

STUDIES ON FOOD INTAKE, DIGESTION AND  
GROWTH OF OREOCHROMIS NILOTICUS

By

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This thesis is dedicated to my children

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ABSTRACT

Factors affecting the daily food intake, digestion and growth of O. niloticus were investigated for fish of varying weights. A preliminary experiment showed that daily food intake increased with increasing feeding frequency up to six meals per day. Further increase in feeding frequency had no significant effect on total daily food intake. This feeding frequency was subsequently used to investigate the relationship between maximum daily food intake and fish weight. Three different models were used to describe this relationship, the best fit being given by  $\text{Log } M = a + b \text{ Log } W$ . Maximum daily food consumption was found to vary between 2.4% and 9.1% b.w. for 200g and 10g fish respectively. However these values appear high and are not recommended for commercial culture situations. The satiation meal size (maximum food ingested in a single meal) for fish of different weights was also investigated and varied between 1.27% and 1.96% b.w. for 200g and 10g fish. A time of 10-15min was found to be adequate to satiate different weights of fish using stomach capacity as the criterion.

The effect of pre-feeding deprivation periods of 24h up to 96h on single meal food intake, liver weight and intestine length for different weights of fish showed that food intake increased significantly with starvation period up to 72 hours. However further increase in starvation period depressed food intake. No significant effect on liver weight was observed, but increasing starvation period decreased the intestine length significantly. Furthermore the study suggested that volume and colour of bile could be used as useful indicators of recent feeding history in fish.

Following initial trials to compare techniques, the method of sequential slaughter was used for all digestion studies in O. niloticus.

Quantitative relationships between gastric evacuation coefficient and certain factors affecting gastric evacuation coefficient and time were established. These factors were temperature, fish weight, meal size, food composition and pre-feeding deprivation periods.

From the stomach evacuation studies the daily food consumption rates for different weights of fish (based on the assumption that appetite is closely related to stomach evacuation time) were calculated and recommended for feeding of O. niloticus in intensive culture systems. The recommended daily food consumption was found to vary between 1.1% and 4% b.w. for 200g and 5g fish respectively.

Digestive enzyme activities were also investigated. It was shown that activity of pepsin-like, trypsin-like and  $\alpha$ -amylases could be increased significantly by increasing the dietary level of protein and carbohydrate. Lipases did not show any change in activity with increasing dietary lipid level from 11% to 18%. Pepsin- and trypsin-like enzyme activities were observed to decrease with increasing fish weight, but  $\alpha$ -amylase activity increased with fish weight. Overall it was shown that ingested food helps regulate digestive enzyme activity and fish deprived of food for between 24h and 96h showed marked reductions in digestive enzyme activities.

Long term trials were also conducted to investigate the effect of feeding frequency, fish weight, feeding rates and food composition

on growth, food utilization and carcass composition. The study showed that the optimum feeding frequency for O. niloticus is twice per day. Increasing the feeding frequency beyond two meals per day results in a significant increase in food intake with no corresponding increase in weight gain or specific growth rate. The relationship between growth and feeding rates showed that specific growth rate increases to various degrees with increasing feeding rate. Comparison between two weights of fish (6.86g and 14.27g) in terms of food intake for maintenance, optimum and maximum growth showed that smaller fish had higher requirements than larger fish. The maximum feeding rates obtained for both weights of fish (6.86g and 14.27g) were in close agreement with the recommended rates derived from the gastric evacuation studies.

Further investigation of the effects on food composition showed a large effect on all nutritional parameters measured. A significant protein sparing effect was observed with increasing dietary level of lipid and carbohydrate.

The results of this study are discussed in relation to the data available for a variety of fish species.

## GLOSSARY

- M = Meal size in (g)
- W = Fish weight in (g)
- h = Hours
- G.R.L. = Gut relative length
- s.v. = Stomach volume (ml)
- s.c. = Stomach capacity  $[\frac{\text{st. volume}}{\text{fish weight}} \times 100]$
- S.E.T. = Stomach evacuation time
- S.E.R. = Stomach evacuation rate (g/h)
- T.E.T. = Total evacuation time
- S.E.C. = Stomach evacuation coefficient
- 
- Y<sub>t</sub> = Stomach contents in dry weight at time (t)
- Y<sub>o</sub> = Stomach contents in dry weight at time (o)
- R<sub>v</sub> = Evacuation coefficient (volume model)
- R<sub>a</sub> = Evacuation coefficient (surface area model)
- R<sub>e</sub> = Evacuation coefficient (exponential model)
- 
- S.G.R. = Specific growth rate (% wt/day)  
The percentage increase in body weight per unit time
- F.C.R. = Food conversion ratio  
The amount of dry food fed per unit live weight gain of fish
- P.E.R. = Protein efficiency ratio  
The weight gain of fish per gram of crude protein consumed
- A.N.P.U. = Apparent net protein utilization  
The apparent efficiency of deposition of dietary protein as body tissue

## 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

With the ever increasing global human population, and through improvement in health care and increasing life expectancy, food shortages, especially in developing countries, are bound to become more severe. Aquaculture can contribute to the alleviation of protein malnutrition as well as diversification of human diet and should be encouraged in Third World countries (Iverson, 1976).

Increasing protein production through the culture of fin fish will require a thorough understanding of the nutrition of the cultivated species. This will maximise conversion of feedstuffs of low nutritional value for direct human consumption, to high quality fish protein (Jauncey, 1982; Jauncey & Ross, 1982).

This thesis attempts to improve understanding of aspects of food intake, digestion and growth in Oreochromis niloticus. This species is the most important representative of the most widely cultivated group of warm, freshwater fin fishes, the tilapias (Bardach et al., 1972; Balarin & Hatton, 1979).

### 1.1 Food Intake

Several methods are available for measurement of food consumption (ingestion) by fish. Direct methods are based on offering items of food of known weight and number to fish and observing how many are eaten (Windell, 1966; Windell et al., 1969; Elliott, 1972). An alternative to observing the amount eaten is to measure the difference

between the quantity of food offered to fish for a period of time and that remaining at the end of the feeding period (Brett, 1971; Wallace, 1973; Grayton & Beamish, 1977; Wootton, 1980) or by feeding the fish to satiation. Immediately after satiation feeding a group of fish are sacrificed and their food intake is determined by measuring their stomach contents (Brett & Higgs, 1970; Elliott, 1972; Peters & Hoss, 1974; Persson, 1979). Elliott (1975a, b) determined maximum daily food consumption of brown trout (Salmo trutta) of various sizes, at various temperatures, by multiplying the weight of food required to satiate the fish in a single meal by the number of meals which could be taken in a day. Rates of gastric evacuation in combination with weight of food in the stomach have also been used to estimate food consumption, especially of fish in the wild (Bajkov, 1935; Darnell & Meiroto, 1962; Windell, 1966; Swanson & Smith, 1973). A field method for estimation of food consumption was devised by Bajkov (1935) based on studies of white fish (Coregonus clupeaformis) sampled from their natural habitat using the following formula:

$$D = \frac{24A}{n}$$

Where D = daily food intake

A = average amount of food in the stomach

n = stomach evacuation time in hours.

However, this method assumes that the rate of digestion is independent of the quantity of food ingested and that feeding is continuous. Noble (1972) estimated the daily food intake of Perca flavescens by determining the average amount of food per stomach and the stomach evacuation time. By multiplying the stomach content by the number

of evacuation periods in 24 hours an estimate of daily food intake was obtained. Elliott & Persson (1978) have developed an equation which describes the amount of food in a fish stomach at a particular time, from which an estimate of food consumption can be obtained. They assumed, on the basis of many other publications, that the rate of gastric evacuation is closely described by an exponential equation and that for a short period the rate of food consumption will be constant. The actual amount of food consumed is given by:

$$c = \frac{(St - So e^{-R_e t}) Rt}{1 - e^{-Rt}}$$

Where  $c$  = food consumption

$R_e$  = stomach evacuation coefficient

$So$  = stomach content at the beginning

$St$  = stomach content at the end of the sampling period ( $t$ , hours)

Consumption of food by fish can also be estimated by indirect methods which are based on the relationship between growth and assimilation of nutrients or the amount of food consumed. The relationship between food intake and growth measured in the laboratory can be used to estimate food consumption from measured growth rates of fish in the natural environment (Baldwin, 1956; Hatanka et al., 1956; Warren & Davis, 1967; Carline & Hall, 1973; Elliott, 1975c; Jobling, 1983).

Gerking (1962, 1971) and Stirling (1971) used the Nitrogen Balance Method to determine food intake of bluegill sun fish and European bass, respectively. The amount of nitrogen lost through the gills, kidney and faeces was estimated; these losses were then added to the gain in nitrogen resulting from growth observed to give the total

nitrogen of the food intake. Ricken (1971) recommended four repeated experiments in a 24 hour period so that the mean rate of nitrogen loss can be estimated.

The daily food consumption rates of fish are influenced by a number of factors which include water temperature, size of fish, food composition, activity and metabolic rate of fish and the rate of passage of food through the digestive canal. Kapoor et al. (1975) review the factors reported to influence food intake by fish and they consider that many of these factors may alter the rate of feeding by altering the rate of digestion of food in the digestive tract.

A change in water temperature has been observed to alter food intake and feeding frequency in several species of fish (Rozin & Mayer, 1961; Brett, 1971; Elliott, 1975a, b; Bassimi, 1978; Gwyther, 1978). Wootton et al. (1980) reported that the food consumption of Casterosteus aculeatus of a single size category increased from 5.6% b.w. to 12.2% b.w. with increasing water temperatures from 6°C to 19°C respectively. It has been suggested that the increase in food intake accompanying an increase in temperature is caused by a rise in metabolic rate and activity (Browen, 1946; Brett & Grove, 1979; Caulton, 1982) and/or a faster evacuation rate of food from the alimentary canal (Jobling et al., 1977; Elliott, 1972; Gwyther, 1978; Gwyther & Grove, 1981).

The weight of the fish also has an important effect on food consumption. It has been found that larger fish eat a greater absolute weight (g) of food per day than smaller fish, but that this meal constitutes a smaller percentage intake as a proportion of their body weight

(Hunt, 1960; Pandian, 1967; Brett, 1971; Lozoides, 1975; Wallace, 1973; Grove & Crawford, 1980; Wootton et al., 1980; Ross & Jauncey, 1981). The fact that food intake is larger the smaller the body size (as % b.w.) is in agreement with the fact that smaller fish have relatively higher metabolic and growth rates (Fry, 1957; Brett & Groves, 1979; Brett, 1979).

Previous history of feeding has also been found to affect feeding rate in fish (Fänge & Grove, 1979). A fish with an empty gut takes food actively whilst as the gut residium from previous food intake increases, feeding becomes less active (Lozoides, 1975; Crawford, 1977). When there is only one meal per day, the amount of food taken is large. As the frequency of feeding increases, the amount of food consumed per meal declines with increases in the total daily ration, but maximum daily ration is soon reached (Ishiwata, 1969; Grayton & Beamish, 1977; Thia-Eng & Seng-Keh, 1978).

The nutritional value of the food, and therefore the total food intake, depends on the relative proportions of dietary protein, lipid and carbohydrate (Windell, 1967; Hilton et al., 1983; Wang et al., 1985). Lee and Putnam (1973) showed that the voluntary food intake of rainbow trout increased in response to a decreased dietary energy content. Hence feeding rate usually increases to the metabolic need or the energy budget of fish as reported by Rozin and Meyer (1961) for Carassius auratus; Grove et al. (1978) for Salmo gairdneri, and Flowerdew and Grove (1979) for Sophthalmus maximus. More recently, Fletcher (1982) and Moctezuma (1982) showed that a compensatory increase in feeding rate of L. Limanda and S. maximus occurred only

when the energy content of the artificial diet was reduced to a low level (4.5 Kcal/g dry weight - 1 Kcal/g). It is evident that, apart from the energy value of the diet, the actual nutrient composition may also modify consumption. The levels of any one of the three major nutrients may significantly affect the feeding rate of fish. When the dietary energy level of the diet was kept constant, there were no significant changes in the food consumption of Ictalurus punctatus when offered diets with a protein range of 15% to 45% (Lovell, 1979). Above 45% dietary protein level, food consumption declined significantly. Wang et al. (1985) reported that daily food consumption was found to be affected by dietary protein or cellulose level in O. niloticus. Not only may high levels of protein reduce food intake, but the digestible energy to percentage protein ratio (DE:P) is an important factor in controlling food consumption in fish (Jobling, 1983). At a particular protein level, the addition of lipid or carbohydrate to the diet, so that the DE:P increased, resulted in decreased food consumption by Ictalurus punctatus (Page & Andrews, 1973; Lovell, 1979). Unless the correct nutritional balance is achieved, reduced food intake following addition of lipid to the diet may result in an overall reduction in protein intake which might reduce the growth rate of fish (Jauncey, 1982).

Food consumption increases with increasing energy demands from physical activities such as swimming and migration. Hunt (1960) investigated voluntary food consumption in wormouth (Chaenobryttus gulosus), gar (Lipidoseus platyrhincus) and bass (Micropterus salmoides). The daily food consumption of the bass amounted to 0.6 times that of wormouth and 0.4 times that of gar. These differences

in the daily food intake were thought to correspond with differences in activity; bass are generally far more active than gar. Activity level is influenced by temperature, but in addition to its effect on activity, temperature influences food intake via its effect on standard metabolic rate. Lowering the ambient oxygen concentration has been found to reduce food intake by Oncorhynchus kisutch (Herman et al., 1962) and Micropterus salmoides (Steward, 1962). Gruber (1960) quoted by Mabaye (1971) noted that tilapia reduced their food intake when dissolved oxygen levels dropped to 1.5 mg/l and that below this feeding ceased, probably because there is insufficient oxygen to support feeding activities.

#### 1.2 Digestion and Gastric Evacuation Time and Rate

Digestion depends on the physical and chemical state of the food consumed, in addition to the type and quantity of digestive enzymes secreted (Barrington, 1957; Kapoor et al., 1975). The three major classes of digestive enzymes found in fish and other vertebrates are proteases, carbohydrases and lipases, which catalyse the breakdown of proteins, carbohydrates and lipids, respectively. By the action of enzymes in the digestive fluids of the alimentary canal, the food components (protein, carbohydrate and lipid) are converted into smaller fragments and simpler molecules, which can be easily absorbed and assimilated. The digestive enzymes which are secreted into the lumen of the alimentary canal originate from the gastric mucosa, the pyloric caeca, the pancreas and the intestinal mucosa (Barrington, 1957; Kapoor et al., 1975; Fänge & Grove, 1979). A number of fishes (e.g.

carp and blennies) lack both a stomach and/or pyloric caeca, so that the proteolytic juices are mainly of pancreatic origin (Fänge & Grove, 1979).

Several factors have been shown to influence digestive enzyme activity in fish, including diet composition (Kawai & Ikeda, 1972; Hofer, 1979; Reimer, 1982), pH of gut contents (Dabrowski, 1979), temperature (Hofer, 1979; Dabrowski, 1979) and the stage of the fish development (Kawai & Ikeda, 1973; Dabrowski, 1979; Lauff & Hofer, 1984).

Rate of digestion was first recognised by Ricker (1946) as having an important bearing on fish production in terms of estimating the daily ration. Food consumption estimates of commercially important fish species in the wild as well as of cultivated fishes have been calculated from digestion rate measurements obtained in the laboratory (Elliott, 1975a, b; Thorpe, 1977; Elliott & Persson, 1978; Diana, 1979; Grove et al., 1985). Such estimates are based on the assumption that food intake is closely related to the available gastric capacity (Brett & Higgs, 1970; Ware, 1971; Elliott, 1975; Grove & Crawford, 1980; Singh & Srivastava, 1985). Rate of digestion is defined as the rate at which food passes from the stomach into the intestine and also along the intestine itself, which is usually measured as gram or mg dry weight of material digested per hour (Kapoor et al., 1975; Windell, 1978; Fänge & Grove, 1979). Digestion is considered complete when the stomach, intestine or the whole digestive tract becomes empty of all measurable food items (Windell, 1978). It is important to note that different terminologies have been used by

different workers when discussing evacuation rates and times from the stomach, intestine and the whole alimentary canal. Some of the common terms in use are 'digestion', 'evacuation', 'emptying', 'removal' and 'clearance' rate. However, most workers have measured and reported the amount of food removed from the stomach over a defined time period. Therefore it would be more appropriate to use evacuation or emptying as terms to avoid any implication of physiological digestion or absorption.

A variety of methods has been used to determine the rate of evacuation in different species of fish. The most commonly used method is to feed groups of fish a known ration, and to determine by sequential sampling the decrease in food content of the stomach after various time intervals. This method involves killing a number of fish at intervals of time after feeding and has been used by many workers (Darnell & Meierotto, 1962; Windell, 1966, 1967; Windell & Norris, 1969; Brett, 1970; Brett & Higgs, 1970; Tyler, 1970; Daan, 1973; Bassimi, 1978; Grove & Crawford, 1980; De Silva & Owoyemi, 1983). However the main disadvantage of this method is that it is wasteful of fish and is not suitable for very small fish due to the difficulties of reliable dissection and removal of stomach contents during the late stage of evacuation.

Another method employed is to force out the contents of the stomach at different time intervals after feeding, by employing a stomach pump (Elliott, 1972; Foster, 1977; Kennedy, 1981; Light et al., 1983; Brodner, 1984). This method is not successful when used with fish which have distinct stomachs with strong sphincter muscles, or long and coiled intestines (Talbot, 1985).

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An x-ray method was devised and used by Molnar & Tolg (1962), which involved the use of x-rays to observe the passage of bony and other hard parts of prey food items from the stomach as digestion proceeded. X-rays have also been used to follow the passage of meals containing barium sulphate as a contrast medium along the alimentary canal (Edwards, 1971, 1973; Jobling et al., 1977; Flowerdew & Grove, 1979; Ross & Jauncey, 1981; Bassimi & Grove, 1985). Sun et al. (1959) reported that test meals containing barium sulphate had an irritant effect on mammals. However, Jobling et al. (1977) and Edwards (1973) reported that this effect was absent in fish, at least up to an inclusion level of 25% barium sulphate in the diet. Most studies which rely on contrast materials in experimental diets usually necessitate force feeding of fish because of palatability problems. More recently, Talbot and Higgins (1983) developed a method for measuring the gastric evacuation rate in juvenile Atlantic salmon (Salmo salar) using iron particles and x-ray imaging. This method involves incorporating a known quantity (5% w/w) of very fine iron particles (in the size range 100 $\mu$  -200 $\mu$ m) in the diet and using an x-ray technique to count the number of Fe particles. This can be related to the quantity of food present in different regions of the gastro-intestinal tract. Since these authors found no problems with the palatability, this method has the advantage of allowing the fish to feed voluntarily. Food consumption and gastric evacuation rate have also been measured using food items labelled with an isotope that is biologically inert and poorly absorbed across the gut wall (e.g.  $Ce\ Cl^{144}_3$ ). The radioactivity in the stomach and intestine can subsequently be measured using a scintillation counter. As the radioactive concentration of the food is known, the amount of food consumed and its subsequent

evacuation can be measured (Peters & Hoss, 1974; Storebekken et al., 1981). These radioisotopic methods have the advantage that fish consume their food voluntarily. The major disadvantage lies with the attendant hazards of handling radioactive substances.

Other methods, used less frequently, to determine digestion rate involve using food labelled with dyes ( $C_{12}O_3$ ) that are biologically inert and poorly absorbed across the digestive system (Nawwab, 1982; Hadjichristophora & Grove, 1983). This method involves measuring the time between the first appearance of labelled food in the faeces and the last labelled evacuation.

A wide range of factors are known to influence both digestion rate and time in fish including temperature, fish size, meal size, food type, feeding history and meal presentation (Kapoor et al., 1975; Windell, 1979; Fänge & Grove, 1979). These factors are often inter-related in control of the rate of digestion. However, due to the variety of methods used in determining digestion rates it is difficult to make direct comparison of the effects of the controlling factors in different species of fish under different experimental conditions.

As fishes are poikilotherms, they depend on temperature for many of their physiological and biochemical processes. In general, it has been demonstrated that fish at low temperatures, within their physiological range, have relatively low evacuation rates (g/h) which tend to increase with rising temperature, reaching a maximum near the upper limit of temperature tolerance for the species (Molnar et al., 1962; Fabian et al., 1963; Brett & Higgs, 1970; Elliott,

1972; Jobling & Davis, 1979; Jobling, 1980; Ross & Jauncey, 1981). Both temperature and digestion rate follow an exponential curve, such that a positive linear relationship exists between temperature and  $\log_e$  digestion rate (Jones, 1974; Persson, 1979, 1981). A complementary negative relationship exists between temperature and  $\log_e$  gastric evacuation time (Windell et al., 1976; Jobling, 1980; Ross & Jauncey, 1981).

A large amount of evidence exists which shows that the digestion rate (g/h) of fish is normally faster with increasing meal size (Windell, 1966, 1969; Tyler, 1970; Beamish, 1972; Elliott, 1972; Flowerdew & Grove, 1979; Jobling & Davies, 1979; Grove & Crawford, 1980; Gwyther & Grove, 1981). In a few extreme cases the increased rate of evacuation is such that the total clearance time at a particular temperature is the same whatever the meal size (Windell, 1966, 1969; Kitchell & Windell, 1968; Brett & Higgs, 1970). In contrast, Steigenberg and Larkin (1974) reported a reduced gastric evacuation rate with increasing meal size for Ptychocheilus oregonensi. However, in the majority of species an increase in meal size does not lead to a complete compensation in gastric evacuation rate so that the larger the meal, the greater the total evacuation time. For instance, a five-fold increase in meal size only trebled the evacuation time in Limanda limanda (Jobling et al., 1977). Windell (1978) suggested that increased evacuation rate can only be achieved by changes in the volume of gastric contents pumped per peristaltic stroke or an increase in the number of strokes per unit time.

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Several authors have shown that for species like S. maximus (Flowerdew & Grove, 1979), Limanda limanda (Jobling et al., 1977), Stizostedium vitreum (Swanson & Smith, 1973) and a variety of gadoid species (Jones, 1974), larger individuals evacuated a given meal (in gram) more rapidly than smaller fish of the same species. This is in contrast to the situation for Lepomis macrochirus (Windell, 1966) and Perca fluviatilis (Persson, 1979, 1980, 1981) where for a given amount of food, evacuation rate and time are unaffected by fish size. However it has been found that stomach volume is directly proportional to fish weight (Jobling et al., 1977; Flowerdew & Grove, 1979; Nawwab, 1982), and that a meal size based on a known proportion of the fish weight should present the same stimulus to the gut. Pandian 1967; Jobling et al., 1977; Flowerdew & Grove, 1979, and Ross and Jauncey, 1981, reported that smaller fish had to evacuate a percentage body weight meal quicker than larger fish although there may be partial compensation in larger fish through increased evacuation rate.

Separate food fractions such as digestible organic matter and indigestible chitin or plant material may show differential movement through the digestive system (Windell, 1966, 1978; Windell et al., 1969; Jones, 1974). The chitinous exoskeletons of invertebrate prey species often remain in the stomach long after the digestible component has been evacuated. This is particularly true for large pieces of chitin which require softening prior to passing through the pyloric sphincter (Kionke & Windell, 1972). In several species of fish dilution of the basal diet with inert material or the offering of a low energy diet has resulted in enhanced evacuation rate (Grove et al., 1978; Flowerdew & Grove, 1979; Jobling, 1980; Hofer et al., 1982;

Hilton et al., 1983). This evidence suggests that there is an inverse relationship between caloric contents and evacuation rate.

Food presentation is a major complication in measuring evacuation rate. The difficulty of getting a large number of fish to consume a measured meal at the same time has been reported by several workers (Kapoor et al., 1974; Windell, 1978). Therefore, Windell (1966), Steigenberger and Larkin (1974), Jobling et al., 1977; Grove et al., 1978; Flowerdew and Grove, 1979; Gwyther and Grove, 1981; Ross and Jauncey, 1981, force fed their fish. Observations by Windell (1966) and Swenson and Smith (1973) showed that force feeding may depress digestion rate (g/hr) when compared with voluntarily feeding fish. Fish are very sensitive to even slight disturbance (Fletcher, 1982).

In many gastric evacuation studies it has been the normal procedure to deprive the fish of food before feeding the test meal (Jobling et al., 1977; Grove et al., 1978; Gwyther & Grove, 1981; Nawwab, 1982). Deprivation periods are known to cause distinct morphological changes in the digestive tract of some species (Windell, 1966; Elliott, 1972; Jobling, 1980). Such morphological changes might be expected to affect the evacuation rate and time (Elliott, 1972; Windell, 1978). An increase in deprivation time causes a decrease in evacuation rate and an increase in the total evacuation time (Laurence, 1971; Jobling, 1980; Talbot, 1985).

### 1.3 Growth Rate

Fish growth rates are determined by the combined effects of food quantity and quality. The quantity of food consumed is regulated through appetite to satisfy the fish energy requirement (Rozin & Mayer, 1961, 1964; Flowerdew & Grove, 1979; Fletcher, 1982).

Efficient production and growth of fish depends on feeding the best possible diets at levels not exceeding the dietary needs. Among environmental factors, temperature is probably the most important single abiotic factor affecting the life of fish (Kinne, 1970; Brett, 1979). The influence of temperature on the growth of fish has been well documented (Brown, 1957; Warren & Davis, 1967; Brett et al., 1969; Niimi & Beamish, 1973; Elliott, 1976; Caulton, 1982; Jobling, 1983; Saclauso, 1985; Hassan, 1986). As has been mentioned earlier (see 1.2) the activities of feeding and digestion are strongly influenced by temperature, which ultimately results in variation in the growth rate. A decrease in temperature therefore may result in a reduction of the feeding, digestion and metabolic rates which in turn will affect the growth rate of fish (Brett, 1970, 1979). Since fish are poikilotherms, their biological activities respond to Vant Hoff's Principle: raising the temperature by 10°C approximately doubles the speed of the reaction (Philips, 1960; Mesk, 1985). Therefore increasing temperature leads to an increase in growth rate until the optimum is reached. Above the optimum growth rate will decline (Andrew et al., 1972; Brett, 1979; Jobling, 1983). The decline in growth rate above the optimum temperature is thought to be caused by an increased maintenance requirement with increasing temperature (Brett, 1979).

Food consumption is the most influential biotic factor affecting the growth of fish (Brett, 1979; Smith, 1982). The importance of optimum feeding rate has been investigated by several workers for different fish species (Elliott, 1975; Huisman, 1976; Brett, 1979; Katonda, 1979; Bryant & Matty, 1981; Macintosh and De Silva, 1984; Hassan, 1986). Wurtsbaugh and Davis (1977) studied the effect of fish size and ration on growth of rainbow trout (Salmo gairdneri) and found that the effect of fish size on growth rate was dependent upon the food consumption rate of fish. These authors reported similar results to those of Brown (1946) and Brett and Shelbourn (1975), that the maintenance ration of fish decreased relatively with increases in fish size. Therefore the efficiency values for the large fish increased with increases of ration size from near maintenance levels then remained largely unchanged or decreased over a wide range of ration sizes, whereas those for the small fish continued to increase with ration increases to the highest level offered. The relationships between values of gross efficiency and food consumption rates are influenced principally by two factors which change with changes in fish size. First, weight specific standard metabolic rate generally decreases with increases in fish weight (Winberg, 1956; Brett & Groves, 1979). Because energy expended in standard metabolism (Averett, 1969; Niimi & Beamish, 1974), the relative maintenance rations are smaller for large fish than for small fish (Brown, 1946; Lee, 1969; Niimi & Beamish, 1974; Brett & Groves, 1979). Second, the maximum ration (as % b.w.) decreases with increasing fish size (Pandian, 1967; Lee, 1969; Brett, 1971; Elliott, 1975; Grove & Crawford, 1980). As consumption rates approach maximum levels, increasing portions of the food may be lost as faeces

(Kinne, 1960; Averett, 1969; Lee, 1969; Kelso, 1972) and respiratory losses may increase due to increase in specific dynamic action (Averett, 1969; Niimi & Beamish, 1974). Consequently, values of gross efficiency depend, in part, on the extent to which feeding rates approach the maximum feeding rates.

#### 1.4 Aims of the Present Study

Tilapia are among the most widely cultivated fish in the world, at present second only to cyprinids (Bardach et al., 1972; Balarin & Hatton, 1979). The species selected for the present study, the "Nile" tilapia (Oreochromis niloticus, Trewavas) is the most important member, from an aquaculture viewpoint, of this tribe (Balarin & Hatton, 1979).

To date studies on digestive physiology in this group are scarce (Ross & Jauncey, 1981; De Silva & Owoyemi, 1983).

The present programme of research was designed to investigate selected aspects of this subject area;

Firstly, techniques for quantifying food intake and evacuation rates were evaluated. The selected methods were then applied to evaluate the effects of the following variables on food intake and evacuation time, viz.

Fish weight  
Feeding frequency  
Pre-feeding starvation periods  
Meal size and gross diet composition.

Longer term trials were also conducted to evaluate the effect of feeding frequency, fish weight, feeding rates and gross dietary composition on selected parameters including growth, food utilisation and carcass composition. The principal aim of the experimental programme carried out was to improve understanding of various aspects of digestive physiology in O. niloticus with a view to improving techniques used in commercial culture of this species. In addition, it was hoped to gain some insight into general mechanisms of feeding and digestion in a warm, freshwater teleost fish.

## 2. MATERIALS AND METHODS

### 2.1 Fish

Genetically pure Oreochromis niloticus, Trewavas in the size range 1-200 grams were obtained from the Institute of Aquaculture hatchery.

For a period of two weeks before commencing each trial, fish were acclimatised to the experimental system. During this period they were fed a commercial trout diet of appropriate pellet size (Ewos-Baker, 49% Crude protein, 7.5% Lipid, 9.75% Ash and 7.3% Moisture). At the start of each experiment the individual weight and fork length of each fish was measured to the nearest 0.1g and 0.1 cm respectively. To facilitate handling fish were anaesthetised in benzocaine at a concentration of 60-70 mg l<sup>-1</sup> according to the method of Ross and Geddes (1979).

### 2.2 Experimental System

Experimental fish were held in a temperature controlled recycling system (Fig. 1). The system consisted of twenty four self cleaning circular tanks, each with a capacity of 50 l. Water was fed to each tank at a rate of 2 l min<sup>-1</sup> from a 115 l header tank. Water drained from the tanks, through central stand pipes, into a series of six 115 l solids settling tanks. The water was then pumped up to a 115 l tank which was filled with gravel to act as a biological filter. From here it flowed to a header tank which was aerated vigorously using airstones. Individual tanks were also aerated using single airstones

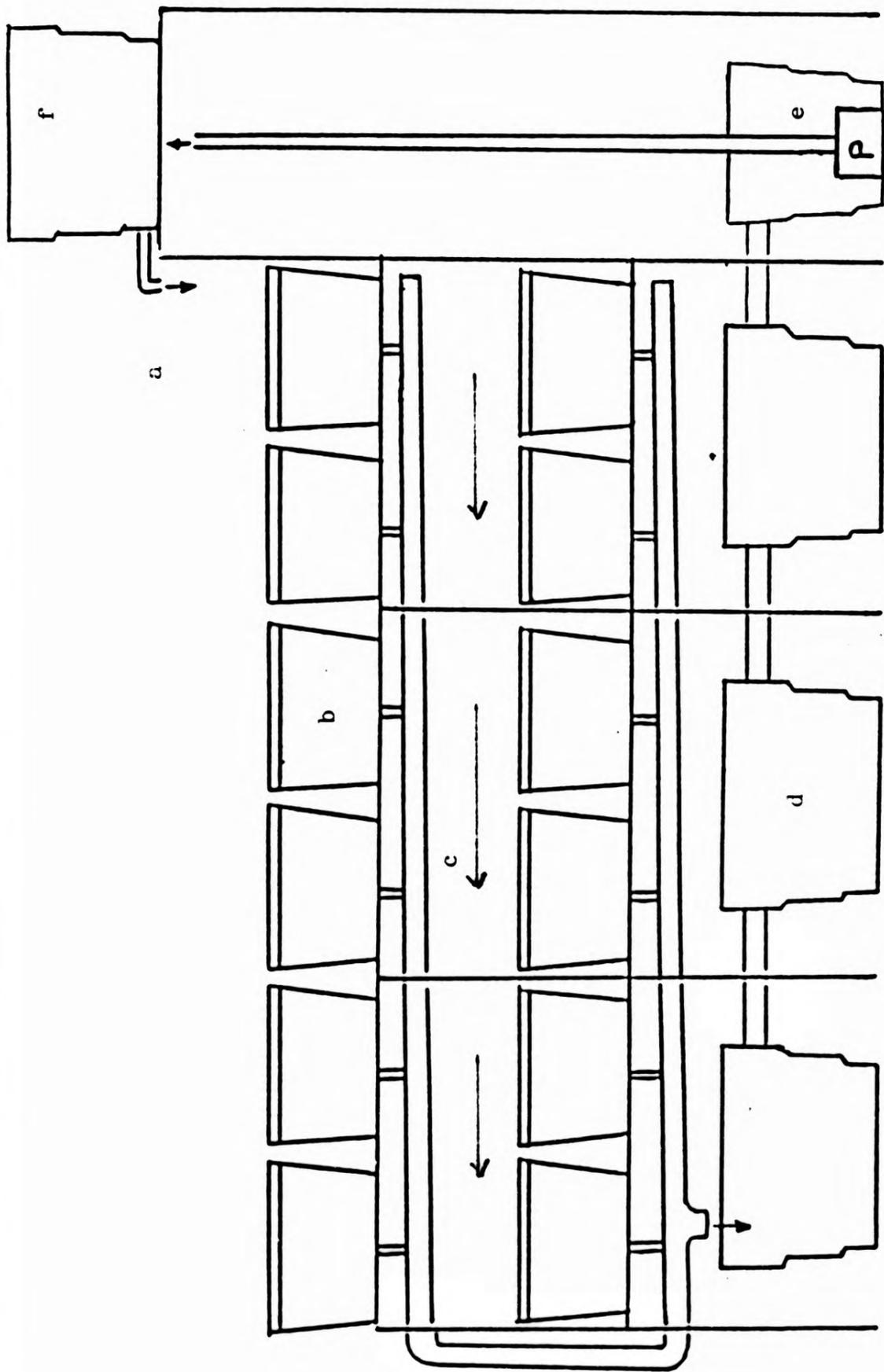


FIGURE 1 Diagrammatic representation of the recirculated water system used in the experiments

- a) Inflow pipe
- b) Experimental tanks
- c) Outflow drain pipe
- d) Settling tank
- e) Pump
- f) Header tank

Direction of water

in order to maintain a minimum level of 90% oxygen saturation. The water was heated by a 3 kw thermostatically controlled immersion heater fitted into the header tank and the temperature was maintained at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . This temperature is close to the optimum temperature for tilapia recorded by several workers (e.g. Cridland, 1962; Huser, 1978). Water losses due to evaporation were made up by a continuous fresh water input of  $0.15 \text{ l min}^{-1}$ . To avoid accumulation of excretory products, 25% of the total water in the system was replaced weekly with pre-warmed fresh water. In this way water quality was maintained within acceptable limits for fish growth (Huet, 1972; Wickins, 1980; Colt, 1981). Samples of water were taken from the system, before the weekly replacement of 25% of the total water volume, and analysed for dissolved oxygen and total ammonia according to the methods given by Stirling (1985). Nitrite was determined by the method of Strickland and Parson (1972) while pH was recorded using an Extech 65 digital pH meter. Table 1 shows the range of values recorded for these water quality criteria measured during the course of the experiment. Throughout the experiment a photo period of 12 hours light and 12 hours darkness was maintained, and fish were always fed during the light period.

### 2.3 Food Intake

The effect of feeding frequency, fish weight, satiation amount and starvation on subsequent food intake of O. niloticus was investigated by measuring the difference between the quantity of food offered to fish for a period of time and the amount of food remaining at the end of the feeding period (Brett, 1970; Grayton & Beamish, 1977; Wootton et al., 1980). The quantity of food consumed was determined by feeding

TABLE 1 Water quality criteria as measured during the course of the experiments

Parameter	Range
pH	6.9 - 7.2
Temperature (°C)	27 - 28
Dissolved oxygen	6.8 - 8 mg/l <sup>-1</sup>
Total ammonia	0.098 - 0.125 mg/l <sup>-1</sup>
Total nitrite	0.029 - 0.033 mg/l <sup>-1</sup>

the fish to satiation, immediately after satiation was judged to have occurred, a group of fish were sacrificed and their food intake was determined by measuring their stomach contents

### 2.3.1 The effect of feeding frequency on food intake

The effect on total daily food intake of feeding O. niloticus at  $27.5^{\circ} \pm 1^{\circ}\text{C}$  to satiation 1, 2, 4, 6 and 8 times per day was investigated. Fish of mean weight  $31.1\text{g} \pm 0.5\text{g}$  were stocked at a level of six fish per tank in 10 experimental tanks, thus giving one replicate of each of five treatments. Fish were fed trout pellets (Ewos-Baker - Omega 4, 49% Crude protein) to satiation at each feeding time during the light period, from 0800h - 2000h, for one week. Satiation was judged to have been achieved when the fish would no longer accept food despite two or three pellets being available for 2-5 min. in the tank. Satiation was generally reached within 10-15 min. of the onset of a feeding period. After an acclimation period of one week to these feeding regimes, the daily amount of food consumed at each feeding frequency by each tank of fish was recorded for a further four days.

### 2.3.2 The effect of fish weight on maximum daily food intake

The effect of fish weight on maximum daily food intake was investigated using a number of different size classes of fish covering the range 10.5-178g (Table 2) at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . Fish were fed trout pellets (49% Crude protein) of appropriate particle size at two hourly intervals during the light photo period from 0800h - 2000h. Fish were fed to satiation as described previously (Section 2.3.1), for

TABLE 2 The weight range of fish used for the determination of maximum daily food consumption at  $27.5^{\circ} \pm 1^{\circ}\text{C}$  of O. niloticus

Weight range (g)	No. of fish	Stocking density
10.5 - 11	20	10 fish/tank
22.5 - 23	20	10 " "
31.5 - 32.6	20	10 " "
43 - 44	20	10 " "
55 - 56	20	10 " "
60 - 61	20	10 " "
90 - 91	10	5 fish/tank
99 - 100	10	5 " "
118 - 120	10	5 " "
148 - 149	10	5 " "
177 - 178	10	5 " "

a period of one week under this feeding regime. After this period of acclimatisation total daily food intake was recorded for a further four days.

### 2.3.3 Maximum food intake in a single meal (satiation amount)

The maximum food intake in a single meal was investigated in four size groups of fish (Table 3). After acclimatisation to the experimental system at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ , the fish were deprived of food for a period of 48h to achieve standard emptiness of the alimentary canal (Jobling, 1974; Grove & Crawford, 1980). All four groups of fish were then fed to satiation with trout pellets of appropriate particle size. Immediately after feeding 15 fish from each size group were sacrificed by a sharp blow on the head and the fork length and weight of individual fish were measured to the nearest 0.1 cm and 0.1g respectively. The alimentary canal was dissected, uncoiled and the total length of the intestine from the pylorus to the anus was measured. The relationship between fish length and length of the intestine (relative gut length) was established according to the method of Al-Hussaini (1953) where

$$\text{R.C.L} = \frac{\text{Total length of intestine in cm}}{\text{Fish length in cm}}$$

The liver weight of individual fish was determined by weighing to the nearest 0.01g on a balance to establish the relationship between fish size and liver weight. Individual stomach contents were removed carefully, weighed to the nearest 0.001g on a Mettler AC100 micro-balance and then dried in an oven at  $105^{\circ}\text{C}$  for 12h to constant dry weight. Thus the wet and dry weights of food consumed by individual fish from each size group were established. The empty stomachs were

TABLE 3 Mean weight ( $\pm$  S.E.), range and fish number used for the determination of maximum food intake in a single meal by O. niloticus at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

Group	Mean weight (g, $\pm$ S.E.)	Range (g)	No. of fish
1	14.8 $\pm$	9.1 - 20.0	15
2	34.47 $\pm$ 2.03	22.6 - 45.9	15
3	73.64 $\pm$ 3.97	51.73 - 99.6	15
4	131.5 $\pm$ 4.3	109.1 - 170.0	15

then ligatured at both ends and the stomach volume of individual fish was measured based on the method of Jobling et al. (1977). A hypodermic needle connected to a water filled burette by narrow gauge polythene tubing (5 mm) was gently inserted into the gastric lumen through the stomach wall (Fig. 2). The stomach volume was determined as the volume of water required to distend the stomach fully at a hydrostatic pressure of 50cmH<sub>2</sub>O(Jobling et al.,1977; Grove et al.,1978).

#### 2.3.4 The effects of periods of starvation on subsequent food intake.

The effects of periods of starvation of 24, 48, 72 and 96 hour duration on subsequent food intake were investigated using four weight classes of fish (Table 4). All four groups of fish were fed commercial trout pellets (49% Crude protein) to satiation. After feeding the fish were deprived of food for 24, 48, 72 or 96h. At the end of each deprivation period 15 fish from each size group were again fed to satiation. Immediately after feeding the fish were sacrificed and the weight, length, intestine length, liver weight, food intake and the stomach volume of each fish were determined as described previously (Section 2.3.3).

#### 2.3.5 The effects of starvation on the gall bladder weight

During the course of the previous experiment variations in gall bladder size and colour were noted. An experiment was therefore designed to investigate the effect of varying periods of starvation on gall bladder size and colour. 90 fish of mean weight 38.8g ± 0.09g were held at 27.5° ± 1°C in 10 experimental tanks. For two weeks fish

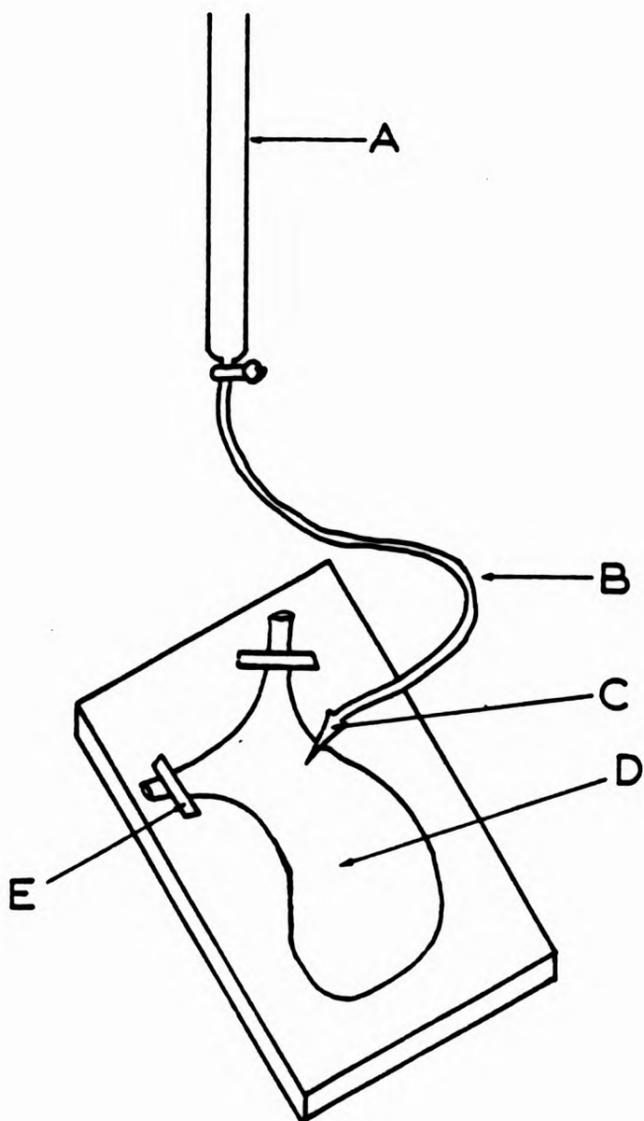


FIGURE 2 Apparatus used for measuring stomach volume

- A a 50ml burette
- B Polystyrene tube
- C Surgical needle
- D Stomach
- E Haemostatic clamps

**TABLE 4** Mean weight ( $\pm$ S.E.), weight range, stocking density and number of fish used to determine the effect of periods of starvation on subsequent food intake at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

Group	Mean weight (g, $\pm$ S.E.)	Range (g)	No. of fish	Stocking density
1	14.78 $\pm$ 0.47	7 - 20.8	60	15
2	36.56 $\pm$ 1.12	24.5 - 54.5	60	15
3	76.32 $\pm$ 1.79	55 - 100	60	15
4	136.5 $\pm$ 2.8	100.5 - 180.5	60	15

were fed to satiation at two hourly intervals during the light photo period of 0800 - 2000h. At the end of this period five fish were sampled at each feeding time and weighed to the nearest 0.1g. The liver and gall bladder were carefully dissected out and weighed to the nearest 0.01g. The remaining 50 fish were deprived of food for 24, 48, 72 or 92h respectively. At the end of each period of starvation a further five fish were sampled and their livers and gall bladders were dissected out and weighed. The remaining fish were then refed after the 96h deprivation time at two hourly intervals and five fish were again sampled after each feeding time and their livers and gall bladders were dissected out and weighed as described previously.

#### 2.4 Gastric Evacuation Studies

Three different techniques were chosen from the literature in order to select the most appropriate method for O. niloticus which minimised stress and suited the species involved. These techniques included the direct method (sequential slaughter), dye technique and the radiography technique.

The aim of the study was to establish some quantitative relationship between the rate of stomach evacuation and the factors affecting evacuation rate and time, such as temperature, fish weight, meal size, food type and feeding history.

2.4.1 Comparison of three different techniques used in the determination of stomach evacuation time

The following techniques were used to select the most appropriate method for subsequent experiments.

2.4.1.1 The use of dyes

This method involves measuring the time between the first appearance of labelled food in the faeces and the last evacuation of labelled food.

18 fish of mean weight 43.5g (S.E.  $\pm$  1.8) were stocked in six experimental tanks. After the standard acclimatisation period of two weeks the fish were deprived of food for 48h to achieve standard emptiness of the alimentary canal. Fish were then fed a single meal (1.5% b.w.) of trout pellets labelled with 1% of the inert marker  $\text{Cr}_2\text{O}_3$  (Nawwab, 1982; Hadjichristophora & Grove, 1983). The inert marker was added to finely ground commercial trout diet (49% Crude protein) at a concentration of 1% w.w. After thorough mixing, sufficient water was added to form a stiff dough and the resulting paste was re-pelleted using a Hobart A200 food mixer and a 2 mm die plate before being dried by air convection at 60°C. The experimental tanks were cleaned after every defaecation time, and the faeces were siphoned out for inspection. The time elapsed between the first and last evacuation of labelled faeces was recorded for each tank.

2.4.1.2 X-radiography technique

This method involves feeding the fish a diet containing barium sulphate or metallic iron powder so that the rate of passage of these

diets through the alimentary canal can be observed by taking radiographs serially (Edwards, 1971, 1973; Flowerdew & Grove, 1979; Ross & Jauncey, 1981; Talbot & Higgins, 1983).

Two groups of fish (Table 5) were held in the experimental tanks at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . After acclimatisation to the experimental tanks and deprivation of food as described previously (Section 2.3.1-2.4.1) one group was force-fed a paste containing barium sulphate and ground trout pellets. This was prepared by grinding trout pellets finely and adding barium sulphate to give a final concentration of 25% (Jobling *et al.*, 1977). Water was added (25 ml/100g) to form a paste which was force-fed to the fish at a rate of 1.5% b.w. using a 5 cm<sup>3</sup> syringe fitted with a short length of polypropylene canula tubing of 2.5 mm outside diameter. The second group of fish was given a meal consisting of trout pellets labelled with metallic iron powder (Talbot & Higgins, 1983). The metallic iron powder was sieved to separate particles in the size range 100-200  $\mu\text{m}$ , which were added to finely ground commercial trout diet to give a concentration of 0.5% w.w. and the mixture was re-pelleted as described previously (Section 2.4.1.2). This diet was fed voluntarily to the fish at a rate of 1.5% of their body weight.

In both groups of fish the passage of food through the alimentary tract was observed radiographically using a Watson Mobilix Ward type X-ray machine. Fish were x-rayed every 2-4 hours or more frequently when the food was about to pass from one alimentary region to another. Prior to taking an x-ray it was necessary to sedate the fish using benzocaine (Ross & Geddes, 1979).

TABLE 5 The weight range, mean weight and number of fish used to evaluate the x-radiography technique

Group	Contrast medium	Mean weight (g)	Size range (g)	No. of fish
1	Barium sulphate	42.02 ± 0.75	36 - 50.7	36
2	Metallic iron powder	43.5 ± 1.8	35 - 60	18

#### 2.4.1.3 Sequential slaughter

This method is a direct method for the determination of gastric evacuation time and rate since it involves killing a group of fish immediately after feeding and at regular intervals thereafter until the stomach is empty (Brett & Higgs, 1970; Tyler, 1970; De Silva & Owoyemi, 1983).

42 fish of mean weight 62.11g (S. E.  $\pm$  0.5) were held in seven experimental tanks at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . The fish were acclimatised to the experimental system for a period of two weeks and during this period they were fed commercial trout pellets (49% Crude protein) at a rate of 2% of their body weight divided into three feeds per day. After this period they were deprived of food for 48h to achieve standard emptiness of the alimentary canal (Jobling et al., 1977; Grove et al., 1985). The fish were then fed to satiation as described previously (Section 2.3.1) with commercial trout pellets. Immediately after satiation feeding 14 fish were sacrificed by a sharp blow on the head. These fish were individually weighed to the nearest 0.1g. The stomach of each fish was then dissected out and the contents were removed and weighed to the nearest 0.001g on a Mettler AC100 micro-balance and then dried in a Gallenkamp oven at  $105^{\circ}\text{C}$  for 12h to constant dry weight. Dry fish with empty stomachs were also noted. At subsequent three hourly intervals a further four fish were sacrificed and their weight and stomach contents were determined as described previously. The experiment was terminated when the stomach was 90-95% empty of food.

#### 2.4.2 Mathematical description of gastric evacuation model

Three mathematical models were used to calculate the coefficient of evacuation and time required for complete evacuation of the stomach. These models are based on three different hypotheses, namely, the volume dependent model (Jobling, 1981), the surface area model (Fänge and Grove, 1979) and the exponential model (Elliott, 1972).

##### (1) Volume Dependent Model (Jobling, 1981)

The volume dependent model assumes that gastric motility is associated with the radial distension of the stomach and that the circumferential tension so developed is proportional to the radius. Since the radius of a cylinder varies with the square root of the volume, then the tension developed in the stomach wall will also be proportional to the square root of the volume of food in the stomach. The volume dependent model can be expressed as:

$$\frac{dy}{dt} = -Ry^{0.5}$$

Where the differential  $dy/dt$  describes the rate of evacuation,  $y$  is the volume of food in the stomach and  $R$  is constant. After integration and linearization it can be mathematically expressed as:

$$Y_t^{0.5} = Y_o^{0.5} - RvT \quad \text{or}$$
$$Rv = \frac{Y_o^{0.5} - Y_t^{0.5}}{T}$$

Where  $Y_t$  = the amount of food in the stomach at time  $t$   
 $Y_o$  = the amount of food ingested in (g)  
 $T$  = the time in hours, and  
 $Rv$  = the slope of the line which represents the stomach evacuation coefficient.

(2) Surface Area Model (Fange and Grove, 1979)

The basis of the surface area model is that digestive enzymes typically act on the surface of a food bolus and that the evacuation of the stomach is assumed to be proportional to the surface area of the food remaining in the stomach, such that:

$$\frac{dy}{dt} = -Ry^{0.67}$$

Where  $dy/dt$ ,  $y$  and  $R$  are as described in (1). After integration and linearization it can be expressed as:

$$y_t^{0.67} = y_o^{0.67} - RaT \quad \text{or}$$
$$Ra = \frac{y_o^{0.67} - y_t^{0.67}}{T}$$

Where  $y_t$ ,  $y_o$ ,  $T$  and  $R$  are as described in (1).

The stomach evacuation time (S.E.T.) from (1) and (2) was calculated assuming that:

$y_t = 0$  at 't' end therefore stomach evacuation time can be calculated as:

$$T = \frac{y_o^{0.5}}{Rv} \quad (\text{volume dependent model})$$

$$T = \frac{y_o^{0.67}}{Ra} \quad (\text{surface area model})$$

(3) Exponential Model (Elliott, 1972)

The exponential model assumes that the rate of stomach evacuation is proportional to stomach fullness. The larger the original volume of the meal then the greater is the initial evacuation rate of the stomach and this rate will then decrease as the stomach empties. Thus the evacuation rate is dependent on the amount of food remaining

in the stomach and can be expressed as:

$$Y_t = Y_0^{-ReT} \quad \text{or}$$

$$\text{Log}_e Y_t = \text{Log}_e Y_0 - ReT$$

Where  $Y_t$ ,  $Y_0$ ,  $Re$  and  $T$  are as described in (1) and (2).

The stomach evacuation time was determined for 99% of food evacuated since 100% of evacuation would theoretically require an infinite amount of time. Therefore the modified equation of Elliott (1972) was used:

$$\text{Stomach evacuation time (h)} = \frac{\text{Log}_e 100 - \text{Log}_3(100-P)}{Re}$$

Where  $P$  = % of evacuation

$Re$  = stomach evacuation coefficient.

### 2.4.3 Factors affecting stomach evacuation time and coefficient

Of the three methods evaluated sequential slaughter was selected as the most suitable method for O. niloticus since it minimised stress to the fish and the actual food ingested could be directly determined. Consequently this method was adopted in all subsequent gastric evacuation studies.

#### 2.4.3.1 Effect of temperature

93 fish of mean weight  $26.9\text{g}(\text{SE}\pm.65)$  were used to investigate the effect of temperature on gastric evacuation time and coefficient in O. niloticus. Fish were stocked in three 80 l experimental glass tanks at a density of 31 fish per tank. Each tank contained a fully submersible Controlomatic aquarium heater to maintain three constant temperatures of 20°, 27° and 35°C. Each tank was also provided with two Eheim electric pumps to act as biological filters and two air stones to maintain a minimum level of 90% O<sub>2</sub> saturation. The water in each tank was changed twice weekly with fresh water of the same temperature. Black polythene sheets were used to partially cover the tanks so that fish could retreat to a zone of low light intensity where they were less disturbed (Elliott, 1972). Throughout the experimental period a photo period of 12h light and 12h darkness was maintained.

Fish were acclimatised for a period of two weeks at these experimental temperatures during which time they were fed commercial trout pellets (49% crude protein) at a rate of 2% b.w. three times daily. After acclimatisation fish were deprived of food for 48h to attain

standard emptiness of the alimentary canal. They were then fed trout pellets to satiation in order to determine the effect of temperature on voluntary food intake and evacuation time and rate. Immediately after feeding 10 fish from each group were sacrificed and were used to determine the food intake in a single meal. Subsequently, a further three fish were killed and weighed at two hourly intervals. The stomach and intestinal contents of each fish were removed separately and weighed to the nearest 0.001g before drying in an oven at 105°C for 12h to constant dry weight. The experiment was terminated after 14h after feeding.

#### 2.4.3.2 Effect of fish weight

It has been shown by several workers (e.g. Jobling et al., 1977; Grove et al., 1978) and also in this study (Section 2.3.3) that stomach volume is directly proportional to body weight. Thus feeding a meal based on the weight of a fish should present a uniform stimulus to the gastrointestinal tract enabling direct comparison between fish of different weights.

Three weightgroups of fish (Table 6) were held in the experimental system at  $27.5 \pm 1^\circ\text{C}$  for two weeks. After acclimatisation and deprivation of food as described previously (Section 2.4.1.3) the fish were fed a commercial trout diet at 1% of their body weight. Immediately after feeding 10 fish from each group were sacrificed to establish the actual meal size ingested. Thereafter a further four fish from each group were sacrificed every two hours and the weight of their stomach and intestine contents was determined. The experiment was terminated when 90%-95% of the stomach contents had been evacuated.

TABLE 6 Mean weight, weight range and number of fish used to determine the effect of fish weight on gastric evacuation time and coefficient

Group	Mean weight (g, $\pm$ S.E.)	Range (g)	No. of fish
1	49.6 $\pm$ 0.96	40 - 60	42
2	97.3 $\pm$ 1.3	83.1 - 110.2	42
3	144.8 $\pm$ 1.11	130 - 157.0	42

#### 2.4.3.3 Effect of meal size

To investigate the effect of meal size on gastric evacuation time and rate, 84 fish of mean weight 28.4g (S.E.  $\pm$  0.39) were held in three experimental tanks at 27.5°C ( $\pm$  1°C). After the standard acclimatisation period of two weeks (Section 2.4.1.3) fish were deprived of food for 48h before the experiment began to achieve standard clearance of the alimentary canal. The fish were then fed commercial trout pellets (Ewos-Baker, 49% crude protein) at 0.5%, 1.00% and 1.5% of their body weight. Ten fish from each group were sacrificed immediately following the meal to determine the actual food intake. At subsequent intervals of two hours a further three fish were sacrificed from each group and their stomach and intestine contents were determined as described previously (Section 2.4.3.1). The experiment was terminated after 12h.

#### 2.4.3.4 Effect of food type

Four experimental diets were formulated to investigate the effect of food type on gastric evacuation time and rate. Herring fish meal (70.16% crude protein, 10.19% lipid) was used as the source of dietary protein to provide the following experimental diets:-

Diet A: Control (35% crude protein, 11% crude lipid,  
20.6% available carbohydrate)

Diet B: High available carbohydrate (35% crude protein,  
10.3% crude lipid, 33% available carbohydrate).  
This diet gives about a 60% increase in available  
carbohydrate over the control diet (A).

Diet C: High lipid diet (35% crude protein, 18.8% crude lipid, 20% available carbohydrate). This diet gives a 70% increase in lipid content over the control diet (A).

Diet D: High protein diet (49% crude protein, 11.3% crude lipid, 16% available carbohydrate). This diet gives a 40% increase in crude protein over the control diet (A).

Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) was included in all the experimental diets at a concentration of 0.5% as an inert marker for the determination of the digestion coefficient (Maynard and Loosli, 1969). The composition of the experimental diets is shown in Table 7. The experimental diets were prepared by mixing all the dry ingredients thoroughly in a Hobart A200 food mixer for a period of 10 minutes. The process was repeated with the addition of oils and water until the binder had been primed. The homogenous paste was then extruded under pressure through a 2 mm die plate, forming long spaghetti-like strands. These were then dried by warm air current in a constant temperature cabinet at  $37^\circ\text{C}$  and subsequently broken into pellets of appropriate size. The dry pellets were sealed in polythene bags and stored at  $-20^\circ\text{C}$  until fed.

Four groups of 58 fish each of mean weight 94.9g (S.E.  $\pm$  1.3) were held in the experimental tanks at  $27.5^\circ \pm 1^\circ\text{C}$  for a period of two weeks. During this time each group was fed one of the four experimental diets twice daily at a rate of 2% b.w. After this period the fish were deprived of food for 48h to achieve standard emptiness of the alimentary tract, they were then fed to satiation in order to determine the effect of food type on maximum food intake. Immediately

TABLE 7 Composition of the four experimental diets

Diet ingredients	Percentage in the diet			
	Control diet A	High carbohydrate B	High lipid C	High protein D
Fish meal	52.00	52.00	52.00	72.00
Cod liver oil	2.00	1.50	5.00	1.00
Corn oil	3.50	3.00	7.50	1.50
Mineral Mix <sup>1</sup>	7.00	3.00	4.00	3.00
Vitamin Mix <sup>2</sup>	2.00	2.00	2.00	2.00
Binder <sup>3</sup>	2.00	2.00	2.00	2.00
$\alpha$ -cellulose	10.40	3.00	7.00	2.00
Cr <sub>2</sub> O <sub>3</sub>	0.50	0.50	0.50	0.50
Dextrin	12.00	23.00	12.00	10.00
Corn starch	8.60	10.00	8.00	6.00
TOTAL	100.00	100.00	100.00	100.00

	Proximate analysis of the diets			
	A	B	C	D
Crude protein	40.80	42.75	41.23	53.30
Crude lipid	8.00	7.00	14.82	7.99
Crude fibre	8.90	1.70	5.80	2.10
Available carbohydrate <sup>4</sup>	24.67	31.94	22.98	18.83
Ash	13.50	11.25	11.28	13.18
Moisture	4.13	5.36	3.89	4.65
Cr <sub>2</sub> O <sub>3</sub>	0.45	0.45	0.43	0.49
Gross energy (Kjoule/g)	16.80	17.20	19.32	18.40

<sup>1</sup> Mineral mix: supplying per Kg diet, MgSO<sub>4</sub>.7H<sub>2</sub>O 5.10g; NaCl 2.40g; KCl 2.00g; FeSO<sub>4</sub>.7H<sub>2</sub>O 1.00g; ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.22g; CuSO<sub>4</sub>.5H<sub>2</sub>O 0.0314g; MnSO<sub>4</sub>.4H<sub>2</sub>O 0.1015g; CoSO<sub>4</sub>.7H<sub>2</sub>O 0.0191g; CaIO<sub>3</sub>.6H<sub>2</sub>O 0.0118g; CrCl<sub>3</sub>.6H<sub>2</sub>O 0.0051g (Tacon et al., 1983)

- 2 Vitamin mix: supplying per Kg of diet, Thiamine HCl 50mg;  
Riboflavin 50mg; Calcium pantothenate 100mg;  
Niacin 200mg; Pyridoxine HCl 40mg; Biotin 6mg;  
Folic acid 15mg; Cyanocobalamin 0.1mg;  
Inositol 200mg; Ascorbic acid 1000mg; Choline  
chloride 400mg; Menadion 40mg; Tocopherol acetate  
400 mg; P-amino-benzoic acid 50mg; Vitamin A  
acetate 2000 IU; Vitamin D<sub>3</sub> 1000IU  
(Tacon et al., 1983)
- 3 Carboxymethyl cellulose
- 4 Available carbohydrate was determined by calculation:  
$$= 100 - (\% \text{ crude protein} + \% \text{ crude lipid} + \% \text{ moisture} + \% \text{ crude fibre} + \% \text{ ash})$$

after this meal 10 fish from each treatment were sacrificed to determine mean food intake. At subsequent time intervals of two hours a further six fish were sacrificed from each group and the wet and dry weight of the stomach and intestinal contents were determined. The dried material was stored in airtight bottles for subsequent analysis to determine the digestion coefficient (Maynard and Loosli, 1969). During the sampling period faeces from each group were collected by siphoning every two hours from the tank, these samples were dried in an oven at 105°C for 12h to constant dry weight, ground to produce a fine powder and stored in airtight bottles for subsequent analysis. The experiment was terminated after 16 hours.

#### 2.4.3.5 Effect of periods of starvation

The effect of periods of starvation was investigated in two groups of fish (Table 8). Fish were held in eight experimental tanks at 27.5° ± 1°C for two weeks during which they were fed a commercial trout pellet (49% crude protein) at a rate of 2% b.w. After acclimatisation, both groups of fish were deprived of food for 24h, 48h, 72h and 96 hours. At the end of each deprivation period the first group of fish were fed to satiation on a commercial trout diet as described previously (Section 2.3.1), the second group of fish were fed the same diet at a rate of 1% of their body weight. Immediately after feeding 60 fish from the first group and 40 fish from the second group were sacrificed to determine the mean food intake after each deprivation period. Thereafter a further 20 fish from the first group and 16 fish from the second group were sacrificed at intervals of five and two hours respectively, and the weight of stomach and intestinal contents were determined as described earlier.

