each rearing tank was aerated separately, in addition to aeration of the header tank, in order to supply adequate dissolved oxygen. Sufficient fresh water was added every day to replace evaporative losses.

4.5 Feed

Two types of diet using purified (2 formulations) and semi-purified (14 formulations) ingredients were prepared in the present study. Fish were acclimatized to diets two weeks prior to each experiment and became accustomed to the new diets within a few days.

Fig 4.2a Diagram of part of System 2, showing (A) 100l header tank, (B) and (C) filters, (D) inlet valve, (E) drain pipes, (F) biological filter tank, (G) submerged pump and (H) crushed shell filter trays. Arrows denote direction of flow.

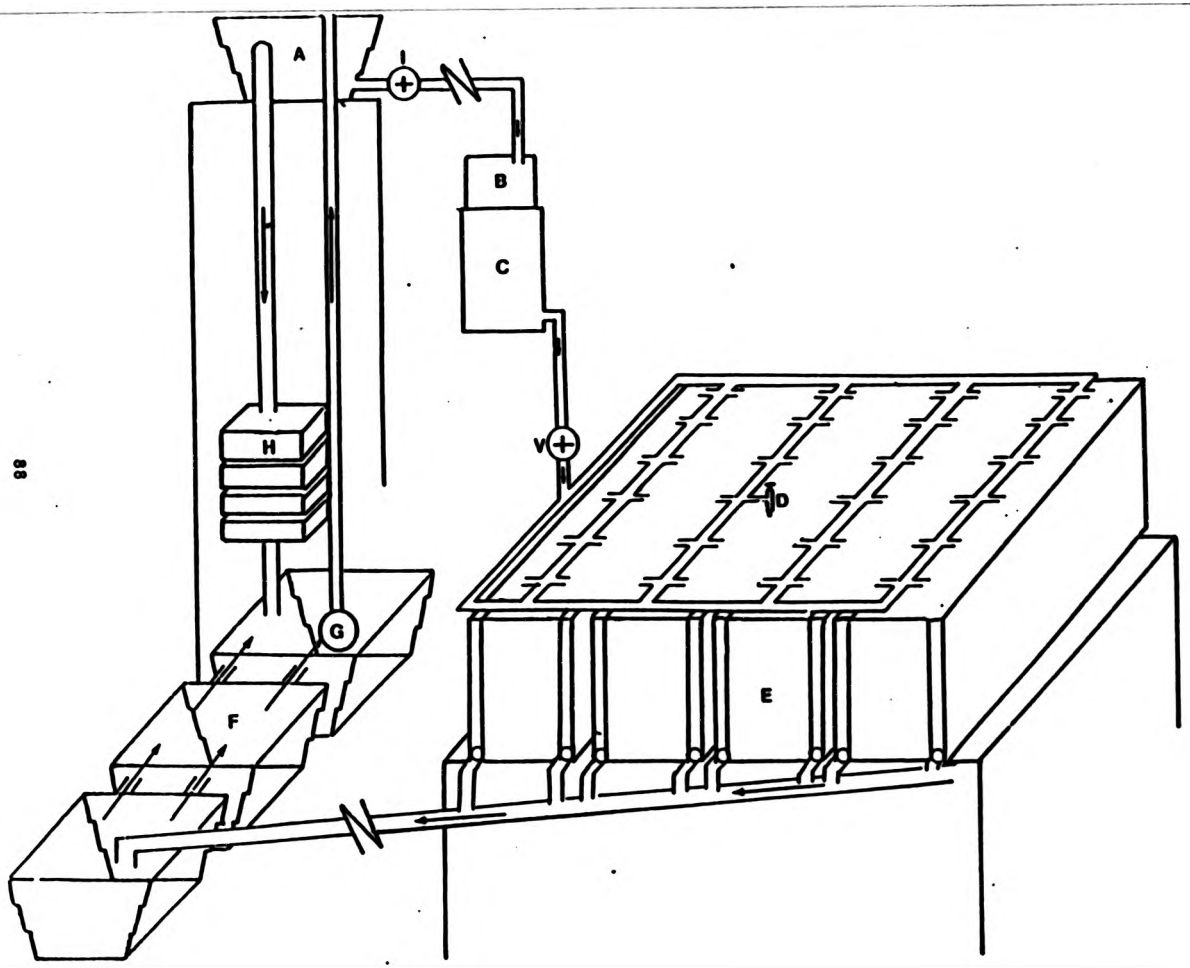

A
I
B
C
H
V
D
G
F
E

Fig 4.2b Individual rows in System 2, showing 12, 21 tanks.

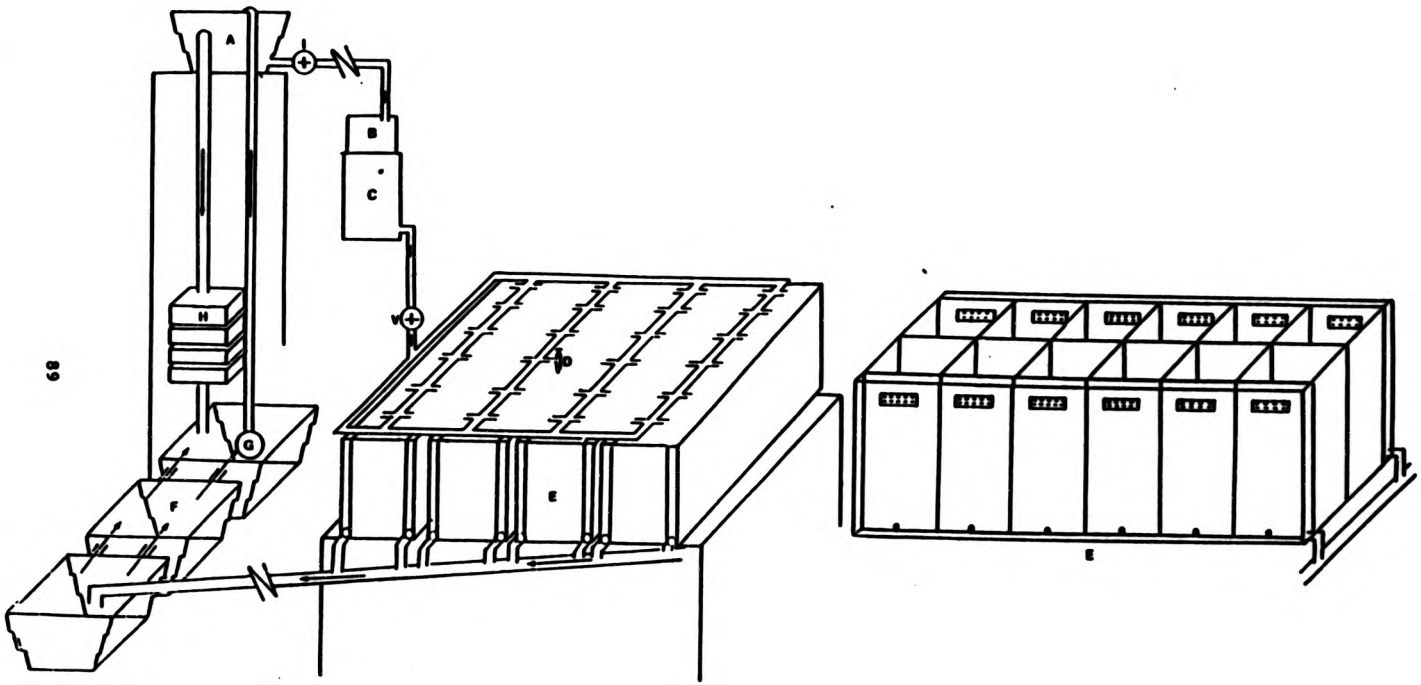

A
B
C
H
G
F
E
E

Fig 4.3 System 3. A recirculating system with 32, 91 tanks.

A Header tank (100 l)

B Overflow

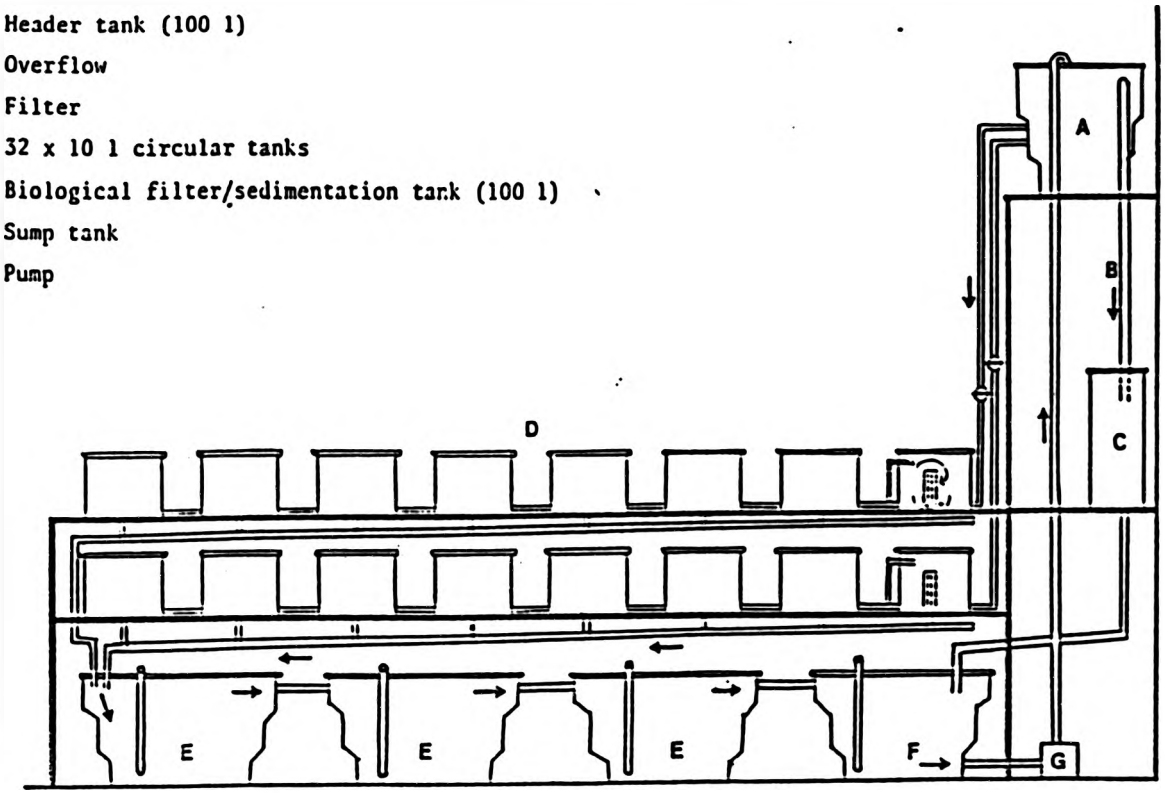
C Filter

D 32 x 10 l circular tanks

E Biological filter/sedimentation tank (100 l)

F Sump tank

G Pump



4.5.1 Feed preparation and storage

All dietary ingredients except herring fish meal were in the purified form (Sigma Co Ltd) and used as supplied. The fish meal was sieved to a particle size of 700 µm. Corn and cod liver oil were dried at 60 °C for 48 hrs prior to formulation of the diets. The solid ingredients were weighed according to the formulation and mixed manually in a closed bucket. The mixture was then transferred to the food mixture bowl (Hobart A400 food mixer) and blended for 30 minutes. Corn and fish oil were added during the blending process. An adequate amount of pre-heated water (70°C; approximately 50% water in purified diets and 30% in semi-purified diets) was added gradually and mixed until a homogeneous texture was obtained. The moist mixture was extruded through the mincer attachment of the Hobart food processor via a 2 mm die plate. The resultant spaghetti-like strand was then dried by a warm air current at 37°C in a drying cabinet. The pellets were subsequently broken into small particles with a manual grinder and sieved to the required particle size. Prior to feeding, a sample of the diet was analyzed for proximate composition and the rest was stored in a deep-freeze at -20°C until required.

4.6 Analytical Techniques

4.6.1 Growth parameters

4.6.1.1 Measurement of growth

Fish were caught with a hand net and anaesthetized in a water bucket containing benzocaine (Ross and Geddes, 1979). They were blotted with tissue paper and weighed to the nearest 0.01g using a top loading balance. (Mettler, PC 4400)

4.6.1.2 Growth indices

Growth in this study was defined either as the rate of change in wet weight or dry weight per unit body weight of fish.

If growth is assumed to be linear over time (i.e. W=kt), relative growth rate can be expressed by the following formula:

$$GR = \frac{(W_f - W_i)}{(T_f - T_i)} \times \frac{1000}{W_i} \quad g/kg/day$$

Where

GR = relative growth rate (assuming linear growth),

W_i = the initial weight in grams at the day T_i,

W_f = the final weight in grams at the day T_f,

Since fish in the present study were fed only 6 days a week, average growth for total period would not indicate the maximum possible growth rate under confined conditions. Therefore, the above index was modified to describe change in weight per unit body weight per day fed, and expressed as follows:

$$GR = \frac{(W_f - W_i)}{[T_f - (T_i + T_{nf})]} \times \frac{1000}{W_i} \quad g/kg/day$$

where, T_{nf} = number of non fed days and others are as above.

If growth is assumed to be exponential over time (i.e. $dW/dt \propto W \Longrightarrow dW/dt=kW \Longrightarrow dW/Wdt=k \Longrightarrow W_f=W_i.e^{kt}$), growth rate can be expressed by the following formula:

$$SGR\ (\%) = \frac{(\ln W_f - \ln W_i)}{(T_f - T_i)} \times 100$$

Where

SGR(%) = specific (relative) growth rate as a percentage (assuming exponential growth),

W_i = the initial weight in grams at the day T_i,

W_f = the final weight in grams at the day T_f,

ln = natural logarithm (base e),

[Note: it is conventional to represent SGR as a percentage. This should not have any effect on statistical analysis, however, as the factor 100 can be considered as a constant.]

As it was found that there was a large variance in growth pattern among treatments, both formulae were used to describe the rate of change in weight in all situations.

4.6 Food utilization parameters

4.7.1 Feeding

In some treatments fish were fed a restricted ration whilst in others they were fed *ad libitum* to determine the maximum possible growth rate. In all instances feeding was restricted to 10 hours per day. In growth experiments, the larger fish (above 15 g in weight) were fed three or four (depending on the experimental design) times per day and the smaller fish (2-14 g in weight) were fed four times per day. When fish were fed restricted rations, the daily allocation was divided into equal portions according to feeding frequency in order to maintain consistency between treatments.

Quantification of *ad libitum* feeding was achieved by weighing a known amount of food the previous day and weighing that remaining in the container after feeding. The difference was taken as the amount fed per day. Attempts were made not to offer extra food to the fish fed an *ad libitum* ration. However, a little excess food was added to chambers after a standard meal (which was based on the amount ingested previous

day) in order to determine satiety. If they failed to consume the food offered, the remaining food was siphoned out, concentrated, and dried at 100°C, and the amount deducted from the offered weight.

Fish were fed 6 days per week and all fish in each treatment were weighed individually (except for the group experiment reported in this thesis) on the seventh day. Data were fed into a prepared computer programme and the amounts of diet fed in restricted rations adjusted accordingly.

4.7.2 Faecal collection

Since most growth trials were conducted in square tanks (Systems 1 and 2), daily cleaning was required. In System 1, faeces concentrated at the edges of each chamber in a tank. Faeces were siphoned out, pooled on a tank basis (faeces from 4 fish), concentrated, dried at 65°C to constant weight and stored in a deep freeze at -20°C. Faeces were collected twice daily (at 8.00 and 19.00 hours) from the second week of the experiment and this continued until the end of the trial. The faeces from half of the small, individually reared fish (2 g in initial weight; in system 2), were pooled for each treatment, as there was insufficient material. No statistical analyses were subsequently carried out.

4.7.3 Digestibility

As total faeces collection was difficult to achieve, digestibility was measured using an inert marker method. Hydrolysis resistant organic matter (Buddington, 1980) and chromic oxide (Furukawa and Tsukahara, 1966) were used as inert markers in purified and semi-purified diets respectively. As 'endogenous' nutrient contribution to the

faeces was not measured, digestibility was given as the apparent digestibility co-efficient according to the following formulae:

(a) Apparent nutrient digestibility co-efficient =

$$100 - \left[100 \times \frac{\text{Nutrient in faeces per unit indicator}}{\text{Nutrient in food per unit indicator}}\right]$$

or by further simplifying:

$$100 - \left[100 \times \left\{\frac{\text{\% indicator in food}}{\text{\% indicator in faeces}} \times \frac{\text{\% Nutrient in faeces}}{\text{\% Nutrient in food}}\right\}\right]$$

(b) Total digestibility co-efficient =

$$100 - \left[100 \times \frac{\text{\% indicator in food}}{\text{\% indicator in faeces}}\right]$$

4.7.4 Indices of food utilisation.

(a) Food Conversion Ratio (FCR)

Food conversion ratio is conventionally defined as the amount of dry food required to produce a unit of live weight of fish.

$$FCR = \frac{\text{Dry food fed (g)}}{\text{Wet weight gain (g)}}$$

(b) Protein Efficiency Ratio (PER)

FCR does not take into account the most expensive and valuable nutrient in the food, protein. However, Protein

Efficiency Ratio (PER) gives a significant insight into protein utilization. The PER is defined as the live weight of fish produced per unit weight of dietary protein .

$$PER = \frac{\text{Wet weight gain (g)}}{\text{Dry protein intake (g)}}$$

(c) Protein conversion efficiency (PCE)

Although PER is a valuable index, it does not consider the variations in carcass composition such as lipid and ash content. Protein conversion efficiency is a measure of the amount of ingested protein retained in the fish body .

$$PCE = \frac{\text{Final body protein - initial body protein}}{\text{Protein intake}} \times 100$$

(d) Growth efficiency (K_1 and K2).

These indices are defined in Chapter 2 and as follows:

$$K_1 = \frac{\text{Energy retained}}{\text{Gross energy ingested}}$$

$$K_2 = \frac{\text{Energy retained}}{\text{Metabolisable energy ingested}}$$

4.7 Biochemical Analysis

4.8.1 Material

In all instances, initial samples of fish were individually weighed and dried. At the end of the experiment, all fish were killed by an over-dose of benzocaine, weighed and dried individually. Fish larger than 15g in were dissected and sexed before drying. Drying was continued to constant weight (approximately 7 days) at 65°C. Dried fish were

individually ground and sealed in labeled, air-tight containers and stored in a deep freeze at -20 'C until proximate analyses could be carried out.

In one experiment, all proximate composition analysis of fish were done on individual animals, but when available time and materials became critical, the following procedure was used for biochemical analysis. For larger fish (above 10 g in weight), males and females were separated, and half of the males were randomly assigned to one pool and the rest to the other. The females were also treated in the same manner. The resultant four groups were separately mixed using a homogeniser. In the experiment conducted on small individual fish, the available materials were the critical factor. Therefore, half of the dried fish from each treatment were pooled and used for subsequent analysis.

4.8.2 Moisture

The moisture content of fish was measured in whole fish dried in an oven in a pre-weighed container at 65'C to constant weight. The fish were immediately ground and a sample placed in an oven at 103'C to determine the difference in drying at a lower temperature. In all instances it was found that there was less than 1 % difference, and so this was not corrected for. The same procedure was followed for drying food for biochemical analysis.

All subsequent analyses were performed on dried samples and duplicate or triplicate samples (further repeated where necessary) from each group of fish, faeces and diets were analyzed as follows.

4.8.3 Crude protein

A microKjeldahl method for determining total nitrogen, modified for use with the Teactor automatic distillation unit (Tecator, Sweden), was used to determine crude protein. Conversion factors of 5.55, 6.38 and 6.25 were used for gelatin, casein and fish meal respectively (Merrill and Watt, 1955).

4.8.4 Crude lipid

Crude lipid was extracted by petroleum ether in a Soxtec solvent extraction system (Tecator, Sweden) with a slight procedural modification. About 0.5 g of faeces, 1 g of food or fish materials were measured to 0.0001 g (Mettler, AC100), wrapped in filter paper and transferred to a clean thimble. The extraction cups were dried in an oven at 103°C for 2 hours, cooled in a desiccator and weighed to 0.00001 g (Oertling, R51). The materials were placed in the extraction unit for 30 minutes in the boiling position followed by an hour in the rinsing position. A blank test was carried out with a filter paper in a similar thimble and data corrected for the difference.

4.8.5 Crude fibre

A fibertec system (Tecator, Sweden) consisting of hot and cold extraction units was used with alkali and acid hydrolysis to determine crude fibre in diets.

4.8.6 Ash

The ash content was measured by burning dry samples in a muffle furnace at 450°C for 12 hours.

4.8.7 Chromic oxide

A wet acidic digestion procedure, as described by Furukawa and Tsukahara (1966), was utilized to determine chromic oxide concentrations in test diets.

4.8.8 Hydrolysis-Resistant Organic Matter, (HROM)

The term hydrolysis-resistant organic matter is used to denote organic matter resistant to acid hydrolysis (mixture of Acetic and Nitric acid) and it was measured by the method of Buddington (1980).

4.9 Determination of combustion energy

The combustion energy value of purified dietary ingredients (namely, casein, dextrin,gelatin, corn oil and cod liver oil) were measured by a Gallenkamp automatic adiabatic calorimeter prior to dietary formulation. The values obtained were;

Casein	24.179 ± 0.051 kj/g (5.78 kcal./g),
Gelatin	22.128 ± 0.066 kj/g (5.29 kcal./g),
Dextrin	17.628 ± 0.030 kj/g (4.21 kcal/g),
Corn oil	39.304 ± 0.019 kj/g (9.394 kcal/g),
Cod liver oil	39.276 ± 0.002 kj/g (9.42 kcal/g).

4.10 Water quality parameters

The following physico-chemical water parameters of the experimental systems were measured fortnightly using standard procedures as given by APHA (1985).

(a) Temperature: by thermometer to an accuracy of 0.1° C.

(b) Dissolved oxygen, (DO): by oxygen meter to 0.01 mg/l (Clandon, YSI Model 57).

(c) pH : with bench pH meter, (WPA, CD 640 digital pH meter).

(d) Total ammonia.

(e) Total nitrite.

All these parameters were found to be within the normal range recommeneded for tilapias (Chervinski, 1982).

4.11 Statistical analysis

All statistical analyses were accomplished using Minitab and SPSS statistical packages. Student t-tests were employed to determine growth differences in male and female fish within treatments and analysis of variance (ANOVA), followed by a multiple range test, was employed to determined the differences among treatments. As Duncan's new multiple range test (Duncan, 1955) is widely used by most fish nutritionists by convention, the same method was utilized in the present study. However, as many statisticians recommend the Tukey multiple range procedure (Zar, 1984), in addition to all Duncan's tests, the Tukey test and Student Newman-Keuls (SNK) test were performed on the data. In all instances Duncan's and SNK procedures showed agreement, although in some cases Tukey test results differed from the former tests. Since there is no agreement among statisticians (Zar, 1984), the results of both Duncan's and Tukey test are presented with some analysis in order to highlight the situation.

CHAPTER 5

RESOURCE ACQUISITION OF *O. niloticus* UNDER DEFINED CONDITIONS.

5.1 Introduction.

Under natural conditions a polyphagous animal will ingest a number of different food items in order to fulfill its nutritional and energy requirements. Under artificial conditions, however, a diet has to be formulated and the growth response assessed. Most studies on nutritional requirments of fishes have been concerned with these aspects, whilst optimality in feed cost is sought through a least cost dietary formulation. For a terrestrial animal, food can be offered *ad libitum* and consumption can be determined with relative ease. In fish, however, quantification of nutritional requirements is not so simple, as the recovery of excess food offered is difficult. Even if quantification could be carried out accurately, it would only represent the short term requirements and these may differ from the long term reqirements. Nutritional energetics studies in fish, therefore, consist of two major parts: one is the study of dietary formulations incorporating measurements of the fish's response to those diets and the other is the quantification of the nutritional requirements. There are a considerable number of studies on the former for tilapias (*eg.* Jauncey, 1982), but information on quantitative requirements is sparse. This chapter focuses on the quantitative nutrient/energy requirements of tilapias and also checks the validity of the conceptual model presented earlier in this thesis.

There are two basic features associated with the present type of study. One is the ratio of building blocks to dietary energy in a formulated diet and the other is the rate of feeding. Of the different types of building blocks, protein

represents the most costly dietary constituent as it is required in larger quantities than the others such as vitamins and minerals. The major focus of this study is, therefore, on the protein requirements of *O.niloticus*. The next section of this chapter investigates the optimum protein to dietary energy ratio in a formulated diet (Investigation 1). This will provide information on the density of protein energy (PE) and non-protein energy (NPE) which should be present in a diet. Once data on the protein-to-energy ratio is available, the protein and energy requirements can be quantified. This has been achieved here by manupulating feeding intensity and results are presented in Investigation 2. With a knowledge of the protein and energy requirements of the fish it is then possible to examine the constraints imposed by food items such as non-digestible bulk present in the food and the effect of the composition of dietary energy on the efficiency of feed utilisation. These aspects are dealt with in Investigation 3.

5.2 Investigation 1: Optimal dietary protein and energy density for tilapias.

5.2.1 Scope.

The dietary protein requirements for fish are dictated by the protein quality and the balance of dietary protein to total energy ratio. Provided that the qualitative requirement is fulfilled by the protein source, the energy balance is considered to be one of the most important aspects in cost-effective feed formulation.

The dietary protein-to-energy ratio has been expressed as mg protein to kcal total energy (PE ratio, *eg.* Garling and Wilson, 1976), protein energy to total energy ratio (PE:TE,

eg. Jobling, 1988)) or as dietary energy (kg/k.cal) to protein (%) ratio (DE/P, *eg.* Wang *et al.*, 1985; Teshima *et al.*, 1985). The PE ratio, mg protein kJ^{-1} was used in this thesis.

