THE FEEDING AND GROWTH OF *TILAPIA RENDALLI* IN RELATION TO ITS AQUACULTURE POTENTIAL

by

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DEDICATION

To my mother, my hero and inspiration.

DECLARATION

I declare that **THE FEEDING AND GROWTH OF *TILAPIA RENDALLI* IN RELATION TO ITS AQUACULTURE POTENTIAL** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

Hlophe Samkelisiwe Nosipho Date

[redacted]

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ABSTRACT

The feeding habits of a macrophagous fish, *Tilapia rendalli*, were investigated at an oligotrophic dam that has no macrophytes, Flag Boshielo Dam. This dam supports a significant population of the macrophagous, *Tilapia rendalli*. The diet of *T. rendalli* was investigated by examining the frequency of occurrence of different food items in the stomach of the fish over a period of twelve months. A size related dietary shift was evident. The diet of juvenile fish (<5 cm) was dominated by zooplankton and the diet of adult fish (>15 cm) was predominantly marginal vegetation, particularly *Cyperus sexangulasris* and *Panicum schinzi*. However, dietary overlaps between the different size groups were evident. The diversity of food items increased with fish size until the fish were 15 cm in length and thereafter declined as the fish predominately fed on marginal vegetation. Scales were used to determine the age of T*. rendalli*. Age at length data was fitted to the Von Bertalanffy growth model, which showed that males grew faster and attained a larger size than females. The growth of *T. rendalli* in Flag Boshielo Dam was comparable to those reported in other dams with macrophytes. It is inferred here that the absence of macrophytes is not a limiting factor in the growth of *T. rendalli* in lentic ecosystems.

The ability of *T. rendalli* to achieve good growth rates when feeding on marginal vegetation prompted a subsequent study where its utilisation of readily available plant diets was evaluated under culture conditions. The culture of macrophagous fish that naturally feed on plant diets may be the solution to reduce the current dependence on fishmeal. Fishmeal is not only expensive, but its supply is not always guaranteed. This study focussed on the growth performance, gastric evacuation rate, gastric transit time and carcass composition of *Tilapia rendalli* fed fresh plants, to determine the extent to which *T. rendalli* can utilise fresh plants. Kikuyu grass (*Pennisetum clandestinum*), cabbage (*Brassica oleracea)*, duckweed (*Lemna minor*), vallisneria (*Vallisneria aethiopica*) and fishmeal pellets (control) were offered *ad libitum* to duplicate groups of *T. rendalli* for 224 days. Specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR) were used to determine the growth performance. Fish fed kikuyu grass attained a significantly (P<0.05) higher SGR and a better FCR than those fed on the other plant diets. Fish fed vallisneria lost weight. The serial slaughter method showed that vallisneria was evacuated significantly ($P<0.05$, ANCOVA) faster and was eaten in significantly ($P<0.05$) higher quantities than the other diets. Kikuyu grass was evacuated much more slowly and eaten in lesser amounts than the other plant diets. The low energy content (14.74 MJ/kg) of vallisneria may explain its faster evacuation and high consumption levels. Digestibility studies indicated that *T. rendalli* is capable of breaking down both cellulose and fibre. Fish fed kikuyu grass had higher protein levels, higher omega-3 fatty acids (25.13%) and higher mineral content than those fed on the other experimental diets. Fishmeal fed fish had the lowest content of the omega-3 fatty acids (2.52%). *T. rendalli* performed better when fed plant diets with higher protein and energy contents.

The good growth performance and carcass quality of *T. rendalli* fed on kikuyu grass, led to another study where the use of kikuyu grass meal as a dietary protein replacement for fishmeal in practical diets for *T. rendalli* was evaluated. To determine the optimum substitution level, kikuyu grass meal was used to replace 20, 40, 60 and 80% of fishmeal in isonitrogenous (CP =16.70%) and isocaloric (GE =15.20 MJ/kg) diets. The test diets were fed to triplicate groups of fish held in 1 m^3 fibre glass tanks at 10 (36 ± 2 g) fish per tank for 60 days. The best specific growth rate (1.60 g/day) and feed conversion ratio (1.86) were recorded for fish fed diets with 20% kikuyu grass meal. The lowest specific growth rate (1.29 g/day) and feed conversion ratio (2.56) were recorded for fish fed diets with 80% kikuyu grass meal. When the level of kikuyu grass meal was more than 20% in the diet, growth performance was reduced. However, there were no statistically significant differences in the growth performance indices measured across the tested diets. The observed reduction in growth for diets containing higher kikuyu grass meal is explained by the decreasing amino acids levels (particularly methionine and lysine) and increasing fibre content. The results from the growth trials suggest that kikuyu grass meal is a suitable protein replacement for the expensive fishmeal in *T. rendalli* practical diets when it constitutes up to 20% of the dietary protein.

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CHAPTER 1: GENERAL INTRODUCTION

Food security remains a major global challenge as the world population continues to increase. Population projections indicate that there will be a 32% rise in the world's population between 1995 and 2020 (Food and Agricultural Organisation (FAO), 2004). Over 97% of this increase will be in the developing world and the greatest portion will be in Africa where the population growth is expected to increase by 70%. Africa is under increasing pressure to ensure food security (FAO, 2004). Chronic hunger is prevalent in Africa where between two and four hundred million people in Sub-Saharan Africa are affected by malnutrition. It is estimated that over 23 million African children are malnourished (World Bank, 2006). South Africa is no exception and measures to reduce malnutrition and hunger need to be taken.

Africa has to find means of meeting the increasing demand for protein for its growing population by considering not only terrestrial animals but also fish. With the turn of the century, more and more people are becoming conscious of their health, and fish is being recommended by nutritionists as a good protein and fatty acid source because of its high ratio of omega 3: omega 6 fatty acids. Aquaculture production is playing an increasing role in satisfying the demand for human consumption of fish. In the past few years, major increases in the quantity of fish consumed come from aquaculture. The average contribution of aquaculture to per capita fish available for human consumption rose from 14% in 1986, to 30% in 1996 and 47% in 2006, and is expected to reach 50% in the next few years (FAO, 2011).

Fisheries provide a source of protein and sustainable income in many parts of Africa. Thirty five million people depend wholly or partially on the fishery resources for their livelihood (World Fish, 2005). However, fish yields from capture-based fisheries seem to have reached or nearing their limits (FAO, 1996). The African populace has always exploited fishery and fish resources as an accepted and relatively cheap form of animal protein. Africa is only second to Asia on its dependence, per capita, on fish as a primary source of protein. Demand for fish has increased beyond production levels in Africa due to population growth in the past 40 years. Recently, there has been an increased interest in aquaculture as a way to increase production to meet this demand.

The development of rural aquaculture could be a possible solution to combat the food crisis in South Africa, leading to improved nutrition and income. The aquaculture industry in South Africa has failed to reach sustainable levels of development. One of the major hindrances to its development is the high production costs, which are mostly feed costs. Finding cheaper alternative feed sources can be an effective way to reduce fish production costs. Too often South African rural aquaculture projects have been initiated by donor funds with exaggerated commercial objectives and expectations, only to be abandoned at the end of the funding period. A different approach has to be adopted where fish farmers are educated on the use of cheaper plant based feed sources and fish species that will effectively utilise this feed.

Tilapia, is an important fish for consumption in developing countries and is widely used in commercial farming systems for intensive aquaculture, and constitutes the third largest group of farmed finfish, after carps and salmonids. Currently, tilapia culture is widely practiced in many tropical and subtropical regions of the world. More than 22 tilapia species are being cultured worldwide. However, hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) is the most commercially cultured tilapia species. Previously, tilapia was consumed mainly in Africa and Asia but nowadays it has been touted as the “new white fish” replacing the depleted ocean stocks, leading to a worldwide demand for the fish (Yue and Zhou, 2008).

The major determinant of a fish species to be used in aquaculture is its growth rate (especially in captivity), its acceptance of artificial feeds immediately after the yolk-sac absorption, resistance to handling stress, ease of reproduction, high fecundity and consumer acceptance. *Tilapia rendalli* meets these requirements; however, its culture in Southern Africa is very limited. Currently, *O. mossambicus* is the most widely cultured tilapia species in South Africa. However, production has not been successful; this may be due to the restricted temperature range in South Africa, as water temperatures are below optimum levels most of the year. Furthermore, *O. mossambicus*’s breeding nature contributes to its low productivity as females stop feeding during mouth brooding. The costs of production continues to escalate, making aquaculture production a non-profitable enterprise. Feed costs represent over 50% of the total variable production costs. It is of primary importance for fish farmers to find inexpensive and efficient feeds. *T. rendalli* is a promising alternative candidate because of its ability to utilise plant protein (Chifamba, 1990) which is relatively cheap when compared to the conventional fishmeal.

The culturing of *T.* r*endalli* is minuscule in South Africa as it is often overlooked as an aquaculture candidate species. This may have been due to its lower growth rate when compared to *O. mossambicus* (Pauly *et al*., 1988). However, *T. rendalli* can play an important role under extensive/semi-intensive fish culture system as it feeds mainly on readily available macrophytes. It has many attributes that make it a good candidate for aquaculture (El-Sayed, 2006) and these include:

- feeding at low trophic levels as they feed largely on macrophytes,
- resistance to stress and disease,
- tolerance to a wide range of environmental conditions such as temperature, low dissolved oxygen levels, high ammonia levels and salinity,
- fairly fast growth and ability to reproduce readily in captivity and
- does not incubate eggs in the mouth, females do not stop feeding when breeding.

Despite these factors, there is very little information in the literature on its aquaculture potential. The paucity of information on this species, which has the potential to play an important role in semi-intensive aquaculture systems, prompted this study whose main focus was to determine the diet of *T. rendalli* in an environment where its preferred diet is not available and to evaluate its utilisation of readily available plant diets under culture environment.

Traditionally, fishmeal has been used as the major protein source in fish feeds because of its nutritional value and palatability. However, due to the limited world supplies and increasing price of fishmeal, fish farmers in the developing world cannot afford the expensive fish feed. Alternative plant protein sources are generally cheaper compared to animal protein sources. The shortage in world production of fish meal (the main conventional protein source) coupled with increased demand for fish meal in feeds for livestock and poultry is likely to reduce the dependence on fish meal as a single protein source in aquafeeds. Fish nutritionists have made several attempts to partially or totally replace fish meal with less expensive, locally available protein sources. The limiting factors in the utilization of plant protein sources by fish are related to diet palatability, digestibility and essential amino acid balance. Fish species, feeding habit and fish size also affect the utilization of plant protein sources by fish.

A wide range of feed sources has been exploited by several researchers in the culture of tilapias. These feed sources include; fishmeal, fishery by-products (Fagbenro, 1994; Wassef *et al.*, 2003) terrestrial animal by-products (Tacon *et al*., 1983) oilseed plants (Tacon *et al*., 1983), aquatic plants (Skillicorn *et al*., 1993; Fasakin *et al*., 1999). An understanding of the feeding biology, physical adaptations, and natural feeding habits is a critical prerequisite when selecting a fish species that can efficiently utilise non-conventional feed sources. A fish species that feeds on a wide range of food sources is preferred. *T. rendalli* is a potential species for aquaculture because of its herbivorous and opportunistic feeding habits.

1.1 Dissertation layout

The feeding and growth of *T. rendalli* was assessed both in a natural water body and in culture in order to determine the aquaculture potential of this species. This dissertation has been divided into seven Chapters, each addressing a step in assessing the aquaculture potential of *T. rendalli*.

Chapter 2

In this chapter, relevant literature on morphological feeding adaptations of *T. rendalli*, its feeding habits in natural water bodies, and its growth potential in such systems is reviewed. Furthermore, literature on the utilisation of different plant based diets and growth of this fish under culture conditions is explored.

Chapter 3

In this chapter, the feeding habits and growth of *T. rendalli* at Flag Boshielo Dam are described. Reference is made to the selection of sampling sites, as well as the description of marginal vegetation found in this dam. The contribution of *T. rendalli* in the total fish population is estimated. Furthermore, the physico–chemical properties of the water in this dam are described.

Chapter 4

This chapter focuses on the utilisation of locally available fresh plant diets by *T. rendalli*. Utilisation is measured by growth performance indices. Moreover, the effect of the plant diets on the carcass composition is determined.

Chapter 5

The factors affecting feeding selectivity, consumption rates, plant digestibility, gastric transit time and gastric evacuation rate of the selected plant diets in *T. rendalli* were explored.

Chapter 6

In this chapter the use of kikuyu grass as fishmeal substitute in practical diets for *T. rendalli* is investigated.

Chapter 7

The aquaculture potential of *Tilapia rendalli* is discussed based on the observed feeding habits and the resultant growth. Recommendations are made on how the ability of *T. rendalli* to utilise plant diets can be enhanced.

CHAPTER 2: LITERATURE REVIEW

2.1 Classification and taxonomy of tilapias

The name tilapia was an effort by A. Smith its author to spell the bushman word for ‘fish’ which began with a click, rendered ‘Til’ (Trewavas, 1982). They represent a large number of freshwater fish within the family Cichilidae. There are three major genuses of tilapia which have been used in aquaculture, namely; *Tilapia, Oreochromis and Sarotherodon.* The genus *Tilapia* was first described by Smith (1840). The genus *Tilapia* was later split based on breeding behaviour and feeding habits, into two subgenera: *Tilapia* (substrate spawners) and *Sarotherodon* (mouth brooders), El-Sayed (2006). The subgenus *Sarotherodon* was raised to a genus and further subdivided into two genera, *Sarotherodon* and *Oreochromis.* This subdivision was based on whether parental females (*Oreochromis*) or males (*Sarotherodon*) or both parental sexes (*Sarotherodon*) perform the mouth brooding behaviour (El-Sayed, 2006).

Tilapias of the genus *Tilapia* guard their eggs and fry. They are generally macrophyte feeders with coarse teeth and less than 12 gill rakers on the lower limb of the first gill arch (Lovell, 1989; Popma and Masser, 1999). The mouth brooding *Sarotherodon* and *Oreochromis* incubate the fertilised eggs and fry in the mouth of the male or female. They are primarily microphagous, feeding on phytoplankton, periphyton and detritus (El-Sayed, 2006). They have fine teeth, more than 14 gill rakers on the lower limb of the first gill arch (Matthew, 1992). The *Sarotherodon* and *Oreochromis* genuses differ in the response of the post larvae to their parents. In *Oreochromis* they respond positively to the retrieving motions of the mother, whereas in *Sarotherodon* neither parents nor young interact positively and the released young form schools and avoid their parents (Fishelson and Yaron, 1983).

The most commonly cultured species of tilapia are *Oreochromis niloticus*, *O. mossambicus* and *O. aureus*, and then *Tilapia rendalli*. *T. rendalli* may be the best candidate for extensive, semi-extensive or polyculture as it feeds on higher plants and has a reasonable growth rate when reared in extensive systems and supplemented with plant material (Chikafumbwa, 1996).

Tilapia rendalli is classified as follows (Skelton, 2001):

Kingdom: Animalia
    Phylum: Chordata
        Class: Actinopterygii
            Order: Perciformes
                Family: Cichlidae
                    Genus: *Tilapia*
                        Species: *Tilapia rendalli*
                            Common name: Redbreast tilapia

2.2 Origin and distribution of tilapias

Tilapias are euphemistically referred to as ‘aquatic chicken’ due to their high growth rates, adaptability to a wide range of conditions, ability to reproduce in captivity and feeding at low trophic levels (El-Sayed, 2006). They have the ability to survive in poor water conditions, feed on a wide range of sources including insect larvae, algae, fish fry, worms, plants, and detritus. Tilapias breed easily with no need for artificial methods (Nandlal and Pickering, 2004).

Tilapias are a fresh water group of fish originating exclusively from Africa excluding Madagascar (Philippart and Ruwet, 1982). Outside Africa, they are also widely distributed in South and Central America, Southern India, Sri Lanka and Lake Kinneret, Israel (Philippart and Ruwet, 1982). Although tilapias inhabit a wide range of ecosystems, they seem to have evolved as riverine fish living in marginal waters and flood plain pools, but they have adapted to lacustrine conditions (El-Sayed, 2006). This makes tilapias ideal for aquculture because although they are a riverine fish they are able to utilise the littoral (which is otherwise poorly utilised) when trapped in a reservoir as they are adapted to lacustrine conditions (Fernando and Holčík, 1991).

It is generally assumed that tilapias evolved from a marine ancestor which penetrated fresh water and that this accounts for the large number of euryhaline species. They can tolerate salinity as low as 0.1 mg NaCl and as high as 100% sea water (Balarin and Hatton, 1979; Lovell, 1989). Tilapia are tolerant to a wide range of water temperatures (9-42.5°C), dissolved oxygen as low as 0.1 mg/l, and an unionized ammonia concentration of 2.4 mg/l (Lovell, 1989; Popma and Masser, 1999).

Tilapias are currently found in various ecological water systems, including slow-moving rivers and flood plain pools and swamps, small shallow lakes, large deep lakes, impounded water bodies, isolated crater lakes, sodalakes, thermal springs and brackish-water lakes (Philippart and Ruwet, 1982; Lowe-McConnell, 2000). These fish are highly adapted to their environments as reflected by their tolerance to a wide range of environmental conditions.

T. *rendalli* was originally described by Boulenger in 1896 from the Sabi/Lundi River system in Zimbabwe. Its distribution and natural habitats are freshwater lakes and marshes. According to Skelton (2001) *T. rendalli* is native to Lake Tanganyika (Tanzania), Malawi basins, Lakes Chilwa and Chuita, South Africa, and Swaziland, but currently has a wide distribution across Africa and the rest of the world (Fig. 2.1).

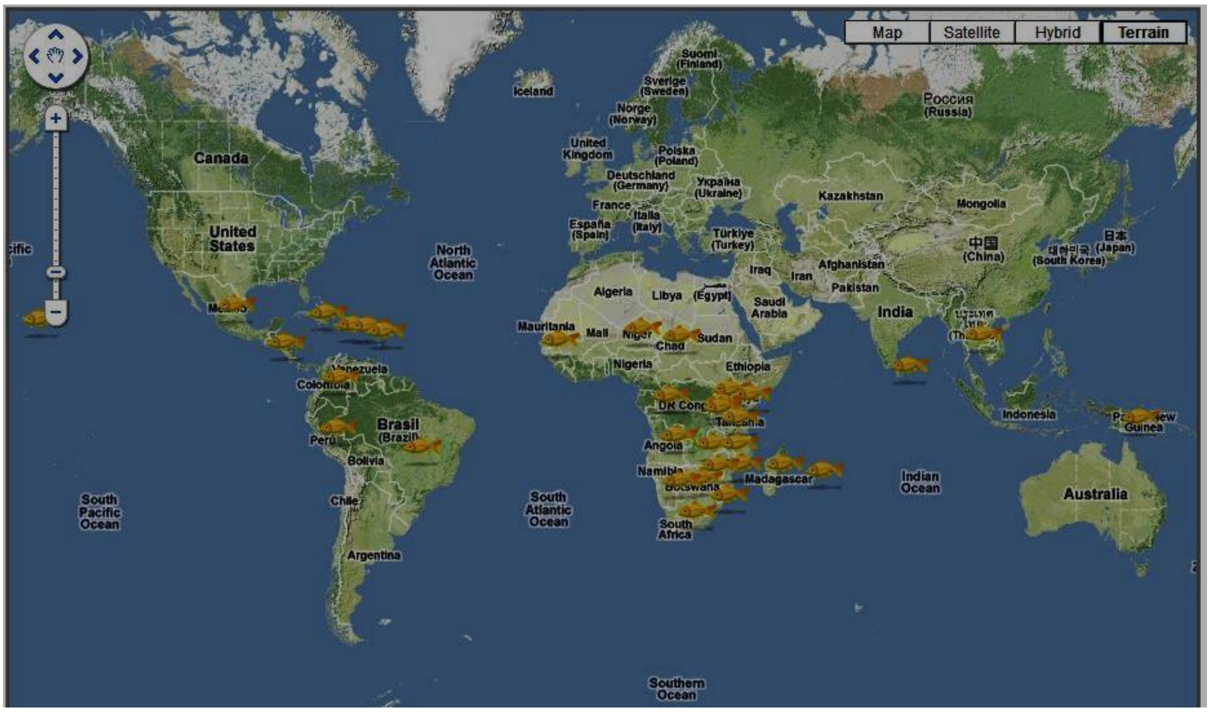


Figure 2.1: World distribution of *Tilapia rendalli* (shown in orange). (http://www.fishwise.co.za/Default.aspx?TabID=110&SpecieConfigId=25132 &GenusSpecies=*Tilapia_rendalli*).

The distribution of *T*. *rendalli* is affected by temperature and salinity. *T*. *rendalli* tolerates a wide range of temperatures (11-41°C), with the optimum being 19-28°C (Philippart and Ruwet, 1982; Behrends and Smitherman, 1984; Skelton, 2001). Juvenile *T. rendalli* are more tolerant to extreme temperatures than adults (Caulton, 1977).

T. *rendalli* naturally occurs in the tropics between 20°N and 20°S (Whitfield and Blaber, 1976). This fish has been introduced successfully into rivers and reservoirs south of the Pongola River in South Africa (>29°S). This however marks the southern boundary of the natural distribution (Whitfield and Blaber, 1976). These authors also suggest that it is because of its inability to tolerate salinities >19‰ that *T. rendalli* never succeeded in colonising the more southern rivers where the temperature regime is still sufficient for its survival and reproduction.