TABLE 8 Mean weights and number of fish used for the determination of the effect of periods of starvation on gastric evacuation time and rate

Group	Mean weight (g, ± S.E.)	Total no. of fish	Deprivation time in (h)	Feeding level	No. of fish sampled after feeding and at intervals	Duration of the experiment in (h)
1			24			
	35.21	140	48	satiation	5 fish/5h	20
	± 0.47		72			
			96			
2			24			
	19.50	120	48	1% b.w.	4 fish/2 h	10
	± 0.29		72			
			96			

## 2.5 Digestive Enzymes

The present study was designed to investigate the effect of food composition, fish size and deprivation period on digestive enzyme activity with the aim of relating the results obtained to gastric evacuation time and rate.

### 2.5.1 Preparation of enzyme extracts

At the end of each experiment three fish from each replicate were sacrificed by an overdose of benzocaine (Ross and Geddes, 1979). The stomach and intestine of each fish were freed from mesenteries dissected out and the contents were discarded. The tissues were then rinsed with distilled water, blotted on filter paper and weighed to the nearest 0.001g on a Mettler PC400 microbalance before being cut into small pieces and homogenised in an electrical homogeniser (Ultra-Turrax type TP18-10) for 10 min. Phosphate buffer (pH 7) was added at a rate of 5 ml for every 1g of tissue and the mixture was homogenised for a further 10-15 min. The homogenised tissue was then centrifuged at 4000-5000 xg in a Minor's centrifuge for 15 min. The resulting supernatant was reserved for the enzyme assays. The corresponding parts of the digestive tracts of these fish were pooled in order to minimise individual differences and to give a sufficient amount of extract to carry out the enzyme assays.

## 2.5.2 Enzyme assays

### 2.5.2.1 $\alpha$ -amylase

The following substrates, reagent and standards were prepared for use in the determination of  $\alpha$ -amylase activity in the intestine of the fish.

#### a) Starch solution (1% w/v)

This was used as a substrate for amylase determination. 1.0g of soluble starch was dissolved in 100 ml of phosphate buffer (pH 6.2).

#### b) Dinitrosalicylic acid reagent

5.0g of 3,5-dinitrosalicylic acid was dissolved in 150 ml of distilled water and 200 ml of 1 N sodium hydroxide by warming to around 60°C. 150g of potassium sodium tartrate was then added and finally the volume was made up to 500 ml with double distilled water.

#### c) Maltose standard

100 mg of D(+) maltose was dissolved in 100 ml of distilled water. 0.2, 0.4, 0.6, 0.8 and 1.0 ml of maltose standard solution was distilled with 0.85, 0.65, 0.45, 0.25 and 0.05 ml of distilled water respectively. 1.0 ml of starch solution and 2.0 ml of dinitrosalicylic acid reagent were added to each tube and mixed thoroughly. The solutions were placed in a water bath at 100°C for 5 min. Finally, the mixtures were cooled to room temperature and the developed

colour was read using a Uican 810 electronic spectrophotometer at a wave length of 546 nm.

$\alpha$ -amylase activity was assayed using soluble starch as a substrate according to the method of Bennfeld (1955). Two replicates of 100  $\mu$ l of the enzyme extract were each incubated with 1.0 ml of 1% starch solution (pH 6.2) for exactly 10 min. at 30°C. The incubation was terminated by the addition of 2.0 ml of 3,5-dinitrosalicylic acid reagent and they were heated in a boiling water bath (100°C) for 5 min. The tubes were then cooled at room temperature and the absorbance of the colour which developed was measured in a spectrophotometer at a wave length of 546 nm. One unit of amylase activity was defined as the amount of enzyme required to liberate one  $\mu$ mole of maltose per minute per gram of tissue at 30°C.

#### 2.5.2.2 Lipase

The following substrates, reagents and standards were prepared for use in the determination of lipase activity in the intestine of fish.

##### a) Olive oil emulsion (pH 7.2)

Olive oil emulsion (Sigma lipase substrate Cat 800) was used as a substrate for the determination of lipase activity. Tris buffer (pH 7.2) were added to two volumes of olive oil emulsion and the mixture was shaken vigorously by hand for 5 min. before use.

b) Copper reagent

75.9 ml of triethanolamene and 23.75 mg of cupric nitrate trihydrate were dissolved in 50 ml of acetic acid and 600 ml of distilled water with the aid of a magnetic stirrer. 250g of sodium chloride was then added and dissolved by further stirring. The mixture was diluted to 975 ml with distilled water and the pH of the solution was adjusted to 8.3 with a few drops of acetic acid or triethanolamine. Finally the volume was made up to 1000 ml with distilled water.

c) Saturated sodium bromide

510g of sodium bromide was dissolved in 500 ml of distilled water with the aid of a magnetic stirrer. The mixture was allowed to stand overnight and then filtered through a glass wool pad.

d) Sodium diethyl dithiocarbonate

100 mg of sodium diethyl dithiocarbonate was added to 60 ml of n-butanol and the volume was made up to 100 ml with chloroform.

e) Oleic acid standard

Oleic acid solution was used as a standard for the lipase activity for each enzyme assay. 1.0 ml of 6.0 mmole oleic acid was diluted with 100 ml of an extraction solution consisting of a mixture of chloroform and heptane in the ratio 3:2.

Lipase activity was assayed using the method of Yang and Biggs (1971) as modified by Mytle and Zell (1975). Two replicates of 100  $\mu$ l of the enzyme extract were each mixed thoroughly with 1.0 ml of the olive oil emulsion (pH 7.2) substrate and were incubated at 30°C for exactly 10 min. The incubation period was terminated by adding 7.0 ml of extraction solution (chloroform and heptane in the ratio 3:2). The samples were then shaken using a mechanical shaker at high speed for 4-5 min. and then centrifuged at 6000  $\times$ g in a Minor centrifuge for 10 min. The upper emulsified layer was removed and discarded as recommended by Yang and Biggs (1971). 1.0 ml of saturated sodium bromide and 3.5 ml of copper reagent were added to 5.0 ml of the supernatant (chloroform and heptane layer) and shaken at low speed for 2-3 min. on the mechanical shaker. The samples were then centrifuged at 2000  $\times$ g for 10 min. 0.5 ml of sodium diethyl dithiocarbonate reagent was added to 3.0 ml of the upper organic layer. The absorbance of the resulting solution was measured in a spectrophotometer at a wave length of 435 nm against a reagent blank. Sample blanks and standards (oleic acid) were included with each enzyme assay. One unit of lipase activity was defined as the amount of enzyme required to liberate one  $\mu$ mole of oleic acid per minute per gram of tissue at 30°C.

#### 2.5.2.3 Protease

The following substrates, reagents and standards were prepared for use in the determination of pepsin-like enzyme in the stomach and trypsin-like enzyme in the intestine of O. niloticus.

a) Haemoglobin solution

Two haemoglobin solutions were prepared to provide pH (2) and (8.2) for the determination of pepsin and trypsin activity respectively.

The pH (2) haemoglobin solution was prepared by dissolving 2.0g of haemoglobin in 100 ml of 0.06 NHCl.

The pH (8.2) solution was prepared by dissolving 2.0g of haemoglobin and 36.0g of urea in 50 ml of double distilled water. 8.0 ml of 1 N sodium hydroxide was added and the mixture was made up to 80 ml with double distilled water. After 30 min. at room temperature 10 ml of molar boric acid and 4.4 ml of 5% calcium chloride was added and the pH of the solution was adjusted to 8.2 with 1 N hydrochloric acid. Finally, the solution was made up to 100 ml by the addition of distilled water.

b) Phenol reagent

10.0g of sodium tungstate, 2.5g of sodium molybdate and 15g of lithium sulphate were dissolved in 10 ml of concentrated hydrochloric acid and 5.0 ml of concentrated orthophosphoric acid. The mixture was then made up to 100 ml with distilled water. Before use one volume of this solution was diluted with two volumes of double distilled water.

c) Tyrosine standard

18.119 mg of L-(-) tyrosine was dissolved in 100 ml of 0.2N hydrochloric acid. 0.2, 0.4, 0.6, 0.8 and 1.0 ml of this solution was diluted with 4.8, 4.6, 4.4, 4.2 and 4.0 ml of 0.2N hydrochloric acid, respectively. 10.0 ml of 0.5N sodium hydroxide was added to each tube. 3.0 ml of diluted phenol reagent was then added with continuous shaking and the absorbance of the developed colour was read in a spectrophotometer at a wave length of 750 nm against a blank containing 0.2N hydrochloric acid.

Pepsin and trypsin activities were assayed using the method of Anson (1938). Two replicates of 200 ul of enzyme extract from the stomach and the intestine were mixed thoroughly with 1.0 ml of pH 2 substrate for pepsin-like enzyme in the stomach and pH 8.2 substrate for trypsin-like enzyme in the intestine. Tubes were incubated at 30°C for 10 min. After this period 2.0 ml of trichloroacetic acid was added and the samples were centrifuged for 10 min. at 4000 xg in a Minor's centrifuge. 2.0 ml of 0.5N sodium hydroxide and 0.6 ml of phenol reagent were added with shaking to 1.0 ml of supernatant. The absorbance was measured in a spectrophotometer at a wave length of 750 nm. The amount of tyrosine liberated was determined using a standard tyrosine curve. One unit of pepsin or trypsin-like enzyme activity was defined as the amount of enzyme required to liberate one umole of tyrosine per minute per gram of tissue at 30°C.

### 2.5.3 The effect of food composition on digestive enzyme activities

The effect of the following four experimental diets on digestive enzyme activities of O. niloticus of mean weight 91.8g ( $\pm 3.5$ ) were investigated.

Diet A: Control (35% crude protein, 11% crude lipid, 20.6% available carbohydrate)

Diet B: High available carbohydrate (35% crude protein, 10.3% crude lipid, 33% available carbohydrate)

Diet C: High lipid (35% crude protein, 18.8% crude lipid, 20% available carbohydrate)

Diet D: High protein (49% crude protein, 11.3% crude lipid, 16% available carbohydrate).

The formulation, preparation and the proximate composition of these diets were described in the gastric evacuation section (2.4.3.4). A total of 40 fish were stocked at a rate of five fish per tank in the experimental system and held at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . Each of the four experimental diets was randomly assigned to eight tanks thus giving one replicate of each treatment. These experimental diets were fed at a rate of 2% b.w. three times daily for three weeks. At the end of this period three fish from each tank were killed by an overdose of benzocaine (Ross and Geddes, 1979) 4-6h after last feeding and their stomach and intestine were immediately dissected out; an enzyme extract was prepared and assays carried out as described in (2.5.2).

#### 2.5.4 The effect of fish weight on digestive enzyme activity

Three groups of 36 fish in the following size ranges were used to investigate the effect of fish weight on digestive enzyme activity.

1. 36.79g  $\pm$  1.5
2. 80.13g  $\pm$  3.9
3. 175.5g  $\pm$  3.0

Fish were stocked at a rate of six fish per tank in each of the six experimental tanks and held at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . Thus one replicate was used for each size group. Fish were fed commercial trout diet (Ewos-Baker, 49% c.p.) at a rate of 1.0% of their body weight three times daily for two weeks. At the end of the feeding trial, three fish from each tank were killed by an overdose of benzocaine (Ross and Geddes, 1979) 4-6h after last feeding and their stomach and intestine were dissected out; an enzyme extract was prepared and assayed as described previously (Section 2.5.2).

#### 2.5.5 The effect of periods of starvation on digestive enzyme activity

Two groups of 18 fish each of mean weight 104g ( $\pm$  2.3) were held in two experimental tanks at  $27.5^{\circ} \pm 1^{\circ}\text{C}$  for two weeks. During this period they were fed commercial trout pellets to satiation three times daily. At the end of the feeding trial, three fish from each tank were sacrificed by an overdose of benzocaine (Ross and Geddes, 1979) 4-6h after last feeding and their stomachs and intestines were dissected out and assayed for enzyme activity. The remaining fish were deprived of food for up to 96h. After 24, 48, 72 and finally 96h deprivation, three fish from each tank were sampled and enzyme

assays were carried out. At the end of the 96h deprivation period the remaining three fish were again fed to satiation in a single meal and 4-6h after last feeding they were sacrificed. Their stomachs and intestines were dissected out, enzyme extracts prepared and assays carried out as described previously (Section 2.5.2).

## 2.6 Growth Studies

This study investigated the effects of feeding frequency, feeding rate, fish weight and diet composition on food intake, growth rate and body composition in hatchery reared O. niloticus.

### 2.6.1 The effect of feeding frequency on food intake, growth and body composition of O. niloticus fed to satiation

An eight week experiment was conducted to investigate the effect of the frequency of feeding of fish fed to satiation on food intake, growth and body composition. Fish were fed one, two, four, six and eight times daily. 215 fish in the weight range 12.5g-19.2g were acclimatised to the experimental system for a period of two weeks at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . During this period they were fed trout pellets twice daily at a rate of 3% of their body weight. After acclimatisation fish were individually weighed and stocked at a density of 20 fish per tank into 10 tanks (one replicate of each of five treatments). Tank weights were balanced so that differences between initial mean weight were minimised. The remaining 15 fish were killed by a sharp blow on the head and stored in a deep freeze at  $-20^{\circ}\text{C}$  for subsequent carcass analysis. Fish were fed a commercial trout diet (Ewos-Baker 49% protein, 7.5% lipid). Pellets were finely ground and 0.5% of

$\text{Cr}_2\text{O}_3$  was added as a digestibility marker before the diet was repelleted as described previously (Section 2.4.1.2). A 12h dark and 12h light photo period was maintained and fish were fed during the light period at times indicated in Table 9. On each feeding occasion fish were fed to satiation (Section 2.3.1). Accurate records of weekly food consumption were obtained by weighing the food containers for each tank so that food consumed was calculated by difference. Any mortalities occurring during the experimental period were also recorded. At weekly intervals throughout the eight week experimental period fish were bulk weighed to the nearest 0.1g without anaesthesia after starvation for 24h. During the final week of the experiment faeces were collected by siphoning from each tank during the first 2-4h after feeding to minimise the leaching of nutrients in water. The faecal samples were dried at  $105^\circ\text{C}$  for 12h to constant weight and stored in airtight bottles until required for analysis of crude protein and  $\text{Cr}_2\text{O}_3$ . On the final day of the experiment fish were individually weighed to the nearest 0.1g after sedation with benzocaine (Ross and Geddes, 1979). 15 fish from each tank were then sacrificed by an overdose of benzocaine (Ross and Geddes, 1979) and stored at  $-20^\circ\text{C}$  for subsequent proximate analysis.

2.6.2 The effect of feeding frequency on the growth and body composition of *O. niloticus* fed a restricted ration (6% b.w.)

The effect of feeding frequency on the growth and body composition of fish fed to a restriction ration was investigated in a five week experiment using fish of mean weight 1.2g (S.E.  $\pm$  0.1). 150 fish were acclimatised to the experimental system at  $27.5^\circ \pm 1^\circ\text{C}$  as



described in the previous section. At the start of the feeding trial 30 fish were sacrificed by administration of an overdose of benzocaine (Ross and Geddes, 1979) and stored at  $-20^{\circ}\text{C}$  for subsequent proximate analysis. The remaining 120 fish were individually weighed to the nearest 0.1g and stocked in six experimental tanks at a stocking density of 20 fish per tank, thus giving one replicate for each treatment. Fish were fed during the 12h light photo period on a commercial trout pellet (Ewos-Baker, 49% c.p.) at a rate of 6% of their body weight divided into two, four and six daily feeds. Thus meal size per feeding was 3%, 1.5% and 1% b.w. respectively per meal. Fish were bulk weighed weekly and their food intake was adjusted according to the new mean body weight. At the end of the five week feeding period fish were individually weighed to the nearest 0.1g after sedation with benzocaine (Ross and Geddes, 1979). 18 fish from each tank were sacrificed by an overdose of benzocaine (Ross and Geddes, 1979) and stored at  $-20^{\circ}\text{C}$  for subsequent proximate analysis.

### 2.6.3 The effect of meal size and fish weight on growth and body composition of *O. niloticus*

The effect of meal size and of fish weight on the growth and body composition of *O. niloticus* was investigated in a seven week growth trial at  $27.5^{\circ} \pm 1^{\circ}\text{C}$ . Two different size groups of fish of mean weights 14.27g (S.E.  $\pm 1.2$ ) and 6.8g (S.E.  $\pm 0.1$ ) were held in the experimental tanks for two weeks to acclimatise them to the experimental system. During this period the feeding regime was as described earlier (Section 2.6.1). At the start of the experiment 15 fish from each size were killed and stored at  $-20^{\circ}\text{C}$  for subsequent proximate analysis.

The remaining fish from each group were individually weighed to the nearest 0.1g and stocked at a rate of 20 fish per tank. One replicate was used for each treatment. Fish were fed three times daily during the 12h light photo period except on the day that the fish were weighed. Fish from each size class were fed the following levels of commercial trout pellets.

<u>Size group</u> ( $\bar{x} \pm$ S.E.)	<u>Feeding level</u> (% b.w.)
1. 14.27g $\pm 1.2$	0%, 1, 1.5, 2, 2.5, 3, 4% b.w.
2. 6.8g $\pm 0.1$	0%, 1, -, 2, -, -, 4, 6% b.w. (Ewos-Baker, 49% crude protein, 7.5% crude lipid)

Fish were bulk weighed weekly and their food intake was adjusted according to their new mean body weight. On termination of the experiment all the experimental fish were individually weighed to the nearest 0.1g after sedation with benzocaine (Ross and Geddes, 1979). 18 fish from each tank were sacrificed and stored at  $-20^{\circ}\text{C}$  for subsequent chemical analysis.

#### 2.6.4 The effect of food type on growth and body composition of *O. niloticus*

Since food composition showed a significant effect on gastric evacuation time and rate it was decided to use the diets prepared previously (Section 2.4.3.4) for an eight week growth trial. Diet effects on growth and body composition of *O. niloticus* of mean weight 1.1g (S.E.  $\pm 0.03$ ) were noted. 190 fish were acclimatised to the experimental tanks for a period of two weeks at  $27.5^{\circ} \pm 1^{\circ}\text{C}$  and during

this period they were fed at 3% of their body weight, three times daily, the following experimental diets:

Diet A: Control (35% crude protein, 11% crude lipid, 20.6% available carbohydrate)

Diet B: High available carbohydrate (35% crude protein, 10.3% crude lipid, 33% available carbohydrate)

Diet C: High lipid (35% crude protein, 18.8% crude lipid, 20% available carbohydrate)

Diet D: High protein (49% crude protein, 11.3% crude lipid, 16% available carbohydrate)

The formulation, preparation and the proximate composition of these were as described previously (Section 2.4.3.4). At the start of the growth trial 30 fish were sacrificed by administration of an overdose of benzocaine and stored at  $-20^{\circ}\text{C}$  for subsequent proximate analysis. The remaining 160 fish were individually weighed to the nearest 0.1g after sedation with benzocaine (Ross and Geddes, 1979) and stocked at a density of 20 fish per tank in eight experimental tanks, thus giving one replicate for each treatment. Fish were fed the four experimental diets at a rate of 6% b.w. three times daily during the 12h light photo period, except on the day that the fish were weighed. Fish were bulk weighed weekly after which their food intake was adjusted to the new mean body weight. During the last week of the experiment faeces from each tank were collected by siphoning during the first 2-4 hours after feeding and were dried in an oven at  $105^{\circ}\text{C}$  for 12h to a constant dry weight. The faecal samples were stored in airtight bottles until required for analysis of digestibility. On termination of the experiment fish were individually

weighed under anaesthesia using benzocaine (Ross and Geddes, 1979). 18 fish from each tank were sacrificed by an overdose of benzocaine and stored at -20°C for subsequent proximate analysis.

## 2.7 Analysis of Experimental Data

### a. Specific growth rate (SGR)

The SGR is the rate of change in weight of fish. It was calculated as the percentage increase in body weight per day as follows:

$$\text{SGR (\%/day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100 \quad (\text{after Brown, 1957})$$

Where

$W_1$  = The initial live body weight (g) at time  $T_1$  (day)

$W_2$  = The final live body weight (g) at time  $T_2$  (day)

### b. Food conversion ration (FCR)

The food conversion ratio is defined as the amount of dry food fed per unit live weight gain of fish. It was calculated as follows:

$$\text{FCR} = \frac{\text{Food fed (dry weight)}}{\text{Live weight gain}}$$

### c. Protein efficiency ratio (PER)

The PER is the gain in weight of fish per gram of crude protein consumed, and gives an indication of the efficiency with which the fish were able to utilize dietary protein. This was calculated as:

$$\text{PER} = \frac{\text{Live weight gain}}{\text{Crude protein fed}} \quad (\text{after Osborne et al., 1919})$$

d. Apparent net protein utilization (ANPU)

Net protein utilization is the apparent efficiency of deposition of dietary protein as body tissue. NPU was determined by the carcass analysis method of Miller and Bender (1955). Since no correction was made for endogenous nitrogen losses during the experiments, results are expressed as apparent NPU. This was calculated as:

$$\text{ANPU} = \frac{\text{Nb} - \text{Na}}{\text{Ni}} \times 100$$

Where

Na = The body nitrogen at the start of experiment

Nb = The body nitrogen at the end of experiment

Ni = The amount of nitrogen consumed

2.8 Chemical Analysis

Triplicate samples of diets and faeces from each experiment were taken and analysed as follows:

a. Moisture

Samples were oven dried at 105°C for 12-24 hours to constant weight.

b. Crude protein

This was determined by the microKjeldahl method for determining total nitrogen modified for use with the Tecator automatic distillation

system. Crude protein content was derived using the indirect method of Munro and Fleck (1969) by measuring the total nitrogen within the sample and converting this value to a crude protein value by multiplying it by the empirical factor of 6.25. This factor is based on the assumption that the average protein contains about 16% N. (AOAC, 1980).

c. Crude lipid

Moisture-free samples were extracted with petroleum ether (40°-60°C) in a soxhlet apparatus for four hours (AOAC, 1980).

d. Crude fibre

Crude fibre was determined by the digestion method with 12.5% H<sub>2</sub>SO<sub>4</sub> and 12.5% NaOH (AOAC, 1980).

e. Ash

This is defined as the inorganic residue left after the complete destruction of the organic matter. 1.0g of samples were ashed in a muffle furnace at 450°C for 24h. (AOAC, 1980).

f. Chromic oxide

The chromic oxide contents of the experimental diets and faeces were determined by the wet digestion method of Furukawa and Tsukahara (1966). Apparent nutrient digestion coefficients were determined using the formulae of Maynard and Loosli (1969).

Apparent dry matter digestion coefficient =

$$100 - \left( 1 - \frac{C}{B} \times \frac{1 - B}{1 - C} \right)$$

Where

C = Cr<sub>2</sub>O<sub>3</sub> in diet (g/100g)

B = Cr<sub>2</sub>O<sub>3</sub> in faeces (g/100g)

Apparent protein digestion coefficient =

$$100 \left( 100 - \frac{\% \text{ Cr}_2\text{O}_3 \text{ in diet}}{\% \text{ Cr}_2\text{O}_3 \text{ in faeces}} \times \frac{\% \text{ Protein in faeces}}{\% \text{ Protein in diet}} \right)$$

## 2.9 Statistical Methods

Statistical comparisons of the results were made using analysis of variance. Mean differences were determined using Duncan's Multiple Range Test (Duncan, 1955). Standard error ( $\pm$  S.E.) was calculated to identify the range of means.

### 3. RESULTS

#### 3.1 Food Intake

##### 3.1.1 The effect of feeding frequency on food intake

Maximum daily food intake of fish mean weight 31.1g held at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$  increased with increasing frequency of feeding up to six feeds per day (Fig. 3), therefore there was no significant difference in food consumption between fish fed six and eight times per day (Table 10). The increase in food intake was found to be significant at the 0.05 level. Although fish were fed to satiation on each feeding occasion the food intake in a single meal was much smaller for the higher feeding frequency regimes than under the lower ones.

##### 3.1.2 The effect of fish weight on maximum daily food intake

Maximum daily food intake for each weight class of fish was found to increase with increasing fish weight (Fig. 4). To establish the mathematical relationship between maximum food intake and fish weight, four different transformations of the data were carried out.

Model 1 - Assumes that food intake is linearly related to body weight

$$M = a + bw \quad (1)$$

Where M = maximum daily food intake  
w = fish weight  
a and b are constants.

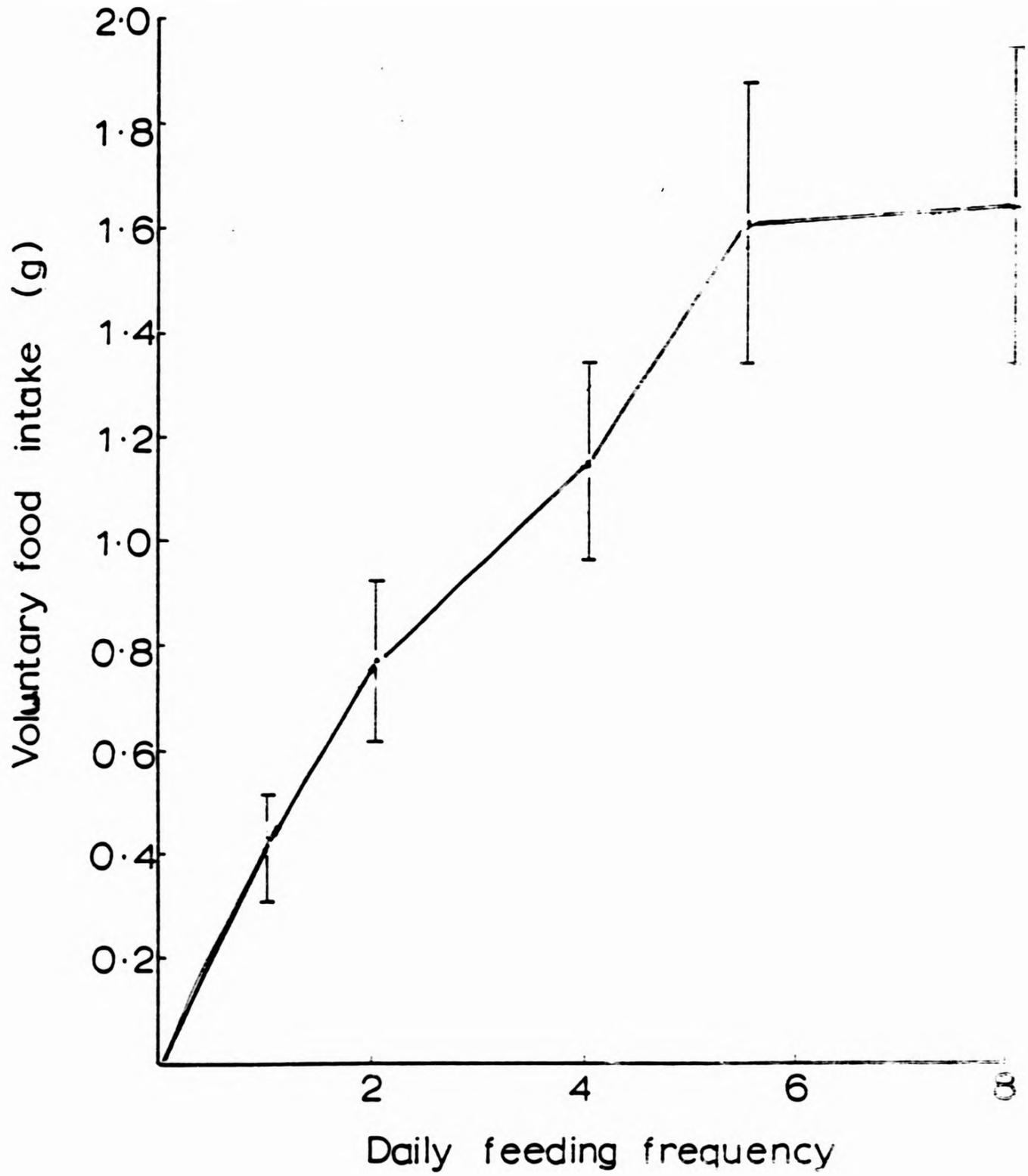


FIGURE 3 The effect of feeding frequency on the maximum daily food intake (weight of fish)

TABLE 10 The relationship between feeding frequency and food intake of O. niloticus fed to satiation at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Daily feeding frequency	1	2	4	6	8
Daily mean food intake (g)	0.403 <sup>a</sup>	0.768 <sup>b</sup>	1.158 <sup>c</sup>	1.615 <sup>d</sup>	1.633 <sup>d</sup>
Food intake g/meal	0.403	0.384	0.2895	0.269	0.204

Mean value with the same superscript are not significantly different at 0.05

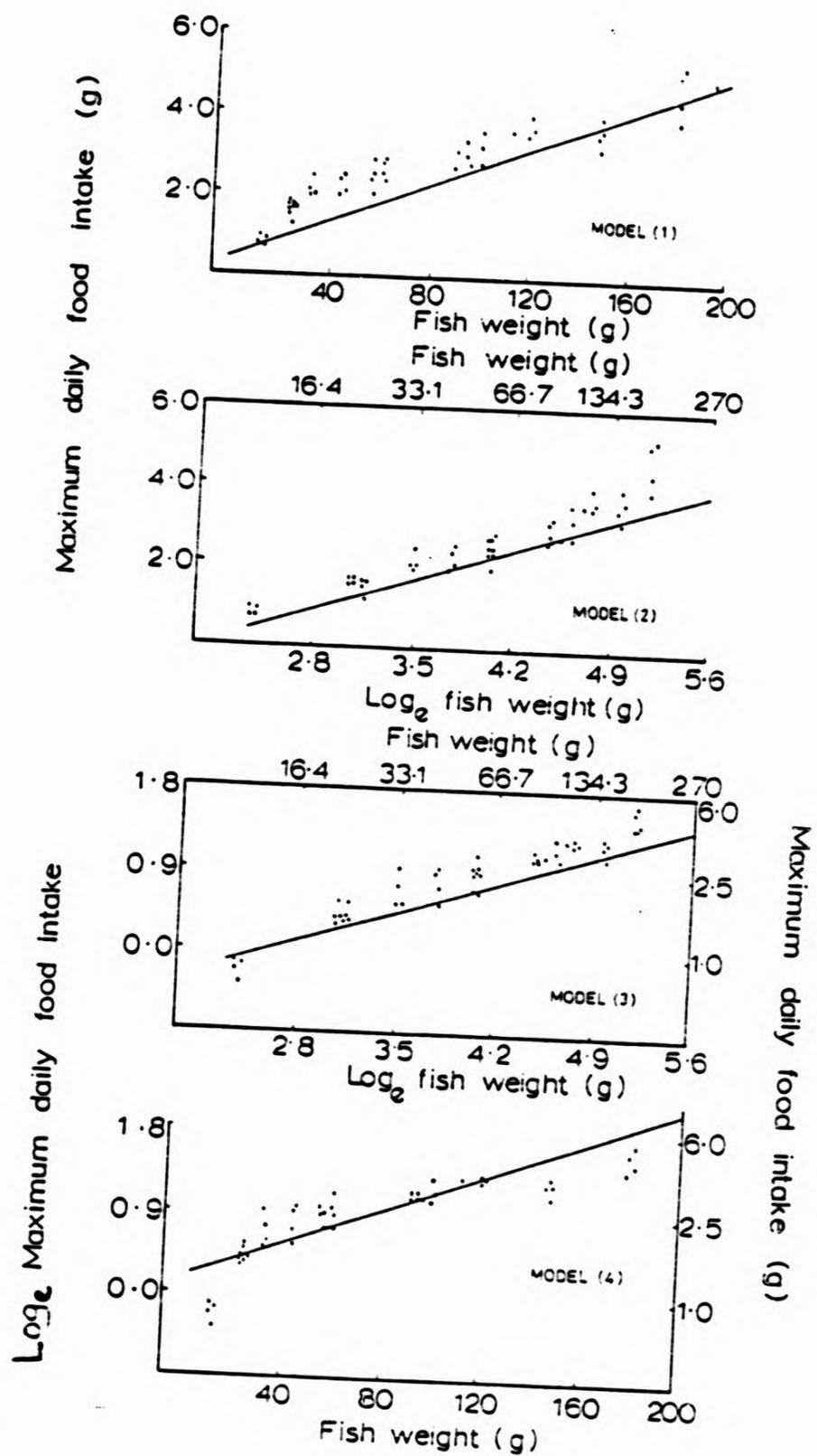


FIGURE 4 The effect of fish weight (g) on maximum daily food intake by *O. niloticus* at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

Model 2 - Assumes that food intake is a linear function of the natural logarithm of body weight

$$M = a + b \text{Log}_e w \quad (2)$$

Model 3 - Assumes that there is a linear relationship between the natural logarithms of both food intake and body weight

$$\text{Log}_e M = a + b \text{Log}_e w \quad (3)$$

Model 4 - Assumes that food intake increases exponentially with body weight

$$\text{Log}_e M = a + bw \quad (4)$$

The success of each transformation of the data was estimated on the basis of two criteria. Firstly, the correlation coefficient ( $r$ ) was used to give a measure of the proportion of the dependent variable explained by the regression line on the independent variable. The closer the value of ( $r$ ) is to 1, the more useful is the fitted equation for predicting the future value of the dependent variable. Secondly, the adequacy of the fitted line was determined by plotting the residuals, which are the differences between the observed values of the dependent and the predicted values for that variate from the equation, against the predicted values (Fig. 5). The more the residual values scattered round zero the greater the adequacy of the line, (Zar, 1974).

Table 11 gives the regression equations, correlation coefficients and significance levels. From both Table 11 and Fig. 5 it is apparent that the best fit for the data was obtained using Model 3 where both

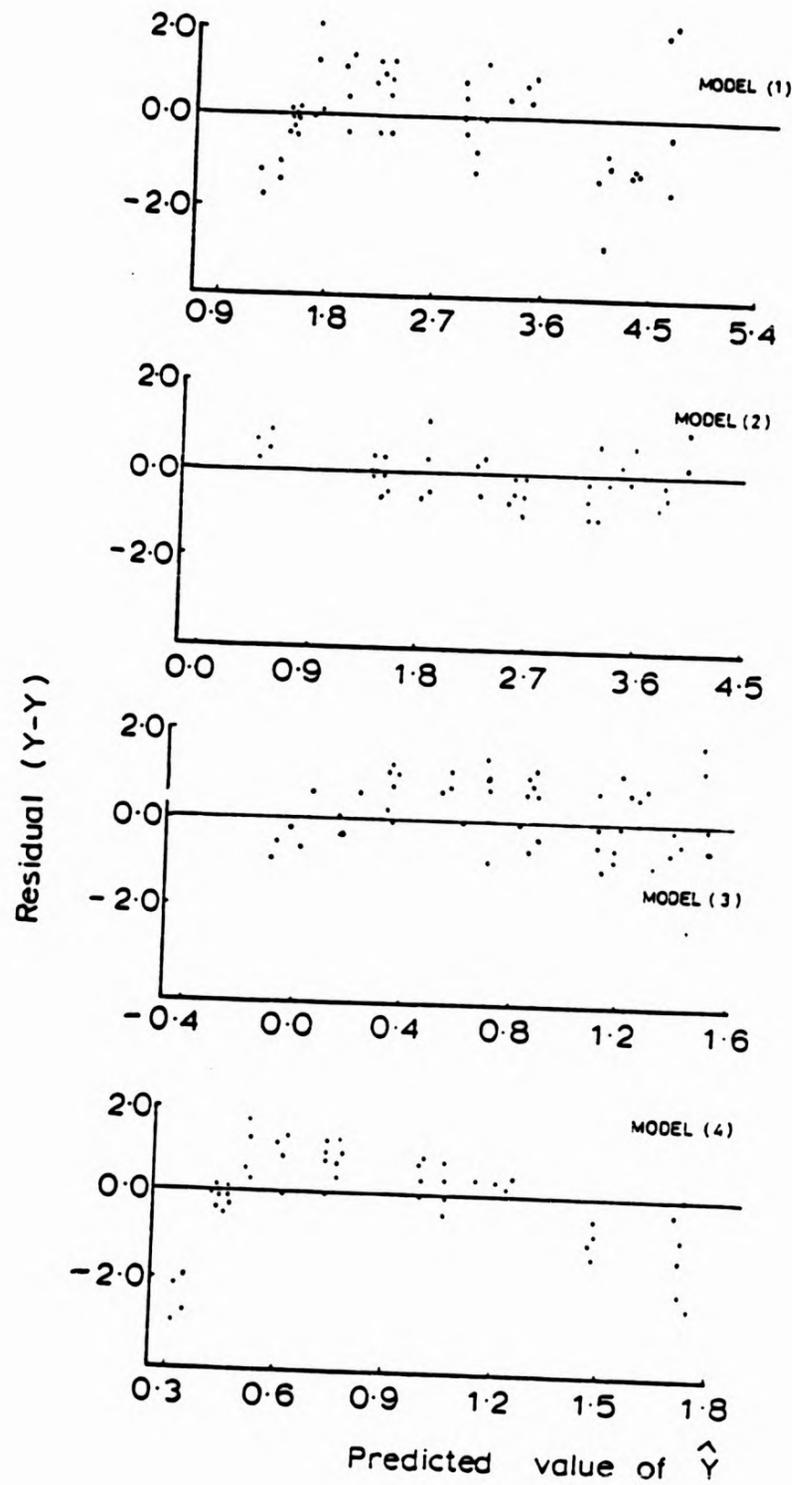


FIGURE 5 Residuals plotted against predicted values  $\hat{Y}$  for the four models used for the relationship between maximum daily food intake and fish weight (g)

TABLE 11 Regression equations and correlation coefficients for the four mathematical models predicting maximum food consumption for different weights of O. niloticus at 27.5°C

Model	Regression equation	Correlation Coefficients	P <
1. $M = a + bw$	$M = 1.10 + 0.0202w$	0.95	0.001
2. $M = a + b\text{Log}_e w$	$M = -2.43 + \text{Log}_e 1.25$	0.942	0.001
3. $\text{Log}_e M = a + b\text{Log}_e w$	$\text{Log}_e M = -1.38 + 0.556\text{Log}_e w$	0.97	0.001
4. $\text{Log}_e M = a + bw$	$\text{Log}_e M = 0.252 + 0.00813w$	0.89	0.001

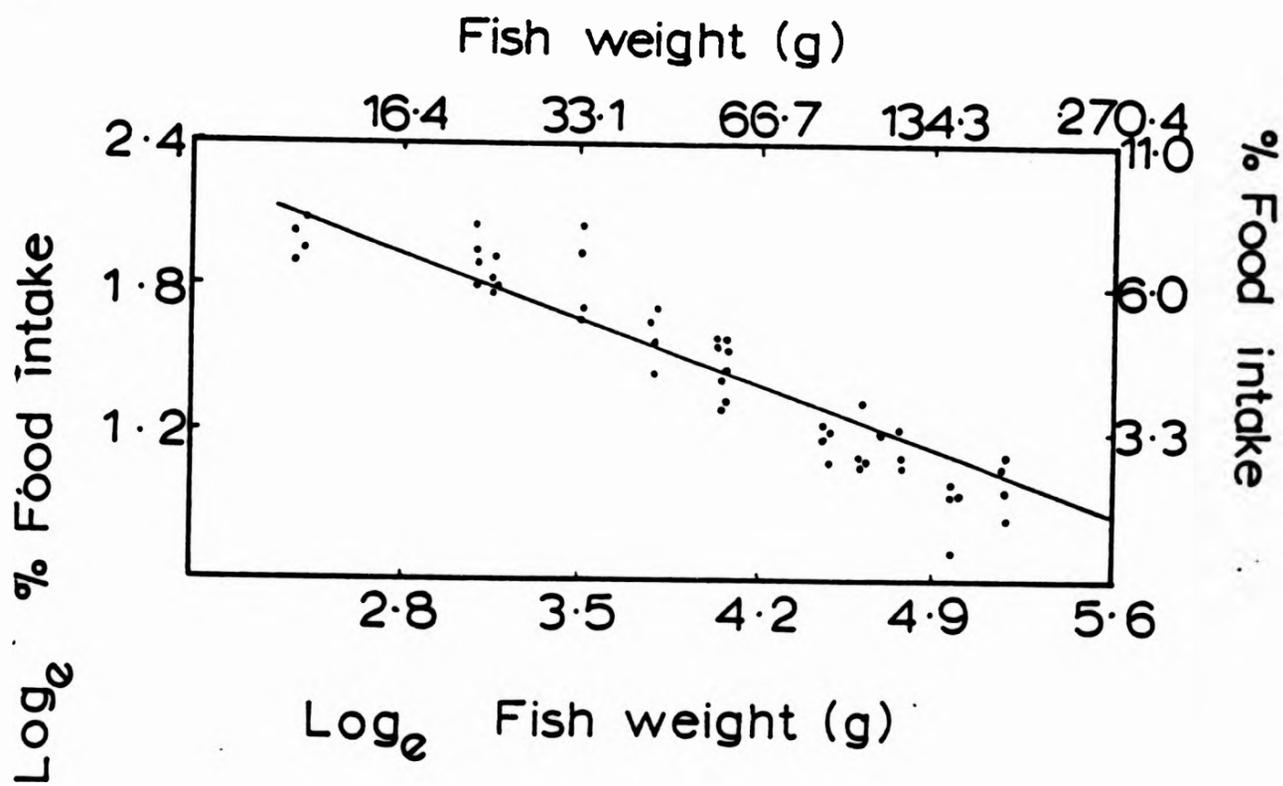


FIGURE 6 The effect of fish weight (g) on maximum daily food intake (% b.w.) by O. niloticus

food intake and fish weight were transformed into natural logarithms. Also from Table 11 and Fig. 4 it can be seen that there is a positive linear relationship between maximum food intake and fish weight; Thus maximum daily food intake increased with fish size over the wide range of fish size investigated. Consequently a 10g fish would only consume 0.91g of food, but this will increase to 4.78g for a 200g fish. Food intake decreases with fish size, however, when expressed on a % body-weight basis (Fig. 6). The regression equation of this relationship was calculated as:

$$\text{Log}_e \text{ maximum daily food intake (\%)} = 3.22 - 0.443 \text{ Log}_e \text{ fish weight (g)}$$

The correlation coefficient of 0.95 was highly significant at 0.001 level. Thus a 200g fish would only consume 2.39% of its body weight per day compared to 9.02% b.w. for 10g fish.

### 3.1.3 Maximum food intake in a single meal (satiation amount)

Before removal of the stomach contents the form of the digestive tract was observed. The digestive tract of O. niloticus is relatively simple (Fig. 7) consisting of small caecal-type stomach and a very long tapering intestine which is characteristic of a herbivorous habit. The buccal cavity contains two types of teeth, the jaw and the pharyngeal teeth which are specialised for shredding coarse material and breaking open cell walls. The action of both sets of teeth increases the surface area to volume of the food which facilitates enzyme-substrate interactions in the stomach. From the buccal cavity the food mass passes to the stomach via a short oesophagus of relatively small diameter. The stomach consists of a simple thin-walled sac

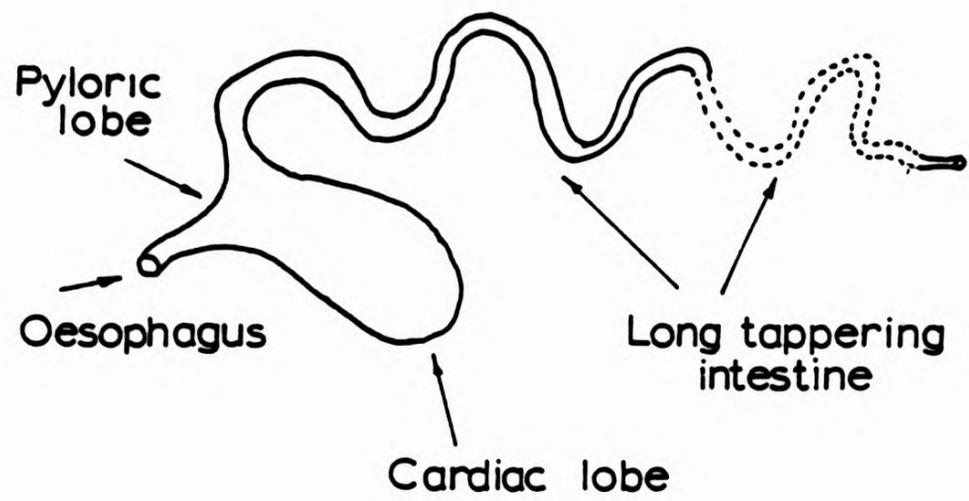


FIGURE 7 Diagrammatic representation of the alimentary canal of O. niloticus

which opens into the intestine via a pyloric sphincter. The intestine consists of many coils and ends in an anal sphincter. The anterior portion of the intestine is short, thin-walled and of greater diameter than the posterior part. The common bile duct opens into the interior part of the intestine immediately behind the pyloric sphincter.

15 fish from each of the four weight classes were used to establish the relationship between fish weight and fish length, intestine length and fish length, and liver weight and fish weight. There was a linear relationship between  $\log_{10}$  fish weight and  $\log_{10}$  fish length (Fig. 8) and the regression equation of the relationship was calculated as:

$$\text{Log}_{10} \text{ fish weight (g)} = -1.62 + 2.97 \text{ Log}_{10} \text{ fish length (cm)}$$

The correlation coefficient of 0.99 was highly significant at the 0.001 level of significance.

There was a positive linear relationship between intestine length and fish length (Fig. 9). The regression equation of this relationship was calculated as:

$$\text{Intestine length (cm)} = -14.5 + 6.86 \text{ fish length (cm)}$$

The correlation coefficient of 0.95 was highly significant at the 0.001 level of significance. Thus the relative gut length increased with increasing fish length from 4.8 for a 7cm fish to 6.14 for a 20cm fish.

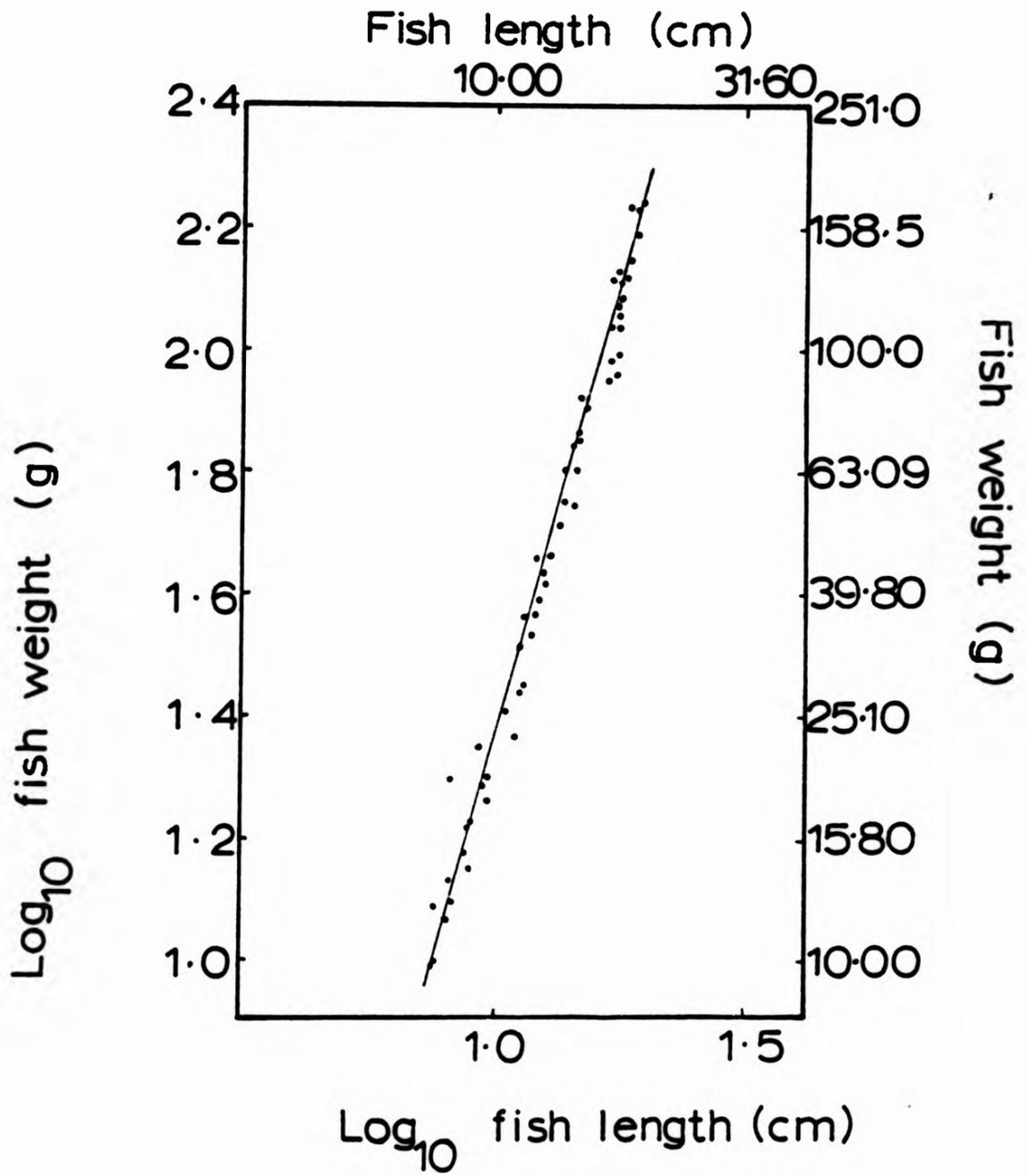


Figure 8 The relationship between  $\text{Log}_{10}$  fish weight (g) and  $\text{Log}_{10}$  fish length (cm)

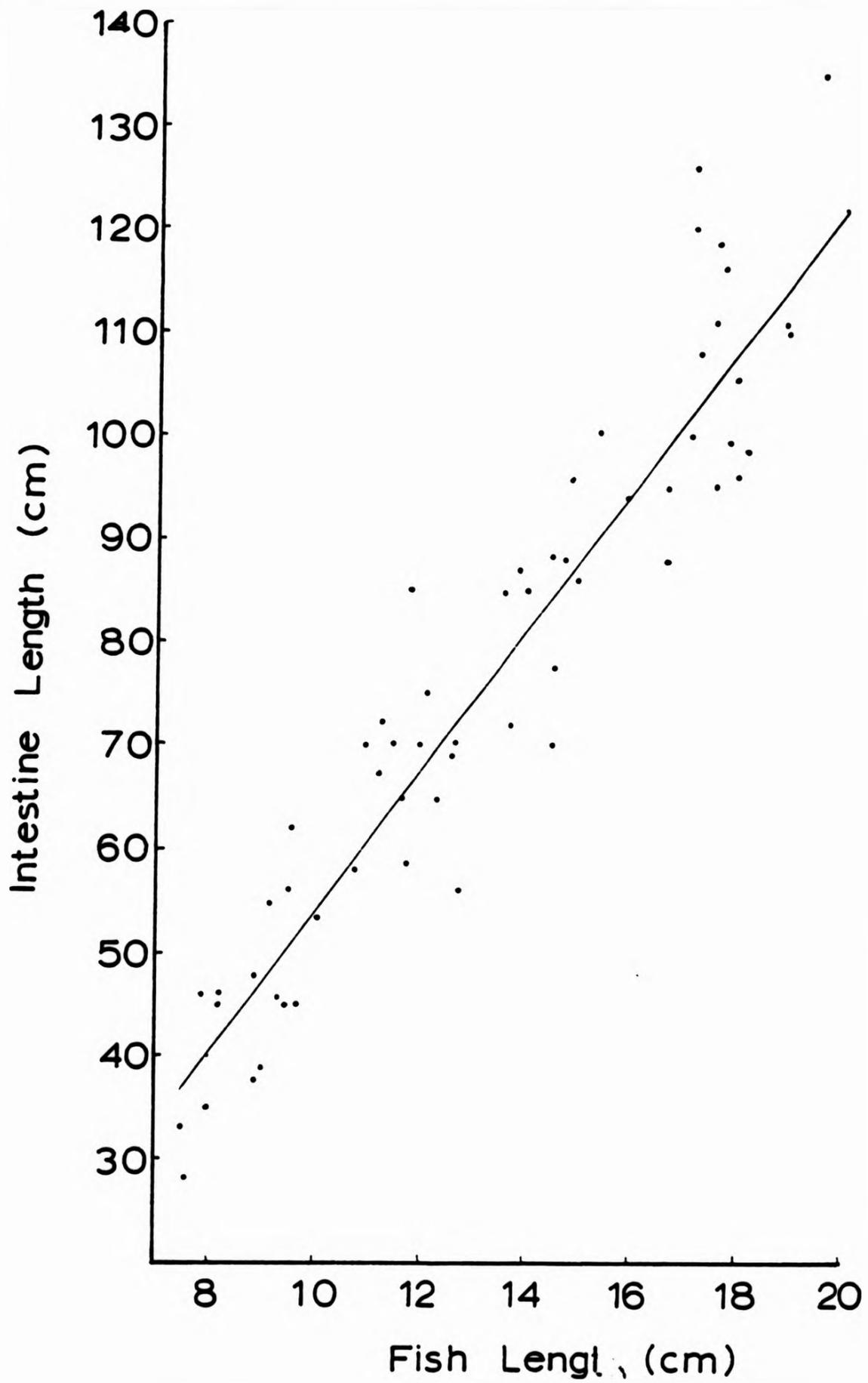


FIGURE 9 The relationship between fish length (cm) and intestine length (cm)

There was also a positive linear relationship between liver weight and fish weight (Fig. 10) and the regression equation of this relationship was calculated as:

$$\text{Liver weight (g)} = 0.0383 + 0.01669 \text{ fish weight (g)}$$

The correlation coefficient of 0.97 was highly significant at the 0.001 level of significance. Thus liver weight increased from 0.205 for a 10g fish to 3.38g for a 200g fish. However, when liver weight was expressed as a percentage of fish weight, it was found to decrease with fish weight from 2.05% for a 10g fish to 1.69% for a 200g fish.

There was a positive linear relationship between fish weight and food intake in a single meal (Fig. 11). The regression equations were calculated as:

$$\text{Food intake in a meal (dry weight)} = 0.173 + 0.01188 \text{ fish weight}$$

$$\text{Food intake in a meal (wet weight)} = 0.75 + 0.044 \text{ fish weight}$$

The correlation coefficients of 0.95 and 0.92 for dry and wet weights respectively, were significant at the 0.001 level. Since the best description of this relationship in the previous experiment (3.1.2) was obtained using Model 3 where both food intake and fish weight were transformed into natural logarithms, the present data were also analysed using Model 3 and the regression equations were calculated as:

$$\text{Log}_e \text{ food intake (dry wt) in g} = -3.60 + 0.856 \text{ Log}_e \text{ fish wt in g}$$

$$\text{Log}_e \text{ food intake (wet wt) in g} = -1.96 + 0.776 \text{ Log}_e \text{ fish wt in g}$$

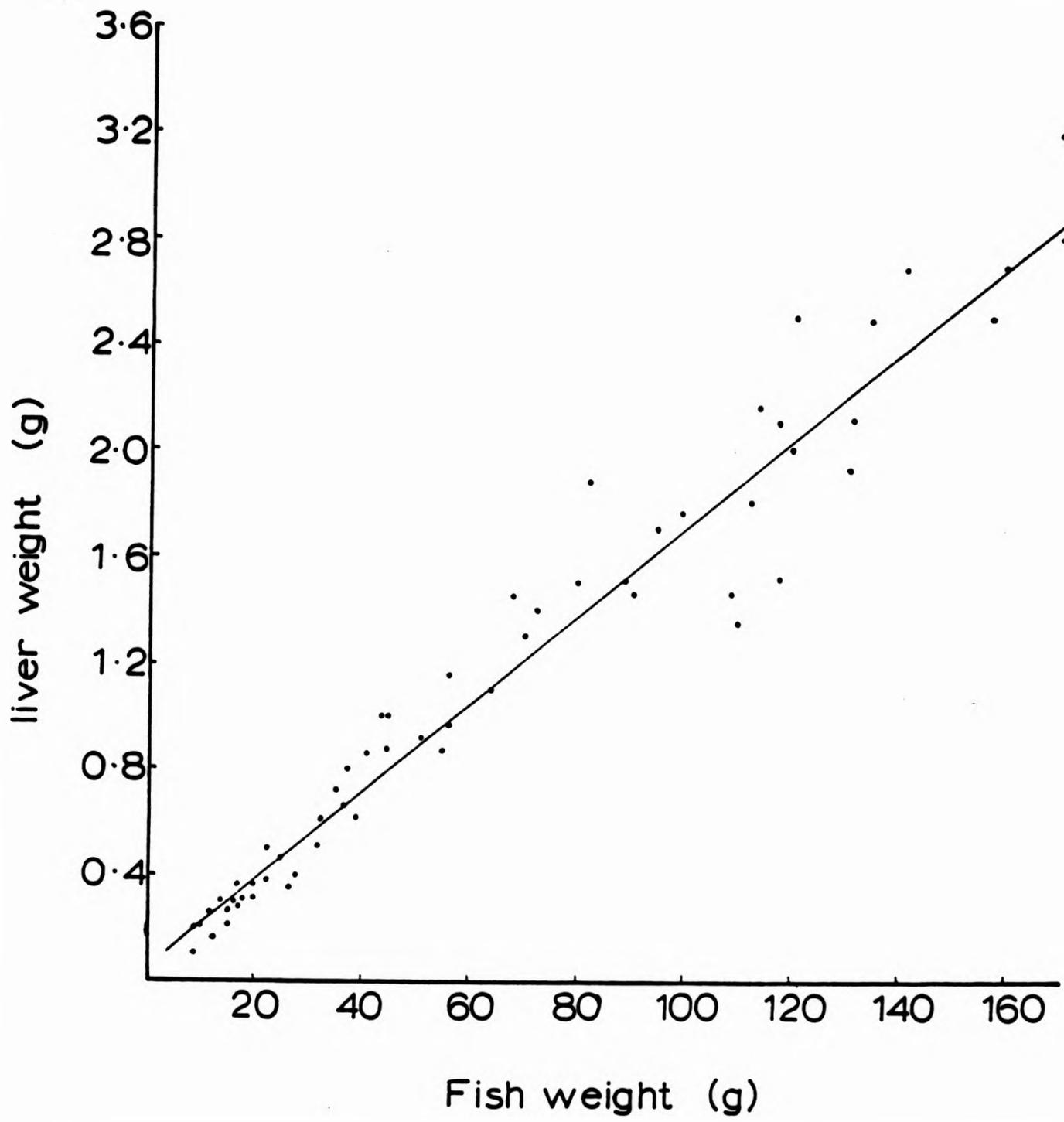


FIGURE 10 The relationship between fish weight (g) and liver weight (g)

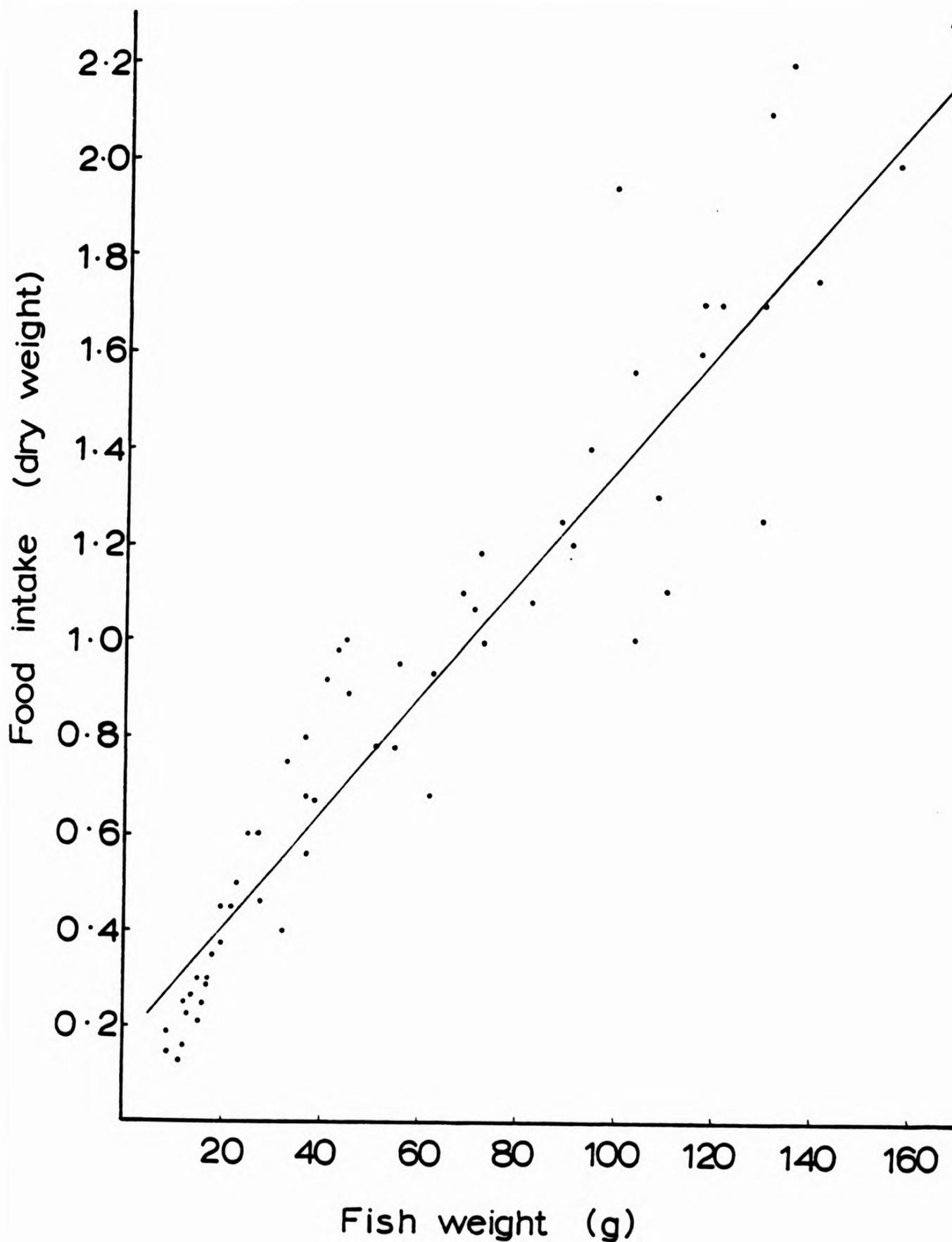


FIGURE 11 The relationship between maximum food intake in a single meal (g) and fish weight (g)

The correlation coefficient of 0.96 and 0.93 for dry and wet weight respectively, were highly significant at the 0.001 level. Thus the maximum food intake in a single meal for a 200g fish was 9.55g on a wet weight basis, equivalent to 2.55g dry weight of food. A 10g fish, however, consumed only 1.19g of food on a wet weight basis equivalent to 0.292g of food on a dry weight basis. Although the food intake was found to increase with fish weight, it decreased with fish weight when expressed as a percentage of their body weight. Thus the maximum food intake in a single meal for a 10g fish was 8.4% b.w. on wet weight basis, equivalent to 1.96% b.w. on dry weight basis, which declined to 4.30% b.w. and 1.27% b.w. on wet and dry weight bases for a 200g of fish.