Although there are a significant number of studies on protein and energy density in formulated diets for tilapias, there appears to be no agreement among different authors (*eg.* Bowen, 1982; Jauncey, 1982; Wang *et al.*, 1985; Teshima *et al.*, 1985). For example, Wang *et al.* (1985) proposed a range of 140-150 DE/P as optimum for tilapia. Teshima *et al.* (1985), however, disagreed and argued that optimum DE/P is approximately 110 for *O. niloticus*. Since the quantification of nutrient requirements cannot be investigated without resolving the above contrversy, an experiment, involving *O. niloticus*, was designed in order to determine the optimum protein to energy ratio. Unfortunately, however, all attempts at group feeding trials were unsuccessful due to high mortality caused by unexpected levels of aggresion in the fish. Hence, it was decided to re-assess the data available in the literature in order to search for the basis of this disagreement.

It was found that after standardising the way in which data was presented, most studies were in fact in very close agreement. The following section presents re-analysed data of protein to energy ratio for tilapias.

5.2.2 Materials and methods.

Most of the published data on the nutrient requirements of tilapias was collected. Data was rejected from those studies in which author(s) had not provided the proximate composition of the diet or if experiments were carried out in manured ponds (where natural food is available) or if experiments were conducted in order to measure responses to

novel souces of protein. The PE ratio (mg protein to dietary energy) is based on mamalian physiological fuel values (Atwater and Bryant, 1903) and is expressed in terms of gross energy. The data were standardised by converting all proximate composition values to a dry a matter basis and to energy terms using mammalian physiological fuel values (MPFV) of 4, 4, 9 kcal g^{-1} (or 16.74, 16.74, 37.66 kJ g^{-1}) for protein, carbohydrate and protein respectively.

A thorough statistical analysis was impossible from the data gathered from the literature survey, as authors provided only the mean value for fish growth in their publications. However, there are two studies in which the wide range of PE ratios were subjected to regression analysis in order to delineate the functional relationship in general.

5.2.3 Results.

A summary of available data is presented in Table 5.1. The mg protein /TE ratio in column 2 of Table 5.1 relates to the highest growth observed by the experimenter. It can be seen that the PE ratio for the highest observed growth from studies 1 - 7 ranged from 17.82 to 23.32 mg protein kJ^{-1} on MPFV basis (15.83 - 19.70 on gross energy basis). The non-significant region for growth for different studies ranged from approximatly 18 - 30 mg protein kJ^{-1}. In general, most studies indicate 22 mg protein kJ^{-1} (range approximately 18 - 25 mg protein kJ^{-1}) as the optimum PE ratio for *O. niloticus* and this region is graphically shown in Fig. 5.1a. Data from Wang *et al.*(1985) are very scattered. However, when regressed, the data from the rising portion of the curve (up to 22 mgP kJ^{-1}) yields a regression coefficient (R^2) of 0.848. This was compared with the data from Jauncey (1982) for *O. mossambicus* which is shown in Fig 5.1b. The rising portion was subjected

TABLE 5.1 Optimum protein and maximum dietary energy ratios for tilapias.

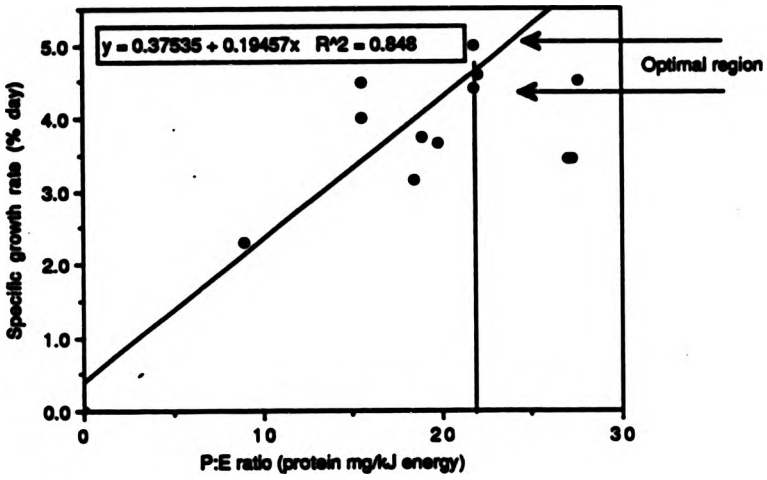
	Species	PE ratio mg protein KJ^{-1}		Initial and final body wt. (g)	S.G.R. (% day^{-1})	Initial and final body lipid (%)*c
		*a	*b			
1	*O. niloticus*	21.72	(18.32)	3.6 - 10.3	5.01 (21 days)	-
2	*O. niloticus*	17.82	(15.83)	6.0 - 19.3	4.11 (21 days)	-
3	*O. niloticus*	22.10	(18.69)	2.41 - 11.04	2.42 (63 days)	20.68 - 22.41
4	*O. niloticus*	18.53	(15.96)	2.4 - 10.07	2.28 (63 days)	20.68 - 22.41
5	*O. niloticus*	22.50	(19.00)	0.56 -	-	-
6	*O. niloticus*	23.32	(19.35)	17.78 - 108.8	2.15 (84 days)	- 11.5
7	*O. niloticus*	21.41	(18.14)	4.43 - 39.31	3.08 (70 days)	9.70 - 19.0
8	*O. mossambicus*	28.19	(23.08)	1.83 - 39.31	3.84 (40 days)	39.60 - 18.04
9	*O. aureus*	31.50	(25.38)	0.73 - 7.1	4.06 (56 days)	- 9.2
10	*T. zillii*	23.92	(19.10)	1.80 - 3.42	3.07 (21 days)	8.51 - 12.17

Notes

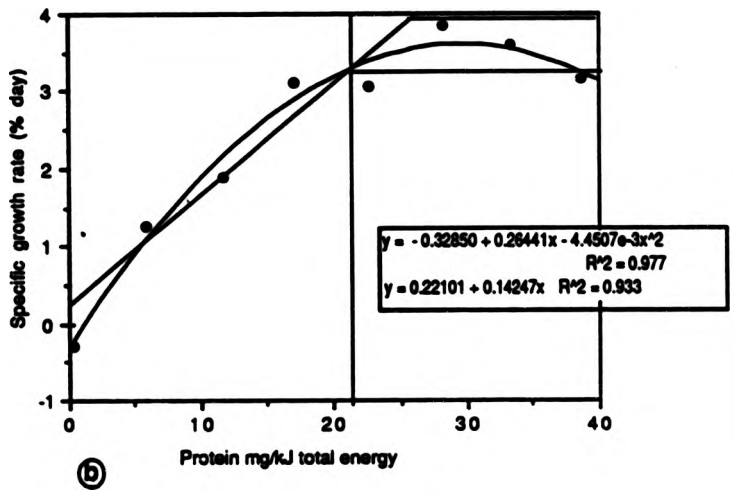
*a Based on mammalian physiological fuel values.
*b Based on gross energy values.
*c Dry weight basis.
1 & 2 Wang *et al* (1985). 33% and 31% protein at 23-24°C.
3 & 4 Anderson *et al* (1984). 36% protein at 26°C.
5 Teshima *et al* (1985). 40% protein at 29°C.
6 Edwards *et al* (1985). 25% protein at 28-36°C - outdoor tanks.
7 Wee and Ng (1986). 35% protein at 27-29°C - outdoor tanks.
8 Jauncey (1982). 42% protein at 27°C.
9 Winfree and Stickney (1981). 35% protein - first feeding fry.
10 Mazid *et al* (1979). 35% protein.

Fig 5.1 (a) Specific growth rate (% day^{-1}) *versus* P:E ratio (mg Kj^{-1} energy) for *O. niloticus* [data from Wang *et al* (1985)].

(b) Specific growth rate (% day^{-1}) *versus* P:E ratio (mg Kg^{-1} total energy) for *O. mossambicus* [data from Jauncey (1982)].


y = 0.37535 + 0.19457x R^2 = 0.848
Optimal region
Specific growth rate (% day)
P:E ratio (protein mg/kJ energy)
0.0
1.0
2.0
3.0
4.0
5.0
0
10
20
30

(a)


y = - 0.32850 + 0.26441x - 4.4507e-3x^2
R^2 = 0.977
y = 0.22101 + 0.14247x R^2 = 0.933
Specific growth rate (% day)
Protein mg/kJ total energy
-1
0
1
2
3
4
0
10
20
30
40

(b)

to linear regression where R^2=0.93 and the second order polynomial regression was found to be an excellent fit (R^2 = 0.977) for the whole range of values. The highest observed growth for *O. mossambicus* by Jauncey (1982) is at a PE ratio of approximately 28 mg kJ^{-1}.

Columns 3, 4 and 5 in Table 5.1 show the initial and final weights of the fish, SGR (% day) and change in body lipid composition during the experimental period respectively. Experimental duration is presented within brackets in Table 5.1, column 4.

5.2.4 Discussion.

As stated above, the PE ratio (mg protein to dietary energy) employed in this thesis is based on mammalian physiological fuel values (Atwater and Bryant, 1903), as the actual metabolisable energy values of different dietary constituents for fishes are still not known. An energy value based on mammalian metabolisable energy will not represent the true metabolisable energy for fish species, as it is known that protein provides more metabolisable energy for fishes than for terrestrial animals. However, the error is absolute rather than relative, and so is relatively unimportant in a comparative study.

The close agreement in values of PE ratio in Table 5.1 for *O. niloticus* suggest that the disagreement between theory and practice is due to pursuing calculations on a wet weight basis and the use of different conversion factors for determining

energy density in the diets (*eg.* Wang *et al.*, 1985: Teshima *et al.*, 1985). Studies 1, 3, and 5-7 in Table 5.1 suggest that the optimum PE ratio for *O. niloticus* is approximately 22 mg protein kJ^{-1} (≈ 19 mg kJ^{-1} gross energy basis). These studies indicate that the optimum PE ratio is independent of feeding rate [*eg. ad libitum* (Wang *et al.* 1985), to 3% BW Anderson *et al.*, 1984)], body size (between 2 and 100g), protein density (from 25 to 40% in the diets used) and temperature (21 - 36 °C). The experiment carried out by Anderson *et al.* (1984) indicates that the PE ratio may be dependent upon carbohydrate source. When dextrin was used as the carbohydrate source maximum growth was obtained at 25% inclusion rate, which is equal to 22.10 mg protein kJ^{-1} and when starch is used 40% carbohydrate was added which yields 18.53 mg protein kJ^{-1}. This may be due to lower digestibility of dietary starch relative to dextrin. Wang *et al.* (1985) also presented a value as low as 17.82 for 6 - 19g fish. No explanation can be given for this observation, although the standard deviation of mean initial and final body weights of their experimental fish suggests that there is no significant difference in growth of fish in the range of 18 - 30 mg protein kJ^{-1} in the experiments.

From the evidence, it seems reasonable to conclude that the optimum PE ratio for *O. niloticus* is approximately 22 mg protein kJ^{-1} for 2-100g fish. It should be emphasised, however, that studies 6 and 7 were carried out in outdoor concrete tanks in the presence of some natural food. Disregarding the small amount of algae present in the tanks, these two studies were selected to demonstrate independence of PE ratio from body weight up to a minimum marketable size (100g) in *O.*

niloticus.

Studies 8 - 10 in Table 5.1 summarise PE ratios for three other species which are widely culture through out the tropics. The PE ratio for *Tilapia zilli* is in close agreement with the value quoted above for *O. niloticus*. The PE ratios for *O. aureus* (Winfree and Stickney (1981) and *O. mossambicus* (Jauncey, 1982) are relatively higher than the value found for nile tilapia. It is apparent that Winfree and Stickney (1981) did not investigate PE ratios below 27 mg protein kJ^{-1} (42% protein). The growth observed is not significantly different from the value cited in Table 5.1, which is 30.50 mg protein kJ^{-1}. This diet contained the lowest protein (34%) and energy density of the experimental diets. Moreover, their data shows that the highest growth obtained from an experimental diet (7.11g at the end of experiment) is four times lower than the control groups of fish fed commercial pellets (30g at the end of the experiment:SGR 5.9% day). This suggests that the experimental diets were either lacking some essential nutrients or that the fish had not consumed their experimental diet. Hence, the results are questionable.

Examination of column 5 of Table 5.1 shows that the body lipid composition increases towards the end of the experiment in all studies cited, except the data for *O. mossambicus* provided by Jauncey (1982). There is approximately 40% lipid (dry weight basis) in the body at the beginning of the experiment, which is probably the highest amount that can be stored in a fish body. As explained in the conceptual model presented earlier, it is expected that such fish will not be able to utilise food of very high non-protein or protein

energy, as they will face an energy storage problem. The optimum strategy under this situation will be to select a diet of intermediary protein and energy density. The intersection of the linear regression and the quadratic regression in Fig. 5.1b shows that the optimum PE ratio for *O.mossambicus* is also probably close to 22 mg protein kJ^{-1}, assuming that this will be the point of deviation from linearity.

One of the most interesting points of the conceptual growth model can be seen from the data provided by both Jauncey (1982) and Winfree and Stickney (1981). Prior to the experiments, the tilapia fry had been fed with commercial trout pellets by Jauncey (1982). Assuming these pellets are similar to Ewos Baker's current pellet formulation (which contains approximately 50% protein, 14% lipid and 16% carbohydrate) used to feed fish fry in the Institute of Aquaculture, Stirling, the PE ratio for this diet would be approximately 31 mg protein kJ^{-1} on MPFV basis, with an energy density of 16.32 kJ g^{-1} (20.2 kJ g^{-1} gross energy). Yet the initial body lipid composition reported by Jauncey (1982) represents the highest percentage of lipid that can be found in the literature for *O. mossambicus*. These results suggest that lipid accumulation in the body is not a sole function of non-protein energy content or undesirable PE ratio. Rather, fish tend to ingest food at levels above S_{MAX} (which is the maximum synthesis capacity of the conceptual growth model) when offered a high energy density food. In addition to non-protein energy (which is only 40% of total gross energy in trout fry pellets), fish convert all excess protein into body lipids, thus reducing its value to humans as a protein source. The

data from Winfree and Stickney (1981) show that when fish are fed *ad libitum* (100 -200 g $kgBW^{-1}$) with a diet of total gross energy ranging from 13.5 - 18.0 kJ g^{-1} (PE ratio 28-31.5 mg protein kJ^{-1}), there is an increase in body lipid levels to 35 - 40% on a dry weight basis. This result contradicts the assumption of rapid digestion made in the conceptual model [termed gastrointestinal overloading by Jobling (1986)] and implies that irrespective of dietary energy density, fish tend to over-ingest food and enhance their body lipid reserves. These results, when compared with the results for *O. niloticus* and *T. zillii* in Table 5.1, show that the lipid accumulation and PE ratio have no direct link, and also indicate that the prediction of over-consumption in the conceptual growth model appears to be correct.

Jauncey and Ross (1982) claimed that utilisation of carbohydrates by tilapias appears, at least superficially, to be similar to channel catfish, *Ictalurus punctatus*. Indeed, this also appears to be true with regard to optimum PE ratio. Garling and Wilson (1976) showed that the optimum PE ratio for channel catfish is 21 mg protein kj^{-1} and found no pathalogical changes in fish liver which is contrary to the general belief that undesirable PE ratios may be responsible for liver abnormalities. Wee and Ng (1986) in a study of nutritional value of cassava root meal for *O. niloticus* (Table 5.1) observed highest growth with fish fed on a diet with 35% protein, 7.35% lipid and 46% nitrogen free extract (NFE), which had a PE ratio of 21.40 mg protein kJ^{-1}. They also did not observe any pathlogical changes in the liver, except non-homogeneous distribution of lipid cells in the liver of fish fed a high

carbohydrate diet relative to the control group of non-fed fish.

The concept of optimum PE ratio is, however, misleading, as the denominator represents the total energy content, including protein energy, in the diet. Garling and Wilson (1976) were cautious about the concept of PE ratio and advocated restricting its use to diets containing adequate total energy. This recommendation is, however, the result of an experimental artifact, as they employed a constant feeding rate (3% body weight), disregarding the protein and energy density of different experimental diets. Notwithstanding the above reason, however, it may be advantageous to express the PE ratio as non-protein energy to protein energy (NPE/PE) because the index in this form will provide information on how many units of non-protein energy should be incoporated for a single unit of protein energy. For example, 22 mg protein kJ^{-1} is a yield of 1.72 NPE/PE, which suggests that every protein energy unit should be supplimented by 1.72 units of non-protein energy (on MPFV basis) in order to obtain the highest growth of *O. niloticus*. This is equivalent to 1.28 of NPE/PE on a gross energy basis. Further elaboration of NPE into carbohydrate energy (CE) and lipid energy (LE) will be considered under Investigation 3.

There is another advantage of indexing protein to non-protein energy as NPE/PE ratio, as it indicates the maximum non-protein energy which can be added to a diet without causing any detrimental effect on growth. It has been hypothesised for many terrestrial animals that gastric motility is controlled by the duodenal osmoreceptors (*eg.* Hunt,

1980) and this may also be true for fish species (Jobling, 1986). These receptors respond to the fraction of digestible energy content in a diet, such as available carbohydrate, protein and lipid (Hunt, 1980). If the above concept is true for fishes, it may be expected that the available nutrient density of food controls the maximum amount of materials that can be processed per unit time. If an unit of NPE or PE generate an equal osmotic pressure, increasing NPE above a limit will adversely affect a fish's ability to obtain maximum building blocks per unit time. Hence, the optimum protein to energy ratio implies the maximum energy to protein ratio, and for tilapias this apears to be approximately 1.72 (1.28 on gross energy basis) with an easily digestible carbohydrate source such as dextrin. Addition of NPE above this limit will cause a reduction in growth due to a fish's inability to obtain enough building blocks for synthesis. Under restricted feeding conditions, if a fish is offered a diet with high NPE, it will accumulate more lipid (*eg.* Wee and Ng, 1986) than a fish offered a diet with insufficient NPE, as fish do not generally expend energy for thermoregulation. On the other hand, if the protein density in a diet is relatively high and if PE produces an equal osmotic pressure to NPE, the protein will be digested rapidly and metabolic pools in the body will become flooded with amino acids. This suggests that there will be nutrients (especially proteinaceous building blocks) in the metabolic pools of the body in excess of S_{MAX}, which is the maximum synthesis capacity per unit time in the conceptual model explained earlier. The extra protein may be converted to lipids. This may be the reason for higher lipid

accumulation under higher protein density, as been indicated by Jauncey (1982) for *O. mossambicus* sustained on trout pellets prior to his experiment. This condition may be avoided by if a fish is offered an extremely high protein diet (which has very little NPE), as the energy for synthesis has to be generated from protein catabolism, but is clearly a wasteful practice.

Summing up the above reasoning, and taking data presented in Table 5.1 into account, it is reasonable to conclude that a PE ratio of 22 to 30 mgP kJ^{-1} will produce maximum growth of fish under restricted feeding conditions. It will be advantageous to present the protein and energy requirements as NPE/PE ratio, as it indicates a fish's capacity to deal with protein and non-protein energy. On an NPE/PE basis, the above range represents 1.72 to 1 on an MPFV basis, or 1.28 to 0.75 on a gross energy basis.

It seems clear now how a diet should be formulated in order to obtain maximum growth for *O. niloticus*, and the following section will investigate their capacity to processed ingested energy.

5.3 Investigation 2:

Protein and energy requirement of *O. niloticus*.

5.3.1 Scope.

There are two ways to determine the energy requirement of a fish. The first is to employ the classical energetic approach and compute the energy requirement, as advocated by Ivlev (1938) and Winberg (1956). The second method is to study the turnover rate of the nutrients in the animal body by relating food consumption to nutrient deposition. Both of these approaches have their own drawbacks with regard to aquatic animals, such as field measurements of metabolic rate with the former, and problems of nutrient leaching from food and faeces with the latter. The second method, however, has a number of advantages over the first, since the first is concerned with total metabolism, or total energy requirement, rather than with some economically important energy fraction, such as protein metabolism or requirement.

The nutrient turnover method has been employed for number of different species of fish by various authors *eg.* Menzel (1960) Bermuda reeffish, *Ephinephalus glutatus*; Pandian (1967) snakehead, *Ophiocephalus striatus*; Brett *et al.* (1969) sockeye salmon, *Oncorhynchus nerka*; Gerking (1971) blugill sunfish, *Lepomis macrochirus*; Gatlin *et al.* (1986) channel catfish, *Ictalurus punctatus*. Although the tilapias are widely cultured, there are only a limited number of studies which have addressed the functional relationship between growth and nutrient intake. Even those studies are contradictory. For example, Birkett (1972) showed a linear relationship between growth and feed intake in *O. mossambicus*, whilst Nawwab (1987) produced a curvilinear relationship between growth and feed intake in *O.niloticus* fed on Ewos Baker's trout pellets.

The present study, therefore, is designed to examine the effects of a wide range of nutrient intakes on growth and growth efficiency and on average body composition of *O. niloticus*. Three experiments are reported under this section:

Experiment 1: Growth of *O. niloticus* with average weight range of 2 - 12g under isolated conditions,

Experiment 2: Growth of *O. niloticus* with average weight range of 2 - 9g under group feeding conditions,

Experiment 3: Growth of *O. niloticus* with average weight range of 18 - 50g under isolated conditions,

The protein and energy requirement for maintanance and for maximum growth were computed from these experiments.

5.3.2 Materials and Methods.

5.3.2.1 Food.

Two purified diets containing 35% protein plus 18.8 kJ g^{-1} gross energy, and 25% protein plus 13.4 kJ g^{-1}, were formulated from purified ingredients (Sigma Co. Ltd). A casein and gelatin 3:1 mixture has been shown to mimic tilapia's amino acid body composition (Teshima *et al.*, 1986), and this was used as the sole protein source in the diets. Dextrin was used as the carbohydrate source, and a 1:1 mixture of cod liver oil and corn oil was used as lipid source. This mixture is expected to fulfill the total saturated and unsaturated fatty acid requirements of tilapias, and has been incorporated at 10% level as recommended by Jauncey and Ross (1982). Vitamin and minerals were supplied at twice the level recommended by Jauncey and Ross (1982) for tilapias in order to avoid limiting factors. Low energy densities were achieved by the additon of cellulose to the diet. Details of dietary formulations are presented in Table 5.2.

Table 5.2 Composition of experimental diets used to determine the protein and energy requirements of *O. niloticus*.

Ingredients	Diet 1 (g/100g)		Diet 2 (g/100g)	
(Dry Weight Basis)				
Casein	27.36		19.53	
Gelatin	8.80		6.30	
Dextrin	37.50		20.40	
Corn oil	5.00		5.00	
Cod liver oil	5.00		5.00	
Vitamin[a]	4.00		4.00	
Mineral mix[b]	4.00		4.00	
α-cellulose	7.34		34.77	
CMC[c]	1.00		1.00	
Proximate composition				
Moisture	7.70		12.72	
(Dry weight basis)	Expected[d]:	Determined[e]	Expected:	Determined
Crude protein	35.00	36.32	25.00	25.10
Crude lipid	10.00	9.85	10.00	9.65
Crude fibre	7.34	6.34	34.77	32.56
Ash	4.25	4.15	4.20	4.55
NFE[f]	-	43.34	-	28.14
Available COH[g]	37.50	-	20.40	-
Energy content(KJ/g)	Calcl.1[h]	Calcl.2[i]	Calcl.1	Calcl.2
1. Gross energy	18.80	20.08	13.43	14.67
USFUL ENERGY				
2. *Digestable energy*	16.63	17.80	11.89	13.07
3. *Phy. fuel value*	15.90	17.04	11.36	12.54
4. *Metabolisable energy*	15.63	16.67	11.25	12.27
Protein/energy ratio (mg protein/KJ)				
1. GE basis	18.62	18.09	18.62	17.11
2. *DE* basis	21.04	20.40	21.03	19.21
3. *PFV* basis	22.00	21.31	22.00	20.01
4. *ME* basis	22.40	21.78	22.23	20.45

a. Vitamin premix: (g/kg of premix) Thiamine(B_1)=2.5, Riboflavin(B_2)=2.5; Pyridoxine (B_6)=2.0; Pantothenic acid= 5.0; Inositol=100.0; Biotin=0.3; Folic acid=0.75; Para aminobenzoic acid=2.5; choline=2.5; Niacin (B_3)=10.0; Cynocobolamin(B_{12})=0.005; Retinol palmitate(A)=100,000 IU; α-tocopherol acetate (E)=20.1; Ascorbic acid (C)=50.0; Menadion (K)=2.0; Cholecalciferol (D_3)= 500,000 IU.

b. Mineral premix: (g/kg of premix) $CaHPO_4.2H_2O$=727.78; $MgSO_4.7H_2O$= 127.50; NaCl=60.00; KCl=50.00; $FeSO_4.2H_2O$=25.00; $ZnSO_4.7H_2O$=5.50; $MnSO_4.4H_2O$=2.54; $CuSO_4.5H_2O$=0.79; $CoSO_4.7H_2O$=0.48; $CaIO_3.6H_2O$=0.30; $CrCl_3.6H_2O$=0.13.

c. Carboxymethylcellulose.

d. Expeted: Expected nutrient composition in the diets.

e. Determined: Results from laboratory analysis.

f. NFE: Nitrogen Free Extract =100-(crude protein+crude lipid+crude fibre+ash). Assumed to be equalent to available carbohydrate.

g. Available COH: available carbohydrate; amount of dextrin added to the diet was assumed tobe equivalent to available carbohydrate in the diets at the dietary formulation stage.

h. Calcl 1: Calculation 1; calculated on the basis of expected nutritional value as in (d).

i. Calcl 2: Calculation 2; calculation was based on the proximate composition of diets as in (e).