2.3 External Morphology of *Tilapia rendalli*

T. *rendalli* (Fig. 2.2) has a typically deep body that is laterally compressed, with a convex head. It has a protruding mouth with prominent bicuspid teeth. Its body and head are covered with relatively large cycloid scales that are not easily dislodged (Ross, 2000; Skelton, 2001). Mature fish are olive green to brown in colour, and have a bright red chest and throat; hence it is commonly known as Redbreast tilapia (Skelton, 2001). *T*. *rendalli* has dark vertical bars. Its caudal fin has a spotted upper half and a red or yellow half. This red or yellow pigment is also present on the anal fin. It has a carmine flush on the lower flanks, behind the pectoral fin, and this is not confined to one sex or to mature fish. The long dorsal fin is spiny and the rear is soft rayed and the anal fins also have hard spines and soft rays. The pectoral and pelvic fins are large and more anterior in an advanced configuration (Ross, 2000). It attains a total length of 400 mm and weight of up to 2 kg (Skelton, 2001) and lives up to 7 years. *T*. *rendalli* have well developed sense organs represented by prominent snares and a clearly visible and interrupted lateral line. The eyes are also relatively large, providing the fish with an excellent visual capability (El-Sayed, 2006).

Figure 2.2: Adult *Tilapia rendalli* (http://www.google.co.za/search?tbm=isch&hl=en&source=hp&biw=1334&bih=527&q=tialpia+rendalli&btnG=Search+Images&gbv=2&oq=tialpia+rendalli&aq=f&aqi=&aql=&gs_sm=s&gs_upl=0l0l0l2785l0l0l0l0l0l0l0l0ll0l0&safe=active)

2.4 The reproductive biology of *Tilapia rendalli*

Tilapia rendalli is a substrate spawner, breeding at temperatures above 21°C (Coward and Bromage, 2000). Breeding pairs clear vegetation to form a nest, excavating several tunnel-like brood chambers in which eggs and larvae are protected. Both parents guard the nest. After hatching, the female moves fry from one brood chamber to another. Juveniles up to 15 mm SL remain within brood chambers. *T. rendalli* raises several broods during summer. It has a high fecundity, laying approximately 5000 to 6000 eggs at each spawning (Coward and Bromage, 2000). The age and size at first maturity vary between strains and these are affected by environmental conditions (Lowe-McConnell, 1982), overfishing (Gwahaba, 1973), food supply and water body area (Duponchelle and Panfili, 1998).

Investigations on the reproductive biology of *Tilapia rendalli* in Lake Parakrama Sanudra in Sri Lanka showed that the overall female to male ratio was 1.1:1.0 (Chandrasoma and De Silva, 1981). These authors also confirmed that *T. rendalli* is a multiple spawner breeding throughout the year in this lake. They reported its fecundity to be between 760-6160 eggs at each spawning. The length and weight at first maturity varied from 18.8-25.8 cm and 126-380 g respectively (Chandrasoma and De Silva, 1981). In Lake Malawi, *T. rendalli* was reported to dig tunnels in their nests and practise communal care of the young. Both traits are considered to be anti-predator adaptations (Ribbink *et al*., 1981).

Kenmuir (1973) observed a breeding pair of *T. rendalli* in a tank under Lake Kariba's climatic conditions. He concluded that *T. rendalli* was a multiple spawner, breeding almost monthly from January to May, but when the water temperature dropped below 20°C in June, breeding stopped. Breeding commenced again in September until December when the temperature was above 20°C. The minimum temperature at which *T. rendalli* was found to breed was 20°C. Kenmuir (1973) concluded that *T. rendalli* could breed at least eight times a year in the lake. It is clear that *T. rendalli* is a prolific breeder, breeding with ease in different systems. These attributes make it a good candidate for aquaculture as there is no need for artificial breeding methods in its culture.

2.5 Feeding biology of *Tilapia rendalli*

In a broad sense, all tilapias are herbivorous and differ from the majority of fish feeding predominantly on small invertebrates or on young or small size fish. Tilapias are only one step from the primary producers (plant life). Opuszynski (1972); Opuszynski and Shireman (1995) classify herbivorous fish as those in which food constitutes more than 50% plant material by weight or volume, at least in some period of their life. Most species are opportunistic, changing their diet to whatever food items are abundant or available. Feeding habits also change with the age of fish and seasons, which may determine food availability.

Tilapias of the genus *Tilapia* are generally macrophyte feeders (Abdel-Malek, 1972; Caulton, 1976; Lowe-McConnell, 1982). *T. rendalli* is a diurnal feeding species and is predominantly herbivorous (Munro, 1967; Uieda, 1984). The juveniles generally feed on plankton and adults on macrophytes, however the adults often ingest algae, phytoplankton, zooplankton, bacteria, benthic invertebrates, insect larvae, fish, vertebrate eggs and detritus attached to the macrophytes they feed on. These attached materials are an important food component for tilapia species (Bowen, 1982).

Most fish experience a size related dietary shift as they all start feeding on zooplankton and later on become specialised (Fernando, 1994). Several authors have confimed the presence of an ontogenetic shift in the diet of *T. rendalli*. The size at which this shift occurs seem to vary in different strains or water bodies. Fryer and Iles (1972) concluded that *T. rendalli* undergoes an ontogenetic shift in diet from omnivory to macrophytophagy at 100-110 mm during the transition from juveniles to adults. Small fish feed on cladocerans, and there is a shift to filamentous algae in fish larger than 50 mm, although they remain opportunistic throughout their life (Munro, 1967; Caulton, 1976). On the other hand, Le Roux (1956) observed a shift away from chironomid larvae at about 130 mm total length (TL). In studies by Brummett (1995) analysis of stomach contents showed that juvenile fish of less than 150 mm TL prefer to feed on filamentous algae, followed by submerged macrophytes such as *Myriophyllum* and *Vallisneria*, and on softer emergent vegetation. Meschiatti and Arcifa (2002) reported that the main dietary item for *T. rendalli* less than 10 mm was rotifers. They observed an increase in the presence of microcrustaceans, aquatic insects and detritus in the diet of intermediate sized fish (11-33 mm) and that at 21-33 mm; the fish started feeding on higher plants.

In some studies however, the dietary shift was not observed. Batcherlor (1978) found no distinction between the diets of *T. rendalli* of various size classes at Doorndraai Dam in South Africa. Weliange and Amarasinghe (2003) did not observe any ontogenetic shift in the diet of *T. rendalli* in three reservoirs in Sri Lanka. They concluded that its feeding habits did not differ with fish size. The apparent absence of the dietary shift in these studies may be because very small fish (<10 mm) were not analysed in these studies. The presence of a dietary shift might permit improvements in the development of low cost aquafeeds for this species. Brummett (2000) working in fertilised ponds found that the ontogenetic shift in this fish was present, but it occurred rather abruptly. Stomach analysis showed the diet was either dominated by detritus or plant material with only a few stomachs containing an intermediate diet.

The diet of *T*. *rendalli* has been studied in different water bodies and this fish was found to be feeding mostly on macrophytes. Weliange *et al*. (2006) reported that macrophytes and detritus were the major food items of *T. rendalli* (6-9 cm) in

Minneriya Lake in Sri Lanka, contributing 59% and 29% respectively. In another reservoir, (Udawalawe) the diet of *T. rendalli* of length 6-9 cm was composed of 65% macrophytes and 18% detritus. In Lake Minneriya, the diet of 12-15 cm fish was predominantly macrophytes (94%). Meschiatti and Arcifa (2002) working in Lake Monte Alegre (Brazil) observed that in areas devoid of macrophytes, juvenile *T. rendalli* consumed a more diversified diet.

Caulton (1977) reported that the diet of *T. rendalli* is strongly related to the abundance of aquatic plants. In Lake Kariba (Zambia/Zimbabwe), during the period of invasion of this reservoir by *Salvinia molesta*, rootlets of this plant appeared in 19.6% of the investigated stomachs, representing 6.7% of the total food taken, while vegetable detritus represented 60%.

In fertilised ponds (Brummett, 2000) also found that the diet of *T. rendalli* of weight less than 25 g was basically detritus. Fish larger than 25 g however targeted higher plants. Juveniles (1-5 g) fed mainly on plankton, and it was found in 54-86% of the stomachs analysed for this size group. This was followed by detritus (46%) and there was no plant material in the diet of this size group. The diet of fish 6-10 g was also dominated by plankton (26-88%) and detritus was 74%. Plant material was still not important in this size group. In diets of fish greater than 21 g, no plankton was found and the principal component was plant material (31-93%) and detritus was 69%.

T. rendalli feeds on submerged marginal vegetation when macrophytes are not present. In studies by Batchelor (1978) the diet of this species, was diverse when flooded marginal vegetation was absent in the winter months. However, in the summer months (December), when the marginal vegetation was flooded, this fish feeds extensively on the inundated marginal vegetation.

Weliange and Amarasinghe (2007) found the diet of *T. rendalli* at the Minneriya reservoir in Sri Lanka to be dominated by plant material. Terrestrial plant leaves constituted 58% of the diet and aquatic plants accounted only for 4%. In smaller quantities were insects, zoo-benthos, insect larvae, and larval stages of Chironomidae, Ephemeroptera and Odonata. Other food items included diatoms, blue greens, green algae, uncommon algae, filamentous algae and fish.

Weliange and Amarasinghe (2003) studied the feeding habits of different freshwater fish in three reservoirs in Sri Lanka. They found that when the water level rose, there was an increase in food availability for macrophyte feeding fish like *T. rendalli* as they fed on submerged marginal vegetation. However, they found that the diet of *T. rendalli* did not exhibit significant variation in dietary habits between the seasons (of rising or receding water level).

In Volta Lake, Ghana, *T. zillii* and *T. rendalli* were found to feed on higher plants (predominantly of terrestrial origin from flooded land, such as grass). These formed 61.4% of the total food eaten (Petr, 1967). Chimbari *et al*. (1996) studied the food selection behaviour of *T. rendalli* under laboratory conditions to determine if this species can be used as biological agents for snail control. *T. rendalli* was found to be primarily herbivorous and would not feed on the snails. Furthermore, the presence of trout food made no difference in the weed-eating behaviour of *T. rendalli*.

T. rendalli has been described as an opportunistic feeder because of its ability to change its eating habits in the absence of a desired diet. Although *T. rendalli* is not a specialised plankton feeder, it has been reported to feed on different species of plankton. Arcifa and Meschiatti (1993; 1996) working in Lake Monte Algre, (a lake poor in macrophytes yet relatively euthrophic) observed that *T. rendalli* adults (166-245 mm) were feeding on the water column and were planktivores. Adults fed mostly on planktonic algae followed by zooplankton. Juveniles (25-65 mm) were found to be feeding on sediment, or in the scarce littoral vegetation and on insects. Algae however were consumed in small amounts. These authors also observed that the plant tissue in the diet of juveniles originated from decayed and not fresh material.

The ability of *T. rendalli* to feed on plankton was also confirmed by Lazzaro (1991). This author studied the diet of *T. rendalli* in Southern Brazilian lakes and reservoirs and classified *T. rendalli* as a planktivorous. In laboratory experiments, Lazzaro (1991) found that the feeding behaviour was fish size dependant and also influenced by the relative abundance of prey. Fish (29-42 mm) shifted from visual feeding on large copepods to filter feeding on small cladocerans and rotifers. This shift in feeding allows *T. rendalli* to optimally exploit patchy distribution of plankton resources.

T. rendalli shows plasticity in its diet when several water bodies with different food resources are compared. It has primarily been classified as a macrophyte feeder. It feeds on detritus and plankton on a lesser scale. *T. rendalli* is one of the species recorded by anglers at Flag Boshielo Dam. Preliminary visits to the dam showed that this dam has no rooted, submerged or floating macrophytes, yet there was a thriving population of *T. rendalli*. The reasons for a thriving population of *T. rendalli* at Flag Boshielo Dam prompted this study since this dam has no macrophytes. One of the key objectives of this study was to determine the diet of *T. rendalli* in a dam that had no macrophytes. Furthermore, the growth performance of this fish under such conditions was evaluated.

2.6 Adaptations for feeding on macrophytes

Plant food is less digestible than animal food because of the cellulose, which is difficult to break down. Herbivorous fish use pharyngeal teeth (pharyngeal mill) situated in the throat, to cut, tear and grind the plant material (De Silva and Anderson, 1995). This process allows for easier peristaltic mixing and increased exposure to digestive enzymes. Herbivorous fish compensate for the shortage of protein in plants (duckweed contains 31%, water hyacinth 24%) as compared to animal food (up to 80% protein) by ingesting higher quantities of food.

The digestion of aquatic macrophytes is not easy as many have fibres consisting of cellulose, hemicelluloses and lignin which are indigestible and these prevent access of intestinal enzymes to the cytoplasmic contents of plant material (Van Dyke and Sutton, 1977). Macrophytes may also have secondary compounds that may reduce grazing. Lodge (1991) listed the major groups of such compounds: alkaloids, flavanoids, steroids, saponins, phenolics (including tannins), and glucosinolates. The digestibility of plant protein decreases with increasing concentrations of tannins (Mitchell, 1974). Plants containing more than 6-7% tannin (dry weight) would result in low in digestibility as to be of little food value; most higher aquatic plants contain less than 2-3% tannin when harvested before maturity (Boyd, 1968) but tannin values tend to increase with age.

To overcome these obstacles, herbivorous fish have developed structural and secretory adaptations. The digestive system of tilapias is relatively simple and unspecialised, consisting of a very short oesophagus connected to a small sac-like stomach and a very long coiled intestine which can reach seven to thirteen times the total fish length (Caulton, 1976; Balarin and Hatton, 1979). *T. rendalli* being macrophyte feeders, have evolved both morphological and physiological adaptations to aid them in ingestion and mastication of plant material. Notable adaptations are bi- and tricuspid teeth on its jaws which aid in the cutting and macerating of macrophytes (De Silva and Anderson, 1995).

T. rendalli has a thin-walled, elastic stomach. In the stomach, gastric acid (hydrochloric acid) is secreted to an unusually low pH, frequently < 1.5 (Bowen, 1976; Perschbacher *et al*, 2010) which facilitates in the lyses of prokaryotic and eukaryotic cell walls to expose the cytoplasm to intestinal enzymes. This acid also activates pepsinogen to pepsin which is the first protease with which the food comes into contact in the digestive tract. This low pH also benefits the fish by facilitating the release of free amino acids from detritus (Bowen, 1980). The low stomach pH accompanies the presence of food; hence HCl release is triggered by the presence of food in the stomach (Randall, 1961). The long intestines reflect the herbivorous feeding of these fish perhaps because vegetable foods are digested less readily than animal sources (El-Sayed, 2006). The herbivorous feeding nature of *T. rendalli* makes it a potential candidate for semi-intensive aquaculture because it can utilize plant material.

2.7 Environmental requirements for the optimum growth of *Tilapia rendalli*

Temperature is one of the most important factors affecting the physiology, growth reproduction and metabolism of tilapia (El-Sayed, 2006). Tilapias are thermophilic fish, known to tolerate a wide range of water temperatures. The optimum temperature for *Tilapia rendalli* is 19-28°C (Skelton, 2001). Philippart and Ruwet (1982) described 11 and 41°C as the lower and upper lethal temperatures for this fish species.

Ammonia is also a very important parameter in the culture of tilapias as most of the nitrogenous wastes of fish are excreted via the gills in the form of ammonia.

Excreted ammonia exists in un-ionised NH_3 form (UIA-N), which is toxic to fish and ionized NH_4^+, which is non-toxic (Chervinki, 1982). The toxicity of ammonia is influenced by DO, CO_2 and pH. The toxicity increases with decreasing DO and decreases with increasing CO_2 (Chervinki, 1982). Although tilapia can tolerate high levels of ammonia compared to other species, for optimum results, the UIA-N concentration should be maintained below 0.1 mg/l (El-Sayed, 2006).

Dissolved oxygen (DO) is another very critical factor affecting fish feeding, growth and metabolism. Ambient DO range produces the best fish performance, while low DO levels limit respiration, growth and other metabolic activities (Tsadik and Kutty, 1987). Tilapia are known to withstand very low levels of DO as low as 0.1-0.5 mg/l and can even survive at zero DO concentration if they are allowed access to surface air (Tsadik and Kutty, 1987). On the other hand, tilapia can tolerate conditions of high oxygen (up to 400%), which usually occurs because of high photosynthesis resulting from phytoplankton and macrophytes blooming (Morgan, 1972).

2.8 Present status of the aquaculture production of *Tilapia rendalli*

Aquaculture production has pushed the demand for consumption of several freshwater species, such as tilapia and catfish as well as for high-value species, such as shrimps, salmon and bivalves (FAO, 2008). Since the mid-1980s, these species have shifted from being primarily wild-caught to being primarily aquaculture-produced, and a strong increase in their commercialization. Aquaculture has also had a major role in terms of food security in several developing countries (FAO, 2008).

The raising of tilapias for food consumption is believed to first have taken place about 2,500 years ago (Chapman, 1992). Tilapia today has become the third most important fish in aquaculture after carps and salmonids (El-Sayed, 2006). The production of cultured tilapia has shown a tremendous increase jumping from 383 654 metric tons in 1990; 1 505 804 metric tons in 2002 to 3 500 000 metric tonnes in 2008 (FAO, 2008). About 16 tilapine species have been farmed for aquaculture production out of which ten species are commercially exploited (FAO, 2008).

The Nile tilapia (*Oreochromis niloticus*), is by far, the most important cultured species, representing more than 80% of total tilapia production. The Mozambique tilapia (*Oreochromis mossambicus*) comes second, followed by the three-spotted tilapia (*Oreochromis andersonii*) then the Redbreast tilapia (*Tilapia rendalli*; El-Sayed, 2006).

T. rendalli is already an important commercial species in South-Central Africa and with the increasing demand for fish protein; it is becoming an important aquaculture species (Mair, 2001). In Malawi, *T. rendalli* is one of the most cultured species where it has been selected together with *O. karongae*, *O. shiranus* and *Dinotoperus* species as potential species for both commercial and small scale farming. This selection was based on growth, ability to breed, withstand stress and marketing value (Nyirenda *et al*., 2000). A review conducted by the FAO (2004) reported that Sub-Saharan countries are lagging behind in aquaculture development; mainly due to lack of a well developed aquaculture fish feed production. Diets in aquaculture are mainly based on conventional feed-stuffs such as fish oils and fishmeal (Goddard, 1996) but these are expensive for small scale semi-intensive farming systems. Attention is now being shifted to the use of resources available on the farm, including the common practice of pond fertilisation.

2.9 The growth of *Tilapia rendalli* in aquaculture systems

The potential of *T. rendalli* to contribute in commercial aquaculture has not been fully exploited. There is very little reported on the growth of *T. rendalli* in intensive culture. In extensive culture systems (ponds) the growth rate of *T. rendalli* is reported by several researchers. Mataka and Kang'ombe (2007) conducted an experiment to determine the effect of substituting maize bran with chicken manure on the production of *T. rendalli* juveniles (10.71 g) in semi-intensive pond culture. They reported a higher specific growth rate (SGR) of 1.18%/day in ponds where 75% maize bran and 25% chicken manure was applied than the 0.87%/day (SGR) where only maize bran was fed. These authors concluded that the use of chicken manure would produce an increased yield if combined with a supplementary feed like maize bran.

In a separate study, Kang’ombe and Brown (2008) reported that fertilisation with chicken manure alone produced low growth rates. The highest growth and SGR was realised in treatments where chicken manure was supplemented with soybean based diets, followed by sunflower based diets and cottonseed cake based diets. The SGR was 3.6; 2.9; 2.5; and 2.1 for soybean, sunflower, cottonseed and chicken manure respectively. The lowest food conversion ratio (FCR) of 1.2 was obtained in soybean based diets followed by sunflower (1.6) and cottonseed (1.9). These authors concluded that the use of low protein diets containing soy would produce better results and increased yield when combined with fertilisation. In another study, Soko and Likongwe (2002) reported an SGR of 0.87%/day in *T. rendalli* fed maize bran in ponds, and that the addition of chicken manure increased the SGR to 1.18%/day.

These studies suggest that *T. rendalli* can be used as an aquaculture species when its aquaculture potential is fully exploited. This fish has demonstrated its ability to utilise plant diets, however this ability has not been fully explored.

2.10 Use of fresh plants in tilapia diets

A review of literature indicates that a number of research studies have been done on various fresh plant diets. In experiments with controlled feeding regimes where fish were fed fresh macrophytes as complete diets in clear water systems (glass or fibre glass tanks) have often yielded very poor or negative growth results. For example, Hajra (1987) reported an SGR of 0.23%/day in grass carp when hornwort was fed *ad libitum* in a fibre glass rearing system. In an earlier study, Venkatesh and Shetty (1978) obtained an SGR of 1.17%/day and an FCR of 94.00 when feeding oxygen weed to grass carp.

In experiments conducted by Setlikova and Adamek (2004) *O. niloticus* exhibited slow growth and even lost weight when fed exclusively on aquatic vegetation. Fish fed *Potamogeton pectinatus* had an SGR of 3.18%/day whilst those fed *Elodea canadensis* had a slightly lower growth rate with an SGR of 2.54%/day and an FCR of 0.34. However, fish fed *Spirodela polyrhiza and Myriophyllum spicatum* lost weight; they reported an SGR of -1.75 and -1.71 and an FCR of -3.78 and -0.35 respectively. In another study, *O. aureus* also lost weight when fed three aquatic macrophytes (*Elodea canadensis, Myriophyllum spicatum* and *Potamogeton gramineous*) which resulted in negative feed conversion efficiency (Okeyo and Montgomery, 1992).

According to Saeed and Ziebell (1986) *T. zillii* (17.9 g mean weight) showed a slow SGR of 0.15%/day when fed *Chara sp ad libitum* in glass tanks. In a separate experiment, the same authors fed *Najas marina* to *T. zillii* (16.7 g mean weight) held in aquaria. The fish attained an SGR of 0.12%. However, when *T. zillii* was fed on *Elodera densa* or *Myriophyllum exasbescens* negative growth was displayed.