The relationship between stomach volume (in ml) and fish weight (in g) was also established for the four size classes of fish investigated. An estimation of stomach volume was attempted for each of the 60 fish used in the determination of the maximum food intake in a single meal. However, due to the fragility of the stomach wall, values were only obtained for a total of 18 fish covering the four size classes. Based on these values a linear relationship between stomach volume (in ml) and fish weight (in g) was established (Fig. 12) with the following regression equation:

$$\text{Stomach volume (ml)} = 0.82 + 0.059 \text{ fish weight (g)}$$

The correlation coefficient of 0.95 was highly significant at the 0.001 level. Thus stomach volume was found to increase from 1.41ml for a 10g fish to 12.62ml for a 200g fish, but its capacity:

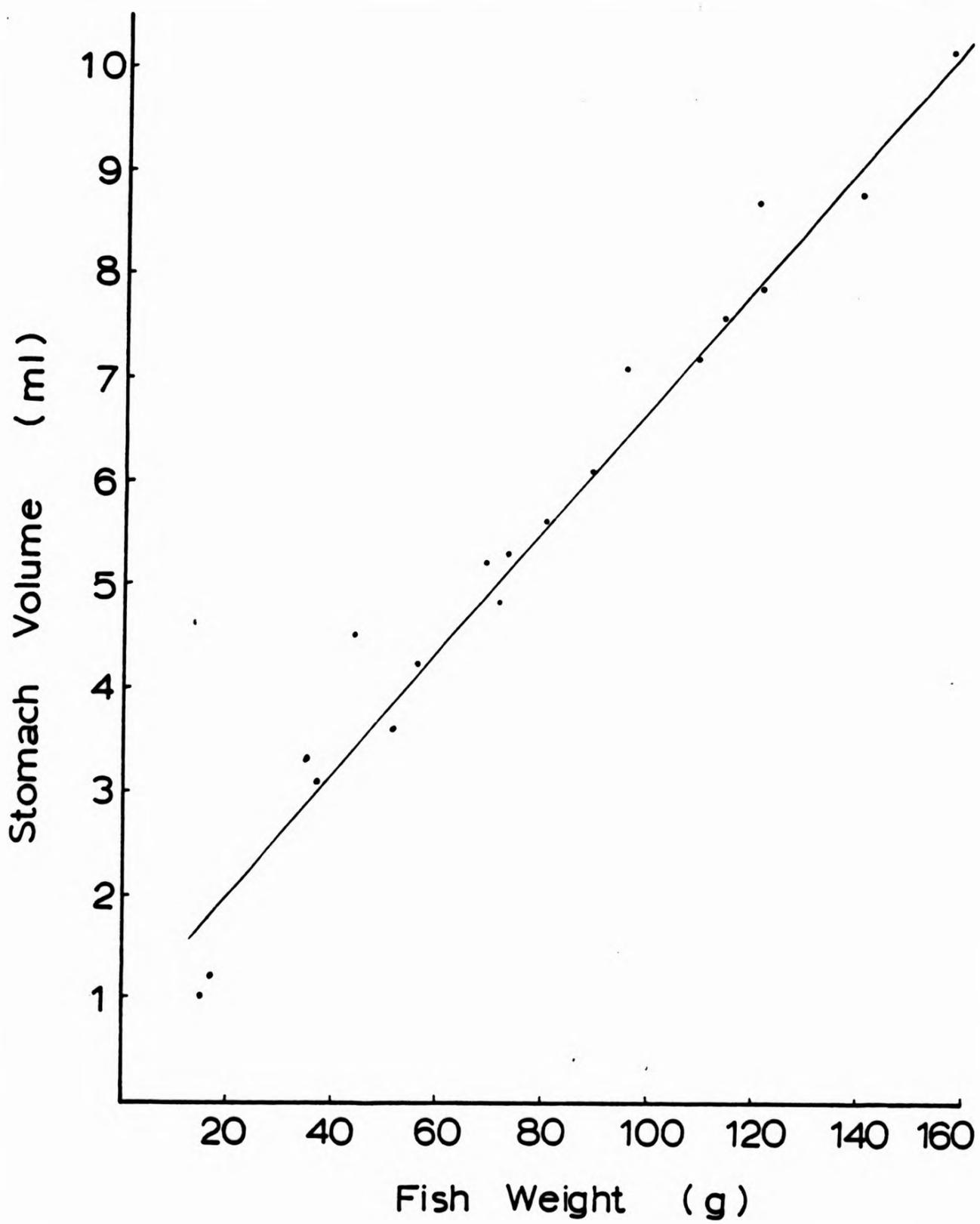


FIGURE 12 The relationship between stomach volume (ml) and fish weight (g)

$$\frac{\text{Stomach volume (ml)}}{\text{Fish weight (g)}} \times 100$$

decreased with fish weight from 14.1% b.w. for a 10g fish, to 6.3% for a 200g fish.

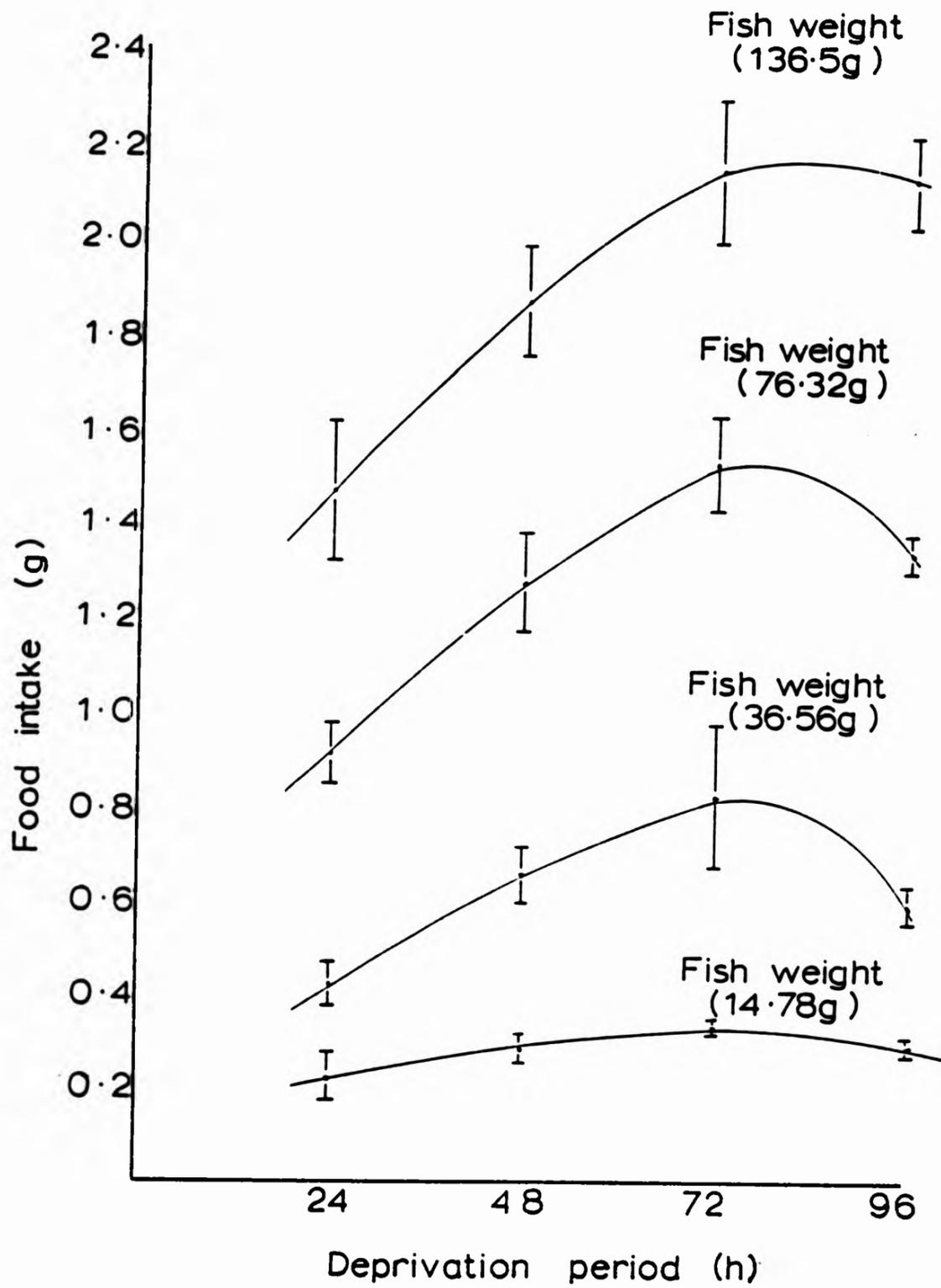
The observed food intake based on stomach contents for each size class, the predicted food intake and stomach volume for the four size classes of fish are presented in Table 12. From this table it can be seen that both food intake and stomach capacity decreased with increasing fish size when expressed as a percentage of their body weights. Also it can be seen that the observed and the predicted food intake (as b.w.) represented 76%-83% of the stomach capacity.

#### 3.1.4 The effect of period of starvation on subsequent food intake

Four size classes of fish were used to establish the effect of starvation period of 24h, 48h, 72h, and 96h duration on subsequent food intake. The mean food consumption in a single meal for each of the four size classes of fish following a period of starvation was found to increase with increasing starvation period from 24h to 72h of food deprivation (Fig. 13). However in each case the food intake by fish starved for 96h was less than that of fish starved for 72h. To establish the mathematical relationship between food intake, fish size and starvation period, the wet and dry weights of food consumed by individual fish after each period of starvation were transformed to natural logarithms (Model 3) and were used to produce the following multiple regression equation:

TABLE 12 Comparison between food intake and stomach capacity for different size classes of *O. niloticus* fed to satiation in a single meal at 27.5°C

Fish size Mean S.E. (Range)	Food intake % b.w. Dry weight		Food intake % b.w. Wet weight		Stomach capacity as % b.w. $\frac{\text{st. vol.} \times 100}{\text{fish wt.}}$	Food intake % bw wet wt. x100 Stomach capacity %	
	Observed	Predicted	Observed	Predicted		Observed	Predicted
14.79 ± 0.88 (9.07-20.0)	1.76	2.36	8.25	9.50	11.44	72.0%	83.0%
34.77 ± 2.03 (22.6-45.9)	1.97	1.69	7.08	6.60	8.25	85.8%	80.0%
73.64 ± 3.97 (51.73-99.6)	1.47	1.42	5.32	5.42	7.01	75.9%	77.32%
131.5 ± 5.3 (109.1-171.31)	1.27	1.32	4.78	4.97	6.52	73.3%	76.23%



**FIGURE 13** The effect of prefeeding starvation periods on food intake for four different sizes of fish at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

$$\text{Log}_e \text{ food intake(dry wt)}(g) = -4.94 + 0.894\text{Log}_e \text{ fish wt}(g) + 0.298\text{Log}_e \text{ starvation period (h)}$$

$$\text{Log}_e \text{ food intake(wet wt)}(g) = -3.25 + 0.902\text{Log}_e \text{ fish wt (g)} + 0.181\text{Log}_e \text{ starvation period (h)}$$

The multiple correlation coefficients of 0.91 and 0.92 were highly significant at the 0.001 level. The fact that the data considered consists of two independent variables, fish weight and starvation period, does not automatically imply that these two variables have a significant effect on the dependent variable, food intake. To establish the influence of these two variables on food intake the 't' test was applied (Zar, 1974). The calculated t values were found to be 32.67 and 6.00 for fish weight and starvation period, respectively. These values both exceed the tabulated value of 3.29 and are thus significant at the 0.001 level. Thus food intake was found to increase with fish weight and starvation period over the wide range of fish sizes investigated. The 't' value also indicated that fish weight is the most potent factor affecting the food intake, whilst starvation period produces a lesser effect. Logically stomach volume will reach a maximum and food intake can not be increased linearly with starvation period independently. Analysis was performed to determine the starvation period beyond which no further increase in food intake occurred. One way analysis of variance revealed that the mean food intake for different weights of fish investigated increased with starvation period (h) up to 72h, further increase in starvation period decreased the food intake, however, this decrease is not significant at the 0.005 level (Table 13).

**TABLE 13** The effect of starvation on subsequent food intake by different weight classes of O. niloticus at 27.5°C ± 1°C

Group	Fish weight $\bar{x}g \pm S.E.$	Deprivation Periods (h)			
		24	48	72	96
1	14.78 ± 0.47	0.218 <sup>a</sup>	0.281 <sup>ab</sup>	0.325 <sup>b</sup>	0.293 <sup>ab</sup>
2	36.56 ± 1.12	0.417 <sup>a</sup>	0.648 <sup>b</sup>	0.85 <sup>c</sup>	0.593 <sup>b</sup>
3	76.32 ± 1.79	0.926 <sup>a</sup>	1.29 <sup>b</sup>	1.53 <sup>b</sup>	1.324 <sup>b</sup>
4	136.5 ± 2.8	1.473 <sup>a</sup>	1.86 <sup>b</sup>	2.16 <sup>b</sup>	2.14 <sup>b</sup>

Mean values with the same superscript are not significantly different (P < 0.05)

The effect of periods of food deprivation on stomach volume was also established. The recorded data of stomach volume of different sizes of fish after each period of starvation were used to produce the following multiple regression equations:

$$\text{Log}_e \text{ stomach volume (ml)} = -3.28 + 0.866 \text{ Log}_e \text{ fish weight(g)} + 0.252 \text{ Log}_e \text{ starvation period (h)}$$

The multiple correlation coefficient of 0.87 demonstrates a directly proportional relationship between these parameters and was found to be significant at the 0.001 level. Calculated 't' values were 15.4 and 4.89 for fish weight and starvation period respectively. These values both exceed the tabulated value of 3.46 and are thus significant at the 0.001 level. Stomach volume of a 10g fish would therefore increase from 0.616 (ml) after 24h of starvation to 0.87 (ml) after 96h deprivation, and that of a 200g fish from 8.24 (ml) to 11.7 (ml) over the same period.

Similar multiple regression analysis was carried out to determine the effect of deprivation time on both intestine length and liver weight. Data for fish length and intestine length after each period of starvation were used to calculate the following multiple regression equation:

$$\text{Intestine length (cm)} = -18.8 + 7.51 \text{ fish length (cm)} - 0.096 \text{ starvation period (h)}$$

The multiple correlation coefficient of 0.95 was highly significant at the 0.001 level. The calculated 't' values were 49.04 and 4.48 for fish length and starvation period respectively, which are both

significant at the 0.001 level. Intestine length increased with increasing fish length after any given period of starvation, but there is a significant decrease in intestine length with increasing starvation period for a given fish length. Thus the intestine length of a 7cm fish would decrease from 31.47cm at 24h of starvation to 24.57cm after 96h of food deprivation, and that of 20cm fish from 129.1cm at 24h starvation to 122.8cm after 96h.

The multiple regression equation relating liver weight to fish weight and deprivation time was calculated as:

$$\text{Liver weight (g)} = 0.0305 + 0.017 \text{ fish weight (g)} - 0.00057 \text{ starvation period (h)}$$

The multiple correlation coefficient of 0.98 was significant at the 0.001 level. The calculated 't' value was 69.65 for fish weight. This value exceeded the tabulated value of 3.29 and was thus significant at the 0.001 level. However the calculated 't' value for the starvation coefficient was 1.31 and this was not significant at the 0.05 level. Thus liver weight increased with increasing fish weight, while starvation periods up to 96h had no significant effect on liver weight for fish in the size range 10g-200g.

Table 14 summarises the effect of starvation period on the subsequent food intake (based on stomach content), stomach capacity, gut relative length and the relative liver weight for the four different weight classes of fish investigated.

TABLE 14 Effect of starvation on food intake, stomach capacity, gut relative length and relative liver weight for different size classes of *O. niloticus* at  $27.5 \pm 1^\circ\text{C}$

Fish weight (g) Mean $\pm$ S.E.	Deprivation time (hr)	Observed food intake as % b.w.		Predicted stomach capacity (%)	Fish length (cm) Mean $\pm$ S.E.	Intestine length (cm)	Gut relative length	Relative liver weight
		Dry	Wet					
1	24	1.60	6.40	5.91	8.63 $\pm$ 0.19	45.60 $\pm$ 2.7	5.29	1.62
	48	1.89	6.71	6.96	8.94 $\pm$ 0.18	41.73 $\pm$ 1.9	4.67	1.60
	72	2.11	6.72	7.65	9.19 $\pm$ 0.21	41.63 $\pm$ 1.5	4.53	1.55
	96	1.91	6.90	8.25	9.08 $\pm$ 0.23	43.90 $\pm$ 1.4	4.83	1.58
2	24	1.16	4.40	5.18	11.70 $\pm$ 0.18	72.60 $\pm$ 2.6	6.21	1.74
	48	1.85	6.70	6.19	11.86 $\pm$ 0.19	60.96 $\pm$ 2.36	5.13	1.78
	72	1.98	6.45	6.71	12.50 $\pm$ 0.18	65.03 $\pm$ 2.5	5.20	1.60
	96	1.60	5.06	7.32	12.19 $\pm$ 0.26	65.83 $\pm$ 2.61	5.40	1.63
3	24	1.25	4.65	4.72	13.90 $\pm$ 0.20	89.20 $\pm$ 8.6	6.42	1.68
	48	1.59	5.89	5.60	14.57 $\pm$ 0.25	89.00 $\pm$ 3.4	6.10	1.75
	72	2.05	6.09	6.20	15.30 $\pm$ 0.16	92.20 $\pm$ 2.95	6.0	1.75
	96	1.71	5.69	6.60	15.70 $\pm$ 0.16	85.33 $\pm$ 2.5	5.4	1.73
4	24	1.09	3.96	4.35	18.50 $\pm$ 0.21	121.80 $\pm$ 4.2	6.58	1.80
	48	1.37	4.96	5.17	18.83 $\pm$ 0.30	117.60 $\pm$ 3.16	6.25	1.63
	72	1.51	5.16	5.70	18.57 $\pm$ 0.11	114.13 $\pm$ 3.4	6.10	1.59
	96	1.53	5.45	6.10	18.67 $\pm$ 0.15	115.16 $\pm$ 3.6	6.17	1.68

### 3.1.5 The effect of starvation on gall bladder weight

Mean gall bladder weight expressed as a percentage of the liver weight for a fish of mean weight 38.8g under a normal feeding regime was 15% of the liver weight (10%-21% liver weight) (Fig. 14). The bile at this time was pale straw coloured and the stomach and the whole intestine were always full of food. The gall bladder weight expressed as a percentage of liver weight increased with increasing periods of starvation to a maximum of  $45\% \pm 6.5$  of the liver weight after 72h of food deprivation (Fig. 15). After 96h of food deprivation there was no further increase in gall bladder weight which suggests that gall bladder had reached its maximum capacity after 72h of food deprivation. Furthermore after 96h of food deprivation the presence of bile was noted in the intestine. However, there was no significant difference ( $P > 0.05$ ) between fish starved for 48h, 72h and 96h in the gall bladder weight (Table 15). With increasing periods of starvation the bile colour became gradually darker, changing from the normal pale straw colour for the first 24h to a light green by 48h and finally after 96h of food deprivation it was dark green. After the fish had been starved for 96h they were again fed normally at two hourly intervals. Within two hours of feeding the gall bladder had decreased in size to around 25% of the liver weight and after a further six hours of normal feeding regime the gall bladder decreased to 14.6% of the liver weight and thus had returned to its normal size for unstarved fish (Fig. 15); the colour of bile by that time had returned to pale straw.

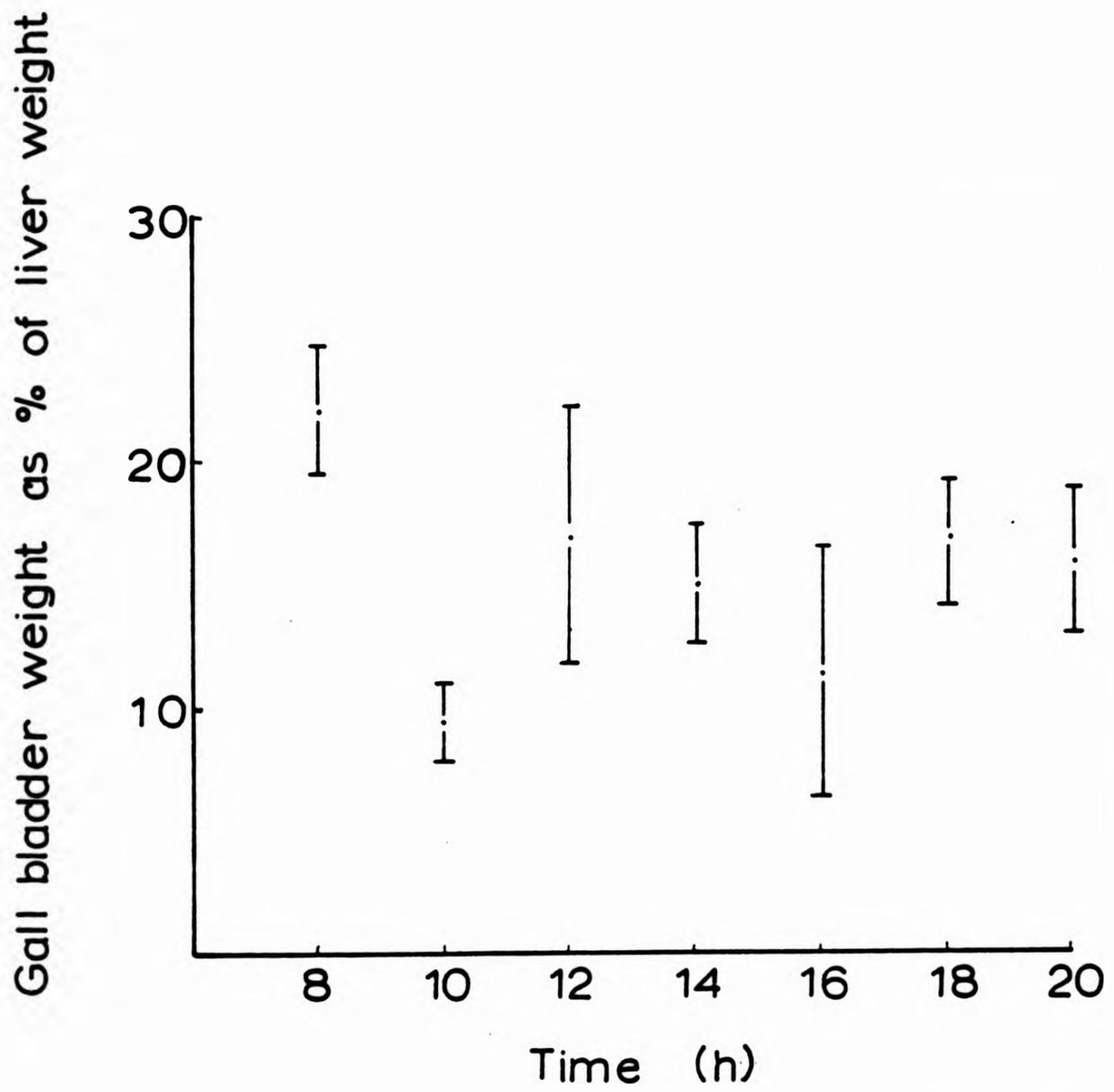


FIGURE 14 Variation in gall bladder weight as percentage of liver weight under a normal feeding regime

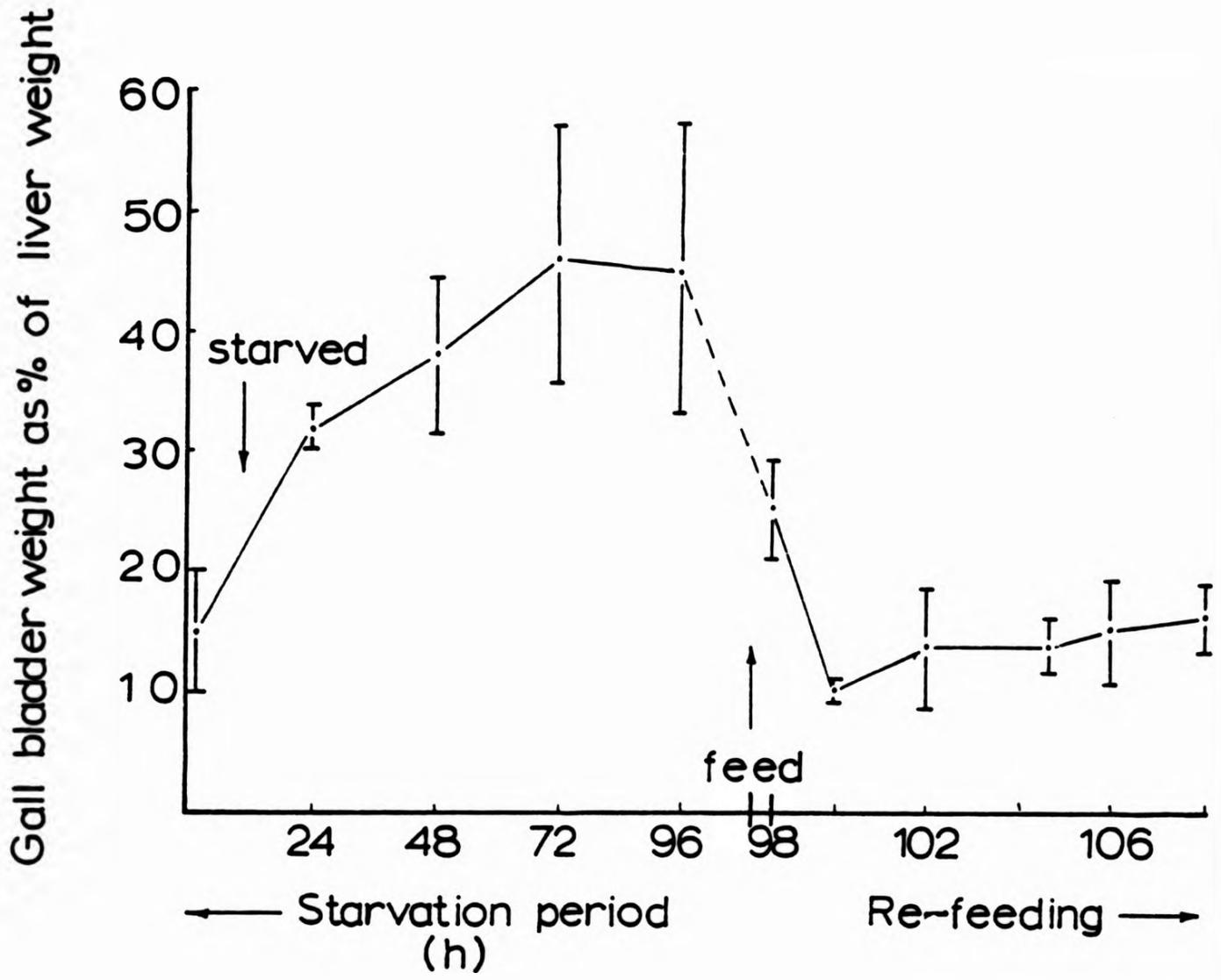


FIGURE 15 Effect of starvation and refeeding on the gall bladder weight (as % of liver weight)

TABLE 15 The relationship between gall bladder weight and starvation periods for O. niloticus at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

	Deprivation Periods (h)			
	24	48	72	96
Gall bladder weight as % of liver weight	31.88 <sup>a</sup> ±1.37	38.12 <sup>ba</sup> ±6.5	44.9 <sup>b</sup> ±6.4	44.8 <sup>b</sup> ±4.7

Mean values with the same superscript are not significantly different at 0.05 level of significance

### 3.2 Gastric Evacuation Studies

#### 3.2.1 Comparison of three different techniques used in determination of gastric evacuation time

##### 3.2.1.1 Use of dye

It was originally intended to isolate fish individually, but unfortunately due to the lack of space during the experimental period and to the loss of appetite when fish were kept individually, fish had to be kept in groups (Section 2.4.1.1).

Two measurements of alimentary evacuation time were attempted:

- (i) the total time required for a meal to be evacuated from the alimentary canal is given by the time in hours elapsed from the ingestion of the chromic oxide labelled meal to the last appearance of green faeces, and
- (ii) the stomach evacuation time is given by the time elapse between the first and last green labelled faeces (Table 16).

However this method (use of dye) did not provide an accurate estimate of food intake by fish or gastric evacuation time due to the leaching of nutrient and the stress effect caused by regular cleaning of tanks, which may depress the gastric evacuation rates (Talbot, 1985).

TABLE 16 Total and stomach evacuation time (h) for O. niloticus fed 1.5% b.w. meal labelled with  $\text{Cr}_2\text{O}_3$

Tank No.	First labelled faeces (h)	Last labelled faeces (h)	S.E.T. (h)
	A	B (=T.E.T.)	(B-A)
1	9	35	26
2	7	47	40
3	5	40	35
4	6	29	23
5	5	48	33
6	10	54	44

#### 3.2.1.2 X-radiography technique

Due to the unpalatability of barium sulphate diet, it was necessary to force-feed the experimental fish. Mortality over the experimental period was 50%. The high mortality rate was mainly due to the tears in the pharyngeal region caused by force-feeding. Tears were also observed in the stomach due to the fragility of the stomach tissue (Plate 1).

To overcome the force-feeding problem another group of fish was fed voluntarily with trout pellets labelled with metallic iron powder (Talbot & Higgins, 1983). It was observed that fish rejected the labelled pellets several times before they were consumed. This was probably due to the hardness of pellets (Jauncey & Ross, 1982). In addition, unlike the barium sulphate diet, the stomach and the intestine cannot be easily differentiated due to the nature of the long coil intestine (Plate 2). Therefore determination of stomach evacuation time was not possible.

#### 3.2.1.3 Sequential slaughter

To compare the results of these trials with other published data (Elliott, 1972; Jobling, 1974; Jobling *et al.*, 1977; Grove & Crawford, 1980), the stomach contents were determined on a dry weight basis, this served to remove the comparison effect of gastric secretion and dietary moisture and made the shape of gastric evacuation more apparent.

PLATE 1 X-radiographs of O. niloticus showing the tears  
in the alimentary canal caused by force-feeding  
1.5% b.w. meal containing 25% barium sulphate



PLATE 2 Comparison between x-radiographs of O. niloticus  
after feeding 1.5% b.w. meals containing  
(A) 25% barium sulphate, and (B) 0.5% metallic  
iron powder at intervals after feeding



A

6 Hours after feeding



B



A

12 Hours after feeding



B

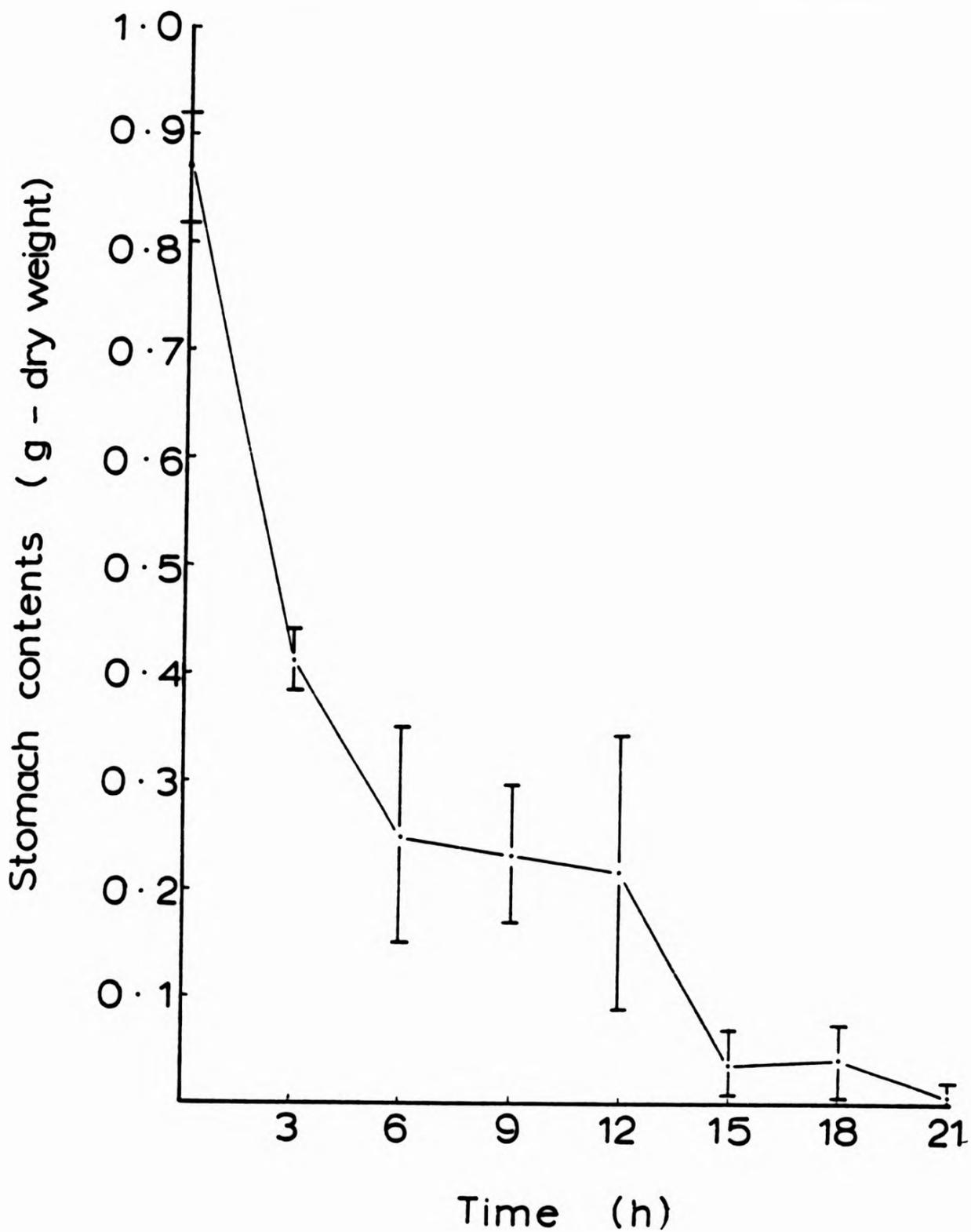
As shown in Fig. 16 the temporal profile of stomach contents is curvilinear. The data of stomach contents after feeding were adequately linearised using three different models (see Section 2.4.2). The regression equations, correlation coefficients and the predicted stomach evacuation times are presented in Table 17. Highest regression coefficients, however, were obtained for the volume dependent model. This model was therefore used in all subsequent gastric evacuation trials.

### 3.2.2 Factors affecting gastric evacuation time and rate (g/hr)

#### 3.2.2.1 Effect of temperature

Fig. 17 shows the stomach evacuation pattern, on a dry weight basis, for each of the three experimental temperatures.

Geometric means of stomach contents at two-hourly intervals after feeding, for fish at each of the experimental temperatures, are linearised using the volume dependent model (2.4.2) in Fig. 18. The regression equations, correlation coefficients and the predicted stomach evacuation times for each of the three experimental temperatures are presented in Table 18. From Fig. 17 and Table 18 it can be seen that an increase in the experimental temperature resulted in a decrease in stomach evacuation time and an increase in stomach evacuation coefficient. The faster evacuation of food from the stomach with increasing temperature can be seen in the increase in steepness of the stomach evacuation graphs (Fig. 18). The time required to evacuate the stomach decreased from 29.9h to 12.33h and the stomach evacuation coefficient



**FIGURE 16** The decline in stomach contents (g, d.w.) after feeding for *O. niloticus* at  $27.5 \pm 1^\circ\text{C}$

**TABLE 17** Regression equation, correlation coefficient and predicted stomach evacuation time for O. niloticus at 27.5°C (± 1°C)

Gastric evacuation model	Regression Equation	Correlation Coefficient	Predicted stomach evacuation time
Volume dependent $\sqrt{Y_t} = \sqrt{Y_0} - R_{\sqrt{t}}$	$Y = 0.818 - 0.0369t$	-0.98	22.16hrs
Surface area model $Y_t^{2/3} = Y_0^{2/3} - R_{at}$	$Y = 0.739 - 0.0368t$	-0.94	20.10hrs
Exponential $\text{Log}_e Y_t = \text{Log}_e Y_0 - R_{et}$	$Y = 0.157 - 0.228t$	-0.93	20.19hrs

$Y_t$  is the amount of food in the stomach at time  $t$

$Y_0$  is the amount of food ingested (g)

$T$  is the time in hours

$R$  is the evacuation rate

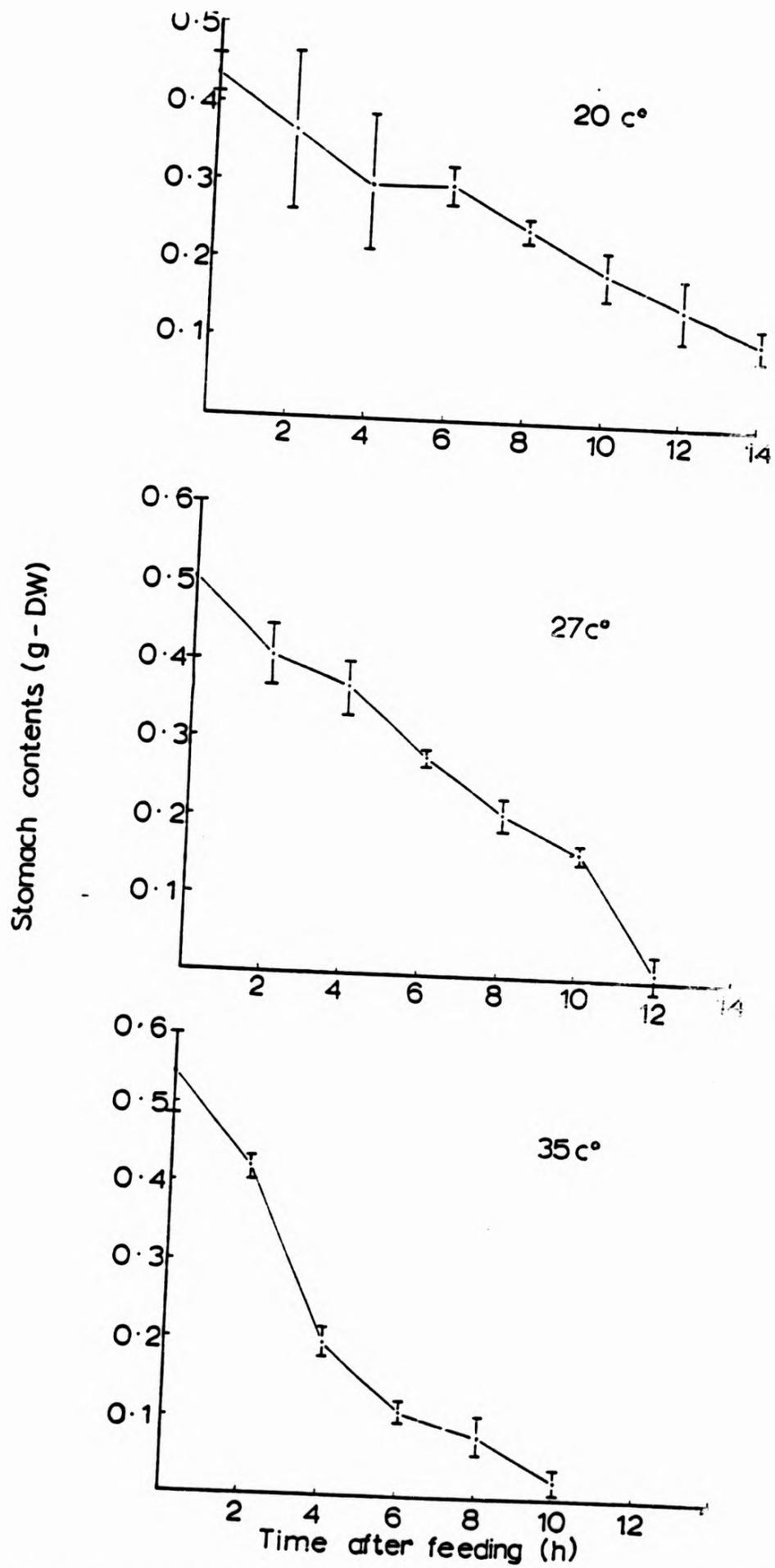


FIGURE 17 The decline in stomach contents with time at the three experimental temperatures ( $^{\circ}\text{C}$ )

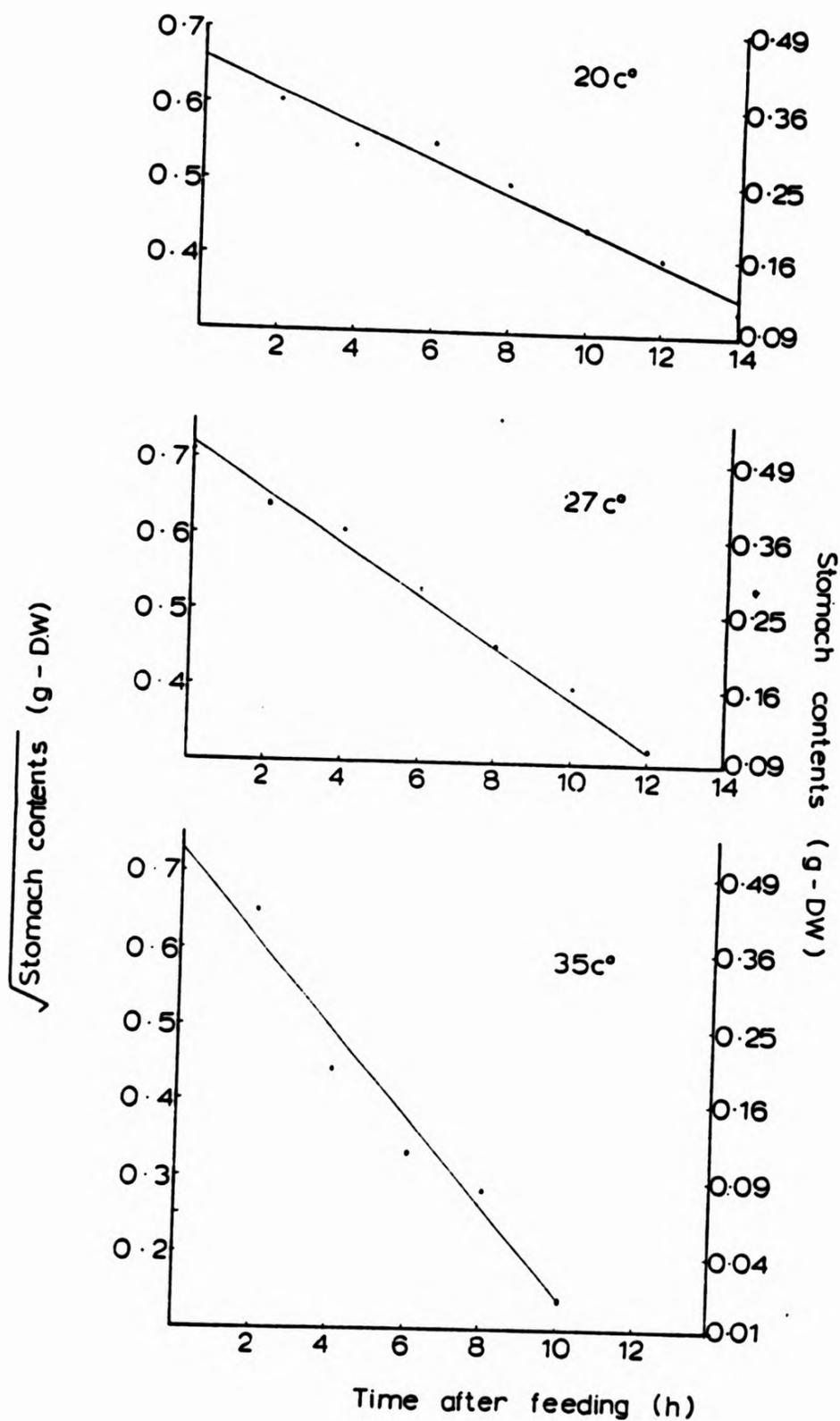


FIGURE 18 Linear regression of volume transformed stomach contents with time for O. niloticus at the experimental temperatures (°C)

**TABLE 18** Regression equations, correlation coefficients and predicted stomach evacuation times (h) for O. niloticus fed to satiation at three different experimental temperatures

Temperature	Regression equations	Corr. coeff.	P	Predicted stomach evacuation time (h)
20°C	$\sqrt{Y_t} = 0.656 - 0.0219T$	-0.989	0.001	29.9h
27°C	$\sqrt{Y_t} = 0.718 - 0.033T$	-0.99	0.001	21.75h
35°C	$\sqrt{Y_t} = 0.726 - 0.0589T$	-0.986	0.001	12.33h

increased from 0.021 to 0.0589 at 20°C and 35°C respectively. To establish the mathematical relationship between stomach evacuation time (h) and temperature (°C) the calculated stomach evacuation time (Table 18) for each experimental temperature was used to plot a graph (Fig. 19). The regression equation of stomach evacuation time (S.E.T.) on temperature was found to be

$$\text{S.E.T.} = 53.25 - 1.17 \text{ Temperature}$$

The correlation coefficient of 0.99 was highly significant at the 0.05 level. As well as S.E.T. it is also possible to evaluate the effect of temperature on stomach evacuation coefficient (S.E.C.) using the method of Jones (1974) by drawing a graph of  $\text{Log}_{10}$  stomach evacuation coefficient against temperature (Fig. 20). The regression equation of  $\text{Log}_{10}$  stomach evacuation coefficient on temperature was found to be

$$\text{Log}_{10} \text{ S.E.C.} = -2.25 + 0.029 \text{ Temperature}$$

The correlation coefficient of 0.99 was significant at the 0.05 level. The effect of temperature on the rate of a physiological process can be expressed in terms of the coefficient  $Q_{10}$ , where  $Q_{10}$  is the increase in rate with a 10°C increase in temperature. In the present experiment the  $Q_{10}$  can be calculated as

$$Q_{10} = 10^{0.029(10)} = 1.95$$

This result can therefore be used to adjust the stomach evacuation coefficient for temperature wherever necessary by using the following relationship

$$R_o = R_c^{0.029(T_o - T_c)}$$

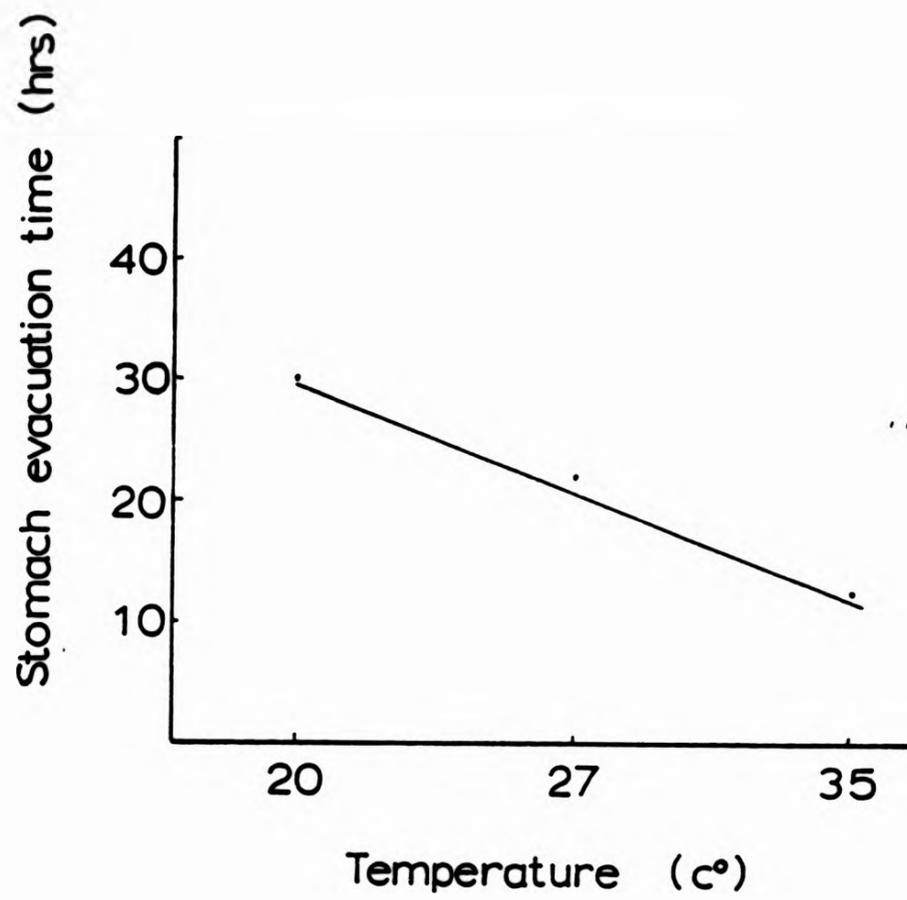


FIGURE 19 The relationship between stomach evacuation time (h) and temperature (°C)

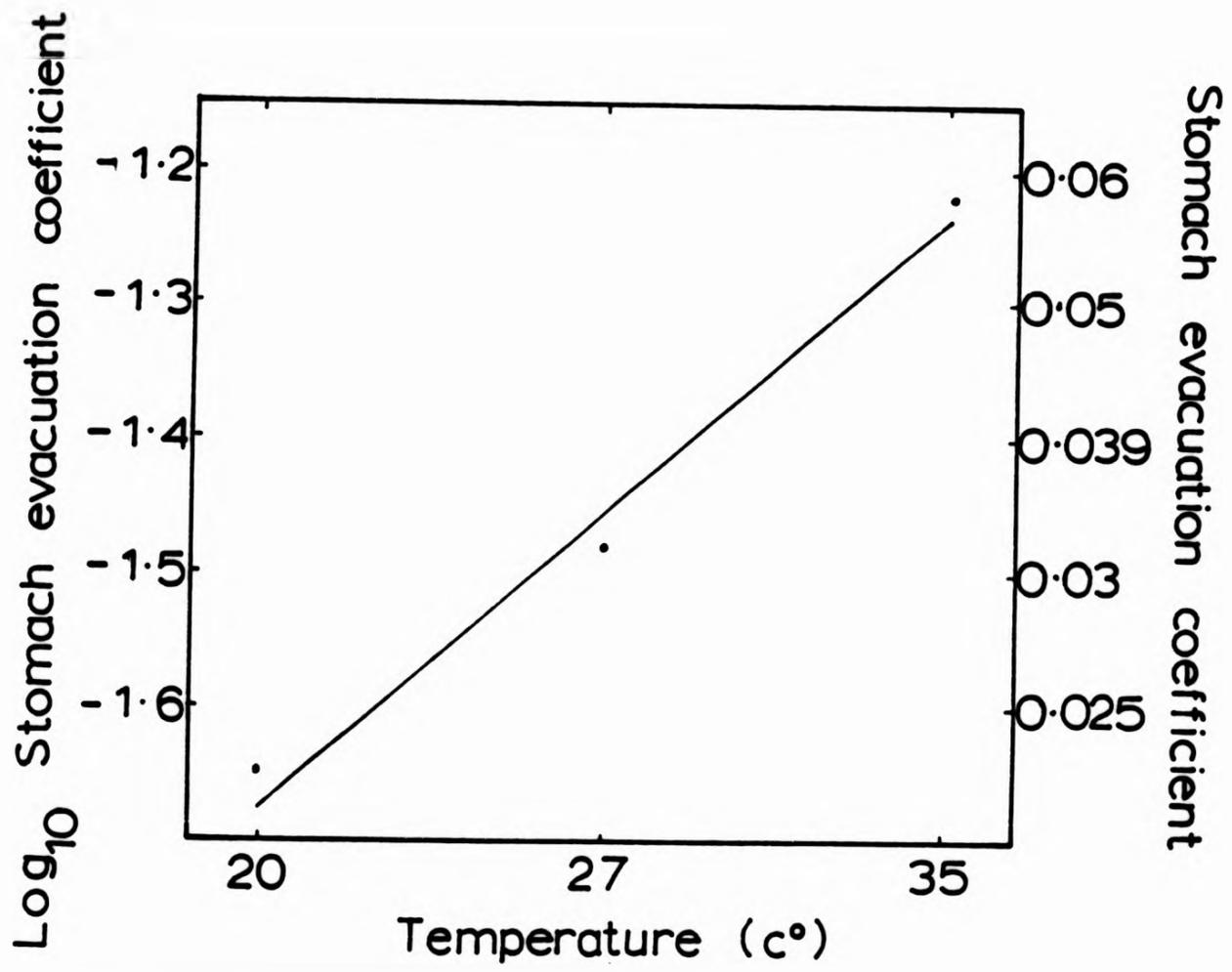


FIGURE 20 The relationship between stomach evacuation coefficient and temperature (°C)

where  $R_o$  and  $R_c$  are the evacuation coefficient of a particular stomach content weight at temperatures  $T_o$  and  $T_c$  respectively.

It is believed that return of appetite is closely related to the percentage of a meal evacuated from the stomach (e.g. Brett, 1970; Grove et al., 1985). The stomach contents at various time intervals after feeding for each of the three experimental temperatures were calculated as a percentage of the initial meal size (stomach contents) and the times required for 50%, 75% and 90% of ingested food to be evacuated were calculated (Fig. 21). From Fig. 21 it can be observed that the time required for 50%, 75% and 90% of ingested food to be evacuated decreases with the increasing experimental temperature. The time required for 50%, 75% and 90% of stomach content to be evacuated was found to decrease from 9h, 14h and 17h to 4h, 6.5h and 8.5h at 20°C and 35°C respectively (Fig. 21).

Passage of food through the intestine as measured by the dry weight of intestinal contents determined at various times after feeding, appears to follow a regular pattern of filling and emptying (Fig. 22). With increasing experimental temperature the rate of filling and emptying of the intestine increased as can be seen from the steepness of the emptying phase (Fig. 22). Estimates of intestinal evacuation time and coefficient at the three experimental temperatures were not possible because of this pattern. The total alimentary canal evacuation times and coefficients were determined by combining the stomach and intestinal dry contents at two hour intervals after feeding for each of the three experimental temperatures. Fig. 23 shows the decline in the total alimentary canal contents with time after feeding plotted

▲ - 35c°

△ - 27c°

■ - 20c°

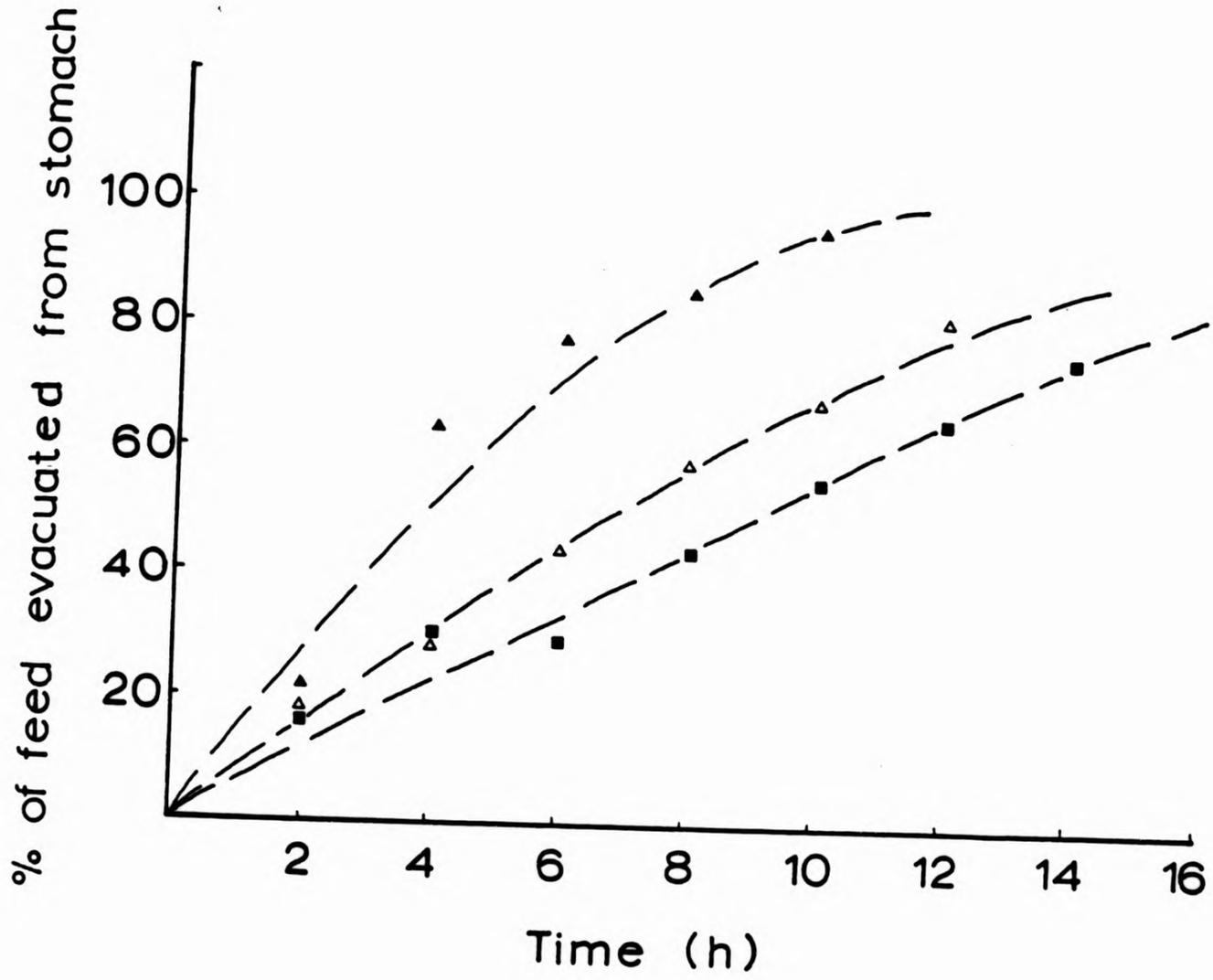
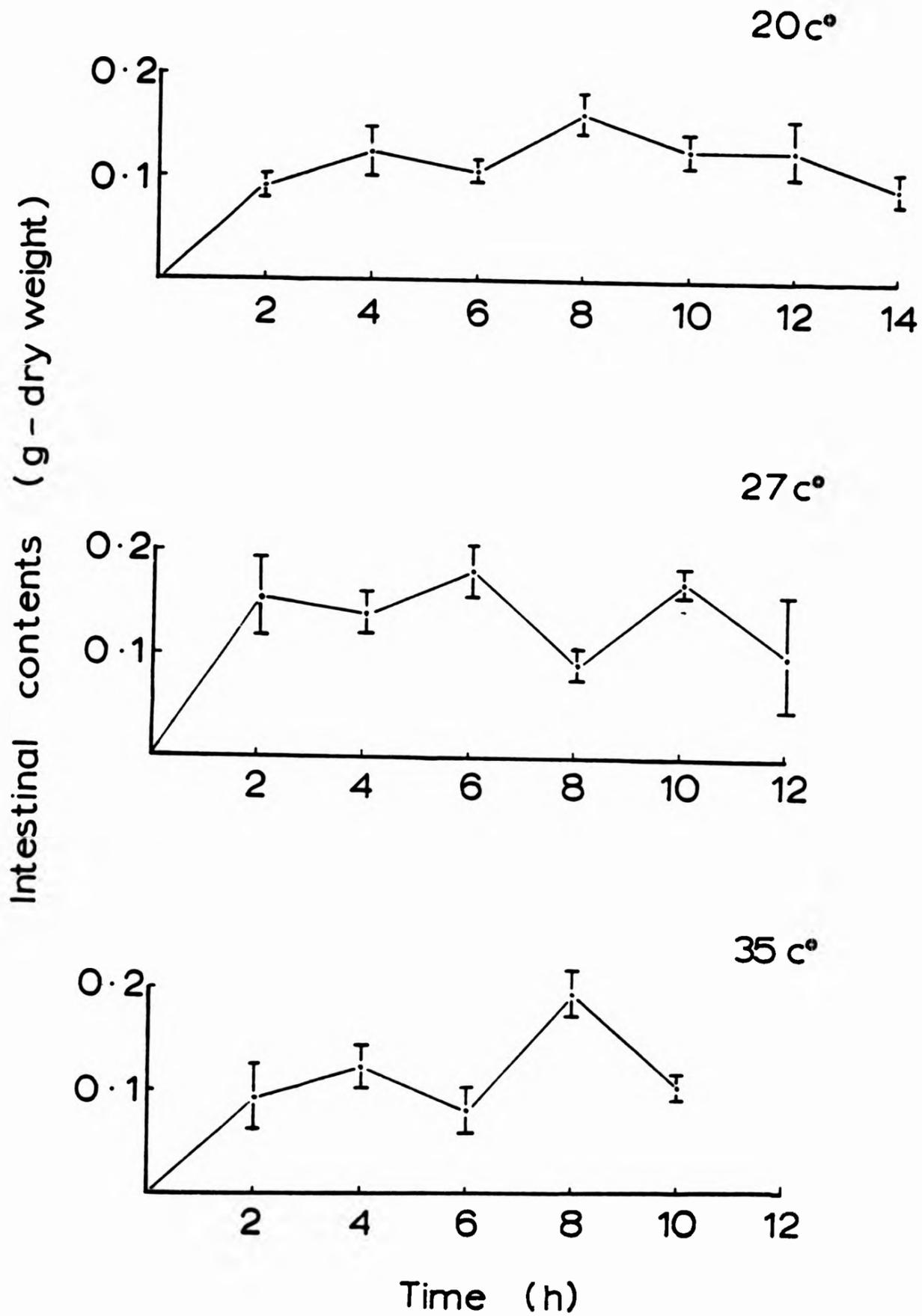


FIGURE 21 Percentage of food evacuated from the stomach at the three experimental temperatures (°C)



**FIGURE 22** The change in the intestinal contents (g, d.w.) with time for *Q. niloticus* at the three experimental temperatures (°C)

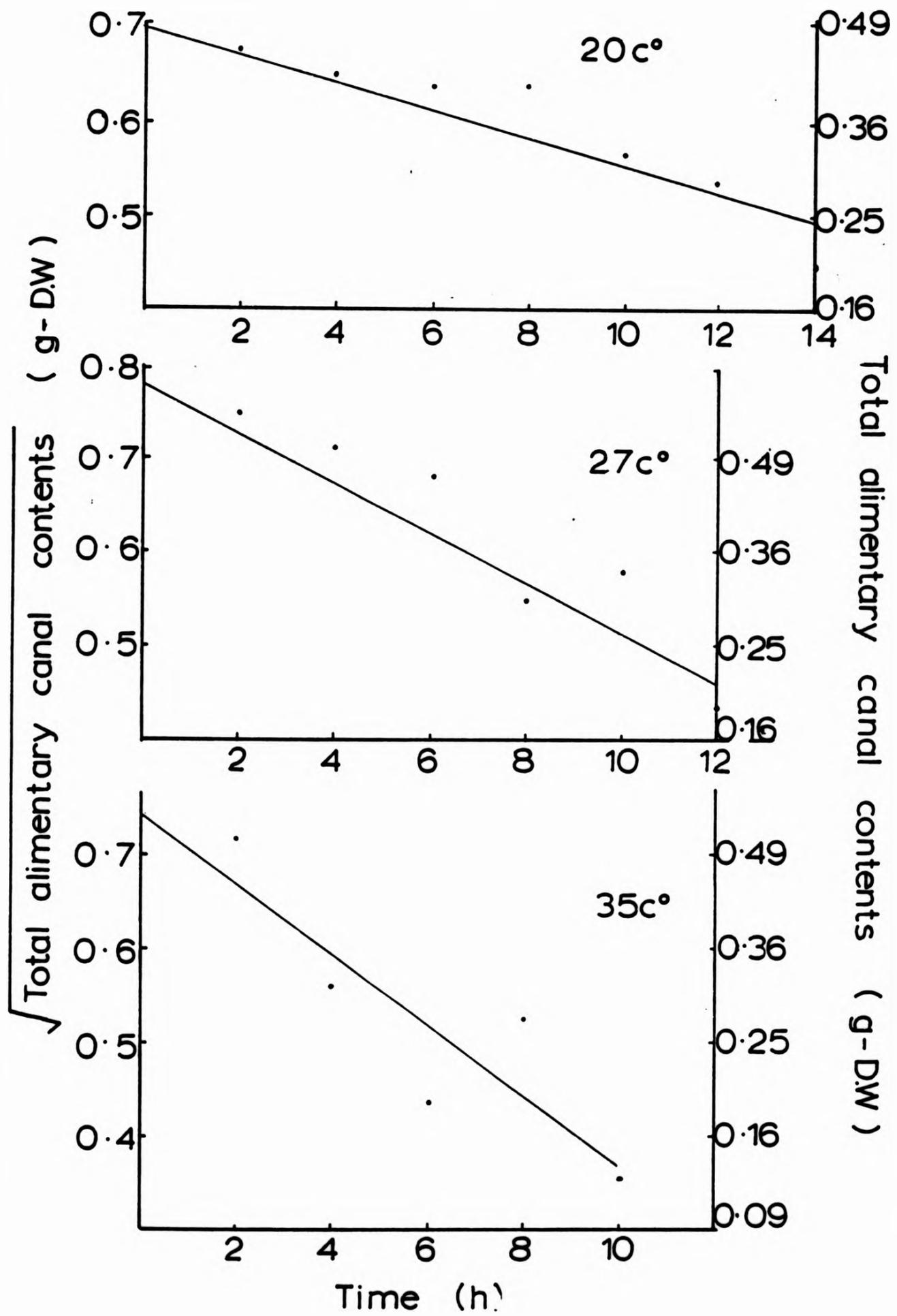


FIGURE 23 Linear regression of transformed total alimentary canal for *O. niloticus* at the three experimental temperatures (°C)

TABLE 19 Regression equations, correlation coefficients and the predicted total evacuation times (h) for *O. niloticus* fed to satiation at three different experimental temperatures (°C)

Temperature	Regression equations	Corr. coeff.	P < 0.0	Predicted total gastric evacuation time (h)
20°C	$\sqrt{Y_t} = 0.702 - 0.0145T$	-0.91	0.001	48.1h
27°C	$\sqrt{Y_t} = 0.771 - 0.0237T$	-0.906	0.001	32.53h
35°C	$\sqrt{Y_t} = 0.741 - 0.037T$	-0.92	0.001	20.00h

after transformation assuming the volume dependent model (2.4.2). The regression equations, correlation coefficients and the predicted total stomach evacuation times at the three experimental temperatures are presented in Table 19. Again it can be observed that increasing the temperature decreased the total evacuation time while the total evacuation coefficient increased (Fig. 23 and Table 19).