1*. Gross energy (GE): energy value s were calculated after determining the heat of combustion of casein, gelatin, corn oil, cod liver oil and dextrin. Analytically determined value were; protein (casein/gelatin mixture)=23.640 kj/g; lipid (corn and cod liver oil mixture)= 39.330 kj/g; carbohydrate (dextrin)= 17.573 kj/g. The same value was used to calculate protein to energy ratio.

2*. Digestible energy (DE): calculated on the basis of protein= 18.828 kj/g; lipid=37.656; carbohydrate=16.736 kj/g as given by Wang *et al*,(1985) for *O. niloticus*.

3*. Physiological fuel value (PFV): based on mammalian physiological fuel values, protein= 16.736 kj/g; lipid= 37.656 kj/g; carbohydrate= 16.736 kj/g (NRC,1981).

4*. Metabolisable energy (ME): energy value of protein (16.736 kj/g) as sited for trout (smith,1971), dextrin (14.602 kj/g) reported for carp (Chiou and Ogino,1975) and of lipid (35.606 kj/g) as used by Jauncey (1982) for *O. mossambicus* were assumed in calculation of ME content of the diets.

Since the digestible or metabolisable energy of all these ingredients was not determined at the initial planing stage of the experiments, dietary energy was balanced using mammalian physiological fuel values. The protein to energy ratio 1.72 NPE/PE (22 mgP kJ^{-1}), which has been demonstrated as optimum for *O. niloticus* in the previous section, was employed. Methods of diet preparation, storage and proximate analysis were as presented in Chapter 4.

5.3.2.2 Experiment 1: Design and analyses.

The objectives of Experiment 1 were to determine the protein and energy requirements and energetic efficiency of *O. niloticus* fingerlings with initial weight of approximately 2g. The experiment was conducted in a warm water recirculating system at 28 °C (details of the recirculating system were given in Section 4.4.2). The fish were stocked in the system two weeks prior to the experiment and held individually in rearing chambers. They were acclimitised for two weeks to Diet 1, which contained 35% protein and 18.8 kJ g^{-1}. At the begining of the experiment, fish were offered Diet 1 at rates of 0, 5, 10, 20, 30, 40, 50, 60, 70, 80 g kg^{-1}BW day^{-1} (g dry food kg^{-1} body weight day^{-1}). There were 10 fish for each treatment and the total experimental duration was 42 days. Except for non-fed fish, all fish survived until the end of the experiment. The fish were fed three times per day for six days per week, and the feeding rate adjusted after weighing individuals on the seventh day. During the second week of the experiment, fish fed 80 g kg^{-1}BW day^{-1} were found to be unable to consume the total ration, and thereafter a considerable fluctuation in feed intake was observed. At the end of the third week, a similar situation was observed with fish fed 70 g kg^{-1}BW day^{-1}.

Attempts were made not to offer food if they failed to consume the fraction offered in the previous meal, and any food remaining was siphoned out, concentrated and dried at 100°C. This was then deducted from the daily the allocation. This procedure, however, could not ensure that the daily allocation had been accurately measured, as the total weight of food used per fish was less than 1g day^{-1} during the entire experiment. The error, therefore, may be considerably larger. Fish fed 60 g kg^{-1} day were also unable to consume the daily ration during the last two days of the experiment. This is ignored, however, as the non-consumed food represents a neglegible fraction of total feed intake. Out of ten starved fishes, only one survived to the end of the experiment.

At the begining of the experiment, a representative sample of fish was sacrificed, dried at 60°C and stored at -20°C until analysis could be carried out. At the end of the experiment, all experimental fish were sacrificed. As the fish were small in size, fish were randomly assigned to three groups and biochemical analysis was carried out as described in Chapter 4.

Faeces were siponed out twice daily (8.00 and 19.00hrs) from the second week until end of the experiment. Faeces were dried at 60°C and feacal waste from five fishes pooled in the subsequent analyses. Hydrolysis-resistant organic matter (HROM) (Buddington, 1980) was used as the digestibility indicator.

Henken *et al.* (1986) showed that the body energy content of African catfish *Clarias gariepinus* can be calculated with reasonable accuracy from proximate analysis using calorific values of 5.65 and 9.45 kcal g^{-1}. The same procedure was employed in the present study for *O. niloticus*.

5.3.2.3 Experiment 2: Design and analyses.

In theory, this experiment was similar to Experiment 1, although the objective was to examine the social or hierarchial effect on the nutrient requirement of *O. niloticus*. The experiment was carried out in a warmwater (28°C) recirculating system, comprising a series of circular fish tanks 9l in volume. Details of the system (System 3) were presented in Section 4.4.3. Before introducing fish into the experimental system, they were acclimitised to the experimental diets in stocking tanks. Fish (initial weight 2g) were introduced to System 3 at a density of 15 fish per tank. Sixteen groups of fish were acclimitised to Diet 1, which contained 35% protein, and another 16 groups to Diet 2, which contained 25% protein. At the begining of the experiment, the stocking density was reduced to 11 fish per tank and the rest were sacrificed in order to determine proximate body composition. Duplicate groups of fish fed Diet 1 were offered food at rates of 5, 10, 20, 30, 40, 50, 60 and 70 g kg^{-1}BW day^{-1}, and groups of fish fed Diet 2 were offered food at rates of 5, 10, 20, 40, 60, 70, 80 and 90 g kg^{-1} day^{-1}. The food ration was adjusted each week, as in Experiment 1.

There were unacceptable levels of aggression, especially among fish fed at higher rates of feeding. Unfortunately, the experiment had to be terminated at the end of third week. All fish were sacrificed at the termination of the experiment, dried at 60°C and stored at -20°C until chemical analysis could be carried out.

It was impossible to quantify the feed intake because of daily mortalities of fish in different treatment groups, and data is presented according to food offered rather than feed intake. No detailed analyses were attempted, as only one fish remained in some treatments at the termination of the experiment.

5.3.2.4 Experiment 3: Design and analyses.

In design, this experiment was similar to Experiment 1, although fish of 18g initial weight were employed. The objective of the experiment was to determine the growth efficiency of fish in the weight range 15 - 75g.

The experiment was conducted in experimental System 1, and comprised forty-eight 20l plastic tanks, each of which had been partitioned into four equal size chambers using 1mm mesh (see Section 4.4.1). A single fish was randomly assigned to each chamber and acclimatised for 2 weeks at 28°C to Diet 1 which contained 35% protein. Fish were fed at rates of 0, 5, 10, 15, 20, 30, 35 g kg^{-1}BW day^{-1} and *ad libitum*, for 6 days per week (for total 42 days). Feed rate was adjusted each week following weighing of individuals. Fish fed *ad libitum* consumed feed at a rate of 50 g kg^{-1}BW during the first week and this reduced to 45 g kg^{-1}BW during the second week. Dramatic fluctuations in feed intake were observed from the end of the third week until the end of the experiment.

There were 16 treatments, and it was expected that the random allocation procedure would result in a representative sex ratio of the parent population. This procedure was addopted due to difficulties encountered sexing fish of 15g in average weight, and the sex was determined at the end of the experiment. The method was found to be satisfactory.

Faeces were collected daily from the end of the first week until the end of the experiment. Faecal waste from four fishes in a single tank were pooled for analysis, yielding four replicate samples per treatment. Hydrolysis-resistant organic matter (HROM) was used as the digestibility marker. Proximate compostion and energy content were determined as described in Experiment 1.

5.3.3 Results.

5.3.3.1 Experiment 1.

Changes in average body weight of young *O. niloticus* (initial weight 2g) fed on Diet 1 containing 35 % protein (with approximately 15.9 kJ g^{-1} energy density) are shown in Fig. 5.2. The growth pattern of the experimental fish changed from linear to exponential within the experimental period, depending on feeding rate. Fish fed lower rations (0 - 30 g kg^{-1}Bw day^{-1}) at first grew in a linear fashion, which gradually changed to an exponential growth pattern. As shown in Fig. 5.2 and Table 5.3, fish fed *ad libitum* feeding regimes had a supressed average growth rate compared with fish fed at rates of 50 - 60 g kg^{-1}BW day^{-1}. As mentioned earlier, the results from the Duncan's test differ from the Tukey test for multiple comparisons. Both are presented in Table 5.3 to describe final weight differences.

The functional relationships between various growth parameters and feed intake are presented in Figs. 5.3 to 5.6. Most of the relationships presented here were subjected to linear regression analysis and the regression equation is given with the relevant figure. Details of statistics are summarised in Table 5.5. Fish fed *ad libitum* were omitted from most regression analyses as they greatly increased the noise associated with the regression equations.

When total weight gain *versus* cumulative feed intake was regressed for fish whose feed intake was measured accurately, there was a linear relationship between wet weight gain and dry feed intake (R^2=0.9, Fig 5.3a). Fig 5.3b summarises the data into different treatments and the standard deviation of feed intake and weight gain is shown by error bars. Fig. 5.3 c shows the relationship between dry weight gain verses total dry food intake.

TABLE 5.3 Feeding rates, initial and final weights (g) of O. niloticus in Experiment 1

FEEDING RATE Feeding rate g kg^{-1} bw d^{-1}	Protein intake g kg^{-1} bw d^{-1}	Initial wt.	Final wt.
5	1.82	$2.06 \pm 0.27^{a\ 1}$	$1.93 \pm 0.16^{a\ 1}$
10	3.63	$2.09 \pm 0.22^{a\ 1}$	$2.38 \pm 0.36^{a\ 1}$
20	7.26	$2.09 \pm 0.24^{a\ 1}$	$4.02 \pm 0.25^{a\ 1}$
30	10.90	$2.09 \pm 0.27^{a\ 1}$	$6.93 \pm 0.56^{b\ 2}$
40	14.53	$2.09 \pm 0.24^{a\ 1}$	8.61 ± 1.12^{bc234}
50	18.16	$2.09 \pm 0.25^{a\ 1}$	10.80 ± 3.02^{cd34}
60	21.79	$2.11 \pm 0.27^{a\ 1}$	12.46 ± 3.21^{d4}
ad libitum 1	22.52	$2.07 \pm 0.27^{a\ 1}$	10.94 ± 2.61^{cd34}
ad libitum 2	18.96	$2.12 \pm 0.20^{a\ 1}$	9.62 ± 1.60^{c34}

Superscripts: letters Duncan's test; Tukey's test ($p < 0.05$)

Fig 5.2 Mean body weight (g) *versus* time (days) for groups of *niloticus* fed different levels of food (Experiment 1).

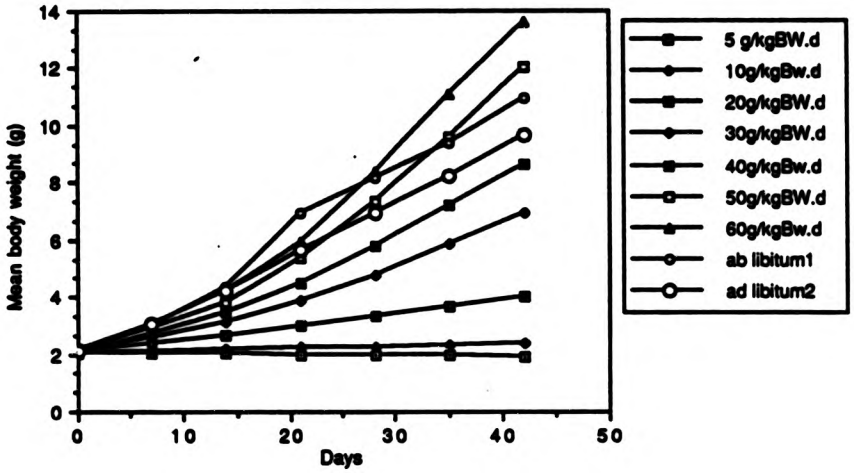
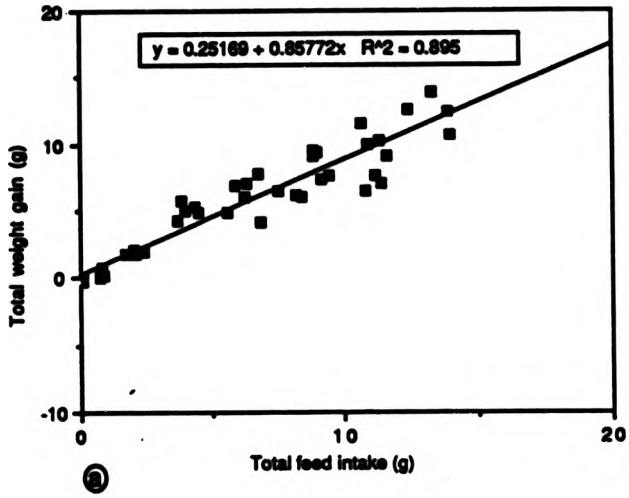

Mean body weight (g)
14
12
10
8
6
4
2
0
0
10
20
30
40
50
Days
5 g/kgBW.d
10g/kgBw.d
20g/kgBW.d
30g/kgBW.d
40g/kgBw.d
50g/kgBW.d
60g/kgBw.d
ab libitum1
ad libitum2

Fig 5.3

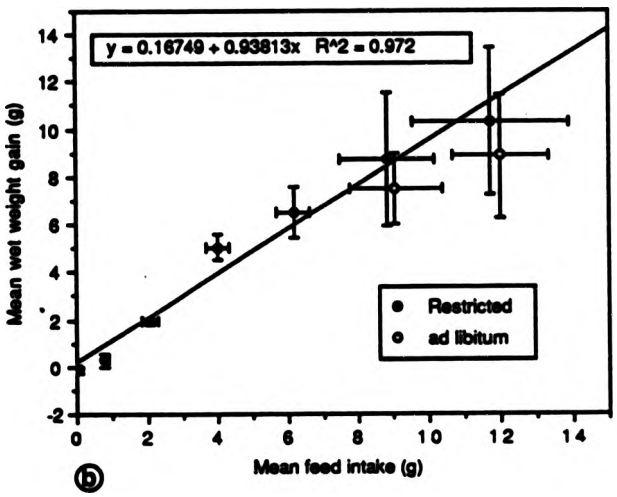
(a) Total wet weight gain (g) *versus* total feed intake (g) over experimental period (Experiment 1).

(b) Mean wet weight gain (g) *versus* mean feed intake (g) over experimental period (Experiment 1). Error bars represent + S.D.

(c) Total dry weight gain (g) *versus* total feed intake (g) over experimental period (Experiment 1).


y = 0.25169 + 0.85772x R^2 = 0.895
Total weight gain (g)
Total feed intake (g)
20
10
0
-10
0
10
20

(a)


y = 0.16749 + 0.93813x R^2 = 0.972
Mean wet weight gain (g)
Mean feed intake (g)
Restricted
ad libitum
14
12
10
8
6
4
2
0
-2
0
2
4
6
8
10
12
14

(b)

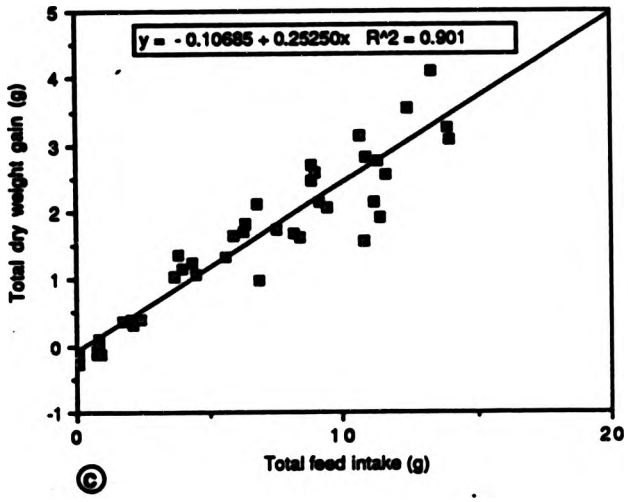
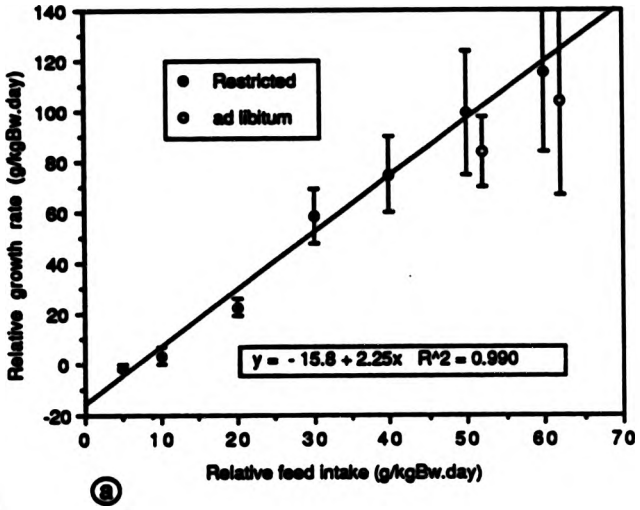

y = - 0.10685 + 0.25250x R^2 = 0.901
Total dry weight gain (g)
Total feed intake (g)
5
4
3
2
1
0
-1
0
10
20

Fig 5.4

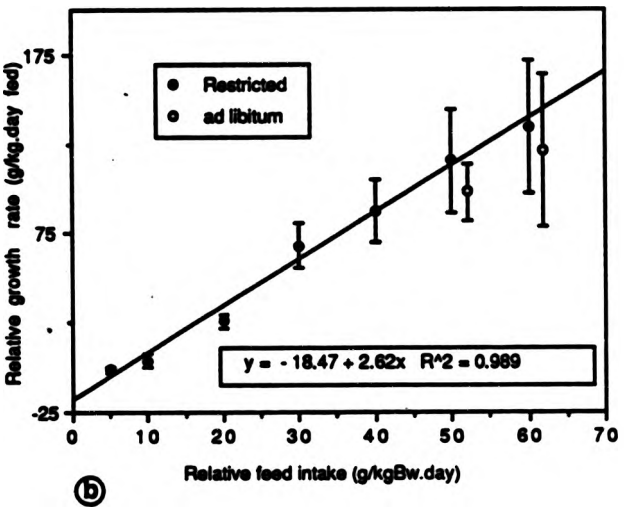
(a) Relative growth rate (g Kg^{-1}BW day^{-1}) *versus* relative feed intake (g Kg^{-1}BW day^{-1}) of groups of fish fed at controlled levels and *ad libitum* (Experiment1).

(b) Relative growth rate (g Kg^{-1}BW day fed^{-1}) *versus* relative feed intake (g Kg^{-1}BW day^{-1}) of groups of fish fed at controlled levels and *ad libitum* (Experiment 1).

(c) Specific growth rate (% day^{-1}) *versus* relative feed intake (g Kg^{-1}BW day^{-1}) of groupsof fish fed at controlled levels and *ad libitum* (Experiment1).
[Error bars represent + S.D. in all figures].


Relative growth rate (g/kgBw.day)
Relative feed intake (g/kgBw.day)
Restricted
ad libitum
y = - 15.8 + 2.25x R^2 = 0.990
140
120
100
80
60
40
20
0
-20
0
10
20
30
40
50
60
70

(a)


Relative growth rate (g/kg.day fed)
Relative feed intake (g/kgBw.day)
Restricted
ad libitum
y = - 18.47 + 2.62x R^2 = 0.989
175
75
-25
0
10
20
30
40
50
60
70

(b)

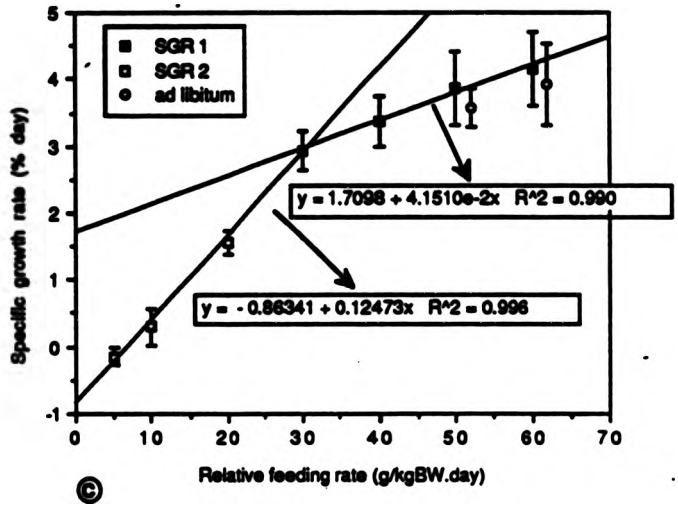
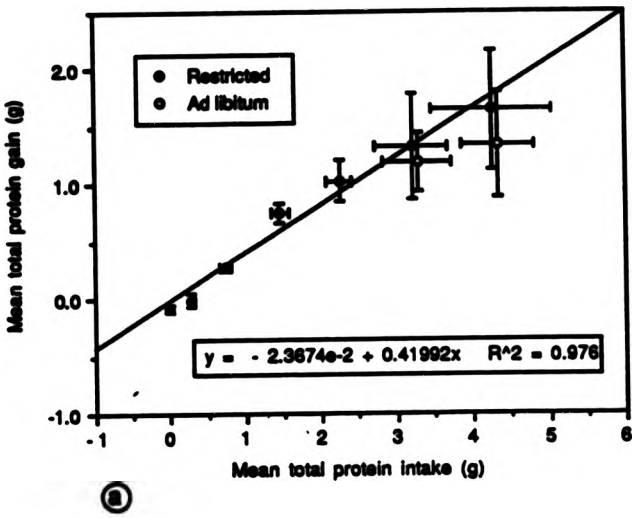

SGR 1
SGR 2
ad libitum
y = 1.7098 + 4.1510e-2x R^2 = 0.990
y = - 0.86341 + 0.12473x R^2 = 0.996
Specific growth rate (% day)
Relative feeding rate (g/kgBW.day)
5
4
3
2
1
0
-1
0
10
20
30
40
50
60
70

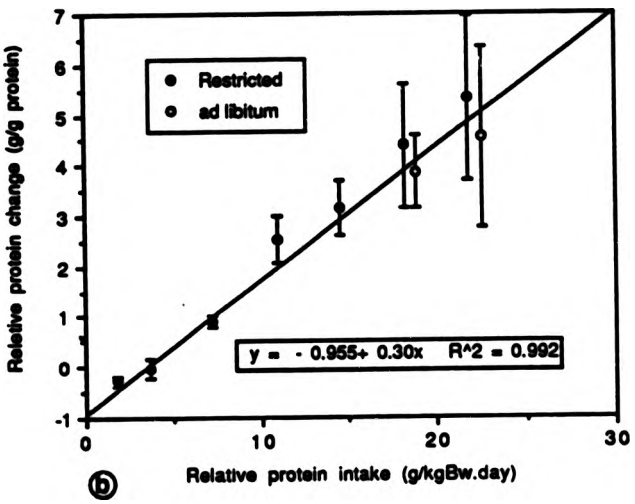
Fig 5.5

(a) Mean protein gain (g) *versus* mean total protein intake (g) (Experiment 1)

(b) Relative protein change ($g\ g^{-1}$ protein) *versus* relative protein intake ($g\ Kg^{-1}\ BW\ day^{-1}$) (Experiment 1).
Error bars represent + S.D.

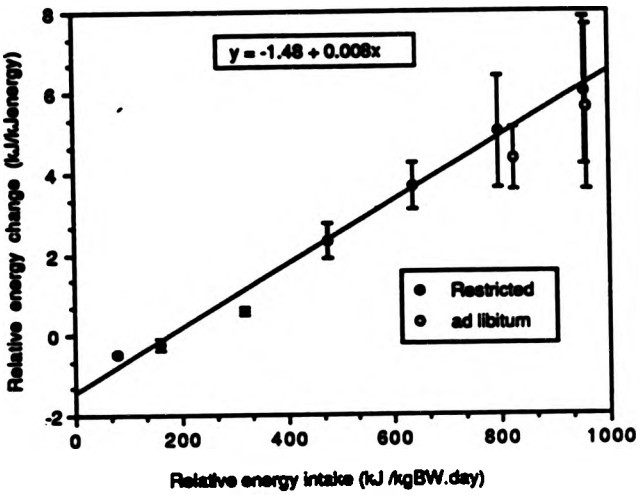

Restricted
Ad libitum
y = - 2.3674e-2 + 0.41992x R^2 = 0.976
Mean total protein gain (g)
Mean total protein intake (g)
2.0
1.0
0.0
-1.0
-1
0
1
2
3
4
5
6

(a)


Restricted
ad libitum
y = - 0.955+ 0.30x R^2 = 0.992
Relative protein change (g/g protein)
Relative protein intake (g/kgBw.day)
7
6
5
4
3
2
1
0
-1
0
10
20
30

(b)

Fig 5.6 Relative energy change (KJ KJ^{-1} energy) *versus* relative energy intake (KJ Kg^{-1} BW day^{-1}) (Experiment 1). Figures based on mammalian fuel values. Error bars represnt + S.D.


y = -1.48 + 0.008x
Restricted
ad libitum
Relative energy change (kJ/kJenergy)
Relative energy intake (kJ /kgBW.day)
8
6
4
2
0
-2
0
200
400
600
800
1000

Relative growth rate *versus* relative feed intake is shown in Figs 5.4a and 5.4b. Figure 5.4a is presented as the relative growth of fish over the experimental period (42 days) and Fig. 5.4b is based upon the calculation of relative growth rate for the number of days on which fish were fed (36 days) in order to determine maxmimum growth capacity. Small *O. niloticus* grew very rapidly and with a mean relative growth rate of 115g kg^{-1}BW day^{-1} (135g kg^{-1}BW day^{-1} on the basis of fed days; SGR 4.15 % day) over the entire experimental period. Maximum growth rate was achieved in this experiment by feeding 60g kg^{-1} day^{-1}, and the corresponding protein intake was 21.8g protein kg^{-1} day^{-1} with an energy intake of approximately 954 kJ kg^{-1} day^{-1}. The functional relationship was linear between feed rates 0 and 60g kg^{-1} day^{-1} (R^2 = 0.88) when regressed with replicates. The coefficient of determination (R^2) increased to 0.97, with minor changes in the regression equation when the regression was performed on the mean relative growth rate. This was, however, not utilised for any statistical analysis.