Experiments have been done with success in feeding *T. rendalli* with Napier grass (*Pennisetum purpureum)*. Chikafumbwa (1996) concluded that feeding *Tilapia rendalli* with Napier grass produced favourable growth rates (SGR 1.29%/day) and was an effective low cost feed for African small holder farmers, and that supplementing the chopped grass was not necessary in pond culture.

Several authors have concluded that duckweed (*Lemna* sp.) has considerable potential for use in tilapia feed as a complete diet in its natural form (Edwards, 1987; Journey *et al*., 1990; Wee, 1991). MacKenzie (1963) observed that *T. rendalli* will feed well on any waste fruit or vegetable matter. Gaigher *et al*. (1984) compared the growth of hybrid tilapia fish (*O. niloticus* X *O. aureus*) on commercial pellets versus duckweed. The fish were cultured at high densities in an experimental recirculating unit for 89 days with duckweed (*Lemna gibba*) or a combination of duckweed and commercial pellets. They concluded that a combination of pellets and *Lemna* gave the best performance, however, when the fish were fed duckweed only, intake rate was low, feed conversion ratio was good (1:1) and relative growth rate poor (0.67% of bodyweight daily). A combination of duckweed and pellets resulted in decreased consumption of duckweed and an increase in growth rate.

Hassan and Edwards (1992) raised tilapia in static-water concrete tanks feeding them two species of duckweed, *Lemna perpusilla* and *Spirodela polyrrhiza* at levels of 0, 25, 50 or 75 g duckweed dry matter (DM) per kg wet weight of fish. The *Spirodela* was poorly consumed whereas *Lemna* was rapidly ingested by fish. In experiments on the utilisation of macrophytes by *O. niloticus* and *T. rendalli* (Micha *et al*., 1988) it was found that *T. rendalli* outperformed *O. niloticus* when fed *Azolla microphylla* only. Although there was 10% mortality in *O. niloticus*, all the *T. rendalli* survived. This may suggest that even though *T. rendalli* does not grow as fast as tilapia of the *Oreochromis* genus, it may be the solution to the current problem of high feed costs in aquaculture because of its ability to utilise higher plants.

The utilisation of any feedstuff is largely dependent on its digestibility. Digestion of food in fish depends on three main factors: Firstly, the ingested food and the extent to which it can be broken down by digestive enzymes; secondly, the activity of the digestive enzymes; thirdly, the length of time the food is exposed to the action of the digestive enzymes. All of these factors are also dependent on a multitude of secondary factors, these include the fish itself, such as fish species, age, size and physiological condition; and the environmental conditions, such as water temperature, and some are related to the food itself, such as its composition, particle size and amount eaten (Hepher, 1988).

2.11 Feeding preference by *Tilapia rendalli*

Herbivorous fish have been shown to feed selectively on available macrophytes (Bryan, 1975; Horn *et al*., 1982; Ojeda and Munoz, 1999). A study by Schwartz and Maughan (1984) showed that the blue tilapia preferred softer macrophytes that are easily broken up and digestible, while *O. mossambicus* preferred small floating macrophytes than large and rooted macrophytes. The main factors reported to affect preference or avoidance of certain macrophytes by herbivorous fish are defensive chemicals in the form of secondary metabolites (Hay, 1992; Cronin and Hay, 1996) and morphological properties or toughness (Watson and Norton, 1985; McShane *et al*., 1994). It has frequently been assumed that the nutrient content is a good index of nutritive value to the consumer. This presumed correspondence is often supported with reference to Horn and Neighbors (1984) who found that *Cebidichthys violaceus* (Girard) assimilated more nutrients from algae that had high nutrient content than algae lower in nutrients.

Nitrogen is a limiting nutrient for many herbivores (Fenchel and Jorgensen, 1977) and is thought to be the nutritional constituent that most frequently influences food choice in terrestrial herbivores (Mattson, 1980; White, 1985). The low concentration of nitrogen, coupled with inter-specific differences in macrophyte nitrogen content, suggests that selection of plants high in nitrogen may be important to individual fitness (Neighbors and Horn, 1991; Bruggemann *et al*., 1994). Energy and protein content are regarded as the most important components in determining nutritive value of a diet (Westoby, 1974; 1977). Items with high structural components, polyphenolic compounds and calcareous material are avoided while food with either high protein or high energy levels or both are favoured (Horn *et al*., 1982).

Preference for some macrophyte species has been investigated by several authors, Prabhavathy and Sreenivasan (1976) reported that grass carp are known to ignore all aquatic vegetation in the presence of oxygen weed. Venkatesh and Shetty (1978) fed two submerged macrophytes (oxygen weed and hornwort) to grass carp and observed that oxygen weed was most readily consumed, the whole plant being eaten in the process. In the case of the hornwort, these authors reported that smaller fish preferred only the leaves, while the bigger fish fed readily on the entire plant. In another study, Mitzner (1978) found that grass carp have a preference for *Najas* and *Potamogeton*. The feeding preferences of the blue tilapia (*Tilapia aurea*) for five aquatic macrophytes were tested by Schwartz and Maughan (1984). These authors found that the order of preference was *Najas guadalupesnis*, *Chara spp*, filamentous algae (predominantly *Cladophora* spp.), *Potamogeton pectinatus* L. and *P. nodosus*.

Tilapia zillii, (a species closely related to *T. rendalli*) also feeds on various types of macrophytes but also shows preference when feeding choices are offered. For example, Buddington (1979) reported that *T. zillii* preferred *Najas guadalupensis* as a food source to *Lemna, Myriophyllum spicatum* and *Potamogeton pectinatus.* Saeed and Ziebell (1986) fed different macrophytes to *T. zillii* and observed that the most preferred macrophyte was *Chara* followed by *Najas marina, Elodea densa* and *Myriophyllum exalbescens*. These authors noted that the coarseness of these macrophytes appeared to have some effect on its consumption by the fish.

In experiments to find out whether *T. rendalli* is a selective feeder giving preference to some plants, Chifamba (1990) fed the fish the following plants: *Ceratophyllum demersum, Lagarosiphon ilicifolius, Vallisneria aethiopica* and *Najas pectinata*. The fish reared in tanks preferred *V. aethiopica, N. pectinata, L. ilicifolius* and

C. demersum respectively. In the wild, the fish feeding preference was: *V. aethiopica, L. ilicifolius, N. pectinata,* and *C. demersum* respectively. In both cases, *C. demersum* was the least preferred food. Chifamba (1990) concluded that the preference of the fish for *V. aethiopica* is explained by its high protein and ash content and that selection was based on palatability and nutritive value.

Other factors such as palatability, abundance of the particular plant, environmental factors, and fish size also play important roles in determining macrophyte preferences. It became important to determine the preference of *T. rendalli* for the different diets used in this study.

2.12 Gut transit time and gastric evacuation

A measure of nutrients taken up by a fish from a macrophyte (nutrient availability) may be a better indicator of food value than a measure of nutrients within the macrophyte (Neighbors and Horn, 1991). Horn (1989) suggested that not all food items are of equal value, because of the differences in macrophyte susceptibility to digestive mechanisms employed by herbivorous fish. This is important, as differences in the ability to digest and assimilate certain macrophytes may influence diet choice. Gut transit time (GTT) can be used in determining the value of a food source because it has been shown that the GTT of different aquatic plants fed to the same fish may vary by several hours (Targett and Targett, 1990).

Food is usually broken down in the stomach of fish through a combination of muscular contractions of the stomach wall and enzymic action in an acid medium (Bromley, 1994). Breakdown products are expelled from the stomach through a process called gastric evacuation. Gastric evacuation is mediated by nervous and hormonal control, including feedback mechanisms (Jobling, 1986).The metabolic rate and hence gastric evacuation rate is a function of environmental conditions (i.e. temperature), species, dietary composition, meal size, fish size and feeding frequency (Dos Santos and Jobling, 1995; Wang *et al*., 1998 and Riche, 2000) and it is one of the prime factors in determining growth rate (Riche *et al*., 2004).

Knowledge of gastric evacuation rate in fish is a necessary tool for both field and laboratory studies concerned with feeding rates, energy budgets and the trophic dynamics of aquatic systems (Sweka *et al*., 2004; Kawaguchi *et al*., 2007). Gastric evacuation rates are an important component in feed management in aquaculture as it has been demonstrated that the quantity of food a fish eats is dependent on stomach fullness, and the time in between subsequent meals is a function of the rate of emptying of the stomach (Holmgren *et al*., 1983; Grove *et al*., 1985). Gastric evacuation rate has proven to be an important parameter for modelling daily feed intake (Jobling, 1981) and can be used to estimate the return of appetite (Riche *et al*., 2004).

Several factors have been found to influence food passage rate. Gastric evacuation rate positively correlates with temperature (Ryer and Boehlert, 1983). These authors reported that evacuation rate is temperature dependent and that faster evacuation rates were observed at high temperatures and slower rates at lower temperatures. As temperature increases above the optimum range the rate of gastric evacuation remains constant and then slows down. Appetite, digestion rate, and amount of secretions produced, all decrease with a decrease in temperature (Ryer and Boehlert, 1983).

In another study, Garber (1983) reported that gastric evacuation rate is affected by dietary moisture. Food particles size and energy content of the food has also been reported to regulate gastric evacuation (De Silva and Anderson, 1995). These authors stated that the greater the energy content, the slower the evacuation rate. Shiau *et al*. (2003) reported that the gastric emptying times decreased as the inclusion level of carboxymethylcellulose was increased in the tilapia diets.

The time of disappearance from the stomach is thought to be a better predictor of the return of appetite. Fish culturists can use GTT to develop appropriate feeding strategies for increasing efficiency in aquaculture. Making food available at an appropriate rate and as soon as appetite has returned can maximize intake and increase feed efficiency (Bromley, 1994; Lee *et al*., 2000; Riche *et al*., 2004; Booth *et al*., 2008). The determination of the optimal feeding schedule of fish is needed for efficient production. Under steady conditions the rate of gastric evacuation equals the rate of ingestion. Daily emptying can be estimated from measurements of stomach fullness and gastric evacuation rates (Hop and Tonn, 1998).

2.13 Replacement of fishmeal in tilapia diets

Dietary protein is always considered to be the most important nutrient component of complete formulated diets (Jauncey, 2000). There have been major studies and efforts which have focussed on the dietary replacement of fishmeal (FM) in tilapia feeds with alternative protein sources. These include fishery and terrestrial animal by-product meals, oilseed meals, aquatic plants, single-cell proteins, legumes and cereal by-products (El-Sayed, 2006). However, Diana (1997) states that fish protein sources are still most reliable in producing rapid tilapia growth as research on substitution of plant protein sources for fish meal has contradictory results.

Soybean meal (SBM) is generally considered to be one of the best readily available plant protein sources (El-Sayed, 2006) because of its protein quality and amino acid profile. In a number of studies, it has been shown that between 67 to 100% of the dietary protein can be supplied in form of SBM in tilapia diets. For example, pre-pressed solvent extracted or full-fat SBM successfully replaced up to 75% of FM in diets fed to *O. nilotcus* fry (Tacon *et al*., 1983) and *O. mossambicus* (Jackson *et al*., 1982). In experiments to test the effect of replacing animal protein with soybean meal, Nyirenda *et al.* (2000) found no significant differences in the growth rate, SGR or FCR in *O*. *karongae* when fed diets containing 0; 5.5 or 10% SBM.

Dietary inclusion of SBM in tilapia feeds seem to be affected by the dietary protein level. Davis and Stickney (1978) fed *O. aureus* SBM based diets at dietary levels ranging from 15 to 36%, and found that SBM impaired fish growth at 15% CP level, but at 36% CP levels, SBM could totally replace FM. These results contradict those reported by Shiau *et al*. (1987) who worked with *O. niloticus* x *O. aureus* hybrids and reported that FM could be partially replaced by SBM within diets containing sub-optimal protein levels (24%), whereas at optimum protein levels (32%) the dietary replacement of FM with 30% SBM significantly depressed fish performance.

Relatively few studies have been conducted concerning the use of aquatic plants as feed ingredients within tilapia feeds and usually yield conflicting results. For example, Almazan *et al.* (1986) and El-Sayed (1992) reported extremely poor performance for *O. niloticus* fingerlings and adults fed *Azolla pinnata*-based diets. Similar results were reported by Micha *et al.* (1988) for *O. niloticus* and *T. rendalli* fed *Azolla* *microphylla*. In contrast, Santiago *et al.* (1988) found that *O. niloticus* fed rations containing up to 42% *A. pinnata* performed better than fish fed a fish meal-based diet.

Duckweed *Lemna* species has also been used as a fishmeal replacer within tilapia feeds. Mbagwu and Andeniji (1988) reported that *Sarotherodon galilaeus* fed a diet (33% protein) containing duckweed *L. paucicostata* as a partial protein source exhibited better growth and feed efficiency than fish fed a commercial (40% protein) diet.

Tavares *et al*. (2008) fed *O. niloticus* fingerlings dried duckweed or its combination with a commercial diet. They found that there was no significant difference in the final weights and SGR of fingerlings fed the commercial diet and those fed 50% commercial diet and 50% dried duckweed. They concluded that dried duckweed can replace up to 50% protein in tilapia fingerling diets without reducing growth.

The growth of *T. rendalli* increased when the dietary protein level was increased (Musuka *et al*., 2009). In the study, dietary protein and water temperature were analysed for effect on the body weight, SGR and FCR of *T. rendalli*. Fish given a diet containing 40% crude protein (CP) at 30°C showed the best growth (220.55%, weight gain), best SGR (1.19%/day) and best FCR (2.74). Fish given a diet containing 30% CP at 22.79°C showed lowest weight gain (75.71%), lowest SGR (58%/day) and highest FCR (4.82). These authors concluded that both temperature and dietary protein had significant effects on the growth and feed utilisation in *T. rendalli*.

The opportunistic feeding behaviour of *T. rendalli* provides an advantage to farmers because it can be reared in extensive, semi-intensive, as well as intensive systems that can be operated with lower feed cost. Even though work has been done on the replacement of fishmeal in tilapia feeds with cheaper protein sources, no work was done on replacing fishmeal in *T. rendalli* feeds using terrestrial grasses yet it has been observed that this fish feeds on inundated terrestrial vegetation during the rainy season.

It is well established that *T. rendalli* feeds from the lower end of the food chain and is well adapted to handling macrophytes, feeding on them in natural systems. In Flag Boshielo Dam there are no macrophytes and it was prudent to investigate the feeding habits and growth of *T. rendalli* in such a dam.

Feed costs constitute more than 50% of the total costs in most aquaculture farms. Protein is the most expensive dietary component particularly if it is animal based. More cost effective plant based protein sources are being increasingly used. The ability of *T. rendalli* to utilise plant diets may be the first step in finding a solution for the high feed costs in tilapia culture. There is paucity of information on the utilisation of fresh plant diets by *T. rendalli* in culture systems. This information is important in evaluating the aquaculture potential of *T. rendalli*. The culture of a species that is naturally adapted to utilise plant diets may result in better growth performance, this may also lead in increases in the level at which plant diets are included in its diet. In this study, the use of a terrestrial grass, kikuyu as a replacement for fishmeal in practical diets of *T. rendalli* was undertaken. No previous studies have been done on the inclusion of kikuyu in tilapia diets. The inclusion of kikuyu grass as a replacement for fishmeal is important in assessing the aquaculture potential of *T. rendalli*.

CHAPTER 3: THE FEEDING AND GROWTH OF TILAPIA RENDALLI AT FLAG BOSHIELO DAM

3.1 INTRODUCTION

Tilapia rendalli is a known macrophyte feeder (Munro, 1967) and can also have a generalised diet becoming opportunistic, changing its diet to whatever food items are abundant or available (Arcifa and Meschiatti, 1996). Its feeding habits also change with the age of fish and seasons (Tripp-Valdez and Arrenguini-Sachez, 2009).

T. rendalli is one of the species recorded by anglers at Flag Boshielo Dam. However, preliminary visits to the dam showed that Flag Boshielo Dam has no rooted, submerged or floating macrophytes, yet it supports a thriving population of the macrophagous *T. rendalli*. The reasons for a presence of *T. rendalli* at Flag Boshielo Dam prompted this study since this dam has no macrophytes.

No pre-impoundment or post-impoundment studies have been published on Flag Boshielo dam. It was important to investigate the physico-chemical parameters of this dam and compile a data base which will serve as a reference for future limnological studies. Fish production is largely determined by the trophic status of the water body. The fisheries potential of this dam is not known. The morphometrical and physico-chemical parameters determined in this study were used to predict the fisheries potential. There are several fish species caught by anglers in the dam. However, as there were no pre-impoundment or post impoundment studies, there is no baseline data on the fish species composition of this dam. The species composition of this dam was also investigated to determine the importance of *T. rendalli* to anglers and also ascertain its potential as a commercial fish species.

The key objective of this chapter was to determine the diet of *T. rendalli* in a dam that had no macrophytes. Furthermore, the growth performance of this fish under these conditions was investigated.

3.2 OBJECTIVES

i) To determine temporal variations in the diet of *Tilapia rendalli* in Flag Boshielo Dam.
ii) To determine size related shifts in the diet of *Tilapia rendalli* in Flag Boshielo Dam.
iii) To determine the growth of male and female *Tilapia rendalli* in Flag Boshielo Dam.

3.3 NULL HYPOTHESES

i) There are no significant temporal variations in the diet of *Tilapia rendalli* in Flag Boshielo Dam.
ii) There are no size related dietary shifts in *Tilapia rendalli* in Flag Boshielo Dam.
iii) There is no significant difference in the growth of male and female *Tilapia rendalli* in Flag Boshielo Dam.

3.4 MATERIALS AND METHODS

3.4.1 Description of the study site

Flag Boshielo Dam (Fig. 3.1) is situated 25 km north east of the town Marble Hall, in the extreme north-western corner of South Africa's Mpumalanga Province.

Figure 3.1: Flag Boshielo Dam (24°49´05´´S; 029°26´39´´E)

Construction of the Flag Boshielo Dam on the Olifants River was completed in 1987 (Clark, 1997). This dam is maintained by the Department of Water Affairs and Forestry. The dam was originally known as the Mokgomo Dam but the name was later changed to Arabie Dam, named after the original farm on which the wall was built. However, in 2001 the name was again changed, to the Flag Boshielo Dam.

The wall of the Flag Boshielo Dam (Fig. 3.1) has a height of 36 m, and a length of 1 225 m. Total catchment area of this dam is 23 712 km^2, with a surface area of 1 288 hectares and a capacity of 185 100 000 m^3. It has a mean depth of 22 m and usually overflows between the months of October and July (Department of Water Affairs and Forestry, 2003).

Flag Boshielo Dam was constructed mainly for irrigation, domestic and industrial supply. Irrigation was initially allocated 18 million m^3 from the dam, however only about 400 ha is currently being irrigated with a monthly water usage of 24 400 m^3. The main crops irrigated are maize and vegetables. Water from Flag Boshielo Dam is supplied to service providers like Lepelle Northern Water for domestic use with an average monthly usage of 366 00m^3. For industrial purposes, water from the dam is supplied to the Labalelo Water User Association which distributes it to several mines including Harvecraft, Mototolo, Marula, and Modikwa.

According to the South African Weather Service, the dam falls within the Northern Transvaal climatic zone (Schulze, 1994), where the rainy season starts in November and peaks in January. However the rainfall in this area is unreliable and severe drought conditions are common.

Several large impoundments situated upstream from the Flag Boshielo Dam have a significant effect on the water level of this dam. The biggest of these is the Loskop Dam, about 85 km upstream on the Olifants River, and the Mkhombo Dam (formerly Rhenosterkop Dam) which can be found about 70 km upstream on the Elands River. The major rivers that feed into the Flag Boshielo Dam are the Olifants River and the Elands River. Several other small non-perennial streams also feed the dam intermittently during periods of high rainfall.

Three sampling sites were selected: (a) dam wall, (b) middle of the dam and (c) the inflow of Flag Boshielo Dam (Fig 3.2).

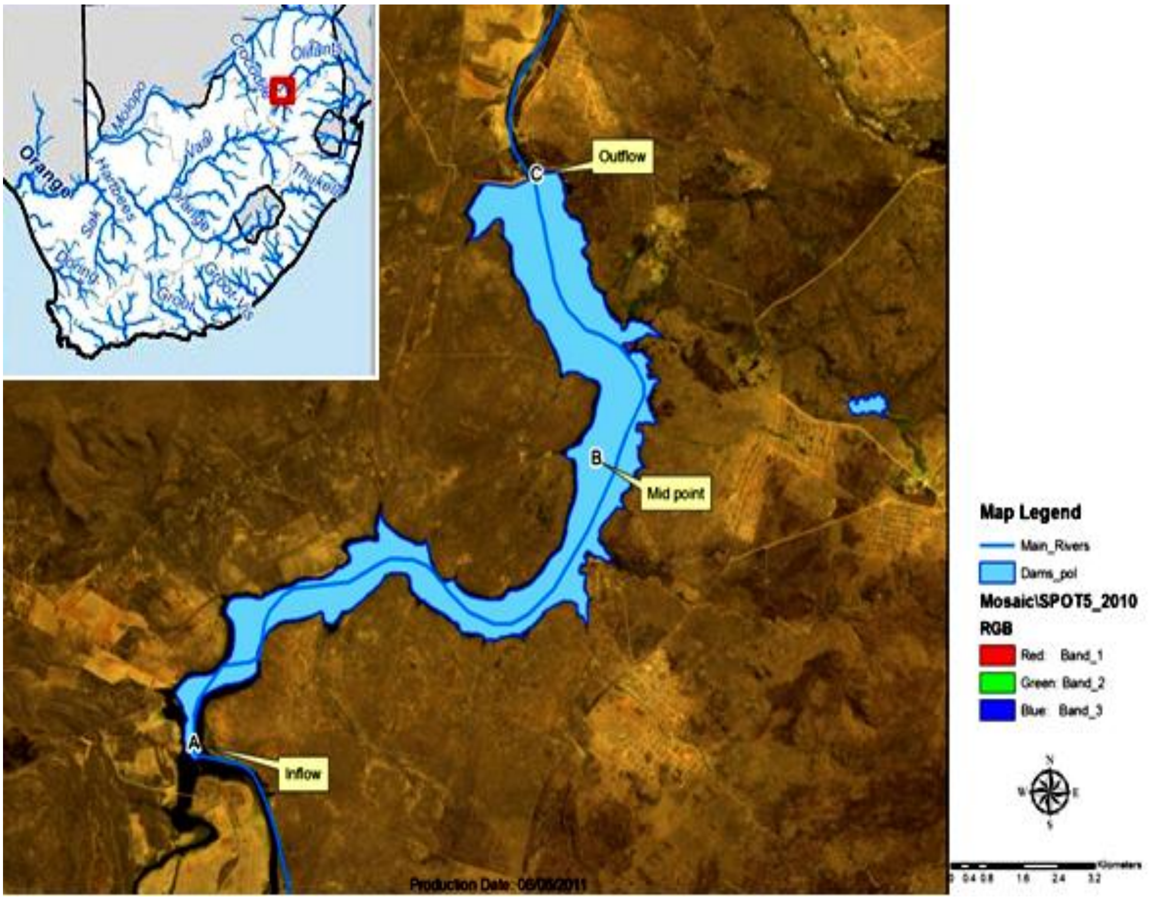


Figure 3.2: Map of Flag Boshielo Dam, showing the three sampling sites.