#### 3.2.2.2 Effect of fish weight

It has been established that stomach volume is proportional to fish weight, thus feeding fish a meal which represents a set proportion of the body weight should present the same stimulus to the gastrointestinal tract. Fig. 24 shows the decline in stomach contents with time after feeding for the three fish weights investigated, each fed a trout diet (49% crude protein) at 1% of their body weight. This data has been transformed according to the volume dependent model and the predicted stomach evacuation times for the three fish weights are presented in Table 20. From Fig. 25 and Table 20 it can be seen that stomach evacuation time and coefficient increased with increasing fish weight. The stomach evacuation time was found to increase from 16.4h to 19.1h for fish of mean weight 49.6g and 144.8g respectively. Similarly stomach evacuation coefficient increased from 0.0359 to 0.055 for fish of mean weights 49.6g and 144.8g respectively.

To determine the mathematical relationship between stomach evacuation time (h) and fish weight (g), a graph between stomach evacuation time and fish weight was plotted (Fig. 26). Stomach evacuation time (S.E.T.) was found to increase linearly with fish weight and the

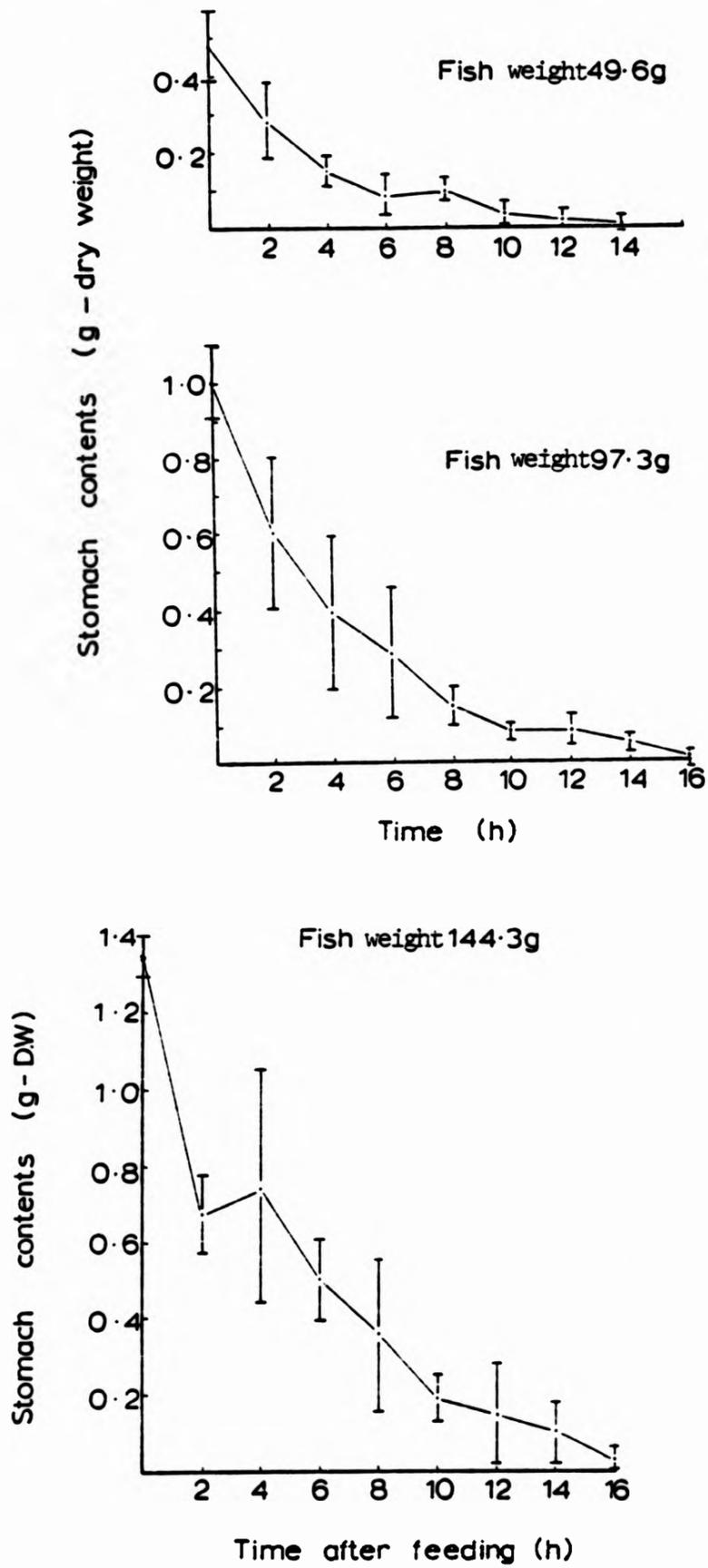
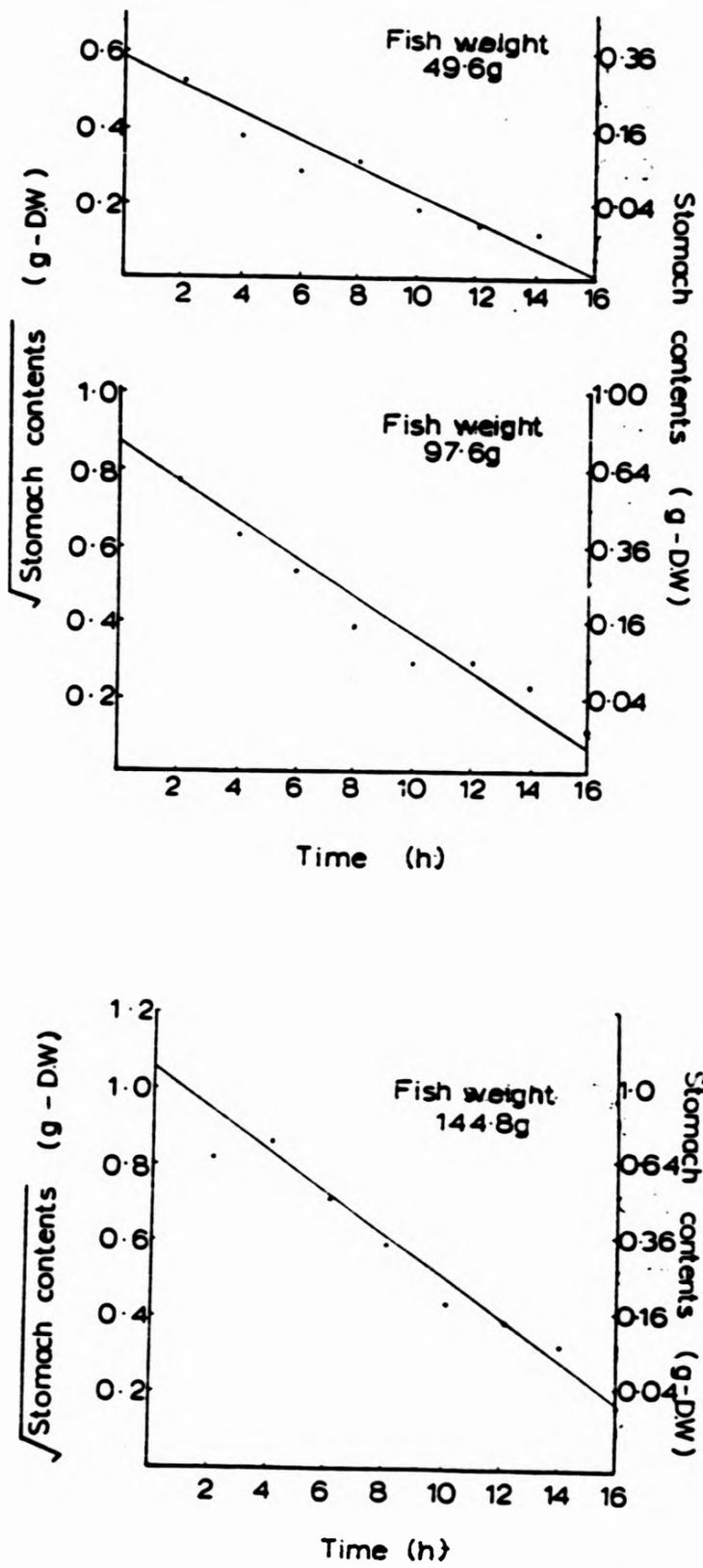


FIGURE 24 The decline in stomach contents (g, d.w.) with time for three different sizes of fish



**FIGURE 25** Linear regression of volume transformed stomach contents (g, d.w.) with time for three different weights of fish (g)

TABLE 20 Regression equations, correlation coefficients and predicted stomach evacuation times (h) for three different weights of O. niloticus fed 1% b.w. at 27.5° ± 1°C

Fish size (g) ± S.E.	Regression equations	Corr. coeff.	P < 0.0	Predicted stomach evacuation time (S.E.T., h)
49.6g ±0.96	$\sqrt{Y_t} = 0.589 - 0.0359T$	-0.96	0.001	16.4h
97.3g ±1.3	$\sqrt{Y_t} = 0.876 - 0.05T$	-0.97	0.001	17.53h
144.8g ±1.11	$\sqrt{Y_t} = 1.05 - 0.055T$	-0.98	0.001	19.1h

regression equation was found to be

$$\text{S.E.T. (h)} = 14.91 + 0.0284 \text{ fish weight (g)}$$

This correlation coefficient of 0.99 was significant at the 0.05 level. Since most workers (e.g. Jobling et al., 1977; Grove et al., 1985) present the relationship between stomach evacuation time (h) and fish size (g) in the natural logarithmic form, to allow comparison the present data was also transformed in this way and the regression equation found to be

$$\text{Log S.E.T. (h)} = 2.285 + 0.131 \text{ Log fish weight (g)}$$

The correlation coefficient of 0.98 was significant at the 0.05 level. As well as S.E.T. it is also possible to establish the effect of fish weight on stomach evacuation coefficient. Stomach evacuation coefficient was found to increase linearly with fish weight (Fig. 27). The regression equation of this relationship was found to be

$$\text{stomach evacuation coefficient} = 0.027 + 0.0002 \text{ fish weight (g)}$$

or its transformation

$$\text{Log S.E.C.} = -4.9 + 0.408 \text{ Log fish weight (g)}$$

The correlation coefficients of 0.96 and 0.99 were significant at the 0.05 level. Data on dry stomach contents were converted to percentage of food evacuated from the stomach and the time required for 50%, 75% and 90% of the meal to be evacuated were derived from Fig. 28. Again the time required for 50%, 75% and 90% of the ingested food to be evacuated from the stomach was found to increase with increasing fish weight. Thus the time required for 50%, 75% and 90%

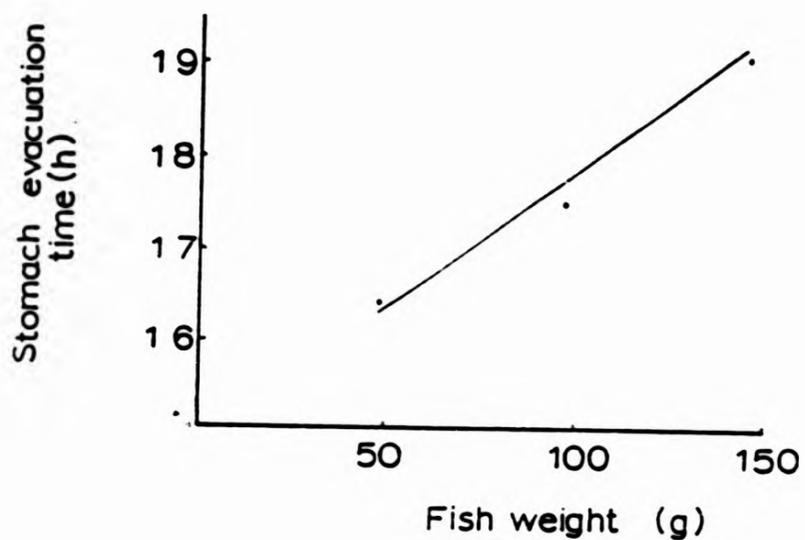


FIGURE 26 The relationship between stomach evacuation time (h) and fish weight (g) for O. niloticus fed 1% of their body weight at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

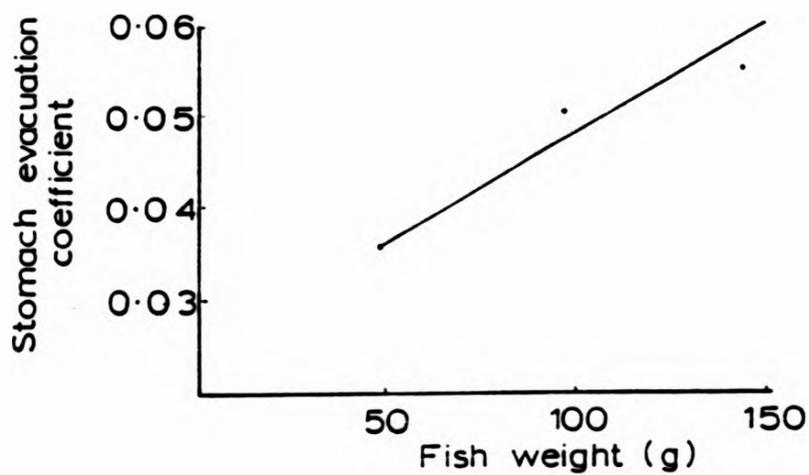
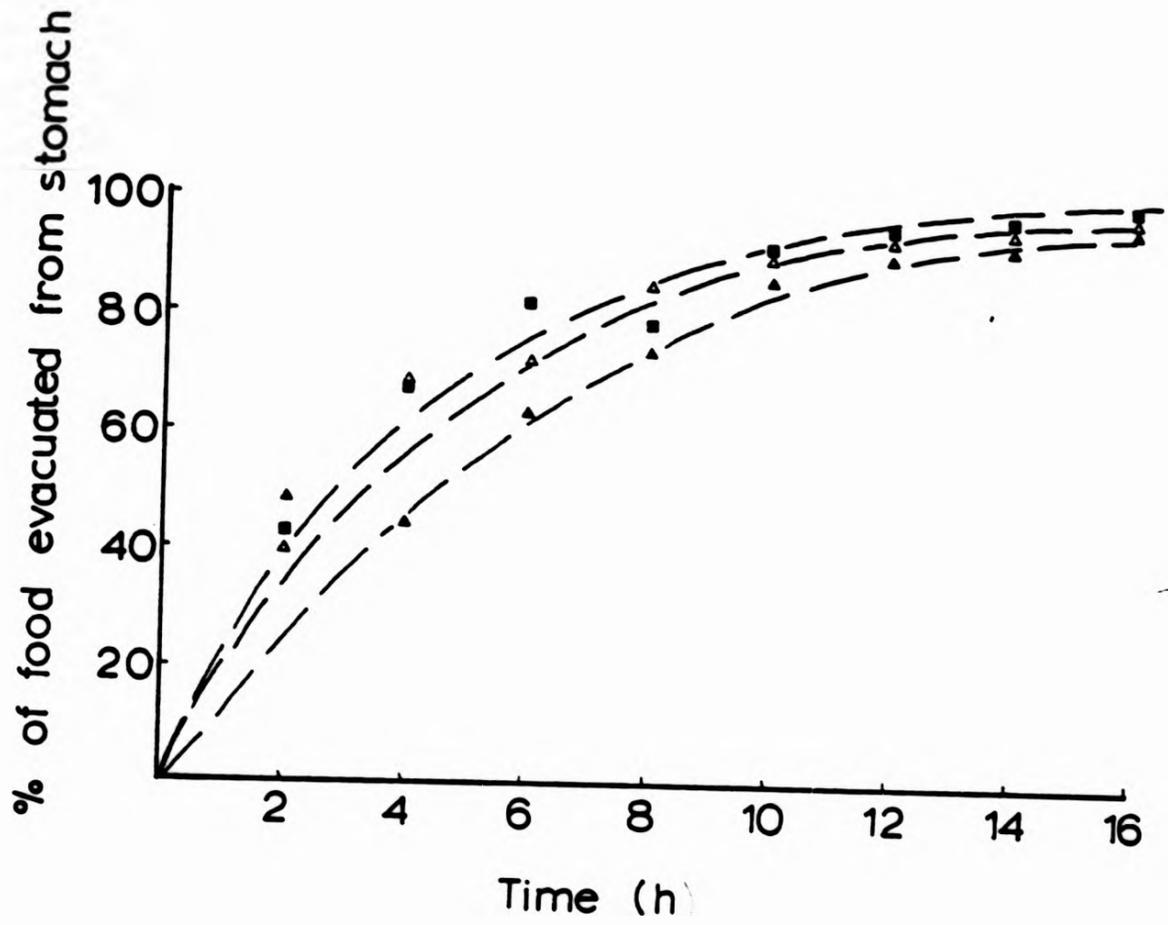


FIGURE 27 The relationship between stomach evacuation coefficient and fish weight (g) for O. niloticus fed 1% of their body weight at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

- - Fish WEIGHT  $49.6 \pm 0.96$
- ▲ - Fish WEIGHT  $97.3 \pm 1.3$
- ▲ - Fish WEIGHT  $144.8 \pm 1.11$



**FIGURE 28** Percentage of food evacuated from the stomach of the three weights of *O. niloticus* at  $27.5 \pm 1^\circ\text{C}$

of the original meal to be evacuated increased from 2.8h, 6h and 9h to 4.5h, 8h and 12h for fish of mean weight 49.6g and 144.8g respectively.

Fig. 29 shows the change in intestinal contents (g dry weight) at various times after feeding for each of the three sizes of fish investigated. Again a cycle of filling and emptying is apparent. The data for stomach contents and intestinal contents were combined and fitted to the volume dependent model (2.4.2) to calculate the total evacuation time (h) and coefficient (Fig. 30). The regression equations, correlation coefficients and the predicted total gastric evacuation times are presented in Table 21. It can be seen from Fig. 30 and Table 21 that increasing fish weight (g) increased the total stomach evacuation time (h). The time required to evacuate the gut totally increased from 27 hours to 31.4 hours for fish of mean weight 49.6g and 144.8g respectively.

### 3.2.2.3 The effect of meal size

Fig. 31 shows the change in dry weight of stomach contents with time after feeding of a single meal of three different sizes (0.5%, 1% and 1.5% of their body weight). This data was transformed according to the volume dependent model (2.4.2) for each meal size (Fig. 32). The regression equation, correlation coefficients and the predicted stomach evacuation times derived from the transformed data are presented in Table 22. From Fig. 32 and Table 22 it can be seen that increasing meal size increased the time and coefficient of stomach evacuation. However, this increase is not proportional to the increase

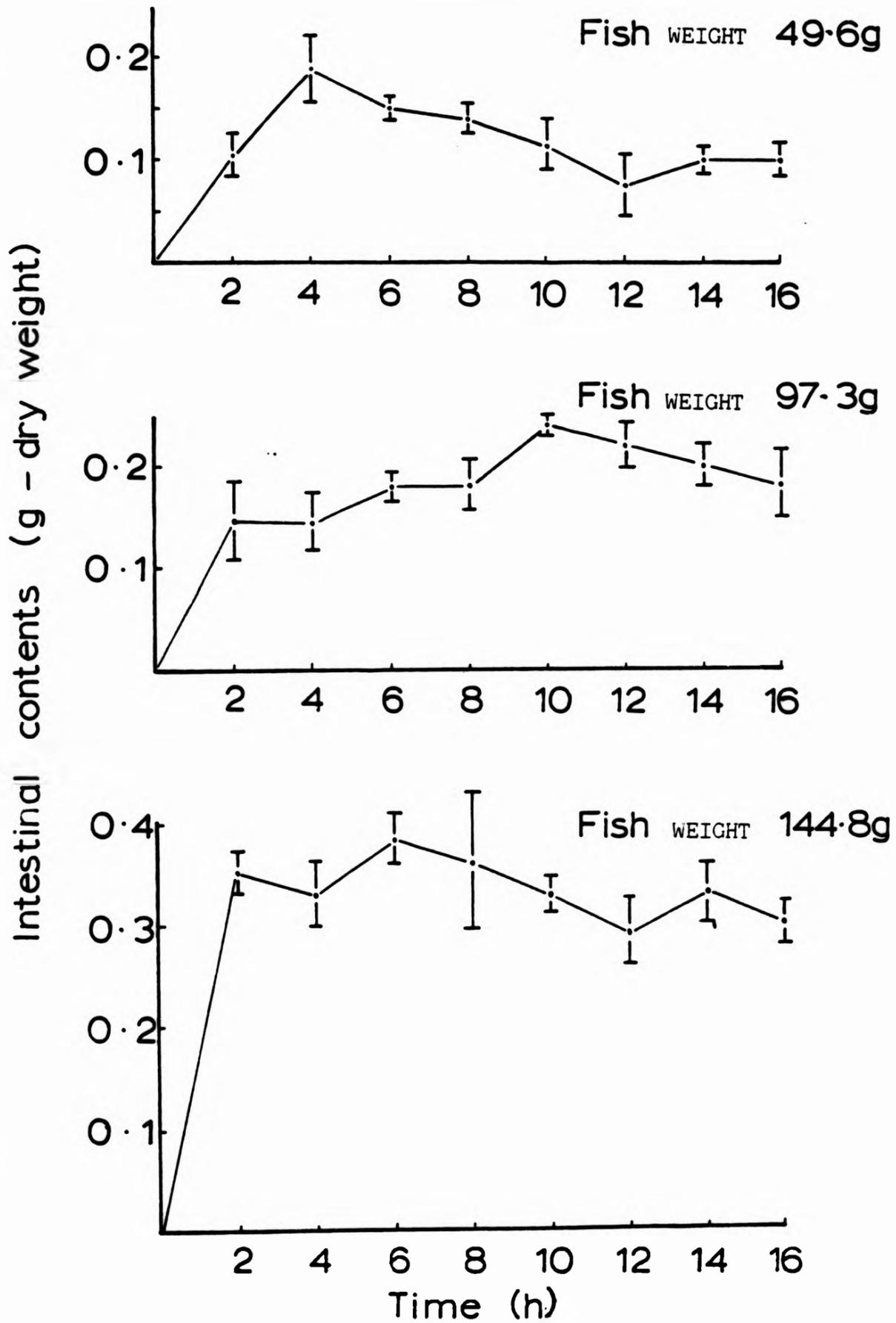


FIGURE 29 The change in the intestinal contents (g, d.w.) with time (h) for the three weights of fish

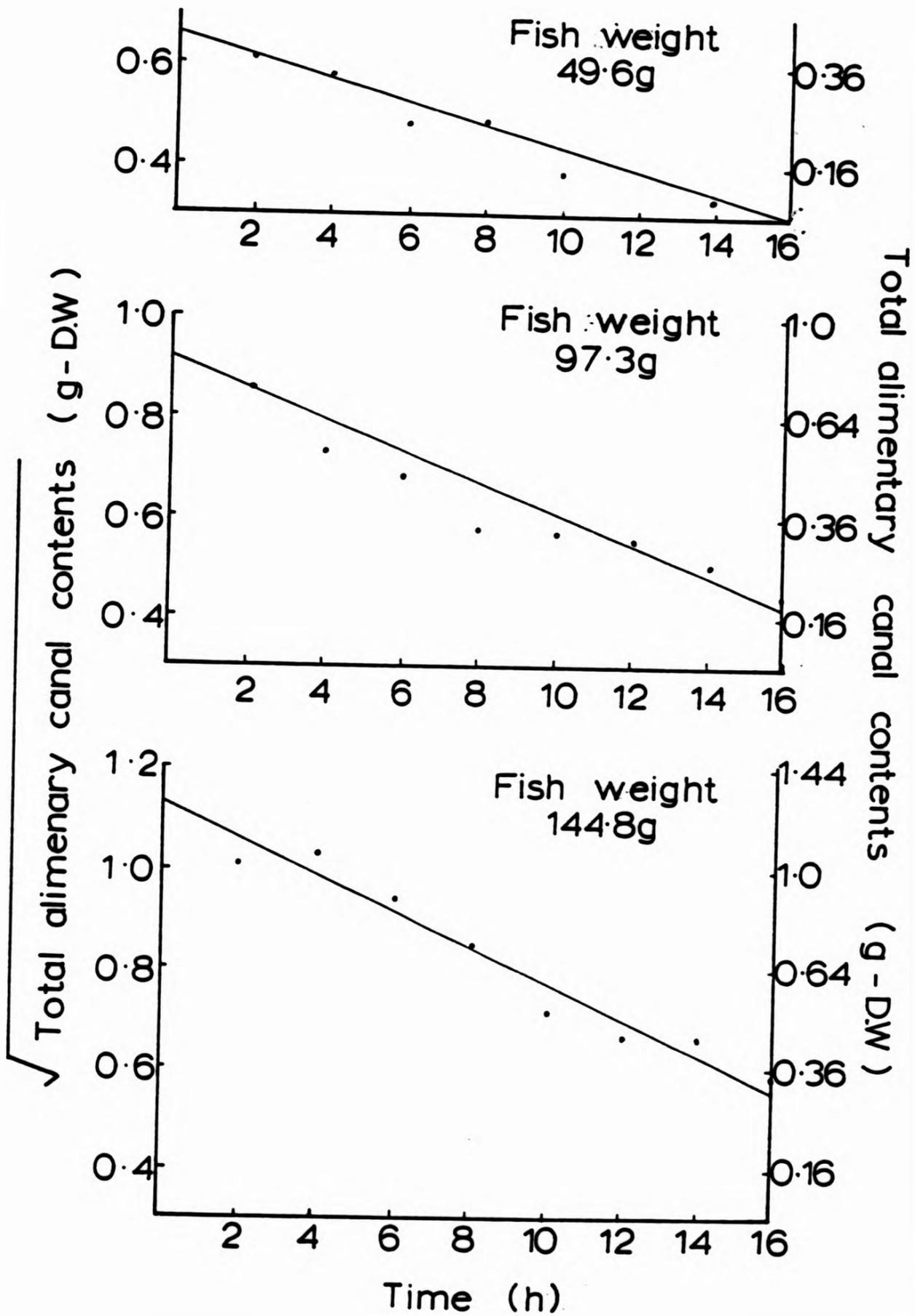


FIGURE 30 Linear regression of volume transformed total alimentary canal contents (g, d.w.) with time (h) for the three different weights of fish

TABLE 21 Regression equations, correlation coefficients and predicted total evacuation time (h) for different sizes of *O. niloticus* fed 1% b.w. at 27.5 ± 1°C

Fish size (g) ± S.E.	Regression equations	Corr. coeff.	P < 0.0	Predicted total gastric evacuation time (T.E.T., h)
49.6 g ±0.96	$\sqrt{Y_t} = 0.6649 - 0.0245T$	-0.97	0.001	27.0h
97.3 g ±1.3	$\sqrt{Y_t} = 0.9081 - 0.031T$	-0.95	0.001	29.3h
144.8 g ±1.11	$\sqrt{Y_t} = 1.135 - 0.0362T$	-0.98	0.001	31.35h

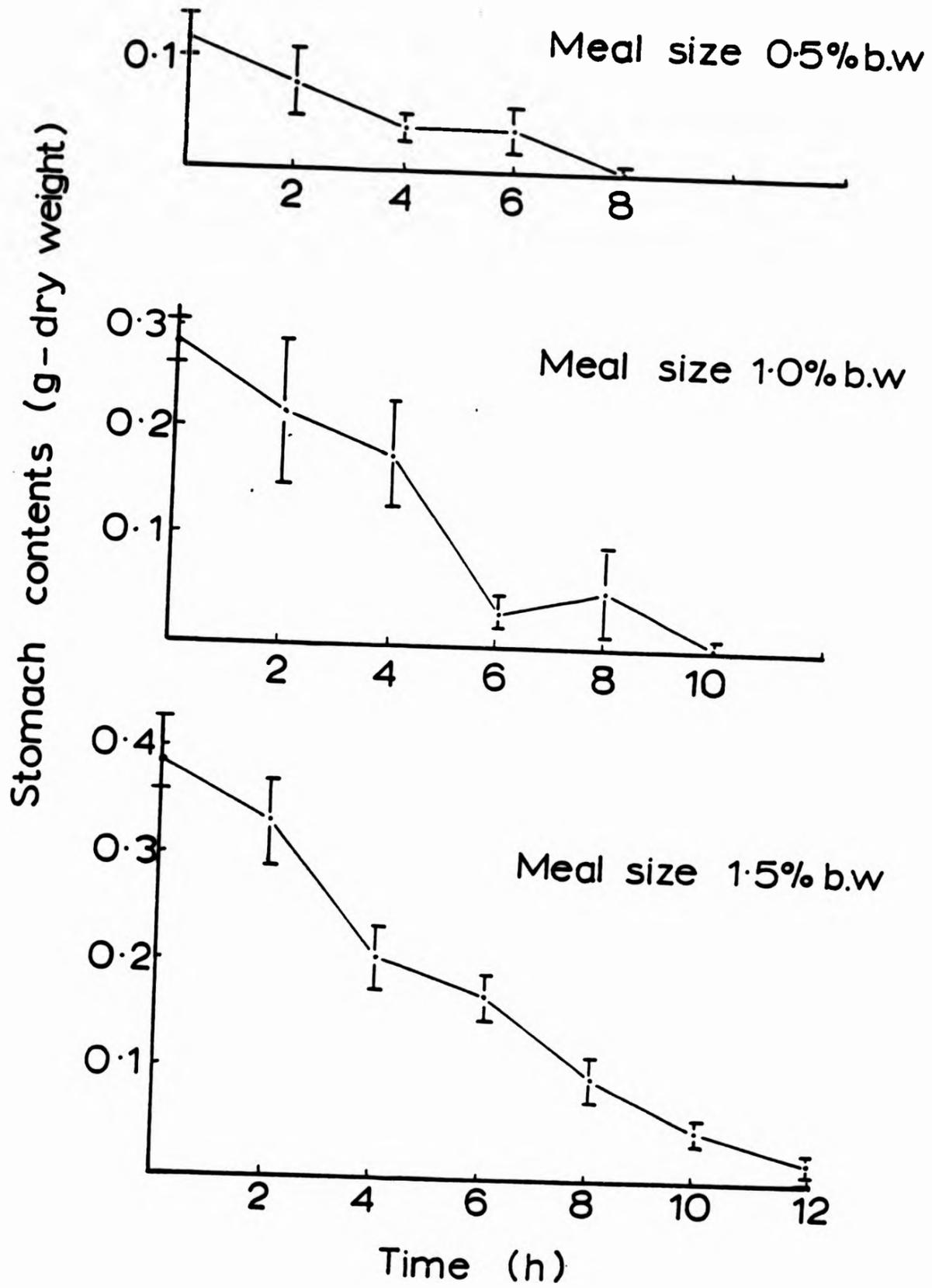
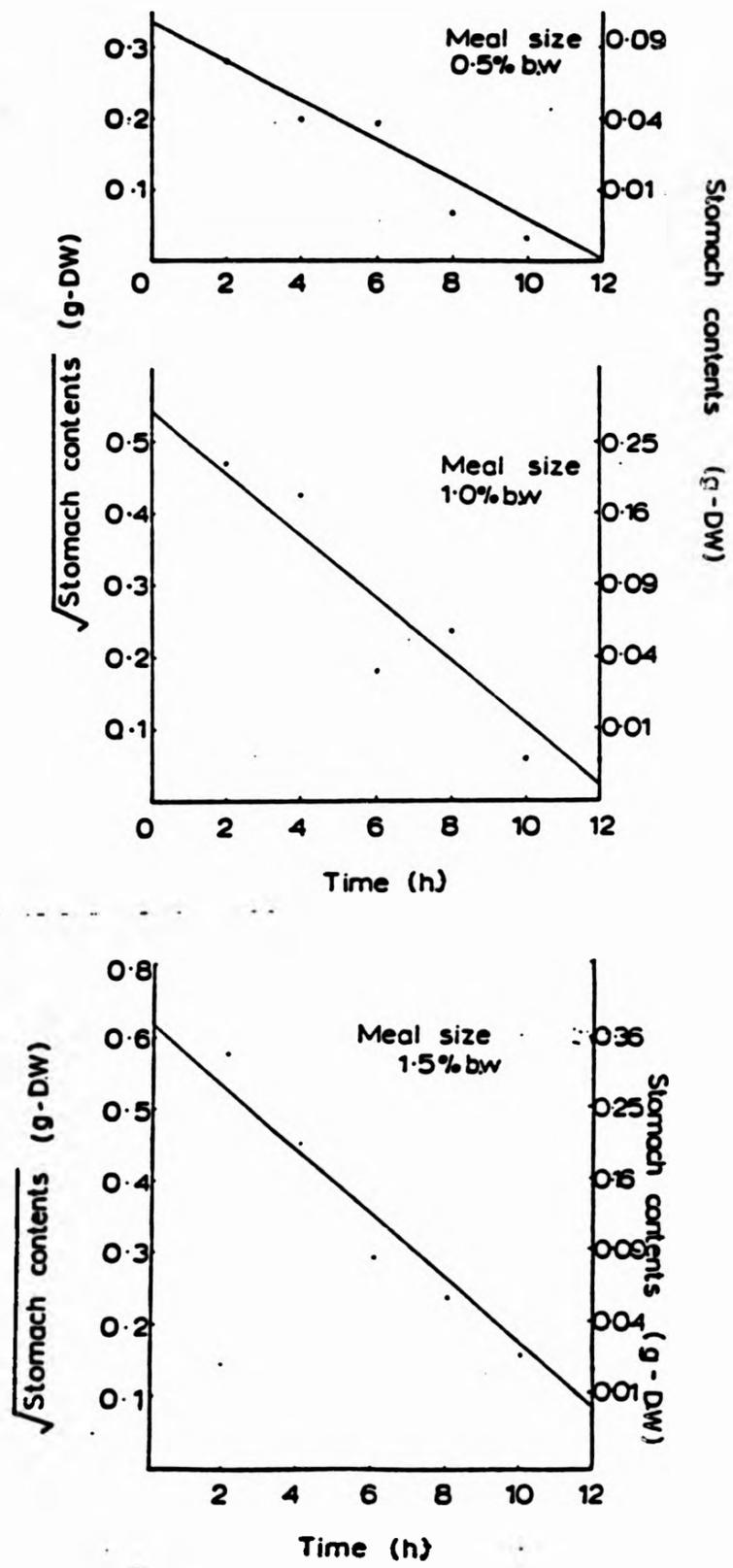


FIGURE 31 The decline in stomach contents (g, d.w.) with time (h) for *O. niloticus* fed 0.5%, 1% and 1.5% of their body weight at  $27.5^{\circ} \pm 1^{\circ}\text{C}$



**FIGURE 32** Linear regression of the transformed stomach contents (g, d.w.) with time for *O. niloticus* fed 0.5%, 1% and 1.5% of their body weight at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

TABLE 22 Regression equations, correlation coefficients and the predicted stomach evacuation times (h) for *O. niloticus* fed 0.5%, 1.0% and 1.5% b.w. at 27.5 ± 1°C

Meal size % b.w.	Regression equations	Corr. coeff.	P < 0.0	Predicted stomach evacuation time (S.E.T., h)
0.5	$\sqrt{Y_t} = 0.336 - 0.0296T$	-0.98	0.001	11.3h
1.0	$\sqrt{Y_t} = 0.54 - 0.036T$	-0.93	0.001	15.0h
1.5	$\sqrt{Y_t} = 0.633 - 0.04T$	-0.96	0.001	15.83h

in meal size. The time required to evacuate the stomach increased from 11.3h to 15.83h for meals of 0.5% and 1.5% body weight respectively. The actual food intakes for the initial 30 fish sampled at zero time, immediately after feeding, were used to calculate their stomach evacuation times (S.E.Ts) using the stomach evacuation coefficients (Table 22). Calculated S.E.Ts are plotted against meal size (g) in Fig. 33. It can be seen that stomach evacuation time increased linearly with increasing meal size and the regression equation was calculated as

$$\text{stomach evacuation time (h)} = 7.845 + 15.39 \text{ meal size (g)}$$

or its transformation

$$\text{Log}_e \text{ S.E.T.(h)} = 2.93 + 0.32 \text{ Log}_e \text{ meal size (g)}$$

The correlation coefficients of 0.9 and 0.91 were significant at the 0.05 level. Further analysis of the S.E.T. data was carried out to determine the exponent for stomach evacuation time in relation to meal size as relative rather than absolute weight. The regression equation was found to be

$$\text{S.E.T.(h)} = 9.77 + 4.14 \text{ meal size \% b.w.}$$

or

$$\text{Log}_e \text{ S.E.T.(h)} = 2.66 + 0.329 \text{ Log}_e \text{ meal size \% b.w.}$$

The correlation coefficients of 0.91 and 0.86 were significant at the 0.05 level.

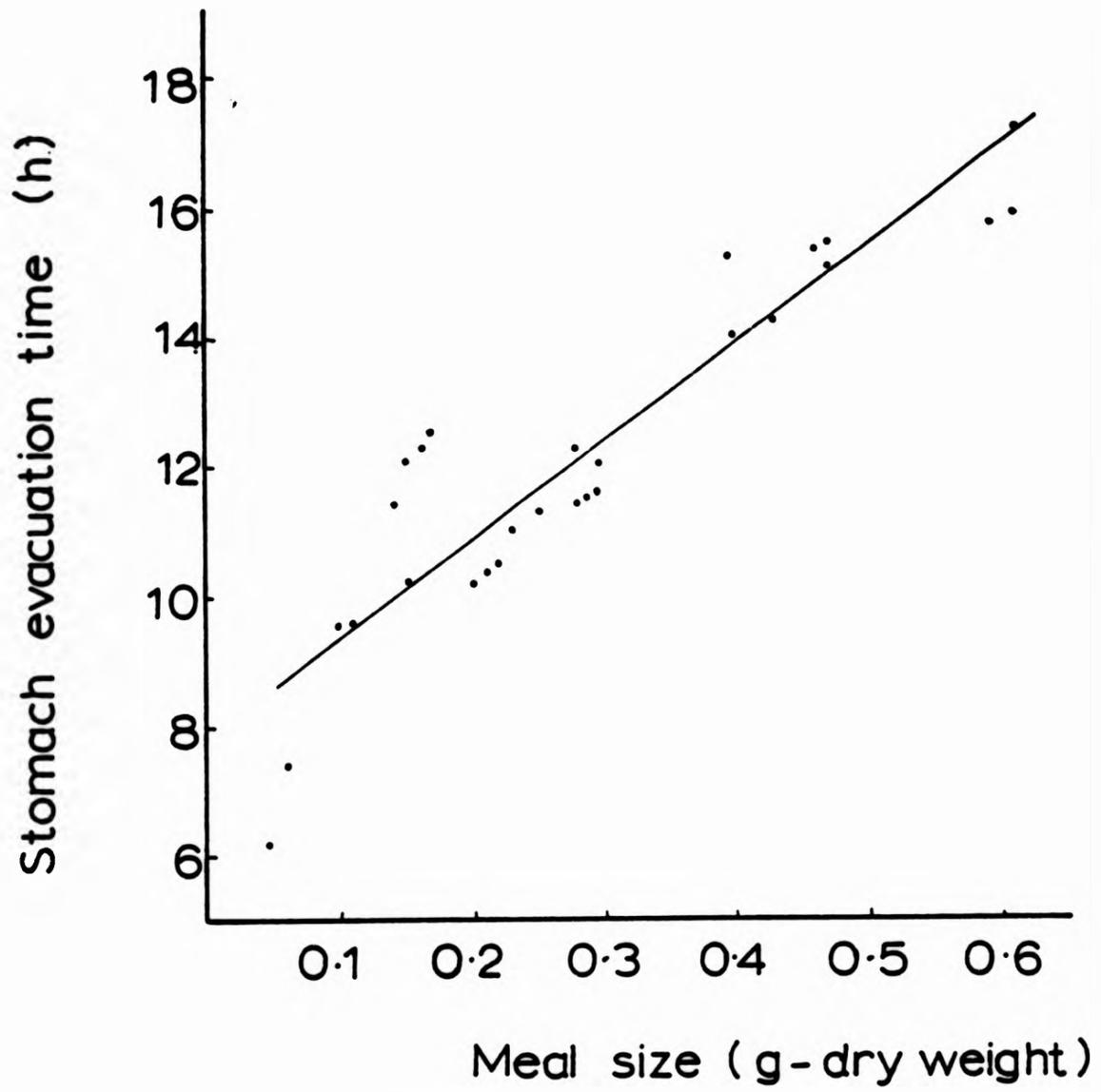


FIGURE 33 The relationship between stomach evacuation time (h) and meal size (g)

The relationship between stomach evacuation rate (g/h) and meal size (g) was obtained assuming that stomach evacuation rate was linear during the first two hours after feeding. The actual food intakes for the 30 fish sampled at zero time were used to calculate the amount of food evacuated during the first two hours after feeding. Calculated stomach evacuation rates for different meal sizes (g) were plotted against meal size (Fig. 34). It can be seen that stomach evacuation rate increased linearly with increasing meal size (Fig. 34). The regression equation of stomach evaluation rate (g/h) on meal size (g) was found to be

$$\text{stomach evacuation rate (g/h)} = 0.0103 + 0.103 \text{ meal size (g)}$$

or

$$\text{Log}_e \text{ S.E.R. (g/h)} = -2.28 + 0.72 \text{ Log}_e \text{ meal size (g)}$$

The correlation coefficients of 0.97 and 0.97 were significant at the 0.05 level.

The time required to evacuate 50%, 75% and 90% of the ingested food increased with increasing meal size (Fig. 35). Fig. 36 shows the intestinal contents at various times after feeding the three meal sizes (0.5%, 1% and 1.5% b.w.). In this Figure a pattern of increase and decrease in the intestinal contents with time is evident. Fig. 37 shows regression lines fitted to data for the whole alimentary canal. The regression equations, correlation coefficients and the predicted total gastric evacuation time for the three meal sizes are presented in Table 23. Total gastric evacuation time was found to increase with increasing meal size from 16.8 to 21.6 with increasing meal size from 0.5% to 1.5% b.w.

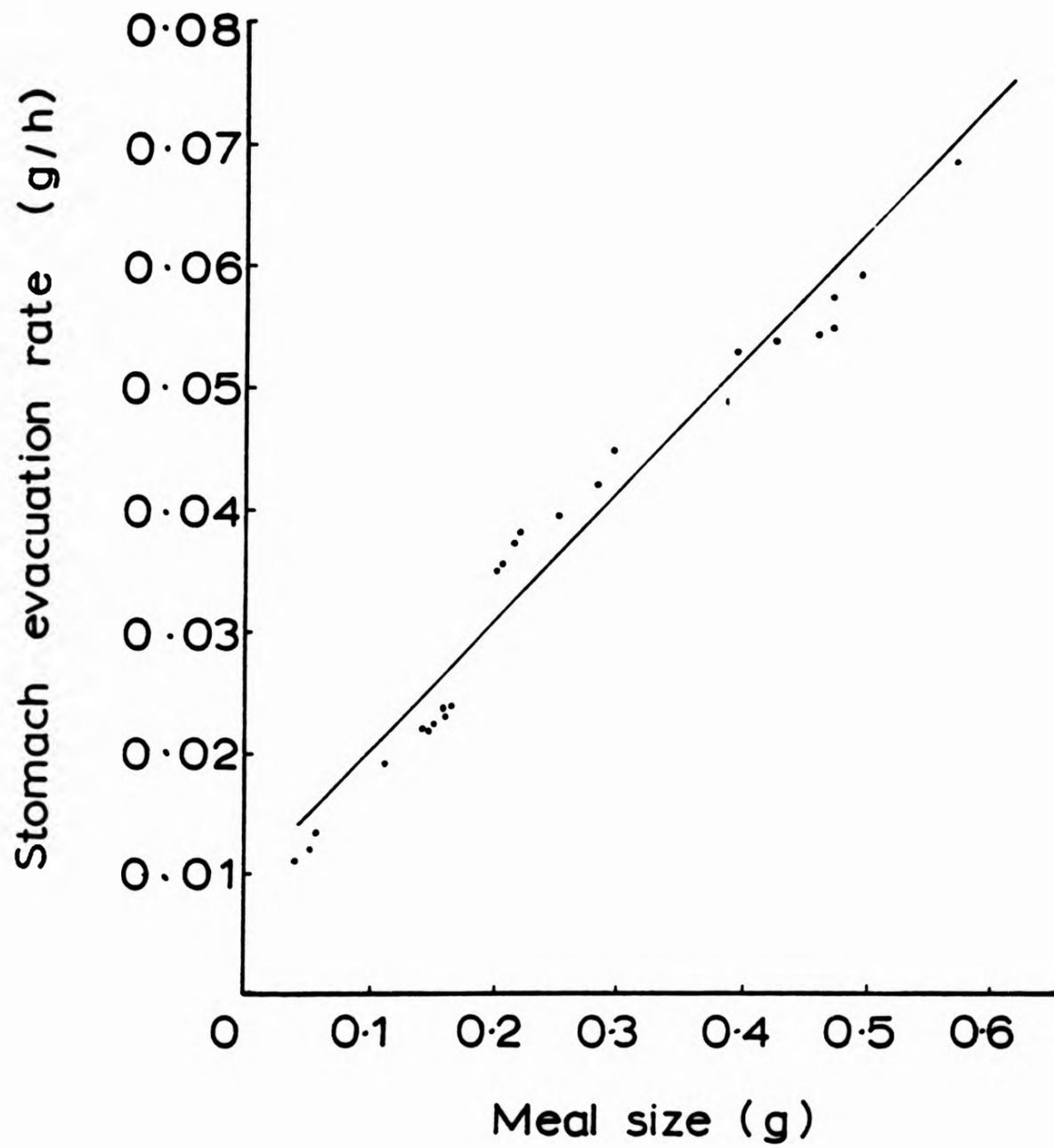


FIGURE 34 The relationship between stomach evacuation rate (g/h) and meal size (g)

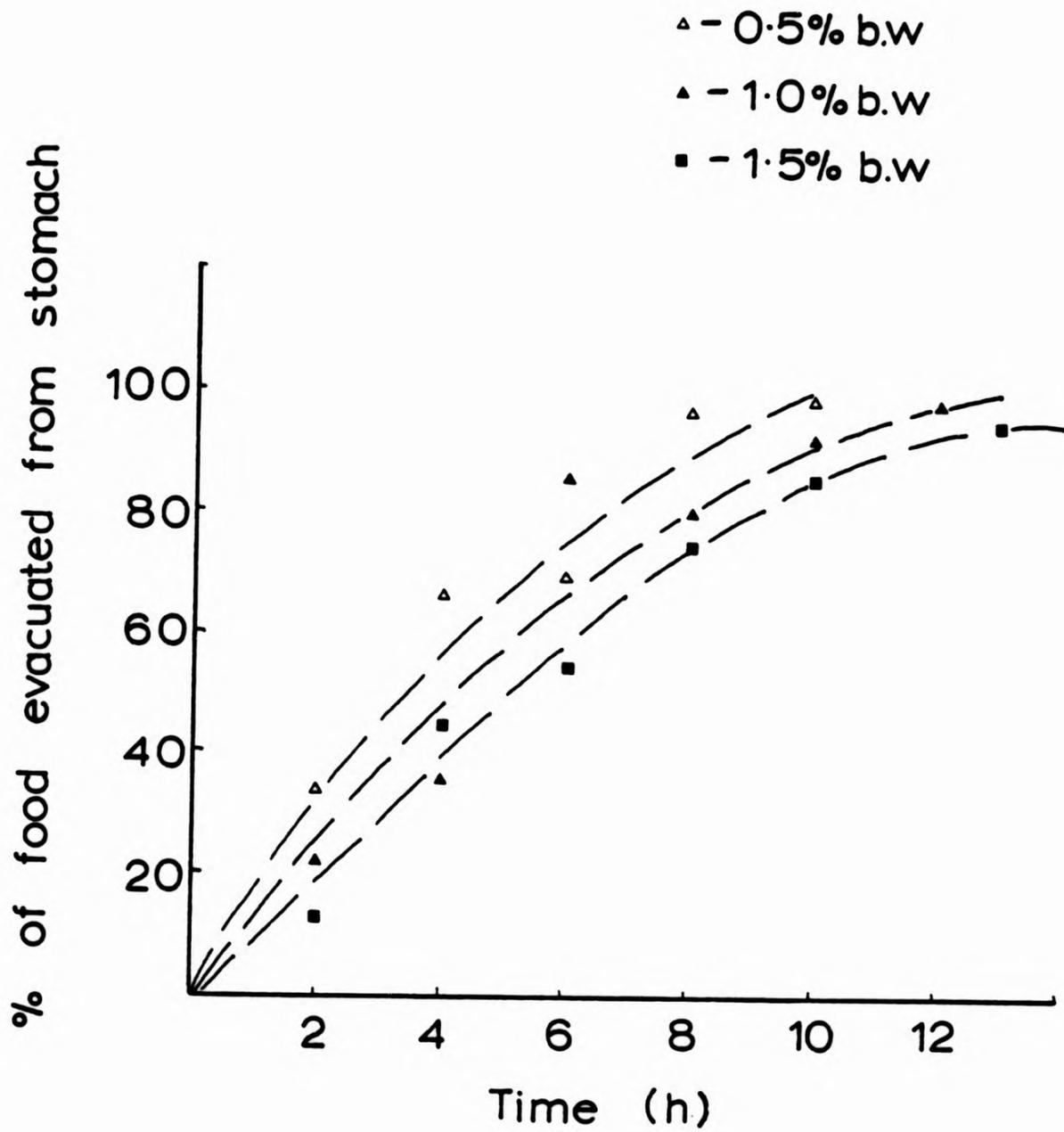


FIGURE 35 Percentage of food evacuated from the stomach of O. niloticus fed 0.5%, 1% and 1.5% of their body weight

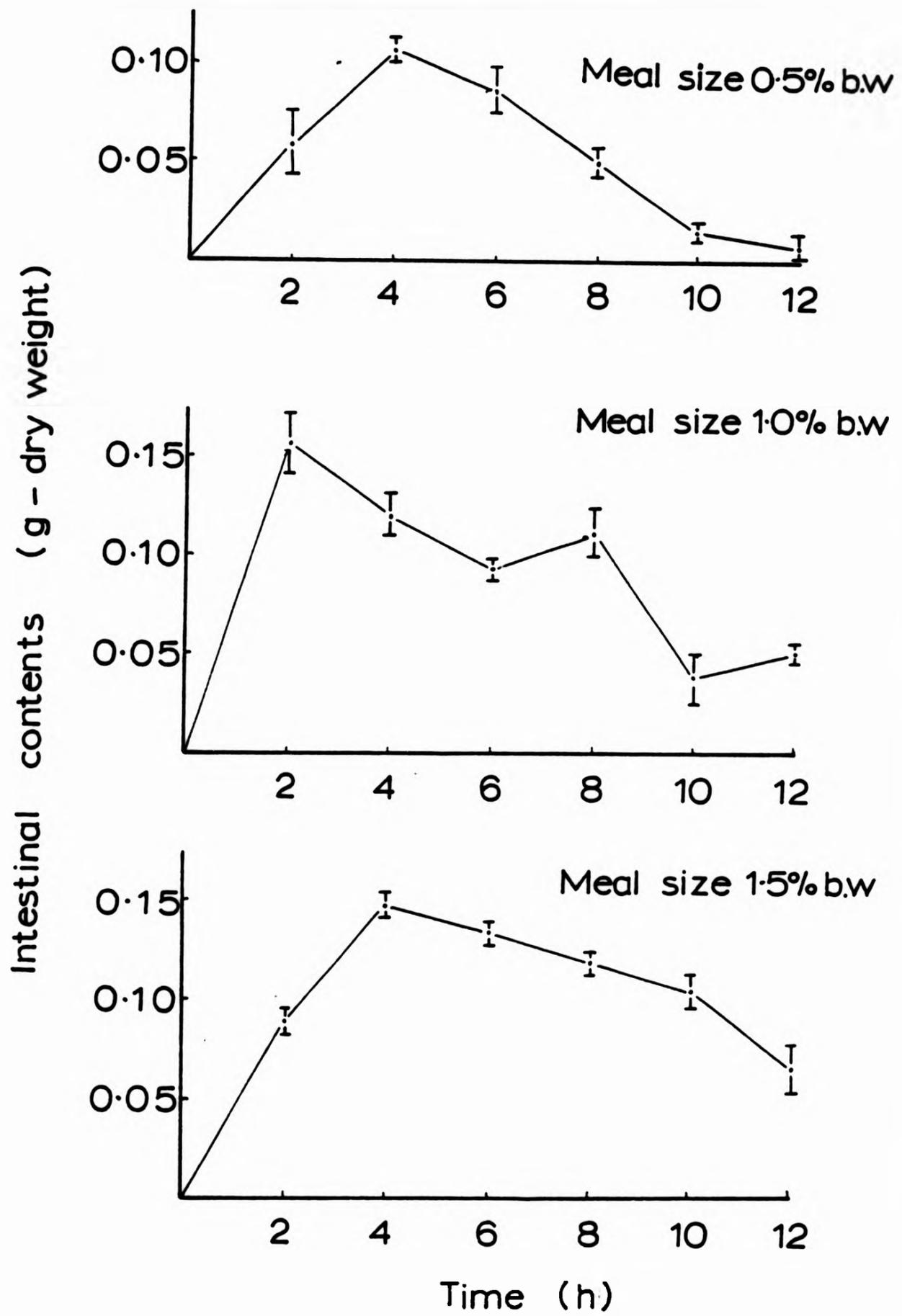


FIGURE 36 The change in the intestine contents (g, d.w.) with time (h) for *O. niloticus* fed 0.5%, 1% and 1.5% of their body weight

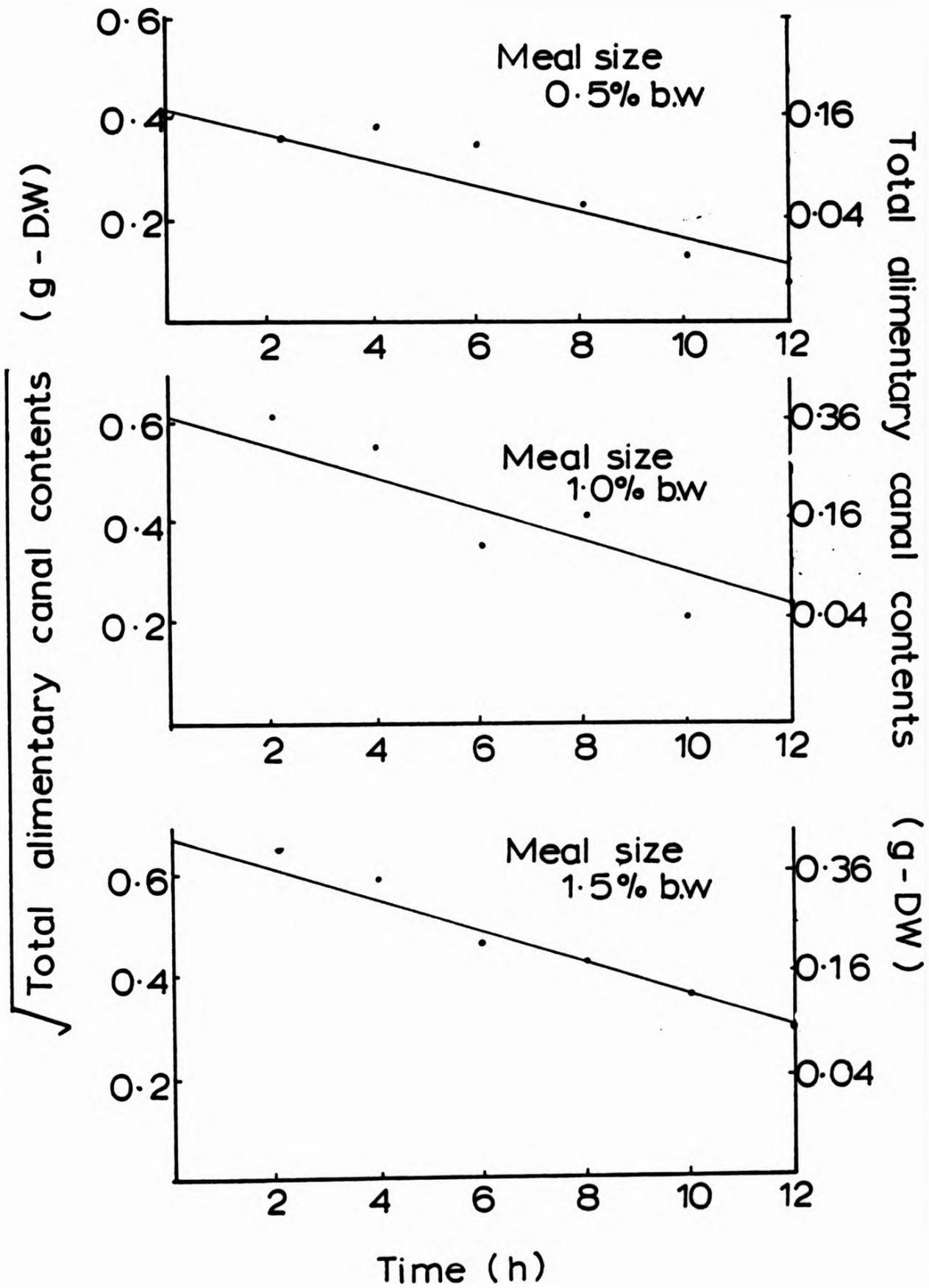


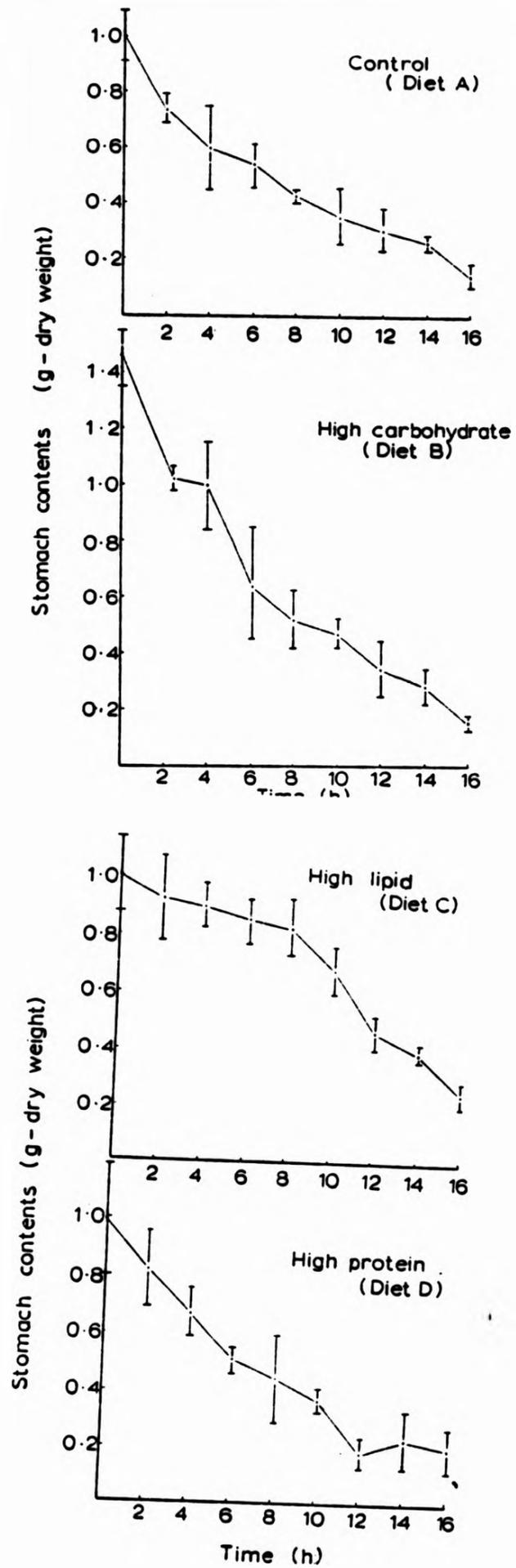
FIGURE 37 Linear regression of volume transformed total alimentary canal contents (g, d.w.) with time (h) for *O. niloticus* fed 0.5%, 1% and 1.5% of their body weight

TABLE 23 Regression equations, correlation coefficients and the predicted total evacuation times (h) for O. niloticus fed 0.5%, 1.0% and 1.5% b.w. at  $27.5 \pm 1^\circ\text{C}$

Meal size % b.w.	Regression equations	Corr. coeff.	P < 0.0	Predicted total evacuation time (T.E.T., h)
0.5	✓ $Y_t = 0.421 - 0.025T$	-0.90	0.001	16.84h
1.0	✓ $Y_t = 0.611 - 0.0329T$	-0.90	0.001	18.6h
1.5	✓ $Y_t = 0.673 - 0.311T$	-0.97	0.001	21.64h

#### 3.2.2.4 The effect of food composition

Fig. 38 shows the stomach evacuation pattern (on dry weight basis) for each of the four experimental diets. From the stomach evacuation curves it can be seen that the initial stomach evacuation rate for the high available carbohydrate diet (B) was faster than the control diet (A) or the high protein diet (D). The high lipid diet (C) showed a slower initial stomach evacuation rate over the eight hours of sampling than the other experimental diets. Stomach evacuation data for each of the four experimental diets was fitted to the volume dependent model (2.4.2) in Fig. 39. The regression equations, correlation coefficients and the predicted stomach evacuation times for each of the four experimental diets are presented in Table 24. The fastest stomach evacuation coefficient and shortest time was achieved with diet B followed by diet D then diet A, while diet C was the slowest (Table 24). Figure 40 shows this data set replotted to allow calculation of the time required to evacuate 50%, 75% and 90% of the ingested food from the stomach. From Fig. 40 it can be seen that the shape of the curve describing the percentage of food evacuated from the stomach at various times after feeding for the high lipid diet differs from the other three experimental diets (A, B and D). This may be explained by the very slow evacuation rate for this diet over the first eight hours of sampling. It can be seen that the shortest times for evacuation of 50%, 75% or 90% of the initial stomach contents were achieved by diet B followed by the control (A) and high protein (D) diets. Figure 41 shows the pattern of the intestinal dry weight content with time after feeding for the four experimental diets. The high protein diet (D) and high available carbohydrate diet (B) in



**FIGURE 38** The decline in stomach contents (g, d.w.) with time (h) for *O. niloticus* fed the four experimental diets (1% b.w.)