The regression curve (Y = -15.9+2.25X) of the relationship between relative growth rate and relative feed intake was utilised to compute the maintanance food requirement of fish maintaining body weight. This resulted in 7.06g kg^{-1} day^{-1} (0.7% BW day^{-1}) as the maintenance requirement of 2-12g fish. The inverse prediction method (Zar, 1984) was used to compute 95% confidence limits, resulting in a value of 5.07. Hence, the range of estimated maintenance requirements is 2-12g kg^{-1} day^{-1}. The observed wide range was the result of the high residual error in the analysis of variance. The corresponding mean protein requirement for maintaining body weight is 2.56g protein kg^{-1} day^{-1} (0.25% BW day^{-1}).

This value may not be correct for fish fed every day. From the regression given in Fig. 5.4b, the corresponding

maintenance requirement can be computed. This gives a value of 7.1g kg^{-1} day^{-1}, and the difference is negligible. When growth is expressed as specific growth rate, however, two patterns emerge (Fig. 5.4c). Fish fed lower rations show a significantly faster rate of growth (at feed rates up to 30g kg^{-1} BW day^{-1}), and thereafter the specific growth rate slows. This may related to different growth patterns which are represented in Fig. 5.2.

When the Y axis represents weight, it does not provide any information on the protein requirements for nitrogen balance in the body. The relationship between mean protein gain and mean protein intake is presented in Fig. 5.5a. The functional relationship is presented as the relative change in body protein [(final protein - initial protein)/initial protein] in Fig. 5.5.b. The regression equation (Y= -0.95 +0.30X) gives a value of 3.16 g protein kg^{-1}Bw day^{-1} which is also within the confidence limits computed earlier. Maximum body protein gain was achieved by feeding 21.8 g protein kg^{-1}Bw day^{-1} and there was a six-fold increase in the protein content of the body.

Total energy required to maintain energy balance can be computed from the relationship between energy change [(final energy - initial energy)/initial energy] and relative energy intake (Fig. 5.6). The resultant regression equation is Y= -1.48 +0.008X, which shows that the energy required to maintain constant body weight is 185 kJ kg^{-1}BW day^{-1}. This is also within the confidence limits computed earlier.

The proximate body composition of the experimental fish is shown in Table 5.4, on a dry matter basis. It is also apparent that the moisture content decreases from 82 to 72% with increasing feeding rate. Relative body lipid content increases from 5% to 28% (Fig 5.7) and the ash content changes from 30% to 12%, each on a dry weight basis. There is a small

decrease in relative protein content at higher feeding rates.

Total digestibility and apparent digestibility of protein are presented in Table 5.7. Faeces were collected over five weeks, using pooled samples of five fish. The variation was, therefore, small between feeding rates 20 and 60g $kg^{-1}BW\ day^{-1}$. There is, however, a significant decrease in protein and total digestibility of fish fed *ad libitum*. There is also a decrease in apparent digestibility at lower feeding rates.

Protein conversion efficiency (PCE) and the calculated growth efficiency values are presented in Table 5.8. There is a large variation in protein utilisation of fish fed lower rations (5 to 10g $kg^{-1}BW\ day^{-1}$). The highest observed protein utilisation efficiency is 52%, at a feeding rate of 20g $kg^{-1}BW\ day^{-1}$ (Fig. 5.8a). The PCE thereafter decreases with increasing feeding rate, although the difference is not significant between feed rates 20 and 60g $kg^{-1}BW\ day^{-1}$.

Growth efficiency was calculated on the basis of expected dietary energy (Table 5.8, K_1^a) and the energy content determined from proximate analysis (K_1^b). The difference between the two calculations is 2%; the real value may lie between the two. Gross growth efficiency increases up to a feeding rate of 30g $kg^{-1}Bw\ day^{-1}$, thereafter reaching a plateau (Fig. 5.8b). The calculated growth efficiency based on mammalian fuel values reaches a plateau at 40%. There is a decrease in growth effeciency with fish fed *ad libitum*.

TABLE 5.4 Growth indices of experimental fish in Experiment 1.

Feeding rate g kg bw^{-1} day^{-1}	Mean total weight gain (g)	Relative growth rate g kg^{-1} bw day^{-1}	g^{-1}/kg. bw day^{-1}	SGR (% day^{-1})
5	0.13 ± 0.12^{a}	1.41 ± 1.20^{a}	1.6387 ± 1.40^{a}	0.15 ± 0.15^{a}
10	0.29 ± 0.29^{a}	3.36 ± 3.33^{a}	3.92 ± 3.88^{a}	0.30 ± 0.28^{a}
20	1.93 ± 0.15^{a}	22.66 ± 3.37^{b}	25.97 ± 3.93^{ab}	1.57 ± 0.18^{b}
30	5.05 ± 0.56^{b}	58.34 ± 10.59^{c}	58.07 ± 12.35^{bc}	2.93 ± 0.30^{c}
40	6.52 ± 1.09^{bc}	74.97 ± 15.34^{cd}	87.46 ± 17.89^{cd}	3.36 ± 0.38^{cd}
50	8.73 ± 2.79^{cd}	99.10 ± 24.70^{ef}	115.64 ± 28.8^{cd}	3.86 ± 0.56^{d}
60	10.33 ± 3.08^{d}	115.3 ± 31.36^{f}	134.49 ± 36.9^{d}	4.15 ± 0.55^{d}
ad lib 1	8.87 ± 2.60^{cd}	103.7 ± 36.5^{f}	121.03 ± 42.6^{d}	3.96 ± 0.61^{d}
ad lib 2	7.50 ± 1.45^{c}	84.05 ± 13.85^{de}	98.06 ± 16.2^{cd}	3.58 ± 0.29^{cd}

(superscripts represent results of Duncan's test ($p<0.05$) for multiple comparisons)

TABLE 5.5 Relationship between growth and feed, protein and energy intake (Experiment 1).

*indicates slopes and intercepts which are significantly different from 0 ($P<0.05$)

RELATIONSHIP	REGRESSION EQUATION	95% C.I. for intercept and t-ratio	95% C.I. for slope and t-ratio	R^2	F value	n
1. Total body weight gain Vs total feed intake	Y=-0.04+0.96X	± 0.55 (0.15)	± 0.09 (22.58)*	0.94	509.97	70 (up to 60 d kg bw day)
2. Total dry weight gain Vs total feed intake	Y=-0.107+0.25X	± 0.12 (1.12)	± 0.03 (19.83)*	0.90	393.23	90 (All fish)
3. Total protein gain Vs total protein intake	Y=-0.05+0.43X	± 0.09 (-1.09)	± 0.09 (21.68)*	0.93	489.84	70
4. Relative growth rate Vs relative feed intake	Y= -15.9+2.25X	± 10.49 (-3.07)*	± 0.29 (15.64)*	0.88	244.51	70
5. Relative protein change Vs relative protein intake	Y= -0.95+0.30X	± 0.51 (-3.77)*	± 0.04 (15.32)*	0.873	234.57	70
6. Relative growth per day fed Vs relative feed intake	Y= -18.6+2.62X	± 12.24 (3.07)*	± 0.34 (15.64)*	0.88	244.51	70
7. Relative energy change Vs relative energy intake	Y= -1.48+0.008X	± 0.57 (5.26)*	0.001 (16.33)*	0.89	266.60	70

TABLE 5.6 Proximate body composition in relation to feed rate. Values are given on a dry weight basis.

FEEDING RATE					
Dry feed $g.kg^{-1}$ bw d^{-1}	Protein intake $g\ kg^{-1}$ bw d^{-1}	Moisture %	Protein %	Lipid %	Ash %
5	1.86	82.31 ± 1.54^{d}	62.75 ± 0.73^{a}	4.69 ± 0.45^{a}	29.95 ± 0.47^{e}
10	3.63	80.0 ± 1.72^{c}	61.50 ± 0.20^{b}	8.76 ± 0.81^{b}	26.34 ± 0.17^{e}
20	7.26	77.72 ± 0.81^{b}	64.10 ± 0.38^{c}	13.31 ± 0.16^{c}	20.73 ± 0.18^{d}
30	10.90	76.24 ± 0.53^{b}	62.25 ± 0.35^{d}	19.74 ± 0.44^{d}	16.13 ± 0.30^{c}
40	14.53	73.89 ± 1.17^{a}	59.30 ± 0.15^{f}	25.76 ± 0.56^{e}	14.41 ± 0.30^{c}
50	18.16	73.46 ± 1.19^{a}	59.49 ± 0.14^{de}	27.62 ± 0.56^{f}	12.44 ± 0.28^{ab}
60	21.79	72.89 ± 1.13^{a}	57.70 ± 1.22^{f}	28.43 ± 0.90^{g}	11.99 ± 0.26^{a}
ad libitum 1	22.52	73.08 ± 1.53^{a}	55.44 ± 0.36^{de}	28.49 ± 0.33^{g}	12.60 ± 0.37^{b}
ad libitum 2	18.96	72.77 ± 1.15^{a}	57.11 ± 0.45^{e}	26.78 ± 0.33^{h}	12.68 ± 0.20^{b}
Initial		75.03 ± 0.88	57.95 ± 0.28	21.65 ± 0.27	19.38 ± 0.31

(superscripts represent the result from Duncan's test (p 0.05) for multiple comparisons)

TABLE 5.7 The apparent digestibility of protein and total dry matter of experimental fish (Experiment 1).

Feed Rate ($g\ kg^{-1} bw\ day^{-1}$)	Apparent protein digestibility	Total digestibility
5	-	-
10	90.40*	72.68*
20	97.24*	88.10*
30	97.86 ± 0.20	89.87 ± 0.34
40	98.01 ± 0.11	89 65 ± 0.55
50	97.34 ± 0.07	89.59 ± 0.50
60	97.42 ± 0.03	90.23 ± 0.07
ad lib (1)	93.17 ± 0.04	87.64 ± 0.24
ad lib (2)	91.32 ± 0.22	87.08 ± 0.65

* pooled samples

TABLE 5.8 Protein conversion efficiency and growth efficiencies of fish in Experiment 1.

Protein intake $g\ kg^{-1}\ bw\ d^{-1}$	Energy intake $kj^{-1}\ kg^{-1}\ bw\ d^{-1}$ ME	GE	PCE 1(%)	$K_1(\%)^a$	$K_1(\%)^b$	$K^2(\%)^a$	$K_2(\%)^b$
1.86	79	94	-374.5 ± 128.7[a]	-503.4± 72.3[a]	-471.3± 67.6	-595.2± 85.4	-555.4± 79.7
3.63	159	188	- 2.95 ± 20.57[b]	-20.29± 12.0[b]	-19.00± 11.2	-23.99± 14.2	-22.39± 13.2
7.26	318	376	36.69 ± 4.46[d]	17.41± 2.85[c]	16.30± 2.67	20.58± 3.37	19.21± 3.15
10.90	477	564	51.86 ± 7.02[d]	35.33± 4.95[d]	33.08± 4.64	41.77± 5.86	33.98± 5.47
14.53	636	752	45.73 ± 4.51[d]	36.66± 3.35[d]	34.32± 3.30	43.35± 4.17	40.45± 3.89
18.16	795	940	45.10 ± 1.71[d]	38.22± 1.44[d]	32.26± 7.97	40.74±10.1	38.02± 9.39
21.79	954	1128	38.08 ± 6.70[d]	32.38± 5.49[d]	30.31± 5.14	38.28±6.49	35.72± 6.06
22.52	994	1166	30.60 ± 8.30[d]	26.71± 6.97[d]	25.01± 6.52	31.58±8.24	29.47± 7.69
18.96	830	982	36.07 ± 3.14[d]	30.06± 2.50[d]	28.14± 2.34	35.54±2.96	33.16± 2.76

a - calculation based on expected energy values in Table 2
b - calculation based on observed energy values based on NFE for carbohydrate
k_1 - gross growth efficiency
k_2 - 2nd order growth efficiency calculated taking mammalian physiological fuel values into account
pce - protein conversion efficiency [(Initial carcass protein-final protein)/protein intake)^{x100}]
± standard deviation

superscripts in the figures represent the result from Duncan's test (p 0.05) for multiple comparisons

Fig 5.7 Body lipid content (% dry matter) *versus* relative feed intake (g kg^{-1}BW day^{-1}) (Experiment 1).

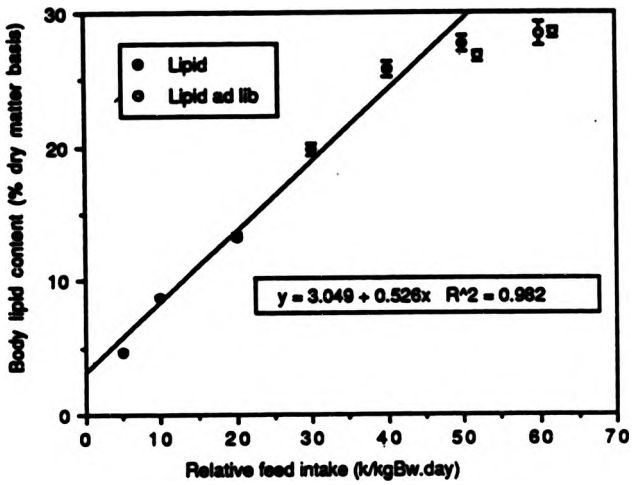
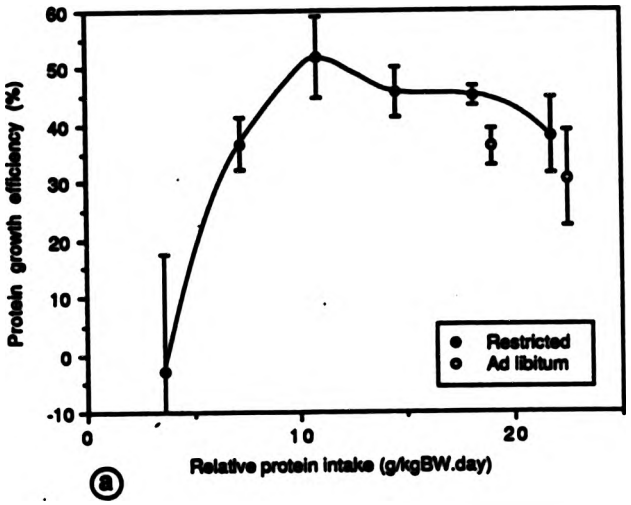

Lipid
Lipid ad lib
y = 3.049 + 0.526x R^2 = 0.982
Body lipid content (% dry matter basis)
Relative feed intake (k/kgBw.day)
0
10
20
30
40
50
60
70

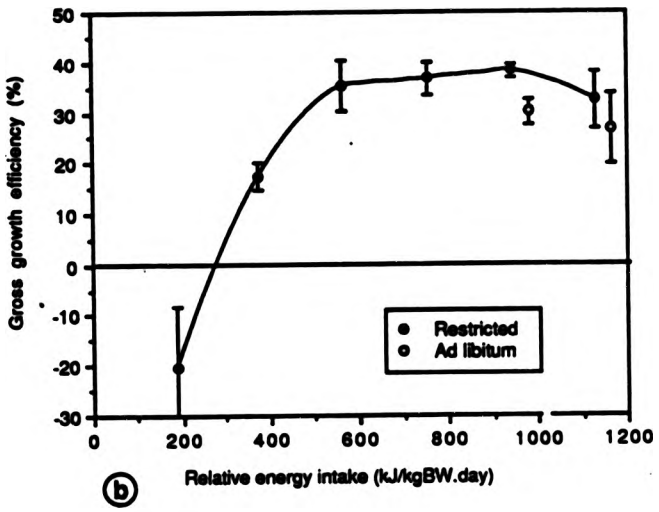
Fig 5.8

(a) Protein conversion efficiency (protein gain/protein intake x 100) (%) *versus* relative protein intake (g kg^{-1}BW day^{-1}) (Experiment 1).

(b) Gross growth efficiency (K_1) (%) *versus* relative energy intake (kJ kg^{-1}BW day^{-1}) (Experiment 1).
Error bars represent S.D.


Protein growth efficiency (%)
60
50
40
30
20
10
0
-10
0
10
20
Relative protein intake (g/kgBW.day)
Restricted
Ad libitum

ⓐ


Gross growth efficiency (%)
50
40
30
20
10
0
-10
-20
-30
0
200
400
600
800
1000
1200
Relative energy intake (kJ/kgBW.day)
Restricted
Ad libitum

ⓑ

5.3.3.2 Experiment 2.

Experiment 2 involved feeding 32 groups of fish Diets 1 and 2 (initial weight 2g). The experiment, however, had to be terminated in the third week due to high mortality resulting from aggression. The survival rates of fish fed 35% protein and 25% protein diets are shown in Fig. 5.9 a and b respectively. Mortality rate increased with feeding rate in both trials. The survival pattern was less marked among fish fed the 25% protein diet.

As a result of the low survival rate, no attempt was made to analyse the data in detail. However, relative and specific growth rates of fish, based upon mean weight of surviving fish, are presented in Fig. 5.10 (35% protein group; details are in Table 5.9) and Fig. 5.11 (25% protein group; details are in Table 5.11). The surviving fish indicate that the maximum growth rate is achieved when the fish are fed at 60 g kg^{-1}BW day^{-1} with the 35% protein diet and 80 g kg^{-1}BW day^{-1} with the 25% protein diet. The corresponding protein intake is 21.7 g protein kg^{-1} day^{-1} for fish fed the 35% protein diet and 20.1 g protein kg^{-1} day^{-1} for fish fed the 25% protein diet.

The proximate body composition of surviving fish is presented in Tables 5.10 and 5.12. The relative change in body composition during the experimental period is similar to that found in Experiment 1.

TABLE 5.9 Mean initial and final body weights, and relative and specific growth rates of fish in Experiment 2 (35% protein diet)

Feed rate (g kg^{-1} BW day^{-1})	Initial weight (g)	Final weight (g)	Relative growth rate (g kg^{-1} BW day^{-1})	S.G.R. (% day^{-1})
0	2.22±0.07^{a}	2.00±0.03^{a}	-4.72±0.91	-0.50±0.10
5	2.33±0.04^{ab}	2.53±0.15^{a}	4.14±3.21	0.39±0.30
10	2.40±0.01^{b}	2.82±0.34^{a}	8.15±9.07	0.72±0.78
20	2.38±0.03^{b}	3.79±0.33^{ab}	28.31±10.6	2.20±0.67
30	2.42±0.07^{b}	5.11±0.70^{bc}	53.30±23.3	3.51±1.11
40	2.42±0.04^{b}	5.62±1.07^{bc}	62.40±27.2	3.92±1.19
50	2.41±0.04^{b}	6.65±0.49^{c}	83.71±10.8	4.82±0.39
60	2.34±0.06^{ab}	6.80±0.92^{c}	90.20±31.0	5.00±1.08
70	2.36±0.03^{ab}	6.88±0.86^{c}	91.00±22.0	5.06±0.76

TABLE 5.10 Proximate body composition (on a dry weight basis) of fish in Experiment 2 (35% protein diet)

Feeding rate g kg^{-1} bw day^{-1}	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
0	55.04 ± 2.17	18.00 ± 0.17	19.80 ± 0.25	78.66 ± 0.12
5	56.27 ± 0.09	18.64 ± 0.76	18.60 ± 3.96	76.98 ± 0.57
10	55.29 ± 0.23	21.67 ± 0.37	18.33 ± 1.24	75.19 ± 0.37
20	55.33 ± 0.64	23.71 ± 0.56	15.78 ± 0.56	74.62 ± 0.28
30	54.54 ± 2.24	26.12 ± 3.26	13.65 ± 0.18	73.94 ± 0.62
40	52.39 ± 0.66	29.83 ± 0.596	12.71 ± 0.02	72.28 ± 0.06
50	51.61 ± 1.89	27.21 ± 2.03	12.99 ± 0.696	72.64 ± 0.26
60	54.91 ± 0.97	28.48 ± 1.91	12.87 ± 0.65	72.55 ± 0.75
70	53.97 ± 1.84	28.38 ± 2.52	12.84 ± 0.32	72.31 ± 0.61
Initial	50.76 ± 0.42	33.17 ± 0.17	13.45 ± 0.25	71.30 ± 0.80

TABLE 5.11 Mean initial and final body weights, and relative and specific growth rates of fish in Experiment 2 (25% protein diet).

Feed rate (g kg^{-1} BW day^{-1})	Initial weight (g)	Final weight(g)	Relative growth rate (g kg^{-1} BW day^{-1})	S.G.R. (% day^{-1})
5	2.80±0.04[a]	2.70±0.001[a]	-1.81±0.37	-0.18±0.04
10	2.75±0.02[a]	2.91±0.00[a]	2.67±0.45	0.26±0.04
20	2.75±0.04[a]	3.92±0.16[b]	20.20±2.59	1.68±0.18
40	2.76±0.01[a]	4.56±0.16[bc]	31.25±4.21	2.40±0.26
60	2.76±0.05[a]	5.81±0.04[c]	52.85±1.70	2.55±0.26
70	2.76±0.07[a]	8.04±0.39[c]	90.99±4.83	5.09±0.17
80	2.79±0.03[a]	8.23±0.35[c]	92.59±10.47	5.14±0.36
90	2.76±0.03[a]	7.31+1.14[c]	78.40+26.10	4.58±0.99

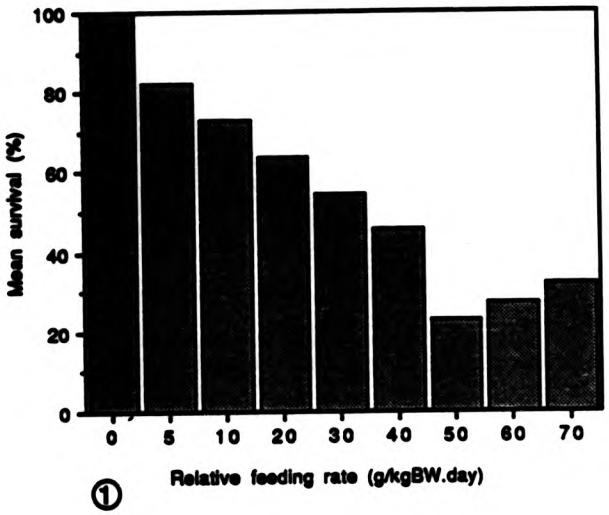
TABLE 5.12 Proximate body composition (on a dry weight basis)of fish in Experiment 2 (25% protein diet)

Feeding rate g kg^{-1} bw day^{-1}	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
5	56.57 ± 1.81	18.02 ± 2.05	19.34 ± 0.72	76.62 ± 0.94
10	57.23 ± 0.47	18.86 ± 0.68	18.21 _ 0.60	76.21 ± 0.26
20	57.34 ± 0.02	20.41 ± 0.75	16.64 ± 0.24	75.195 ± 0.44
40	53.18 ± 0.03	26.66 ± 1.04	14.44 ± 0.45	72.85 ± 0.17
60	51.46 ± 0.69	30.01 ± 0.28	13.55 ± 0.06	71.92 ± 0.01
70	53.32 ± 0.77	28.34 ± 1.30	14.54 ± 1.07	71.77 ± 0.51
80	51.62 ± 0.86	28.25 ± 0.82	13.25 ± 4.5 $^{-03}$	71.15 ± 0.72
90	54.11 ± 0.68	29.21 ± 1.20	13.53 ± 0.25	72.06 ± 0.48

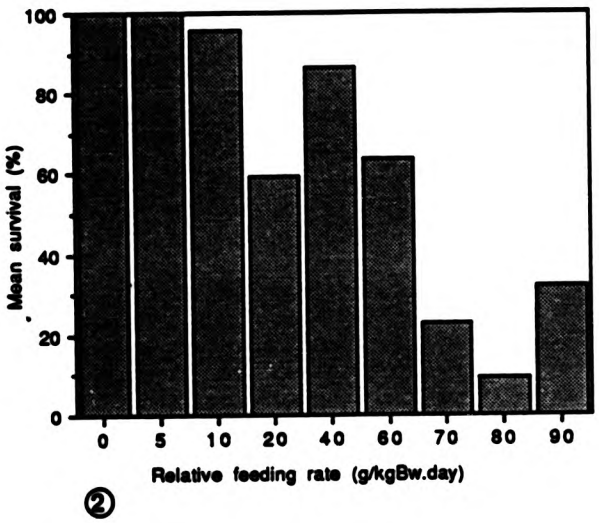
Fig 5.9

(a) Mean survival (%) *versus* relative feed rate (g kg^{-1} BW day^{-1}) (Experiment 2: 35% protein fed group).

(b) Mean survival (%) *versus* relative feed rate (g kg^{-1} BW day^{-1}) (Experiment 2: 25% protein fed group).