The three sampling sites were selected because of their distinct habitant characteristics. The dam wall site (Fig. 3.3A) was the deepest site. The middle site (Fig. 3.3B) was characterised by a number of drowned trees, while the inflow site (Fig. 3.3C) was shallow, more turbid with some rocky parts.

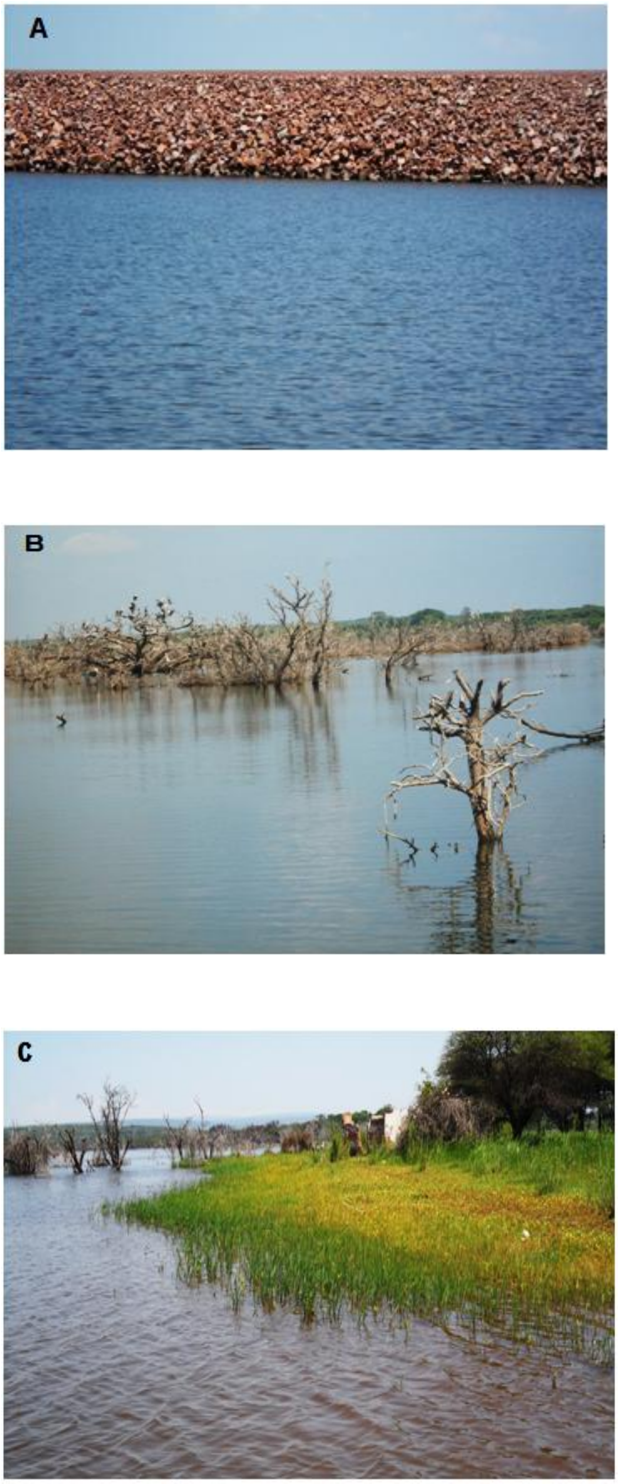


Figure 3.3: A photographic representation of the three sampling sites at Flag Boshielo dam; (A) Dam wall site; (B) Middle site, characterised by drowned trees; (C) inflow site.

3.4.2 Physico-chemical parameters of Flag Boshielo Dam

Water samples were taken monthly at all the sampling sites; this was done in the morning (0800 hours). Water transparency was measured on site using a Secchi disk. The disk was lowered slowly into the water to the point where the four quadrants on the Secchi disk were no longer distinguishable. This point would be taken as the Secchi disk depth. This was done at the 3 sampling sites. Water samples from each site were collected at 0.5 m below the water surface and analysed for chlorophyll *a*, salinity, turbidity, conductivity, nitrogen, phosphorus, aniions and cations.

Chlorophyll a determination

Chlorophyll *a* was analyzed using the method described in American Public Health Association (APHA; 1995). Two 1 L water samples were collected at 0.5 m depth at each of the sampling sites and immediately placed on ice and transported to the laboratory for the determination of chlorophyll *a*. Upon arrival at the lab, each sample was passed through an aspirator connected filter. The filter was then transferred into glass containers filled with 5 ml of 90% methanol.

Samples were then placed in a bath of water maintained at 75°C for 4 minutes to destroy the cell integrity and free the chlorophyll molecules. Containers were then wrapped in aluminium foil and placed in the refrigerator for 18 hours. The extract was then transferred into centrifuge tubes and centrifuged at 4000 rpm for 10 minutes to remove any remnants of the filter. The supernatant was removed and absorbencies were read using an HACH DR/200 spectrophotometer at 650 and 665 nm, methanol was used as a blank. Chlorophyll *a* was calculated according to the formula:

$C_a = 15.65\ A_{650} - 7.340 A_{665}$

where: C_a = chlorophyll *a*

pH

pH was determined according to US standard Methods 4500 H^+B. This method is based on the principle of electrometric pH measurement which is determined by the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode.

Total alkalinity as $CaCO_3$

Total alkalinity was determined according to the US Standard Methods 2320 B. This method is based on the principle that hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end point pH used. A 0.02N sulfuric acid is titrated to an end point of pH 4.5 using methyl red as an indicator.

Nitrate (mg/l NO_3)

Nitrate was measured following procedures of the Merck Spectroquant Method. The principle that concentrated sulphuric acid nitrate ions react with a benzoic acid derivative to form a red nitro compound was used. This compound was then used to determine nitrate photometrically.

Nitrite (mg/l NO_2)

Nitrite was measured according to the US Standard Methods 4500 NO_2B. This method is based on the principle that in an acid solution, nitrite ions react with sulfanillic acid to form a diazonium salt, which in turn reacts with N-(1-naphtyl) ethelynediamine dihydrochloride to form a red-violet azo dye. Then this dye was determined phothometricaly.

Ammonia (mg/l NH_4^+)

Ammonia was determined according to the EPA 350.1 and APHA 450-NH_3D methods. This method is based on the principle that in strongly alkaline solution ammonium nitrogen is almost entirely present as ammonia, which reacts with a chlorinating agent to form monochloramine. This in turn reacts with thymol to form a blue indophenol derivative that was then determined photometrically.

Orthophosphate (mg/l $PO_4^{3-)}$

Orthophosphate was determined according to the US Standard Method 4500-PE. The principle that in sulphuric solution, orthophosphate ions react with molybdate ions to form molybdophosphoric acid, is the bases of this method. Ascorbic acid reduces the molybdophosphoric acid to phosphomolybdenum blue (PMB) that is determined photometricaly.

Determination of cations and anions

These elements were determined by procedures in the US Standard Method 3120 B. The basis of this method is the measurement of emission of light by an optical spectroscopic technique. Samples were nebulized and the aerosol produced was transported to the plasma torch where excitation occurs. Characteristic emission spectra are produced by radio frequency inductively coupled plasma (ICP). The spectra are then dusersed by a grating spectrometer and the intensities of the lines are monitored by a detector. The signals from the detector are processed and controlled by a computer system. A suitable background correction technique is used to compensate for variable background contributions to the determination of the trance elements.

Dissolved oxygen and temperature profiles

Dissolved oxygen and temperature readings were taken only at the centre of the dam (site B) where the water depth was more than 20 m. Temperature (°C) and dissolved oxygen (mg/l) were measured at 1 m intervals from the water surface up to 20 m depth using a hand held YSI (556 MPS) water quality multi parameter instrument (Fig. 3.4).

Figure 3.4: Hand held YSI meter used for measuring water quality parameters.

3.4.3 Fisheries yield potential of Flag Boshielo Dam

The following indices were used to estimate the fisheries yield potential of the dam:

a) Yield (Y) was estimated according to Henderson and Welcome (1974);

$Y = 8.7489\ \mathrm{MEI}^{0.3813}$

where; Morpho-edaphic index (MEI) = $\frac{\text{Conductivity}}{\text{Mean depth}}$

b) Knoeshe and Barthelme's (1998) model was used to predict the Fisheries Yield Potential (FYP) based on primary production (PP).

$\mathrm{FYP} = (1.8 \pm 0.9)\ \mathrm{PP}/10$

where; $\mathrm{PP} = 148 \log \mathrm{TP} - 39.6$ and

TP = total phosphorus

3.4.4 Composition of marginal vegetation at Flag Boshielo Dam

Line transects were used to determine the frequency of occurrence of the marginal vegetation. Marginal vegetation was surveyed along a 35 m line transect at the shore. The frequency of occurrence of the different species along the rope was recorded. Marginal vegetation was identified to species level.

3.4.5 Fish species composition at Flag Boshielo Dam

Fish sampling was done monthly between February, 2010 and February, 2011. Fish were caught using gill nets with stretched mesh sizes of 30, 50, 70, 90, 110, 130 and 150 mm, siene nets, rod and line and by electro-fishing. These different methods were used to ensure that all size groups of fish are represented in the study. Immediately after capture, the total length (TL), standard length (SL) were measured to the nearest mm and mass (g) was determined using a three digit battery operated field balance (Mettler Toledo: JL6001 GLA00). This was done for all fish caught and the fish were then indentified to species level.

Live fish were released back into the dam except for *T. rendalli*. The condition factor (K) was calculated for *T. rendalli* from the relationship:

$K = 100\ W/\ L^{b}$

where: W = body weight in grams,

L = standard length in centimetres and

b = regression coefficient

3.4.6 Establishing the diet of *Tilapia rendalli* at Flag Boshielo Dam

All *T. rendalli* were immediately killed by placing them on ice. The stomach from each fish caught was removed (Fig 3.5). Each fish stomach was awarded an index of fullness from 0 to 1, following procedures described by Hynes (1950), with slight modifications on the score values. An empty stomach scored 0; a stomach that was quarter full, 0.25; half full stomach, 0.5; three quarters full, 0.75 and a full stomach was scored 1. The stomach was then fixed in 10% formalin in individually marked plastic sample bottles.

Figure 3.5: Dissection and removal of stomach for content analysis

In the laboratory, formalin was replaced with 70% ethanol. Each stomach was opened longitudinally and the entire gut contents were carefully transferred to a petri dish with 10 ml distilled water. Only the portion between the oesophagus and the first major bend of the small intestine just after the stomach was used. This was done because digestion is less advanced in this portion and the food items were identifiable. Stomach contents were viewed under a microscope at a magnification of 50X. Frequency of occurrence indicates the presence or absence of an item (Hyslop, 1980), and is calculated from the number of fish in which a particular food item occurred, in relation to the total number of stomachs analysed. The frequency of occurrence was calculated for each identified food item as done by Hyslop (1980):

$$\% \text{ occurrence } = \frac{\text{number of stomachs with food item}}{\text{total number of stomachs analysed}}$$

The overlap of food items among the different size groups was determined using the Schoener index of similarity (Colwell and Futuyma, 1971) based on percentage frequency of occurrence:

$C_{jk} = 1 – ½ \sum_i / P_{ij} - P_{ik}$

where: C_{ji} = resource (food type) overlap between species j and k
P_{ij} and P_{ik} = proportions of the i^{th} resource used by species j and k.

The diversity of food items among different size groups was determined using the Shannon diversity index (Shannon and Weaver, 1949):
$H' = n \log n - \sum (f_i \log f_i)$
where: H'= Shannon's diversity index
n= total number of frequencies
f_i= occurrence frequency of the item

3.4.7 Aging *Tilapia rendalli* using scales

Five scales were removed above the lateral line, anterior to the dorsal fin from each fish, using a pair of tweezers. Scales were placed into marked envelops then stored for analysis. In the laboratory, the scales were soaked in distilled water and brushed with a tooth brush. Once dry, scales were mounted on a scale reader and the number of annuli was counted. The following criteria were used in the identification of annuli;

- Areas where circuli fuse or form anastomoses (Payne, 1976; Barger, 1990).
- Clear zones that were devoid of circuli (Cohen, 1991).
- Areas where circuli were crowded together.

The assumption used for identification of annuli was that a true annulus can be traced completely around the scale and generally exhibits crossing over in the posterior portion of the lateral fields. Age was assigned to each fish according to the number of complete annuli.

Age validation

The distance on the scale (in mm) from the last annulus to the scale margin (marginal increment) was measured. Measurements on scale images were made to the nearest 1 mm. The distance on the scale from the most recent annuli to the anterior edge of the fish was measured in the scales of 1-3 year old fish.

3.4.8 Determination of growth

The ELEFAN I module implemented in FiSAT (FAO, 2004) was used to calculate parameters for the generalized Von Bertalanffy growth function (Von Bertalanffy, 1938) for length (*L*) at age (*t*) and *K* (Pauly, 1982) for *T. rendalli* at the Flag Boshielo Dam.

$L_t = L_\infty$ [1- exp [-K (t – t_o)]]

where: L_t = length at time t

K = growth coefficient

L_∞= average size the stock of fish would reach if they were allowed to grow indefinitely

t_o = hypothetical age of the fish at zero length.

3.4.9 Growth performance index

Moreau *et al*., (1986)'s index of growth performance (ϕ), was used to compare the growth of male and female *T. rendalli* in Flag Boshielo Dam.

$\phi = \log K + 2 \log L_\infty$

where; K and L_∞ are parameters from the von Bertalanffy equation

ϕ = index of growth performance.

3.4.10 Determination of natural mortality

Pauly's empirical formula was used to determine natural mortality (Pauly, 1980):

$\text{Log (M)} = -0.0066 - (-0.279) \log (L_\infty) + 0.6543 \log (K) + 0.463 \log (\overline{T})$

where: M = natural mortality,

K and L_∞ are growth parameters from VBGF

$\overline{T}$ = annual mean temperature

3.4.11 Statistical analysis

One-way analysis of variance (ANOVA) on the Statistical Package and Service Solutions (SPSS version 17.0) was used to determine if the Physco-chemical properties of the water at Flag Boshielo Dam were significantly different between the sampling sites. The Shannon-Weiner diversity index was subjected to one-way ANOVA to determine if there were any statistically significant size related shifts in the diet of *Tilapia rendalli* in Flag Boshielo Dam. Chi^2 was used to determine if there were statistically significant differences in the occurrence of the different food items in the different seasons of the year in *T. rendalli* in Flag Boshielo Dam. The Shannon-Weiner diversity index was used to determine the diversity of the food items in each season.

3.5 RESULTS

3.5.1 Physco-chemical properties water at Flag Boshielo Dam

The phosphorus concentration was low at all the sites. It ranged from 0.044 mg/l at the inflow to <0.001 at the middle site (Table 3.1). The orthophosphate levels were also consistently low (<0.2 mg/l) at all the sites. Sampling site had no statistically significant difference ($P>0.05$, ANOVA) in the phosphorous and orthophosphate levels at Flag Boshielo Dam.

Secchi disk readings were lowest (0.40 m) at the inflow site followed by the middle site with a reading of 0.58 m; the dam wall had the highest with 0.60 m (Table 3.1). These values correspond with the turbidity levels which were also highest at the inflow with an average of 3.03 Nephelometric Turbidity Units (NTU), and the middle had an average of 3.00 NTU and the lowest turbidity levels were recorded at the dam wall with 2.17 NTU. Relative conductivity was highest at the centre (41.58 mS/m), followed by the dam wall (41.35 mS/m) and the inflow had the lowest conductivity of 40.80 mS/m. There were no significant differences ($P>0.05$, ANOVA) in the Secchi disk readings, turbidity and relative conductivity between the sampling sites (Table 3.1).

The averages for ammonia (mg/l NH_3^+) and nitrite (mg/l NO_2^-) were <0.2 mg/l for all sampling points. Nitrate levels varied slightly with the inflow found to have an average of 0.20 mg/l NO_3^- with the middle and dam wall sites both having averages of 0.16 mg/l NO_3^-. The dissolved oxygen levels were relatively high; the dam wall had the highest amount of dissolved oxygen with an average of 8.47 mg/l and the inflow had the lowest level at 6.73 mg/l. These parameters were also not significantly different ($P>0.05$, ANOVA) between the three sampling sites (Table 3.1).

Sulphate concentrations varied between the sampling sites. The inflow had a minimum concentration of <5 mg/l and a maximum concentration of 114 mg/l. The same pattern was observed at the centre, where the minimum concentration was <5 mg/l and the maximum concentration was 116 mg/l. However, the sulphate concentration did not show great variation at the dam wall site as the minimum was 105 mg/l and the maximum 116 mg/l. Chloride concentrations were highest at the dam wall (25 mg/l) followed by the inflow (24 mg/l) and the lowest concentration was at the centre (23.2 mg/l). The overall fluoride concentrations were <0.5 mg/l. The dam wall site had a concentration of 0.47 mg/l followed by the inflow (0.40 mg/l) and the centre had the lowest concentration of 0.37 mg/l. One-way ANOVA showed that no statistically significant differences (P>0.05) in the sulphate, chloride, and fluoride levels, between sites (Table 3.1).

Calcium was the most abundant cation. The highest value was recorded at the dam wall with an average of 23.07 mg/l, followed by the centre (18.90 mg/l) and the lowest level was 18.04 mg/l at the inflow. The concentration of magnesium varied between 11.38 mg/l in the inflow, 14.6 mg/l at the centre and 14.97 mg/l at the dam wall. Potassium was also highest at the dam wall (5.38 mg/l) followed by the inflow (5.33 mg/l) and the lowest potassium levels (4.08 mg/l) were recorded at the centre (Table 3.1). Sodium levels were markedly higher at the inflow (13.3 mg/l), its concentrations at the centre and the dam wall were equal at 0.92 mg/l. Chlorophyll *a* values were highest at the dam wall (1.25 mg/m^3) and lowest at the inflow (1 mg/m^3). Sampling site had no statistically significant (P>0.05, ANOVA) effect on these cations measured at Flag Boshielo Dam.

Table 3.1: Water quality parameters (± STD deviation) of Flag Boshielo Dam

Water constituents	Inflow	Middle	Outflow
pH	7.205±1.36	8±1.11	7.665±1.65
Turbidity NTU	3.03±2.97	3±3.18	2.17±0.78
Conductivity(EC)S/m	40.8±9.55	41.58±6.58	41.35±6.01
Total Alkalinity $CaCo_3$	61.33±36.77	57.33±33.94	68±14.14
Nitrate (mg/l NO_3^-)	0.2±0.00	0.16±0.07	0.16±0.13
Nitrite (mg/l NO_2^-)	<0.2±0.00	<0.2±0.00	<0.2±0.00
Ammonia(mg/l NH_3^+)	<0.2±0.00	<0.2±0.00	<0.2±0.00
Orthophosphate(mg/l PO_4^{3-}	<0.2±0.00	<0.2±0.00	<0.2±0.00
Phosphorous (mg/lP)	0.044± 0.04	<0.001±0.00	0.016±0.03
Sulphate (mg/l SO_4^{2-})	75±77.07	76±78.49	111±7.78
Chloride (mg/l Cl^-)	24±10.61	23.2±8.48	25±5.66
Fluoride (mg/l F^-)	0.4±0.28	0.37±0.28	0.47±0.14
Calcium (mg/l Ca)	18.04±7.92	18.9±8.20	23.07±3.11
Magnesium (mg/l Mg)	11.38±4.14	14.6±7.07	14.97±4.24
Potassium (mg/l K)	5.334±3.44	4.083±1.99	5.38±1.24
Sodium (mg/l Na)	13.3±13.68	0.922±7.46	0.922±0.38
Secchi Disk (m)	0.4±0.14	0.58±0.04	0.6±0.00
Chlorophyll *a* (mg/m^3)	1±0.28	1.2±0.28	1.25±0.35

Temperature and dissolved oxygen profiles

The average temperature at all the sampling sites was relatively uniform throughout the water column in winter (July). In summer (December), however, surface temperature was higher with a steep drop at 6-8 m depth (Fig 3.6).