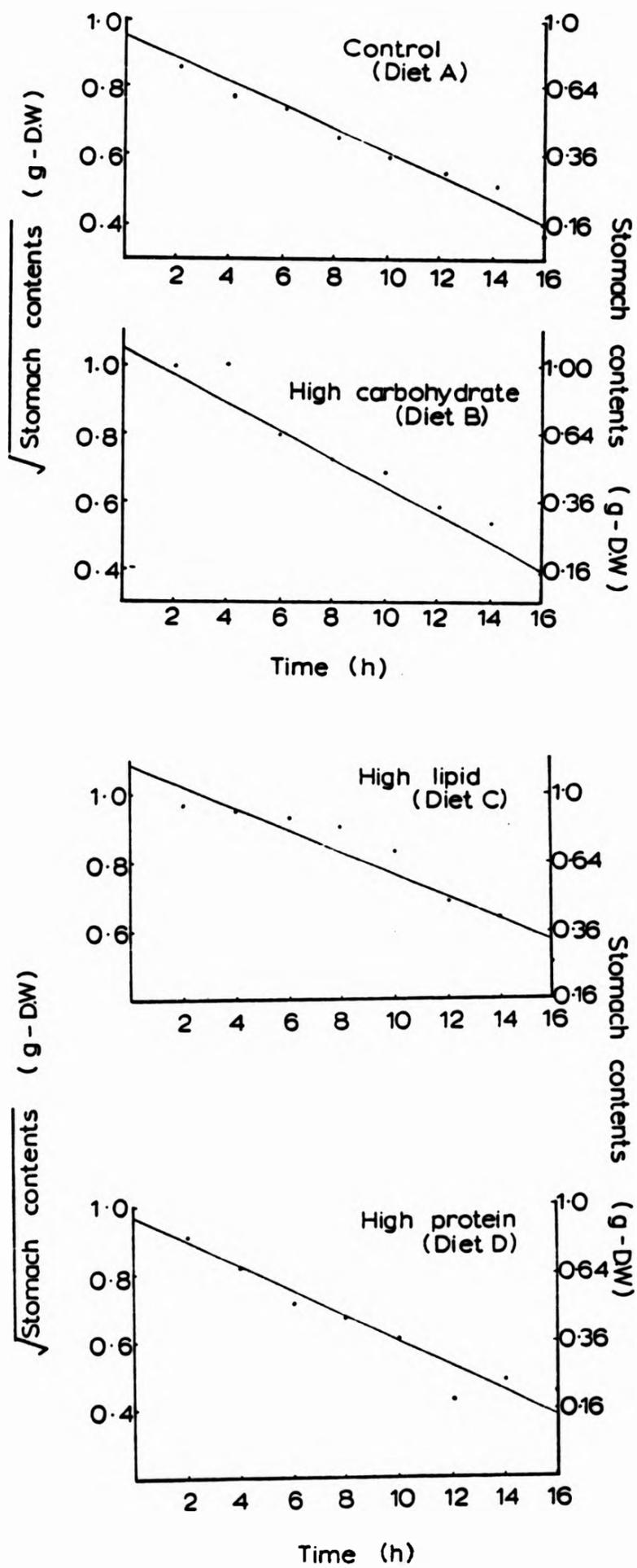
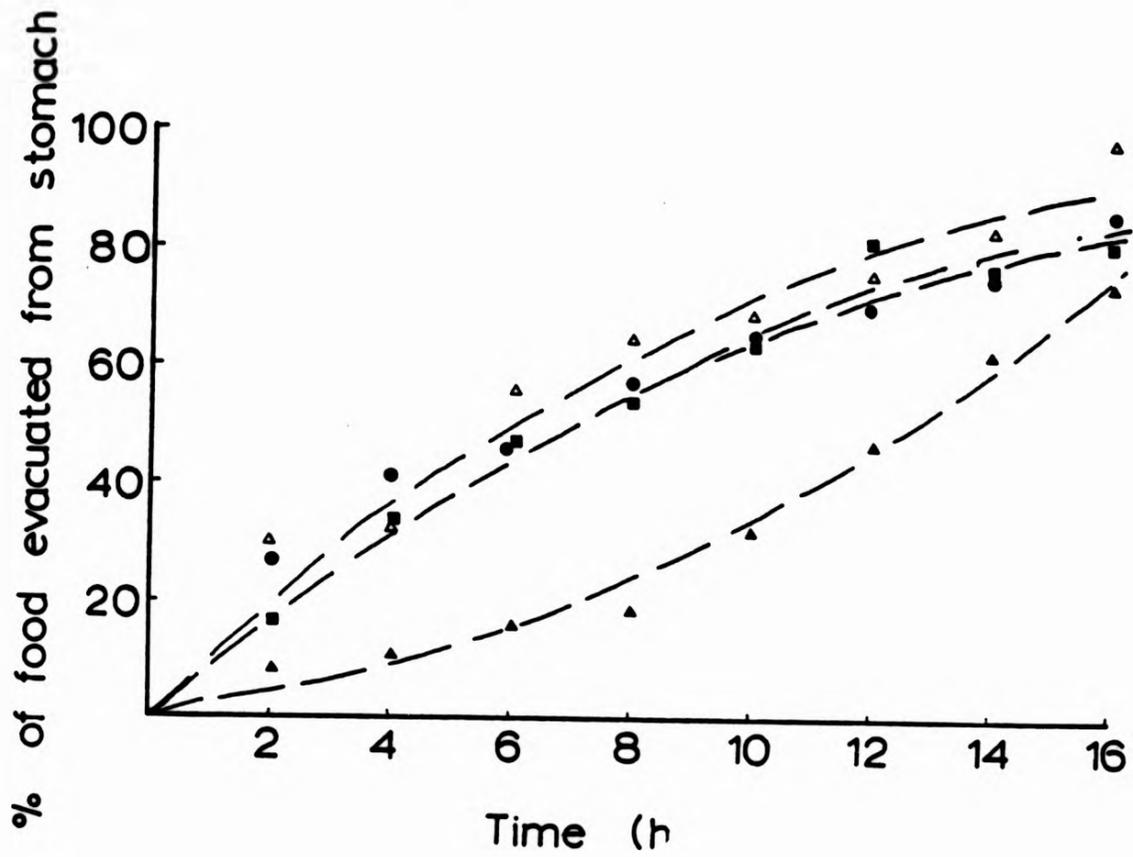


FIGURE 39 Linear regression of volume transformed stomach contents (g, d.w.) with time (h) for *O. niloticus* fed the four experimental diets (1% b.w.)

TABLE 24 Regression equation, correlation coefficients and the predicted stomach evacuation times (h) for diets of different composition fed to O. niloticus to satiation at  $27.5 \pm 1^\circ\text{C}$

Diets	Regression equations	Corr. coeff.	P < 0.0	Predicted stomach evacuation time (S.E.T., h)
Control diet (A)	$\sqrt{Y_t = 0.95 - 0.035T}$	-0.985	0.001	27.14h
High available carbohydrate diet (B)	$\sqrt{Y_t = 1.145 - 0.047T}$	-0.98	0.001	24.4h
High lipid diet (C)	$\sqrt{Y_t = 1.07 - 0.0305T}$	-0.95	0.001	35.08h
High protein diet (D)	$\sqrt{Y_t = 0.963 - 0.036T}$	-0.97	0.001	26.7h

- - Control diet (A)
- △ - High available carbohydrate diet (B)
- ▲ - High lipid diet (C)
- - High protein diet (B)



**FIGURE 40** Percentage of food evacuated from the stomach of *O. niloticus* fed the four experimental diets

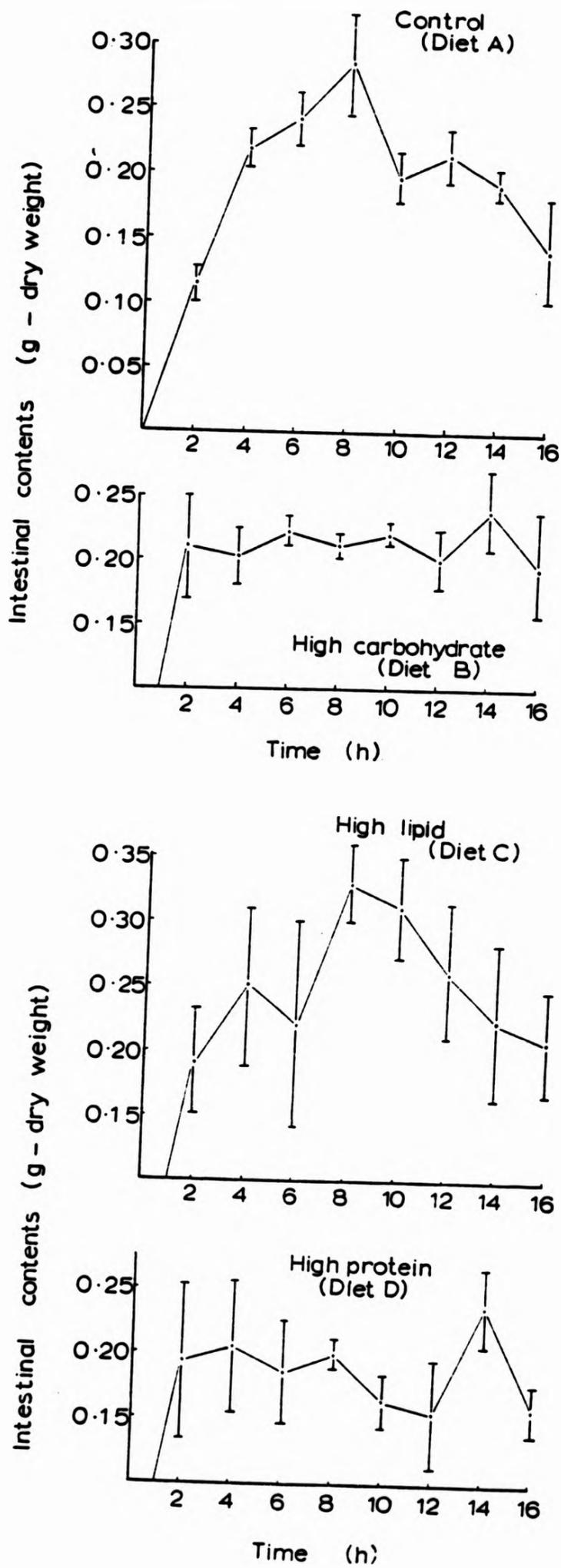


FIGURE 41 The change in the intestinal contents (g, d.w.) with time for *O. niloticus* fed the four experimental diets

general show similar patterns of filling and emptying with time after feeding. The control diet (A) showed an increase in the intestinal content over the first eight hours after feeding, thereafter it declined sharply to increase slightly again by 12h. The high lipid diet ((C) showed an increase in the intestinal content over the first four hours after feeding then a decline to increase again by the eighth hour before decreasing steadily over the sixteen hours of the experiment. The decline in intestinal contents for fish fed the high lipid diet (C) after the first four hours of sampling could explained by the evacuation or digestion of the previous food remaining in the intestine.

Data for the whole alimentary canal contents (2.4.2) transformed to the volume dependent model are shown in Fig. 42. The regression equations,, correlation coefficients and the predicted total evacuation times for the four experimental diets are presented in Table 25. From this table it can be observed that total evacuation time (T.E.T.) decreased and total evacuation coefficient increased compared to the control diet (A), with incorporation of high levels of available carbohydrate (B) and, to a lesser extent, with incorporation of high level of protein (D). Incorporation of high levels of lipid in the diet C increased the total evacuation time and decreased the total evacuation coefficient.

The apparent protein and dry matter digestion coefficients for each of the experimental diets based on intestinal samples at two hourly intervals after feeding and in faecal samples were determined (Table 26). The apparent and dry matter digestion coefficients

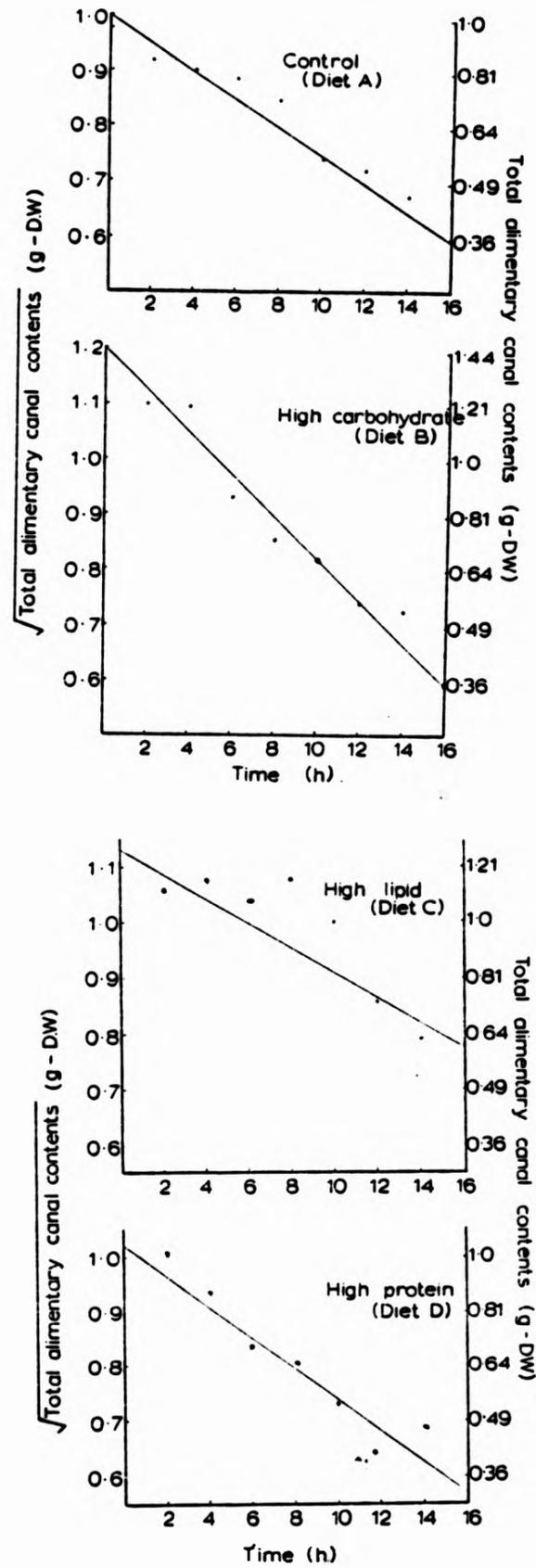


FIGURE 42 Linear regression of volume transformed total alimentary canal contents (g, d.w.) with time for O. niloticus fed the four experimental diets

TABLE 25 Regression equations, correlation coefficients and the predicted total evacuation times (h) for diets of different composition fed to *O. niloticus* to satiation at  $27.5 \pm 1^\circ\text{C}$

Diets	Regression equations	Corr. coeff.	P < 0.0	Predicted total gastric evacuation time (T.E.T., h)
Control diet (A)	$\sqrt{Y_t} = 1.01 - 0.0262T$	-0.97	0.001	38.5h
High available carbohydrate diet (B)	$\sqrt{Y_t} = 1.19 - 0.0371T$	-0.99	0.001	32.16h
High lipid diet (C)	$\sqrt{Y_t} = 1.13 - 0.0218T$	-0.83	0.001	51.8h
High protein diet (D)	$\sqrt{Y} = 1.02 - 0.028T$	-0.96	0.001	36.4h

TABLE 26 The apparent protein and dry matter digestion coefficients for the four experimental diets in the intestine and faeces of *O. niloticus* at different intervals after feeding

Time after feeding	Control diet (A)		High available carbohydrate diet (B)		High lipid diet (C)		High protein diet (D)	
	D.M.D.C.	A.P.D.C.	D.M.D.C.	A.P.D.C.	D.M.D.C.	A.P.D.C.	D.M.D.C.	A.P.D.C.
2h	29.51	36.80	17.8	26.2	32.8	39.2	33.3	50.7
4	24.22	32.50	13.2	20.5	19.65	32.9	27.95	44.3
6	29.34	35.0	36.99	46.2	32.9	47.9	47.32	64.97
8	35.34	41.9	47.91	55.8	31.8	37.5	47.4	58.14
10	30.72	40.2	51.6	63.6	48.4	59.4	48.4	69.8
12	47.75	53.2	46.8	58.1	46.4	55.0	46.43	63.99
14	57.7	60.74	52.6	61.7	51.6	62.5	51.62	72.8
16	61.2	67.0	57.4	64.9	60.9	70.3	70.3	80.2
Faeces	70.61	81.1	79.8	89.0	76.1	86.5	83.5	93.6

D.M.D.C. = Dry matter digestion coefficient

A.P.D.C. = Apparent protein digestion coefficient

generally increased with time in each of the four experimental diets over the period of sampling. The apparent protein digestion coefficient and the dry matter digestion coefficient for diets A, B and C were similar, whilst the high protein diet (D) showed higher values for apparent protein digestibility.

3.2.2.5 The effect of starvation and refeeding on stomach evacuation time and coefficient

Fig. 43 shows the mean food intake at time zero after feeding to satiation for four groups of fish previously starved for 24h, 48h, 72h and 96 hours and the decline in stomach contents with time for 20 hours after feeding. The relationship between the quantity (dry weight) of food remaining in the stomach and time interval after feeding appears to be curvilinear and has been linearised using the volume dependent model (2.4.2) in Fig. 44. The regression equations, correlation coefficients and the predicted stomach evacuation times after different periods of starvation of fish refed to satiation are presented in Table 27. From Fig. 44 and Table 27 it can be seen that an increase in the prefeeding starvation period resulted in an increase in the stomach evacuation time and a decrease in the stomach evacuation coefficient. With a decrease in starvation period there is a faster evacuation of food from the stomach, as can be seen from the increase in steepness of the slopes of the stomach evacuation graphs (Fig.44). The time to complete evacuation of the stomach increased from 20.9h after 24 hours of food deprivation to 39.97h after 96 hours of starvation. Fig. 45 shows the times required to evacuate 50%, 75% and 90% of the ingested food from the stomach. The time required to evacuate

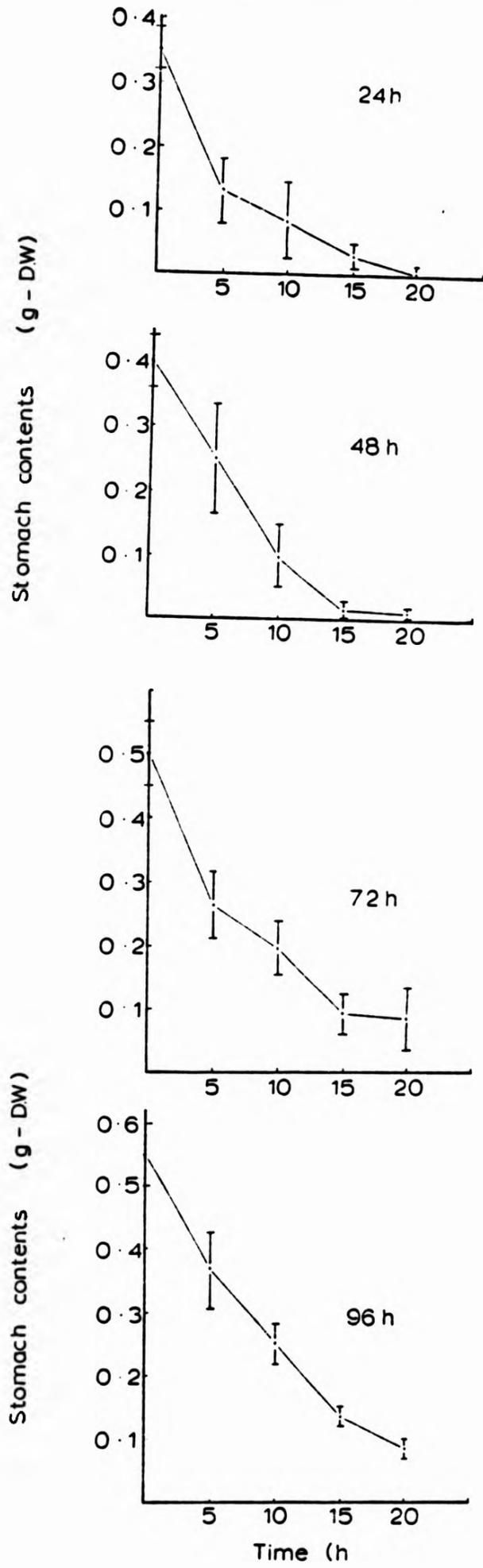


FIGURE 43 The decline in the stomach contents (g, d.w.) with time (h) after refeeding to satiation following starvation periods of 24, 48, 72 and 96 (h)

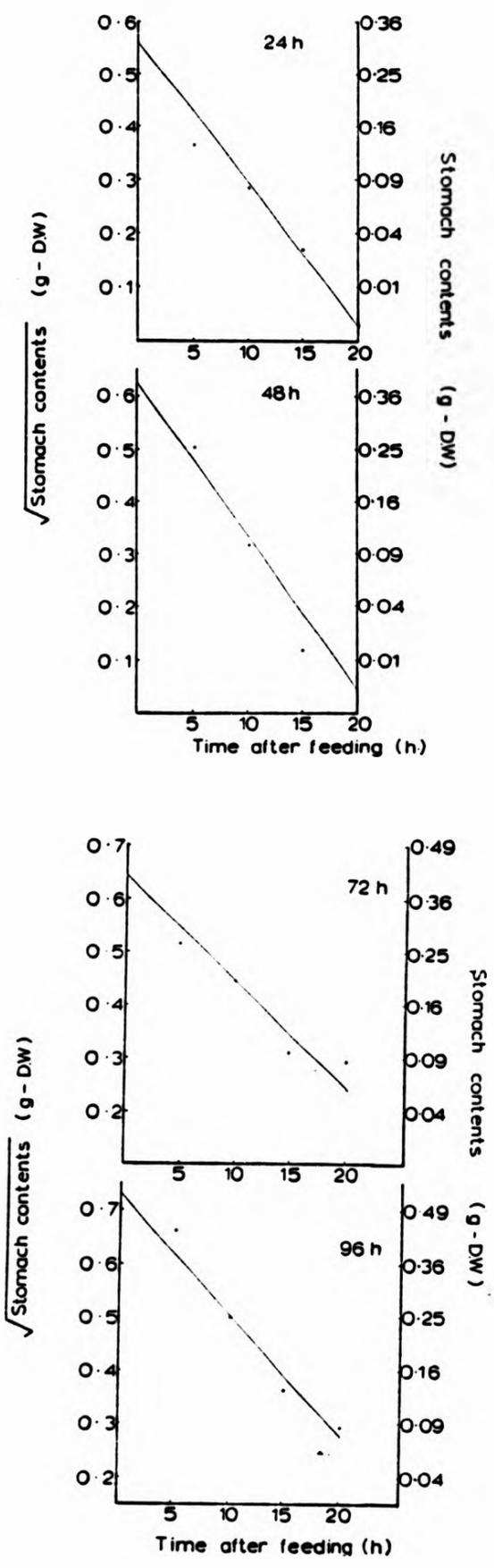


FIGURE 44 Linear regression of volume transformed stomach contents (g, d.w.) with time (h) after refeeding to satiation following starvation periods of 24, 48, 72 and 96 (h)

TABLE 27 Regression equations, correlation coefficients and the predicted stomach evacuation times (h) for *O. niloticus* fed to satiation after different periods of starvation at  $27.5 \pm 1^\circ\text{C}$

Starvation period	Regression equations	Corr. coeff.	P < 0.0	Predicted stomach evacuation time (S.E.T., h)
24h	$\sqrt{Y_t = 0.551 - 0.0263T}$	-0.99	0.001	20.95h
48h	$\sqrt{Y_t = 0.625 - 0.029T}$	-0.98	0.001	21.6h
72h	$\sqrt{Y_t = 0.66 - 0.0208T}$	-0.97	0.001	31.7h
96h	$\sqrt{Y_t = 0.729 - 0.022T}$	0.996	0.001	31.9h

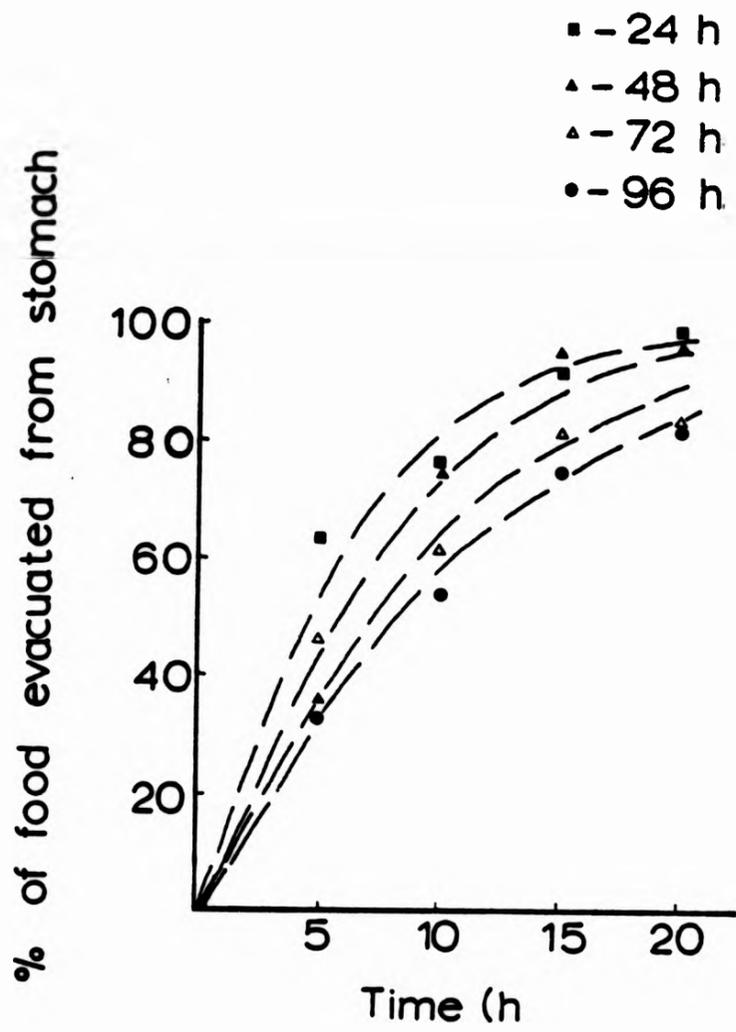


FIGURE 45 Percentage of food evacuated from the stomach of O. niloticus after refeeding to satiation following periods of starvation of 24, 48, 72 and 96 (h)

food from the stomach increased with increasing prefeeding starvation time. The time required to evacuate 50%, 75% and 90% of the stomach increased from 5h, 8.8h and 13h to 8h, 15.5h and 23h with increasing starvation period from 24 hours to 96 hours. Evacuation proceeded most rapidly during the first five hours after feeding, thereafter the rate decreased steadily. The amount of food evacuated during the last five hour interval (15-20h after feeding) is less than half that evacuated during the first five hours (0-5h after feeding) for each prefeeding deprivation period.

A pattern of filling and emptying of dry matter intestinal content was evident for each deprivation group (Fig. 46) as noted in Sections 3.2.2.1-3.2.2.4. Stomach and intestinal content data were combined and linearised according to the volume dependent model (2.4.2) to calculate the total evacuation time and coefficient (Fig. 47). The regression equations, correlation coefficients and the predicted total evacuation times after different periods of starvation are shown in Table 28. From Fig. 47 and Table 28 it can be seen that total evacuation time increased with increasing prefeeding deprivation time, while total evacuation coefficient decreased. The time required to evacuate the gut totally increased from 26.4h to 44h with increasing prefeeding deprivation time from 24 to 96 hours.

A subsequent determination of the effect of varying periods of deprivation on gastric evacuation when all groups were refed to 1% of their respective body weights was conducted (Fig. 48). This data was transformed to the volume dependent model (2.4.2) and linear regression analysis conducted (Fig. 49). The regression equations,

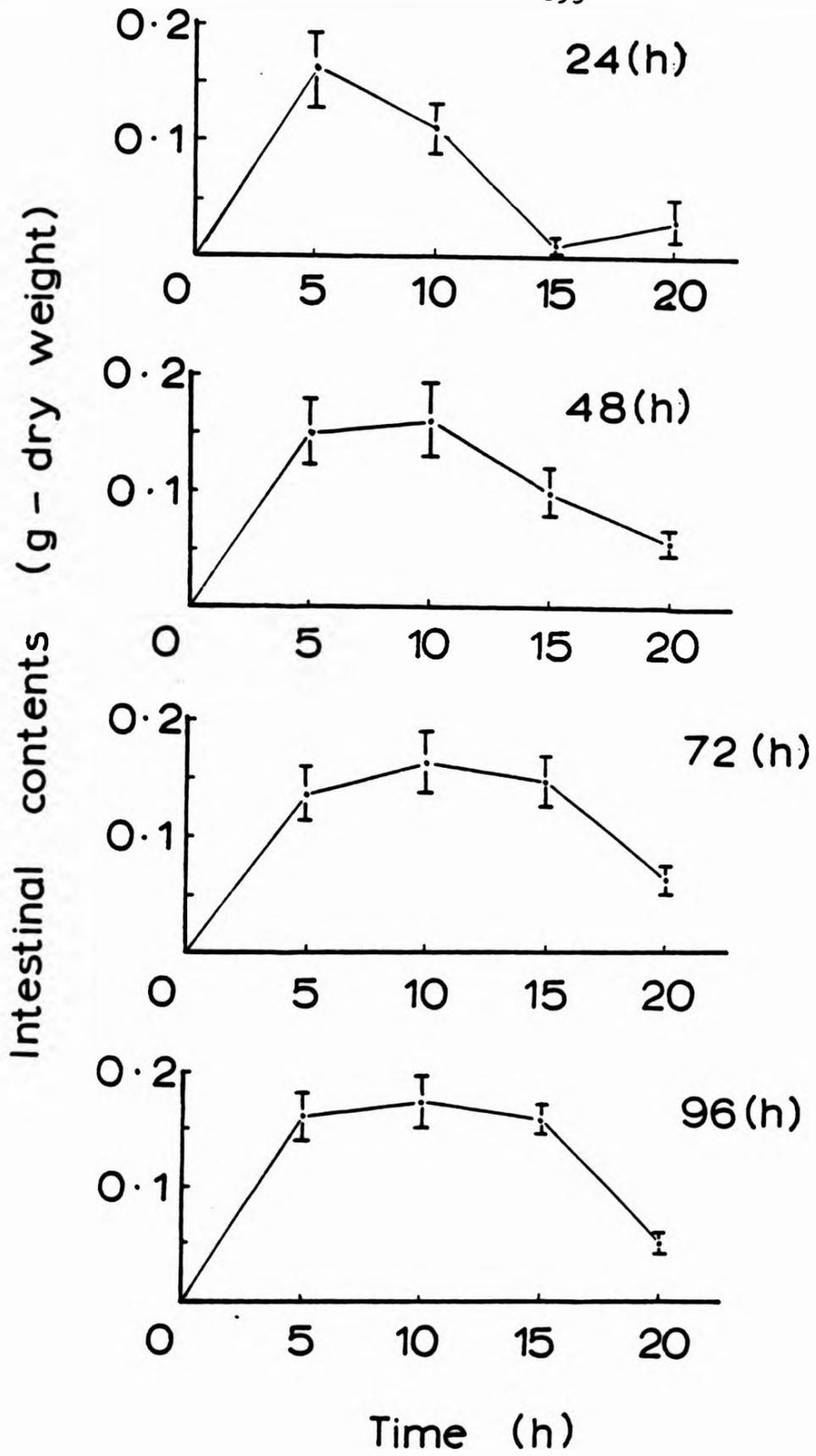


FIGURE 46 The change in the intestinal contents (g, d.w.) with time (h) after refeeding to satiation following periods of starvation of 24, 48, 72 and 96 (h)

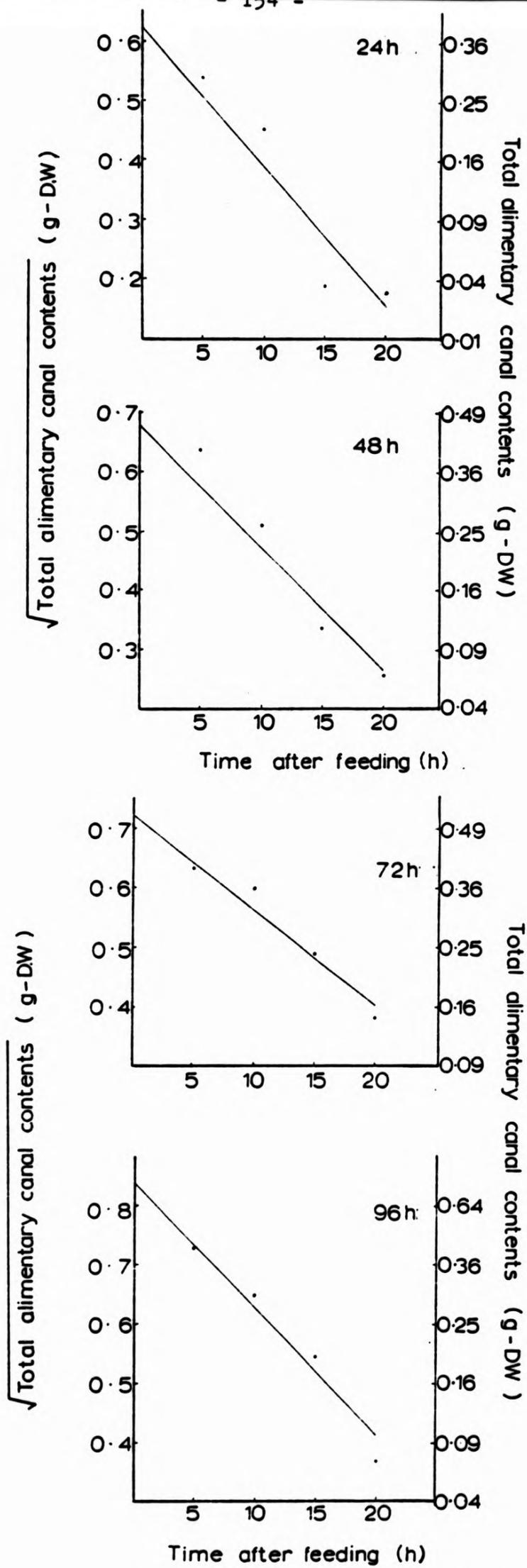


FIGURE 47

Linear regression of volume transformed total alimentary canal contents (g, d.w.) with time for *Q. niloticus* after refeeding to satiation following periods of starvation of 24, 48, 72 and 96 (h)

TABLE 28 Regression equations, correlation coefficients and predicted total evacuation times (h) for O. niloticus fed to satiation after different periods of starvation at  $27.5 \pm 1^\circ\text{C}$

Starvation period	Regression equations	Corr. coeff.	P < 0.0	Predicted total evacuation time (T.E.T., h)
24h	$\sqrt{Y_t} = 0.626 - 0.237T$	-0.96	0.001	26.4h
48h	$\sqrt{Y_t} = 0.686 - 0.0212T$	-0.97	0.001	32.35h
72h	$\sqrt{Y_t} = 0.72 - 0.16T$	-0.98	0.001	45.0h
96h	$\sqrt{Y_t} = 0.791 - 0.018T$	-0.96	0.001	44.0h

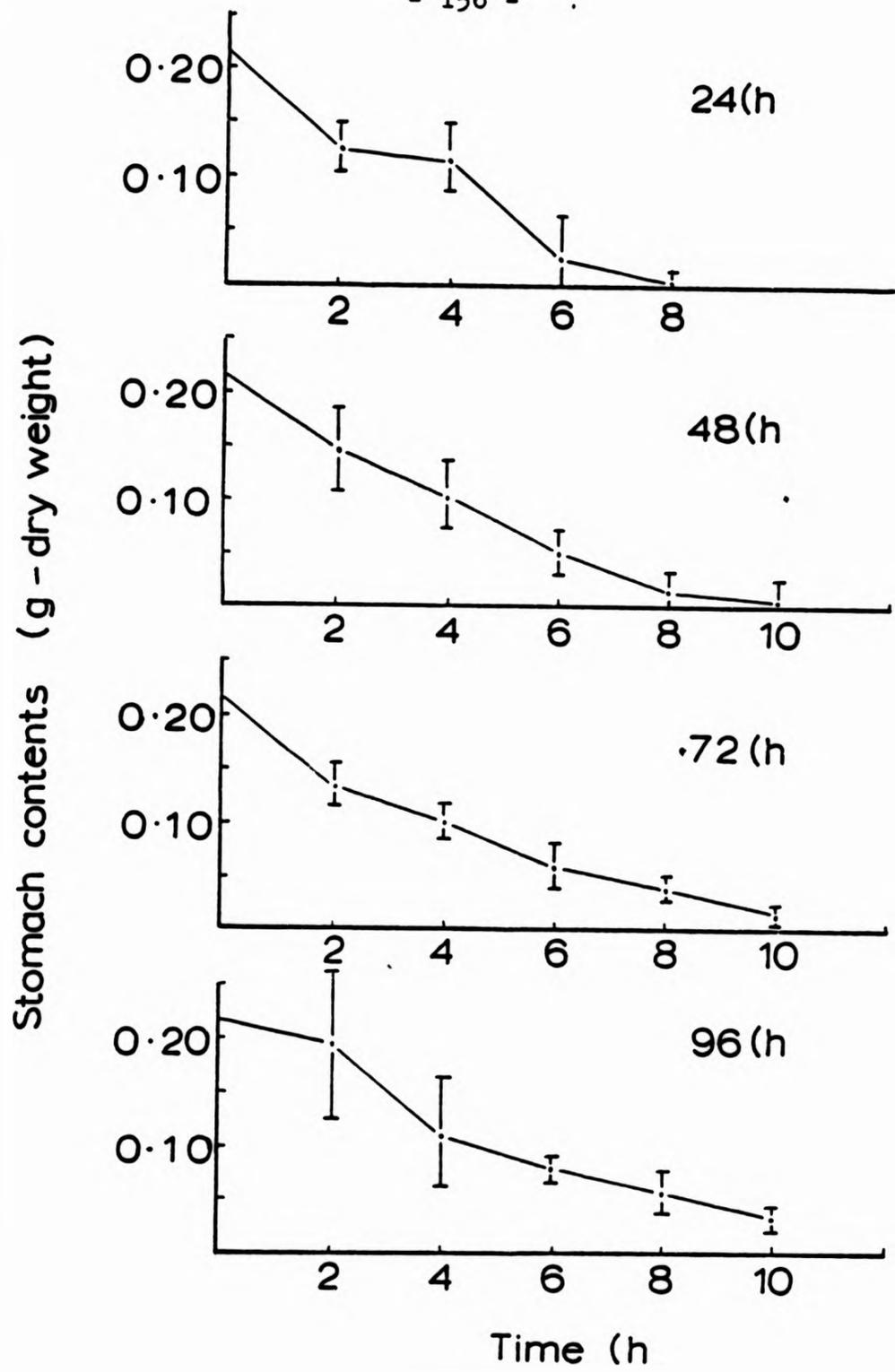
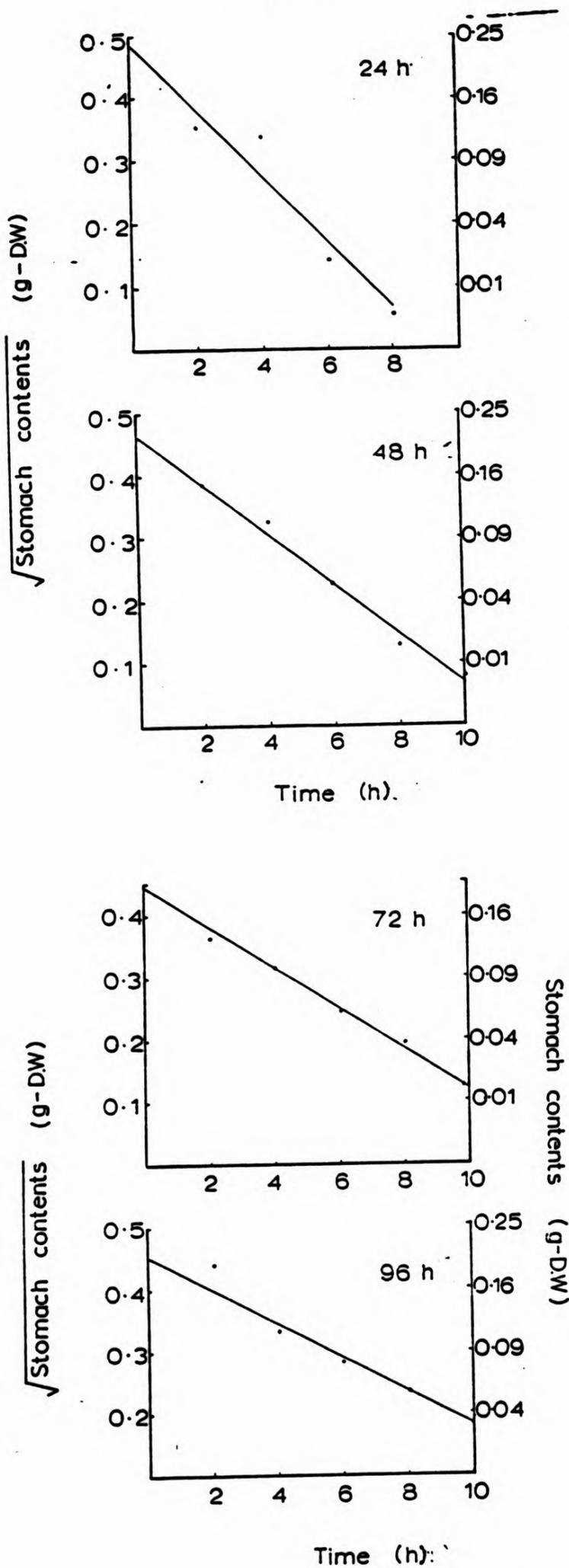


FIGURE 48 The decline in the stomach contents (g, d.w.) with time (h) after refeeding at 1% b.w. following prefeeding starvation periods of 24, 48, 72 and 96 (h)



**FIGURE 49** Linear regression of volume transformed stomach contents (g, d.w.) with time (h) after refeeding at 1% b.w. following prefeeding starvation periods of 24, 48, 72 and 96 (h)

correlation coefficients and the predicted stomach evacuation times after different periods of starvation and refeeding with 1% b.w. are presented in Table 29. Fig. 50 shows the times required to evacuate 50%, 75% and 90% of ingested food from the stomach. Similar results to the previous experiment were obtained, increasing prefeeding starvation periods led to increased stomach evacuation time and decreased stomach evacuation rate (g/h) (Table 29). To establish the mathematical relationship between stomach evacuation time and starvation period, the calculated stomach evacuation time (Table 29) was plotted against starvation period (Fig. 51) and the regression equation was calculated as

$$\text{S.E.T. (h)} = 7.015 + 0.096 \text{ starvation period (h)}$$

The correlation coefficient of 0.99 was significant at the 0.05 level. As well as S.E.T. it is also possible to evaluate the effect of starvation on stomach evacuation coefficient. There was a negative relationship between stomach evacuation coeff. and starvation period (Fig. 52). The regression equation of this relationship was calculated as

$$\text{stomach evacuation coefficient} = 0.0567 - 0.00031 \text{ starvation period (h)}$$

The correlation coefficient of 0.97 was significant at the 0.05 level. Fig. 53 shows the change in intestinal dry matter content for each of the deprivation periods versus time. Again a cycle of filling and emptying is evident (see 3.2.2.1). The stomach and intestinal contents were combined and transformed using the volume dependent model (2.4.2) to calculate total gastric evacuation times (Fig. 54). The regression equations, correlation coefficients and the predicted total

TABLE 29 Regression equations, correlation coefficients and the predicted stomach evacuation times (h) for *O. niloticus* fed 1% b.w. after different periods of starvation at 27.5 ± 1°C

Starvation period	Regression equations	Corr. coeff.	P < 0.0	Predicted stomach evacuation time (S.E.T., h)
24h	$\sqrt{Y_t} = 0.475 - 0.0513T$	0.97	0.001	9.26h
48h	$\sqrt{Y_t} = 0.466 - 0.0399T$	0.996	0.001	11.7h
72h	$\sqrt{Y_t} = 0.447 - 0.032T$	0.995	0.001	13.97h
96h	$\sqrt{Y_t} = 0.469 - 0.029T$	0.99	0.001	16.17h

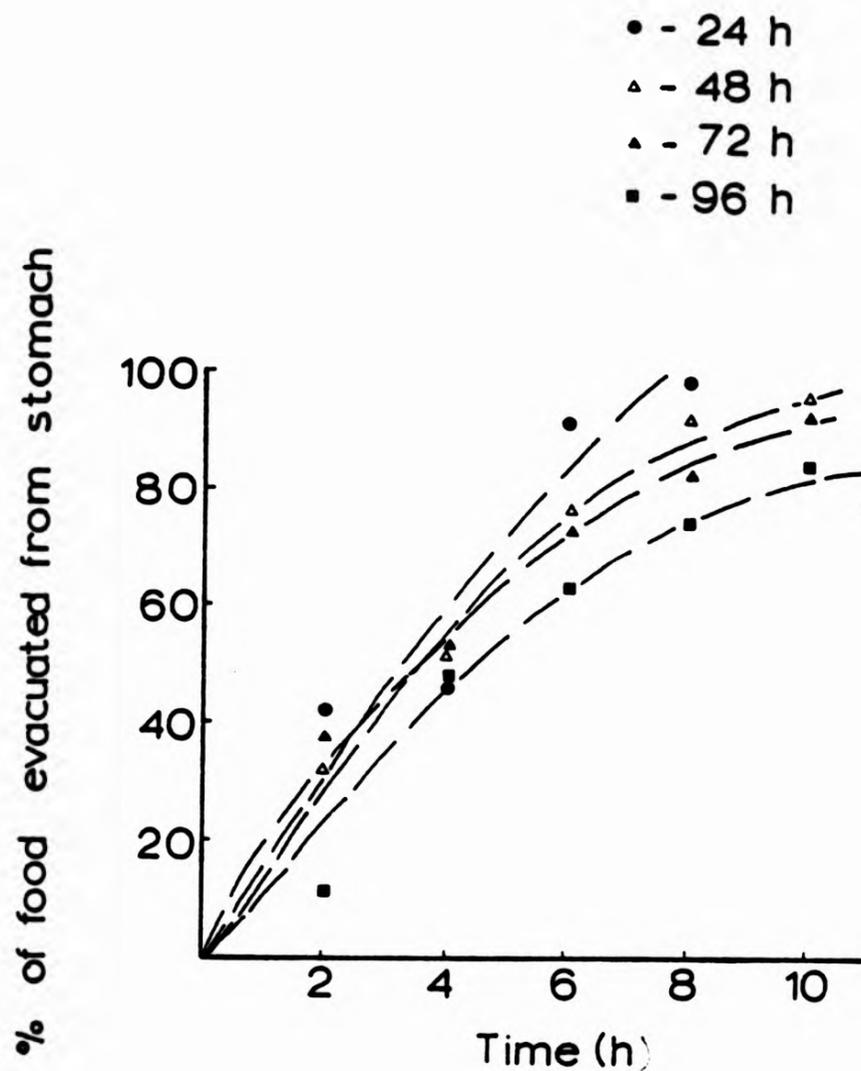


FIGURE 50 Percentage of food evacuated from the stomach of O. niloticus after refeeding at 1% b.w. following prefeeding starvation periods of 24, 48, 72 and 96 (h)

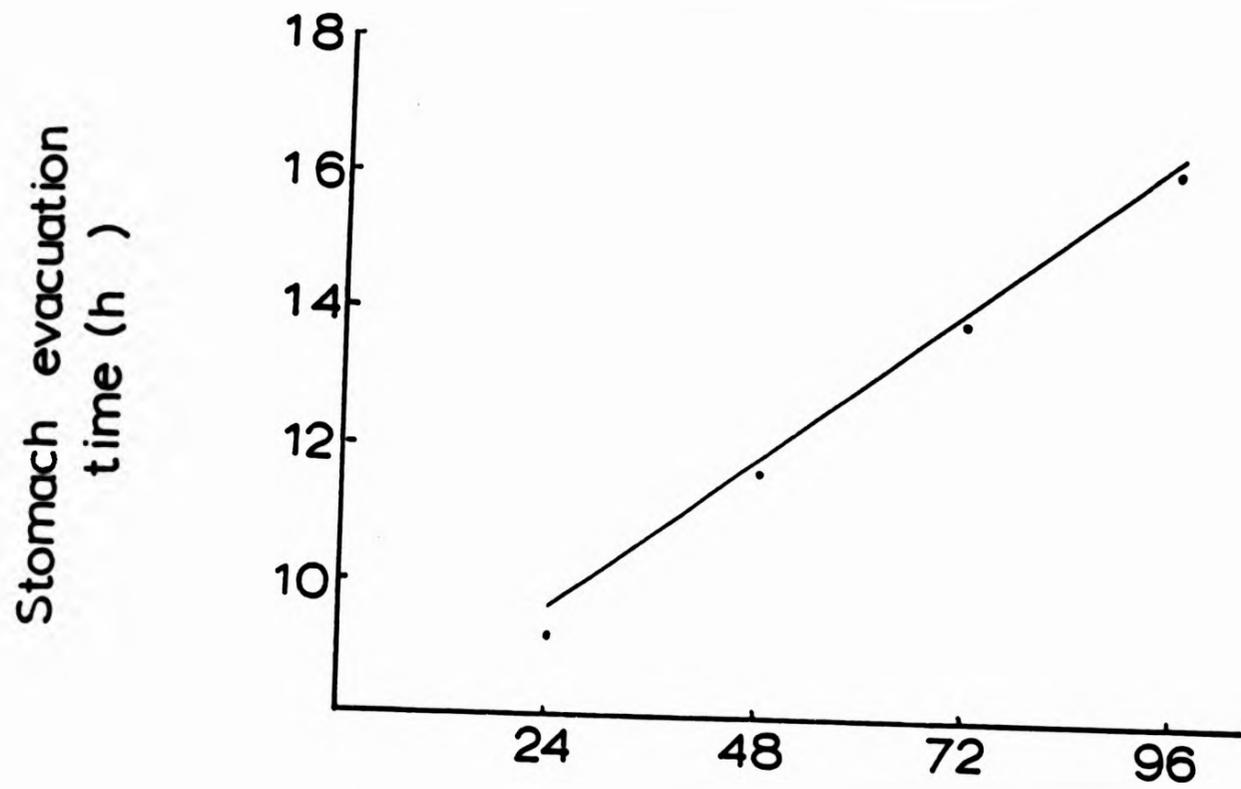


FIGURE 51 The relationship between stomach evacuation time and prefeeding starvation periods (h)

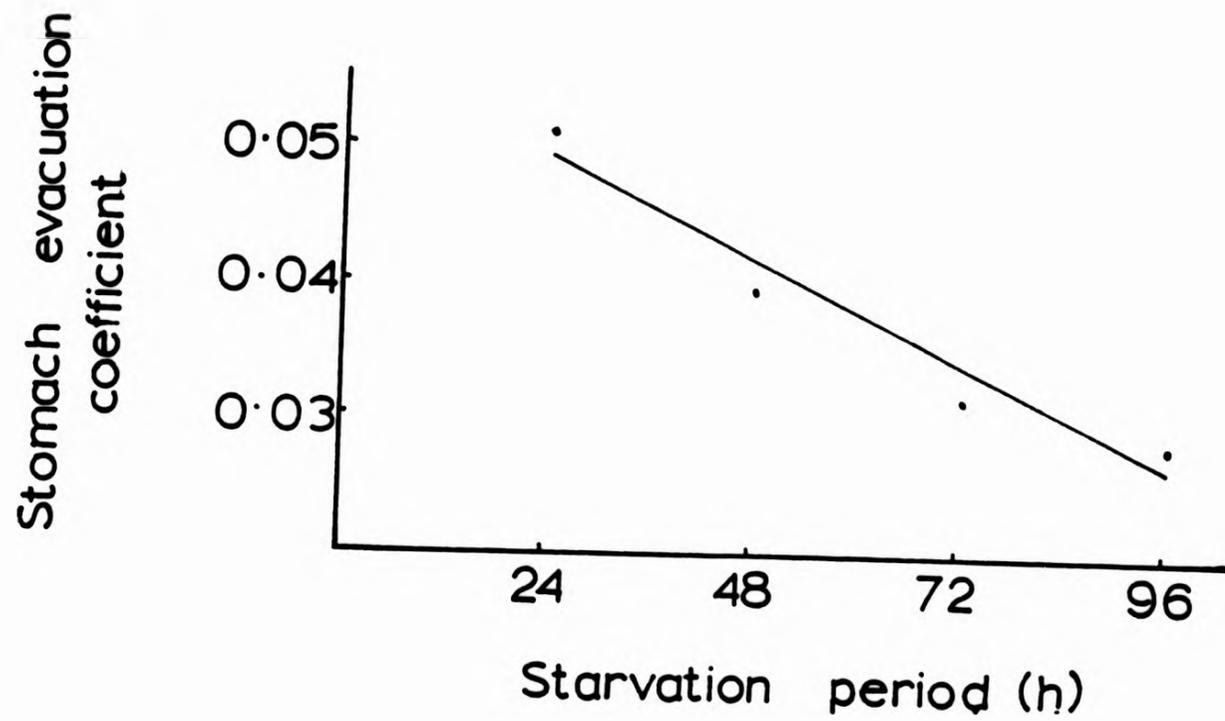


FIGURE 52 The relationship between stomach evacuation coefficient and prefeeding starvation periods (h)

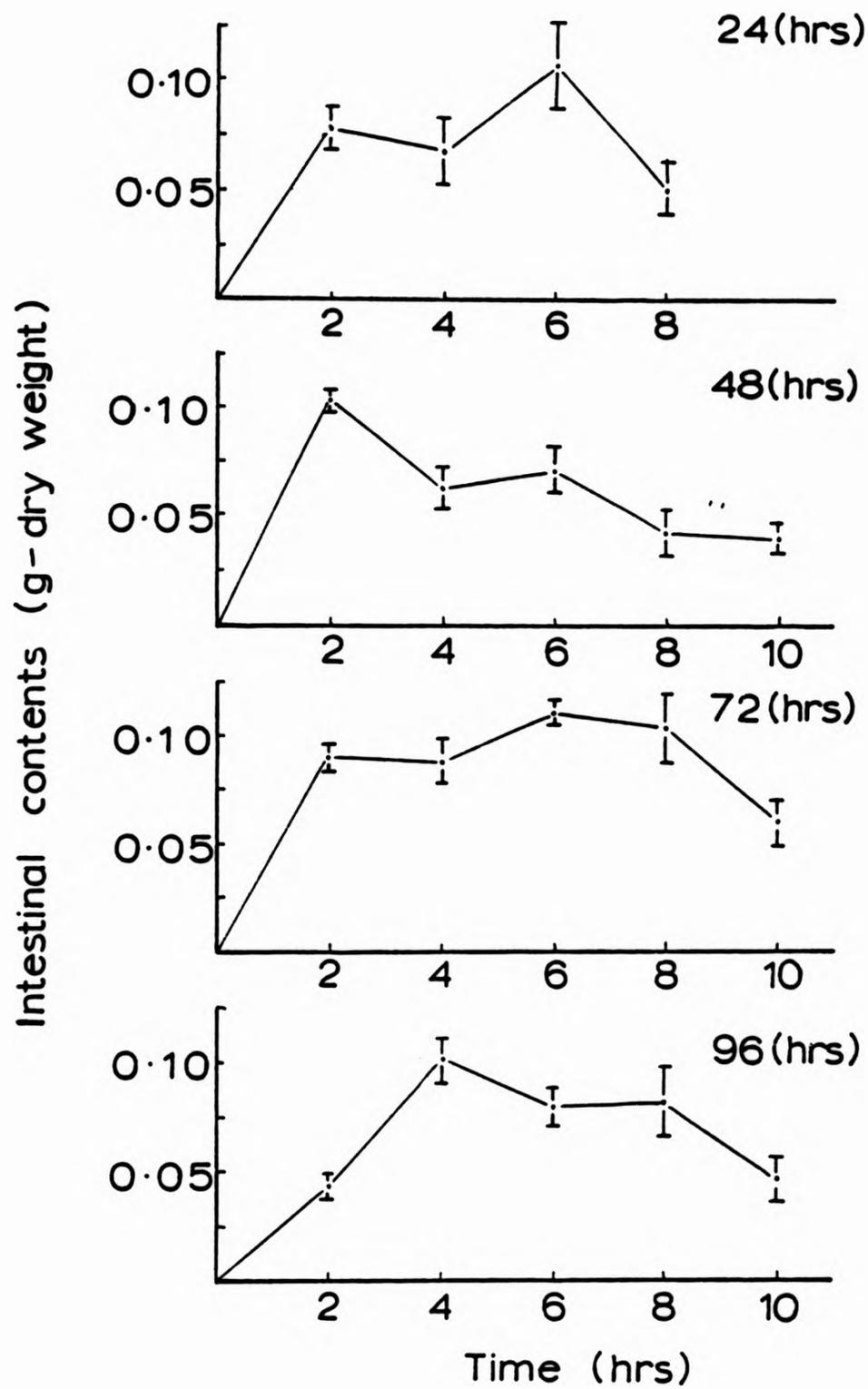
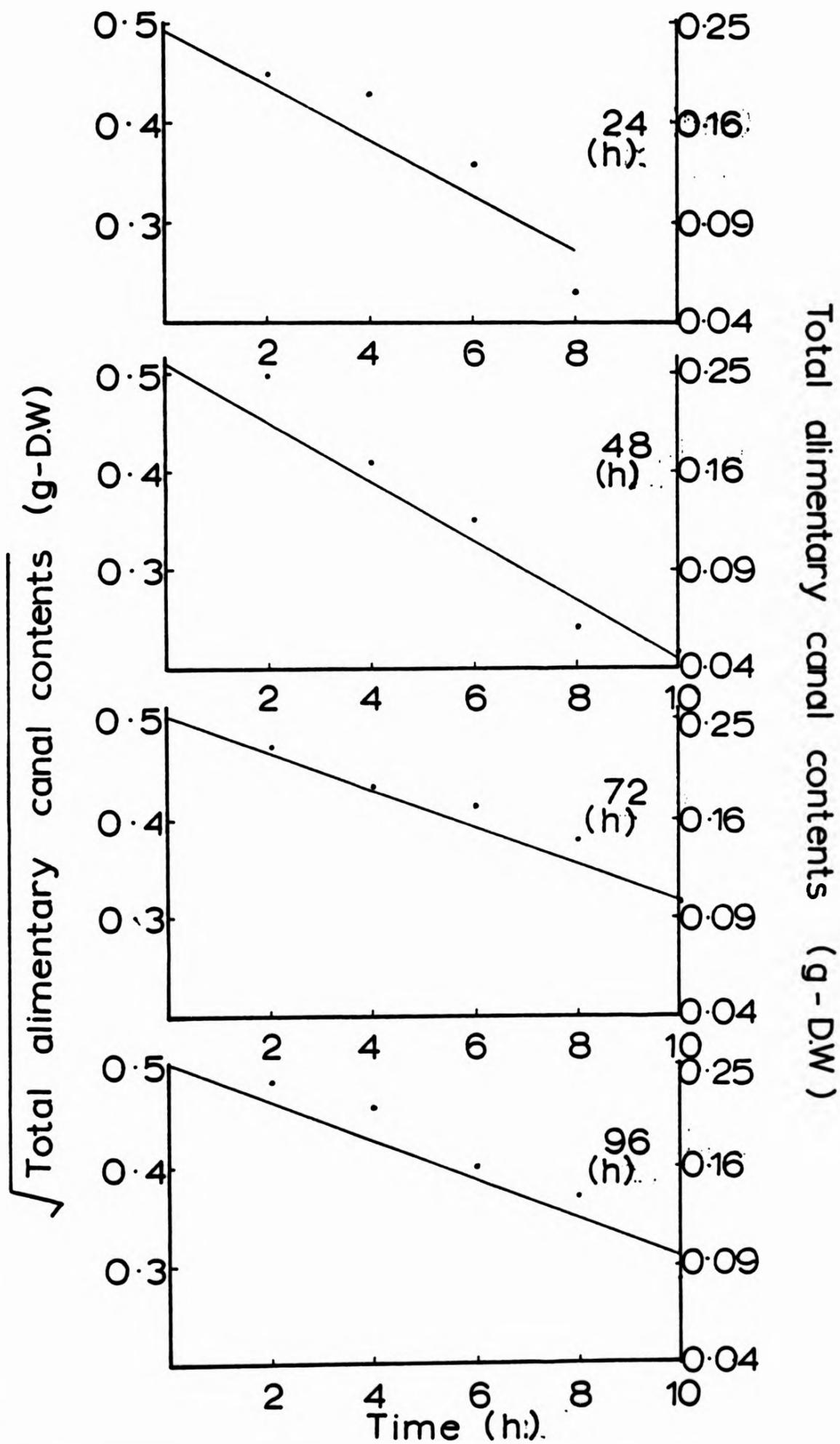


FIGURE 53 The change in the intestinal contents (g, d.w.) with time (h) after refeeding at 1% b.w. following prefeeding starvation periods of 24, 48, 72 and 96 (h)



**FIGURE 54** Linear regression of volume transformed total alimentary canal contents (g, d.w.) with time (h) after refeeding at 1% b.w. following prefeeding starvation periods of 24, 48, 72 and 96 (h)

**TABLE 30** Regression equations, correlation coefficients and predicted total evacuation times (h) for *O. niloticus* fed 1% b.w. after different periods of starvation at 27.5° ± 1°C

Starvation period	Regression equations	Corr. coeff.	P < 0.0	Predicted total gastric evacuation time (T.E.T., h)
24h	$\sqrt{Y_t} = 0.496 - 0.0279T$	-0.922	0.001	17.8h
48h	$\sqrt{Y_t} = 0.51 - 0.029T$	-0.96	0.001	17.6h
72h	$\sqrt{Y_t} = 0.494 - 0.0178T$	-0.99	0.001	27.7h
96h	$\sqrt{Y_t} = 0.503 - 0.018T$	-0.92	0.001	27.9h

gastric evacuation times are presented in Table 30. From Fig. 54 and Table 30 it can be seen that the T.E.T. increased from 17.8h to 27.9h with increasing deprivation time from 24 to 96 hours.