100
80
60
40
20
0
Mean survival (%)
0
5
10
20
30
40
50
60
70
Relative feeding rate (g/kgBW.day)

①

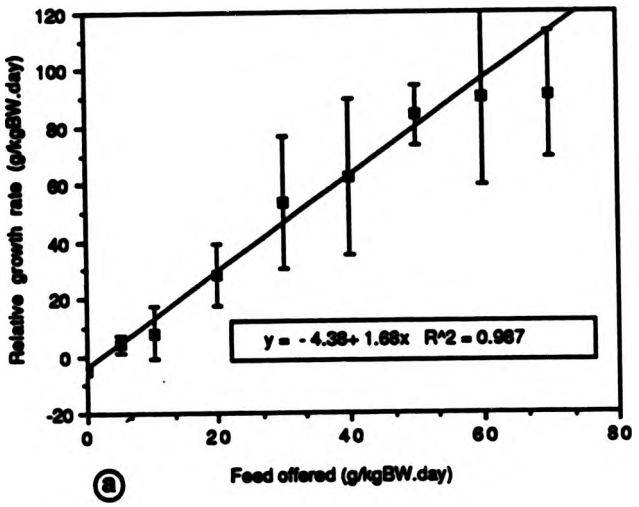

100
80
60
40
20
0
Mean survival (%)
0
5
10
20
40
60
70
80
90
Relative feeding rate (g/kgBw.day)

②

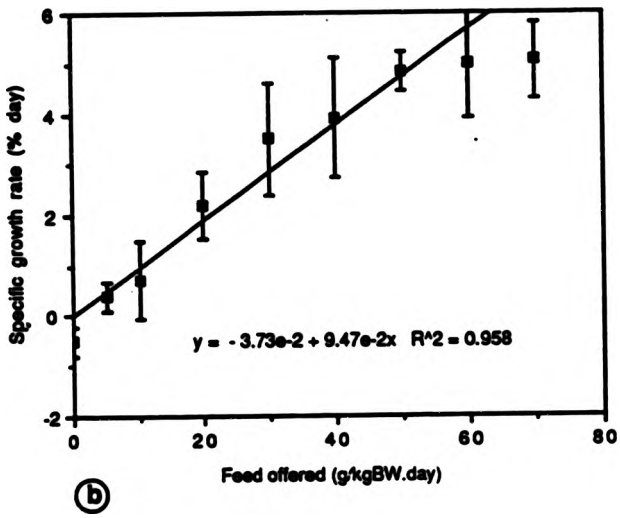
Fig 5.10

(a) Mean relative growth rate (g kg^{-1}BW day^{-1}) *versus* feed offered (g kg^{-1}BW day^{-1}) (Experiment 2: 35% protein diet).

(b) Specific growth rate (%) *versus* feed offered (g kg^{-1}BW day^{-1}) (Experiment 2: 25% protein diet).
[Error bars represent S.D.]


Relative growth rate (g/kgBW.day)
120
100
80
60
40
20
0
-20
0
20
40
60
80
y = - 4.38+ 1.68x R^2 = 0.987
Feed offered (g/kgBW.day)

(a)

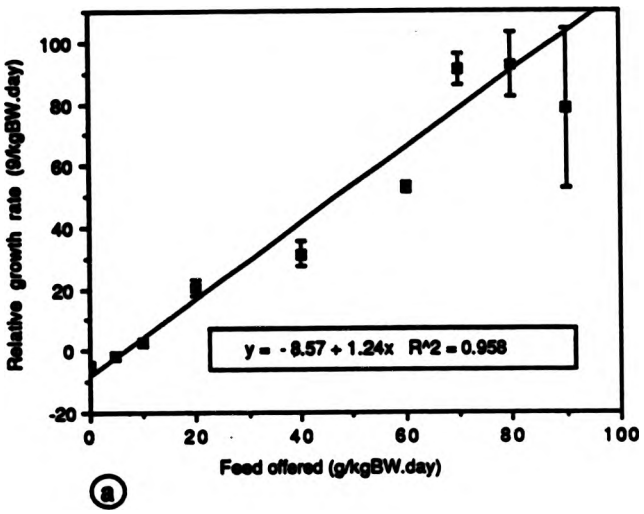

Specific growth rate (% day)
6
4
2
0
-2
0
20
40
60
80
y = - 3.73e-2 + 9.47e-2x R^2 = 0.958
Feed offered (g/kgBW.day)

(b)

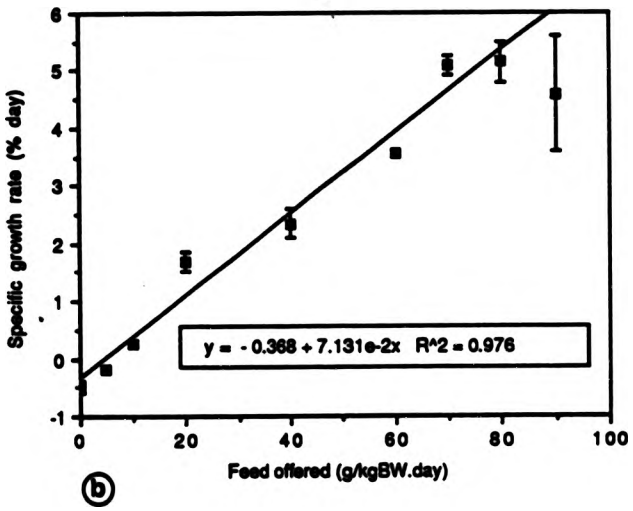
Fig 5.11

(a) Relative growth rate (g kg^{-1}BW day^{-1}) *versus* feed offered (g kg^{-1}BW day^{-1}) (Experiment 2: 25% protein diet).

(b) Specific growth rate (% day^{-1}) *versus* feed offered (g kg^{-1}BW day^{-1}) (Experiment 2: 25% protein diet). Error bars represent S.D.


Relative growth rate (g/kgBW.day)
100
80
60
40
20
0
-20
0
20
40
60
80
100
Feed offered (g/kgBW.day)
y = - 8.57 + 1.24x R^2 = 0.958

(a)


Specific growth rate (% day)
6
5
4
3
2
1
0
-1
0
20
40
60
80
100
Feed offered (g/kgBW.day)
y = - 0.368 + 7.131e-2x R^2 = 0.976

(b)

5.3.3.3 Experiment 3.

O. niloticus, with an average weight of 18 g, were fed varying rates (from 5g kg^{-1}BW day^{-1} to *ad libitum*) of Diet 1, containing 35% protein, in order to quantify the nutrient requirements of fish for on-growing. The changes in mean body weight during the experimental period are presented in Fig. 5.12. There were sixteen fish for each treatment, except for the non-feeding group, which contained eight fish. The fish were fed individually over a six-week period.

The mean initial weights and final weights of males, females and all fish are summarised in Table 5.13. There were no significant differences in initial or final weights between males and females, except for the fish fed at a rate of 10g kg^{-1} bw day^{-1}. At the end of the experiment, the sex ratio of the fish was found to be 1:1. Total wet weight gain and cummulative feeding rate are presented in Fig.5.13a, and the relationships between mean weight and mean food intake of the fish in different treatments are shown in Fig. 5.13 b. Error bars in all figures represent standard deviations. There is a direct relationship between total weight gain and total food intake (R^2=0.93) and mean weight gain and mean food intake (R^2=0.996).

Both relative and specific growth rates are linear over the range of feed intakes from 0 to 35%. The maximum growth rate of this size range of fish was achieved by feeding 35 g kg^{-1}Bw day^{-1}, and the corresponding protein intake was 12.7g protein kg^{-1}Bw day^{-1}. The highest observed relative growth rate was 46 g kg^{-1}Bw day^{-1} over the experimental period, and 54 g kg^{-1}Bw day^{-1} when calculated on the basis of days fed (36 days) (Table 5.14 and Figs. 5.14 a. and b). The fish fed *ad libitum* rations showed a slightly reduced growth rate, and associated wide variations in both feed intake and rates of growth. When

TABLE 5.13 Mean initial & final weights(g) &+S.D. of fish in relation to feeding rate (Experiment 3).

Feeding rate dry food g kg^{-1} bw day^{-1}	Initial wt. male	Initial wt. female	Initial wt. average	Final wt. male	Final wt. female	Overall average
0	$18.938^{a} \pm 3.40$ n = 4	17.539 ± 3.37 n = 4	18.24 ± 3.35 n = 8	$17.29^{a1} \pm 3.34$	$15.38^{a1} \pm 2.89$	$16.34^{a1} \pm 3.18$
5	$18.835^{a} \pm 1.65$ n = 8	18.085 ± 2.31 n = 8	18.46 ± 1.98 n = 16	$19.84^{a12} \pm 1.85$	$19.05^{ab1} \pm 2.19$	$19.45^{a12} \pm 2.00$
10	$20.074^{a} \pm 2.49$* n = 8	17.015 ± 1.71* n = 8	18.54 ± 2.59 n = 16	$26.99^{b2} \pm 1.98$*	$21.44^{b12} \pm 2.65$*	$24.22^{b23} \pm 3.65$
15	$18.030^{a} \pm 3.58$ n = 9	18.583 ± 2.11 n = 7	18.27 ± 2.95 n = 16	$28.60^{b2} \pm 3.81$	$29.44^{c23} \pm 2.39$	$28.97^{c3} \pm 3.19$
20	$19.807^{a} \pm 1.86$ n = 6	18.307 ± 2.27 n = 10	18.87 ± 2.19 n = 16	$39.02^{c34} \pm 6.92$	$34.20^{c3} \pm 5.19$	$36.01^{d4} \pm 6.16$
25	$18.839^{a} \pm 2.54$ n = 8	18.060 ± 2.45 n = 8	18.45 ± 2.44 n = 16	$45.09^{cd3} \pm 5.47^{4}$	$44.58^{d4} \pm 5.98$	$44.84^{e5} \pm 5.54$
35	$18.791^{a} \pm 1.77$ n = 10	18.132 ± 1.13 n = 6	18.54 ± 1.55 n = 16	$55.78^{e} \pm 5.62$	$51.57^{e4} \pm 4.43$	$54.20^{f6} \pm 5.47$
ad libitum	$18.631^{a} \pm 2.42$ n = 9	18.676 ± 1.60 n = 7	18.65 ± 2.04 n = 16	$48.29^{d45} \pm 10.99$	$50.80^{e4} \pm 11.09$	$49.39^{g56} \pm 10.74$

(n = sample size; superscript letters = Duncan's test; numbers = Tukey's test; * indicates the groups of fish where ma and females are significantly different in weight) $\pm$ S.D.

TABLE 3.14 Total weight gain, relative growth rate and specific growth rate of fish in relation to feeding rate (Experiment 3).

Feed rate (g kg^{-1} BW d^{-1})	Mean total wt. gain (g)	Relative growth rate (g g^{-1} BW d^{-1})	(g kg^{-1} BW dy^{-1}fed)	S.G.R. (% dy^{-1})
0	-1.770 ± 1.275^{a}	-2.591 ± 1.444^{a}	-3.023 ± 1.383^{a}	-0.1700 ± 0.1599^{a}
5	0.[illegible] ± 3.005^{a}	1.315 ± 1.345^{a}	1.534 ± 0.571^{a}	0.1146 ± 0.1271^{b}
10	5.[illegible] ± 1.24^{b}	7.[illegible] ± 3.071^{b}	8.580 ± 3.58^{b}	0.6311 ± 0.2269^{c}
15	10.[illegible] ± 2.136^{c}	14.40 ± 0.04^{c}	16.80 ± 4.71^{c}	1.1150 ± 0.2620^{d}
20	17.14 ± 4.48^{d}	21.49 ± 4.36^{d}	25.07 ± 5.09^{d}	1.5201 ± 0.2814^{e}
30	26.[illegible] ± 4.38^{e}	34.41 ± [illegible]^{e}	40.15 ± 7.35^{e}	2.1150 ± 0.[illegible]^{f}
50	36.[illegible] ± 4.36^{f}	47.[illegible] ± 7.74^{f}	55.[illegible] ± 8.33^{f}	2.5602 ± 0.2413^{g}
Ad libitum	30.71 ± 10.20^{b}	39.4[illegible] ± 13.37^{b}	46.03 ± 15.60^{g}	2.279 ± 0.491^{f}

(superscripts represent the result from Duncan's test (p 0.05) for multiple comparisons)

Fig 5.12 Mean body weight (g) *versus* time (days) for groups of fish fed at different rates on a 35% protein diet (Experiment 3).

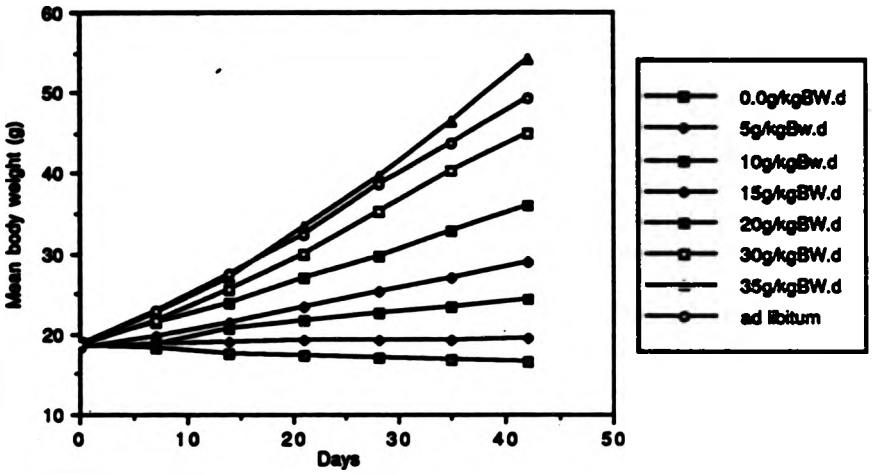
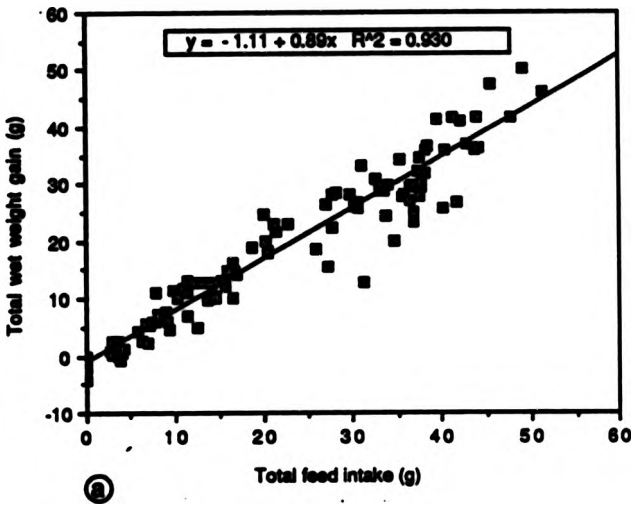

Mean body weight (g)
60
50
40
30
20
10
0
10
20
30
40
50
Days
0.0g/kgBW.d
5g/kgBw.d
10g/kgBw.d
15g/kgBW.d
20g/kgBW.d
30g/kgBW.d
35g/kgBW.d
ad libitum

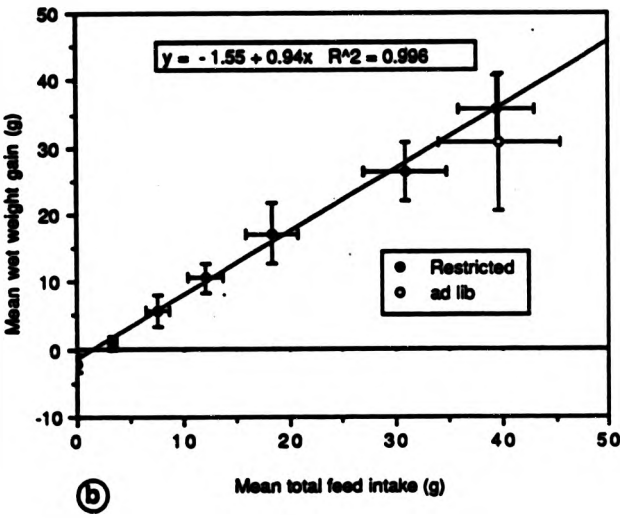
Fig 5.13

(a) Total wet weight gain (g) *versus* total feed intake (g) of all fish (Experiment.3).

(b) Mean wet weight gain (g) *versus* mean total feed intake (g) of fish fed at restricted rates and *ad libitum* (Experiment 3). Error bars represent S.D.


y = -1.11 + 0.89x R^2 = 0.930
Total wet weight gain (g)
Total feed intake (g)
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60

(a)

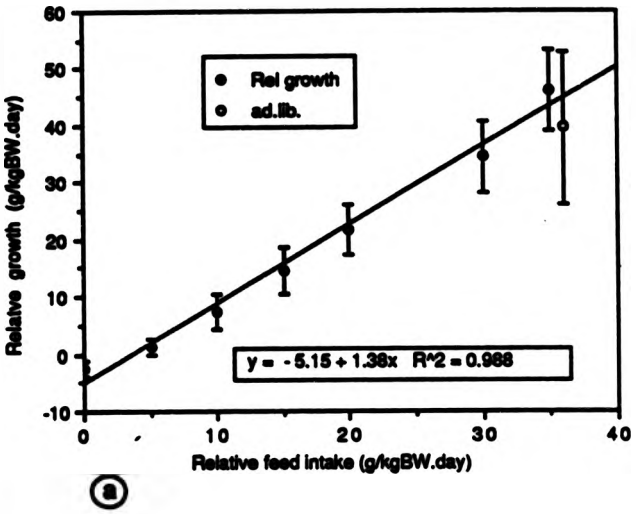

y = -1.55 + 0.94x R^2 = 0.996
Mean wet weight gain (g)
Mean total feed intake (g)
Restricted
ad lib
50
40
30
20
10
0
-10
0
10
20
30
40
50

(b)

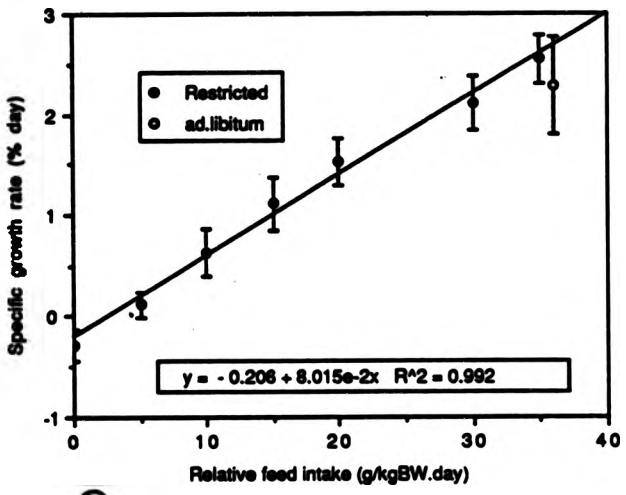
Fig 5.14

(a) Relative growth rate (g kg^{-1}BW day^{-1}) *versus* relative feed intake for fish fed at restricted rates and *ad libitum* (g kg^{-1}BW day^{-1}) (Experiment 3).

(b) Specific growth rate (% day^{-1}) *versus* relative feed intake for fish fed at restricted rates and *ad libitum* (g kg^{-1}BW day^{-1}) (Experiment 3).
Error bars represent S.D.


Rel growth
ad.lib.
y = -5.15 + 1.38x R^2 = 0.988
Relative growth (g/kgBW.day)
Relative feed intake (g/kgBW.day)
60
50
40
30
20
10
0
-10
0
10
20
30
40

(a)


Restricted
ad.libitum
y = -0.206 + 8.015e-2x R^2 = 0.992
Specific growth rate (% day)
Relative feed intake (g/kgBW.day)
3
2
1
0
-1
0
10
20
30
40

(b)

regressed with replicates, the regression equations for relative growth rate *versus* relative feed intake were as follows: females Y= -6.16+1.39X (R^2=0.92): males Y=-5.57+1.42X (R^2=0.92); all fish Y=-5.93+1.39X (R^2=0.92). Maintainence ration was calculated by inverse prediction (Zar, 1984), the value for females being 4.43 (4.7), for males 4.27 (7.3) and for all fish 4.27 (4.8) g kg^{-1}BW day^{-1}. The bracketed figures represent 95% confidence limits, and they are large due to the high residual error. If the calculation is done on the basis of a regression equation between mean relative growth rate and relative feed intake, the result is 3.73 (12) g kg^{-1}BW day^{-1}. When the computation is performed using the specific growth rate equation (Y=-4.184+0.079X), the result is 2.38 (0.15) g kg^{-1}BW day^{-1}. This implies that maintenance ration can be defined differently, depending upon the regression equation used. The relative growth rates of males and females *versus* relative feed intake are presented in Fig. 5.15.

Gain in body protein *versus* cumulative protein intake is presented in Fig. 5.16a, and the relationship between mean protein gain and mean protein intake of fish in different treatments is shown in Fig. 5.16b. The relationships, expressed either way, are linear, the R^2 values being 0.94 and 0.99 respectively. When the relationship is expressed as relative protein change, the resultant regression equation is Y=-0.37+0.23X (Fig 5.17), and the required protein intake to maintain the nitrogen balance in the body is approximately 1.6 g protein kg^{-1}BW day^{-1}.

The relationship between energy change and relative energy intake is shown in Fig. 5.18. There is a linear relationship between the two variables (Y=-0.689+0.007X; R^2=0.95), showing that the energy required to maintain body energy is approximately 98.4 kJ kg^{-1}BW day^{-1}. Details of statistics are given in Table 5.15.

Fig 5.15 Relative growth rate (g kg^{-1}BW day^{-1}) *versus* relative feed intake for male and female fish fed at restricted rates and *ad libitum* (g kg^{-1}BW day^{-1}) (Experiment 2). Error bars represent S.D.

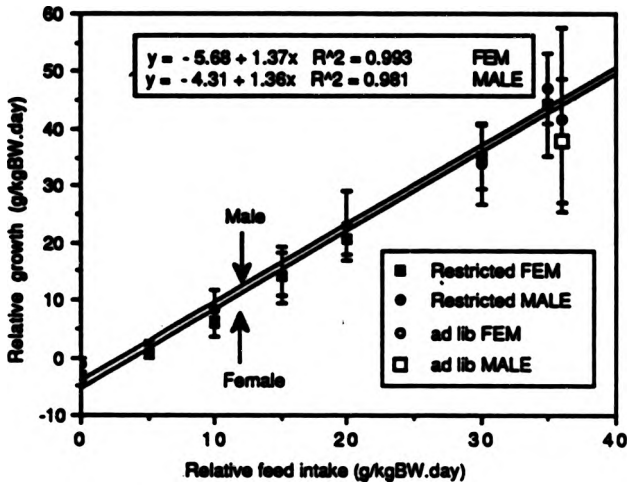
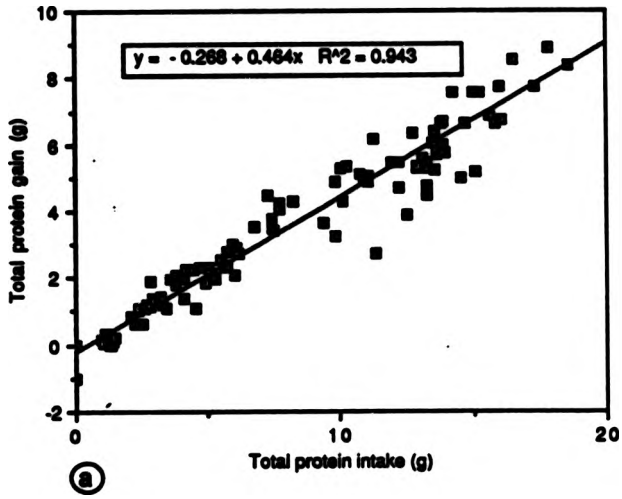

y = - 5.68 + 1.37x R^2 = 0.993 FEM
y = - 4.31 + 1.36x R^2 = 0.981 MALE
Male
Female
Restricted FEM
Restricted MALE
ad lib FEM
ad lib MALE
Relative growth (g/kgBW.day)
Relative feed intake (g/kgBW.day)
60
50
40
30
20
10
0
-10
0
10
20
30
40

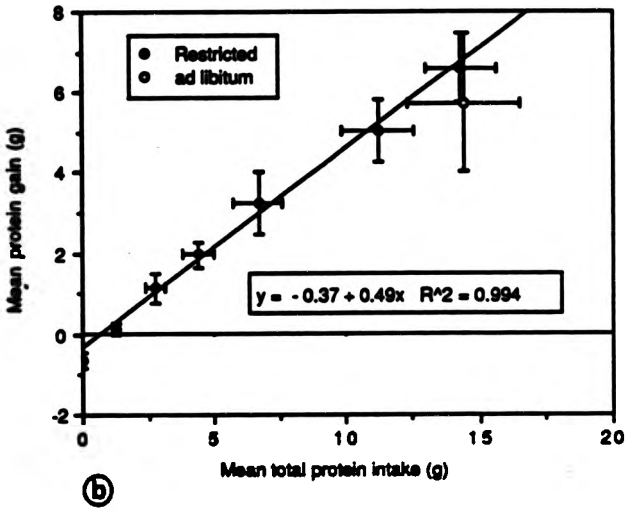
Fig 5.16

(a) Total protein gain (g) *versus* total protein intake (g) over experimental period (all fish) (Experiment 3).