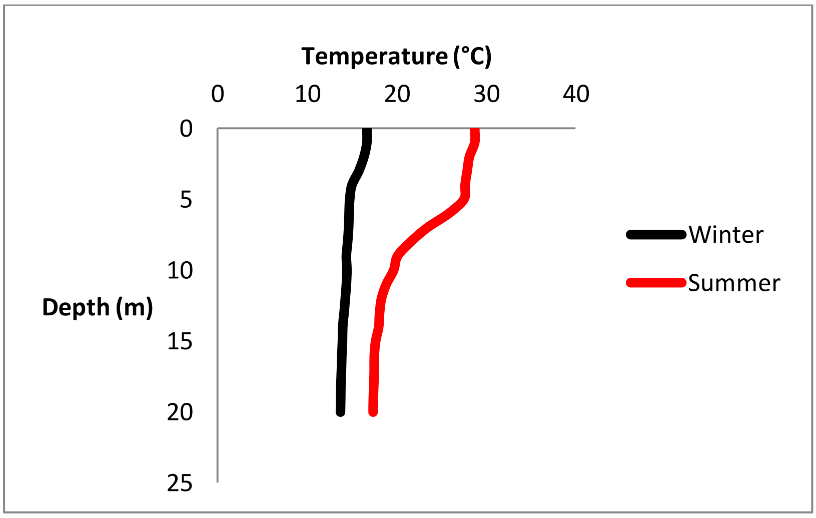


Figure 3.6: Temperature depth profile taken at the centre of the dam

The dissolved oxygen was lower in the upper layers during summer (December); dissolved oxygen was well distributed throughout the water column in winter (July) (Fig. 3.7).

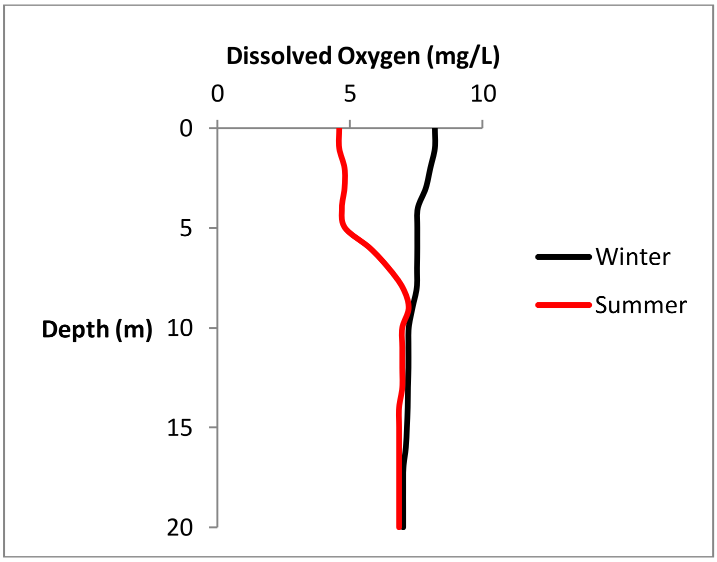


Figure 3.7: Dissolved oxygen profiles taken at the centre of the dam

3.5.2 Fisheries yield potential of Flag Boshielo Dam

a) Based on the Morpho-edaphic index (MEI), the fisheries yield potential of Flag Boshielo Dam was predicted to be:

= 11.0618 kg/ha/yr.

b) Based on primary production, the minimum and maximum fisheries yield potential of Flag Boshielo Dam was estimated to be:

Minimum FYP = **18.9708 kg/ha/yr**

Maximum FYP = **56.9123 kg/ha/yr.**

3.5.3 Composition of marginal vegetation at Flag Boshielo Dam

Cyperus sexangularis (Fig. 3.8A) was the most dominate type of marginal vegetation along the shores of the dam, constituting about 52% of the total marginal vegetation (Fig. 3.9). *Cyperus sexangularis* is a perennial species with six-angled scabrid culms. Its inflorescences are terminal with dense clusters of small flowers. *Ludwigia stolonifera* (Fig. 3.8B) was the second most abundant plant contributing 30% of the total vegetation (Fig. 3.9). This is a perennial creeping herb, rooting at the nodes, was often found floating on the dam. *Panicum schinzii* (Fig. 3.8C), contributed 5% (Fig. 3.9) of the marginal vegetation at Flag Boshielo Dam. *Panicum sichinzii* is a glabrous annual grass of terrestrial origin.

Other vegetation types present at Flag Boshielo Dam included *Bulbostylis hisdula* (Fig. 3.8D). *Bulbostylis hispidula* is also known as hairsedge, it accounted for 3% of the total vegetation (Fig. 3.9). This annual herb forms clumps and takes the form of a small, upright tuft of green herbage growing close to the ground. It has several stems surrounded by thin leaves.

Digitaria ciliaris (Fig. 3.8E), also known as summer grass, is an annual tufted grass with flat leaf blades hairy on the lower surface. This grass is also of terrestrial origin and frequently grows on lawns, lowland pastures, field crops, plantation crops, open ground and disturbed areas (Holm *et al*., 1977). It constituted 2% (Fig. 3.9) of the marginal vegetation at Flag Boshielo Dam. *Cynodo dactylon* (Fig. 3.8F) also known as couch grass is a perennial grass, forming thick mats by means of stolons and rhizomes. It constituted 3% of the marginal vegetation at the Dam (Fig. 3.9).

Figure 3.8: A) *Cyperus sexangularis*, B) *Ludwigia stolonifera*, C) *Panicum schinzii*, D) *Bulbostylis hisdula*, E) *Digitaria ciliaris*, F) *Cynodo dactylon*, G) *Paspalum distichum* and H) *Paspalum scrobiculatum*.

Grasses of the *Paspalum* genus were represented by two species; *P. distichum* (Fig. 3.8G) and *P. scrobiculatum* (Fig. 3.8H). These species constituted 4% and 1%

respectively (Fig. 3.9). *P. distichum* is a perennial deciduous grass with green flowers in late summer.

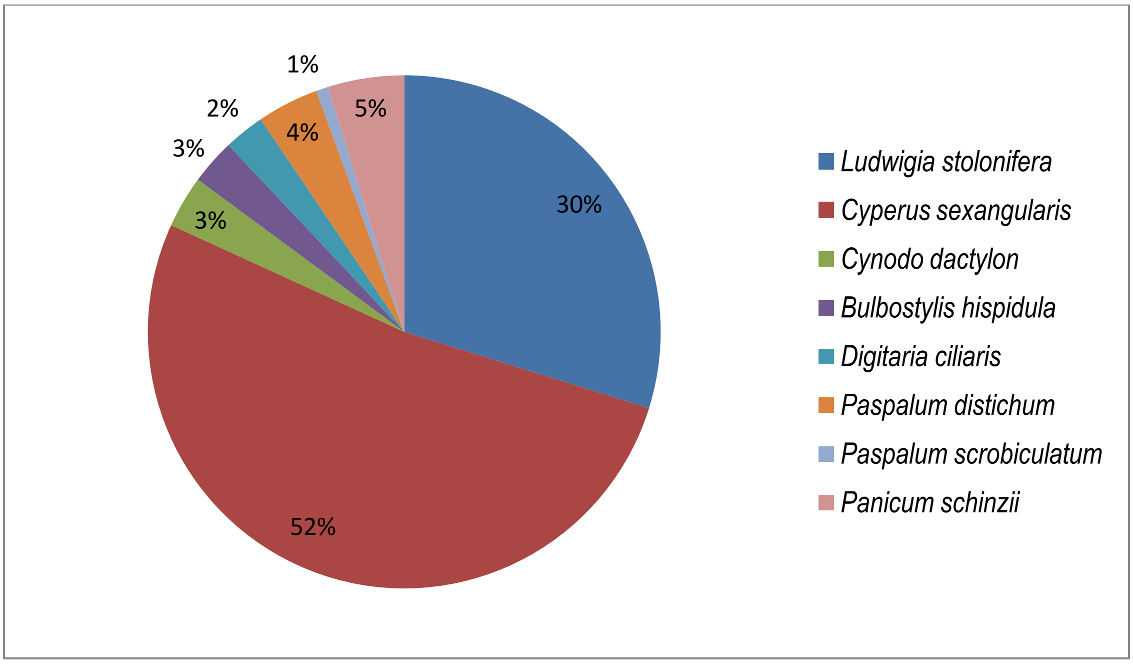


Figure 3.9: Percent frequency of occurrence of marginal vegetation sampled at Flag Boshielo Dam

3.5.4 Fish species composition at Flag Boshielo Dam

Ten fish species were identified at Flag Boshielo Dam. *Hypophthalmichthys molitrix* dominated the fish community in terms of weight (29.0%) followed by *Labeo rosae* (22.7%), *Cyprinus carpio* (18.3%), *Oreochromis mossambicus* (11.6%), *Clarias gariepinus* (7.2%), *Labeobarbus marequensis* (4.2%) and *Schilbe intermedius* (3.9%). *Tilapia rendalli* contributed 2.3% by weight to the total fish sampled (Fig. 10). Other fish species recorded were *Micropterus dolomieu* and *Barbus matozzi* which contributed 0.43 and 0.24 % respectively. In terms of numbers however, *Labeo rosae* was the most dominate fish species followed by *Labeobarbus marequensis*, *Hypophthalmichthys molitrix* and *Schilbe intermedius* respectively (Fig. 3.10).

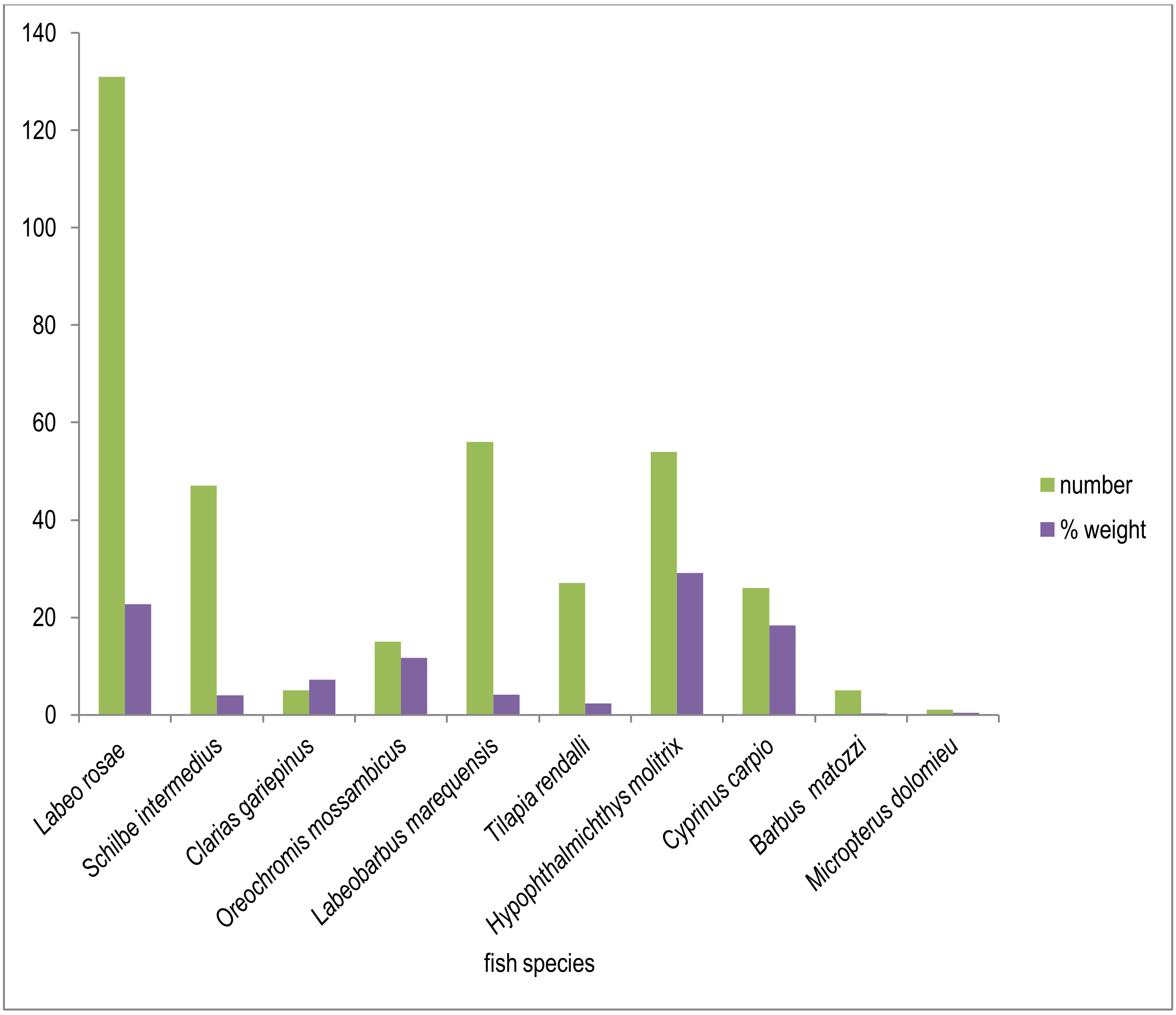


Figure 3.10: Fish species composition in weight and numbers, at Flag Boshielo Dam, South Africa.

3.5.5 Size related dietary variations in *Tilapia rendalli* at Flag Boshielo Dam

The stomach content analysis of *T. rendalli* showed that marginal vegetation and detritus were the most common food items across all size groups. A change in the diet with increasing size was apparent (Table 3.2).

Cyperus sexangulasris and *Panicum schinzi* were the major types of marginal vegetation found in stomachs of *T. rendalli*. The importance of marginal vegetation in the diet increased with increasing fish size. Fish <2 cm generally did not feed on marginal vegetation as it was found only in 6 percent of the stomachs analyzed (Table 3.2).

It was observed that *T. rendalli* starts feeding on marginal vegetation between 2 and 5 cm (SL) and its frequency of occurrence increased tenfold (Table 3.2). Marginal vegetation was found in 60% of the stomachs in this size range. At 5-9 cm (SL), *T. rendalli* were feeding mostly on marginal vegetation and it was found in 95.18% of stomachs of fish between 5 and 9.9 cm. Marginal vegetation was found in all the stomachs of fish >15 cm. (Table 3.2).

Algae was found in the diet of *T. rendalli* at Flag Boshielo Dam, the 10-14.9 cm group had the highest (57.9) percentage of stomachs containing algae. The larger fish >20 cm had the lowest (11.1) percentage of stomachs containing algae (Table 3.2).

Zooplankton was the major food of *T. rendalli* fry (<2cm) and was found in all the stomachs analysed for this size group. The importance of this food item decreased with increasing fish size, ranging from 100% in fry to 11.11% in adult (>20 cm) fish (Table 3.2).

Chironomids were also part of *T. rendalli*'s diet at Flag Boshielo Dam. It was found in 30% of the stomachs of fry (<2 cm), in half the stomachs of the 2-5 cm size group, and 54.2% of 5-9.9 cm fish had fed on chironomids (Table 3.2). The 15-19.9 cm size group had the highest chironomid frequency of occurrence as 75% of stomachs analyzed in this size group had chironomids.

T. rendalli fed on juveniles and smaller teleosts. However, *T. rendalli* did not start predating on other fish until they were >5 cm (Table 3.2). Teleosts accounted for 29.4% in the diet of the 5-9.9 cm size group. The occurrence of teleosts in the diet increased to 32.6% in the diet of the 10-14.9 cm size group. The >20 cm size group had the highest frequency (35%) of stomachs found with fish remains (Table 3.2).

Detritus was a major component in the diet of *T. rendalli* at Flag Boshielo Dam (Table 3.2). It was found in more than 80% of the stomachs analysed in all size groups except in the stomachs of fry (<2 cm) where it was found only in 25% of the stomachs analysed.

T. rendalli at Flag Boshielo dam also fed on insects. These were found in >60% of fish >5 cm. The stomachs of fry (<2 cm) however, did not contain any insects, this implies that fish in this size group had not started feeding on insects. Fish >20 cm had the highest percent occurrence (65%) of insects in the stomachs analysed (Table 3.2).

The Shannon-Weiner diversity index (H') in the diet of fry (<2 cm) was 0.18 and was significantly lower (p<0.05, ANOVA) than that of the other size groups. The diversity increased with increasing fish size until at about 19 cm. The highest diversity of 0.99 was calculated for the 15-19.9 cm size group. The diversity decreased to 0.75 in *T. rendalli* >20 cm (Table 3.2). However, the diversity indices (H') were not significantly different (p>0.05, ANOVA) among the other size groups (2-5 cm to >20 cm).

Table 3.2: Food types expressed as % frequency in different length groups of *Tilapia rendalli* at Flag Boshielo Dam

Length groups (SL)	<2 cm	2- 5 cm	5-9.9 cm	10-14.9 cm	15-19.9 cm	20-30 cm
n	55.00	40.00	83.00	58.00	82.00	16.00
Marginal vegetation*	6.00	60.00	95.20	97.40	100.00	100.00
Algae	35.00	42.00	49.40	57.90	50.00	11.10
Zooplankton	100.00	75.00	67.50	57.90	50.00	11.10
Chironomids	30.00	50.00	54.20	52.60	75.00	50.20
Teleosts	0.00	0.00	29.40	32.60	34.50	35.00
Detritus	25.00	80.00	90.40	100.00	87.50	88.90
Insects	0.00	58.00	63.90	65.80	62.50	65.00
H'	0.18^a	0.76^b	0.82^b	0.83^b	0.99^b	0.75^b

*Marginal vegetation consisted mostly of (*Cyperus sexangulasris* and *Panicum schinzi*).
Diversity indices (H') with different superscripts are significantly different (ANOVA, P=0.05).

3.5.6 Dietary overlap among different size groups of *Tilapia rendalli*

The dietary overlap was highest (86%) between fry (<2 cm) and fingerlings (2.0-5.0 cm). This was followed by the overlap between 15.0-19.9 cm and 20-30 cm sized *T. rendalli* at 83% (Table 3.3). The lowest (54%) dietary overlap was observed between the 2.0-5.0 cm and the 2.0-9.9 cm size groups. The overlap between the 5.0-9.9 and 10-14.9 cm size group was 62% while that of 10-14.9 and 15-19.9 cm size groups was 77% (Table 3.3).

Table 3.3: Values of food overlap (Schoener index of similarity) for *Tilapia rendalli* at Flag Boshielo dam

Length (cm)	2.0-5.0	5.0-9.9	10.0-14.9	15.0-19.9	20.0-30.0
<2.0	0.86				
2.0-5.0		0.54			
5.0-9.9			0.62		
10.0-14.9				0.77	
15.0-19.9					0.83

3.5.7 Seasonal variations in the diet of *Tilapia rendalli* at Flag Boshielo Dam

Marginal vegetation was found in 89.42% of the stomachs analysed in summer, in autumn 72.00% had marginal vegetation and in spring 68.00%. Season had a statistically significant effect (Chi^2, $P<0.05$) on the occurrence of marginal vegetation in stomachs of *T. rendalli* analysed (Table 3.4).

The occurrence of filamentous algae differed significantly with season (Chi^2, $P<0.05$). Filamentous algae were consumed mostly in summer, (45.88%). There was however, none found in the stomachs analysed in autumn. In spring 20.00% of stomachs analysed had filamentous algae.

The occurrence of zooplankton in the stomachs of *T. rendalli* was also significantly (Chi^2, $P<0.05$) effected by season. Zooplankton was mostly consumed in summer and was found in 62.00% of the stomachs. There was a reduction in zooplankton occurrence in *T. rendalli* stomachs in autumn (38.46%) and spring (33.00%).

Teleosts were also found in the stomachs of *T. rendalli* analysed. Season had a significant effect (Chi^2, $P<0.05$) on the frequency of occurrence of teleosts in *T. rendalli* stomachs. The frequency of occurrence of teleosts was 38.67, 36.92 and 22.90% in spring, autumn and summer respectively.

The frequency of occurrence of detritus was significantly different (Chi^2, $P<0.05$) with season, it was highest in spring (found in all the stomachs analysed). In summer and autumn its frequency of occurrence was 70.25 and 69.20% respectively.

The Shannon-Weiner diversity index showed that the diet of *T. rendalli* was most diverse in summer with a diversity index of 0.81, followed by autumn (0.79) and lowest (0.76) in spring (Table 3.4).

Table 3.4: Percent frequency of occurrence of food items in the stomach contents of *Tilapia rendalli* at Flag Boshielo Dam

Item	Spring	Summer	Autumn	Chi^2
n	94.00	127.00	73.00	
Marginal vegetation	72.00	89.42	68.00	19.19
Filamentous algae	0.00	45.88	20.00	194.25
Zooplankton	38.46	62.01	33.00	97.19
Chironomids	69.20	50.18	33.00	79.19
Teleosts	36.92	22.90	38.67	136.85
Detritus	69.20	70.25	100.00	18.34
Insects	41.58	86.67	69.20	45.39
H'	0.76	0.81	0.79	

NB: Tabulated $Chi^2 = 5.99$

3.5.8 Stomach fullness in *Tilapia rendalli* at Flag Boshielo Dam

Fifty five percent of the stomachs analysed in January were full, whilst in February and March, the percentages decreased to 42 and 30% respectively (Fig. 3.11). There were only 26% full stomachs in April and by June; all specimens caught had empty stomachs. In September, the percentage of full stomachs increased to 23%. The number of full stomachs increased in October (38%), and more than half of the stomachs analysed were full by November (57%). The percentage of full stomachs peaked in December (68%; Fig. 3.11). The degree of stomach fullness was highest in summer and low during winter. A two way analysis of variance showed that season had a significant effect on stomach fullness ($P<0.05$). Similarly, the number of empty stomachs was highest in June, during winter (Fig. 3.12) and lowest in the summer months (December-March). Area of capture on the other hand, had a statistically insignificant effect on stomach fullness ($P>0.05$, ANOVA).