### 3.2.3 Calculation of daily food intake from stomach evacuation time

As it appeared impractical to design a specific experiment to establish the relationships between stomach evacuation time, meal size and fish weight an attempt was made to combine data from other experiments to this effect. S.E.Ts were calculated for initial fish (sampled at zero time) from various experiments using their corresponding stomach evacuation coeffs. (Table 31). The derived data sets in Table 31 were analysed and gave the following solution to multiple regression analysis;

$$\text{Log}_e \text{ S.E.T. (h)} = 3.97 + 0.473\text{Log}_e \text{ meal size (g)} - 0.191\text{Log}_e \text{ fish weight (g)}$$

The multiple correlation coefficient of 0.87 was significant at the 0.001 level. The calculated 't' values were found to be 12.43 and 4.24 for meal size (g) and fish size (g), respectively, which were both significant at the 0.001 level. The exponent describing the change in Log S.E.T. with Log fish weight is negative, so that when meal size is expressed in grams, stomach evacuation time will decrease with increasing fish weight. Further analysis of the S.E.T. data was carried out to determine the exponent for stomach evacuation time in relation to meal size as relative rather than absolute weight. The multiple regression equation was found to be

$$\text{Log}_e \text{ S.E.T. (h)} = 1.79 + 0.476\text{Log}_e \text{ meal size (\%b.w.)} + 0.282\text{Log}_e \text{ fish weight (g)}$$

TABLE 31 Calculated stomach evacuation time (h) for different weights of O. niloticus fed different meal sizes (g)

Fish weight (g)	Meal size (g)	Stomach evacuation time (h)	Source
24.7	0.43	16.43	
22.4	0.312	13.99	
20.5	0.22	11.76	
16.2	0.16	10.03	Effect of 48h starvation period (fish refed 1% b.w) (Section 3.2.2.5)
17.2	0.2	11.2	
18.9	0.19	10.92	
15.1	0.15	9.71	
16.9	0.16	10.02	
15.6	0.14	9.38	
23.4	0.24	12.28	
36.5	0.57	22.9	
28.9	0.38	18.68	
30.1	0.295	16.5	Effect of Temperature (27°C) (Section 3.2.2.1)
25.6	0.72	25.7	
26.5	0.56	22.7	
28.6	0.48	20.99	
27.5	0.38	18.7	
30.2	0.48	20.99	
28.1	0.12	25.7	
25.5	0.1724	14.0	
25.7	0.161	13.5	
22.1	0.14	12.6	Effect of meal size (0.5% b.w.) (Section 3.2.2.3)
21.0	0.1	10.6	
22.3	0.11	11.2	
21.0	0.15	13.1	
21.2	0.147	12.95	
35.7	0.17	13.77	
21.0	0.05	7.55	
27.8	0.10	10.6	

Table 31 (cont'd)

Fish weight (g)	Meal size (g)	Stomach evacuation time (h)	Source
33.3	0.4724	19.09	
29.3	0.23	13.3	
32.7	0.217	12.9	
25.1	0.218	12.98	Effect of meal size (1% b.w.)
28.5	0.21	12.73	(Section 3.2.2.3)
34.1	0.456	18.76	
28.9	0.202	12.5	
25.3	0.284	14.8	
31.3	0.284	14.8	
31.5	0.251	13.92	
36.8	0.493	16.77	
37.6	0.499	15.4	
35.3	0.383	12.97	Effect of meal size
28.3	0.282	12.95	(1.5% b.w.)
24.6	0.297	13.29	(Section 3.2.2.3)
25.1	0.287	13.06	
31.7	0.61	19.05	
25.9	0.283	12.97	
28.9	0.43	15.99	
21.0	0.491	17.1	
46.0	0.197	12.37	
60.0	0.757	24.17	
52.0	0.526	20.19	Effect of fish weight (49.3g)
46.0	0.45	12.41	(Section 3.2.2.2)
49.0	0.33	15.91	
53.0	0.63	22.6	
54.0	0.622	21.97	
52.0	0.54	20.3	
49.0	0.41	17.83	
45.0	0.36	17.0	

Table 31 (cont'd)

Fish weight (g)	Meal size (g)	Stomach evacuation time (h)	Source
85.0	0.732	17.11	
110.0	1.34	23.15	
105.0	1.00	20.0	
97.0	1.04	20.4	Effect of fish weight (97.3g) (Section 3.2.2.2)
92.0	1.06	20.6	
99.0	0.89	18.87	
87.0	0.82	18.1	
110.0	1.04	20.39	
105.0	1.1	20.98	
95.0	1.0	20.0	
150.0	1.43	21.7	
149.0	1.34	21.05	
157.0	1.38	21.4	
136.0	1.21	20.0	Effect of fish weight (144.8g) (Section 3.2.2.2)
138.0	1.44	21.8	
136.0	1.37	21.28	
148.0	1.12	19.2	
147.0	1.52	22.42	
150.0	1.28	20.57	
147.0	1.31	22.89	
39.3	0.51	24.63	
32.7	0.35	20.4	
30.0	0.24	16.89	Effect of 48h starvation period (fish refed to satiation) (Section 3.2.2.5)
33.3	0.41	22.1	
32.4	0.29	18.5	
31.2	0.4	21.81	
38.1	0.49	24.13	
35.6	0.42	22.35	
36.0	0.35	20.4	
30.8	0.32	19.51	

Table 31 (cont'd)

Fish weight (g)	Meal size (g)	Stomach evacuation time (h)	Source
40.6	0.88	32.3	
36.9	0.4	21.81	Effect of 48h starvation period (fish refed to satiation) (Section 3.2.2.5)
30.0	0.32	19.5	
32.8	0.39	21.53	
30.3	0.33	19.81	
66.0	0.85	24.98	
70.0	1.07	28.0	
69.8	0.998	27.07	Sequential slaughter (Section 3.2.1.3)
59.2	0.869	25.26	
63.4	0.679	22.26	
70.0	0.809	22.33	
61.5	0.759	24.37	
70.0	1.098	23.6	
60.2	0.706	22.77	
55.0	0.864	22.8	
55.8	0.85	25.8	
65.2	0.73	24.98	
63.5	0.8	23.2	
66.2	0.82	24.5	

The multiple correlation coefficient of 0.86 was significant at the 0.001 level. The calculated 't' values were found to be 12.54 and 12.12 for meal size (% b.w.) and fish size (g), respectively, which are both significant at the 0.001 level. It is worth noting that for a given meal size (% b.w.) the S.E.T. is shorter the smaller the fish and that this trend is opposite to that obtained when meal sizes are expressed in absolute weight (g). These relationships were used to calculate S.E.T.s for fish of different weights fed to satiation (Table 32). Satiation meal sizes for these fish were obtained from 3.1.3. Daily food intake for these fish were calculated using the formula of Elliott (1972)

$$\text{Log}_{10}D = \text{Log}_{10}24 + \text{Log}_{10}S - \text{Log}_{10}T$$

Where D = daily food intake in g

24 = number of hours in a day

S = maximum amount ingested in a meal

T = time required to evacuate that meal.

Table 32 shows calculated daily food intakes based on stomach evacuation time. However these calculated daily food intakes do not take into account the loss of food during the feeding period. It must be borne in mind that this data is derived from actual measurement of stomach contents and is therefore for ingested food. No account is taken of losses occurring during feeding such as leaching, uneaten food, etc. In order to achieve these rates of food ingestion actual feeding rates would need to be slightly higher.

TABLE 32 The calculated daily food intake for different sizes of O. niloticus calculated from stomach evacuation time at  $27.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Fish weight (g)	Satiation meal (g)	Stomach evacuation time (h)	Daily food intake	
			(g)	% b.w.
5	0.108	12.94	0.20	4.0
10	0.196	14.39	0.33	3.3
20	0.35	16.60	0.51	2.6
30	0.502	18.20	0.66	2.2
40	0.64	19.38	0.79	1.97
50	0.78	20.40	0.92	1.84
60	0.91	21.20	1.03	1.72
70	1.04	21.92	1.14	1.63
80	1.16	22.56	1.24	1.55
90	1.29	23.17	1.34	1.49
100	1.41	23.68	1.43	1.43
110	1.53	24.17	1.52	1.38
120	1.65	24.63	1.61	1.34
130	1.76	25.06	1.69	1.30
140	1.87	25.42	1.76	1.26
150	1.99	25.85	1.85	1.23
160	2.11	26.24	1.93	1.21
170	2.23	26.60	2.00	1.18
180	2.33	26.90	2.10	1.15
190	2.44	27.20	2.15	1.13
200	2.55	27.50	2.23	1.11

### 3.3 Digestive Enzymes

#### 3.3.1 Effect of food composition on the digestive enzyme activities

The effects of food composition on digestive enzyme activity are presented in Table 33. It can be seen that the pepsin-like enzyme activity in the stomach (Table 33) showed significant variation with food composition. The high protein diet (Diet D) produced significantly ( $P < 0.05$ ) the highest enzyme activity followed by the high lipid diet (Diet C); the high available carbohydrate diet (Diet B) and the control diet (Diet A) were the lowest and were insignificantly different ( $P < 0.05$ ).

Trypsin-like activity in the intestine (Table 33) also showed significant ( $0.05$ ) variation with diet (Table 33). The high protein diet (Diet D) again ranked highest followed this time by the high available carbohydrate diet (Diet B) and, finally, the high lipid diet and the control diet (D, A) which were insignificantly different.

$\alpha$ -amylase activity (Table 33) was significantly ( $P < 0.05$ ) higher in intestines of fish fed the high available carbohydrate diet (Diet B) whilst there was no significant variation between the other experimental diets.

Lipase activity did not show any significant variation ( $P < 0.05$ ) in the intestine with the experimental diets (Table 33).

TABLE 33 The effect of food type on digestive enzyme activities in *O. niloticus* fed four experimental diets at 27.5°C

Enzyme	Control diet (A)	Carbohydrate rich diet (B)	Lipid rich diet (C)	Protein rich diet (D)	S.E.
Pepsin-like enzyme in stomach (pH 2)	3.533 <sup>1a</sup>	3.45 <sup>a</sup>	3.79 <sup>b</sup>	4.95 <sup>c</sup>	0.044
Trypsin-like enzyme in the intestine (pH 8.2)	5.597 <sup>1a</sup>	5.99 <sup>b</sup>	5.41 <sup>a</sup>	6.46 <sup>c</sup>	0.072
$\alpha$ -amylase in intestine (pH 6.2)	27.37 <sup>2a</sup>	46.76 <sup>b</sup>	27.78 <sup>a</sup>	27.11 <sup>a</sup>	0.270
Lipase in intestine (pH 7.2)	1.255 <sup>3a</sup>	1.263 <sup>a</sup>	1.284 <sup>a</sup>	1.23 <sup>a</sup>	0.0522

<sup>1</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu$ mol of tyrosine/min/g of tissue at 30°C

<sup>2</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu$ mol of maltose/min/g of tissue at 30°C

<sup>3</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu$ mol of oleic acid/min/g of tissue at 30°C

±S.E. is standard error of the mean

Mean values with the same superscript are not significantly different ( $P < 0.05$ )

### 3.3.2 The effect of fish weight on digestive enzyme activity

Both pepsin-like activity in the stomach and trypsin-like enzyme activity in the intestine decreased significantly ( $P < 0.05$ ) with increasing fish weight (Table 34).  $\alpha$ -amylase activity in the intestine was found to increase significantly ( $P < 0.05$ ) with increasing fish weight (Table 34) and lipase activity was significantly higher in the smaller fish group (Table 34).

### 3.3.3 The effect of starvation on digestive enzyme activity

During the first 48h of starvation pepsin-like enzyme activity in the stomach decreased and then remained constant and was not elevated on refeeding (Table 35). Trypsin-like enzyme activity in the intestine decreased during the first 72h of starvation and then remained constant up to 96h (Table 35) and was elevated on refeeding, but not to the 'normal feeding' level (Table 35).  $\alpha$ -amylase activity in the intestine decreased progressively over the 96h of starvation and was slightly elevated on refeeding (Table 35). Lipase activity remained constant for the first 72h of starvation and then fell sharply at 96h and was slightly raised after refeeding (Table 35).

TABLE 34 The effect of fish weight on digestive enzyme activities in O. niloticus at 27.5°C ( $\pm 1^\circ\text{C}$ )

Enzyme	Fish size(1)		Fish size(2)		Fish size(3)		$\pm$ S.E.
	37.79	1.5	80.13	3.9	175.45	3.6	
Pepsin-like enzyme in stomach (pH 2)	6.50 <sup>1a</sup>		5.76 <sup>b</sup>		4.568 <sup>c</sup>		0.12
Trypsin-like enzyme in the intestine (pH 8.2)	7.884 <sup>1a</sup>		6.92 <sup>b</sup>		6.24 <sup>c</sup>		0.103
$\alpha$ -amylase in the intestine (pH 6.2)	21.18 <sup>2a</sup>		26.6 <sup>b</sup>		28.09 <sup>c</sup>		0.274
Lipase in the intestine (pH 7.2)	1.42 <sup>3a</sup>		1.244 <sup>b</sup>		1.22 <sup>b</sup>		0.051

<sup>1</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of tyrosine/min/g of tissue at 30°C

<sup>2</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of maltose/min/g of tissue at 30°C

<sup>3</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of oleic acid/min/g of tissue at 30°C

$\pm$  S.E. is standard error of the mean

Mean values with the same superscript are not significantly different ( $P < 0.05$ )

TABLE 35 The effect of starvation on digestive enzyme activities in *O. niloticus* at 27.5°C ( $\pm 1^\circ\text{C}$ )

Enzyme	Normal feeding	Starvation period (h)			Refed	$\pm$ S.E.	
		24	48	72			96
Pepsin-like enzyme in the stomach (pH 2)	6.39 <sup>1a</sup>	6.166 <sup>ba</sup>	5.84 <sup>cb</sup>	5.525 <sup>c</sup>	5.46 <sup>c</sup>	5.65 <sup>c</sup>	0.114
Trypsin-like enzyme in the intestine (pH 8.2)	7.94 <sup>1a</sup>	7.25 <sup>b</sup>	5.283 <sup>c</sup>	3.876 <sup>d</sup>	3.784 <sup>d</sup>	5.06 <sup>c</sup>	0.153
$\alpha$ -amylase in the intestine (pH 6.2)	25.79 <sup>2a</sup>	23.713 <sup>b</sup>	17.82 <sup>c</sup>	16.617 <sup>c</sup>	11.803 <sup>d</sup>	16.04 <sup>c</sup>	0.589
Lipase in the intestine (pH 7.2)	1.3 <sup>3a</sup>	1.48 <sup>a</sup>	1.533 <sup>a</sup>	1.45 <sup>a</sup>	0.800 <sup>b</sup>	1.13 <sup>c</sup>	0.065

<sup>1</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of tyrosine/min/g of tissue at 30°C

<sup>2</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of maltose/min/g of tissue at 30°C

<sup>3</sup> One unit of activity is defined as the amount of enzyme required to liberate one  $\mu\text{mol}$  of oleic acid/min/g of tissue at 30°C

$\pm$  S.E. is standard error of the mean

Mean values with the same superscript are not significantly different ( $P < 0.05$ )

### 3.4 Growth Studies

#### 3.4.1 The effect of feeding frequency on the food intake, growth, and body composition of *Q. niloticus* fed to satiation

##### Food Intake

Mean food intake over the eight weeks of the experimental period increased significantly ( $P < 0.05$ ) with increasing feeding frequency up to a maximum when fish were fed six times per day. Thereafter there was no significant difference in food intake between fish fed six times or eight times daily (Table 36). Thus an individual fish fed eight times per day consumed 57.72g of food during the eight week trial whilst fish fed once per day had a mean individual food intake of only 30.96g. Although fish were fed to satiation on each feeding occasion the food intake in a single meal was much smaller under the high frequency feeding regimes than the lower ones. Fish fed once a day consumed 0.65g of dry food in a single meal but those fed eight times per day consumed only 0.15g of food in a single meal (Table 36). The amount of food consumed by fish at the various feeding frequencies was calculated as percent body weight (% b.w.) of food consumed per fish per day for each weekly interval of the experimental period, and fish weight was calculated as the mean weight for each of these intervals (Table 37). In each case the food intake as % b.w. decreased as the mean weight of fish increased. Food consumption was found to be clearly significantly different ( $P < 0.05$ ) between groups fed at different frequencies at the start of the experiment. Over

TABLE 36 Food consumption of *O. niloticus* (16.1g) fed to satiation at different feeding frequency at  $27.5 \pm 1^\circ\text{C}$

Food consumption (g)	Feeding frequencies/day				
	1	2	4	6	8
Total food intake by an individual fish over a period of 8 weeks	30.96 <sup>a</sup>	43.85 <sup>b</sup>	49.61 <sup>c</sup>	55.38 <sup>d</sup>	57.72 <sup>d</sup>
Total food intake by an individual fish per week	3.87 <sup>a</sup>	5.48 <sup>b</sup>	6.20 <sup>c</sup>	6.92 <sup>d</sup>	7.22 <sup>d</sup>
Mean daily food intake for an individual fish	0.645 <sup>a</sup>	0.913 <sup>b</sup>	1.03 <sup>c</sup>	1.15 <sup>d</sup>	1.203 <sup>d</sup>
Mean weight of food consumed by an individual fish in a single meal	0.645	0.46	0.26	0.19	0.15

Figures in the same row with the same superscript are not significantly different ( $P < 0.05$ )

TABLE 37 Food consumption and fish weight at different feeding frequencies during the experimental period of 8 weeks

Feeding frequencies	1		2		3		4		5		6		7		8	
	FI <sup>±</sup>	FS	FI	FS	FI	FS	FI	FS	FI	FS	FI	FS	FI	FS	FI	FS
1	4.03 <sup>a</sup> ± 1.12	18.07 ± 1.06	2.75 <sup>a</sup> ± 0.06	20.56 ± 1.51	2.67 <sup>a</sup> ± 0.02	23.17 ± 1.78	2.43 <sup>a</sup> ± 1.14	26.11 ± 1.09	2.4 <sup>a</sup> ± 0.05	29.14 ± 1.1	2.36 <sup>a</sup> ± 0.08	30.66 ± 1.2	2.19 <sup>a</sup> ± 1.1	33.9 ± 1.3	1.98 <sup>a</sup> ± 0.01	37.26 ± 1.2
2	5.13 <sup>b</sup> ± 1.05	20.47 ± 1.61	3.24 <sup>b</sup> ± 1.19	24.58 ± 1.98	2.94 <sup>ba</sup> ± 0.31	28.21 ± 1.39	2.79 <sup>ba</sup> ± 1.11	31.32 ± 1.9	2.61 <sup>ba</sup> ± 0.05	34.42 ± 2.4	2.45 <sup>ba</sup> ± 0.05	36.64 ± 2.4	2.6 <sup>ba</sup> ± 1.2	40.35 ± 3.0	2.35 <sup>a</sup> ± 1.15	44.72 ± 2.16
4	5.55 <sup>c</sup> ± 1.31	20.8 ± 1.53	3.92 <sup>c</sup> ± 1.02	24.75 ± 1.49	3.26 <sup>cb</sup> ± 1.04	28.96 ± 1.23	2.89 <sup>b</sup> ± 1.12	32.1 ± 1.43	2.9 <sup>cb</sup> ± 1.2	35.54 ± 1.86	2.75 <sup>cba</sup> ± 0.05	37.86 ± 1.16	2.7 <sup>b</sup> ± 1.13	41.24 ± 1.27	2.29 <sup>a</sup> ± 1.17	46.08 ± 2.6
6	6.09 <sup>d</sup> ± 1.09	21.2 ± 1.5	4.3 <sup>c</sup> ± 1.10	25.71 ± 1.63	3.64 <sup>c</sup> ± 1.09	29.34 ± 1.74	3.16 <sup>b</sup> ± 1.11	33.72 ± 1.85	2.93 <sup>c</sup> ± 1.12	37.43 ± 1.99	2.86 <sup>cb</sup> ± 0.04	40.4 ± 1.75	2.8 <sup>b</sup> ± 1.04	43.76 ± 1.78	2.45 <sup>a</sup> ± 1.06	51.6 ± 1.3
8	6.5 <sup>e</sup> ± 1.3	21.91 ± 1.98	4.15 <sup>c</sup> ± 1.35	26.53 ± 1.53	3.45 <sup>c</sup> ± 1.22	30.16 ± 1.99	3.11 <sup>b</sup> ± 1.04	34.82 ± 1.85	3.17 <sup>c</sup> ± 0.06	37.68 ± 1.34	3.08 <sup>c</sup> ± 0.05	39.37 ± 1.32	2.99 <sup>b</sup> ± 1.07	42.32 ± 1.03	2.48 <sup>a</sup> ± 0.05	50.66 ± 1.04

± S.E. = Standard error

FI = Food intake as % b.w. per day

FS = Fish size in grams

Mean values in each column with the same superscripts are not significantly different (P < 0.05)

the next six weeks differences in food consumption decreased and by the eighth week no significant differences were observed (Table 37).

To establish the mathematical relationship between food intake (as % b.w.) and fish weight (in g) at each feeding frequency, the calculated food intake (% b.w.) at each interval was analysed for the effect of fish weight. At each feeding frequency a negative relationship was found to exist between food intake and fish weight (Table 38), indicating a decrease in maximum daily food intake with increasing fish weight.

#### Growth, Food Utilization and Carcass Composition

All the experimental fish grew well on the commercial trout diet and mortality throughout the experimental period was negligible. 100% survival was achieved for fish fed 4, 6 and 8 times daily while 90% and 95% survival was achieved for fish fed once and twice daily, respectively. The growth responses are presented in Tables 39 and 40, and illustrated graphically in Fig. 55. Mean initial weight (Table 39) did not vary significantly between treatments ( $P < 0.05$ ). However, by the end of the first week groups fed between two and eight times per day were significantly ( $P < 0.05$ ) heavier than those fed once per day. This difference was maintained until the end of the eighth week, when the final weight of fish fed once per day was only 37.3g compared with 44.7g-50.66g for fish fed between two and eight times daily. These were not significantly different ( $P < 0.05$ ) (Table 39). On a general basis,

TABLE 38 Regression equations expressing the relationships between fish weight in grams and food intake as percentage body weight at different feeding frequencies

Feeding frequency	Regression equations	Correlation coefficient	n	P<
1	$\text{Log}_e M = 3.49 - 0.078 \text{Log}_e W$	-0.92	8	0.001
2	$\text{Log}_e M = 4.06 - 0.87 \text{Log}_e W$	-0.90	8	0.001
4	$\text{Log}_e M = 4.59 - 0.99 \text{Log}_e W$	-0.96	8	0.001
6	$\text{Log}_e M = 4.64 - 0.97 \text{Log}_e W$	-0.97	8	0.001
8	$\text{Log}_e M = 4.83 - 1.02 \text{Log}_e W$	-0.94	8	0.001

M = food intake as % body weight

W = fish weight in grams

TABLE 39 The mean body weight of *O. niloticus* in grams at successive weekly intervals fed to satiation at different feeding frequencies

Week	Feeding frequencies					±S.E.
	1	2	4	6	8	
0	15.77 <sup>a</sup> ± 0.13	16.00 <sup>a</sup> ± 0.10	16.17 <sup>a</sup> ± 0.26	15.99 <sup>a</sup> ± 0.28	16.40 <sup>a</sup> ± 0.10	0.16
1	18.07 <sup>a</sup> ± 0.06	20.47 <sup>b</sup> ± 0.61	20.80 <sup>b</sup> ± 0.53	21.20 <sup>b</sup> ± 0.50	21.91 <sup>b</sup> ± 0.98	0.505
2	20.56 <sup>a</sup> ± 0.51	24.58 <sup>b</sup> ± 0.98	24.75 <sup>b</sup> ± 0.49	25.71 <sup>b</sup> ± 0.63	26.53 <sup>b</sup> ± 0.53	0.65
3	23.17 <sup>a</sup> ± 0.78	28.21 <sup>b</sup> ± 0.39	28.96 <sup>b</sup> ± 1.23	29.34 <sup>b</sup> ± 0.74	30.16 <sup>b</sup> ± 0.99	0.872
4	26.11 <sup>a</sup> ± 1.09	31.32 <sup>b</sup> ± 1.90	32.10 <sup>b</sup> ± 0.43	33.92 <sup>b</sup> ± 0.85	34.82 <sup>b</sup> ± 1.85	1.37
5	29.14 <sup>a</sup> ± 1.10	34.42 <sup>b</sup> ± 2.40	35.54 <sup>b</sup> ± 0.86	37.43 <sup>b</sup> ± 0.99	37.68 <sup>b</sup> ± 0.34	1.36
6	30.66 <sup>a</sup> ± 1.20	36.64 <sup>b</sup> ± 2.40	37.86 <sup>b</sup> ± 1.16	40.40 <sup>b</sup> ± 0.75	39.37 <sup>b</sup> ± 0.32	1.39
7	33.99 <sup>a</sup> ± 1.30	40.35 <sup>b</sup> ± 3.00	41.24 <sup>b</sup> ± 1.27	43.76 <sup>b</sup> ± 0.78	42.32 <sup>b</sup> ± 0.03	1.62
8	37.26 <sup>a</sup> ± 1.20	44.72 <sup>b</sup> ± 2.16	46.08 <sup>b</sup> ± 2.60	51.60 <sup>b</sup> ± 1.30	50.66 <sup>b</sup> ± 1.04	1.78

S.E. Standard error of the mean calculated from the residual mean square in the analysis of variance

Figures in the same row with the same superscripts are not significantly different (P < 0.05)

- - Fed once daily
- ◻ - Fed twice daily
- ◼ - Fed four times daily
- ▲ - Fed six times daily
- △ - Fed eight times daily

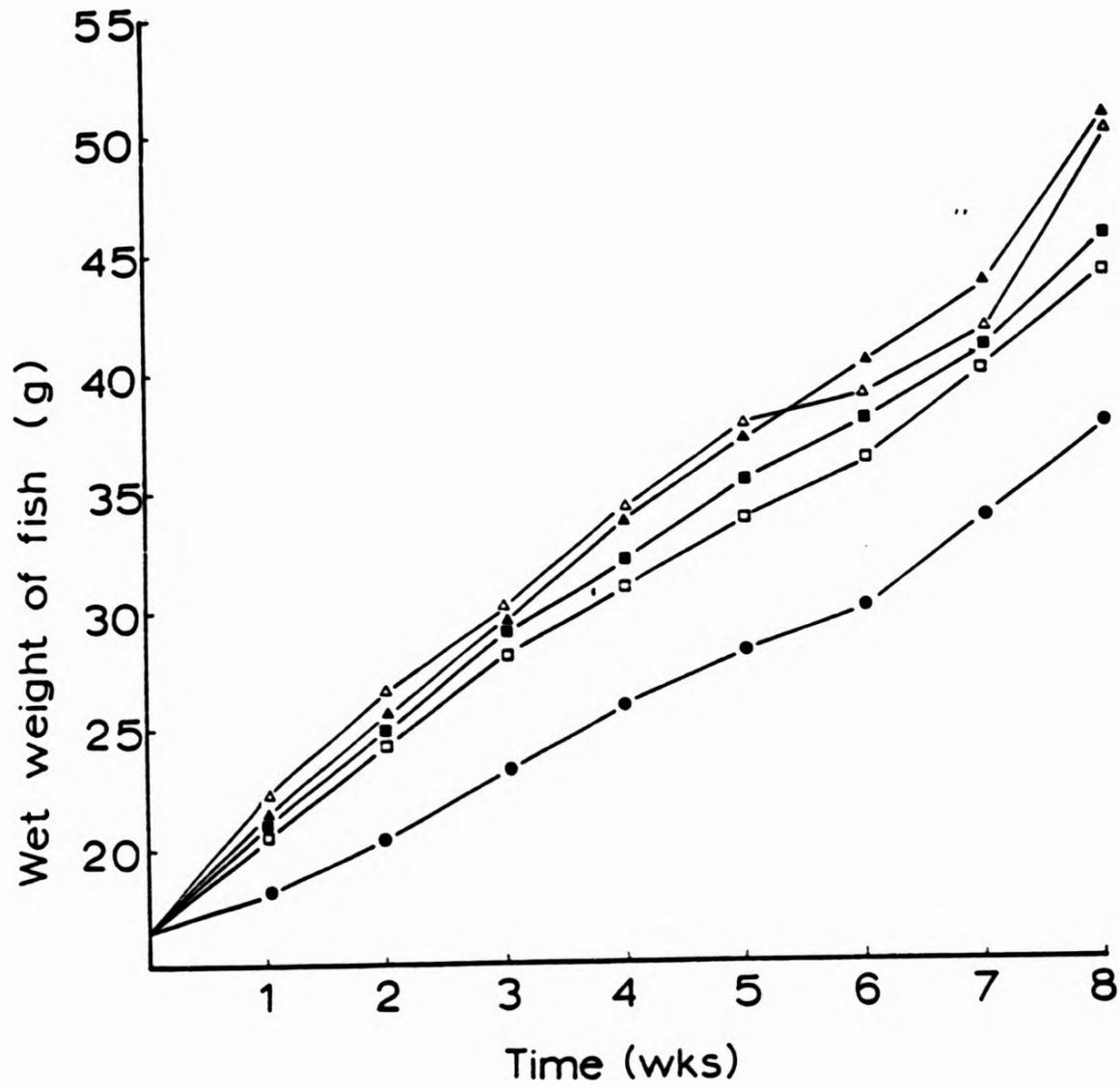


FIGURE 55 Growth of *O. niloticus* fed to satiation between 1 and 8 times daily

final percentage body weight, daily weight gain and specific growth rate all increased with increasing feeding frequency up to six feeds per day and thereafter decreased slightly (Table 40a). Percentage weight gain and daily weight gain increased from 136.3% and 383.8 mg/day for fish fed six times a day and decreased slightly to 208.8% and 611.8 mg/day for fish fed eight times per day. Similarly, specific growth rate (S.G.R.) increased from 1.54 to a maximum of 2.09 for fish fed six times a day and thereafter declined to 2.01 for fish fed eight times per day (Table 40a). The best food conversion ratio (F.C.R.) was achieved by the group fed once per day followed by groups fed 2, 6, 4 and 8 times per day. The levels of protein utilization were calculated in terms of protein efficiency ratio (P.E.R.) and apparent net protein utilization (A.N.P.U.%). The P.E.R. and A.N.P.U. values for the different treatment groups are presented in Table 40a. The protein efficiency ratios obtained for the various feeding regimes were similar to the equivalent food conversion ratios. The P.E.R. values ranged between 1.21 and 1.42, the highest value achieved by the group fed once per day followed by groups fed 2, 6, 4 and 8 times per day.

The highest A.N.P.U. value (29.27%) was observed for the group fed once per day, followed by the group fed 6 times per day (25.89%) with the lowest value (22.65%) for the group fed four times per day.

Protein and dry matter digestion coefficients were found to decrease slightly with increasing feeding frequency. The highest protein

TABLE 40a Growth, feed utilization and carcass composition of *O. niloticus* fed to satiation at different feeding frequencies

Parameter	Feeding frequencies					±S.E.
	1	2	4	6	8	
Mean initial weight (g)	15.77 <sup>a</sup>	16.00 <sup>a</sup>	16.17 <sup>a</sup>	15.99 <sup>a</sup>	16.40 <sup>a</sup>	0.16
Mean final weight (g)	37.26 <sup>a</sup>	44.72 <sup>b</sup>	46.08 <sup>b</sup>	51.58 <sup>b</sup>	50.66 <sup>b</sup>	1.78
Weight gain (%)	136.29	179.50	184.90	222.57	208.90	
Specific growth rate	1.54 <sup>a</sup>	1.84 <sup>b</sup>	1.87 <sup>b</sup>	2.09 <sup>b</sup>	2.01 <sup>b</sup>	0.068
Food intake mg/day/fish	645.00 <sup>a</sup>	913.50 <sup>b</sup>	1033.10 <sup>c</sup>	1153.80 <sup>d</sup>	1202.50 <sup>d</sup>	
Weight gain mg/day/fish	383.75	512.85	534.10	635.50	611.80	
Food conversion ratio	1.68	1.78	1.93	1.82	1.96	
Protein efficiency ratio	1.42	1.34	1.23	1.31	1.21	
Apparent net protein utilization	29.27	24.20	22.65	25.89	22.79	
Dry matter digestion	70.87	70.39	67.33	67.45	65.40	
Protein digestion coefficient	86.35	80.60	76.79	79.50	74.25	

TABLE 40b Carcass composition (% wet weight)

Treatments	Initial	Final				
		1	2	4	6	8
Moisture	75.00	73.49 <sup>a</sup>	73.11 <sup>a</sup>	72.63 <sup>ab</sup>	71.57 <sup>b</sup>	72.46 <sup>b</sup>
Gross protein	16.10	18.73 <sup>a</sup>	17.39 <sup>b</sup>	17.59 <sup>b</sup>	18.61 <sup>a</sup>	17.94 <sup>b</sup>
Gross lipid	3.70	3.33 <sup>a</sup>	3.44 <sup>a</sup>	3.91 <sup>b</sup>	4.26 <sup>c</sup>	3.67 <sup>d</sup>
Ash	4.79	5.05 <sup>a</sup>	5.00 <sup>a</sup>	4.91 <sup>a</sup>	4.22 <sup>b</sup>	4.62 <sup>c</sup>

Mean values for components with the same superscripts are not significantly different at 0.05 level of significance

and dry matter digestion coefficients were observed for the group fed once per day followed by the group fed four times per day.

Proximate carcass composition for the initial and final samples of fish are presented in Table 40b. Carcass moisture contents for final samples decreased significantly ( $P < 0.05$ ) with increasing feeding frequency up to six times per day then increased slightly for eight feeds per day. Carcass lipid contents (Table 40b) increased significantly ( $P < 0.05$ ) with increasing feeding frequency up to a maximum of 4.26% for fish fed six times per day and decreased significantly ( $P < 0.05$ ) to 3.67% for fish fed eight times per day. Highest carcass crude protein contents (Table 40b) were achieved by the groups fed one and six times per day (18.73 and 18.61) and were significantly ( $P < 0.05$ ) different from the other groups. Carcass ash contents decreased significantly with increasing feeding frequency up to six times per day (4.22%) and thereafter increased significantly ( $P < 0.05$ ) to 4.62% for fish fed eight times per day (Table 40b).

In order to obtain a measure of the effect of feeding frequency on the population size distribution the coefficient of variance ( $\frac{\text{Variance}}{\text{Mean}^2}$ ) of the fish population at the start and end of the experiment was calculated. At the start of the experiment fish were selected from a population of mean weight 16.1g and the coefficient of variance varied between 0.02 and 0.025 so that the experimental groups were of more or less uniform size. By the end of the experiment the c.v. for different rearing groups under different feeding regimes was found to increase with increasing feeding frequency and vary between 0.104 and 0.183 (Table 41).

TABLE 41 Changes in coefficients of variance (c.v.) for different groups of fish fed to satiation at different feeding frequencies

Feeding frequency	Initial fish weight	c.v.	Final weight	c.v.	Survival rate (%)
1	15.77	0.025	37.26	0.112	90.0
2	16.00	0.026	44.72	0.167	95.0
4	16.17	0.023	46.08	0.118	100
6	15.99	0.023	51.58	0.183	100
8	16.40	0.020	50.66	0.104	100

3.4.2 The effect of feeding frequency on growth and body composition of fish fed a restricted ration

The growth responses of groups of fish fed 6.0% b.w. daily in two, four or six feeds per day are presented in Tables 42 and 43a. The mean initial weight (Table 42) was not significantly ( $P < 0.05$ ) different between treatments and similarly the average final weights at the end of the five week trial were not significantly ( $P < 0.05$ ) different. Similar results were obtained for percentage weight gain, food conversion ratio and protein utilization (Table 43a).

Proximate carcass analysis of the initial and final fish samples are presented in Table 43b. The carcass moisture contents decreased significantly ( $P < 0.05$ ) with increasing feeding frequency. The pattern displayed by carcass lipid content was the inverse of that found for carcass moisture. Carcass lipid contents increased significantly ( $P < 0.05$ ) with increasing feeding frequency up to a maximum of 5.25% for the group fed six times per day. There were no significant differences ( $P < 0.05$ ) in the carcass protein and carcass ash contents between the treatments (Table 43b).

The coefficient of variance at the start and the end of the five week growth trial is presented in Table 44. It can be observed that the c.v. increased markedly with increasing feeding frequency; c.v. increased from 0.027 and 0.03 to 0.19 and 0.226 for fish fed once and six times per day, respectively.

TABLE 42 Growth of O. niloticus at successive weekly intervals fed a daily ration of 6% b.w., 2, 4 and 6 times per day

Week	Feeding frequencies		
	2	4	6
0	1.06g ± 0.10	1.13g ± 0.08	1.14g ± 0.06
1	1.46 ± 0.11	1.37 ± 0.05	1.38 ± 0.04
2	1.78 ± 0.11	1.71 ± 0.03	1.80 ± 0.06
3	2.36 ± 0.13	2.34 ± 0.08	2.38 ± 0.03
4	3.24 ± 0.20	3.32 ± 0.15	3.43 ± 0.04
5	4.63 ± 0.13	4.60 ± 0.27	4.75 ± 0.08

Standard error of mean

TABLE 43a Growth, feed utilization and carcass composition of *O. niloticus* fed 6% body weight at three feeding frequencies

Parameter	FEEDING FREQUENCIES DAILY			±S.E.
	2	4	6	
Mean initial weight (g)	1.06 <sup>a</sup>	1.13 <sup>a</sup>	1.14 <sup>a</sup>	0.04
Mean final weight (g)	4.63 <sup>a</sup>	4.59 <sup>a</sup>	4.75 <sup>a</sup>	0.18
Weight gain (%)	336.79	306.2	316.7	
Specific growth rate	4.202	4.03	4.08	
Food intake (mg/day/fish)	119.7	121.11	122.25	
Weight gain (mg/day/fish)	102.0	98.9	103.1	
Food conversion ratio	1.17	1.196	1.18	
Protein efficiency ratio	2.04	1.99	2.02	
Apparent net protein utilization	38.48	36.41	36.6	

TABLE 43b Carcass composition (% wet body weight)

	Initial		Final		±S.E.
Moisture	79.1	74.65 <sup>a</sup>	74.3 <sup>ab</sup>	73.58 <sup>b</sup>	.22
Crude protein	11.7	17.22 <sup>a</sup>	17.00 <sup>a</sup>	16.65 <sup>a</sup>	0.301
Crude lipid	4.2	3.78 <sup>a</sup>	4.48 <sup>b</sup>	5.253 <sup>c</sup>	0.135
Ash	5.08	3.28 <sup>a</sup>	3.11 <sup>a</sup>	3.28 <sup>a</sup>	0.09

Mean values with the same superscripts are not significantly different (P < 0.05)

TABLE 44 Change in coefficient of variance (c.v.) for different groups of fish fed a daily ration 6% b.w., 2, 4 and 6 times per day

Feeding frequencies	Initial mean weight	c.v.	Final mean weight	c.v.
2	1.06g	0.027	4.63g	0.19
4	1.13g	0.03	4.6g	0.199
6	1.14g	0.031	4.75g	0.226

3.4.3 The effect of meal size and fish weight on growth and body composition

The growth responses of two groups of fish fed different ration sizes (as % b.w.) are presented in Tables 45a and 46b and shown graphically in Figures 56 and 57. The initial average weights of fish in both weight classes were not significantly ( $P < 0.05$ ) different between treatments (Tables 45a, 46a). Mean final weights were found to increase significantly ( $P < 0.05$ ) with increasing meal size (as % b.w.)

The mean final weight for the large fish (14.27g) increased significantly ( $P < 0.05$ ) with ration size up to 3% b.w., thereafter it decreased significantly ( $P < 0.05$ ) with ration at 4% b.w. The group on 0% b.w. lost weight markedly. Percentage weight gain, daily weight gain and specific growth rate all increased generally with increasing meal size up to 3% b.w., thereafter decreasing with further increase in meal size (4% b.w.).

Although the ration sizes were not similar for the smaller weight group of fish investigated, the mean final weights for the small weight fish (6.8g) increased significantly ( $P < 0.05$ ) with increasing meal size up to 4% b.w. and remained relatively unchanged with further increase in meal size up to 6% b.w. (Table 46a). Percentage weight gain, daily weight gain and specific growth rate all increased with ration size.

To determine the maintenance requirement, optimum and maximum ration for both groups of fish, graphs of specific growth rate

TABLE 45a. Food utilization and carcass composition of *O. niloticus* fed different ration levels  
(Mean weight 14.27g)

Parameter	0%	1%	1.5%	2%	2.5%	3%	4%	± S.E.
Mean initial weight (g)	14.09 <sup>a</sup>	13.9 <sup>a</sup>	14.15 <sup>a</sup>	14.00 <sup>a</sup>	13.99 <sup>a</sup>	14.71 <sup>a</sup>	14.8 <sup>a</sup>	0.24
Mean final weight (g)	11.60	21.27 <sup>a</sup>	26.15 <sup>ba</sup>	32.6 <sup>cb</sup>	37.3 <sup>c</sup>	51.65 <sup>d</sup>	41.8 <sup>c</sup>	2.4
Weight gain (%)	-17.67	53.02	84.81	132.86	166.6	251.12	182.4	
Specific growth rate	- 0.396	0.87	1.25	1.73	2.00	2.56	2.12	
Food intake (mg/day/fish)	-	170.25	279.3	413.0	569.65	880.00	986.5	
Weight gain (mg/day/fish)	-50.8	150.4	244.9	379.6	475.7	753.87	551.02	
Food conversion ratio	-	1.13	1.14	1.1	1.19	1.17	1.77	
Protein efficiency ratio	-	2.11	2.09	2.19	1.98	2.04	1.32	
Apparent net protein utilization	-	28.05	33.58	35.77	34.65	36.84	21.9	

TABLE 45b Carcass composition (% wet weight)

	Initial	Final				± S.E.	
Moisture	72.1	79.55 <sup>a</sup>	75.31 <sup>b</sup>	73.47 <sup>c</sup>	72.16 <sup>d</sup>	72.77 <sup>c</sup>	0.31
Crude protein	16.8	12.71 <sup>a</sup>	15.6 <sup>b</sup>	16.47 <sup>c</sup>	17.19 <sup>d</sup>	16.60 <sup>c</sup>	0.15
Crude lipid	5.1	1.23 <sup>a</sup>	3.34 <sup>b</sup>	4.08 <sup>c</sup>	4.96 <sup>d</sup>	5.11 <sup>d</sup>	0.14
Ash	4.16	6.19 <sup>a</sup>	5.17 <sup>b</sup>	4.68 <sup>c</sup>	4.52 <sup>c</sup>	4.17 <sup>d</sup>	0.115

Mean values with the same superscripts are not significantly different at 0.05 level.

TABLE 46a Growth, feed utilization and carcass composition of *O. niloticus* fed different ration levels (Mean weight 6.86g ±.12)

Parameter	0%	1%	2%	4%	6%	±S.E.
Mean initial weight (g)	6.86 <sup>a</sup>	7.06 <sup>a</sup>	6.79 <sup>a</sup>	6.74 <sup>a</sup>	6.91 <sup>a</sup>	0.13
Mean final weight (g)	5.40	8.91 <sup>a</sup>	17.40 <sup>b</sup>	22.02 <sup>c</sup>	24.03 <sup>c</sup>	0.63
Weight gain (%)	-21.28	26.20	156.25	226.71	247.76	
Specific growth rate	- 0.49	0.472	1.92	2.46	2.54	
Food intake mg/day/fish	-	78.00	209.35	476.05	796.75	
Weight gain mg/day/fish	-29.79	37.65	216.68	315.00	349.37	
Food conversion ratio	-	2.075	0.966	1.514	2.285	
Protein efficiency ratio	-	1.15	2.46	1.57	1.044	
Apparent net protein utilization	-	11.77	38.30	25.90	18.65	

TABLE 46b Carcass composition (% wet weight)

	Initial	Final					±S.E.
Moisture	71.95	80.32 <sup>a</sup>	76.14 <sup>b</sup>	74.21 <sup>c</sup>	72.96 <sup>cd</sup>	72.20 <sup>d</sup>	0.46
Crude protein	15.80	11.44 <sup>a</sup>	14.64 <sup>b</sup>	15.64 <sup>c</sup>	16.34 <sup>d</sup>	17.27 <sup>e</sup>	0.185
Crude lipid	4.22	0.739 <sup>a</sup>	2.89 <sup>b</sup>	3.29 <sup>c</sup>	4.37 <sup>d</sup>	5.08 <sup>e</sup>	0.097
Ash	4.48	6.077 <sup>a</sup>	5.38 <sup>b</sup>	4.93 <sup>c</sup>	4.39 <sup>d</sup>	4.39 <sup>d</sup>	0.06

Mean value with the same superscripts are not significantly different at 0.05 level of significance

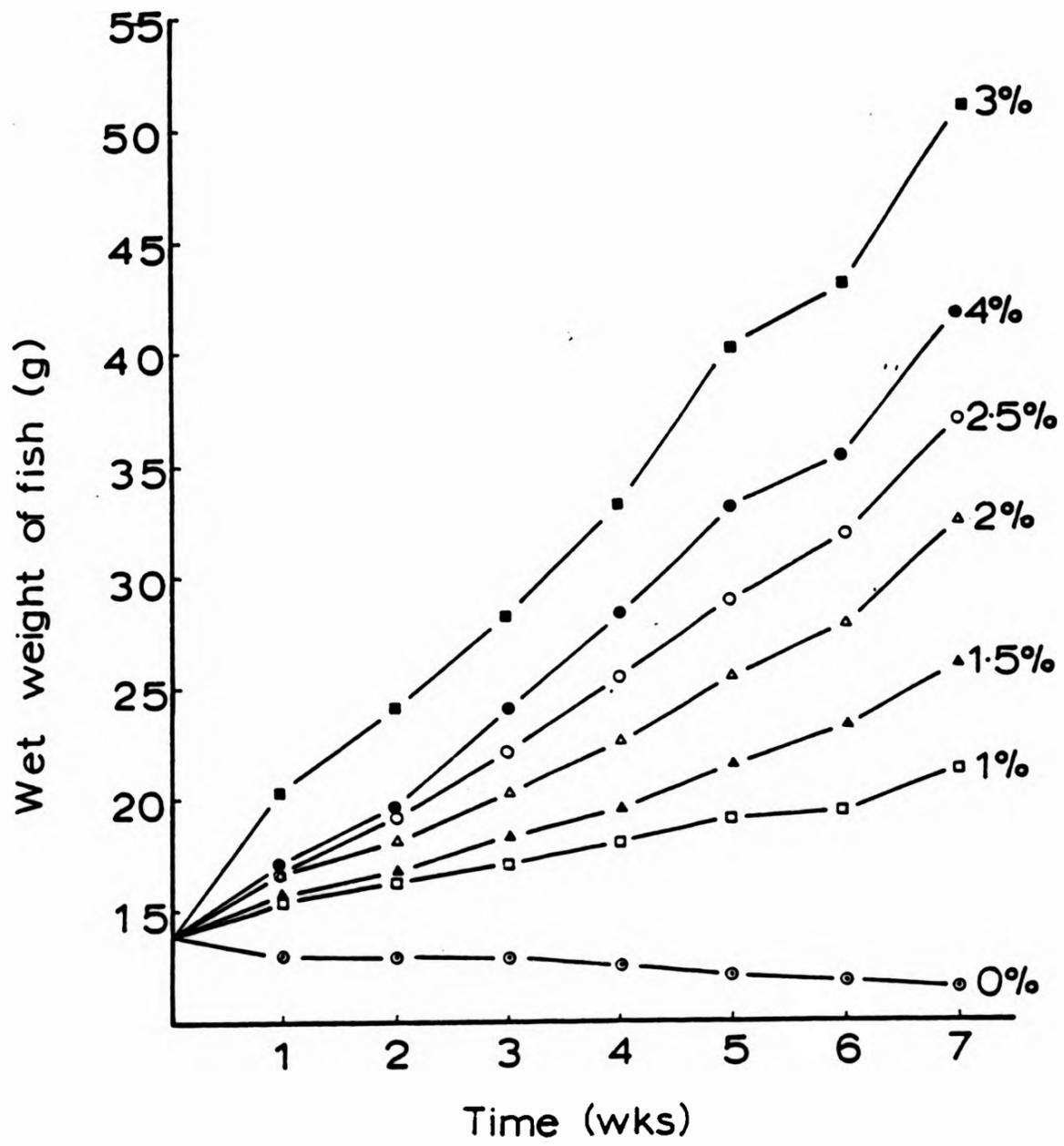
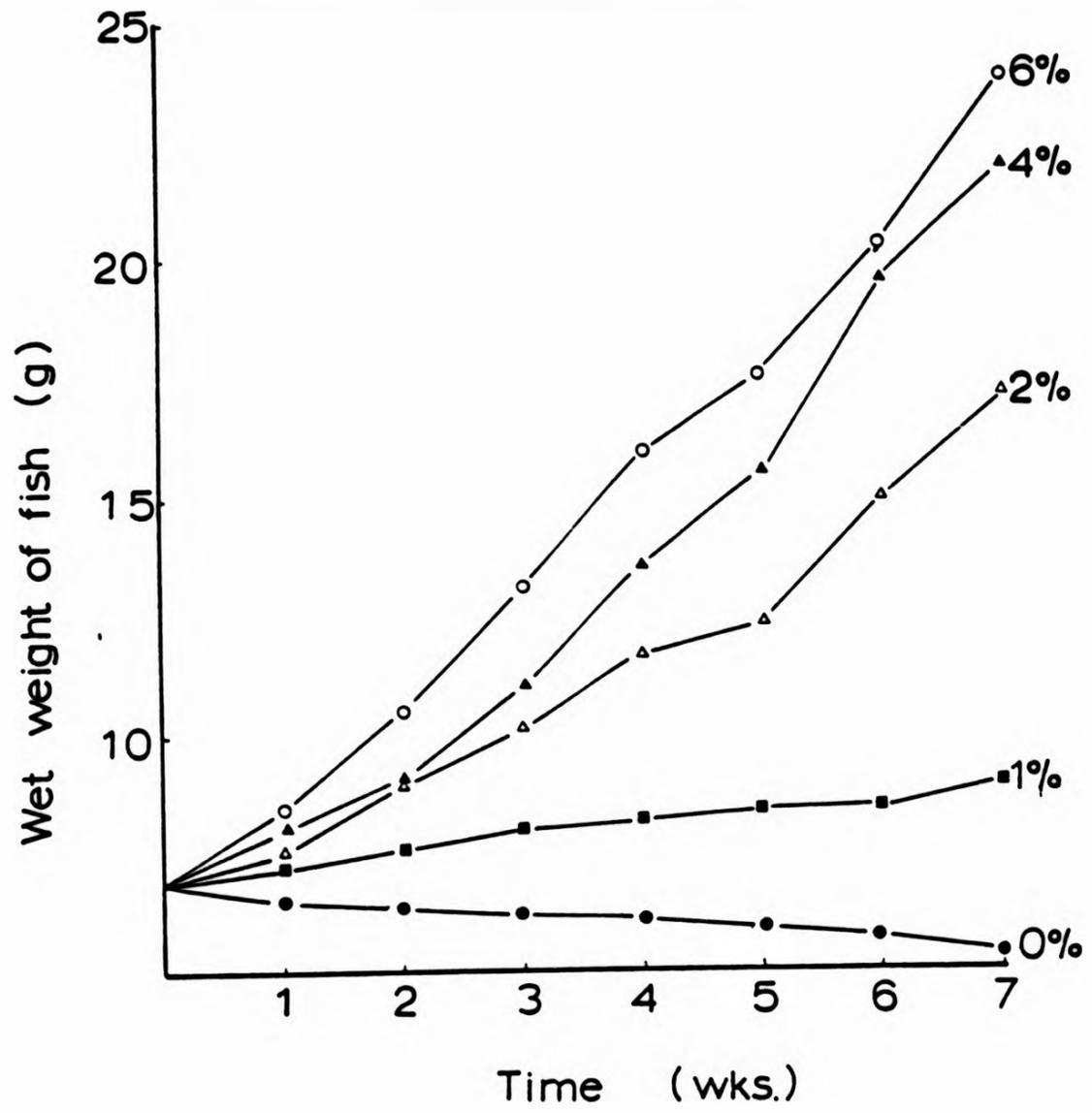


FIGURE 56 Growth of *O. niloticus* (14.27g) at different feeding rates



FIGUR 57 Growth of *O. niloticus* (6.86g) at different feeding rates

against ration size (as % b.w.) were plotted (Figures 58, 59). From these figures it can be seen that maintenance, optimum and maximum rations for smaller fish (Fig. 59) were higher than for larger fish (Fig. 58).

The food conversion ratio (Table 45) for larger fish was relatively unchanged with increasing meal size up to 3% b.w., the lowest F.C.R. for large fish was observed in the group fed 2% b.w. and the highest F.C.R. was obtained in the group fed 4% b.w. The highest P.E.R. was obtained in the group fed 2% b.w. (2.19) and the lowest in the group fed 4% b.w. (1.32). The highest A.N.P.U. was recorded for the group fed 3% b.w. followed by the group fed 2% b.w., and the lowest value was obtained by the group fed 4% b.w. (Table 45a).

The lowest F.C.R. for small fish was observed in the group fed 2% b.w. followed by the group fed 4% b.w., and the highest F.C.R. was obtained for the group fed 6% b.w. (Table 46a).

P.E.R. (Table 46a) for different meal sizes reflected the corresponding F.C.Rs. Protein efficiency ratio ranged between 1.04 and 2.46, the highest value being obtained by the group fed 2% b.w. and the lowest by the group fed 6% b.w. Apparent net protein utilization (Table 46a) tended to reflect observed P.E.Rs. The highest A.N.P.U. value (38.3%) was achieved by the group fed 2% b.w. followed by the group fed 4% b.w. (25.9). The lowest A.N.P.U. was observed for the group fed 1% and 6% b.w.

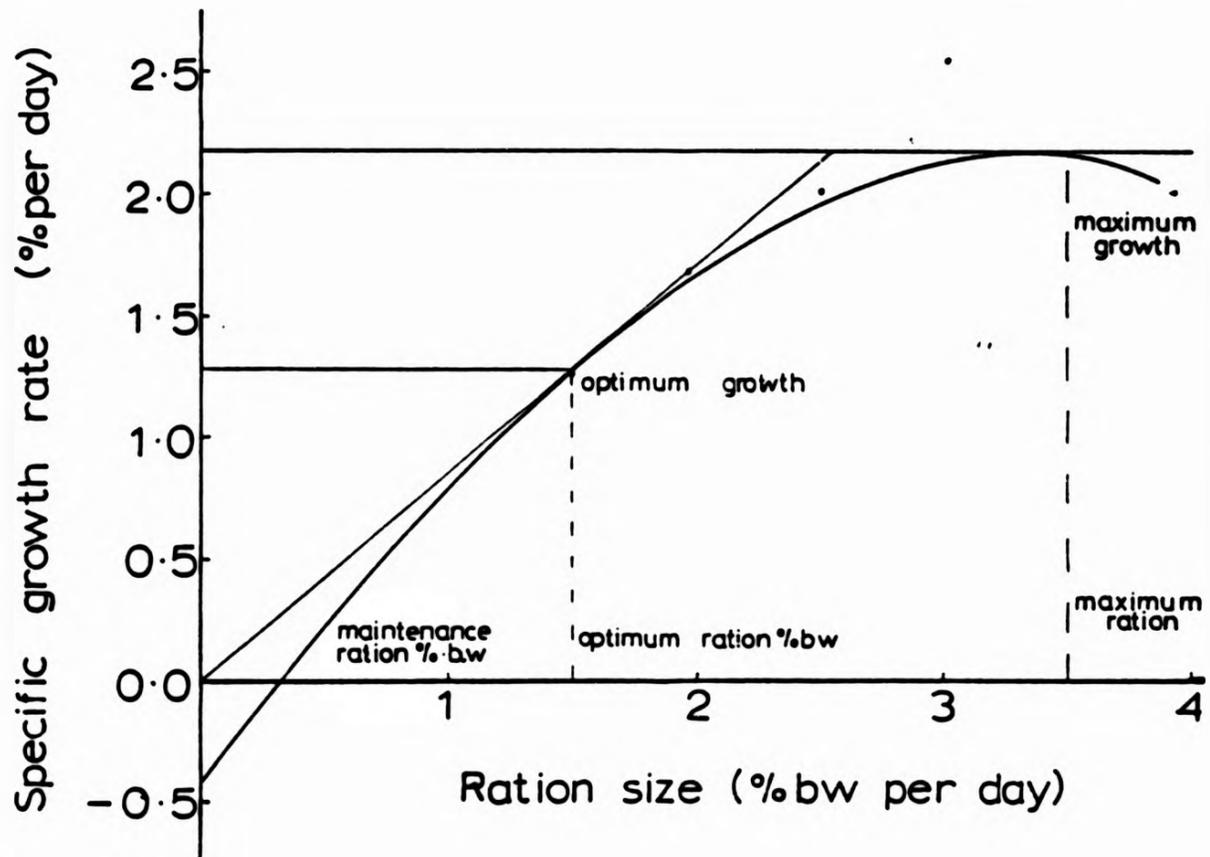


FIGURE 58 The relationship between specific growth rate and feeding rates of O. niloticus (14.27g)

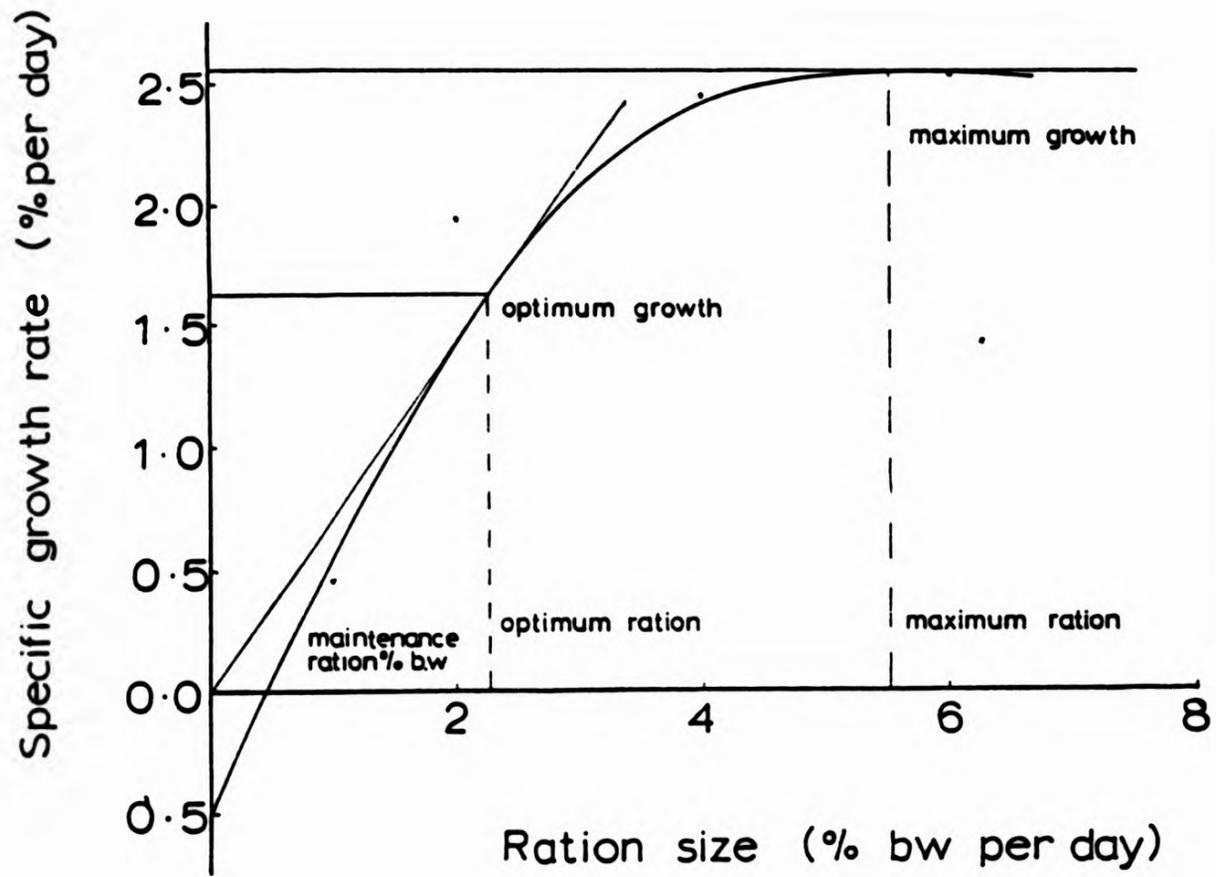


FIGURE 59 The relationship between specific growth rate and feeding rates of O. niloticus (6.86g)

Proximate carcass analyses of fish in both size classes are presented in Table 45b and 46b. Moisture contents (Table 45b) of the final carcass sample of larger fish decreased significantly with increasing meal size. The pattern displayed by protein and lipid contents of final carcass samples were the inverse of that found for carcass moisture. The carcass protein increased significantly ( $P < 0.05$ ) to a maximum of 17.55% for the group fed 3% b.w., thereafter declining significantly ( $P < 0.05$ ) to a value of 16.6% at 4% b.w. Carcass lipid content increased significantly ( $P < 0.05$ ) to a maximum of 5.11% thereafter remaining relatively unchanged with further increase in meal size (Table 45b). Carcass ash contents of the final samples decreased significantly with increasing meal size up to 3% b.w. and then remained unchanged (Table 45b).

Proximate carcass analyses of smaller fish (6.80g), (Table 46b) showed similar trends. Carcass moisture content decreased and the carcass lipid and protein contents increased significantly ( $P < 0.05$ ) with increasing meal size, while carcass ash content decreased significantly ( $P < 0.05$ ) with increasing meal size up to 4% b.w. thereafter remaining constant (Table 46b).

To determine the effect of fish weight and feeding rate on the carcass chemical composition, the data in Tables 45b and 46b for both size classes fed comparable ration sizes were analysed. Two way analyses of variance (Table 47) revealed that both fish weight and feeding rate had a significant effect on carcass chemical composition. Thus with increasing fish weight or feeding rate carcass lipid and carcass protein

TABLE 47 Summary of 2-way analysis of variance of data on carcass moisture, protein, lipid and ash content as a function of feeding rate (% b.w.) and fish weight

MOISTURE					
Source	D.F.	S.S.	M.S.	F	P
Between feeding rate (% b.w.)	3	355.525	118.508	141.42	<.05
Between fish weight	1	5.247	5.247	6.26	<.05
Interaction	3	0.909	0.303	0.361	<.05
Error	40	33.51	0.838	-	-
TOTAL	47	395.191	-	-	-

PROTEIN					
Source	D.F.	S.S.	M.S.	F	P
Between feeding rate (% b.w.)	3	133.347	44.449	209.67	<.05
Between fish weight	1	5.971	5.971	28.16	<.05
Interaction	3	3.099	1.033	4.87	<.05
Error	40	8.482	0.212	-	-
TOTAL	47	150.900	-	-	-

LIPID					
Source	D.F.	S.S.	M.S.	F	P
Between feeding rate (% b.w.)	3	85.6231	28.541	549.92	<.05
Between fish weight	1	3.9998	3.9998	77.1	<.05
Interaction	3	0.4909	0.1636	3.15	<.05
Error	40	2.0766	0.0519	-	-
TOTAL	47	92.1904	-	-	-

ASH					
Source	D.F.	S.S.	M.S.	F	P
Between feeding rate (% b.w.)	3	25.3356	8.4452	182.79	<.05
Between fish weight	1	0.9138	0.9138	19.78	<.05
Interaction	3	1.3360	0.4453	9.64	<.05
Error	40	1.8461	0.0462	-	-
TOTAL	47	29.4316	-	-	-

content increased significantly ( $P < 0.05$ ), whilst carcass moisture decreased significantly ( $P < 0.05$ ).

The relationship between carcass lipid, carcass protein and carcass moisture was investigated (Figs. 60 and 61). The regression equations correlating carcass lipid and carcass protein with carcass moisture were calculated as:

$$\% \text{ carcass lipid} = 41.00 - 0.5 \text{ carcass moisture } (\%)$$

$$\% \text{ carcass protein} = 61.52 - 0.62 \text{ carcass moisture } (\%)$$

The correlation coefficients of 0.98 and 0.96 for carcass lipid and carcass protein contents, respectively, were significant at the 0.001 level. These correlations, regardless of fish weight or feeding rate, enable both the carcass lipid and carcass protein to be predicted with reasonable accuracy from a simple moisture determination under the experimental conditions used here.