(b) Mean protein gain (g) *versus* mean protein intake (g) in groups of fish fed restricted diets and *ad libitum* (Experiment 3).
Error bars represent S.D.


y = - 0.268 + 0.464x R^2 = 0.943
Total protein gain (g)
Total protein intake (g)
10
8
6
4
2
0
-2
0
10
20

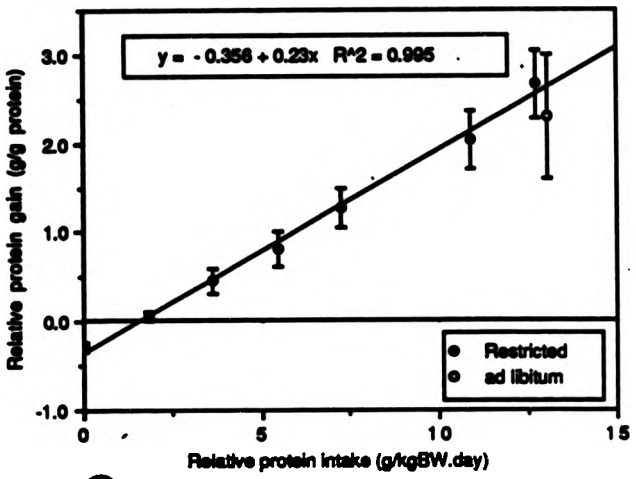
(a)


Restricted
ad libitum
y = - 0.37 + 0.49x R^2 = 0.994
Mean protein gain (g)
Mean total protein intake (g)
8
6
4
2
0
-2
0
5
10
15
20

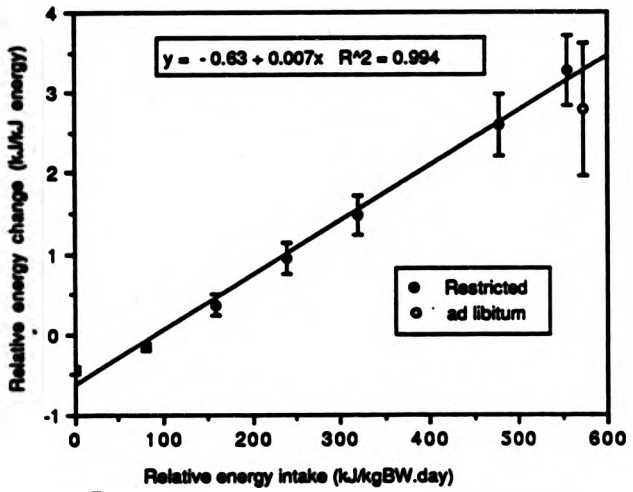
(b)

Fig 5.17 (1) Relative protein gain (g g^{-1}protein) *versus* relative protein intake (g kg^{-1}BW day^{-1}) (Experiment 3).

Fig 5.18 (2) Relative energy change (kJ kJ^{-1}energy) *versus* relative energy intake (kJ kg^{-1}BW day^{-1}) over experimental period (Experiment 3).
Error bars represent S.D.


y = - 0.356 + 0.23x R^2 = 0.995
Relative protein gain (g/g protein)
3.0
2.0
1.0
0.0
-1.0
0
5
10
15
Relative protein intake (g/kgBW.day)
Restricted
ad libitum

①


y = - 0.63 + 0.007x R^2 = 0.994
Relative energy change (kJ/kJ energy)
4
3
2
1
0
-1
0
100
200
300
400
500
600
Relative energy intake (kJ/kgBW.day)
Restricted
ad libitum

②

The proximate body compositions of fish fed on different rations are presented in Table 5.16. Moisture content decreases from 80% to 70% with increasing feeding rate. Lipid content, on a dry weight basis, reached a plateau at 27% (Fig. 5.19) and an inverse relationship is observed between the ash and lipid content in the body.

The apparent digestibilities for protein, lipid and total dry matter are presented in Table 5.17. The apparent protein digestibility is as high as 97% for all feeding rates, and the differences are negligible. The apparent digestibility value for lipid is approximately 98% at all feeding rates and the total digestibility is around 90%, irrespective of feeding regime. There is a slight decrease in apparent digestibility at both extremes of the feeding regime.

Protein conversion efficiency and the calculated growth efficiency values are presented in Table 5.18, and the functional response is shown in Figs. 5.20 and 5.21. Protein conversion efficiency reaches a plateau as protein intake exceeded 5.45 g protein $kg^{-1}BW\ day^{-1}$. Protein efficiency, however, was reduced in fish fed *ad libitum*. Growth efficiency also follows this pattern, and reaches a plateau at approximately 40%, calculated on the basis of gross energy. When calculations were performed on the basis of mammalian physiological fuel values, growth efficiency reached a plateau at around 45%, and feeding efficiency decreased in fish fed *ad libitum*.

5.4.3 Discussion.

Precision and accuracy are the most desirable prerequisites for any experiment. There is, however, a considerable variation in the growth performence observed in the present study. The standared error for the intercept and

TABLE 5.15 Relationship between growth and feed, protein and energy intake (Experiment 3).
* indicates slopes and intercepts which are significantly different from . (p 0.05)

RELATIONSHIP	REGRESSION EQUATION	95% C.I. for intercept and t-ratio	95% CL for slope and t-ratio	R^2	F value	n
1. Total feed intake Vs total gain	Y= -1.39+0.937X	± 0.85	± 0.18	0.96	2276.82	103
2. Total protein gain Vs total protein intake	Y= -0.303+0.49X	± 0.138	± 0.17	0.97	3098.94	103
3. Relative growth rate Vs relative feed intake						
(a) males	Y= -5.57+1.42X	±2.692 (4.14)*	± 0.12 (23.10)*	0.91	533.75	51
(b) females	Y= -6.16+1.39X	±2.388 (5.27)*	±0.115 (24.15)*	0.92	583.30	51
(c) ALL	Y= -5.93+1.41X	±1.76 (-6.67)*	±0.08 (33.48)*	0.92	1120.61	102.
4. Relative protein change Vs relative protein intake	Y= -0.378+0.23X	±0.09 (8.52)*	±0.011 (39.92)*	0.94	1593.21	102
5. Relative energy growth Vs relative	Y= -0.689+0.007X	0.02 (13.46)*	0.001 (45.46)*	0.98	2066.41	102

contd..

TABLE 5.15 contd..

RELATIONSHIP	REGRESSION EQUATION	95% C.I. for intercept and t-ratio	95% for slope and t-ratio	R^2	F value	n
6. Specific growth Vs relative feed intake	Y= -0.18+0.08X	± 0.09 (4.18)*	± 0.004 (38.17)*	0.94	1456.60	103
7. Dry weight gain Vs relative feed intake	Y= -1.65+0.36X	± 0.38 (8.57)*	0.02 (39.73)*	0.94	1578.18	103

TABLE 5.16 proximate body composition of fish on dry weight basis in relation to food and protein intake (Experiment 3).

Feed Rate ($g\ kg^{-1}\ BW\ d^{-1}$)					
Dry food	Protein	Moisture	Protein	Lipid	Ash
		%	%	%	%
0	0	80.56±1.86f	55.49±1.3a	6.08±2.95a	36.20±2.07f
5	1.82	77.61±1.66e	60.52±1.19d	7.75±1.64b	28.75±2.25e
10	3.63	74.99±1.5d	59.92±1.54d	14.57±1.72c	23.51±1.48d
15	5.45	73.68±1.60c	57.95±1.25bc	19.87±1.35e	19.97±1.15c
20	7.26	72.21±1.8b	57.86±1.66bc	23.88±2.10f	21.39±0.79[b]
30	10.9	71.19±1.22a	58.20±1.69c	26.47±1.30g	15.77±1.19ab
35	12.71	70.86±1.83a	57.70±1.96bc	26.79±1.42b	16.82±2.32b
Ad Libitum (36.0)	13.08	70.63±1.78a	56.88±1.42b	27.43±1.79g	14.63±1.26a
Initial		77.18±2.86	58.01±0.56	18.32±0.70	21.39±0.79

TABLE 5.17 The apparent digestibilities of protein, lipid and total dry matter of experimental fish (Experiment 3).

Feed Rate ($g\ kg^{-1} bw\ day^{-1}$)	Protein digestibility	Lipid digestibility	Total digestibility
5	95.20 ± 0.15	-	88.25 ± 0.71
10	97.31 ± 0.31	99.05 ± 0.91	90.29 ± 0.86
15	97.85 ± 0.10	99.04 ± 0.08	90.53 ± 0.48
20	91.79 ± 0.11	99.43 ± 0.04	90.84 ± 0.12
30	96.92 ± 0.14	97.03 ± 0.93	90.14 ± 0.25
35	97.05 ± 0.66	98.06 ± 0.35	89.83 ± 0.51
ad lib	96.53 ± 0.30	98.47 ± 0.13	89.20 ± 0.95

TABLE 5.18 Protein conversion efficiency and growth efficiency of fish in Experimen 3

Feeding rate: Protein intake g kg^{-1} bw day^{-1}	Feeding rate: Gross energy intake kg/kg^{-1} bw day^{-1}	Protein conversion efficiency (PCE) (%)	Growth efficiency K_1^a (%)	K_1^b	K_2^a	K_2^b
1.84	94	12.19 ± 11.13 ^{c}	-21.20 ± 6.77 ^{a}	-19.85 ± 6.34	-25.39 ± 8.11	-23.39 ± 7.47
3.63	188	40.89 ± 10.44 ^{cd}	21.60 ± 6.96 ^{b}	20.22 ± 6.52	25.87 ± 8.34	23.83 ± 7.68
5.45	282	45.09 ± 8.97 ^{de}	33.78 ± 6.39 ^{c}	31.63 ± 5.98	40.45 _ 7.65	37.27 ± 7.05
7.25	376	48.14 ± 6.16 ^{e}	40.14 ± 4.62	37.59 ± 4.33	48.07 ± 5.54	44.29 ± 5.10
10.90	564	45.01 ± 5.59 ^{cde}	38.64 ± 4.40 ^{d}	36.18 ± 4.12	46.27 ± 5.27	42.63 ± 4.85
12.71	658	46.17 ± 3.52 ^{de}	39.52 ± 2.76 ^{d}	37.00 ± 2.588	42.322 ± 3.310	43.601± 3.050
13.08	677	39.16 ± 708 ^{c}	34.62 ± 5.61 ^{c}	32.42 ± 5.25	41.46 ± 6.71	38.20 ± 6.19

a = based on expected energy b = based on determined energy from proximate analysis

K_1 = gross growth efficiency K_2 = 2nd order growth efficiency based on mammalian metabolic energy values

superscripts in figures are result from Duncan's test.

Fig 5.19 Body lipid content (% dry weight) *versus* relative feed intake (g kg^{-1} BW day^{-1}) (Experiment 3).

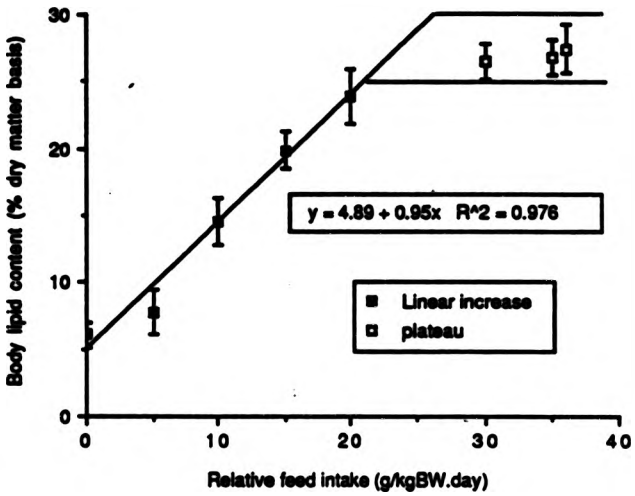
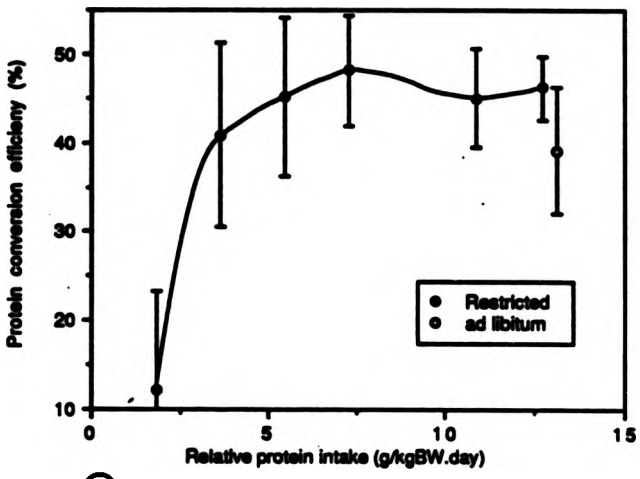
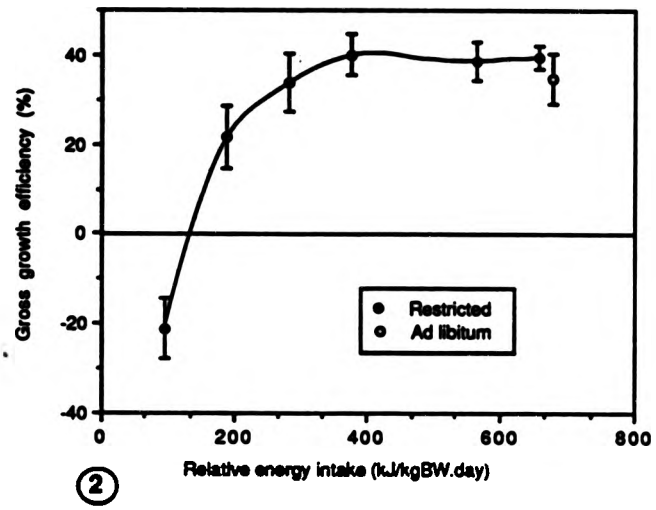

Body lipid content (% dry matter basis)
30
20
10
0
0
10
20
30
40
Relative feed intake (g/kgBW.day)
y = 4.89 + 0.95x R^2 = 0.976
Linear increase
plateau

Fig 5.20 (1) Protein conversion efficiency (%) *versus* relative protein intake (g kg^{-1} BW day^{-1}) (Experiment 3).

Fig 5.21 (2) Gross growth efficiency (%) *versus* relative energy intake (kJ kg^{-1} BW day^{-1}) (Experiment 3).
Error bars represent S.D.


Protein conversion efficiency (%)
50
40
30
20
10
0
5
10
15
Relative protein intake (g/kgBW.day)
Restricted
ad libitum

①


Gross growth efficiency (%)
40
20
0
-20
-40
0
200
400
600
800
Relative energy intake (kJ/kgBW.day)
Restricted
Ad libitum

②

slopes of regression curves ranges from 5 to 15% amongst the variables considered. As a result, the confidence limits computed for the intercept and slope are larger than might have been desired in some functional relationships (Tables 5.5 and 5.15). Likewise, when the inverse prediction method was used to compute the confidence limits of the independent variable, large confidence limits often resulted (*eg.* those associated with computed maintenance requirements). A regression curve is usually more reliable in the middle region. Predictions at either end of the curve tend to be less reliable, as confidence limits increase at both ends. In studies of nutrient turnover, however, interest lies principally with the ends of the curves (*i.e.* the minimum food requirement for maintenance and the maximum requirement for highest growth). Large confidence limits, however, were anticipated at the outset of the present study, given the wide range of weights of the available experimental fish. It is probably because researchers have observed large variability associated with their data that there is no published study in which confidence limits for maintenance ration have been computed (*eg.* Gerking, 1971; Stirling, 1972). The present study is, apparently, the first to do this.

The wide variability in measured responses may have resulted from:

- differences in initial sizes among experimental fish;
- the level of aggresion in fish, even after separation by transparent nets or plastic materials into relatively small chambers;
- the short duration of the experiments;
- the possibility that the assumption that the body composition of experimental fishes was similar to that of initial fish samples was erronious;
- possible errors in chemical analyses and the use of conversion factors to determine the energy density of food and fish.

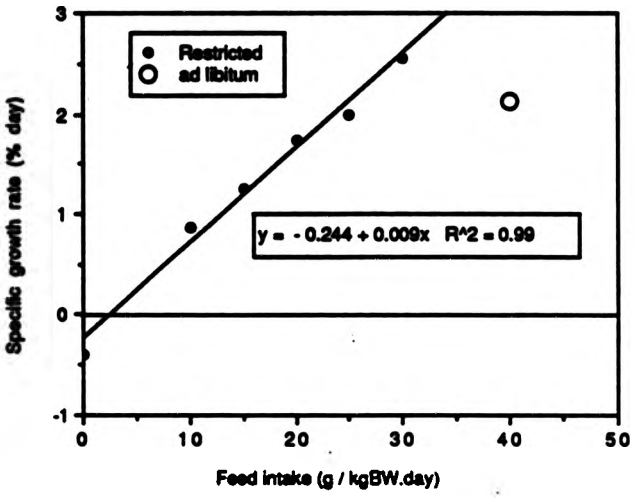
Another factor that could have contributed to variability is that some females released eggs during the experiment, and this was not taken into account in computing growth responses.

If it had been possible to rigidly control all those factors, more consistent results might have been obtained. The objective of the present study, however, was not to measure any particular value (*eg.* maintenance requirement) very accurately, but rather to check the validity of the basic relationships explained in the conceptual model of feed intake and growth. This is considered to be a prerequisite to accurately defining particular values. The results indicate that this goal has been achieved from the three experiments reported in this section.

The functional relationship between feed intake and growth of *O. niloticus* is linear, irrespective of the way the data is presented. The absolute or relative feed/protein/energy intake versus absolute or relative wet weight gain, dry weight gain, relative growth rate or protein growth, always yielded a linear functional relationship. The present study agrees with the functional relationship between feed intake and growth for *O. mossambicus* given by Birkett (1972) and disagrees with the curvi-linear relationship presented by Nawwab (1987) for *O. niloticus* fed on commercial trout pellets. The data presented by Nawab (1987) in his Ph.D thesis has been re-assessed and is shown in Fig. 2.22. Even though the curve fitted by eye is curvilinear for the fish (initial weight 14g), the mean SGR *versus* relative feed intake yields a linear relationship (R^2=0.99) up to fish fed 3% BW per day, with a decline in growth in fish fed *ad libitum*. It is reasonable to conclude, therefore, that the fuctional relationship between growth and feed intake is linear for *O. niloticus* fed formulated diets.

The above results imply that the fraction of waste produced by fish is linearly related to ingestion. The data on apparent digestibility of protein, lipid and total dry

Fig. 5.22 Specific growth rate *versus* feed intake(g kg^{-1}BW day^{-1}) of *O.niloticus* fed on a diet of trout pellets [data from Nawwab (1988)].


Restricted
ad libitum
y = - 0.244 + 0.009x R^2 = 0.99
Specific growth rate (% day)
Feed intake (g / kgBW.day)
3
2
1
0
-1
0
10
20
30
40
50

matter indicates that they are independent of feed intake up to 60g $kg^{-1}BW\ day^{-1}$ for small fish (2-12 g) and up to 35g $kg^{-1}BW\ day^{-1}$ for larger fish (ranging in weight between 18-50g). The apparent digestive efficiencies for protein and lipid are as high as 97% and 99% respectively. The total dry matter digestibility of diet 1, which contains 35% protein, reached an average value of 90% in both size groups of fish. Since the diet contained 7% cellulose, this implies that the carbohydrate digestibility must be as high as the values for protein and lipids. This result agrees with the apparent digestibility values reported by Wang *et al.* (1985), which are 99%, 95% and 97% for protein, crude starch and lipid respectively. In the present study, however, a decrease in apparent digestibility at highest and lowest rations was observed. The low digestibility observed at lowest ration levels may be attributed to one of two reasons. First, the fraction of the nutrients from endogenous excretion in faeces of fish fed the lower rations will be high compared with those fed on increased rations. Second, tilapia can filter feed on detrital or bacterial particles as small as 1µm (Beveridge *et al.*, in press). Contributions to the diet from these sources may be higher at lower feeding rates, and this may alter the ratio of indicator to undigested fraction considerably, resulting in changes in apparent digestibility. The fish fed *ad libitum* also showed lower digestibility. This may have been due to an increase in rate of gastric motility and there may also have been contamination of the faeces by food.

These results indicate that when fed a readily digestible diet, such as a purified diet, *O. niloticus* belongs to the group of fishes categorised under Digestive Strategy 1 in the conceptual growth model. This implies that faecal production and the net rate of obtaining energy are linear functions of feed intake.

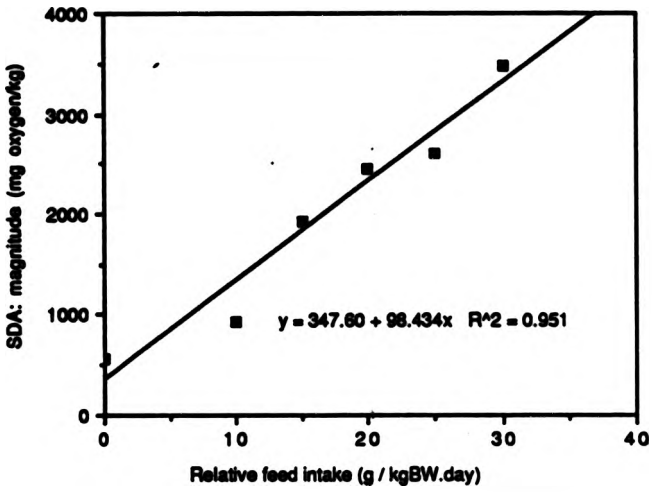
Since growth is a linear function of ingestion, it follows that the non-retained fraction of absorbed energy

should also be a linear function. This prediction can be investigated by considering the specific dynamic action (SDA) of fish, assuming that SDA represents a major fraction of the cost of growth. Fig. 5.23 shows the relationship between oxygen consumption and feed intake of *O. niloticus*. Unpublished data was obtained from Dr. L.G. Ross (Institute of Aquaculture, Stirling) for fish of average weight 50g fed a diet containing 31% protein. Oxygen consumption increases linearly with feed intake, from 5 to 35 g kg^{-1}Bw day^{-1} (R^2=0.97). This implies, as predicted in the conceptual model, that the cost of growth is a linear function of feed intake, assuming SDA represents a major fraction of cost associated with growth.

Excretory losses were not measured in the present study and no data were found in the literature regarding the rate of excretory losses from absorbed energy for *O. niloticus*. However, due to the fact that nutrient retention is a linear function of nutrient intake, the material losses from absorbed energy should similarly be related to absorbed energy in a linear fashion.

In the conceptual model presented earlier in this thesis, it was predicted that a fish would not be able to consume more feed than it could process per unit time. The model also predicted that if fish consumed more feed than this, reduced growth may result. Data from all three experiments reported in this section demonstrate a reduction in growth of fish fed *ad libitum*. Support for this prediction is also apparent in the data from Nawwab (1987) for *O.niloticus* (Fig.5.22). This shows reduced growth resulting from excessive feeding on commercial trout pellets. Similar reductions associated with excessive feeding can be seen from data for many other fish species [*eg.* sockeye salmon (Brett *et al.*, 1969), European bass (Stirling, 1972), common carp (Huisman, 1974), thick-lipped mullet (Flowerdew and Grove, 1980), channel catfish, (Gatlin *et al.*, 1986) and cod (Jobling, 1988)]. It is interesting to note that

Fig 5.23 Relationship between feed intake (g kg^{-1}bw d^{-1}) and SDA as measured by oxygen consumption (mg O_2 kg^{-1} d^{-1}) in 50g *O niloticus* (Dr LG Ross, unpublished data).


4000
3000
2000
1000
0
SDA: magnitude (mg oxygen/kg)
y = 347.60 + 98.434x R^2 = 0.951
0
10
20
30
40
Relative feed intake (g / kgBW.day)

all those studies involved feeding with formulated diets.

There was a slight slight reduction in digestibility for fish fed *ad libitum*. The results for proximate composition reveal an initial linear relationship between relative lipid content of the body and feed intake, which reaches a plateau value at approximately 28 to 30%, whilst body moisture content decreases from 80 to 70% with increasing feed intake. A wide fluctuation in feed intake was also apparent in fish fed *ad libitum*, and as a result, the total amount of feed intake was less in the the group fed Schedule *ad libitum* 2 in Experiment 1. This suggests that the *a priori* expectation concerning the growth response to *ad libitum* feeding was correct. The increase in lipid content, however, did not reach a value as high as 40% on a dry weight basis, as had been reported for *O. mossambicus* by Jauncey (1982). This implies that the lipid storage problem, discussed in detail in Section 3.3.3.1, is not entirely responsible for the observed growth reduction. The fish might have faced a power rating problem which amplified wear and tear to the system.

Jobling (1986) postulated that a formulated diet of high energy density and small particle size caused a rapid rate of gastric evacuation, resulting in a lower food conversion efficiency than with natural food. A similar theory was postulated in the conceptual model presented in the present study (Section 3.3.3) and was termed rapid digestion. Jobling (1988) extended this argument to cod, and claimed that reducing feeding rate does not solve the problem of lower conversion efficiency, and that moist diets must be utilised in their culture. In the present study with *O. niloticus*, a purified diet with easily digestible ingredients is utilised. Particle size was less than 1mm for all ingredients used. There is a linear relationship between food or protein intake and the observed growth responses. This implies that indices such as food conversion ratio (FCR = dry food fed/wet weight gain) and protein efficiency ratio (PER = wet weight gain/dry

protein intake) are constant. The FCR for individuals observed in the present study ranged from 0.8-1.6 and the PER was approximately 2 - 3. No significant differences resulted from varying feeding rate, except at the lowest feeding rate, which resulted in a negative efficiency. The present study, however, showed that reduced growth efficiency is related to over-consumption of food with higher energy density, rather than rapid digestion *per se*. Results from Experiments 1, 2 and 3 also show that better growth rates can be achieved by controlling feed intake rather than by feeding *ad libitum*. The concept of gastrointestinal overload, therefore, does not appear to be true, at least for *O. niloticus*. Further research on these aspects are, however, urgently needed before arriving at a final conclusion.

A comparison of the data from this study with those for other fish species and terrestrial animals may be interesting. It is apparent, however, that when data are presented in terms of specific growth rate (SGR) (% day^{-1}), there are two patterns of growth with respect to relative feed intake. This can be attributed to a change in growth pattern from linear at lower feeding rates to exponential at higher feeding rates. It is interesting to note that the SGR for smaller *O. niloticus* in Nawwab's (1987) data also shows two patterns of growth in relation to relative feed intake. This shows that one should exercise caution when presenting data, as the assumptions made regarding exponential growth may not be true. The assumptions cannnot be statistically verified as the working range of independent variables is relatively narrow, and linear as well as curvi-linear relationship may both yield high correlation coefficients.