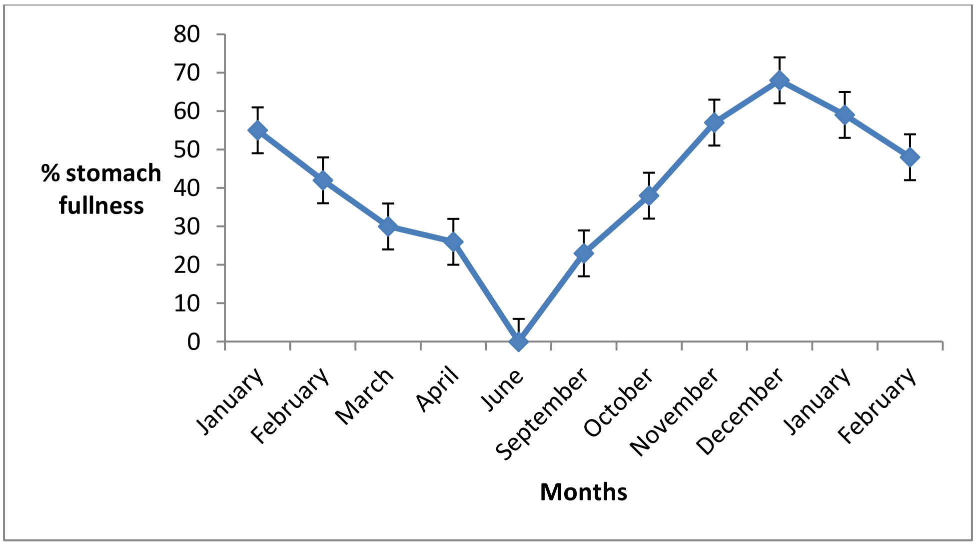


Figure 3.11: Percentage (%) full stomachs of *Tilapia rendalli* for the months surveyed at Flag Boshielo Dam. Error bars represent standard error of the mean.

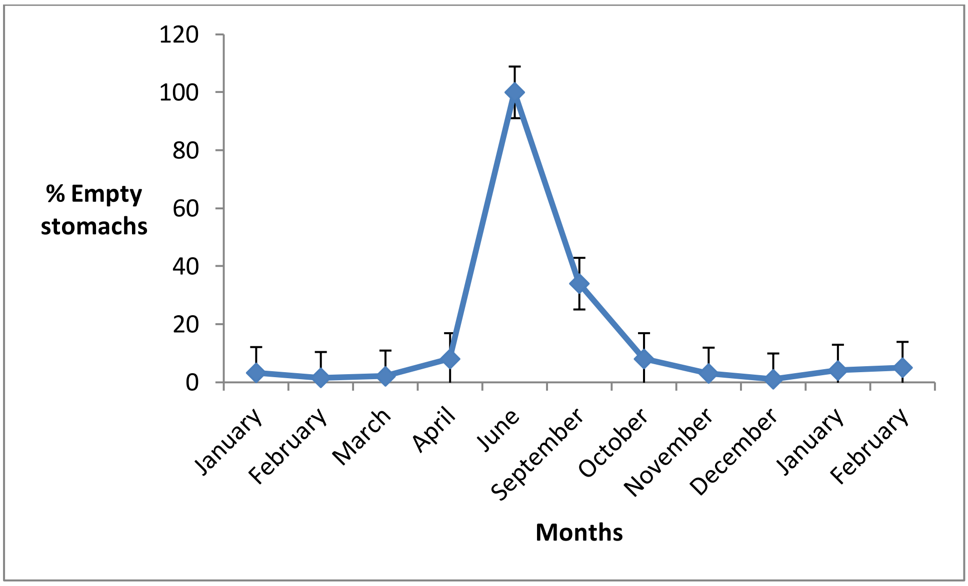


Figure 3.12: Percentage (%) empty stomachs of *Tilapia rendalli* for the months surveyed at Flag Boshielo Dam. Error bars represent standard error of the mean.

3.5.9 Length weight relationship

A scatter plot of weight against length showed that *T. rendalli* at Flag Boshielo Dam was positively allometric in its growth (Fig. 3.13). The “a” and “b” values obtained from the regression of the length against weight were 0.33 and 3.03 respectively.

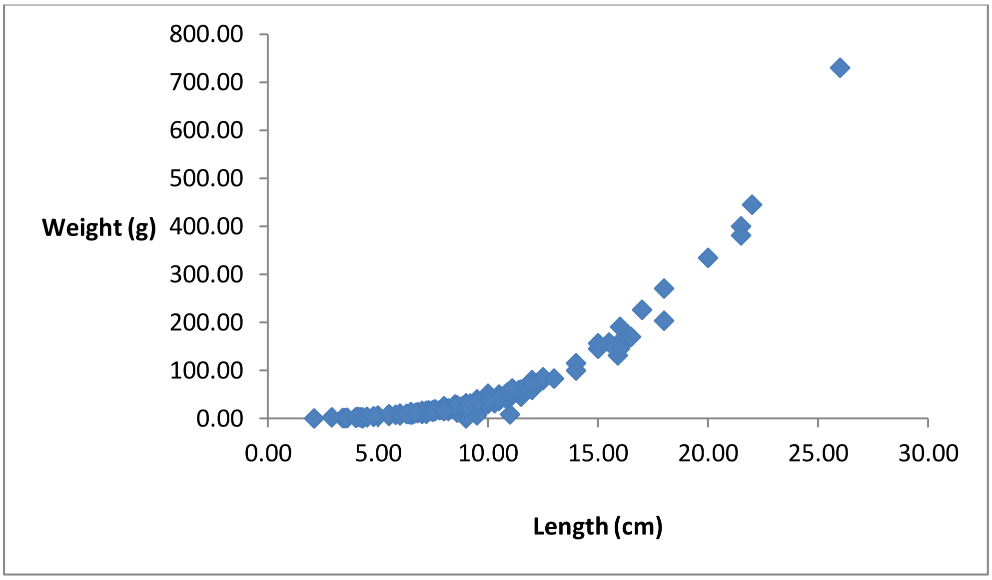


Figure 3.13: Length weight relationship of *Tilapia rendalli* from Flag Boshielo Dam

The condition factor of *T. rendalli* at Flag Boshielo Dam was highest in summer (1.27) followed by spring (1.18; Fig. 3.14). The lowest condition factor values were recorded in winter (1.01). However, the differences in condition factor across the seasons were not significant (ANOVA, P>0.05).

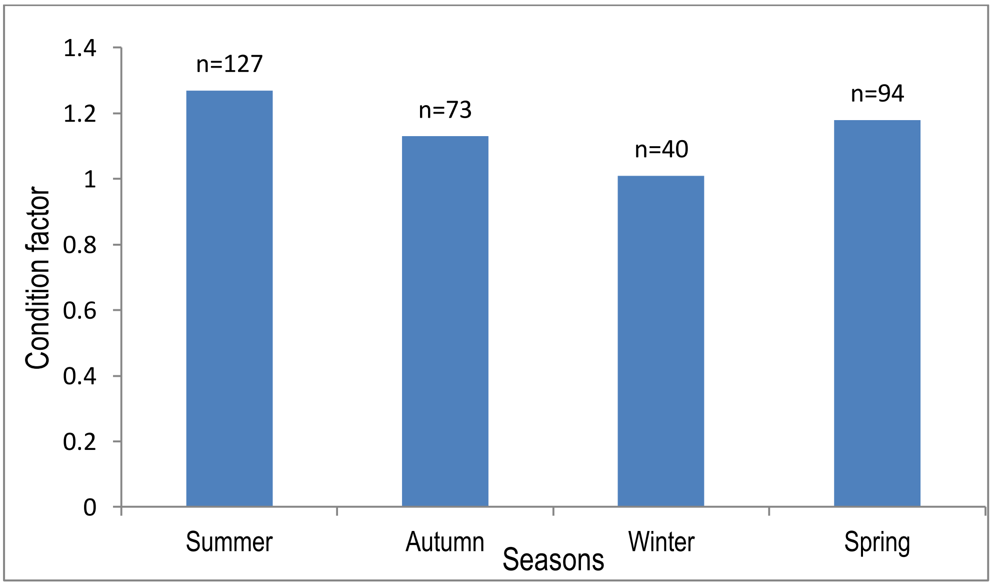


Figure 3.14: Condition factor of *Tilapia rendalli* at Flag Boshielo Dam

3.5.10 Age validation

Monthly measurement of the distance between the last annuli and the scale margin show that minimum scale growth occurred between October and November. During this period, the distance between the last annuli and the scale margin was shortest (Fig.3.15).

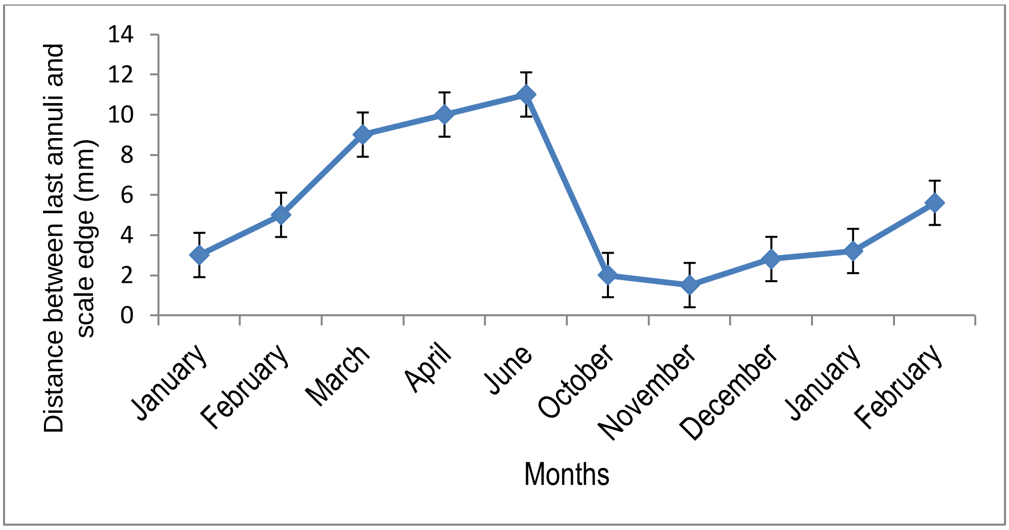


Figure 3.15: Mean monthly distance beyond last annuli of 1 year old *Tilapia rendalli* collected from Flag Boshielo Dam. Vertical bars represent standard error of the mean

3.5.11 Growth of *Tilapia rendalli* at Flag Boshielo Dam

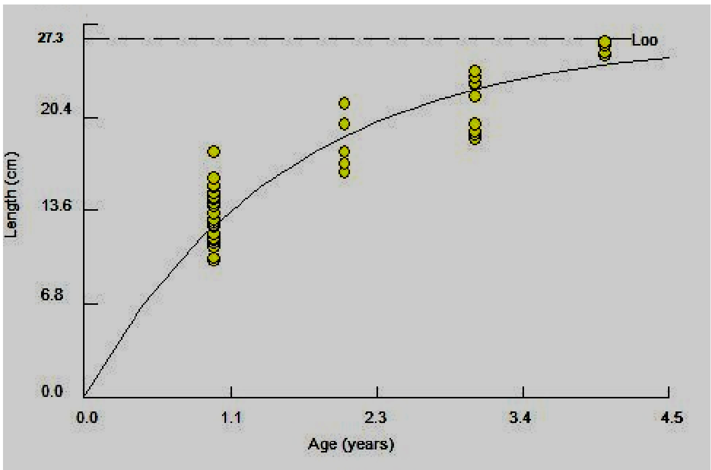
The von Bertalanffy function was calculated for *T. rendalli*; the maximum length was 28 cm (TL) for a 4 year old fish (Fig. 3.16). The growth model for females was:

$L_t = 26.25\ [1\text{- exp}^{[-0.65\ (t-0)]}]$,

For male fish the growth model was:

$L_t = 27.18\ [1\text{- exp}^{[-0.70\ (t-0)]}]$.

a)



b)

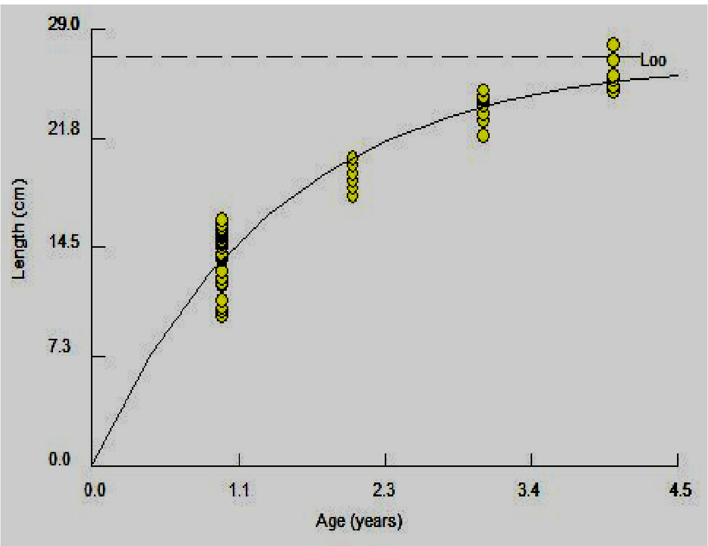


Figure 3.16: Observed length-at-age data for (a) female and (b) male *Tilapia rendalli* at Flag Boshielo Dam established using the Von Bertalanffy growth model.

The growth performance indices (ϕ) were 2.64 and 2.68 for female and male *T. rendalli* at Flag Boshielo Dam respectively. Natural mortality was found to be 1.249 for females and 1.298 for males.

3.7 DISCUSSION

Physco-chemical properties water at Flag Boshielo Dam

The major nutrients that contribute to eutrophication are phosphorous measured as orthophosphate (PO_4^{3-}) and nitrogen as nitrate (NO_3^-). The levels of both nutrients were low at all the sampling sites at Flag Boshielo Dam. Furthermore, spatial variation had no statistically significant effect ($P>0.05$, ANOVA) on the level of both orthophosphate and nitrate.

Phosphorous is one of the major nutrients limiting the productivity of natural waters (Hart *et al*., 1992). Levels between 0.01-0.03 mg/l P may limit growth of water plants and plankton, which provide food for fish. The mean of 0.02 mg/l P recorded at Flag Boshielo Dam may explain the absence of macrophytes. Flag Boshielo Dam has low levels of orthophosphate and according to Wetzel (1983) and DWAF (1996) this dam is classified as oligotrophic. Anthropogenic sources of phosphate include fertilizers and pesticides used in agriculture, animal wastes, domestic and industrial effluents (Dallas and Day, 2004; DWAF, 1996). There is very little farming activity, human settlements and industrial work in the catchment area of Flag Boshielo Dam. This may explain the low levels of phosphate in the dam. The average nitrogen concentration was equally low (<0.2 mg/l) and this is consistent with the oligotrophic status of Flag Boshielo Dam.

Morphology is also important in the trophic status of a water body. The main aspects are mean depth, volume and surface area. Shallow lakes are more productive than deep lakes because they do not stratify and the nutrients remain in circulation and are accessible to plants. Lakes with smaller volume experience a major impact of nutrient loading from the catchment and are also usually more productive. Flag Boshielo Dam is a relatively deep (mean depth of 22 m) and large (surface area of 1 288 ha) water body. This is another factor that contributes to its oligotrophic status.

There are a number of South African reservoirs that are also regarded as oligotrophic, also having no macrophytes. These include the Piet Gouws Dam on the Ngwaritsi River, which had ammonia and nitrate concentrations ranging from 0.08 to

0.3 mg/l and 0.125 to 1 mg/l respectively. Tzaneen Dam (Nicolaai, 2009), Strekfontein Dam (Dorgeloh *et al*., 1993) have also been classified as oligotrophic.

The chlorophyll *a* readings were consistently low (1.2 mg/l) at all sampling sites. These low values imply that there is low primary production which may result in low populations of fish species feeding on phytoplankton and low zooplankton. The low (0.5 m) Secchi disk readings (transparency) imply that light penetration is very limited in this dam. This could further explain the absence of rooted and submerged macrophytes and the resultant low population of macrophyte feeding fish like *T. rendalli.* This in the first study to look at the secchi disc readings and chlorophyll *a* values at Flag Boshielo Dam, it is difficult to know if these values are going up or down. However, low secchi disc readings (10-100 cm) have been recorded at Sterkfontein Dam (Dorgeloh *et al.*, 1993), Mnjoli Dam, Swaziland (Khumalo, 2006) mainly due to the inflow of turbid water.

pH was near neutral at all sampling points and according to Antoine and AI-Saadi (1982) these values are within the range for inland waters (pH 6.5-8.5). A pH range of 6.09-8.45 is ideal for supporting aquatic life including fish (Boyd and Lichtkoppler, 1979). Industry effluents, mine drainage and acid rain are the main types of pollution that may lead to acidification of waters (DWAF, 1996; Davies and Day, 1998).

The fisheries yield potential based on the morpho-edaphic index was found to be 11.06 kg/ha/yr. The minimum fisheries yield potential based on primary production, was 18.97 kg/ha/yr and the maximum was predicted to be 56.91 kg/ha/yr. According to Marshall and Maes (1994) yields from tropical shallow, managed reservoirs average 30-150 kg/ha/yr, while deep reservoirs average 10-50kg/ha/yr.The low predictions of the fisheries yield potential at Flag Boshielo Dam may be influenced by the low nutrient levels and low mean temperatures observed. The water temperature influences the productivity of lakes and ultimately fish yield observed. Mean depth determines to a large extent the temperature regime of lakes, deep lakes generally being colder than shallow lakes. Other factors that affect fish yield include annual phytoplankton production and total phosphorous concentration (Dowing *et al*., 1990). Flag Boshielo Dam being an oligotrophic dam with low nutrient levels, can not support a large fish population, resulting in the low fisheries potential predicted.

Temperature profile

Inland waters of South Africa have a temperature of 5-30°C (DWAF, 1996). The lowest temperature recorded in winter at Flag Boshielo Dam was 13°C, whilst the highest was 28.7°C in summer. Flag Boshielo Dam like most deep South African reservoirs showed a typical warm monomictic pattern with distinct summer stratification (November-April) and holomictic winter circulation between May and October. Lake Tzaneen in the Limpopo province showed a similar temperature regime (Nicolaai, 2009). Other reservoirs in South Africa have been found to be monomictic, such as Roodeplaat Dam (Walmsley *et al*. 1978), Spioenkop and Wagendrift Dams in KwaZulu-Natal (Hart, 1999; 2001).

Dissolved oxygen profile

Flag Boshielo Dam showed an orthograde oxygen profile in summer typical of oligotrophic lakes which undergo thermal stratification. The oxygen content of the epilimnion was lower in the summer months, leaving higher concentrations of oxygen in the hypolimnion. This may be a result of limited oxygen consumption in the bottom sediment. Dissolved oxygen mixing started in April, and well oxygenated water was found throughout the water column during winter (May-August). A minimum of 5 mg/l dissolved oxygen is necessary to sustain aquatic life activities (Alabaster and Lloyd, 1982). At Flag Boshielo Dam, water with dissolved oxygen of >5 mg/l was found at greater depths even during the summer stratification. This means that this dam has enough dissolved oxygen to support aquatic life throughout the year. At no time did anaerobic conditions develop at Flag Boshielo Dam. The main reason might have been the low nutrient levels in this dam and low microbial activity using less dissolved oxygen at the bottom sediment.

Fish species composition at Flag Boshielo Dam

The fish species composition at Flag Boshielo Dam was numerically dominated by *Labeo rosae*. *Hypophthalmichthys molitrix* dominated the species composition in terms of weight. *Cyprinus carpio* was the third most abundant fish species in terms of weight. All of these species belong to the family Cyprinidae. It is important to note that outside *Labeo rosae*, the other two dominant species are exotic. The temperature regimes at Flag Boshielo Dam seem to favour these species with a wide temperature tolerance range. *Cyprinus carpio* has been reported to have an inhibitory effect on the development of aquatic macrophytes because of its feeding habit of digging into the bottom mud. This increases the turbidity of the water thereby blocking the penetration of light and growth of aquatic macrophytes (Huet, 1972; Crivelli, 1983). This may have contributed to the low secchi disc readings and absence of macrophytes in this dam.

The low chlorophyll *a* levels were used as circumstantial evidence for low phytoplankton levels in this dam. However, despite the low phytoplankton levels, there is a thriving population of *Hypophthalmichthys molitrix* which is a specialised plankton feeder. Its abundance in this dam implies that it may be euryphagous. The numbers of *Oreochromis mossambicus*, another plankton feeder were however less than half those of *H. molitrix*, this may imply that *O. mossambicus* is not as efficient as *H. molitrix* in feeding on the little phytoplankton in Flag Boshielo Dam.

Tilapias favour lentic water bodies. In most African lakes, tilapias dominate the inshore fish fauna (Moyo, 1994). However, in Flag Boshielo Dam the fish fauna was dominated by cyprinids. This is probably explained by the temperature regime prevalent in this dam, as the temperatures were below 20°C for 8 months. Tilapias are warm water species and thrive at an optimum temperature of 28°C. Both *C. carpio* and *H. molitrix* have a wide temperature range. *Labeo rosae* is essentially a Limpopo river system fish, although it extends southwards into the Inkomati and Phongolo river systems in South Africa (Skelton, 2001).