#### 3.4.4 The effect of diet composition on growth and body composition of *O. niloticus*

The growth responses of the experimental groups are presented in Table 48a and illustrated graphically in Fig.62. Mean initial weights (Table 48a) did not vary significantly between treatments ( $P < 0.05$ ). Mean final weights increased significantly ( $P < 0.05$ ) with progression from the control diet (Diet A) to the high protein diet (Diet D). Percentage weight gain, specific growth rate and daily weight gain ranked the treatments  $D > C > B > A$ . Food conversion ratio (Table 48a) improved with progression from control diet (A) to the high protein

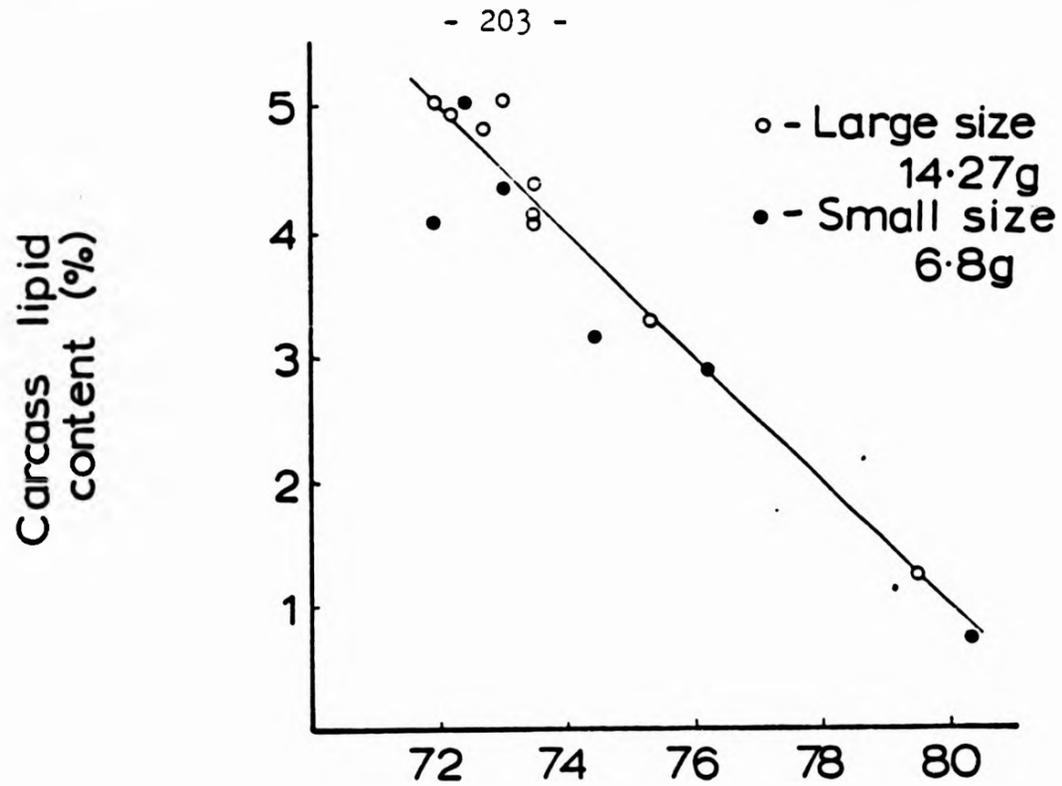


FIGURE 60 The relationship between carcass lipid and carcass moisture contents of O. niloticus at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

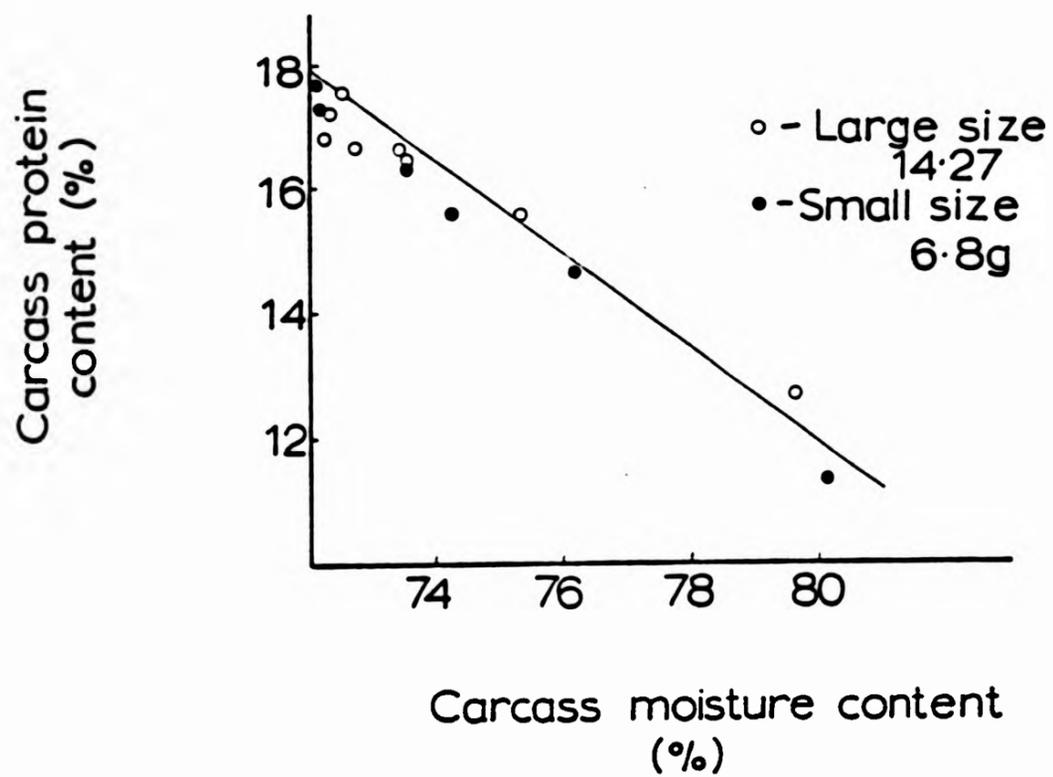


FIGURE 61 The relationship between carcass protein and carcass moisture contents of O. niloticus at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

TABLE 48a Growth, food utilization and carcass composition of *O. niloticus* fed four different experimental diets

Parameter	DIET A Control	DIET B High carbohy.	DIET C High lipid	DIET D High protein	±S.E.
Mean initial weight (g)	1.07 <sup>a</sup>	1.02 <sup>a</sup>	1.09 <sup>a</sup>	1.12 <sup>a</sup>	0.04
Mean final weight (g)	6.09 <sup>a</sup>	7.12 <sup>ab</sup>	7.8 <sup>b</sup>	9.48 <sup>c</sup>	0.39
Weight gain (%)	449.16	598.04	615.59	746.43	
Specific growth rate	3.09	3.44	3.57	3.88	
Food intake (mg/day/fish)	161.00	175.00	184.00	215.6	
Weight gain (mg/day/fish)	89.6	108.9	119.8	149.3	
Food conversion ratio	1.79	1.61	1.54	1.44	
Protein efficiency ratio	1.59	1.69	1.84	1.48	
Apparent net protein utilization	27.5	30.64	32.19	26.91	
Dry matter digestibility	65.38	67.85	72.95	67.55	
Apparent protein digestibility	80.49	85.42	86.5	85.45	

TABLE 48b Carcass composition (% wet weight)

	Initial	Diet A	Diet B	Diet C	Diet D	±S.E.
Moisture	78.6	74.12 <sup>a</sup>	73.9 <sup>a</sup>	73.18 <sup>a</sup>	74.08 <sup>a</sup>	0.22
Crude protein	11.4	16.34 <sup>a</sup>	17.17 <sup>ab</sup>	16.7 <sup>ab</sup>	17.37 <sup>b</sup>	0.15
Crude lipid	4.28	5.69 <sup>b</sup>	5.12 <sup>a</sup>	7.02 <sup>c</sup>	5.18 <sup>a</sup>	0.095
Ash	5.14	3.69 <sup>a</sup>	3.7 <sup>a</sup>	3.65 <sup>a</sup>	3.72 <sup>a</sup>	0.08

Figures in the same row with the same superscript are not significantly different (P < 0.05)

- ▲ - Control diet (A)
- - High carbohydrate diet (B)
- △ - High lipid diet (C)
- - High protein diet (D)

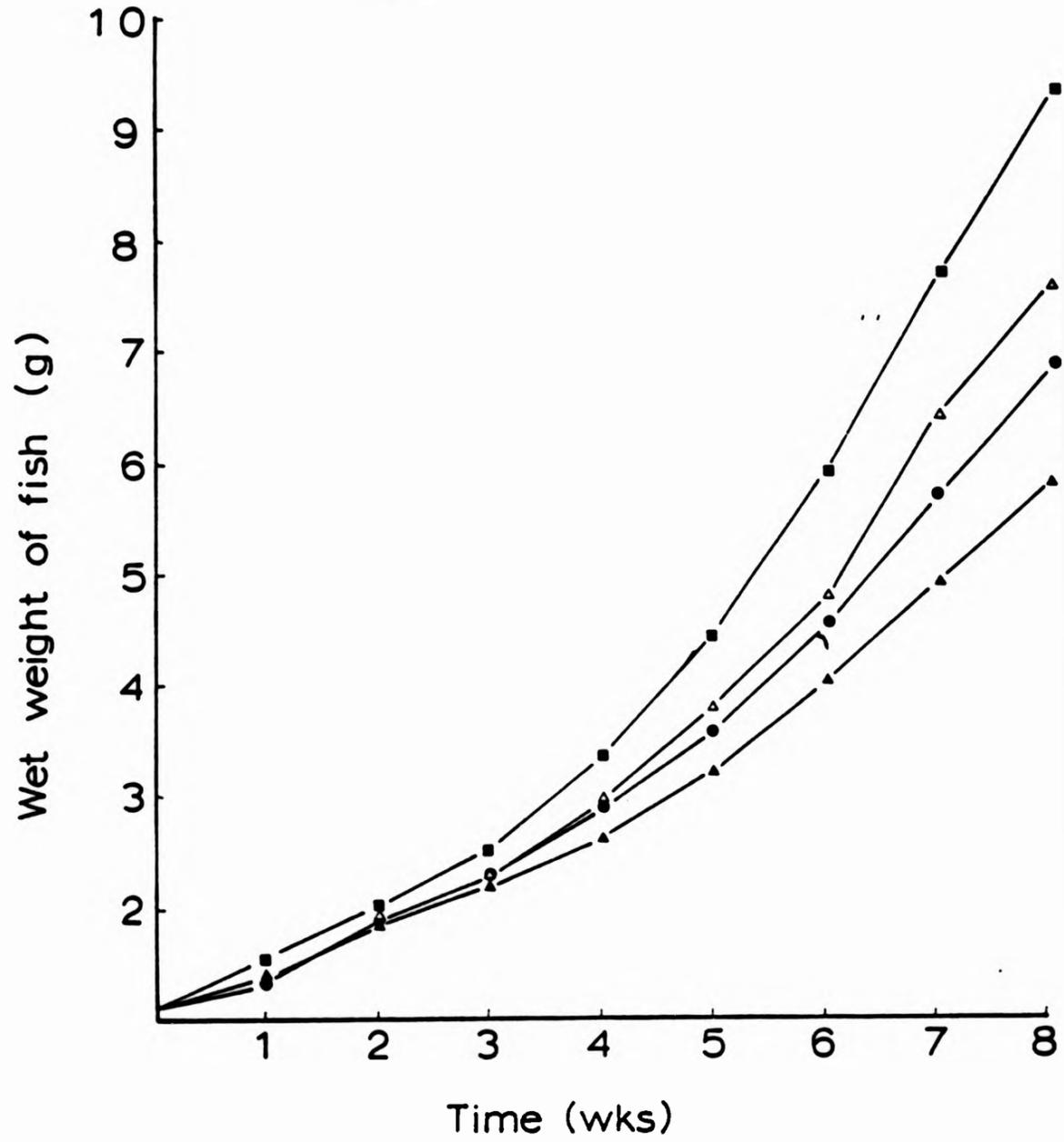


FIGURE 62 Growth of *O. niloticus* fed four experimental diets at  $27.5^{\circ} \pm 1^{\circ}\text{C}$

diet (D). Protein efficiency ratio was highest for the high lipid diet (Diet C) followed by Diets B, A and the high protein diet (D). Apparent net protein utilization showed the same trend (Table 48a).

Dry matter digestion coefficient was highest for the high lipid diet (72.95) followed by the high carbohydrate diet, high protein diet and control diet. Apparent protein digestion coefficient was highest for the high lipid diet followed by the high protein diet, the high available carbohydrate diet and the control diet.

Proximate carcass analysis for initial and final samples are presented in Table 48b. No significant ( $P < 0.05$ ) differences in carcass moisture or ash contents were observed between the treatments (Table 48b). Carcass protein and lipid content were lower in the initial sample and showed differing trends in the final samples. Carcass protein contents showed little variation with the high protein diet (Diet D) giving a significantly ( $P < 0.05$ ) higher value than the control diet (Table 48b). Carcass lipid content showed significant ( $P < 0.05$ ) differences between treatments; the highest value was obtained for the group fed the high lipid diet and the lowest by groups fed the high available carbohydrate diet and high protein diet (Table 48b).

#### 4. DISCUSSION

##### 4.1 Food Intake

The quantity of food ingested by O. niloticus fed to satiation was increased by increases in feeding frequency up to six feeds per day. Further increase in feeding frequency (to eight feeds per day) had no significant ( $P < 0.05$ ) effect on total daily food intake (Fig. 3 and Table 10). This finding is in general accord with earlier observations made by other workers in a variety of species (Andrew & Page, 1975; Grayton & Beamish, 1977; Sampath, 1984; Singh & Srivastava, 1984).

Charles et al. (1984) reported that food intake in Cyprinus carpio (0.173mg) increased to a maximum at three feeds per day and that any further increase in feeding frequency reduced the total daily food intake. It was suggested that this reduction in food intake with increasing feeding frequency may be due to the limited capacity of the foregut in carp as most of the food remained unevacuated. However no such reduction in total daily food intake at high feeding frequency was observed in the present study (Table 10).

When O. niloticus were fed a single daily meal the food intake per meal as a proportion of the body weight was large. As the frequency of feeding increased so the food intake per meal declined, with, however, an increase in total daily ration (Table 10). The reduction in food intake per meal with increasing feeding frequency may be explained by consideration of evacuation rates and times. Food

intake is governed by hunger or satiation level which, in turn, depend upon the amount of food remaining in the stomach. Consequently the food intake in a single meal will be related to the quantity of food remaining in the gastrointestinal tract from the previous meal. This, in turn, will be related to the inter-feeding deprivation period and to gastric evacuation rate and time with maximum <sup>occurring</sup> meal size/when stomach capacity is at its greatest, after completion of gastric evacuation (Brett & Higgs, 1970; Ware, 1972; Elliott, 1975; Singh & Srivastava, 1985; Grove et al., 1985).

The mathematical relationship between maximum daily food intake in tilapia fed an artificial diet is worthy of consideration with respect to the present study. A systematic approach to modelling this relationship was adopted because there is no well established theory which allows prediction of maximum daily food intake from fish weight in tilapia fed on a complete artificial diet (Moriarty & Moriarty, 1973; Caulton, 1978). Both empirical and theoretical arguments suggest that the relationship between maximum daily food consumption and fish weight should be of the form:

$$M = aw^b$$

or in linear form:

$$\text{Log}_e M = a + b \text{Log}_e W$$

Where M = food intake (g)

W = fish weight

a and b are constants

Analysis of data for O. niloticus shows that maximum daily food intake increased with fish weight in a linear fashion (see Section 3.1.2).

Daily food intake, expressed in g per fish, increased with increasing fish body weight (Fig. 4) but when expressed as a proportion of the body weight (% body weight) food intake was inversely proportional to fish weight (Fig. 6). Fish of 10g consumed 9.1% of their body weight per day whilst fish of 200g consumed only 2.4% body weight per day. By extrapolation from the relationship derived in Fig. 4 between fish weight and food intake, first feeding tilapia fry should have a maximum food intake of 48% b.w./day. This is in reasonable agreement with the recommendation of 36% b.w./day made by Macintosh and Sampson (1985) for O. mossambicus and O. niloticus fry. Ross and Jauncey (1981) reported a maximum daily food intake of 9.0%-8.2% b.w./day for 20g and 40g fish respectively, at 30°C, which is slightly higher than have been predicted in the present study for similar size (6.7%-4.9% b.w.).

In contrast to the foregoing Moriarty and Moriarty (1973) derived an equation for the daily food intake of O. niloticus in Lake George which predicts a daily food intake of 4% b.w. for 10g fish and 1.5% b.w. for 200g fish. In addition, Caulton (1978) derived an equation for T. rendalli fed fresh C. demersum at 30°C which predicted a daily food intake of 12g of fresh C. demersum for 100g of fish or an equivalent of 1g dry mass of food. The predicted feeding level from these two studies are much lower than in the present investigation, which could be explained by the higher water contents (80%-90%) of the natural food used in their investigation, therefore bulking may have limited the food intake compared to the dry pelleted feed used in the present investigation or it could be explained by the differences in the technique used for the estimation of the food intake since Moriarty and Moriarty derived the daily food intake from gastric evacuation times.

As well as maximum daily food intake, satiation meal size (maximum voluntary food intake in a single meal, g) will also increase linearly with increasing body weight (Fig. 11). However, when expressed as a relative proportion of the body weight (% b.w.) maximum daily food intake decreased from 1.96% b.w. to 1.27% b.w. for 10g and 200g fish respectively. The fact that the daily food intake and the satiation meal size are relatively larger (as % b.w.) for smaller fish is in agreement with the fact that smaller fish have relatively higher metabolic and growth rates than larger fish (Fry, 1957; Brett, 1979; Caulton, 1982). These results are consistent with those of other workers on other species (e.g. Ishiwata, 1968; Brett, 1971; Grove & Crawford, 1980; Wootton et al., 1980). Elliott (1975) reported that satiation meal size for Salmo trutta decreases from 1.0% b.w. to 0.5% b.w. for 10g and 200g fish respectively. Comparison of results for satiation meal size in the current investigation with those of other workers on tilapia cannot be made since no such studies appear in the literature.

Several workers have reported that stomach volume and food digestibility may determine the amount of food ingested voluntarily by fish (Western, 1971; Ware, 1972; Singh & Srivastava, 1985). In the present study stomach volume was measured by water replacement (Jobling, 1974; Jobling et al., 1977). An almost linear relationship was found between maximum stomach volume (ml) and fish weight (g) in the size range investigation (Fig. 12). These results are in general accord with earlier observations made by other workers in a variety of fish species (Jobling et al., 1977; Grove et al., 1978; Flowerdew

& Grove, 1979; Huebner & Langton, 1982). Although absolute stomach volume increases almost linearly with fish body weight (as ml/fish), relative stomach (ml/g for weight) decreases. Relative stomach volume was 0.141ml/g in 10g fish which fell to 0.06ml/g in the largest fish (200g) used in this study. The decrease in relative stomach volume with increasing fish body weight correlates well with satiation food intake levels expressed as % b.w. (Table 12). A satiation meal in the present study represents 72%-83% of the stomach capacity (Table 12). The feeding periods of 10-15min per meal (see Section 2.3.1) was therefore adequate to satiate the different weights of fish using stomach fullness as the criterion. The satiation time (the time from the start of feeding to cessation of voluntary food intake) may vary with species. Satiation times of 44.44min for Hetero pneustes fossilis (Singh & Srivastava, 1985), 42.58min for Salmo gairdneri (Grove et al., 1978), 15min for Fugi vermiculatus and Stephanolipis cirrhifer (Ishiwata, 1968), and 60min for Oncorhynchus herka (Brett, 1971) have been recorded. Many factors are responsible for variation in food consumption by fish and consequently can affect the satiation meal size and satiation time. These factors include ambient water temperature, size of fish, gut capacity, type of food ingested, activity of fish and the rate of food passage through the alimentary canal (see Sections 1.1 and 1.2). It is possible that many of these factors may alter rates of food consumption by altering the rate of digestion of food in the digestive tract.

Amongst environmental factors, temperature has the greatest effect on food intake and optimal feeding frequency in several fish species (Edwards, 1971; Elliott, 1972, 1975; Jobling et al., 1977). Wootton

et al. (1980) reported that the food consumption of Gasterosteus aculeatus of a single size category increased from 5.6% b.w. to 12.2% b.w. with increasing water temperature from 6°C to 19°C respectively. It has been suggested that the increase in food intake accompanying an increase in temperature is caused by a rise in metabolic rate (Brett & Groves, 1979; Caulton, 1982) and/or a faster evacuation rate of food from the stomach and intestine which in turn increase food intake by increasing the feeding frequency (Tyler, 1970; Jobling & Davies, 1979; Persson, 1979; Jobling, 1980; Gwyther & Grove, 1981; present study, Section 3.2.2).

The nutritional value of the food, and therefore the total food intake, depends on the relative proportion of protein, lipid and carbohydrate (Windell, 1967; Jobling, 1980a; Davies, 1984). Rozin and Mayer (1961) and Grove et al. (1978) found that both goldfish and rainbow trout, respectively, increased their intake in response to dilution of their food with non-nutritive kaolin. More recently Hilton et al. (1983) and Davies (1984) reported that rainbow trout increased their food consumption with increasing dietary fibre by increasing either stomach volume or gastric evacuation rate. The increased food intake could be achieved by increasing feeding frequency caused by a more rapid rate of evacuation (Adron et al., 1973). It has been suggested that when diets were diluted the frequency of feeding increased in order to maintain a relatively constant level of energy consumption to satisfy the energy requirement of fish (Lee & Putnam, 1973; Jobling, 1981; Grove et al., 1985). However no attempt was made to determine the effect of food type on food consumption for O. niloticus, but the results obtained from gastric evacuation (see

Section 3.2.2.4) could indicate such increases in food consumption with increasing carbohydrate level in the diet.

Maximum voluntary food intake by fish is assumed to be an objective measure of appetite and the time required for return of appetite is assessed by examining the relationship between food deprivation time and voluntary food intake (Brett, 1971; Elliott, 1975; Grove et al., 1978; Grove & Crawford, 1980). Voluntary food intake in O. niloticus was affected by deprivation time (h) in the present study. With increasing starvation period (h) food intake increased up to 72h. of starvation, after which it declined slightly although not significantly ( $P < 0.05$ ) (Fig. 13 and Table 13). This increase in food intake is thought to indicate development of a systematic deficit during food deprivation which is corrected when food later becomes available (Ince & Thorpe, 1976; Weatherley & Gill, 1981; Storch & Juario, 1983). Increased food intake only occurs up to a limited deprivation time beyond which food intake may decrease or even fail altogether (Rozine & Mayer, 1961; Bilton & Robsin, 1973; Hadjichristophora, 1980) as was observed in the present study (Table 13, Fig. 13). Randolph and Clemens (1978) indicated that channel catfish (Ictalurus punctatus) consumed 10%-17% more food after one day of food deprivation while food intake declined to about 36% of the normal intake after 5-7 days of starvation. Grove and Crawford (1980) showed that full appetite returned 5-8 hours after a satiation meal in a stomachless fish (Blennius pholies) and that food intake was the same whether the deprivation time was for 5-8 hours or 48 hours. Reduced food intake after long deprivation periods may reflect a progressive decrease in metabolism (Smith, 1935; Javid & Anderson, 1967; Brett & Zala, 1975;

Chowdery, 1977). It has been known for some time that there is usually a gradual reduction in oxygen consumption during starvation. Beamish (1964) and Jobling (1980) showed that for fish this reduction is due to a decrease in spontaneous activity as well as a decrease in standard metabolism.

Morphological change in the digestive tract may account for the reduction in food intake after long periods of deprivation. Windell (1966) cited in Brett (1971) noted that food intake of bluegill sun fish (Lepomis macrochirus) increased to a maximum after four days of starvation and then decreased, accompanied by some degenerative changes in the pyloric caeca after 10 days of starvation. Similar morphological changes have been reported by Elliott (1972) for brown trout (Salmo trutta) and Jobling (1980) for plaice (Pleuronectes platessa). However the stomach volume of O. niloticus in the present study was found to increase with starvation period (Table 14). This increase in stomach volume could indicate the looseness of stomach muscle which may affect the gastric evacuation rate and time (see Section 3.2.2.5). In contrast to stomach volume, intestine length was found to decrease with starvation periods (see Section 3.1.4, Table 14). Similar reductions in intestine length with starvation has been reported by Angelescu and Gneri (1949) cited in Fange and Grove (1979). Jobling (1980) reported a reduction in gut relative size from 3.07% b.w. to 1.78% b.w. after 35 days of starvation for Pleuronectes platessa. Similar reduction in gut relative size has also been reported for Salmo gairdneri by Weatherley and Gill (1981). The relative length of the gut was determined in the present study and found to vary between 4.8 and 6.1 which is in close agreement with the

Al Hussaini (1947) value of 6.3 for O. niloticus and the Hofer and Schiemer (1981) value of 6.8 for O. mossambicus. The relative length of the gut is well correlated with the varying feeding habits of fish. Carnivorous fish have the shortest intestine: Omnivorous and herbivorous fish have the largest (Al Hussaini, 1947; Barrington, 1957; Kapoor et al., 1975). Hsu and Wa (1979) reported that relative gut lengths for Japanese eel and snakehead vary between 0.46 and 0.68 compared to 6.29 for O. mossambicus.

Period of starvation did not have any effect on liver weight in the present study (see Section 3.1.4, Table 14) which suggests that during this period most of the energy demand has been derived from the lipid and glycogen from the muscle and viscera (Ince & Thorpe, 1976). Ince and Thorpe (1976) state that

"of particular interest is the observation that extra-hepatic lipid deposits in the intestinal region become greatly reduced before any significant changes in liver lipid can be measured".

Nagai and Ikeda (1973) reported that in Cyprinus carpio held without food for 22 days there was appreciable depletion of liver lipids, while the glycogen was untouched.

Love (1975, 1980) and Talbot and Higgins (1982) reported that gall bladder weight and colour may be a good indication of feeding history in fish. In actively feeding fish the gall bladder is nearly empty of bile, during deprivation the weight of the gall bladder increases and the colour of bile changes from pale straw to dark green or dark blue according to the deprivation period. The gall bladder

weight of O. niloticus under a normal feeding regime in the current investigation varied between 10%-21% of the liver weight (Fig. 14) and the colour of bile was usually pale straw. The weight of the gall bladder increased with starvation period and reached a maximum after 72 hours (Table 15); over this period the colour of the bile changed to dark green, which returned to normal 4-6 hours after refeeding the fish (Fig. 15). Bile is produced by the liver and passes either directly or via the gall bladder to the intestine where its role in digestion is to solubilise dietary fat (Fange & Grove, 1979). The colour of the bile may be due to resorption of water and salts by the gall bladder epithelium (Diamond, 1962; Mackay, 1975) which would concentrate organic substances in the bile. The results of gall bladder weight and colour in the present study are in general accord with observations made by other workers on other species of fish (Love, 1975, 1980; Western, 1972; Talbot & Higgins, 1982; Rana, personal communication).

#### 4.2 Gastric Evacuation

The mathematical description of the rate at which food leaves the fish stomach is disputed. Some workers, (e.g. Hunt, 1960; Swenson & Smith, 1973) observed a linear relationship between the amount of food remaining in the stomach after feeding and time; Data of this sort allow the calculation of true rate of stomach evacuation as the rate of evacuation of food does not change during the course of evacuation. Others, (e.g. Windell, 1966; Elliott, 1972; Grove & Crawford, 1980; Davies, 1984) found this relationship to be curvilinear and

so the rate of evacuation therefore changes. In the present study a curvilinear relationship was found between the amount of food remaining in the stomach and time after feeding (Fig. 16) and a linear transformation using the three models (2.4.2) fit the data well. Overall the volume dependent model gave a good fit for the data (Table 17).

The relationship between stomach evacuation coeff. and temperature for O. niloticus was found to be exponential (Fig. 20) which is in accordance with the findings of Jones (1974) and Persson (1979, 1981) working on Gadus morhua and Perca fluviatilis respectively. The  $Q_{10}$  was calculated to be 1.96 and it is encouraging to note the closeness of this figure to the generally accepted value of 2 for biological activities (Mesk, 1985). A similar value of 1.92 was obtained by Ross and Jauncey (1981) for tilapia (O. niloticus x O. aureus). Fabian et al. (1963) reported that digestion by fish at different temperatures changed in compliance with an exponential or power function and suggested that digestion in the stomach was simple decomposition by pepsin and hydrochloric acid. However, the processes of gastric digestion and evacuation are complex, and depend on the inter-relationships between secretion, the activity of the digestive enzymes and the muscular activity of the stomach. Rapid evacuation of the stomach at high temperatures, resulting from a temperature dependent increase in gastric peristalsis, would result in a lower digestive efficiency unless there was a concomitant increase in the activity and secretion of digestive enzymes. Smit (1967) has pointed out that changes in temperature within the physiological range can affect digestion rate (g/hr) by at least three dependent processes namely enzymatic activity, gastric secretion and gut motility. Buchs (1954) showed that in trout,

pepsin activity in vitro increased with increasing temperature up to 35°-40°C, which is well above the upper lethal temperature for this species. Similar observations were reported by Trofimova (1973) and Hofer (1979) for common carp and roach respectively. Smit (1967) working on Ictalurus nebulosus, found that the rate of gastric juice secretion is not only temperature dependent, but that temperature also affected the pH of the gastric juice. The gastric response to increase in temperature resulted in a decrease in pH of gastrointestinal secretions. An increase in gut motility with temperature was reported for Fundulus heteroclitis (Nicholls, 1931). Kapoor et al. (1975) also considered that temperature affects digestion rate by influencing rates of intestinal absorption and the rate of foraging and feeding.

As stomach evacuation rate increased with increasing temperature for O. niloticus, the stomach and total gut evacuation time decreased (Table 18,19) thus giving a negative correlation between stomach evacuation time and temperature (Fig. 19). For example, a 27g fish fed to satiation would have a stomach evacuation time of 29.9h at 20°C compared to only 12h at 35°C. The stomach evacuation time (h) decreased with increasing temperature to the exponent 1.17 and the stomach evacuation coefficient increased with temperature to the exponent 0.029. These results are in agreement with those of other workers for a variety of fish species (Brett & Higgs, 1970; Elliott, 1972; Molnar & Tolg, 1972; Gerald, 1973; Bassimi, 1978; Flowerdew & Grove, 1979; Jobling & Davies, 1979; Persson, 1979, 1981; Jobling, 1980; Ross & Jauncey, 1981; Hershley & McDonald, 1985).

Food intake is governed by hunger or satiation level which in turn depends to a certain extent on the amount of food remaining in the stomach (Ware, 1972; Grodin, 1981) and it is thus assumed that return of appetite is closely related to gastric evacuation time (Elliott, 1975; Flowerdew & Grove, 1979; Grove & Crawford, 1980; Gwyther & Grove, 1981; Grove et al., 1985; Singh & Srivastava, 1985). The present study indicated that 75% of appetite returns in 6.5h at 35°C compared to 14h at 20°C (Fig. 21), which suggests that increasing temperature leads to an increase in feeding frequency resulting in an overall increase in daily food intake. Similar observations were made by Gwyther and Grove (1981) who pointed out that the increase in daily food intake with increasing temperature in Limanda limanda is primarily brought about by an increase in feeding frequency rather than by an increase in food intake in a single meal.

Jobling et al. (1977) and Flowerdew and Grove (1979) suggested that in investigations of the effects of fish weight on stomach evacuation it is appropriate to compare meals of equal stimulus by feeding the fish a given percentage of their body weight since the volume of the stomach increases linearly with increasing fish weight. When O. niloticus in the weight range 49 to 148 were fed 1% of their body weight in a single meal, gastric evacuation time for smaller fish was shorter than the larger ones thus demonstrating that gastric evacuation time increased with increasing fish weight (Table 20, Fig. 25). When Jobling et al. (1977) fed Limanda limanda a ration expressed as a percentage of body weight, it was found that larger fish took significantly longer to evacuate the stomach. Pandian (1967), Gerald (1973) and Ross and Jauncey (1981) also used this approach and

confirmed that smaller fish evacuate a given percentage body weight meal in a shorter period than do larger fish. The stomach evacuation time for O. niloticus was found to vary, as fish weight increased to the power of 0.131 and stomach evacuation coefficient was found to vary, as fish weight increased to the exponent 0.408. These results are in agreement with those of other workers for a variety of fish species (Pandian, 1967; Bassimi, 1978; Flowerdew & Grove, 1979; Gwyther & Grove, 1981), although Jobling (1980) observed that the rate of gastric evacuation in plaice was relatively unchanged by fish size and Persson (1981, 1982) reported similar findings for perch, Perca fluviatilis and roach, Rutilus rutilus.

In O. niloticus it was found that at 27.5°C the time required to complete the evacuation of the stomach increased with increasing meal size (Table 22, Fig. 32). The digestion rate in O. niloticus was also found to increase with meal size raised to the power of 0.72 (Fig. 34). Several workers have also reported that the rate of digestion of larger meals fed to a given size of fish at a defined temperature is greater than for a smaller meal (Hunt, 1960; Bassimi, 1978; Jobling & Davies, 1979; Gwyther & Grove, 1981). Barrington (1957) and Fänge and Grove (1979) suggested that because of the relatively larger surface area available to the digestive enzymes for smaller food items, fish digest small meals more rapidly than large meals and small prey more quickly than larger prey. However in studies by Windell (1966), Kitchell and Windell (1968) and Person (1981) for Lepomis macrochirus, Lepomis gibbosus and Perca fluviatilis respectively, it was demonstrated that the total evacuation time at a particular temperature was the same whatever the meal size. By contrast

Steigenberg and Larkin (1974) reported a reduced gastric evacuation rate with increasing meal size for Northern squawfish (Ptychochirulus oregonensi). In the majority of species however, an increase in meal size does not lead to a complete compensation in gastric evacuation rate (g/h) so that the larger the meal the greater the gastric evacuation time. Previous investigations of gastric evacuation time for several species of fish have shown that gastric evacuation time increased in proportion to meal size. Beamish (1972) found that a four-fold increase in meal size fed to Micropterus salmoides doubled the time required to empty the stomach, while Jobling et al. (1977) found that a five-fold increase in meal size led to a three-fold increase in gastric emptying time in Limanda limanda. The increased stomach evacuation time in the present study was not directly proportional to the increase in meal size, since a larger meal stimulated a more rapid emptying of food from the stomach during the early stage than a smaller meal. Hunt and McDonald (1954) and Hunt and Knox (1968) reported similar findings for higher vertebrates, in that the larger the meal the higher the initial rate of emptying from the stomach. Hunt and Knox (1968) concluded that these more rapid early stomach evacuation rates are caused by an increase in the stroke volume of a constant frequency of gastric pump under the influence of radial gastric distension. Similar observations have been reported by Jobling et al. (unpublished data) cited in Jobling et al. (1977) for Pleuronectes platessa and Salmo gairdneri, where increasing meal size increased the amplitude but not the frequency of gastric peristaltic contractions. The gastric evacuation time in the present study was found to vary with variation in meal size to the power 0.32 in the case of meal size expressed in absolute weight (g) and to the power

0.33 when it is expressed as a percentage body weight. Furthermore gastric evacuation rate was found to vary with meal size to the power 0.72. These results are in general accord with the majority of published data for a variety of fish species (Hunt, 1960; Pandian, 1967; Windell et al., 1969; Elliott, 1972; Swenson & Smith, 1973; Bassimi, 1978; Flowerdew & Grove, 1979; Jobling & Davies, 1979; Gwyther & Grove, 1981).

Hess and Rainwater (1939) were the first to suggest that the type of food organism may affect the rate of stomach evacuation in fish and this has been subsequently supported by the observations of Reimer (1957) on rainbow trout. Separate food fractions such as digestible matter and indigestible chitin or plant materials, may show differential movement through the stomach (Pandian, 1967; Windell, 1969; Jones, 1974; Windell, 1978; MacDonald et al., 1982; Medved, 1985). The chitinous exoskeletons of invertebrate prey species often remain in the stomach long after the digestible component has been evacuated. This is particularly true for large pieces of chitin which require softening prior to passing through the pyloric sphincter (Kinoka & Windell, 1972).

Variation in dietary levels of lipid, carbohydrate and protein had a marked effect on both gastric evacuation rates and times in O. niloticus (Table 24, Fig. 39). Compared to the control ration which contained only 11% lipid, the gastric evacuation coefficient of the high lipid diet (18% lipid) was significantly slower. Thus increasing the dietary lipid from 11% to 18% decreased the stomach evacuation coefficient from 0.035 to 0.03. Hunt (1960) considered

that dietary lipid has an inhibitory effect on gastric evacuation rate, since this nutrient can modify the overall rate at which the organic component passes from the stomach. Windell (1967) and Hofer et al. (1982) reported that meal worms, which contain 35% lipid, were evacuated more slowly by both Lepomis macrochirus and Rutilus rutilus, in comparison to other natural food items. By contrast Windell et al. (1972) observed no differences in gastric evacuation rate (g/h) of Salmo gairdneri fed diets containing lipid in the range 6.5 to 14.5%. The slowing of gastric evacuation rate (g/h) in O. niloticus fed the high lipid diet (Diet C) may be due to the higher calorific value of this diet. In higher vertebrates an increase in the dietary calorific level has been shown to reduce gastric evacuation rate (Hunt & Knox, 1968). Windell (1967) suggested that the presence of fat in the food may increase gastric evacuation time as a result of the release of a hormone similar to introgastron from the intestinal wall. In mammals this hormone is known to inhibit gastric motility (Hunt & Knox, 1968).

In several teleosts (Salmo gairdneri, Scophthalmus maximus, Pleuronectes platessa and Rutilus rutilus) dilution of a basal diet with inert material or the ingestion of low energy diets results in enhanced gastric evacuation rates (Grove et al., 1978; Flowerdew & Grove, 1979; Jobling, 1980; Hofer et al., 1982). Grove et al. (1978) demonstrated that gastric evacuation time decreases when experimental diets are diluted with kaolin. These authors also showed that rainbow trout trained to operate demand feeders providing food diluted with kaolin compensated by eating more, and that the increased food intake was achieved by increased feeding frequency as a result of a more

rapid gastric evacuation rate (g/h). Similar observations were made by Grove et al. (1985) for Scophthalmus maximus. In the present study increasing the level of carbohydrate in the diet increased the gastric evacuation rate (g/h) and consequently decreased gastric evacuation time (Fig. 39 and Table 24). Similarly Lee and Putnam (1973) and Hilton et al. (1983) reported that gastric evacuation time was much faster in rainbow trout fed high levels of dietary carbohydrate. It is possible that the rapid gastric evacuation rate of O. niloticus fed a high carbohydrate diet is a physiological adjustment designed to permit increased consumption of low energy diets by increasing the feeding frequency which in turn increases daily food intake to satisfy energy requirements (Grove et al., 1985). The gastric evacuation rate for a high protein diet (49%) in the present study was not significantly different from the control ration containing 35% protein (Table 24). This suggests that increasing the dietary protein level up to 49% did not have any effect on stomach evacuation coeff. or time (Table 24). It thus appears that the dietary lipid and carbohydrate level of the diet have the most pronounced effect on gastric evacuation rate.

In general the apparent protein and dry matter digestion coefficients of intestine for the four experimental diets were found to increase with time after feeding (Table 26). The apparent protein digestion coefficient for all four diets was found to exceed that of the dry matter, this was expected since protein is the major component of the diet and is relatively more assimilable than the remaining component of the diet. The variation in the apparent protein and dry matter digestion in the intestinal contents for each of the

experimental diets with time could, in part, be explained by individual variation between fish. It is also possible that the chromic oxide passed through the alimentary canal at a slightly faster rate than the organic matter in the meal, since it was observed that the colour of the faeces on first appearance was slightly darker green than subsequent faecal products. There are several reports assessing chromic oxide as an inert indicator for digestibility studies in fish (Bowen, 1978; De Silva & Owoyemi, 1983). Leavitt (1985) showed that chromic oxide at levels of up to 1% of the diet does not suppress dry matter intake and reported that there was differential passage of the chromium pigment into the faeces. However the present study was not specifically designed to study rates of gut passage of chromic oxide and can only be regarded as indicative of differential passage of the chromium through the gut. Variation in digestion coefficients might be explained, in part, by the profile of the intestinal flow rates after feeding. Intestinal contents with time showed a regular pattern of filling and emptying (Fig. 41). Since it is known that there is little or no absorption of nutrient via the stomach wall (Fange & Grove, 1979; Bowen, 1983), the arrival of the newly evacuated material into the intestine will result in cycles in nutrient levels following the pattern of filling and emptying which will in turn affect the intestinal digestion coefficients recorded. The apparent protein digestion coefficient for the control diet (Diet A), the high carbohydrate diet (Diet B) and high lipid diet (Diet C) were found to be lower than for the high protein ration (Diet D) (Table 26). This variation may be explained by the endogenous faecal nitrogen excretion which, when low protein diets are fed, represents a large proportion of the total nitrogen in the faeces and hence the digestibility of

protein in a low protein diet will tend to be underestimated. It has been suggested that the protein digestion coefficient is more or less constant and independent of dietary protein level when correction for endogenous faecal nitrogen excretion has been made (Nose, 1967; Ogino & Chan, 1973). The total dry matter digestion coefficients were found to be lower in the control diet (D) and high lipid diet (Diet C) than in the high protein diet (Diet D) and high carbohydrate diet (Diet B). This might be due to the poorer digestibility of the non-protein portion of the diet containing higher level of indigestible carbohydrate which may interfere with processes of protein digestion and absorption (Rychly & Spannhof, 1979; Smith, 1971; Jobling, 1981, 1983; Hilton et al., 1983; Davies, 1984). The recorded apparent protein and dry matter digestion coefficients in the present study were higher than have been reported for other species of cichlid by Moriarty and Moriarty (1973), Caulton (1978), Bowen (1979, 1981) and De Silva and Perra (1983). Comparison between results of the present study on digestion coefficients and those presented in the literature can not easily be made as a consequence of variation in the reliability of different markers used by different workers under different experimental conditions using different diets. As most of the work carried out with cichlid fish involves using wild fish and natural diets (Moriarty & Moriarty, 1973; Bowen, 1979, 1981) direct comparisons of results is not possible.

In many gastric evacuation studies it has been the normal procedure to deprive fish of food prior to feeding the test meal and then holding the fish without food until the test meal is totally evacuated (Windell, 1966; Bassimi, 1978; Grove et al., 1978; De Silva

& Owoyemi, 1983; Grove et al., 1985). Unfortunately, many workers have neglected to report the duration of this fasting period and its effect on evacuation (Windell, 1978). Food deprivation is known to cause distinct morphological changes in the alimentary canal of some species (Windell, 1966; Elliott, 1972; Jobling, 1980) which in turn might be expected to affect the gastric evacuation rate and times.

When O. niloticus were deprived of food for varying periods of between 24 and 96 hours and subsequently fed either to satiation or a fixed ration (1% b.w.) the response was a decrease in stomach evacuation coeff. which thus increased evacuation times (Tables 27, 29 and Figs. 44, 49). These results are consistent with those of other workers (Windell, 1966; Goddard, 1974; Jobling, 1980; Talbot et al., 1984). In contrast to the present study, Elliott (1972) found that starvation periods of up to seven days prior to feeding brown trout did not affect subsequent evacuation rates, however starvation periods of ten days or more did significantly reduce the evacuation rates and increased the evacuation time in Salmo trutta. The reduced stomach evacuation coeff. after periods of starvation in the present study could, in part, be explained by the reduction in digestive enzyme activities (see Section 3.3.3) and possibly by a reduction in motor activity as reflected in increased gastric evacuation time (Goddard, 1974; Jobling, 1980; present study). However, as absorption efficiency is unaffected by fasting (Jobling, 1980) it appears that increased food retention time may be a compensatory mechanism to allow adequate digestion and absorption of food following long term deprivation of food as has been suggested by Elliott (1972) and Jobling (1980).

Windell (1978) argued that gastric motility once initiated, remains relatively constant and the phenomenon of food passage in fishes is similar to higher vertebrates. The intestinal flow rates in the present investigation for all the experiments carried out showed a regular pattern of filling and emptying, which suggests that the digesta pass into the intestine in more or less finite quanta and are retained in the intestine for some time for further digestion and absorption before the next quantum arrives. Similar observations on flow rates of intestinal contents have been reported by Moriarty and Moriarty (1973) and De Silva and Owoyemi (1983) for tilapia. These results on the intestinal flow rates support the model describing the gastrointestinal involvement in food intake by Jobling (1984) which suggests that the emptying of food from the stomach is not a continuous smooth process, but occurs in a pulse-like fashion.

From the gastric evacuation studies conducted, an attempt was made to calculate the daily food intake by different sizes of O. niloticus (Table 32). It is apparent that feeding levels based on this data were slightly lower than those reported by other workers for tilapia (Moriarty & Moriarty, 1973; Caulton, 1982; Macintosh & De Silva, 1982 cited in Jauncey & Ross, 1982). The present estimations of food intake were based on the actual food present in the stomach without any correction made for the losses of food during the feeding period and may therefore underestimate the feeding levels necessary to ensure this level of intake. In order to obtain these levels of feed, the feeding rate would be slightly higher especially for smaller fish sizes as most of the

gastric evacuation studies were carried out on larger fish and daily food intakes for smaller sizes were predicted from them.

#### 4.3 Digestive Enzymes

In the present study it was observed that  $\alpha$ -amylase activity was higher than protease activity (Tables 33, 34, 35) which is in general accord with the statement of Al Hussaini (1947), Barrington (1957), Kapoor et al. (1975) and Hsu and Wu (1979) that the production of digestive enzymes is correlated with feeding habits. In general carnivorous fish exhibit higher digestive protease activity than herbivorous fish which tend to have higher amylase activity. Volya (1966) investigated the proteolytic, lipolytic and amylolytic activities of extracts from the alimentary canal of carnivorous and herbivorous fish and reported that highest proteolytic and lipolytic activities were found in the carnivorous fish and lowest proteolytic activity, but highest amylolytic activity, were found in herbivorous fish. Agrawal et al. (1975) reported that carbohydrase activity is higher in herbivorous fish (Labeo rohita) than in the carnivorous fish (Wallago atta) or omnivorous fish (Clarius batrachus), whilst high protease activity was found in carnivorous fish.

All digestive enzymes showed an increase in activity when fish were fed diets high in the corresponding substrate (except the high lipid diet, C). The high protein diet resulted in high peptic and tryptic activities in the stomach and intestine, the high available carbohydrate diet resulted in the highest activity of intestinal

$\alpha$ -amylase. The high lipid diet, however, did not produce any significant increase in activity of intestinal lipase (Table 33). Several previous studies have indicated that there is no lipase adaptation to increasing fat content in the diet. Chesley (1934), Naguse (1964) and Mukhopadhyay (1977) have all reported that there was no correlation between the fat content in the diet and lipase activity, no explanation of this phenomenon was attempted by these authors. The failure of lipase activity to increase in response to increasing dietary lipid content in the present study may be due to differences in the time required for each type of secretion to become adapted to a particular diet or to differences between species. Reimer (1982) reported that lipase activity increased with increasing dietary fat contents for an Amazonian fish (Matrincha melanopterus). The stimulatory effects of food upon digestive enzyme activity can be additive, but obviously there will be a limit beyond which enzyme activity cannot be stimulated any further. In the present experiment the control diet (A) contained approximately optimal levels of all nutrients (Jauncey & Ross, 1982). It is possible that greater effects on digestive enzyme activity, including lipase, would have been detected if diets containing low levels of each of the substrates (protein, lipid and carbohydrate) had been included in the study.

In the present study intestinal  $\alpha$ -amylase activity was increased in fish fed a high available carbohydrate diet (Table 33). This is in agreement with the results of Nagas (1964) who found increased  $\alpha$ -amylase activity in O. mossambicus fed a high carbohydrate diet. Similar results have been reported by Kawai and Ikeda, 1972; Shimeno

et al. (1981) for carp (Cyprinus carpio) and Reimer (1982) for Matrincha melanopterus.

Increased dietary protein stimulated intestinal trypsin-like protease activity in the present study (Table 33). This has also been reported for O. mossambicus (Nagas, 1964), carp and rainbow trout (Kawai & Ikeda, 1972, 1973) and Matrincha melanopterus (Reimer, 1982). The increased stomach pepsin-like activity with increased dietary protein contents found in the present study was not found by Nagas (1964) in O. mossambicus or by Reimer (1982) in M. melanopterus. However, Kawai and Ikeda (1973) reported increased pepsin-like activity with increasing dietary protein content in rainbow trout in agreement with the present observations.

The effect of fish size on the activities of digestive enzyme in O. niloticus was investigated (Table 34). Pepsin-like and trypsin-like activities decreased significantly ( $P < 0.05$ ) with increasing fish size (Table 34).  $\alpha$ -Amylase activity increased significantly ( $P < 0.05$ ) with increasing fish size, whilst lipase activity was little changed being significantly highest ( $P < 0.05$ ) in the smallest size group (Table 34). Reduced activities of pepsin-like and trypsin-like enzymes with increasing fish size may be as a consequence of lower food intake (as % b.w.) in larger fish. Comparison of results for the effect of fish size on digestive enzyme activities in the current investigation with those of other workers on tilapia cannot be made, since no such studies appear in the literature. The reduced activities of pepsin-like and trypsin-like activities in the present study with increasing fish size (Table 34) are in general accord with

observations by Morishita et al. (1964) on carp (Cyprinus carpio) and Hofer and Uddin (unpublished data cited in Hofer & Uddin, 1985) for Rutilus rutilus. These authors reported trypsin-like activity to be negatively correlated with fish weight. In contrast to the present study, Clark et al. (1985) and Buddington (1985) reported increased activity of pepsin-like and trypsin-like enzymes with increasing fish size for Solea solea and Acipenser fulvescens respectively.

Increased  $\alpha$ -amylase activity with increasing fish size was observed in the present study (Table 34) and has also been reported for Abramis brama (Kuzmina, 1980), Rutilus rutilus Hofer & Uddin, 1985) and Cyprinus carpio (Kawai & Ikeda, 1973). This is in contrast with the observations of Buddington (1985) for Acipenser fulvescens where  $\alpha$ -amylase activity was reduced with increasing fish size. The differences may be explained by differences between species in their feeding habits (Kapoor et al., 1975). However, comparison between the results of the present study with those of other workers cannot easily be made, because of differences in methodology with respect to extraction of enzymes, incubation times, substrates, fish size and species. In addition, many different units have been used to express enzyme activity by different authors.

Starving O. niloticus for periods of 24, 48, 72 and 96 hours resulted in general reductions in the activity of the digestive enzymes and it was also apparent that ingested food serves as a stimulus to enzyme secretion (Table 35). These reductions in digestive enzyme activities correlate with the results of the effect of prefeeding

starvation periods on gastric evacuation rate and time obtained in the previous section (3.2.2.5). It appears that reduced gastric evacuation rate (g/h) and prolonged gastric evacuation time with increasing starvation period could be an adaptive mechanism to maximise the utilization of food ingested after a period of starvation. This explanation has been suggested by Elliott (1972), Windell (1978) and Jobling (1980). The results of the present study are in general agreement with the observations of Thomas and Nation (1984) for Scapteriscus acletus, who observed reductions in the level of all digestive enzymes after starvation.

#### 4.4 Growth Studies

Mean daily food intake of O. niloticus fed to satiation were raised by increasing feeding frequency only up to six meals per day. At higher frequencies of feeding no differences in total daily food intake was observed (Table 36), in general accord with earlier observations made in the present study (Section 3.1.1).

In the present study as the experiment progressed daily food intake at each feeding frequency was found to decrease (as % b.w.) (Table 37). This is not unexpected as maximum daily food intake is known to decrease with increasing fish weight (Pandian, 1967; Grove & Crawford, 1980; present study, 3.1.2).

Total daily food consumption was significantly different ( $P < 0.05$ ) for each of the feeding frequencies at the start of the

experiment (Table 37). During the course of the experiment these differences diminished and by the end of the experiment there were no significant differences between daily food intake of fish fed at different frequencies (Table 37). It is likely that this indicates the presence of an adaptive mechanism; Fish fed less frequently gradually increased their satiation meal size by increasing their stomach capacity, thus becoming hyperphagic. Unfortunately no measurements of stomach volume were performed in this investigation to support this theory, however the results obtained in Section 3.1.4 indicated that fish increased their stomach volume in response to increasing intervals between feeding. Evidence for this proposed hyperphagia may also be obtained by comparison of directly measured food intake in this long term study (Table 37) with predicted levels of food intake from single measurements of food intake (Section 3.1.3). For example, the predicted food intake from a single meal measurement is 1.8% b.w. for a 15g fish compared to 4% b.w. obtained from the present study (Table 37). Daily food consumption in the present investigation is higher than predicted from single measurements of food intake in Section 3.1.3 supporting this theory. There have been few long-term studies into the effects of feeding pattern on growth and food intake in fishes which indicate the development of hyperphagia (Tyler & Dunn, 1976; Jobling, 1982; Singh & Srivastava, 1984). Brown (1957) reported that brown trout respond to reduced feeding frequency by increasing food intake. When fish were allowed only restricted access to food they ingested so much more that they bulged markedly after each meal. Fish fed more frequently never showed this phenomenon. Rainbow trout (Salmo gairdneri) also respond to reduced feeding frequency by displaying hyperphagia. Grayton & Beamish (1977) state that:

"rainbow trout allowed to consume food to satiation at one meal per day ingested significantly less than fish fed either three or six meals per day during the first ten days of the experiment, during the remaining 20 days differences among the three treatments were not significant".

which indicates the development of hyperphagia during the last 20 days.

The present study demonstrates, under controlled conditions, that feeding frequency does not directly affect body composition or growth; changes in these parameters are more likely to be a function of the total quantity of food consumed. Feeding frequency does, however, influence the food intake of fish. This relationship is such that food intake increases with feeding frequency to some maximum. The lowest frequency at which this is achieved may be termed the optimum feeding frequency. Taking the maximum value of the daily rate of growth as 100% it is proposed that the optimum frequency of feeding is that which produces 85% of this maximum growth rate (Ishiwata, 1969). The present study reveals that fish grew well, showed good food utilization and that optimum growth rate was achieved when fish were fed to satiation at two meals per day. Although fish fed once per day grew well and showed better food conversion, the overall growth rate was significantly lower (Table 40) than for fish fed at higher feeding frequencies, principally as a reflection of lower food consumption during the first five weeks of the trial (Table 37). Feeding frequencies greater than two meals per day did not yield corresponding increases in growth rate, but tended to slightly increase food conversion (Table 40). Increased food conversion ratios at higher feeding frequencies indicate that the food ingested was less efficiently utilised. It is possible that incomplete

digestion led to reduced utilization of the fish food ingested. The dry matter and apparent protein digestion coefficients in the present study (Table 40) were found to decrease with increasing feeding frequency supporting this explanation. Dawes (1930) found, in Pleuronectes platessa, that a second meal taken a short interval after the first, caused food to leave the stomach and pass down the alimentary canal more quickly. Under such circumstances digestion of food may well be less efficient. Fletcher (1982) reported that under a multiple feeding regime a meal was evacuated faster when followed three hours later by a second meal in dab (Limanda limanda). Similar observations have been reported by Laurence, 1971; Kelso, 1972; and Noble, 1973. Food remaining in the alimentary canal for a longer period of time may allow for more complete enzymatic degradation resulting in more efficient digestion and absorption (Windell, 1978; Jobling, 1980).

The 'optimum' feeding frequency for O. niloticus in the present study is taken as two meals per day since it resulted in 85% of the maximum growth rate observed. Optimum feeding frequency may be expected to vary with species, size, temperature and physical and chemical composition of the diet. The optimum feeding frequency is once every two days for Epinephelus tavinna (Thia-Eng & Song-Keh, 1978), twice a day for Ictalurus punctatus (Andrew & Page, 1975), once a day for Channa striatus and Salvelinus alpinus (Sampth, 1984; Jobling, 1983) and continuous feeding for Clarius lazera (Hogendoorn, 1981).

Since increasing the feeding frequency results in a significant increase in food intake, with no corresponding increase in weight

gain or specific growth rate beyond two meals per day, it seems that a portion of food was wasted at the higher feeding frequencies either by leaching of nutrients or by incomplete digestion, as has been mentioned earlier. It is further suggested that the feeding rate rather than feeding frequency affects the growth of O. niloticus directly. It is presumed that physical bulk limited the food intake in the group fed once per day. This suggestion is supported by a further experiment which showed that when groups of O. niloticus were fed equal amounts of food (6% b.w.) divided into 2, 4 and 6 meals per day, no statistically significant differences were observed between the growth rates of the different treatments (Table 42). These results are consistent with observations made by Teshima et al. (1986) with O. niloticus, who reported that neither growth rate nor food conversion ratio were affected by feeding frequency, whilst feeding rate significantly affected growth rate, food conversion and protein efficiency.

The frequency with which food is consumed appeared to affect body composition of O. niloticus (Table 40). With satiation feeding those groups fed more frequently tended to deposit more lipid in their carcasses whilst exhibiting decreasing moisture and ash contents since overall food intake is greater. In the restricted feeding experiment no significant differences in carcass protein or ash contents between treatments were observed (Table 43). However, lipid contents were found to increase significantly ( $P < 0.05$ ) with increasing feeding frequency. These results are consistent with the observations made by Grayton and Beamish (1977) and Jobling (1982) for rainbow trout and plaice, respectively, who reported an increase in carcass lipid content with increasing feeding frequency.

O. niloticus, of approximately similar sizes, when reared together in both feeding frequency experiments exhibited a definite hierarchy. The populations initially had small coefficients of variance (c.v.) at the start of both experiments, which tended to increase by the end of the experiment (Tables 41 and 44). An increase in the c.v. with time is indicative of growth suppression of certain individuals within a population. Suppression of the growth of small individuals may be principally due to disproportionate food acquisition by subdominant fish caused by the aggression of a few dominant fish. The establishment of size hierarchies may be caused by direct competition for food (Magnuson, 1962; Eaton & Farley, 1972). In the case of competition, size hierarchies are established when food supply is limiting and few individuals monopolise the available food to the exclusion of others. When adequate food is available the size hierarchy effect is either reduced or abolished (Magnuson, 1962; Eaton & Farley, 1972). However, the present study clearly shows the development of size hierarchies even when fish were fed more frequently (Tables 41 and 44). This suggests that size hierarchies may develop because smaller fish are inhibited from feeding in the presence of larger fish (Purdom, 1974). Brown (1946) suggested that smaller fish suffer from social stress in the presence of larger ones and that increased production of adrenocortic trophic hormone (A.C.T.H) in these individuals inhibits their growth. A similar phenomenon has been reported for brown trout (Brown, 1957), carp (Wohlfarth & Moav, 1972; and Hassan, 1986), Channa striatus (Sampath & Panidan, 1980), Arctic char (Jobling & Wandsvik, 1983) and tilapia (Saclauso, 1985). The results of the present study indicate that social interaction between individuals led to increased size variation which was inde-

pendent of food availability. The fact that growth of smaller O. niloticus is inhibited in the presence of larger fish suggests that under commercial culture conditions O. niloticus might benefit from regular grading.

The effect of feeding rate on growth and body composition of two size classes of O. niloticus was investigated (Figs. 56 and 57). The relationship between growth and feeding rate shows that specific growth rate increased to varying degrees with increasing feeding rate in both size classes of fish examined (Tables 45, 46). However, specific growth rates for the larger size group (14.27) reached a maximum at 3% b.w., feeding rates above 3% b.w. led to a decline in growth rate. Huisman (1976), Meske (1985) and Hassan (1986) for carp, Shell (1969) for O. mossambicus, Andrew and Page (1972), Reddy and Katne (1979), Andrew (1979) and Arunachalam and Reddy (1981) for catfish observed a fall in growth rate with high levels of feeding. The decline in growth rate at high feeding rates could be explained by the observations made by Paloheimo and Dickie (1965, 1966a, b). These authors showed that the metabolic rate of fish increases 4-5 times as food ration increases from the maintenance level. Working on C. striatus, Vivekanandon (1976) reported a 3.5 times increase in specific dynamic action (S.D.A.) as the ration was increased from maintenance to the maximum level. Similar observations have been made by Arunachalam and Reddy (1981) with catfish (Mystus vitatus). Thus it may be concluded that excessive feeding would induce a high metabolic rate, resulting in a significant reduction in the amount of energy available for growth. In contrast to the present study Brett et al. (1969) found with sockeye salmon (Oncorhynchus nerka)

that increasing feeding rate (1.5% to excess) for a 13g fish increased the growth rate up to an asymptote beyond which it remained fairly constant.

Increasing the feeding rate from 4% b.w. to 6% b.w. for the smaller size group of fish evaluated in the present study did not result in a significant increase in body weight (Table 46). This suggests that maximum growth rate for this group occurred with a ration size between 4% and 6% b.w. Comparison of data in Tables 45 and 46 (large and small fish groups, respectively) shows that smaller fish had higher specific growth rates, at each comparable feeding rate, which is in general accord with observations made by Elliott (1976), Wurtsbaugh and Davis (1977) and Katonda (1979), who reported a decrease in growth rate with increasing fish size. Smaller size group fish fed 1% b.w. (Table 46) showed a reduction in specific growth rate, high food conversion ratio, lower P.E.R. and lower apparent protein utilization than larger fish fed 1% b.w. (Table 45). This may be attributed to the higher maintenance requirement for smaller fish (Fig. 59). Comparison between smaller and larger sizes of fish in terms of food intake for maintenance, optimum and maximum growth revealed that smaller fish had higher requirements than larger fish (Figs. 58, 59), which suggests a higher metabolic rate for smaller fish (Fry, 1957; Brett & Groves, 1979). Fish starved in both size classes lost weight during the experimental period (Tables 45, 46). Weight losses during the experimental period were higher in smaller than in larger fish (Tables 45, 46). These losses in weight may be due to the catabolism of fat and protein tissue stores as observed in Lepomis macrochirus (Savitz, 1971), Mystus vittatus

(Arunachalam & Reddy, 1981), Heteropneustes fossilis (Reddy & Katne, 1979), O. mossambicus (Pandian & Raghuraman, 1972) and rainbow trout (Reinitz, 1983).

Although many workers have published analysis of the body composition of fish, few have examined the change in body composition in relation to body size and ration size (Parker & Vanstane, 1966; Brett et al., 1969; Elliott, 1976). Variation in ration size had significant effects ( $P < 0.05$ ) on carcass chemical composition in both size classes of O. niloticus examined (Tables 45 and 46). For both size classes carcass protein and carcass lipid contents increased significantly ( $P < 0.05$ ) with increasing ration size (Tables 45b and 46b); there was a concomitant decrease in carcass moisture and ash contents. Increasing food intake is thought to have resulted in increased energy storage in the carcass in the form, principally, of tissue lipids. These observations are in general agreement with those of other workers (Groves, 1970; Elliott, 1976; Brett et al., 1969; Reinitz, 1983) who reported similar increases in both carcass protein and carcass lipid with increasing feeding rates.

In O. niloticus there was a striking relationship between carcass lipid, carcass protein and carcass moisture contents - an increase in the proportion of one led to a decrease in the other (Figs. 60 and 61), so that the sum remained approximately constant. This relationship has been described in the literature by several workers in a variety of species (Love, 1960; Iles & Wood, 1965; Brett et al., 1969; Niimi & Beamish, 1974; Elliott, 1976; Caulton & Bursell, 1977).