It was found in the present study that the highest growth rates are achieved by feeding approximately 21.8 g protein kg^{-1}BW day^{-1} for fish in the weight range 2 -12g. This is comparable with data provided by Jauncey (1982) for *O. mossambicus*, suggesting 25 g protein kg^{-1}BW day^{-1} for fish

weighing between 1 and 8g. The recommendations for feeding rate proposed by Jauncey and Ross (1982) would also seem to be correct. The maximum protein requirement for highest growth of *O. niloticus* in the weight range 18 to 50g, however, was found to be 12.8 g protein kg^{-1}BW day^{-1} in the present study. The above authors' recommendation that a diet containing 30 - 35% protein, fed at a rate of 6% BW day^{-1}, was appropriate for fish weighing 1-50g would thus appear to be an over-estimation. Such a feeding rate should be restricted to fish in the 2-10g weight range. Amending Jauncey and Ross's (1982) recommendations, the following daily feeding rates would be appropriate: for fish weighing between 1 and 10g, 6% Bw; 11 to 35g, 4% Bw; and 36 to 75g, 3% Bw.

Comparisons between the protein requirement for fish with data available for terestrial animals in the literature was made. The data shows that there is a close similarity between the chicken and *O. niloticus*: protein requirement for young birds is 21 -12 g kg^{-1}BW day^{-1}, and this is similar to the protein requirements of *O. niloticus* found in the present study. Protein requirements are also similar for rats (24 g kg^{-1}BW day^{-1}) and lambs (16 g kg^{-1}BW day^{-1}), but are higher than those observed for calves (3 g kg^{-1}BW day^{-1}). The growth rate of the latter, however, is 10 times less than for young *O.niloticus* (Bowen, 1987).

The feed required to maintain constant body weight of *O. niloticus* fed diet 1 containing 35% protein is approximately 7g kg^{-1} BW day^{-1} and 2.38 to 4.27g kg^{-1} BW day^{-1}, depending upon the regression equation used, for fish in the 2 to 12g and 18 to 50g weight ranges respectively. These values resulted in a protein requirement for maintainence of body weight of 2.56g protein kg^{-1} BW for small fish, and 0.9 - 1.5g protein kg^{-1} BW for larger fish. The latter range is comparable to the data for channel catfish (8 - 30g in weight range) of 1- 1.32g protein kg^{-1} BW day^{-1} (Gatling *et al*, 1986), and slightly lower than the value for rainbow trout of a similar weight range of

1.74g protein kg^{-1} BW day^{-1} (Kaushik and Luquet, 1987). When the maintanance ration was calculated from the regression curve of Fig. 5.22 (Nawwab, 1987), the resultant value is 2.57 g kg^{-1}BW day^{-1}. Since the commercial trout pellets in his study contained 49% protein, this gives 1.3 g protein kg^{-1}BW day as the maintenance ration for *O. niloticus*, thus supporting the above figures. The present study indicates, however, that great caution should be exercised when determining the maintenance requirements of fish using the nutrient turnover method, as the the value depends upon the functional relationship used in the determination.

The corresponding energy required to maintain body weight for *O niloticus* is 109 and 38-68 kJ kg^{-1}BW day^{-1} for smaller and larger groups respectively. The latter values correspond with those for channel catfish (63 kJ kg^{-1}BW day^{-1}).

The protein requirements for protein balance in the present study are 3.16 and 1.6 g protein kg^{-1}Bw day^{-1} for fish in weight ranges 2-12 g and 16-50 g respectively. No value can be found in the literature for small fish of other species. The value presented by Gatlin *et al.* (1986) for channal catfish is 1.07, which is slightly lower than the value found in the present study. The value given by Gatling *et al* (1986), however, is questionable, as the data showed that the protein requirement for maintaining constant body weight is 1.32, whilst that for maintaining protein balance is 1.07.

The major discrepancy between the present study and others is in the energy required for the maintenance of energy balance in the body, which was higher than expected. It was observed that fish in both size groups mobilised lipid for energy yielding processes in order to retain protein, resulting in higher lipid losses. The resultant regression equations were $Y = -1.48+0.008X$ for 2-12g fish, and $Y = -0.689+0.007X$ for fish ranging from 18-50g. The resultant energy requirements for maintenance, therefore, are 185 and 98.4 kj kg^{-1}BW day^{-1} for smaller and larger size groups

respectively. No data can be found in the literature for other fish species of similar 2-12 g size. The results for larger fish were somewhat higher reported for other fish species. For example, Gatlin *et al* (1986), utilising the nutrient turnover method, reported a value of 71.12 kJ kg^{-1} day^{-1} for channel catfish and this is lower than the value found in the present study for 18 - 50g *O niloticus*. With the aid of indirect calorimetry, Caulton (1978) found that *Tilapia rendalli* expend 45.03 kJ kg^{-1} day^{-1} on maintenance at 23 °C. If a Q_{10} value of 2.3 is assumed (Ross and Ross, 1983), this gives a value of approximately 70 kJ kg^{-1}BW day^{-1} at 28°C.

The above evidence suggests that the energy requirements have been over-estimated in the present study. However, determination of maintainence requirements by a method employing starved fish and a method employing active fish may not necessarily yield similar values. Hogendoorn (1983), in a study of African catfish, also found that the maintenance requirements calculated from nutrient turnover method are higher value than those obtained by indirect calorimetry. The relatively high value obtained in the present study cannot be completly attributed to possible errors of using conversion factors for energy determination, as Gatlin *et al* (1986) used similar conversion factors. Another contributing factor may be the level of aggression observed among *O.niloticus*, even after separating them with transparent nets and plastic materials. Further studies, therefore, are necessary before arriving at a definitive answer.

The protein conversion efficiencies (PCEs) determined in the present study range from 45 to 50% for fish fed 10.9 to 21.8g protein kg^{-1}BW day^{-1}, and 5.45 to 12.7g protein kg^{-1}BW day^{-1} for 10 to 12g and 18 to 50g fish respectively. This indicates that the protein retention efficiency does not change with body weight over the size range investigated. These values are higher than the value of 25% calulated from the data of Jauncey (1982) for *O. mossambicus*, but are similar

to the figure of 40% calculated from the data for *O. niloticus* from De silva and Perera (1984). This is also in agreement with the value for channel catfish of 45% (Gatling *et al*, 1986), 48% for common carp (Murai *et al*, 1986) and close to the value for rainbow trout of 40% obtained by Ogino *et al* (1976). The PCE for *O. niloticus* is higher than for many terrestrial animals (*e.g.* calf 24%, lamb 30%; NRC, 1981), but is comparable to chicken (44 - 57%. Nijkamp *et al.*, 1974). These results indicate that fish are neither superior nor inferior to chickens with regard to protein utilisation, as was suggested by Nijkamp *et al* (1974).

First order (K_1) and second order (K_2) growth efficiencies (total energy conversion or retention efficiencies) increased after maintenance requirements were fulfilled, to a plateau of approximately 35% for gross growth efficiency (K_1) and 40% for K_2, which are calculated from mammalian physiological fuel values. Calculations were based on the expected food composition and the energy values determined by proximate analysis. The energy density of food calculated from proximate analysis will utilise NFE as the available carbohydrate, possibly yielding a higher energy value. However the calculated value of energetic efficiency showed that the difference between the two calculations is only 2-3%, and the true value is likely to be some intermediate value. Since the calculated growth efficiencies in the present study are comparable to those for many other fish species [*eg.* Bermuda reef fish *Ephinephalus glutatus* K_1 = 18-42% (Menzel, 1960), snakehead *Ophicephalus striatus* K_1 = 43% (Pandian, 1967), sole *Pleuronectus plattessa* K_1 = 34% (Edwards *et al.*, 1969) channel catfish *Ictalurus punctatus* K_2 = 31 - 53% (Gatlin *et al.*, 1986), it is reasonable to conclude that the procedure employed in the present study has not seriously affected the determination of growth efficiency.

Many authors conventionally present data on proximate body composition on a wet weight basis and discuss their data

in terms of relative composition (on % basis). The proximate body composition data in the present study are utilised only to delineate the reduction in moisture and increase in lipid content. On the other hand, moisture determination in the present study was carried out on whole body weights of individual fish. Since a figure for moisture content was available for each fish, any error associated with that figure may enhance the variability among computed values in data analyses of individual fish. For this reason, data were presented on a dry weight basis for all experiments in order to maintain consistency among presentations. As there were no differences between male and female body composition, data were presented for all fish without regard to sex.

The major problem encountered throughout the study was the uncontrollable aggression among experimental fish. When the fish were reared in stock tanks, even though agressive fish were present, non-aggressive fish could seek refuge by hiding among other fishes. The mortality rate was, therefore, not as severe as in experimental fish. Once the experimental fishes were sorted out, in order to ensure a close agreement in body weight among indivuduals in different treatments, and the stocking density was reduced, aggression became uncontrollable. A further two studies which have not been reported in this thesis had to be abandoned after 3-4 weeks, as only single fish remained in some treatments. Tilapias are known to be a very active as well as very agressive fish species. Chen and Prowse (1964) showed that living space of *O. mossambicus* affects growth rate through social hierarchial effects when food is not a limiting factor. This was given as the reason for stunting among pond fish populations in the Fisheries Station, Mallacca, Malaysia. Dominant-subordinate relationships under aquarium conditions appear to affect the growth rate of *Tilapia zilli* through disproportionate food acquisition by surbodinates (Koebele, 1985). Mishirgi and Kubota (1978) showed that territoriality in *O. niloticus* is not

related to maturity, but rather that fish are hyper-aggressive. They also suggested that an increase in space would reduce aggresiveness. Aggresive behaviour, as with other behaviour patterns, requires both the appropriate internal physiological state and an external releasing stimulus (Stringer and Hoar, 1955). The exact nature of the stimuli was not determined in the present study, although it was observed that aggression was highest among fish fed on optimal rations (see Figs. 5.9a and b). Further studies would be advantageous, as the growth response may be significantly affected by this behaviour pattern.

Experiment 2 suggests that differences in energy or protein density do not appear to affect growth rates achieved by fish. This aspect has not been studied in *O. niloticus* in detail. The following section investigates the effects of food composition on growth response.

5.4 Investigation 3.

5.4.1 Scope.

There are an infinite number of combinations that can be utilised in dietary formulations to form a diet for fish. Such formulations provide guidelines or recipes for nutritionists or feed manufacturers to produce a diet from available ingredients whilst incurring the lowest cost. However, there may be a range of combinations that a fish can utilise more efficiently and this may be species specific. Hence, a study of constraints imposed by dietary composition is important in animal husbandry as this will provide information about best possible use of available feed materials.

There are several studies concerned with the effect of varying levels of lipid and carbohydrate on fish growth (*eg.* Garling and Wilson, 1977). However, very little information seems to be available for tilapias (Jauncey and Ross, 1982).

Investigation 1 provided information on the PE/NPE ratio in dietary formulations, whilst Investigation 2 quantified the upper limits of synthesis capacity. The objectives of this investigation were to highlight the effects of the composition of non-protein energy (*i.e.* carbohydrate energy and lipid energy) and total energy density on the growth response of *O. niloticus* under confined conditions.

Investigation 3 comprised nine experiments, in which one experiment aimed to measure the growth response of *O. niloticus* for different dietary compostion, whilst the others set out to investigate the effects of food composition and energy density on the rate of gastric evacuation.

Experiment 2 showed that the differences in energy or protein density do not appear to affect the growth rates achieved by the fish. This aspect has not been studied for *O. niloticus* in detail. The following section investigates the effects of food composition on growth response.

5.4.2 Materials and methods.

5.4.2.1 Diets.

When a diet is prepared on a percentage composition basis, there are two ways to ways to fulfill this task; one gives the ratio of materials, in which the dietary formulation is presented by taking all dietary constituents into account. The other gives the ratio of energy-yielding substances (*i.e* protein, lipid and carbohydrate), which is defined as the fraction of energy-yielding constituents relative to total energy yielding substances [*eg.* protein/ (protein+lipid+ carbohydrate)] This is termed the PLC ratio (P=protein, L=lipid and C=carbohydrate) in the present study. If one desires to keep both the NPE/PE ratio and the energy density constant in a diet, this can be achieved only by changing all

three fractions at the same time as shown in Table 5.19. If the objective is to study the effect of energy density on growth response, the best way is to formulate a diet in which both the NPE/PE ratio and the L:C ratio are kept constant, whilst the energy density is changed. If gastric evacuation is controlled by osmotic receptors in the upper intestine, it is desirable to keep a constant lipid to carbohydrate ratio, as the reduction in osmotic pressure will be proportional to changing energy density. This is termed dietary dilution in this thesis.

Fourteen diets were prepared using semi-purified ingredients according to the ratios shown in Table 5.19. The following steps were taken in the formulations: the ratio of NPE/PE was maintained at 1.72 (PE ratio 22 mg protein kJ^{-1}) in all diets. The protein level (34%) and energy density (15.5 kJ g^{-1}) were kept constant in seven diets on the basis of dry material ratio [diets D1.(34) to D7.(34)]. Lipid levels changed from 5 to 20% and carbohydrate from 47.1 to 13.35% as shown in Table 5.19.

From the above seven diets, two diets with low (5% : diet 1.(34) and high (15% : diet 4.(34) lipid level were selected. These two diets have lipid (L) to carbohydrate (C) ratios of 5.8 : 54.7 and 20.4 : 33.4 respectively (on PLC basis). The energy density of diet D1.(34) changes from 15.5 to 13, 10 and 7 kJ metabolisable energy g^{-1}, according to the ratios of dry materials shown in Table 5.19. The metabolisable energy density of diet D4.(34) changed from 15.5 to 18, 13, 10 and 7 kJ g^{-1}. This was achieved by either reducing or increasing the amounts of indigestible filler used, as shown in Table 5.20. Cellulose was utilised as the filler.

The information generated from Investigations 1 and 2 were utilised in this investigation in diet preparation and feeding. Many difficulties were experienced in the preparation

TABLE 5.19 Theoretical dietary formulation of diets used in Investigation 3. All values except energy density (mammalian physiological fuel value; KJg^{-1}) and PE ratio (mg protein KJ^{-1}) are expressed as % dry weight.

DIET No.	Protein	Lipid	COH	ENERGY	P (%)	L	C	P+L+C	PE ratio	NPE/PE
D.1(34)*	34.00	5.00	47.10	15.46	39.49	5.81	54.70	86.10	22.00	1.72
D.2(34)	34.00	7.50	41.48	15.46	40.97	9.04	49.99	82.98	22.00	1.72
D.3(34)	34.00	10.00	35.85	15.46	42.58	12.52	44.90	79.85	22.00	1.72
D.4(34)	34.00	12.50	30.23	15.46	44.31	16.29	39.40	76.73	22.00	1.72
D.5(34)	34.00	15.00	24.60	15.46	46.20	20.38	33.42	73.60	22.00	1.72
D.6(34)	34.00	17.50	18.98	15.46	48.24	24.83	26.93	70.48	22.00	1.72
D.7(34)	34.00	20.00	13.35	15.46	50.48	29.70	19.82	67.35	22.00	1.72
D.8(28)	28.60	4.20	39.61	13.00	39.49	5.81	54.70	72.42	22.00	1.72
D.9(22)	22.00	3.24	30.48	10.00	39.49	5.81	54.70	55.71	22.00	1.72
D.10(15)	15.40	2.26	21.33	7.00	39.49	5.81	54.70	38.99	22.00	1.72
D.11(40)	39.60	17.47	28.65	18.00	46.20	20.38	33.42	85.72	22.00	1.72
D.12(28)	28.60	12.62	20.69	13.00	46.20	20.38	33.42	61.91	22.00	1.72
D.13(22)	22.00	9.70	15.91	10.00	46.20	20.38	33.42	47.62	22.00	1.72
D.14(15)	15.40	6.79	11.14	7.00	46.20	20.38	33.42	33.33	22.00	1.72

COH = CARBOHYDRATE

* figures in parenthesis are nominal protein content (% dry weight)

TABLE 5.20 Diet formulation and proximate composition in Investigation 3. All values, except P E ratio (mg protein KJ^{-1}) and energy density (mammalian physiological fuel value; KJg^{-1}), are expressed as dry weight.

DIET No.	* INGREDIENTS									PROXIMATE COMPOSITION						
	Fish meal	Dextrin	Fish oil	Corn oil	Vitamin	Mineral	Cellulose	CMC	Gelatin	Crude Protein	Crude Lipid	Fibre	Ash	NFE	Cr_2O_3	Energy	PE ratio
D.1 (34)*	42.80	47.10	0.00	0.72	2.00	4.00	0.34	2.00	1.00	34.20	4.60	0.43	9.65	51.11	0.41	16.00	21.36
D.2 (34)	42.80	41.50	0.00	3.22	2.00	4.00	3.47	2.00	1.00	33.47	7.20	3.26	9.40	46.66	0.39	16.12	20.76
D.3 (34)	42.80	35.85	0.72	5.00	2.00	4.00	6.59	2.00	1.00	33.20	9.71	6.45	9.48	41.15	0.39	16.10	20.62
D.4 (34)	42.80	30.22	1.97	6.25	2.00	4.00	9.72	2.00	1.00	33.46	12.26	9.42	9.48	35.39	0.38	16.14	20.73
D.5 (34)	42.80	24.60	3.22	7.50	2.00	4.00	12.84	2.00	1.00	33.08	15.18	13.59	9.46	28.68	0.36	16.05	20.61
D.6 (34)	42.80	18.98	4.47	8.75	2.00	4.00	15.96	2.00	1.00	33.36	17.44	17.69	9.29	22.22	0.44	15.87	21.02
D.7 (34)	42.80	13.35	5.72	10.00	2.00	4.00	19.09	2.00	1.00	33.21	19.83	21.61	9.48	15.86	0.37	15.68	21.18
D.8 (28)	35.82	39.61	0.00	0.62	2.00	4.00	14.95	2.00	1.00	28.53	3.81	13.44	8.56	45.66	0.39	13.85	20.60
D.9 (22)	27.26	30.48	0.00	0.51	2.00	4.00	32.74	2.00	1.00	22.07	3.15	31.53	7.54	35.71	0.38	10.85	20.40
D.10 (15)	18.69	21.33	0.00	0.39	2.00	4.00	50.58	2.00	1.00	16.52	2.13	48.41	6.89	26.05	0.47	7.93	20.84
D.11 (40)	50.10	28.65	3.72	8.73	2.00	4.00	0.00	1.79	1.00	40.10	17.26	0.42	10.39	31.83	0.45	18.54	21.63
D.12 (28)	35.82	20.69	2.73	6.31	2.00	4.00	25.44	2.00	1.00	28.24	12.44	25.76	8.74	24.83	0.4	13.57	20.82
D.13 (22)	27.26	15.95	2.12	4.85	2.00	4.00	40.82	2.00	1.00	22.22	9.57	39.27	7.64	21.30	0.42	10.89	20.41
D.14 (15)	18.69	11.14	1.52	3.40	2.00	4.00	56.25	2.00	1.00	14.78	6.10	55.40	6.35	17.37	0.37	7.68	19.25

* figures in parenthesis are nominal protein content (% dry weight)

of Diet 2 (Table 5.2, p120), due to the fact that gelatin and cellulose form hard pellets which are very difficult to break into small particles without altering the diet composition. It was decided, therefore, to use fish meal as the protein source in this experiment. Diets 1 and 2 (Table 5.2), however, proved to have excellent stability (*i.e.* even after 24h, intact pellets remain in the water), probably because of the inclusion of gelatin. It was decided, therefore, to replace 1% of the fish meal protein with gelatin, in addition to the inclusion of 2% carboxymethyl cellulose in diets. Dextrin was utilised as the carbohydrate source. Dietary lipid was added in a ratio of 1 : 1 fish oil to corn oil. Since fish meal contains a fraction of lipid, diets with lower lipid contents were supplemented by only corn oil up to desired lipid level. Since some formulations contained only a negligible amount of cellulose, Cr_2O_3 was used as the digestibility marker and added to the diets at 0.5% level.

The ratios of dry ingredients used in the formulations and the proximate compositions of diets are presented in Table 5.20. Mammalian physiological fuel values were utilised in the energy density determinations. Feed preperation and storage is as described previously.

5.4.2.2 Evaluation of growth performance.

The experiment was conducted in a warm water recirculating system (Experimental system 1), maintained at a temperature of 28 °C, comprising fourty-eight 20l plastic tanks, each of which had been divided into four equal-sized chambers using 1mm mesh. A total of 168 fish of 14g mean intial weight were randomly assigned to each chamber two weeks prior to the experiment and allowed to acclimitise to the sustem and to the diets. Three tanks, comprising four fish

each, were randomly assigned to each treatment and there were 12 fish in each treatment. The mean weight of fish at the beginning of the experiment was 18g.

The results obtained from Experiment 3 were utilised to determine feeding rate. Fish were fed at the rate of 12 g protein kg^{-1}BW day^{-1} and the corresponding metabolisable energy content of food fed was approximately 550 kJ kg^{-1}BW day^{-1}. The experiment was designed so that the total dry matter that could be ingested depended upon the protein and energy densities of the diets. For example, fish fed lowest protein and energy density (D10 and D14) were offered 78.6g kg^{-1} day^{-1}, whilst fish fed on the diet with highest protein and energy density (D11) were offered only 30.6g kg^{-1} day^{-1}. Fish were fed six days per week for a total of 40 days. Preliminary studies indicated that to consume equal amounts of protein and energy, fish fed low energy (7 kJ kg^{-1}BW day) diets should be offered food at least four times a day. The frequency of feeding in all diets, therefore, was maintained at four times per day in order to maintain consistency among treatments. Fish were individually weighed each week and the feeding rate adjusted accordingly.

Faeces were collected daily, from the end of the first week until the end of the experiment. The proximate composition and energy density of dietary ingredients, food, faeces and fish were determined as described in the previous experiment.

5.4.2.3 Gastric evacuation studies.

Five diets were selected according to their composition and energy density. These were diets D11.(40), D6.(34), D12.(28), D13 (22) and D14.(15). The energy density in these diets changed from 18 to 7 kJ g^{-1},thus totalling five separate experiments.

Experiments were carried out in two adjacent recirculating systems (28°C), collectively referred to as Experimental system 3, which consisted of a series of circular tanks, 91 in volume. Details of the system were presented in Section 3.3.3. Fish of average weight 75g were stocked individually in each tank, two weeks prior to each trial, and fed at a rate of 12 g protein kg^{-1} day^{-1} for two weeks. At the end of two weeks, fish were offered food at a rate of 10g kg^{-1} (1% BW) in the morning (8.00 hrs), and the rate of gastric evacuation measured by serial slaughter method. This involved measuring the remaining stomach contents after sacrificing numbers of fish at pre-defined or random intervals.

Thirty minutes after offering food, three fish were killed with an overdose of benzocaine, dissected, and the dry matter content of the remaining food determined by drying digesta in an oven at 103 °C for 12 hours. Every two hours thereafter, three fish were randomly selected and the same procedure carried out until the stomachs were found to be completely empty. All five trials were carried out within a total period of 12 weeks and similar- sized fish were used in each trial.

5.4.3 Results.

The growth rates of *O.niloticus* fed on different diets are presented in Table 5.21. The highest observed growth in terms of total weight gain, relative growth rate over the experimental period or on the basis of number of days fed, and specific growth rate, was among fish fed diet D13.(22), which contained 22% protein and 10 kJ g^{-1} metabolisable energy, on the basis of mammalian physiological fuel values. Problems with statistical procedures were once again encountered in this experiment. According to Tukey's test, only fish groups fed diets D13.(22) and D6.(34) were significantly different at 0.05 (F = 1.98) level on the basis of total weight gain. Analysis using Duncan's test, however, concluded that the growth rates of fish fed diet D13.(22) were different from those fed diets D1, 2, 4, 6 - 9, and also suggested that the weight gain among fish fed diet D5 differed from that of those fed diet D6. According to Tukey's test, neither the relative growth rates nor the specific growth rates of fish in various treatments were significantly different. However, Duncan's procedure concluded that the rate of growth of fish fed diet D13 was significantly different from groups fed diets D2, 4, 6 - 9. The growth rate of fish fed diet D10, which contained 16% protein, was significantly higher than groups fed diets D2, 4, 6 and 7, all of which contained 34% protein. Analysis by Duncan's test also showed that the growth rate of the diet D5-fed group was different from groups fed diets D6 and D7 [F-ratios for relative and specific growth rates are 2.206 ($P<0.01$) and 2.086 ($P<0.01$) respectively]. Hence, the interpretation of the data from this experiment is entirely dependent upon the statistical procedure employed.

It is interesting to note that fish with the highest observed rates of growth had been offered diets containing 22%

Table 5.21 Initial mean weights (g), final mean weights (g) and total mean weights (g) and growth indices for groups of fish fed on different diets in Investigation 3.