Size related variation in the diet of *Tilapia rendalli* at Flag Boshielo Dam

The diet of *T. rendalli* at Flag Boshielo Dam varied between the different size groups. Fry <2 cm (SL) fed almost exclusively on zooplankton. At this size, the stomach is not fully developed and the fish are unable to efficiently utilise plant diets as there is no acid secretion. Most fish experience a size related dietary shift as they all start feeding on zooplankton and later on become specialised. The importance of zooplankton in the diets of post larval fish has been examined by Whitfield (1983), he reviews literature that shows zooplankton to be the major nutritional source of larval fish in most aquatic environments and suggests that this is due to the higher energy of zooplankton relative to other organisms of equivalent size. It is generally accepted that the first food of post larval fish consists at least partially of zooplankton

(Fernando, 1994). The findings of this study confirm results by several authors: Meschiatti and Arcifa (2002); El-Sayed (2006); Adeyemi *et al*. (2009) and Elnady *et al*. (2010) who reported that tilapias feed on an animal based protein diet at a juvenile stage. However, Batchelor (1978); Weliange and Amarasinghe (2003), reported that there was no distinction in the diet of *T. rendalli* of different size groups. This contradiction is probably explained by the fact that the transition from a zooplankton based diet to a plant based diet is abrupt (Brummett, 2000) and these authors did not focus on the diet of fish <2 cm.

In the current study, it was observed that *T. rendalli* started feeding on marginal vegetation between 2 and 5 cm and the importance of the marginal vegetation increased with fish size. The ability of *T. rendalli* to utilise plant based diets at such a small size (2-5 cm) is very important in culture systems. This implies that at this stage, the morphological adaptations for the digestion of plant diets have developed. Under aquaculture conditions, plant based diets can be introduced early, thus reducing feed cost and making the enterprise more profitable.

Flag Boshielo Dam is oligotrophic and devoid of any macrophytes. A review of literature indicates that adult *T. rendalli* feeds on macrophytes (Philippart and Ruwet, 1982; Chifamba, 1990; El-Sayed, 2006). In Flag Boshielo Dam, the preferred diet of *T. rendalli* is absent and the fish were opportunistically feeding on other plant diets particularly marginal vegetation. Brummett (1995) indicated that juvenile fish of less than 15 cm preferred filamentous algae followed by marginal vegetation. According to Lowe-McConnell (1987), Winemiller (1991) and Winemiller and Kelso-Winemiller (2003) cichlid fish like *T. rendalli* are opportunistic feeders during periods when resources are scarce reverting to a more specialized diet when resources are plenty. Fryer and Iles (1972) reported that *T. rendalli* is a herbivore and planktivore in lakes in Madagascar, where both juveniles (<10 cm) and adults had similar diets. Phillipart and Ruwet (1982) considered *T. rendalli* as a macrophyte feeder. In a South American reservoir in Brazil, this species was reported to be a detritivore or detritivore-herbivore (Arcifa *et al*., 1991). In the current study however, no phytoplankton was found in adult fish. Furthermore, the low chlorophyll *a* levels indicate that the dam has low phytoplankton levels.

The results of this study contradict those of Lazzaro (1991); Arcifa and Meschiatti (1993; 1996) who reported that *T. rendalli* was a planktivore, feeding in the water column. This was not observed in the current study probably because of the low phytoplankton levels at Flag Boshielo Dam. It is however important to note that *T. rendalli* is not adapted to utilization of phytoplankton because of its low gill rakers (8-10) count.

The herbivorous feeding nature of *T. rendalli* is clearly demonstrated by the fish's ability to utilise macrophytes or marginal vegetation in water bodies devoid of macrophytes such as Flag Boshielo Dam. This unique ability of *T. rendalli* to feed on higher plant diets makes it more suitable for culture than other herbivorous species such as the widely cultured *O. mossambicus*. This implies that *T. rendalli* can be cultured in low input systems and fed on higher plants. The culture of *T. rendalli* on low cost plant diets may be the solution in reducing the high feed costs and increase production.

T. rendalli is able to use other feed resources in the absence of macrophytes. These catholic feeding habits make this species an excellent candidate for aquaculture where a combination of feed sources may be used to maximise production.

The feeding overlaps which occurred between different size groups of *T. rendalli* suggest a degree of intra-specific competition. The highest overlap was observed between the <2 cm and 2-5 cm size groups. This implies that although fish >2 cm had started including other food items in their diet; zooplankton was still a major component. The low overlap coefficient between the 2-5 cm and the 5-9.9 cm confirms the assertion that as *T. rendalli* grows its diet becomes more specialized and focused towards higher plants. *T. rendalli* has the ability to utilise higher plants because it possesses bi-cuspid teeth and pharyngeal teeth which enable it to truncate and break down plant material. It is also one of the few species that is able to secrete hydrochloric acid in the stomach which helps to further break down the cell walls and expose its contents to digestive juices in the digestive tract.

Seasonal variations in the diet of *Tilapia rendalli*

Environmental changes may also have an impact on the dietary choices available to the fish. These include changes in water temperature, and abiotic factors such as precipitation, circulation and stratification. At Flag Boshielo Dam, the diet of *T. rendalli* changed with season. All the major food items were abundant in summer probably because of the higher water temperatures and increased primary production. However, in spite of the abundance of other food items in the environment, marginal vegetation was still the most preferred item as it appeared in 89% of stomachs analysed in summer. This is a result of the increasing water level and inundation of new areas of marginal vegetation. However, it is of paramount importance to note that this species fed on any food they came across in summer as its diet was more diverse during this season.

The observed changes in the diet of *T. rendalli* with season are in agreement with earlier reports. Weliage and Amarasinghe (2003) reported that fish change their feeding habits seasonally due to the temporal variation in food availability. The change of the diet from summer to winter may be caused by low food availability and in these situations, the fish will feed on whatever is available and abundant (Caulton, 1977; Chifamba, 1990). Flecker and Feifarek (1994) also indicated that diet changes might be due to seasonal modification in the species community. Munro (1967) also stated that the diet of fish varies due to environmental changes.

The ability of *T. rendalli* to switch its diet and feed opportunistically on other resources in the absence of the preferred diet (macrophytes) makes this species an ideal aquaculture candidate. This will enable farmers to include different feed sources in its diet for maximum production.

Feeding activity and condition factor of *Tilapia rendalli* at Flag Boshielo Dam

The percentage of full stomachs was highest in December, which is midsummer. This period coincided with increased water level when a larger area of marginal vegetation was inundated. The high percentage of full stomachs may also be attributed to increased appetite and metabolic rate as the water temperature increased. These results are indicative of a higher feeding intensity in summer when food supply was abundant. A decrease in water temperature in autumn was followed by a corresponding decrease in the number of full stomachs and fish condition. There was a further decrease in the percentage of full stomachs towards winter. By June (midwinter), all the stomachs examined were empty. This may be a result of the low metabolic rate and reduced appetite of fish during the low winter temperatures. As reported in this study, *T. rendalli* stopped feeding in winter and lost condition. These low winter temperatures in South Africa affect the aquaculture production of warm water species such as *T. rendalli* as these fish stop feeding and loose condition during this time. This leads to longer production periods to produce minimum weight required for the market.

Factors influencing fish condition factor include growth phase, season, degree of stomach fullness, gonad maturity, sex, health status. The condition factor of *T. rendalli* at Flag Boshielo Dam was highest in summer when there was abundant marginal vegetation available to the fish. This is confirmed by the high percentage of full stomachs in this season. As the water temperature decreased, *T. rendalli* stopped feeding probably because of low metabolic rates. The condition factor of *T. rendalli* also decreased and was lowest in winter; this was evidenced by the high number of empty stomachs during this period. The marked decline in condition factor which occurs in autumn and winter suggests that *T. rendalli* greatly reduces food intake in the cold winter months.

The seasonal variations observed in the feeding habits of *T. rendalli* are especially important in determining its aquaculture potential. *T. rendalli* did not feed in winter when the water temperature was low (reduced metabolic processes) and the marginal vegetation was not available because of the drawdown. This resulted in reduced body condition during the winter period. In South Africa, the winter period is long (3-4 months) the reduced feeding in winter leads to poor growth, resulting in low production levels.

It is clear that feeding in *T. rendalli* was greatly reduced in the cold winter months. This has serious implications on pond culture in South Africa where the water temperature is not regulated. When the fish stop feeding in winter, it will lose condition thereby delaying the time taken to reach acceptable market size. This delay will also result in increased production costs and affect the profitability of the enterprise. There are places however, in the Limpopo province such as the Vhembe district where summer temperatures are high (average 28°C). The culture of *T. rendalli* is recommended in such places. The Vhembe district also experience moderate winter temperatures (average 18℃). This implies that all year feeding is possible in this district, although at a lower growth rate because according to El-Sayed (2006) tilapias stop feeding between 15°C-16°C.

Growth performance of *Tilapia rendalli* at Flag Boshielo Dam

Scales were successfully used for the age determination of *T. rendalli*. The rings on the scales were validated as being true annuli by marginal increment analysis which showed that the distance between the last annuli and the scale edge was shortest in the spring months, (October/November). This is the time in which the annulus formed.

Annulus formation in *T. rendalli* at Flag Boshielo Dam coincides with the onset of rains and increased water temperature in this region. This indicates that annulus formation may be a response to these environmental ques. This implies that high temperatures associated with the beginning of the hot season are an important factor in annulus formation. The time for annulus formation also coincided with the onset of rainfall and an increase in water inflow into the dam. Reduced conductivity associated with increased inflow at the beginning of the rainy season may be another important que in the formation of annuli. Furthermore, the onset of rains is usually associated with the onset of breeding activities Garrod, (1959).

The results on annulus formation confirm the findings of several authors who worked with other tilapia species. Mehanna (2004) and Hadi (2008) also reported that annual rings formed in early spring after the first rains in *T. zillii* and *O. aureus* respectively. On the contrary, Weyl and Hecht (1998) reported that a single opaque band was formed in the otoliths of both *T. rendalli* and *O. mossambicus* during winter in Lake Chicamba, Mozambique. Lagler (1956) stated that fluctuations in the growth rate of fish in separate bodies of water and in different areas of the same impoundment are normal and may vary from year to year.

T. rendalli in this study displayed sexual dimorphism with males growing at a higher rate and to a larger asymptotic size than females. This is in agreement with studies done by Fryer and Iles (1972) and Batchelor (1978). This implies that to maximise growth performance and increase production of this fish in culture, it may be important to focus on all male production.

Tilapias generally exhibit a rapid linear growth in the first year of life, Fryer and Iles (1972) also reported that under natural conditions the growth achieved in the first year of life by several species of *Tilapia* is from about 9 to 12 cm. This represents adaptations to adverse conditions for growth as a result of selection for low growth rates and relatively early maturation. This may also represent an evolved adaptation to reduce its vulnerability to the environment. The asymptotic length (L_∞) of 32.27 cm observed in this study is slightly higher than the L_∞= 28.25 cm reported by Moreau *et al*. (1986), for the same species. However, it is comparable to the L_∞= 33.10 cm obtained by Batchelor (1978) who worked with *T. rendalli* in the same province. Kolding *et al*. (1996) reported a higher L_∞ of 35.5 cm in *T. rendalli* in the Bangweulu Swamps (Okavango Delta). The growth rate in fish is a function of mean water temperature and food availability in the water body. The Okavango Delta has higher annual mean temperatures (32°C) than the mean of 22°C recorded in Flag Boshielo. Pauly *et al*. (1988) reported that the mean growth performance of *T. rendalli* in the wild was 2.45. The mean growth performance of *T. rendalli* at Flag Boshielo Dam was 2.66. This implies that despite the lack of macrophytes at Flag Boshielo Dam, *T. rendalli* attained growth rates that were comparable to those obtained in other water bodies.

Furthermore, the growth performance index (ϕ) of *T. rendalli* in nature (2.45) was found to be comparable to that of the widely cultured *O. mossambicus* (2.48), Pauly *et al* (1988). Under aquaculture conditions however the growth performance indices (ϕ) were 2.60 for *T. rendalli* and 3.14 for *O. mossambicus* respectively (Pauly *et al*., 1988). It is important to note that Pauly (1988)'s results were taken from different studies and the performance was not directly compared. Direct comparison of these two species has not been done.

From this chapter, it can be concluded that *T. rendalli* does not only feed on macrophytes but also on marginal vegetation some of which was of terrestrial origin. To determine the potential of this species as a candidate for aquaculture, laboratory experiments had to be conducted to measure its growth on plant diets in captivity. One of the most significant growth parameters in commercial aquaculture is the efficiency of conversion of food into fish flesh; this is measured by the food conversion ratio (FCR).

CHAPTER 4: UTILISATION OF FRESH PLANT DIETS BY *TILAPIA RENDALLI*

4.1 INTRODUCTION

The macrophagous feeding nature of *T. rendalli* in different water bodies has been explored in Chapter 2. It was also shown that *T. rendalli* opportunistically feeds on other plant diets, particularly marginal vegetation in the absence of macrophytes. The absence of macrophytes at Flag Boshielo Dam did not affect the growth rates of *T. rendalli* when compared to other water bodies (Chapter 3).

The ability of *T. rendalli* to feed on marginal vegetation of terrestrial origin and attain acceptable growth rates prompted a subsequent study where its utilisation of macrophytes, terrestrial grass and a commonly available vegetable was determined. The opportunistic feeding nature of *T. rendalli* makes it a suitable aquaculture species because it can be reared in extensive, semi-extensive or intensive production systems feeding on low cost plant diets. Chikafumbwa (1996) and Brummett (2000) reported that terrestrial vegetation and agricultural crop residues have a potential to be used in the culture of *T. rendalli* in ponds.

As already highlighted in Chapter 1, the culture of *T. rendalli* in South Africa is minuscule, and tilapia culture is dominated by *O. mossambicus*. However, overall tilapia production has failed to reach satisfactory levels. The main reason is that aquaculture has become unprofitable because of the high production costs especially the cost of feed. Traditionally, fishmeal has been used as the major protein source in fish feeds because of its nutritional value and palatability. However, due to the limited world supplies and increasing price of fishmeal, fish farmers in the developing world cannot afford the expensive fish feed. Alternative plant protein sources are generally cheaper compared to animal protein sources. Furthermore, feeding herbivorous fish such as *T. rendalli* on expensive fishmeal protein may not be necessary.

To satisfy the protein needs of the growing population, it is fitting to consider a fish species that can be fed solely or largely on plant diets in extensive or semi-extensive culture. This relatively low capital approach can be adopted by rural subsistence farmers to improve fish production and ensure food security.

One of the primary objectives of this chapter is to evaluate the growth of *T. rendalli* when fed on readily available fresh plants. The plants under investigation are a common lawn grass; kikuyu (*Pennisetum clandestinum*), an abundant vegetable; cabbage (*Brassica oleracea*), a common floating weed; duckweed (*Lemna minor*) and vallisneria a submerged macrophyte (*Vallisneria aethiopica*). Commercial fishmeal pellets were used as a control.

- Kikuyu grass (*Pennisetum clandestinum*), is relatively abundant in South Africa, and is often used in livestock production (Marias *et al*., 1987) because of its high nutritive value and ease of production.
- Cabbage (*Brassica oleracea*), is an inexpensive vegetable, widely cultivated and available throughout the year in South Africa. Cabbage leaves are usually discarded as a kitchen or garden waste.
- Duckweed (*Lemna minor*), a floating macrophyte of the Lemnaceae family, has been widely used for cattle, poultry, swine and fish feeding, with favourable results (Skillicorn *et al*., 1993). Duckweeds have received much attention for use in aquaculture because of their potential to remove mineral contaminates from water, their attractive nutritional properties, ease of production and rapid growth.
- Vallisneria (*Vallisneria aethiopica*) is easy to establish, although it’s an exotic plant, it is already found in a number of dams across South Africa. It was selected for this experiment because *Tilapia rendalli* feed mostly on submerged macrophytes in nature.

Dietary protein is considered the most important nutrient supporting fish growth, and is critical for herbivorous fish feeding on low protein foods (Jauncey and Ross, 1982). The combination of essential amino acids as opposed to total protein plays an important role in fish growth (Siddiqui *et al*., 1988). The nutrient composition and amino acid profiles of the diets used in this study were determined. Low winter temperature is a serious constraint in tilapia production in South Africa, as these fish stop feeding at water temperatures below 16°C (El-Sayed, 2006). The effect of seasonal change in water temperature on the consumption rate of the different food items by *T. rendalli* in aquaculture systems is also investigated in this chapter.

4.2 OBJECTIVES

i) To determine the growth performance of *Tilapia rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

ii) To determine the effect of temperature change on the feeding rate of *T. rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

iii) To determine the carcass composition of *Tilapia rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

4.3 NULL HYPOTHESIS

i) There is no significant difference in the growth performance parameters of *Tilapia rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

ii) Temperature has no significant effect on the feeding rate of *T. rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

iii) There is no significant difference in carcass composition of *Tilapia rendalli* fed fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria.

4.4 MATERIALS AND METHODS

The experiments for utilisation of fresh plant diets were conducted at the Aquaculture Research Unit at the University of Limpopo, South Africa.

4.4.1 Growth performance of *Tilapia rendalli* fed fresh plant diets

The herbivorous nature of *T. rendalli* was shown in chapter 3. In this chapter, we explore the growth performance of *T. rendalli* on selected plants. An evaluation of the use of fresh plant diets as a sole feed for *T. rendalli* will give an indication as to which plant can be used in the culture of *T. rendalli* in ponds. Furthermore, the ability of *T. rendalli* to continue feeding on the selected plant diets in winter is investigated.

The utilisation of kikuyu grass, vallisneria, cabbage, duckweed and commercial fishmeal pellets (control) by *T. rendalli* was determined in the laboratory. This experiment was conducted in a 30 m x 10 m greenhouse in ten rectangular (1 m^3) fibre glass tanks (Fig. 4.1). The tanks were supplied with a continuous flow of water and re-circulated through a bio-filter before being reintroduced to the experimental tanks. A completely randomised design experiment was set up. There were four treatments and a control and this was duplicated for each feed type. Each tank was stocked with 25 mixed-sex sub-adult *T. rendalli* of mean weight 64±2 g.

Figure 4.1: Fibre glass tanks used in feeding experiments

Kikuyu grass, vallisneria and duckweed were harvested fresh each morning and cabbage was bought fresh from a local market. All plant diets with the exception of duckweed were chopped into 1 cm pieces. Duckweed and vallisneria were blotted dry with a paper towel to remove excess water. All test diets were fed *ad libitum* each morning. All fish from each tank were weighed once every four weeks. This experiment lasted for 224 days.

The following growth performance indices were calculated:

Specific growth rate (SGR; g/day), calculated according Winberg (1956).

$$\mathrm{SGR} = \left[\frac{\mathrm{In}\ W_{t-}\ \mathrm{In}W_0}{t}\right] \times 100\%$$

where: W_t = final body weight (g)

W_0= initial body weight (g)

t = time feeding period (days)

$$\text{Feed conversion ratio (FCR)} = \frac{\text{food consumed (g)}}{\text{mass gained (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Increase in body mass (g)}}{\text{Protein consumed (g)}}$$

$$\text{Standing crop} = \frac{\text{total mass (g)}}{\text{area (m}^3\text{)}}$$

$$\text{Yield increment} = \frac{\text{mass (g)}}{\text{area (m}^3\text{)}}$$

$$\text{Production} = \frac{\text{yield per area (g/m}^3\text{)}}{\text{time (days)}}$$

4.4.2 Feed consumption in relation to temperature

During the growth experiments, the water temperature was not controlled; it was allowed to fluctuate with the changes in seasons. At flag Boshielo Dam, *T. rendalli* did not feed in winter (Chapter 3). The influence of seasonal temperature variations and food consumption was further explored. Daily food consumption was recorded and temperature was monitored on a daily basis.

4.4.3 Water quality monitoring

Daily measurements of temperature (°C), dissolved oxygen (mg/l) and pH were taken using a handheld YSI (556 MPS) multi-meter. Air was continuously supplied using a cyclone blower and diffused through air stones in each tank.

4.4.4 Effect of experimental diets on water quality in static systems

At the end of the feeding trial, the recirculation of the water was stopped for 30 days. This was done in order to determine the effect of the different diets on the water quality. The fish were fed each diet to satiation for the duration of the experiment. After the 30 days, water samples were collected in 1 L sample bottles and stored at -20°C until they were analysed. pH was determined, using the US Standard method 4500 H^+B through the determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode. Bicarbonate alkalinity was determined using the US Standard method by titrating 2320 B, 0.02 N sulphuric acid to an end point of pH 4.5, using methyl red as an indicator. Calcium (Ca) and magnesium (Mg) were determined using the US Standard method 3120 B, based on the emission of light by an optical spectroscopic technique. Total hardness was determined by calculation from the Mg and Ca determination. Ammonia was determined photometrically using Standard methods EPA 305.1 and APHA 4500 NH_3D. Orthophosphate was analysed using US Standard method 4500 PE, based on the theory that, in sulphuric solution orthophosphate ions react with molybdate ions to form molybdophophiric acid. Ascorbic acid was used to reduce this to phosphomolybdenum blue (PMB) that was then determined photometrically. Nitrate was determined using a Merk Spectroquant Method, with concentrated sulphuric acid and benzoic acid derivative. Determination of nitrite was done photometrically using US Standard Methods 4500 NO_2B.

4.4.5 Determination of proximate composition of experimental diets

All diets used in this study were analysed for crude protein, crude fat, crude fibre, carbohydrates, energy, ash, moisture and minerals, following the procedures stipulated by the Association of Official Analytical Chemists (AOAC International, 2003). Dry matter was determined by freeze-drying each sample for 72 hours. Nitrogen content of the dry matter of the feed and animal tissue was determined using a LECO FP2000 Nitrogen Analyser using the Dumas combustion with protein content calculated as % nitrogen x 6.25. Lipid content was assessed by Soxhlet extraction of the freeze-dried samples with petroleum ether at 40-60°C. Ash was determined by burning the samples in a muffle furnace at 550°C for 4 hours. Gross energy was analysed for using a DDS isothermal CP 500 bomb calorimeter.