Several workers have reported that when salmonids were starved there was an immediate decrease in carcass lipid content followed by a gradual decrease in carcass protein contents, whilst water content showed a considerable increase (Idler & Clemens, 1959; Phillips et al., 1966; Reinitz, 1983). Similarly in O. niloticus in the present study water content was higher in the starved groups of both size classes. Increases in carcass moisture content of fish due to starvation have also been observed in O. mossambicus (Pandian & Raghuraman, 1972), Gambusia affinis (Katne, 1977), Mystus vittatus (Arunachalam & Reddy, 1983) and Salmo gairdneri (Reinitz, 1983). In fish the primary energy sources during starvation appear to be derived from the catabolism of fat and skeletal muscle protein by the process of gluconeogenesis (Simpson, 1965; Kamara, 1966; Ince & Thorpe, 1976). This may explain the observations in the present study. Carcass lipid contents of the starved groups (Tables 45 and 46) decreased more than carcass protein, suggesting a greater emphasis on the former as dietary energy source (Livingston & Posten, 1966; Pandian & Raghuraman, 1972; Reinitz, 1983). The decrease in carcass lipid and protein contents was more pronounced in the smaller fish than in the larger ones, possibly as a consequence of their higher metabolic rates (Brett & Groves, 1979; Caulton, 1982).

In the experiment evaluating the effect of four feeds of widely varying composition on growth and body composition of O. niloticus (Table 48) diet composition had a large effect on all the nutritional parameters measured. Increasing the carbohydrate, lipid and protein contents of the diets to levels higher than the control diet (A) tended to improve the growth rate and food conversion of fish fed these diets

(Table 48a). Lower protein efficiency ratios and utilization were observed in the group fed the high protein diet (49%).

A decrease in protein utilization with increasing dietary protein levels has been reported for many species including Tilapia zillii (Mazid et al., 1979), O. mossambicus (Jauncey, 1982), O. niloticus (Wang et al., 1985; De Silva & Perera, 1985), Cyprinus carpio (Ogino & Saito, 1970), Morone saxatilis (Millikin, 1982), and Seriola quinqueradiata (Tekeda et al., 1975). Highest P.E.Rs and A.N.P.Us were obtained for the group fed the high lipid diet (Diet C) followed by the group fed the high carbohydrate diet (Diet B) (Table 48a). As fish eat to satisfy their energy requirement (Lee & Putnam, 1973; Page & Andrews, 1973; Fletcher, 1982) it seems that increasing the level of lipid or carbohydrate in the diet increased the energy to protein ratio (DE:P).

More efficient use of protein, in terms of utilization, occurred in the high lipid diet (Diet C) followed by the high carbohydrate diet (Diet B) (Table 48a). Thus in a diet with a high energy content in relation to the percentage of dietary protein, less protein is wasted as an energy source, which in turn means that less protein would need to be consumed to meet the protein requirement. Increasing the dietary lipid or carbohydrate content appears to spare dietary protein for growth in O. niloticus resulting in improved protein utilization and reduced catabolism of dietary protein as an energy source. Similar sparing effects have been demonstrated in numerous species including rainbow trout (Reinitz et al., 1978; Bergot, 1979; Beamish & Medland, 1986), channel catfish (Murray et al., 1977; Dupree

et al., 1979; William & Stickney, 1980), yellowtail (Shimeno et al., 1985), turbot (Adron et al., 1976), sea bass (Alliot et al., 1978) and carp (Jauncey, 1979; Ufodik & Matty, 1983). Contrary to the present study Shimeno et al. (1978) reported poor growth and food conversion in carp fed diets containing 10%-20% carbohydrate compared to the control fish receiving no carbohydrate. Murai et al. (1985) reported that increasing lipid contents in the diet of carp did not improve the growth or the protein utilization, but only increased the carcass lipid proportionately to the supplemented level, which contrasts with the present observations. However this might be explained by the higher level of  $\alpha$ -cellulose in their diet which might interfere with the digestion of protein.

Proximate carcass analysis of fish fed the four experimental diets (Table 48b) shows that increasing the proportion of dietary lipid (Diet C) resulted in a significant increase in carcass lipid content, with a slight decrease in carcass moisture. Similar responses to increased dietary lipid levels have been previously reported (Dupree, 1969; Page & Andrews, 1973; Reinitz et al., 1978; Jauncey, 1979; Reinitz & Hitzel, 1980; Reinitz, 1983; Millikin, 1983; Murai et al., 1984) implying that dietary energy excess to requirement is stored in carcass lipid as an energy source. Carcass protein content was highest in fish fed the high protein diet (Diet D), but varied little between the other three diets (Table 48b). Similar trends have been reported by other workers (Cowey et al., 1972; Satia, 1974; Takeda et al., 1975; Millikin, 1983). Carcass moisture and ash contents of fish fed the four experimental diets did not vary significantly

( $P < 0.05$ ) although carcass moisture was slightly lower in fish fed the high lipid diet (Diet C).

High levels of body fat have been historically regarded as detrimental to the health of fish (Phillips & Podoliak, 1957). However, recent studies (Lee & Wales, 1973; Reinitz et al., 1978; Jauncey, 1979; Shimeno et al., 1985) indicate that high dietary, and consequently carcass lipid levels, are not detrimental to fish as long as lipid quality is good. High carcass lipid contents may protect fish from the effect of starvation by providing a readily mobilised energy source (Bilinski, 1963; Savitz, 1971; Ince & Thorpe, 1976). An optimal balance between dietary protein, lipid and carbohydrate levels will result in improved utilization of dietary protein in anabolic, rather than catabolic, biochemical pathways.

#### 4.5 Estimation of Daily Food Intake

Preliminary investigation of the effect of feeding frequency on daily food consumption by O. niloticus fed to satiation showed that maximum food intake occurred when fish were fed six meals per day (Table 10). This feeding frequency was subsequently employed to investigate the relationship between daily food intake and fish weight (3.1.2). Maximum voluntary food intake was found to vary between 2.4% and 9.0% of the body weight per day (dry food/whole wet fish) for 200g and 10g fish respectively. As food consumption is a primary growth influencing factor (Brett, 1979; Jobling, 1983) the effect of feeding frequency on daily food intake, food utilization and growth was further investigated (3.4.1). A long term study was

conducted with approximately 16g fish to establish the accuracy of the maximum daily food intake values obtained in Section 3.1.2. This long term study showed that increasing the feeding frequency resulted in a significant increase in food intake with no corresponding increase in weight gain or specific growth rate beyond two meals per day (Table 40a). It appears therefore, that a proportion of food presented was wasted at higher feeding frequencies either as a result of incomplete ingestion or incomplete digestion of the food fed. It is likely that if a fairly constant percentage of the food fed at each feeding time was wasted (unconsumed) then more food in total (per day) would be wasted at higher feeding frequencies leading to an apparent increase in total daily food intake. Incomplete digestion may lead to reduced utilization of ingested food, particularly with increasing meal size or feeding frequency (Fletcher, 1982). Feeding frequency may affect the process of digestion and absorption of nutrients through an effect on gastrointestinal evacuation rates. Stimulation of evacuation by ingestion of meals more frequently reduces the time for which food is exposed to the processes of digestion and absorption and will result in reduced food utilization.

Several authors have reported that the percentage loss of nutrients in the faeces increased with increasing ration size (Kinne, 1960; Pandian, 1967). Pandian and Raghuraman (1972) reported that at the maximum food consumption rate for O. mossambicus a greater percentage of undigested food was evacuated in the faeces and that the specific dynamic action (S.D.A.) was double that found at the optimum feeding rate. This suggests that not only is part of the food ingested not absorbed, but that food which is absorbed presents a greater metabolic

demand in terms of energy requirements. Food remaining in the alimentary canal for a longer period of time will allow for more efficient enzymatic degradation resulting in more efficient digestion and utilization of the ingested food (Windell, 1978; Jobling, 1980). The maximum daily food intakes for different weights of tilapias obtained in Section 3.1.2 appear high and are therefore not recommended for feeding O. niloticus in commercial culture situations.

Predicted daily food intakes at optimum feeding frequencies in the present study were found to vary between 2.1% b.w. and 4.2% b.w. for 20g and 45g of fish respectively. Daily food consumption estimates for commercially important fish species in the wild as well as of cultivated fishes have been made from digestion time and rate measurements in the laboratory (Elliott, 1975a, b; Thorpe, 1977; Elliott and Persson, 1978; Diane, 1979; Grove et al., 1985). Such estimates are based on the assumption that food intake is closely related to the available gastric capacity. Voluntary food intake (appetite) is presumed to be zero when the stomach is full and becomes greater than zero with decreasing stomach contents. Several reports have shown that appetite in fish is inversely related to stomach fullness (Brett and Higgs, 1970; Ware, 1972; Elliott, 1975a, b; Grove and Crawford, 1980). The relationship between stomach filling and evacuation will depend on the feeding regime, including period of prefeeding starvation. Starvation periods are known to affect the subsequent consumption of food and evacuation time and rate (Windell, 1966; Jones, 1974; Elliott, 1972; Talbot, 1985; present study). Talbot (1985) argued that estimates of daily food intake from gastric evacuation studies could be affected by the experimental methods

used to calculate the evacuation time and rate. Most of the laboratory studies on gastric evacuation require handling and starving the fish before and after the experimental meal is offered. Talbot (1985) recommended the use of sophisticated methods based on demand feeders or labelled food since these methods provide greater flexibility in experimental feeding regimes and allow appetite to be studied in a more direct way under natural conditions.

In the present study, following initial trials to compare different techniques, the method of sequential slaughter was used for all digestion studies in O. niloticus (3.2.1). This required starving of fish before and after the experimental meal was offered. From the digestion studies an attempt was made to calculate daily food consumption for different fish weights based on the assumption that appetite is closely related to stomach evacuation time (Table 32). The calculated daily food intake was found to vary between 4.0% and 1.1% b.w. per day for 5g and 200g fish respectively. To evaluate the accuracy of these feeding levels a long term trial was initiated with two different weights of fish (6.8g and 14.3g). This study showed that maximum growth rates occurred at feeding rates of 3.5% b.w. per day and 5.4% b.w. per day for 14.3g and 6.86g of fish respectively (Figs. 58, 59). Furthermore the growth response of fish of mean weight 14.3g decreased when the feeding level was further increased to 4% b.w. per day. These feeding rates are in a close agreement with those calculated from the gastric evacuation studies for similar weights of fish (Table 32). It appears that the starvation period employed in the gastric evacuation studies had no significant effect on calculation of daily food intake. The feeding

rates presented in Table 32 can therefore be suggested for feeding of O. niloticus in intensive culture systems. It must be borne in mind that these recommended feeding rates are based on actual dry food present in the stomach without any correction being made for losses of food during the feeding period (non-ingested). In order to correct for this it can be assumed that the feeding rate required would be slightly higher than predicted, especially for smaller sizes of fish where higher proportions of food wastage are likely.

5. Summary Conclusion

1. Daily food intake increased with increasing feeding frequency up to six meals per day. Further increase in feeding frequency had no significant effect on total daily food consumption.
2. Maximum daily food intake was found to increase with fish weight raised to the power of 0.56 and it varied between 2.4% and 9.1% b.w. per day for 200g and 10g fish respectively.
3. Satiation meals varied with fish weight raised to the power 0.86. The time to reach satiation (10-15min) was found to be adequate to satiate the different sizes of fish using the stomach capacity as the criterion.
4. Different weights of fish respond to starvation periods (h) by increasing their food intake up to 72h of starvation.
5. No significant effect on liver weight was observed with starvation period, but intestine length decreased significantly with starvation.
6. Gall bladder weight and colour could be used as a useful indicator of recent feeding history in O. niloticus.
7. Increasing the experimental temperature will decrease the stomach evacuation time and increase stomach evacuation coefficient. Stomach evacuation time was found to vary with temperature raised to the power -1.17 and stomach evacuation coefficient increased with temperature raised to the power 0.029.

8. For any given meal size, expressed as % b.w., the larger the fish the longer is the time required to evacuate that meal. Stomach evacuation time increased with fish size raised to the power 0.13.
9. For a fish of given weight the larger the meal (as % b.w.), the longer the time to complete stomach evacuation. Stomach evacuation time increased with meal size raised to the power 0.33.
10. The rate of stomach evacuation as g/h is faster the larger the meal (g). Stomach evacuation rate was found to vary as meal size raised to the power 0.72.
11. Variation in dietary levels of lipid, carbohydrate and protein had a marked effect on both stomach evacuation rate and time.
12. Increasing the prefeeding deprivation time led to a significant increase in stomach evacuation time (h) and to a decrease in stomach evacuation coefficient. Stomach evacuation time increased with starvation to the power 0.096 and stomach evacuation coefficient decreased to the power -0.00031.
13. The daily food intake based on stomach evacuation time for different weights of O. niloticus varied between 4.00% b.w. and 1.1% b.w. for 5g and 200g fish, respectively.
14. Food composition had a significant effect on digestive enzymes activities (except lipase).

15. Pepsin-like and trypsin-like enzyme activity decreased with increasing fish size, but  $\alpha$ -amylase activity increased with fish size.
16. Ingested food helps regulate digestive enzyme secretion and fish deprived of food for 24 hrs to 96 hrs showed marked reduction in their digestive enzyme activities.
17. Long-term study showed that optimum feeding frequency for O. niloticus is two feeds per day and that fish respond to lower feeding frequency by becoming hyperphagic.
18. Specific growth rate (% per day) increased to various degrees with increasing feeding rate (% b.w.) for both sizes of fish investigated. Smaller fish (6.86g) showed higher requirement than larger ones in terms of food intake for maintenance, optimum and maximum growth. Furthermore the maximum feeding rate for both sizes of fish is in close agreement with what has been estimated from gastric evacuation studies supporting the recorded level of daily feeding.
19. Diet composition showed a large effect on all nutritional parameters measured. A significant protein sparing effect was observed with increasing dietary level of lipid and carbohydrate.

REFERENCES

- ADRON, J. W., BLAIR, A., COWEY, C. B. and SHANKS, A. M. (1976).  
Effects of dietary energy level and dietary energy source on  
growth, food conversion and body composition of turbot  
(Scophthalmus maximus). Aquaculture, 7: 125-132
- ADRON, J. W., GRANT, P. T., and COWEY, C. B. (1973). A system for  
the quantitative study of the learning capacity of rainbow  
trout and its application to the study of food preferences and  
behaviour. J. Fish. Biol. 5: 625-636
- AGRAWAL, V. P., SASTRY, K. V. and KAUSHAB, S. K. S. (1975).  
Divestive enzymes of three teleost fishes. Acta. Physiol. Acad.  
Sci. Hungarica, Tomus (46)2: 93-98
- AL-HUSSAINI, A. H. (1947). On the functional morphology of the  
alimentary tract of some fish in relation to differences in  
their feeding habits. Quart. J. Micr. Sci. 90: 323-354
- AL-HUSSAINI, A. H. and KHOLY, A. A. (1953). On the functional  
morphology of the alimentary tract of some omnivorous teleost  
fish. Proc. Egypt. Acad. Sci. 9: 17-39
- ALLIOT, E., PASTOURE, A., and NEDELEC, J. (1978). Etude de l'apport  
calorique et du rapport calorico-azote dans l'alimentation du  
Bar (Dicentrachus labrax). Influence sur la croissance et la  
composition corporellein. EIFAC Symposium on Fin Fish Nutrition  
and Food Technology, 10, Hamburg, June 1978. Dic. EIFAC/78: 122pp
- ANDREW, J. W. (1979). Some effects of feeding rate on growth, food  
conversion and nutrient absorption of channel catfish.  
Aquaculture, 16: 243-246
- ANDREW, J. W. and PAGE, J. W. (1975). The effects of frequency of  
feeding on culture of catfish. Trans. Am. Fish. Soc. 104(2):  
317-321

- ANDREWS, J. W. and STICKNEY, R. R. (1972). Interaction of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. Trans. Am. Fish. Soc. 1: 94-99
- ANSON, M. L. (1938). The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. J. Gen. Physiol. 22: 79-89
- A.O.A.C. (1980). Official Methods of Analysis, 13th ed., Association of Official Analytical Chemists, Washington DC: 1018pp
- ARUNACHALAM, S. and REDDY, S. R. (1981). Interactions of feeding rates on growth, food conversion and body composition of the freshwater cat fish (Mystus vittatus). Hydrobiologia, 78: 25-32
- AVERETT, R. C. (1969). Influence of temperature on energy and material utilization by juvenile coho salmon. PhD thesis, Oregon State University, Corvallis
- BAGGE, O. (1977). Meal size and digestion in cod (Gadus morrhua) and sea scorpion (Myoxocephalus scorpius). MeddrDanm. Fisk-og Havunders, 7: 437-446
- BAJKOVE, A. D. (1935). How to estimate the daily food consumption of fish under natural conditions. Trans. Am. Fish. Soc., 65: 288-289
- BALARIN, J. D. and HATTON, J. P. (1979). Tilapia - A guide to their biology and culture in Africa. University of Stirling, Stirling, Scotland: 174pp
- BALDWIN, N. W. (1956). Food consumption and growth of brook trout at different temperatures. Trans. Am. Fish. Soc., 86: 323-328

- BARDACH, J. E., RYTHER, J. H. and McLARNEY, W. O. (1972).  
Aquaculture: The Farming and Husbandry of Freshwater and Marine Organisms. Wiley-Interscience, New York.
- BARRINGTON, E. J. W. (1957). The alimentary canal and digestion.  
In: The Physiology of Fishes (Brown, M. E., ed), Vol. 1:  
109-161. Academic Press, New York
- BASSIMI, R. A. (1978). Feeding and growth in plaice (Pleuronectes platessa). PhD thesis, University College of Wales
- BASSIMI, R. A. and GROVE, D. J. (1985). Gastric emptying rate in  
Pleuronectes platessa. J. Fish. Biol., 26: 545-552
- BEAMISH, F. W. H. (1964). Respiration of fishes with special emphasis  
on standard oxygen consumption. II. Influence of weight and  
temperature on respiration of several species. Can. J. Zool.  
42: 177-178
- BEAMISH, F. W. H. (1972). Ration size and digestion in largemouth  
bass, Micropterus salmoides. Can. J. Zool., 50: 153-164
- BEAMISH, F. W. H. and MEDLAND, T. E. (1986). Protein sparing effect  
in large rainbow trout (Salmo gairdneri). Aquaculture, 55:  
35-42
- BENNFELD, P. (1955). Amylase,  $\alpha$  and  $\beta$ . In: Methods of Entomology  
(Colowick, S. P., ed), Vol. 1: 149-150. Academic Press,  
New York
- BERGOT, F. (1979). Carbohydrate in rainbow trout diet: Effects of  
the level and source of carbohydrate and the number of meals  
on growth and body composition. Aquaculture, 18: 157-167
- BEUKEMA, J. J. (1968). Predation by the three-spined stickleback  
(Gasterosteus aculeatus): The influence of hunger and  
experience. Behaviour, 31: 1-126

- BILINSKI, E. and GARDNER, L. J. (1963). Effect of starvation on free fatty acid level in blood plasma and muscular tissues of rainbow trout. J. Fish. Res. Board Can., 25: 1555-1560
- BILTON, H. T. and ROBBINS, G. L. (1973). The effect of starvation and subsequent feeding on survival and growth of Fulton channel sockeye salmon fry (Oncorhynchus nerka). J. Fish. Res. Board Can., 30: 1-5
- BOWEN, S. H. (1978). Chromic acid in assimilation studies - a caution. Trans. Am. Fish. Soc., 107: 755-756
- BOWEN, S. H. (1979). Chromic oxide in food assimilation studies. Comments. Trans. Am. Fish. Soc., 108: 650-652
- BOWEN, S. H. (1981). Digestion and assimilation of periphytic detrital aggregate by Tilapia mossambica. Trans. Am. Fish. Soc., 110: 239-245
- BOWEN, S. H. (1982). Feeding, digestion and growth - qualitative consideration. In: The Biology and Culture of Tilapias. Pullin, R. S. V. and Lowe-McConnell, R. H. (eds.). ICLARM (Manila) Proc. Int. Conf. on the Biology and Culture of Tilapias (1980).
- BRETT, J. R. (1971). Satiation time, appetite and maximum food intake of sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Board Can., 28: 409-415
- BRETT, J. R. (1979). Environmental factors and growth. In: Fish Physiology (Hoar, W. S., Randall, D. J. and Brett, J. R., eds.) Vol. VIII: 599-675. Academic Press, New York.
- BRETT, J. R. and GROVES, T. D. D. (1979). Physiological energetics. In: Fish Physiology (Hoar, W. S., Randall, D. J. and Brett, J. R., eds.), Vol. VIII. Academic Press, New York.

- BRETT, J. R. and HIGGS, D. A. (1970). Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Board Can., 27: 1767-1779
- BRETT, J. R. and SHELBOURN, J. E. (1975). Growth rate of young sockeye salmon (Oncorhynchus nerka), in relation to fish size and ration level. J. Fish. Res. Board Can., 32: 2103-2110
- BRETT, J. R., SHELBOURN, J. E. and SHOOP, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Board Can., 27: 1767-1779
- BRETT, J. R. and ZELA, C. A. (1975). Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon under controlled conditions. J. Fish. Res. Board Can., 32: 2479-2486
- BRODUER, R. D. (1984). Gastric evacuation rate for two foods in the black rock fish (Sebastes melanops). J. Fish. Biol., 24: 287-298
- BROWEN, M. E. (1946). The growth of brown trout (Salmo trutta). II. The growth of two-year old trout at a constant temperature. J. Exp. Biol., 22: 130-144
- BROWEN, M. E. (1957). Experimental studies on growth. In: Fish Physiology (Browen, M. E., ed.), Vol. 1. Academic Press, New York.
- BRYANT, P. L. and MATTY, A. J. (1980). Optimisation of Artimia feeding rates for carp larvae (Cyprinus carpio). Aquaculture, 11: 203-212
- BUCHS, S. (1954). Die proteolyse im Turnagen. Zeitschrift fur vergleichende physiologie, 36: 165-175

- BUDDINGTON, R. K. (1985). Divestive secretions of lake sturgeon (Acipenser fulvescens) during early development. J. Fish. Biol., 26: 715-723
- CARLIN, R. F. and HALL, J. D. (1973). Evaluation of a method for estimating food consumption rates of fish. J. Fish. Res. Board Can., 30: 623-629
- CAULTON, M. S. (1978). The importance of habitat temperature for growth in the tropical cichlid Tilapia rendalli. J. Fish. Biol., 13: 99-122
- CAULTON, M. S. (1982). Feeding, metabolism and growth of Tilapias: Some quantitative considerations. In: The Biology and Culture of Tilapias (Pullin, R. S. V. and Lowe-McConnell, R. H., eds.). ICLARM Conf. Proc. 7: 432pp. International Center for Living Aquatic Resources Management, Manila, Philippines.
- CAULTON, M. S. and BURSELL, E. (1977). The relationship between changes in condition and body composition in young Tilapia rendalli. J. Fish. Biol., 11: 143-150
- CHARLES, P. M., SEBASTIAN, S. M., VICTOR, M. C. and MARIAN, M. P. (1984). Effect of feeding frequency on growth and food conversion of Cyprinus carpio fry. Aquaculture, 40: 293-300
- CHESLEY, L. C. (1934). The concentration of proteases, amylase and lipase in certain marine fish. Biol. Bull., 66: 133-144
- CLARK, J., MacDONALD, N. L. and STARK, J. R. (1985). Metabolism in marine flat fish. II. Protein digestion in Dover sole (Solea solea) Comm. Biochem Physiol. B., 81: 217-222
- COLT, J. E. and ARMSTRONG, D. A. (1981). Nitrogen toxicity to crustaceans, fish and molluscs. Bio-engineering Symposium for fish culture. (FCS publ. 1): 34-47

- COWEY, C. G., POPE, J. A., ADRON, J. W. and BLAIR, A. (1972).  
Studies on the nutrition of marine flat fish. The protein  
requirement of plaice (Pleuronectes platessa). British J.  
of Nutrition, 28: 447-456
- CRAWFORD, C. (1977). Food consumption and digestion in Blennius  
pholis. MSc thesis, University of Wales.
- CRIDLAND, C. (1962). Laboratory experiments on the growth of  
Tilapia spp. The effect of light and temperature on the  
growth of T. zillii in aquaria. Hydrobiologia, 20: 155-166
- DAAN, N. (1973). A quantitative analysis of the food intake of  
North Sea cod, Gadus morhua. Neth. J. Sea Res., 6: 479-517
- DABROWSKI, K. (1979). The role of proteolytic enzyme in fish digestion.  
In: Cultivation of fish fry and its live food (Styczniska-Jurewicz,  
E., Backiel, T., Jaspersand, E., and Persoone, G. (eds.)). Eur.  
Maricult. Soc., Bredene, Belgium. Special Publ. 4: 107-126
- DARNELL, R. M. and MEIEROTTO, R. R. (1962). Determination of feeding  
chronology in fishes. Trans. Am. Fish. Soc., 91: 313-320
- DAVIES, S. J. (1984). The nutritional value of dietary fibre for  
rainbow trout (Salmo gairdneri). PhD thesis, Institute of  
Aquaculture, University of Stirling, Scotland.
- DAWES, B. (1930). The absorption of fats and lipoids in plaice.  
J. Mar. Biol. Assoc. UK, 17: 75-102
- DE SILVA, S. S. and OWEYEMI, A. A. (1983). Effect of dietary quality  
on the gastric evacuation and intestinal passage in Sarotherodon  
mossambicus fry. J. Fish. Biol., 23: 347-355

- DE SILVA, S. S. and PERERA, M. K. (1985). Effect of dietary protein level on growth, food conversion and protein use in young Tilapia nilotica at four salinities. Trans. Am. Fish. Soc., 114: 584-589
- DIAMOND, J. M. (1962). The mechanism of water transport by the gall bladder. J. Physiol. London, 161: 503-527
- DIANA, J. S. (1979). The feeding pattern and daily ration of a top carnivore, the northern pike (Esox lucius). Can. J. Zool., 57: 2121
- DUNCAN, D. V. (1955). Biometrics, Vol. 11: 1-42
- DUPREE, H. K., GRUGLITZ, E. J., HALL, A. S. and HOULE, C. R. (1979). Effect of dietary lipids on growth and acceptability of channel catfish. In: Fine fish nutrition and fish feed technology (Halver, J. E. and Tiews, K., eds.), Vol. 2: 87-103. Heenemann, Berlin, Federal Republic of Germany
- EATON, R. C., and FARLEY, R. D. (1974). Growth and the reduction of depensation of zebra fish (Brachydanio nerio) reared in the laboratory. Copeia, 1: 204-209
- EDWARDS, D. J. (1971). Effect of temperature on rate of passage of food through the alimentary canal of the plaice, Pleuronectes platessa L. J. Fish. Biol., 3: 433-439
- EDWARDS, D. J. (1973). The effect of drugs and nerve section on the rate of passage of food through the gut of plaice. J. Fish. Biol., 5: 441-446
- ELLIOTT, J. M. (1972). Rates of gastric evacuation in brown trout, Salmo trutta. Freshwater Biol., 2: 1-18
- ELLIOTT, J. M. (1975). The growth rate of brown trout (Salmo trutta) fed on reduced rations. J. Animal Ecol., 44: 823-842

- ELLIOTT, J. M. (1975a). Weight of food and time required to satiate brown trout, Salmo trutta. Freshwater Biol., 5(1): 51-64
- ELLIOTT, J. M. (1975b). Number of meals in a day, maximum weight of food consumed in a day and maximum rate of feeding for brown trout, Salmo trutta. Freshwater Biol., 5: 287
- ELLIOTT, J. M. (1976). Body composition of brown trout (Salmo trutta) in relation to temperature and ration size. J. Animal Eco., 45: 273-289
- ELLIOTT, J. M. and PERSSON, L. (1978). The estimation of daily rates of food consumption for fish. J. Anim.Ecol., 47: 977
- FABIAN, G., MOLNAR, G. and TOLG, I. (1963). Comparative data and enzyme kinetic calculations on changes caused by temperature in the duration of gastric digestion of some predatory fishes. Acta. Biol. Acad. Sci. Hung. 14: 123-129
- FANGE, R. and GROVE, D. J. (1979). Digestion. In: Fish Physiology (Hoar, W. S., Randall, D. J. and Brett, J. R., eds.), Vol. VIII: 161-260. Academic Press, New York and London.
- FLETCHER, D. J. (1982). Digestion and the systemic control of appetite in the dab (Limanda limanda). PhD thesis, University College of North Wales.
- FLOWERDEW, M. W. and GROVE, D. J. (1979). Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, Scophthalmus maximus (L.) using radiography. J. Fish Biol., 14: 229-238
- FOSTER, J. R. (1977). Pulsed gastric lavage: an efficient method of removing the stomach contents of live fish. Prog. Fish. Cult., 39: 166

- FRY, F. E. J. (1957). The aquatic respiration in fish. In: The Physiology of Fish (Brown, M. E., ed.), Vol. 1: 1-63
- FURNKAWA, A. and TSUKAHARA, H. (1966). On the acid digestion method for determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jap. Soc. Sci. Fish., 32: 502-506
- GERALD, V. M. (1973). Rate of digestion in Ophiocephalus punctatus. Bloch. Comp. Biochem. Physiol., A46: 195-205
- GERKING, S. D. (1962). Production and food utilization in a population of blue gill sunfish. Ecol. Monographs, 32: 31-38
- GERKING, S. D. (1971). Influence of rate of feeding and body weight on protein metabolism of blue gill sunfish. Physiol. Zool., 44: 9-19
- GODDARD, J. S. (1974). An x-ray investigation of the effects of starvation and drugs on intestinal motility in the plaice, Pleuronectes platessa. Ichthyologia, 6(1): 49-58
- GODIN, J. G. J. (1981). Daily pattern of feeding behaviour, daily rations and diets of juvenile pink salmon (Oncorhynchus gabuscha) in two marine bays of British Columbia. Can. J. Fish. Aqu. Sci., 38: 10-15
- GRAYTON, B. D. and BEAMISH, F. W. H. (1977). Effect of feeding frequency on food intake, growth and body composition of rainbow trout (Salmo gairdneri). Aquaculture, 11: 159-172
- GROVE, D. J. and CRAWFORD, C. (1980). Correlation between digestion rate and feeding frequency in the stomachless teleost, (Blennius pholis L.). J. Fish Biol., 16: 235-247

- GROVE, D. J., LOIZIDES, L. G. and NOTT, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in Salmo gairdneri. J. Fish Biol., 12: 507-516
- GROVE, D. J., MOCTEZUMA, M. A., FLETT, H. R., FOOTT, J. S., WATSON, T. and FLOWERDEW, M. W. (1985). Gastric emptying and the return of appetite in juvenile turbot (Scophthalmus maximus) fed on artificial diets. J. Fish Biol., 26: 339-354
- GROVES, T. D. (1970). Body composition changes during growth in young sockeye (Oncorhynchus nerka) in fresh water. J. Fish. Res. Board Can., 27: 929-942
- GWYTHHER, D. (1978). The control of appetite in the dab (Limanda limanda). PhD thesis, University College of Wales.
- HADJICRISTOPHORA, M. (1980). Studies on digestion in the green turtle (Chelonia mydas). MSc thesis, University of North Wales.
- HADJICRISTOPHORA, M. and GROVE, D. J. (1983). A study of appetite, digestion and growth in juvenile green turtle (Chelonia mydas) fed on artificial diets. Aquaculture, 30: 191-201
- HASSAN, M. R. (1986). Husbandry factors affecting survival and growth of carp (Cyprinus carpio) fry and an evaluation of dietary ingredients available in Bangladesh for the formulation of a carp fry diet. PhD thesis, University of Stirling, Scotland.
- HATANKA, M., KOSAKA, M. and SATO, Y. (1956). Growth and food consumption in plaice, Part II. Tohoku J. Agric. Res., 7: 151-162
- HERMAN, R. B., WARREN, C. E. and DANDOROFF, P. (1962). Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Am. Fish. Soc., 91: 155-167

- HERSHLEY, A. E. and McDONALD, M. E. (1985). Diet and digestion rates of slimy sculpin (Cottus cognatus) in an Alaskan Arctic Lake. Can. J. Fish. Aqu. Sci., 42: 483-487
- HESS, A. D. and RAINWATER, J. H. (1939). A method for measuring for preference of trout. Copeia, 3: 154-157
- HILTON, J. W., ATKINSON, J. L. and SLINGER, S. J. (1983). Effect of dietary fibre on the growth of rainbow trout, Salmo gairdneri. Can. J. Fish. Aqu. Sci., 40: 81-84
- HOFER, R. (1979). The adaptation of digestive enzymes to temperature, season and diet in roach (Rutilus rutilus) and rudd (Scardinius erythrophthalmus). Proteases. J. Fish Biol., 15: 373-379
- HOFER, R. (1982). Protein digestion and proteolytic activity in the digestive tract of an omnivorous cyprinid. Comp. Biochem. Physiol., 72: 55-63
- HOFER, R., FORSTNER, H. and RETTENWANDER, R. (1982). Duration of gut passage and its dependence on temperature and food consumption in roach (Rutilus rutilus): Laboratory and field experiments. J. Fish Biol., 20: 289-299
- HOFER, R. and NEWRKLA, P. (1983). Determination of gut passage time in tilapia fry (O. mossambicus) under laboratory and field conditions. In: Proc. International Symposium on Tilapia in Aquaculture, Tel Aviv University, Tel Aviv, Israel, 5-13 March 1983
- HOFER, R. and SCHIEMER, F. (1981). Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia (Berl), 48: 342-345
- HOFER, R. and UDDIN, A. N. (1985). Digestive processes during the development of the roach (Rutilus rutilus). J. Fish Biol., 26: 683-689

- HOGENDOORN, H. (1981). Controlled production of the African catfish (Clarias lazara). IV. Effect of feeding regime in fingerling culture. Aquaculture, 24: 123-131
- HSU, Y. and WU, J. (1979). The relationship between feeding habits and digestive protease of some freshwater fishes. Bull. Inst. Zool. Academia Sinica, 18(1): 45-53
- HUEBNER, J. D. and LANGTON, R. W. (1982). Rate of gastric evacuation for winter flounder (Pseudeopleuronectes americanus). Can. J. Fish. Aqu. Sci., 39: 356-360
- HUET, M. (1972). Text Book of Fish Culture. Page Brothers Ltd., Norwich, UK
- HUISMAN, E. A. (1976). Food conversion efficiencies at maintenance and production for carp (Cyprinus carpio) and rainbow trout (Salmo gairdneri). Aquaculture, 9: 259-273
- HUNT, B. P. (1960). Digestion rate and food consumption of Florida gar, warmouth and largemouth bass. Trans. Am. Fish. Soc., 89(2): 206-210
- HUNT, J. N. and KNOX, M. T. (1968). Regulation of gastric emptying. In: Handbook of Physiology (Code, C. F., ed.), Sect. 6, Vol.4: 1917-1935. Am. Physiol. Soc., Washington DC
- HUNT, J. N. and MacDONALD, I. (1954). The influence of volume on gastric emptying. J. Physiol. (London), 126: 459-474
- HUSER, N. J. (1978). Temperature requirements of Tilapia zillii. Calif. Fish and Game, 63: 228-233
- ILES, T. D. and WOOD, R. J. (1965). The fat/water relationship in North Sea herring and its possible significance. J. Mar. Biol. Ass. UK, 45: 353-366

- INCE, B. W. and TH PE, A. (1976). The effect of starvation and force-feeding on the metabolism of the northern pike, Esox lucius. J. Fish Biol., 8: 79-88
- ISHIWATA, N. (1969). Ecological studies on the feeding of fishes. VII. Frequency of feeding and satiation amount. Nippon Suisan Gakkaishi, 35: 979-984
- JAUNCEY, K. (1979). Growth and nutrition of carp in heated effluents. PhD thesis, University of Aston, Birmingham: 202pp
- JAUNCEY, K. (1982). Carp (Cyprinus carpio) nutrition - a review. In: Recent Advances in Aquaculture (Muir, J. F. and Roberts, R. J. (eds.)). Croom Helm, London and Canberra: 215-263
- JAUNCEY, K. and ROSS, B. (1982). A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture, University of Stirling, Scotland: 111pp
- JAVID, M. Y. and ANDERSON, J. M. (1967). Influence of starvation on selected temperature of some salmonids. J. Fish. Res. Board. Can., 24: 1515-1519
- JOBLING, M. (1980a). Gastric evacuation in plaice, Pleuronectes platessa L: effects of dietary energy level and food consumption. J. Fish. Biol., 17: 187-196
- JOBLING, M. (1980b). Gastric evacuation in plaice, Pleuronectes platessa. J. Fish. Biol., 17: 547-551
- JOBLING, M. (1981a). Dietary digestibility and the influence of food components on gastric evacuation in plaice (Pleuronectes platessa). J. Fish. Biol., 19: 29-36
- JOBLING, M. (1981b). Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. J. Fish. Biol., 19: 245-257

- JOBLING, M. (1982). Some observations of the effects of feeding frequency on the food intake and growth of plaice (Pleuronectes platessa). J. Fish. Biol., 20: 431-444
- JOBLING, M. (1983). Effect of feeding frequency on food intake and growth of Arctic charr (Salvelinus alpinus). J. Fish. Biol., 23: 177-185
- JOBLING, M. and DAVIES, P. S. (1979). Gastric evacuation in plaice, Pleuronectes platessa L: effects of temperature and meal size. J. Fish Biol., 14: 539-546
- JOBLING, M., GWYTHYR, D. and GROVE, D. J. (1977). Some effects of temperature, meal size and body weight on gastric evacuation time in the dab, Limanda limanda (L). J. Fish Biol., 10: 291-298
- JOBLING, M. and WANDSVIK, A. (1983). An investigation of factors controlling food intake in Arctic charr (Salvelinus alpinus). J. Fish Biol., 23: 397-404
- JONES, R. (1974). The rate of elimination of food from the stomachs of haddock, Melanogrammus aeglefinus, cod, Gadus morhua, and whiting, Merlangius merlangius. J. Cons., Cons. Int. Explor. Mer., 35(3): 225-243
- KAMARA, S. K. (1966). Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (Gadus morhua). J. Fish. Res. Board Can., 23: 975-982
- KAPOOR, B. G., SMIT, H., and VERIGHINA, I. A. C. (1975). The alimentary canal and digestion in teleosts. Adv. Mar. Biol., 13: 109-239
- KATONDA, K. I. (1979). Energy storage, maintenance requirements and body water in Blennius pholis fed different daily rations. MSc thesis, University College of North Wales.

- KAWAI, S. and IKEDA, S. (1972). Studies on digestive enzymes of fishes. II. Effect of dietary change on the activities of digestive enzymes in carp intestine. Bull. Jap. Soc. Sci. Fish., 38(3): 265-270
- KAWAI, S. and IKEDA, S. (1973a). Studies on digestive enzymes of fishes. III. Development of the digestive enzymes of rainbow trout after hatching and the effect of dietary changes on the activities of digestive enzyme in the juvenile stage. Bull. Jap. Soc. Sci. Fish., 39(1): 819-823
- KAWAI, S. and IKEDA, S. (1973b). Studies on digestive enzymes of fishes. IV. Development of the digestive enzymes of carp and Black Sea bream after hatching. Bull. Jap. Soc. Sci. Fish., 39(8): 877-881
- KELSO, J. R. M. (1972). Conversion, maintenance and assimilation for walleye (Stizostedion vitreum) as affected by size, diet and temperature. J. Fish. Res. Board Can., 29: 1181-1192
- KINNE, O. (1970). Temperature: animals, invertebrates. In: Marine Ecology (Kinne, O., ed.), Vol. 1. Environmental factors, Part I. Wiley-Interscience, London.
- KIONKA, B. C. and WINDELL, J. T. (1972). Differential movement of digestible and indigestible food fractions in rainbow trout, Salmo gairdneri. Trans. Am. Fish. Soc., No.1: 112-115
- KITCHELL, F. J. and WINDELL, J. T. (1968). Rate of gastric digestion in pumpkin seed sunfish (Lepomis gibbosus). Trans. Am. Fish. Soc., 97: 489-492
- KUZMINA, V. V. (1980). Seasonal and age-related changes in  $\alpha$ -amylase activity in the bream (Abramis brama). J. Ichthyol., 20: 105-110

- LAUFF, M. and HOFER, R. (1984). Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture, 37: 335-346
- LAURENCE, G. C. (1971). Digestion rate of larval largemouth bass. New York Fish and Game Journal, 18
- LEAVITT, D. F. (1985). An evaluation of gravimetric and inert marker techniques to measure digestibility in the American lobster. Aquaculture, 47: 131-142
- LEE, D. J. and PUTNAM, G. B. (1973). The response of rainbow trout to varying protein energy ratios in a test diet. J. Nutr., 103: 916-922
- LEE, D. J. and WALES, J. H. (1973). Observed changes in rainbow trout fed varying levels of casein-gelatine mixture and herring oil in experimental diets. J. Fish. Res. Board Can., 30: 1017-1020
- LIGHT, R. W., ADLER, P. H. and ARNOLD, D. E. (1983). Evaluation of gastric lavage for stomach analyses. North Am. J. Fish. Mgmt., 3: 81
- LOVE, R. M. (1960). Water content of cod (Gadus callarias) muscle. Nature, London, 185: 692
- LOVE, R. M. (1975). The Chemical Biology of Fishes. Academic Press, New York: 547pp
- LOVE, R. M. (1980). The Chemical Biology of Fishes. Academic Press, London: 943pp
- LOVELL, R. T. (1979). Factors affecting voluntary food consumption by channel catfish. In: Fine fish nutrition and fish feeds technology (Halver, J. E. and Tiews, K., eds.), Vol. 1: 555-564. Heenemann, Berlin, Federal Republic of Germany.

LOZOIDES, L. G. (1975). Studies on digestion and appetite in rainbow trout (Salmo gairdneri). MSc thesis, University of Wales.

MacDONALD, J., WAIWOOD, K. G. and GREEN, R. H. (1982). Rates of digestion of different prey in Atlantic cod (Gadus morhua), Ocean pout (Macnozoarces americanus), winter flounder (Pseudopleuronectes platenoides). Can. J. Fish. Aqu. Sci., 39(5): 651-659

MACINTOSH, D. J. and DE SILVA, S. S. (1984). The influence of stocking density and food ration in fry survival and growth in Oreochromis mossambicus and O. niloticus female x O. aureus male hybrids reared in a closed circulated system. Aquaculture, 345-358

MACINTOSH, D. J. and SAMPSON, D. R. T. (1985). Tilapia culture: Hatchery methods for O. mossambicus and O. niloticus, with special reference to all-male fry production. Institute of Aquaculture, University of Stirling, Stirling, Scotland: 154pp

MACKAY, W. C. (1975). Effect of temperature on fluid absorption by teleost gall bladder. Comp. Biochem. Physiol., 50A: 383-385

MABAYE, A. B. E. (1971). Observation on the growth of Tilapia mossambica fed on artificial diets. Fish. Res. Bull. Zambia, 2: 379-396

MAGNUSON, J. J. (1962). An analysis of aggressive behaviour, growth and competition for food and space in medaka (Oryzias latipes). Can. J. Zool., 40: 313-363

MAYNARD, L. A. and LOOSLI, J. K. (1969). Animal nutrition, 6th ed. McGraw Hill Book Company.

- MAZID, M. A., TANAKA, Y., KATAYAMA, T., ASADUR, R. M., SIMPSON, K. L. and CHICHESTER, C. O. (1979). Growth response of Tilapia zillii fingerling fed isocaloric diets with variable protein levels. Aquaculture, 18: 115-122
- MEDVED, R. J. (1985). Gastric evacuation in the sand bar shark, (Carcharhinus plumbeus). J. Fish Biol., 26: 239-253
- MESK, C. (1985). Fish Aquaculture. Pergamon Press, Oxford: 237pp
- MILLIKIN, M. R. (1982). Effects of dietary protein concentration on growth, feed efficiency and body composition of age-0 striped bass. Trans. Am. Fish. Soc., 11: 373-378
- MILLIKIN, M. R. (1983). Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. Trans. Am. Fish. Soc., 112: 185-193
- MOCTEZUMA, M. A. (1982). Feeding, digestion and assimilation efficiency in young turbot (S. maximus). MSc thesis, University College of North Wales.
- MOLNAR, G. and TULG, I. (1962). Relation between water temperature and gastric digestion of largemouth bass (Micropterus salmoides). J. Fish. Res. Board Can., 19: 1005-1012
- MORIARTY, C. M. and MORIARTY, J. W. (1973). Quantitative estimation of the daily ingestion of phytoplankton by Tilapia nilotica and Haplochromis nigripinnis in Lake George, Uganda. J. Zool. London, 171: 15-23
- MORIARTY, D. J. W. (1973). The physiology of digestion of blue-green algae in the cichlid fish Tilapia nilotica. J. Zool. London, 171: 25-39
- MORISHITA, T., NODA, H. and KITAMIKADO, M. (1964). On the activity of the digestive enzymes in cultured fish. J. Fac. Fish. Pret. Univ., 6: 239-246

- MUIR, B. S. and NIIMI, A. J. (1972). Oxygen consumption of the euryhaline fish aholehole (Kuhlia sandvicensis) with reference to salinity, swimming and food consumption. J. Fish. Res. Board Can., 29: 67-77
- MUKHOPADHYAY, P. K. (1977). Studies on the enzyme activities related to varied pattern of diet in the air-breathing cat fish. Hydrobiologia, 52: 235-237
- MURAI, T., AKIYAMA, T., TAKEUCHI, T., WATANABE, T. and NOSE, T. (1985). Effects of dietary protein and lipid levels on performance and carcass composition of fingerling carp. Bull. Jap. Soc. Sci. Fish., 51(4): 609-617
- MURRAY, M. W., ANDREW, J. W. and DE LOACH, H. L. (1977). Effect of dietary protein and environmental temperature on growth, food conversion and body composition of channel catfish. J. Nutr. 107: 272-280
- MYRTLE, J. F. and ZELL, W. J. (1975). Simplified photometric copper-soap method for rapid assay of serum lipase activity. Clin. Chem., 21: 1469-1473
- NAGAI, M. and IKEDA, S. (1973). Carbohydrate metabolism in fish. IV. Effect of dietary composition on metabolism of acetate- $U^{14}$ -C and L-alanine- $U^{14}$ -C in carp. Bull. Jap. Soc. Sci. Fish., 39: 633-643
- NAGAS, G. (1964). Contribution to the physiology of digestion in Tilapia mossambica digestive enzymes and the effects of diets on their activity. Z. vergl. physiol., 49: 270-284
- NAWWAB, A. R. (1982). Food consumption and digestion in sea-scorpion (Taurulus bubalis). MSc thesis, University of Wales.

- NICHOLLS, J. V. V. (1931). The influence of temperature on digestion in Fundulus heteroclitus. Contrib. Can. Biol. Fish., 7: 45-55
- NIIMI, A. J. and BEAMISH, F. W. H. (1973). Bioenergetics and growth of largemouth bass (Micropterus salmoides) in relation to body weight and temperature. Can. J. Zool., 52: 447-456
- NOBLE, R. L. (1973). Evacuation rates of young yellow perch, Perca flavescens. Trans. Am. Fish. Soc., 102(4): 443-454
- NOSE, T. (1967). On the metabolic faecal nitrogen in young rainbow trout. Freshwat. Fish. Res. Lab. Tokyo, 17: 97-105
- OGINO, C. and CHAN, M. S. (1973). Protein nutrition in fish. IV. Biological value of dietary protein in carp. Bull. Jap. Soc. Sci. Fish., 36: 250-254
- OGINO, C. and SAITO, K. (1970). Protein nutrition in fish. I. The utilization of dietary protein by young carp. Bull. Jap. Soc. Sci. Fish., 36: 250-254
- OSBORNE, T. B., MENDEL, L. B. and FERRY, E. L. (1919). A method for expressing numerically the growth promoting value of proteins. J. Biochem., 37: 223-229
- OWEN, T. C. and WIGGS, A. J. (1971). Thermal compensation in the stomach of the brook trout. Comp. Biochem. Physiol., 40B: 465-473
- PALOHEIMO, J. E. and DICKIE, L. M. (1965). Food and growth of fishes. I. A growth curve derived from experimental data. J. Fish. Res. Board Can., 22: 521-542

- PALOHEIMO, J. E. and DICKIE, L. M. (1966a). Food and growth of fishes. II. Effect of food and temperature on the relation between metabolism and body weight. J. Fish. Res. Board Can., 12: 869-908
- PALOHEIMO, J. E. and DICKIE, L. M. (1966b). Food and growth of fishes. III. Relation among food, body size and growth efficiency. J. Fish. Res. Board Can., 23: 1209-1248
- PANDIAN, T. J. (1967). Intake, digestion, absorption and conversion of food with fishes Megalops cyprinoides and Ophiocephalus striatus. Mar. Biol., 1: 16-32
- PANDIAN, T. J. and RAGHURAMAN, R. (1972). Effects of feeding rate on conversion efficiency and chemical composition of the fish Tilapia mossambica. Mar. Biol., 12: 129-136
- PANDIAN, T. J. and VIVEKANADAN, E. (1976). Effects of feeding and starvation on growth and swimming activity in an oblicatory air-breathing fish. Hydrobiologia, 49: 33-39
- PARKER, R. R. and VANSTANE, W. E. (1966). Changes in chemical composition of central British Columbia pink salmon during early sea life. J. Fish. Res. Board Can., 23: 1353-1384
- PERSSON, L. (1979). The effects of temperature and different food organisms on the rate of gastric evacuation in perch, Perca fluviatilis. Freshwat. Biol., Vol. 9: 99-104
- PERSSON, L. (1981). The effects of temperature and meal size on the rate of gastric evacuation in perch, (Perca fluviatilis) fed on fish larvae. Freshwat. Biol., 11: 131-138
- PERSSON, L. (1982). Rate of food evacuation in roach (Rutilus rutilus) in relation to temperature, and the application of evacuation rate estimates for studies on the rate of food consumption. Freshwat. Biol., 12: 203-210

- PETERS, D. S. and HOSS, D. E. (1974). A radioisotopic method of measuring food evacuation time in fish. Trans. Am. Fish Soc., 103: 626-629
- PHILLIPS, A. M., LIVINGSTON, D. L. and POSTEN, H. A. (1966). The effect of changes in protein quality, caloric sources and calorie levels upon the growth and chemical composition of brook trout. N.Y. State Dep. Conserv. Fish. Res. Bull., 29: 6-7
- PHILLIPS, A. M., Jr. and PODOLIAK, H. A. (1957). The nutrition of trout. III. Fats and minerals. Prog. Fish. Cult., 19: 68-75
- PURDOM, C. E. (1974). Variation in fish. In: Sea Fisheries Research. (Hardon Jones, F. R., ed.), London. Elek Science.
- RANDOLPH, K. N. and CLEMENS, H. P. (1978). Effect of short-term food deprivation on channel catfish and implications for culture practice. Prog. Fish. Cult., 40(2): 48-50
- REDDY, S. R. and KATRE, S. (1979). Growth rate and conversion efficiency of the air-breathing catfish (Heteropneustes fossilis) in relation to ration size. Aquaculture, 18: 35-40
- REIMER, G. (1982). The influence of diet on the digestive enzyme of the amazon fish. J. Fish. Biol., 21: 637-642
- REIMERS, N. (1957). Some aspects of the relation between stream food and trout survival. Calif. Fish Game, 43: 43-69
- REINITZ, G. (1983). Relative effect of age, diet and feeding rate on the body composition of young rainbow trout (Salmo gairdneri). Aquaculture, 35: 19-27
- REINITZ, G. and HITZEL, F. N. (1980). Formulation of practical diets for rainbow trout (Salmo gairdneri) based on desired performance and body composition. Aquaculture, 19: 243-252

- REINITZ, G., L., ORME, L. E., LEMM, C. A. and HITZEL, F. N. (1978). Influence of varying lipid concentration with two protein concentrations in diets for rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 107: 751-754
- RICKER, W. E. (1946). Production and utilization of fish population. Ecol. Monogr., 16: 373-391
- RICKER, W. E. (1971). Method for assessment of fish production in freshwaters (Ricker, W. E., ed.). IBP Handbook No. 3, Blackwell Publication, Oxford.
- ROSS, B. and JAUNCEY, K. (1981). A radiographic estimation of the effect of temperature on gastric emptying time in Sarotherodon niloticus (L.) x S. aureus (Steindachner) hybrids. J. Fish. Biol., 19: 333-344
- ROSS, L. G. and GEDDES, J. A. (1979). Sedation of warmwater fish species in Aquaculture research. Aquaculture, 16: 183-186
- ROZIN, P. and MAYER, J. (1961). Regulation of food intake in the goldfish. Am. J. Physiol., 201: 968-974
- RYCHLEY, J. and SPANNHOF, L. (1979). Nitrogen balance in trout. 1. Digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture, 16: 39-46
- SACLAUSO, C. A. (1985). Interaction of growth with social behaviour in Tilapia zillii raised in three different temperatures. J. Fish. Biol., 26: 331-337
- SAMPATH, K. (1984). Preliminary report on the effects of feeding frequency in Channa striatus. Aquaculture, 40: 301-306
- SAMPATH, K. and PANDIAN, T. J. (1980). Effects of density on food utilization and surfacing behaviour in the obligatory air-breathing fish (Channa striatus). Hydrobiologia, 68(2): 113-117

- SATIA, B. P. (1974). Quantitative protein requirements of rainbow trout. Prog. Fish. Cult., 36: 80
- SAVITZ, J. (1971). Effect of starvation on body protein utilization of bluegill sunfish (Lepomis macrochirus) with a calculation of caloric requirement. Trans. Am. Fish. Soc., 100: 18-21
- SHELL, E. W. (1969). Relationship between rate of feeding, rate of growth and rate of conversion in feeding trials with two species of tilapia, Tilapia mossambica and Tilapia nilotica. FAO Fish. Rep., 44(3): 411-415
- SHIMINO, S., HOSOKAWA, H. and TAKEDA, M. (1978). The importance of carbohydrate in the diet of a carnivorous fish. FAO/EIFAC/78 Symp., E15: 1-32
- SHIMINO, S., TAKEDA, M., TAKAYAMA, S., FUKUI, A., SASAKI, H. and KAJIYAMA, H. (1981). Adaptation of hepatopancreatic enzymes to dietary carbohydrate in carp. Bull. Jap. Soc. Sci. Fish., 47(1): 71-77
- SIMPSON, J. H. (1965). Comparative aspects of the control of glycogen utilization in vertebrate liver. Comp. Biochem. Physiol., 15: 187-195
- SINGH, R. P. and SRIVASTAVA, A. K. (1984). Effect of feeding frequency on the growth, consumption and gross conversion efficiency in the siluroid catfish (Heteropneustes fossilis). Bamidgeh, 36
- SINGH, R. P. and SRIVASTAVA, A. K. (1985). Satiation time, gastric evacuation and appetite revival in Heteropneustes fossilis (Bloch) (Siluriformes: Pisces). Aquaculture, 49: 307-313
- SMIT, H. (1967). Influence of temperature on the rate of gastric juice secretion in the brown bullhead (Ictalurus nebulosus). Comp. Biochem. Physiol., 21: 125-132

- SMITH, L. S. (1982). Introduction to Fish Physiology. TFH Publications Inc.
- SMITH, R. R. (1971). A method for measuring digestibility and metabolizable energy of fish feeds. Prog. Fish. Cult., 33(3): 132-134
- STEIGENBERG, L. W. and LARKIN, P. A. (1974). Feeding activity and rates of digestion of northern squawfish (Ptychocheilus oregonensis). J. Fish. Res. Board Can., 31(4): 411-420
- STEWART, N. E. (1962). Quoted from Warren and Davies, 1967.
- STIRLING, H. P. (1977). Growth, food utilization and effect of social interaction in the European bass (Dicentrarchus labrax). Marine Biol., 40: 173-184
- STIRLING, H. P. (1985). Water Analysis for Aquaculturists. Institute of Aquaculture, University of Stirling, Stirling, Scotland: 139pp
- STORCH, V. and JUARIO, J. V. (1983). The effect of starvation and subsequent feeding on the hepatocytes of Chanos chanos fingerlings and fry. J. Fish Biol., 23: 95-103
- STOREBAKKEN, K., AUSTRENG, E. and STOENBERG? K. (1981). A method for determination of food intake in salmonids using radioactive isotopes. Aquaculture, 24: 133
- STRANGE, C. D. and KENNEDY, G. J. A. (1981). Stomach flushing of salmonids: A simple and effective technique for the removal of stomach contents. Fish. Mgmt., 12: 9
- STRICHLAND, J. D. H. and PARSONS, T. R. (1972). A Practical Handbook of Seawater Analysis, Bull. No.167, 2nd edition. Fish. Res. Board Can., Ottawa: 310pp

- SUN, D. H. C. , SHAY, H. A. and WOLOSHINTT, J. (1959). Effect of tricyclamol on gastric emptying and intestinal transit. Am. J. dig., 4: 282-288
- SWANSON, W. A. and SMITH, L. L. (1973). Gastric digestion, food consumption, feeding periodically and food conversion efficiency in walleye (Stizostedion vitreum). J. Fish. Res. Board Can., 30(9): 1327-1336
- SYLVESTER, J. R. (1972). Effect of starvation time on the amount of food eaten by coho salmon. Prog. Fish. Cult., 34(3): 160-161
- TAKEDA, M., SHIMENO, S., HOSOKAWA, H., KAJIYAMA, H. and KAISYO, T. (1975). The effect of dietary calorie-to protein ratio on the growth, food conversion and body composition of young yellow-tail. Bull. Jap. Soc. Sci. Fisheries, 41(4): 443-447
- TALBOT, C. (1985). Laboratory methods in fish feeding and nutritional studies. In: Fish Energetics, New Perspectives (Tytler, P. and Calow, P., eds.). Croom Helm, London.
- TALBOT, C. and HIGGINS, P. J. (1983). A radiographic method for feeding studies on fish using metallic iron powder as a marker. J. Fish Biol., 23: 211-220
- TALBOT, C., HIGGINS, P. J. and SHANKS, A. M. (1984). Effect of pre- and post-prandial starvation on meal size and evacuation rate of juvenile Atlantic salmon (Salmo salar). J. Fish Biol., 25: 551
- TESHIMA, S., KANAZAWA, A. and UCHIYAMA, Y. (1986). Effect of several protein sources and other factors on the growth of Tilapia nilotica. Bull. Jap. Soc. Sci. Fish., 52(3): 525-530
- THIA-ENG, C. and SENG-KEH, T. (1978). Effects of feeding frequency on growth of young estuary grouper (Epinephelus tauvina) cultured in floating net cages. Aquaculture, 14: 31-47

- THOMASE, K. K. and NATION, J. L. (1984). Protein, amylase and lipase activity in the mid gut and hind gut of the cricket and mole cricket. Comp. Biochem. Physiol., 79A(2): 297-304
- THROPE, J. E. (1977). Daily ration of adult perch, Perca fluviatilis during summer in Loch Leven, Scotland. J. Fish. Biol., 11: 55
- TROFIMOVA, L. N. (1973). Dynamics of common proteolytical activity in the digestive tract of carp and its dependence on incubation temperature. Sbornik nauc. trwd. VNIIPRCh 10: 170-181
- TYLER, A. V. (1970). Rate of gastric emptying in young cod. J. Fish Res. Board Can., 27: 1177-1189
- TYLER, A. V. and DUNN, R. S. (1976). Ration, growth and measure of somatic and organ condition in relation to meal frequency in winter flounder, with hypotheses regarding population homeostasis. J. Fish. Res. Board Can., 33: 63-75
- UFODIKE, E. B. C. and MATTY, A. J. (1983). Growth responses and nutrient digestibility in mirror carp (Cyprinus carpio) fed different levels of cassava and rice. Aquaculture, 31: 41-50
- VIVEKANDAN, E. (1976). Effect of feeding on the swimming activity and growth of Ophicephalus striatus. J. Fish Biol., 8: 289-292
- VOLYA, G. (1966). Some data on digestive enzyme in some Black Sea fishes and a micromodification of a method for the identification of trypsin, amylase and lipase. In: Fisiologia morskikyzhitvotnykh Nauka.
- WALLACE, J. C. (1973). Observation on the relationship between food consumption and metabolic rate of Blennius pholis. Comp. Biochem. Physiol., A45: 293-305

- WANG, K-W, TAKEUCHI, T. and WATANABE, T. (1985). Optimum protein and digestible energy levels in diets for Tilapia nilotica. Bull. Jap. Soc. Sci. Fish., 51: 141-146
- WANG, K., TAKEUCHI, T. and WATANABE, T. (1985). Effect of dietary protein levels on growth of Tilapia nilotica. Bull. Jap. Soc. Sci. Fish., 51(1): 133-140
- WARE, D. M. (1971). Predation by rainbow trout (Salmo gairdneri): the influence of hunger, prey density and prey size. J. Fish. Res. Board Can., 29: 1193-1201
- WARREN, C. E. and DAVIS, G. E. (1967). Laboratory studies on the feeding, bioenergetics and growth of fish. In: The Biological basis of freshwater fish production (Gerking, S. D., ed.): 175-214. Blackwell Scientific Publications, Oxford.
- WEATHERLEY, A. H. and GILL, H. S. (1981). Recovery growth following periods of restricted rations and starvation in rainbow trout (Salmo gairdneri). J. Fish Biol., 18: 195-208
- WESTERN, J. R. (1971). Feeding and digestion in two cottid fishes, the freshwater Cottus gobio and the marine Enophris bubalis. J. Fish Biol., 3: 225-246
- WICKENS, J. F. (1980). Water quality requirements for intensive aquaculture. A review in: Aquaculture in Heater Effluents and Recirculation Systems. Proc. World Symp. Tiews, K. (ed.). H Heeneman GmbH, Berlin: 17-37
- WILLIAM, L. Y. and STICKNEY, R. R. (1980). Growth of caged channel catfish fingerlings reared on diets containing various lipids. Prog. Fish. Cult., 42: 24-26
- WINBERG, G. G. (1956). Rate of metabolism and food requirements of fishes. Transl. Ser. Fish. Res. Bd. Can., 194: 253

WINDELL, J. T. (1966). Rate of digestion in the blue gill sunfish, Lepomis macrochirus. Invest. Indiana Lakes and Streams, Vol. 7, No.6: 185-214

WINDELL, J. T. (1967). Rates of digestion in fishes. In: The biological basis of freshwater fish production (Gerking, S. D., ed.). Blackwell Scientific Publications, Oxford and Edinburgh: 495pp

WINDELL, J. T., FOLTZ, J. W. and SARAKON, J. A. (1978). Effect of fish size, temperature and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, Salmo gairdneri. Trans. Am. Fish. Soc., 107(4): 613-616

WINDELL, J. T. and NORRIS, D. O. (1969). Gastric digestion and evacuation in rainbow trout. Proc. Fish Cult., Vol.31: 20-26

WINDELL, J. T., NORRIS, D. O., KITCHELL, J. F. and NORRIS, J. S. (1969). Digestive response of rainbow trout, Salmo gairdneri, to pelleted diets. J. Fish. Res. Board Can., Vol.26, No.7: 1801-1812

WOHLFARTH, G. and MOAV, R. (1972). The regression of weight gain on initial weight in carp. 1. Methods and Results. Aquaculture, 1: 7-28

WOOTTON, R. J., ALLEN, J. R. and COLE, S. J. (1980). Effect of body weight and temperature on the maximum daily food consumption of Gasterosteus aculeatus and Phoxinus phoxinus: selecting an appropriate model. J. Fish. Biol., 17: 695-706

WURTSBAUGH, W. A. and DAVIS, G. E. (1977). Effect of fish size and ration level in the growth and food conversion efficiency of rainbow trout (Salmo gairdneri). J. Fish. Biol., 11: 99-104

YANG, J. S. and BIGGS, H. G. (1971). Rapid reliable method for measuring serum lipase activity. Clin. Chem., 17: 512-518

ZAR, H. H. (1974). Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ: 620pp