Diet	Initial weight (g)	Final weight (g)	Total weight gain (g)	Relative growth rate: g kg^{-1} BW day	Relative growth rate: g kg^{-1} BW day fed	SGR % day^{-1}
D.1 (34)[1]	17.13 ± 3.41	39.11 ± 10.90	22.32 ± 9.68	33.70 ± 12.95	38.66 ± 14.85	2.097 ± 0.57
D.2 (34)	17.49 ± 3.34	36.31 ± 6.23	18.81 ± 4.25	28.0 ± 7.18	43.34 ± 8.24	1.877 ± 0.33
D.3 (34)	18.07 ± 3.47	40.73 ± 11.35	23.21 ± 8.68	33.20 ± 8.68	38.08 ± 9.96	2.104 ± 0.38
D.4 (34)	17.99 ± 3.22	36.83 ± 7.77	18.84 ± 6.39	27.31 ± 9.56	31.32 ± 10.96	1.821 ± 0.46
D.5 (34)	17.34 ± 2.91	43.58 ± 6.65	25.79 ± 6.09	38.87 ± 13.38	44.58 ± 15.35	2.319 ± 0.50
D.6 (34)	17.72 ± 3.92	33.86 ± 6.86*	16.34 ± 5.69*	24.96 ± 9.75	28.63 ± 11.19	1.696 ± 0.52
D.7 (34)	18.31 ± 3.13	37.21 ± 7.78	18.90 ± 6.48	26.87 ± 10.14	30.82 ± 11.64	1.797 ± 0.48
D.8 (28)	17.78 ± 3.84	37.46 ± 7.13	19.67 ± 6.81	30.07 ± 14.14	34.49 ± 16.22	1.920 ± 0.620
D.9 (22)	17.82 ± 4.19	37.18 ± 7.28	19.37 ± 7.70	29.96 ± 13.45	34.37 ± 15.43	1.906 ± 0.69
D.10 (15)	15.85 ± 2.32	41.69 ± 9.95	25.84 ± 8.11	41.40 ± 8.78	47.49 ± 10.07	2.445 ± 0.33
D.11 (40)	19.04 ± 3.79	42.53 ± 11.25	23.50 ± 9.42	32.03 ± 11.07	36.74 ± 12.70	2.032 ± 0.52
D.12 (28)	19.33 ± 3.48	43.36 ± 10.90	24.04 ± 10.76	33.26 ± 16.21	38.15 ± 18.60	2.041 ± 0.72
D.13 (22)	18.38 ± 3.19	48.84 ± 15.02*	30.45 ± 12.64*	41.70 ± 14.51	47.83 ± 16.65	2.417 ± 0.59
D.14 (15)	17.17 ± 2.91	38.61 ± 8.05	21.44 ± 6.38	32.50 ± 10.39	37.28 ± 11.91	2.063 ± 0.45

* denotes groups which are significantly different ($P<0.05$) using Tukey's test.

[1] figures in parenthesis are nominal protein content (% dry weight)

protein, whilst the second highest had been fed on a diet containing 16% dietary protein with approximately 2% lipid. This indicates that *O. niloticus* can tolerate up to 50% indigestible filler in their diets, at least over short time periods (*i.e.* 40 days in this experiment), when the dietary protein density is as low as 15 - 16%. This experiment also demonstrates that the ability of *O. niloticus* to obtain sufficient protein and total energy for maximum growth from diets containing metabolisable energy as low as 7 kJ g^{-1}.

The average body composition of *O. niloticus* under different dietary treatments is shown in Table 5.22. Variability within treatments is negligable, as three fishes were homogenously mixed (4 replicates for biochemical analyses for each treatment). Statistical procedures were not employed for this reason. However, it is clear from Table.5.22 that relative body lipid content decreases with low enegy diets as well as with high carbohydrate diets. The relative protein content is also generally higher than with diets with lower lipid content.

The apparent digestibilty coefficients for protein, lipid and nitrogen free extract (NFE) are presented in Table 5.23. Variability is again less within treatments, probably as a result of the long period of faecal production and the fact that data were pooled. Very high faecal production was associated with diets containing higher cellulose content, and this condition forced tanks to be cleaned more than twice per day. It was not possible to collect sufficient faeces from fish fed diets with low cellulose content, and the results are presented on pooled samples for all fishes within treatments. The small differences among treatments, therefore, is ignored and no statistical analyses were undertaken.

Protein digestibilty on average approximated 85%, and this shows that the combination of casein and gelatin is

TABLE 5.22 Mean (± 1 SD) proximate body composition of groups of *O. niloticus* grown on various diet formulations in Investigation 3.

Diet No.	Moisture % dry wt.	Protein % dry wt.	Lipid % dry wt.	Ash % dry wt.
Initial	69.71 ± 2.53	49.79 ± 1.63	30.07 ± 2.26	18.04 ± 0.52
D1 (34)	70.80 ± 1.53	54.21 ± 0.43	24.47 ± 0.75	17.28 ± 1.32
D2 (34)	69.95 ± 1.05	53.61 ± 0.68	24.52 ± 1.23	16.27 ± 0.97
D3 (34)	69.63 ± 1.51	53.30 ± 0.82	29.42 ± 0.56	15.38 ± 0.89
D4 (34)	71.12 ± 0.58	53.30 ± 0.91	28.62 ± 0.88	16.16 ± 0.97
D5 (34)	69.19 ± 1.37	51.81 ± 0.79	29.98 ± 1.01	16.34 ± 0.97
D6 (34)	70.54 ± 3.31	52.03 ± 1.10	30.49 ± 2.41	15.42 ± 1.03
D7 (34)	69.79 ± 1.49	51.52 ± 0.56	31.79 ± 1.34	15.48 ± 0.95
D8 (28)	70.62 ± 1.29	54.98 ± 1.19	27.00 ± 0.96	15.33 ± 0.66
D9 (22)	70.34 ± 0.79	53.83 ± 0.82	28.16 ± 0.84	17.01 ± 0.50
D10 (15)	70.67 ± 1.21	56.00 ± 0.58	24.65 ± 0.99	17.17 ± 0.87
D11 (40)	69.19 ± 2.05	51.81 ± 0.30	32.51 ± 0.83	14.61 ± 0.77
D12 (28)	69.20 ± 1.41	51.93 ± 1.10	29.53 ± 1.37	16.19 ± 0.85
D13 (22)	69.81 ± 1.04	52.51 ± 0.43	28.69 ± 2.54	17.23 ± 0.95
D14 (15)	69.66 ± 1.16	51.14 ± 0.77	31.23 ± 0.40	16.19 ± 0.78

TABLE 5.23 Digestibility, FCR and PER indices for groups of fish grown in Investigation 3. (* Pooled samples).

Diet No.	Protein % dry wt.	Lipid % dry wt.	NFE % dry wt.	FCR	PER
D1 (34)	84.23*	99.99*	96.29	$1.546^{b} \pm 0.227$	$1.921^{a} \pm 0.304$
D2 (34)	81.40*	91.14*	93.79*	$1.694^{b} \pm 0.218$	$1.785^{a} \pm 0.249$
D3 (34)	82.85 ± 3.51	95.40 ± 1.07	92.25 ± 2.06	$1.527^{b} \pm 0.286$	$2.015^{a} \pm 0.341$
D4 (34)	85.40 ± 1.28	97.73 ± 0.19	92.32 ± 0.74	$1.674^{b} \pm 0.196$	$1.802^{a} \pm 0.220$
D5 (34)	89.36 ± 0.72	98.79 ± 1.414^{-3}	92.01 ± 1.53	$1.386^{b} \pm 0.187$	$2.207^{a} \pm 0.278$
D6 (34)	84.58 ± 0.61	99.05 ± 0.29	84.44 ± 1.37	$1.809^{b} \pm 0.456$	$1.724^{a} \pm 0.395$
D7 (34)	87.94 ± 1.23	97.83 ± 0.05	83.66 ± 1.19	$1.717^{b} \pm 0.212$	$1.771^{a} \pm 0.206$
D8 (28)	87.29 ± 1.23	93.87 ± 0.71	91.09 ± 0.59	2.305 ± 1.213	$1.809^{a} \pm 0.850$
D9 (22)	87.10 ± 1.68	94.68 ± 0.68	93.49 ± 0.73	2.627 ± 0.839	$1.865^{a} \pm 0.668$
D10 (15)	86.78 ± 2.20	97.04 ± 1.18	81.95 ± 0.68	2.662 ± 0.235	$2.286^{a} \pm 0.205$
D11 (40)	82.47*	99.99*	91.01	1.285 ± 0.251	$1.892^{a} \pm 0.334$
D12 (28)	87.54 ± 0.78	98.11 ± 0.26	87.09 ± 1.39	1.765 ± 0.170	$2.020^{a} \pm 0.200$
D13 (22)	88.93 ± 0.23	97.37 ± 0.35	84.03 ± 0.45	1.972 ± 0.454	$2.360^{a} \pm 0.490$
D14 (15)	86.04 ± 1.05	96.76 ± 0.32	87.70 ± 1.03	3.367 ± 0.480	$2.039^{a} \pm 0.305$

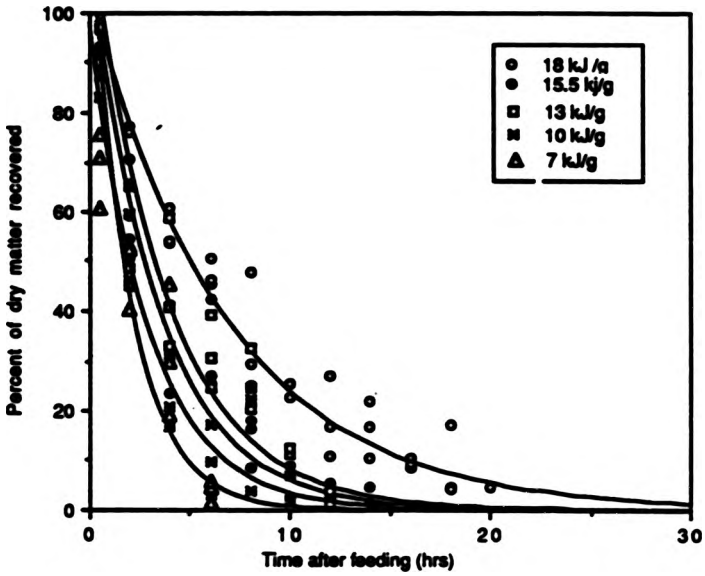
superior to fish meal in terms of digestibilty. Digestibility of lipid is as high as an average of 98%, and the digestibility of nitrogen free extract also reaches a value of 85 - 90%. It was noticed, however, that there was a cosiderable leaching of lipids from diets containing more than 15% lipid.

The data for five gastric evacuation studies are summarised in Fig.5.24. There is a considerable variation in gastric evacuation rate within treatments. However, the objective of the study was not to generate an exact mathematical description of the gastric evacuation model for tilapias, but rather to study the relative shape of the curve and the time taken to evacuate the stomach completely following a single meal. The total time taken to evacuate a single meal was found to be a function of nutrient density. The time taken to evacuate a complete meal in relation to energy density was:

18 kJ g^{-1} : 20 - 22 hrs
15 kJ g^{-1} : 16 - 18 hrs
13 kJ g^{-1} : 14 - 16 hrs
10 kJ g^{-1} : 12 - 14 hrs
7 kJ g^{-1} : 6 - 8 hrs

In fact, there were 10 gastric eveacuation trials in total (2 purified diets and 8 semipurified diets, including those above) with different energy density. However, time does not permit presentation of all data, or more vigorous mathematical analysis.

Fig. 5.24. Gastric evacuation rate, expressed as % mean dry matter recovered, *versus* time after feeding (hrs) for groups of fish fed on diets of different energy density (Investigation 3).


Percent of dry matter recovered
100
80
60
40
20
0
0
10
20
30
Time after feeding (hrs)
18 kJ /g
15.5 kj/g
13 kJ/g
10 kJ/g
7 kJ/g

5.4.4 Discussion.

Statistics is known as the mathematics of uncertainty. Unfortunately, however, some statistical procedures tend to increase uncertainty rather reduce it. Many statisticians (*eg.* Zar, 1984) have expressed concern that using Newman-Keuls test tends to provide more significant results than Tukey's test. This was found when the present data were subjected to Newman-Keuls analysis and the results from Newman-Keuls procedeure and Duncan's agreed in all cases. If one favours Tukey's procedure for its robustness, it can be concluded that *O. niloticus* is able to utilise dietary lipid within the range employed in this study in such a way that each lipid energy unit can replace 2.25 carbohydrate energy units. Garling and Wilson (1977) reported a similar finding for channel catfish and the this evidence once again shows the similarity in energy utilisation between these two species. Contrary to the common belief that carbohydrate increases body lipid, Table 5.22 indicates that carbohydrate in fact decreases average body lipid. This should not be surprising, as it is probable that dietary lipid is more lipogenic than carbohydrate since the cost of lipid deposition is relatively lower (Reeds *et al.*, 1982).

The present study also provides empirical evidence for the remarkable ability of tilapias to thrive on comparatively high levels of indigestible matter. Fish fed on a diet diluted with 50% cellulose ingested all food offered, and grew at a marginally higher rate than fish fed a diet with higher dietary protein density. Bromley and Adkins (1984) reported an increase in dry matter intake of rainbow trout, up to a dietary dilution of 30% cellulose, and observed rapid rates of growth. *Tilapia zilli* in the natural enviromnment thrive on aquatic weeds containing 30% cellulose, and Bowen (1979)

postulated that the rapid growth of *Oreochromis mossambicus* in lake Sabaya was a result of consumption of detritus containing less than 14% protein. Bowen (1982) claimed that the tilapias in nature consume food of less than 14% protein content. These observations are substantiated by the present study.

The results, however, show the unsuitability of percentage as a unit to express the protein or energy requirements. Fish grew equally well within the range of 16 - 40% protein when they were offered the same amount of protein $kg^{-1}BW\ day^{-1}$. Hence, the expression of the protein requirement for *O. niloticus* as 16% or 40%, is nonsensical, unless the feeding rate is specified. A similar observation has been reported for carps and for rainbow trout by Ogino (1980), in which he observed that by doubling feeding rate from 2 to 4% $BW\ day^{-1}$, the protein density in a diet for carp and rainbow trout can be decreased from 60 to 30%. However, *O. niloticus* seems to have a unique ability to extract protein from food when the protein density is much lower.

O. niloticus' ability to extract protein at low protein density is because it can increase the rate of gastric evacuation, without any detrimental effect on digestion. By reducing energy and nutrient density by 50% (approximately 16 to 8 kJ metabolisable energy g^{-1}), fish emptied their stomachs within approximately half the time. These results agree with the concept that gastric evecuation is controlled by the energy density of the diet (Jobling, 1986). It was also apparent that the pattern of total dry matter evacuation is more linear in form with low energy density diets. However, the data generated in the present study should be subjected to a thorough investigation before arriving at a final conclusion.

The present experiment may be critisised on several grounds. One major point was its short duration (40 days).

Many other similar studies, however, have been carried out for only 6 - 10 weeks duration. The problem of unequal initial size of fish (approximately 16-20g in weight range) resulted in a large variability in weight gain.

Chapter 6

DISCUSSION

It is often said that the objective of a scientific study should be oriented towards discovering new facts, rather than attempting to reduce available information to a few mathematical equations. This may well be true, provided that most of the basic concepts in the field under study are well defined and understood. Hence, one may proceed to make new discoveries by selecting a relatively narrow field of study. By contrast, the literature on fish biology is extremely rich in factual or empirical data, but as was shown in Chapter 2, current concepts on growth and feeding relationships of fish still highly confusing. This probably has much to do with the fact that the literature in fish biology does not possess a unifying theory explaining the growth phenomenon. This is the principle problem of concern in the present study, and it appears to be the this seems to be the first attempt to link all this observations and various concepts into a single explanatory model of fish growth.

Although the complexity is relative and dependent upon the eye of the observer rather the system itself, it was decided to simplify as much as possible the way in which concepts would be presented in this thesis. It was realised, however, that the process of simplification is far more time-consuming than the abbreviation of concepts to a few mathematical equations. On the other hand, the mathematical manipulation of the system is always been undertaken in order that predictions hidden in apparent complexity emerge (*eg.* Sibly and Calow, 1986, 1987). Whenever predictions are simple and straight-forward, mathematical tools were omitted.

Once the basic concepts were clarified at the planing stage (these are presented in Chapter 3), the experimental

part of the present study was designed as follows. It was decided to study the relative requirement of building blocks (especially protein) and energy that *O. niloticus*. The functional relationship between growth and feed intake was then investigated. The intent was to use this relationship to quantify the nutrient requirements of *O. niloticus*., so that it would then be possible to assess the constraints imposed by food items such as indigestible filler on fish. The knowledge of nutritional requirements and the constraints imposed by food items by their composition could then be employed in a study of more interesting relationship such as that between natural foodstuffs (*eg.* algae) and growth. Although the experiments exploring this theme were carried out, the results are not presented here due to lack of available time. The data presented in this thesis are, therefore, incomplete, although the experimental presented have been selected to illuminate and clarify basic concepts presented in the growth model in chapter 3.

From the outset, the present study attempted to clarify the controversy on relative protein requirement or PE ratio for tilapias. Two experiments were carried out to study in this aspect. Unfortunately, however, the level of aggression in aquarium *O. niloticus* population resulted in the failure of all group experiments. As a consequence, a decision was taken to re-analyse the available data from the literature, and it was found that the data closely agrees. It was normal practice to present the data on proximate composition on wet weight basis. However, this procedure is misleading in a comparative study, since the increasing number of variables make difficult to compare data with many other animal species.

It was pointed out that the advantage of presentation of protein and energy ratio as NPE/PE (= 1.72 for *O niloticus*; non-protein energy/protein energy) because that it indicates the

number of energy units that can be added to a diet without any detrimental effect on growth. It was also shown that NPE can be further analysed into LE/CE (lipid energy/ carbohydrate energy) in terms of mammalian physiological values. A value of 2.25 was calculated for tilapias fed diets containing 5 - 20% dietary lipids.

The unsuitability of the percentage as a unit of quantifying nutrient requirement was recognised at the project planing stage, and experiments are designed accordingly. Bowen (1987) presented a similar argument whilst this thesis was in preparation. His argument, however, aimed at only to clarify the misconception commonly expounded in the fish literature that fish have a comparatively high protein requirement. The argument in this thesis developed further.

The percentage requirement undoubtedly advantageous in the least cost feed formulation. However, it has no real meaning beyond this, since the concept implicitly implies *ad libitum* feeding, as in terrestrial animals. It was shown in the present study that fish tend to overconsume food of higher density for a short period of time and results in retarded growth and poor utilisation of food offered. This discovery has special importance for aquaculture, since it is not possible to observe whether fish have consumed the given meal or not. The upper limit for synthesis, therefore, has to be determined in order to minimise the cost and maximise the production per unit time.

It was postulated that growth retardation may be the results of energy storage problem and/or power rating problem that fish is faced under *ad libitum* feeding. Jobling's (1988) claim that the retarded growth and poor utilisation of 'offered food' are solely problems characteristic to formulated diet is disputed in the present study. It was shown that this is a result of over-consumption and not related to

over-digestion *per se*. The problem can overcome with *O. niloticus* if restricted feeding regime is employed. However, the way in which a restricted feeding regime can be established in practice is another problem, as the dominant fish may, anyhow, over-consume high energy density diets, at least for short period. These concepts, however, remain open to criticism and further detailed studies are deemed necessary.

This study also highlights a close resemblance between *O. niloticus* with channel catfish in terms of NPE/PE (or P:E) ratio, protein and carbohydrate utilisation *etc.*, and also shows that there is a similarity between protein requirement of *O. niloticus* and chicken. These results, together with those of Bowen (1987), may further help to dispel the common mis-beleif that the fish require more protein than mammals and birds.

The remarkable ability of *O. niloticus* to tolerate high cellulose diets (as high as 50%) are presented in this thesis. Fish achieved this by accelerating gastric evacuation rate, and the digestion model presented in the chapter 3 (modified after Sibly, 1981) seem to be applicable for *O. niloticus*. Unfortunately, however, the time constraints on completion of the thesis meant a detailed analysis had to be abandoned. Data were presented only to demonstrate that the model is sufficient to explain the gastric digestion in fishes.

The introduction of variety of concepts from other related field to fish biology in the present study may appear unnecessary. There are a considerable number of gastric evacuation models for fish, but all are designed to help estimate food consumption in nature. Sibly's (1981) model is, however, different from those as it contributes to greater understanding of growth, growth efficiency and feed intake relationship as discussed in Chapter 3.

It is shown for *O. niloticus* that the functional

relationship of feed intake verses growth is linear, irrespective to the way of data presentation. This implies that when a formulated diet was offered, waste production is a linear function of feed intake. So called specific dynamic action has been shown a linear function of feed intake, and it has been postulated that this may be responsible for major part of cost of growth. This implies that one of the most important concepts in nutritional energetics is absorbed energy.

The concept of absorbed energy can be utilised in developing predictive models. The present study, however, is not aimed at validation of the concept presented here, as all the data necessary cannot be generated in a single, short-term study. The aim of this section is, rather, to demonstrate the usefulness of this approach in order to provide a tool for production management and also to define goals for future research.

Growth can be expressed as:

Growth = Absorbed Energy - Dissipated Energy [6.1]

As mentioned earlier ingested energy can be expressed as a function of body weight.

$$IE = iW^{n}$$

Suppose a fish ingests at a level immediately below S_{sat}, then, irrespective of the digestive strategy, a constant fraction of energy will be absorbed. Fish will breakdown part of the protein and liberate NH_3. The energy value for liberated NH_3, therefore, has to be corrected (*i.e.* metabolisable energy). Since structural material synthesis is a linear function of absorbed energy (and hence metabolisable energy), this can be estimated from a growth vs absorbed energy curve or by using standard laboratory calorimetry.

Therefore:

Corrected absorbed energy (metabolisable energy)= $a(iW^n) = hW^n$ [6.2]

Over a sufficient period of time, the rate of food intake (and hence hW^n) is proportional to metabolic body size (Kleiber, 1961). Hence, the exponent $n \approx b$ which is from metabolic body size.

Dissipated energy comprises heat costs of maintenance and induced metabolic activities, which can be expressed as a factor of standard metabolic rate.

Total heat cost = $d(aW^b) = a_1W^b$

The heat cost must be estimated on the basis of absorbed energy equivalent. In order to accomplish this task a new growth efficiency for absorbed energy which is similar to Kleiber's partial efficiency is defined.

$$K_A = G/(A - M_n)$$

where K_A is the growth coefficient of absorbed energy or metabolisable energy, G = growth, A = absorbed energy and M_n = maintenance energy and the K_A is a constant.

The efficiency of energy utilisation for maintenance and growth may be different. However, if the difference is assumed to be negligible due to the small body size of fishes, the growth coefficient K_A can be used to estimate the absorbed energy equivalent of heat cost.

Absorbed energy equivalent of heat cost = a_1W^b/K_A [6.3]

K_A can be expressed as a function of body weight similar to K_3.

$$K_A = pW^{-r}$$

Substituting in equation [6.3]

Absorbed energy equivalent of heat cost = $a_1W^b/pW^{-r} = kW^m$ [6.4]

where $k = a_1/p$ and $m = (b + r)$.

The metabolic turnover rate (Kleiber, 1975) is expressed as $(M/W) = aW^{b-1}$, which indicates the fraction of body energy

given off as heat. There is evidence that oxygen consumption and growth rate are linearly related (Jobling, 1985). In other words, the total heat cost and the energy retained are linearly related. Hence the fraction of heat cost from the body plus ingested energy should be directly proportional to the fraction retained over a period of time. This implies the exponent b-1 metabolic turnover rate should be equal (or close) to exponent r from growth efficiency (K_1) vs body weight relationship. The exponent 'm' of absorbed energy equivalent of heat cost is, therefore, close or equal to 1. In fact, there is empirical evidence that in cod, *Gadus morhua* $K_1 = 0.73W^{-0.15}$ (Jones, 1976) and metabolic rate $Q = aW^{0.87}$ (in a starved or fed state, Saunders, 1963); $Q = 1W^{0.82}$ (Edwards, 1972).

Substituting equations [6.2] and [6.4] to equation [6.1] and expressing the growth term in a differential equation form:

$$dW/dt = hW^n - kW^g$$

This is the Von Bertalanffy equation and, according to the above reasoning, the exponent n is equal to the exponent from metabolic body size and m is equivalent or close to 1. This development may be an encouragement for many fisheries scientists as most authors unable to provide a physiological justification to Von Bertalanffy curve in its original form (*eg.* Paloheimo and Dickie, 1965; Ursin, 1967; Ziaka, 1973; Pauly, 1979; Pauly and Silvert, 1987). The above development, however, partially agrees with Zaika (1973) and Sibly and Calow's (1985) interpretations and provides a method to calculate four parameters in the equation. Parameters n, h, m and k can be calculated from the rates of feed intake, metabolic rate and growth efficiency verses body weight relationship. If m=1 a general solution can be found as integrated by Pauly (1979) and m =/1 numerical integration

techniques has to be utilised. In this way, the Von Bertalanffy equation can be utilised with a physiological meaning.

Winberg (1984) claimed that if the Von Bertalanffy equation is presented in the form of 2.22:

$$dW/dt = hW^{1-\mu} [1 - (W_t/W_\infty)^{\mu}],$$

it has no connection to balance processes of assimilation and dissimila-tion. When expressed in this form, however, it has a clear biological meaning. If equation 2.22 is presented in the form of:

Growth = Absorbed energy X retarding factor.

According to the above reasoning, $hW^{1-\mu}$ is proportional to metabolic rate and therefore $|1-\mu| \propto |b|$ and $h \alpha a$. Therefore:

metabolic rate = $chW^{1-\mu} = aW^{1-\mu}$

metabolic turnover rate = $aW^{-\mu}$

metabolic turnover time = $(1/a)W^{\mu}$.

The ratio of metabolic turnover time

$$= (1/a)W_t^{\mu}/(1/a)W_\infty^{\mu} = (W_t/W_\infty)^{\mu}$$

Metabolic turnover time indicates the number of days (or time) required to give off the total chemical energy content of the body (Kleiber, 1975).

In order to avoid the risk of death by starvation newborn animals should grow at a faster rate. It is known that the exponent of metabolic rate vs body weight $\propto$ 1 for newborn fish. Hence $dW/dt = hW$ suggests exponential growth. The fraction $[1 - (W_t/W_\infty)^{\mu}]$ which is determined by metabolic turnover time, changes from 1 (for newborn fish) to 0 for mature animals, when growth ceases.

This thesis has attempted only to clarify the concept of fish growth and its efficiency. However, the above interpretation of Von Bertalanffy formula may facilitate further work in this field. As the Von Bertalanffy formula prove to be an excellent fit for many fishes, this may be an

immense important to aquaculture in prediction of growth. Hence, further clarifications and improvements of the concepts presented in this thesis appears to be promising for years to come.

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