4.4.6 Determination of amino acid composition and limiting amino acids

The amino acid composition of the diets was determined using a Beckman Amino Acid Analyser System 6300. The method which uses lysine as a reference amino acid, and the requirements for all other essential amino acids (EAA) expressed as a percentage of lysine was used. This was necessary to identify limiting amino acids in the different diets. Lysine was chosen because lysine is normally the first limiting amino acid in most feedstuffs. Thus if one knows the dietary lysine requirement and the whole-body amino acid composition of an animal, then one should be able to estimate the dietary requirement for the remaining EAA of the animal relative to the lysine requirement. The ratio of each amino acid to lysine was calculated, a ratio of less than 1 means that the particular amino acid was limiting in the diet.

4.4.7 Carcass composition

A sample of fish (2 males and 2 females) was collected from the initial batch before the start of the experiment this sample was also analysed for proximate composition. These results were used a bases on which results of the final carcass composition after feeding the experimental diets were compared. The rationale for the determination of carcass composition was to ascertain if the different diets had an effect on the composition of different nutrients in the carcass, especially fatty acid composition.

The fish samples were cleaned of all internal organs, deboned and de-scaled, then frozen at -20°C until they were analysed for proximate and amino acid composition. At the end of the feeding experiment, samples of four fish were taken from each treatment. The internal organs were removed from all samples, and then samples were deboned and de-scaled. Fish samples were then frozen at -20°C until they were analysed for proximate composition of the carcass. Nitrogen content of the animal tissue was determined using a LECO FP2000 Nitrogen Analyser using the Dumas combustion with protein content calculated as % nitrogen x 6.25. Fat content was assessed by Soxhlet extraction of the freeze-dried samples with petroleum ether at 40°C. Ash was determined by burning the samples in a muffle furnace at 550°C for 4 hours. Gross energy was established with a DDS isothermal CP 500 bomb calorimeter. Gas chromatography was used for fat analysis. Non-volatile fatty acids were chemically converted to the corresponding volatile methyl esters. The resulting volatile mixture was then analyzed by gas chromatography.

4.4.8 Statistical analysis

One-way analysis of variance (ANOVA) on the Statistical Package and Service Solutions (SPSS version 17.0) was used to determine significant differences ($P < 0.05$) on SGR, FCR, PER, standing crop, yield increment and the total production of *T. rendalli* fed duckweed, vallisneria, cabbage, kikuyu grass and fishmeal pellets. Tukey’s test was used to separate means where significant differences ($P < 0.05$) were found. A regression analysis was done on the consumption of the different experimental diets in relation to water temperature and the analysis of covariance (ANCOVA) was used to test if there were significant differences in the regressions.

4.5 RESULTS

4.5.1 Utilisation of the different experimental diets by *Tilapia rendalli*

Fish that were fed on the commercial fishmeal pellets grew significantly better (P<0.05, ANOVA) than those fed exclusively on plant diets. Fish fed vallisneria consistently lost weight; its feeding was terminated after 90 days (Fig. 4.2).

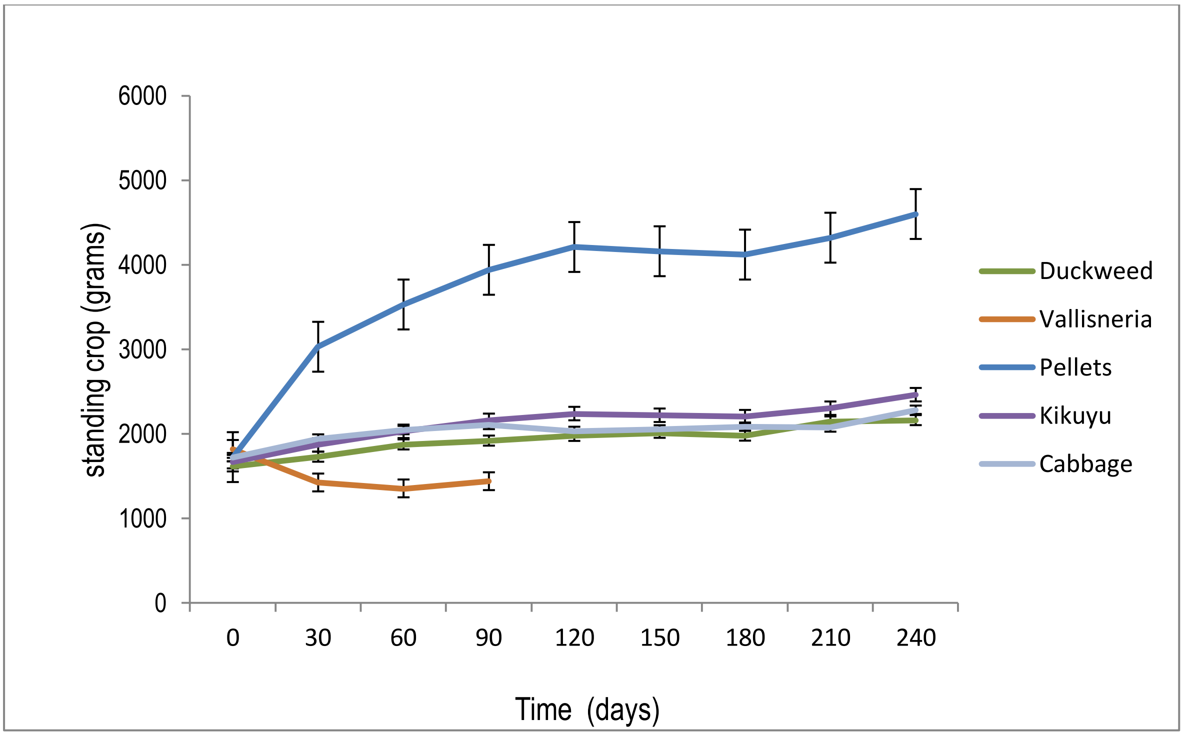


Figure 4.2: Standing crop of *Tilapia rendalli* fed different experimental diets

There were significant differences (P< 0.05, ANOVA) in the SGR of *T. rendalli* fed duckweed, vallisneria, kikuyu, cabbage and commercial fishmeal pellets. The best SGR was observed in fish fed fishmeal pellets (2.14%), followed by fish fed kikuyu grass (0.173%), duckweed (0.130%), cabbage (0.126%) and fish fed vallisneria lost weight (-0.105%). Tukey's post-hoc test showed that all means for SGR were significantly different among the diets tested (P<0.05; Table 4.1).

Similarly, significant differences (P<0.05, ANOVA) were observed in the FCR of *T. rendalli* fed fishmeal pellets, kikuyu grass, duckweed, cabbage and vallisneria. The FCR values confirmed the growth rates observed, fishmeal pellets gave the lowest FCR (1.2). Among the plant diets, kikuyu gave the lowest FCR (14.7) followed by duckweed (20.8) and cabbage (51.6) while the FCR of *T. rendalli* fed on vallisneria was negative (-0.11). Tukey's post-hoc test showed that all FCR means were significantly different ($P<0.05$; Table 4.1).

PER values also differed significantly ($P<0.05$, ANOVA) among fish fed the different test diets. The PER values also followed the same trend as the growth rates, fish fed fishmeal pellets showed the best PER (3.99), again followed by those fed kikuyu grass (0.26), duckweed (0.18) and cabbage (0.17) respectively. *T. rendalli* fed vallisneria showed a negative PER (-0.41). Tukey's post-hoc analysis showed that there were no significant differences ($P>0.05$) in the PER of fish fed duckweed and cabbage (Table 4.1).

Significant differences ($P<0.05$, ANOVA) were also observed in the yield increment (g/m^3) of *T. rendalli* fed the experimental diets. Fish fed fishmeal pellets gave the highest yield (1949 g/m^3). Among the plant diets, kikuyu grass gave the best yield of 792 g/m^3, followed by duckweed (700 g/m^3) and cabbage (560 g/m^3) respectively. The yield increment was negative in *T. rendalli* fed vallisneria (Table 4.1).

Results on total production followed the same trend as those observed on the other growth performance indices. Fish fed fishmeal pellets gave the highest production (8.7 $g/m^3/d$), again followed by those fed kikuyu grass (3.5 $g/m^3/d$), duckweed (3.1 $g/m^3/d$), then cabbage (2.5 $g/m^3/d$). In fish fed vallisneria, the production was negative (-5877 $g/m^3/d$). Tukey's post-hoc tests showed that there were no significant differences ($P>0.05$) in production in fish fed duckweed and cabbage (Table 4.1).

Table 4.1: Growth performance of *Tilapia rendalli* fed on different experimental diets. Data are mean values ±SE

Diet	Yield (g/m^3)	Production ($g/m^3/d$)	PER	FCR	SGR (%/day)
Pellets	1949.0±1.00^a	8.700±0.05^a	3.991±0.05^a	1.120±0.01^a	2.140±0.05^a
Kikuyu	792.0±3.50^b	3.536±0.02^b	0.257±0.01^b	14.728±0.30^b	0.173±0.01^b
Vallisneria	-382.0±1.00^c	-5877±1.50^c	-0.411±0.09^c	-7.762±0.07^c	-0.105±0.06^c
Cabbage	560.0±2.00^d	2.500±0.15^b	0.167±0.08^d	51.607±0.06^d	0.126±0.08^d
Duckweed	700.0±2.00^e	3.125±0.04^b	0.175±0.04^d	20.816±0.08^e	0.161±0.05^e

NB: Figures in the same column with different superscripts are significantly different ($P<0.05$, ANOVA).

4.5.2 Feed consumption in relation to temperature

T. rendalli generally consumed high quantities of the plant diets than the fishmeal pellets (Fig. 4.3). After the feeding of vallisneria was terminated, cabbage was consumed in higher quantities compared to the other diets, followed by duckweed then kikuyu grass. Fishmeal pellets were consumed at much lower rates than all the plant diets.

Daily consumption of all the experimental diets was adversely affected by decline in water temperature as winter approached. Food consumption was lowest in winter and the consumption rates increased again in spring. The consumption of kikuyu grass declined from 168 g/day in March to 51 g/day in July (Fig. 4.3). In August, daily kikuyu consumption increased as the water temperatures increased, by October kikuyu daily consumption was 136 g/day. The same trend was observed for the other plant diets, duckweed consumption was declined from 216 g/day in March to 102 g/day in July, increasing again to 269 g/day in October. The decline in consumption was more acute in fishmeal pellets as daily consumption rates reached 2 g/day in July from 56 g/day on March.

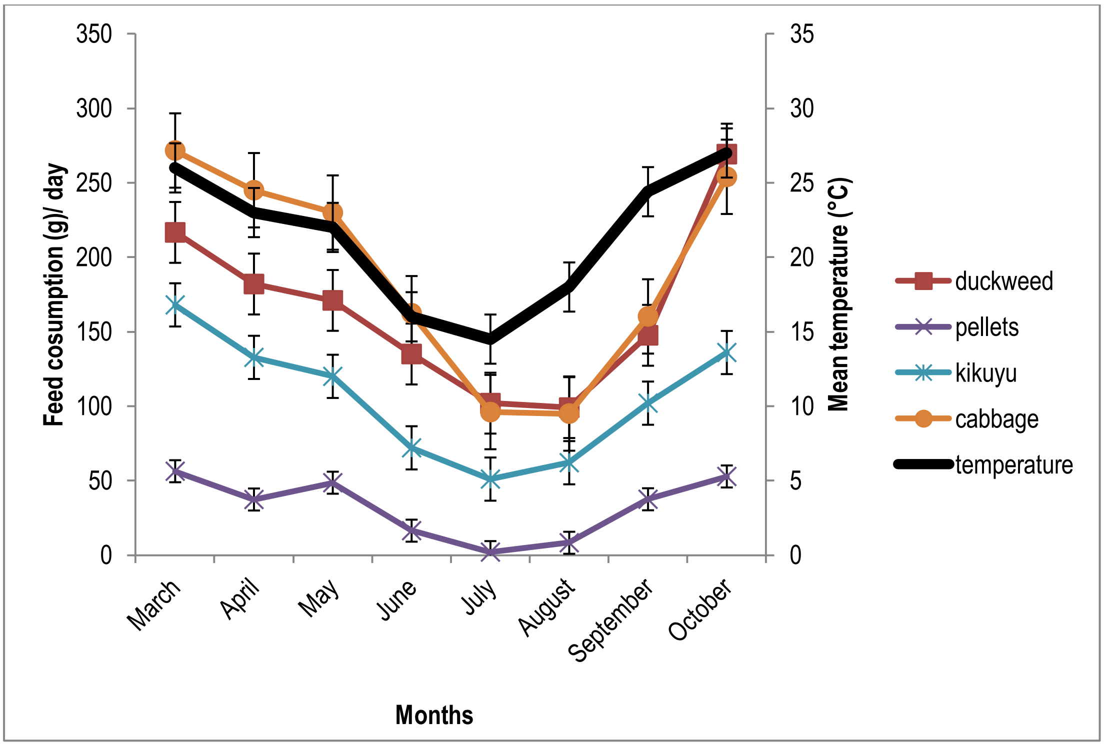


Figure 4.3: Mean monthly feed consumption in relation to mean water temperature

The relationship between food consumption for all the experimental diets and change in water temperature was represented by a linear regression. The linear regression of the consumption of fishmeal pellets on water temperature is shown in Fig. 4.4. Using the linear regression below, the temperature at which *T. rendalli* stops feeding on fishmeal pellets is 13.58°C.

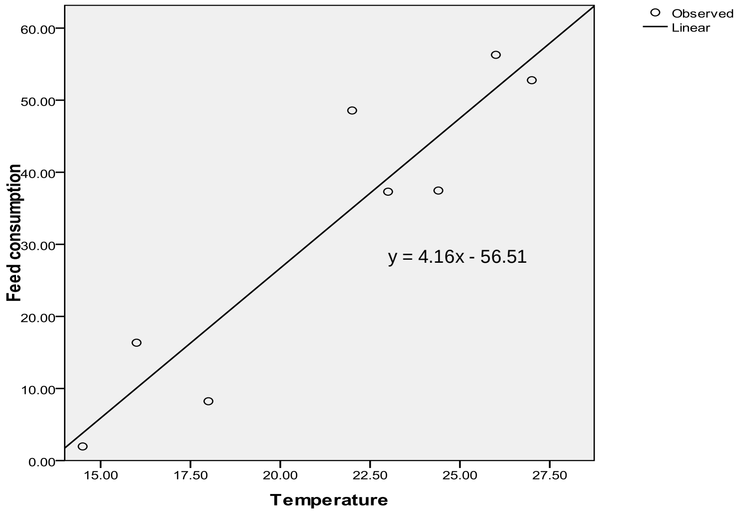


Figure 4.4: Regression of feed consumption and water temperature in *Tilapia rendalli* fed commercial pellets.

The regression of the consumption of kikuyu grass on water temperature was also linear (Fig. 4.5). This regression equation show that the temperature at which *T. rendalli* stops feeding on kikuyu grass is 8.00°C.

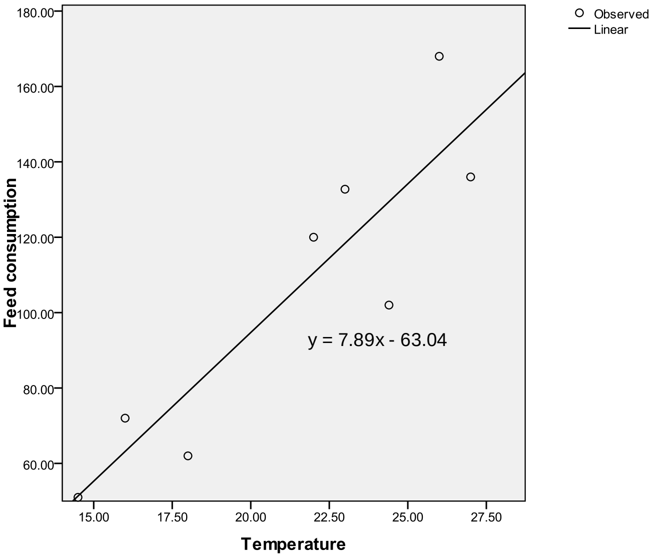


Figure 4.5: Regression of feed consumption and water temperature in *Tilapia rendalli* fed kikuyu grass.

The linear relationship between the consumption of duckweed and water temperature is shown below (Fig. 4.6). Based on this regression, the temperature at which *T. rendalli* stops feeding on duckweed is 5.79°C.

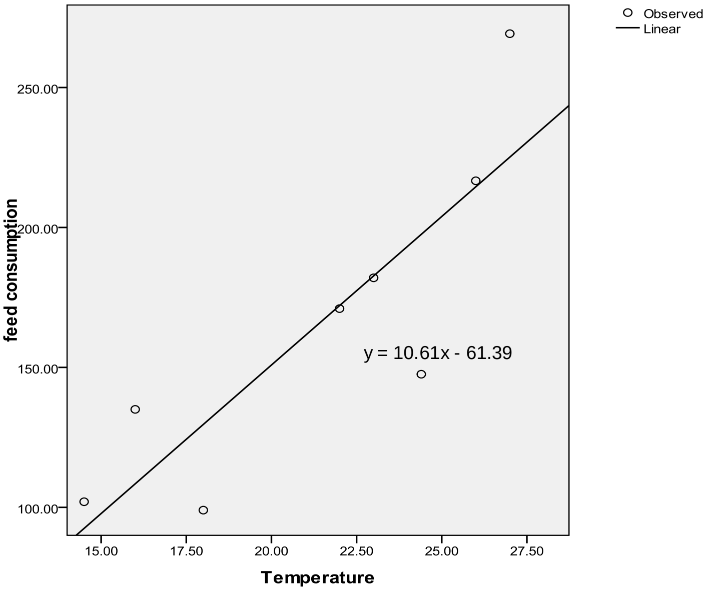


Figure 4.6: Regression of feed consumption and water temperature in *Tilapia rendalli* fed duckweed.

Figure 4.7 represents the linear relationship between cabbage consumption and water temperature, and the predicted temperature at which *T. rendalli* stops feeding on cabbage is 6.00°C.

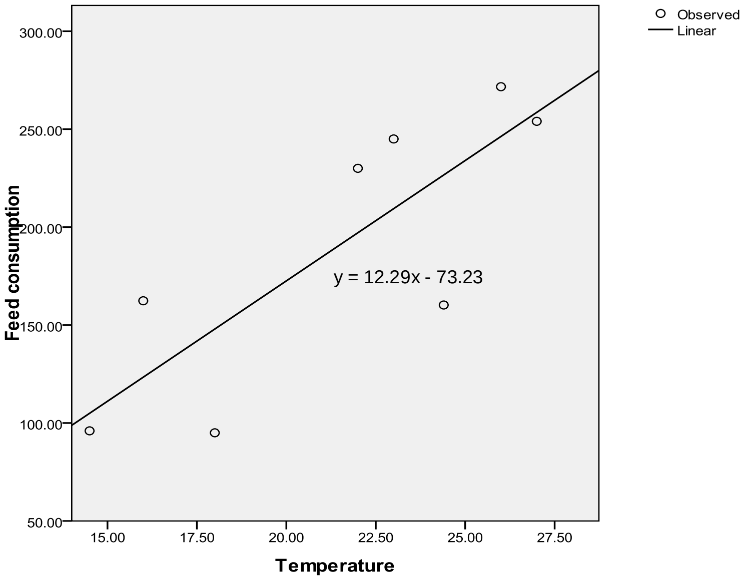


Figure 4.7: Regression of feed consumption and water temperature in *Tilapia rendalli* fed cabbage.

The analysis of covariance (ANCOVA) showed that the slopes of the regressions for all the different diets are not significantly different (P>0.05).

4.5.3 Nutritional composition of diets

The nutritional composition of the experimental diets was analysed to determine if the observed differences in growth performance were affected by the different nutritional composition of the experimental diets. The crude protein values were different between all diets. Fishmeal pellets had the highest protein content of 34.43% and duckweed had the highest protein content of 27.42% among the plant diets, followed by kikuyu grass (26.40%), vallisneria (12.79%) while cabbage had the lowest protein level of 11.67 (Table 4.2).

Kikuyu grass had the highest energy content (17.99 MJ/kg), followed by fishmeal pellets (17.00 MJ/kg), then duckweed (16.43 MJ/kg) and cabbage (16.09 MJ/kg). The lowest energy level (14.74 MJ/kg) was found in vallisneria (Table 4.2).

The fat content was highest in kikuyu grass (4.63%), followed by duckweed (4.26%), fishmeal pellets (2.97%), vallisneria (1.77%) and cabbage (1.43%) respectively (Table 4.2).

Vallisneria had the highest ash content (13.84%), followed by kikuyu grass (12.49%), duckweed (12.26%), fishmeal pellets (10.78%) and cabbage had the lowest ash content (6.06%; Table 4.2).

Duckweed and vallisneria had the highest moisture content (93.57% and 93.15% respectively), cabbage had a moisture content of 91.78% and kikuyu grass had the lowest moisture content among the plants diets. The moisture content of fishmeal pellets was 7.80% (Table 4.2).

Fishmeal pellets had the lowest fibre (2.30%) and kikuyu grass had the highest fibre content (18.58%) among the plant diets, followed by vallisneria (14.72%) and cabbage (11.71%). Duckweed had the lowest fibre content among the plants (10.37%; Table 4.2).

Table 4.2: Proximate composition of the experimental diets expressed as percent dry matter

Diet	*Protein	Gross energy (MJ/kg)	Ash	Moisture	Fat	Crude fibre
Duckweed	27.42	16.43	12.26	93.57	4.26	10.37
Kikuyu	26.40	17.99	12.49	82.85	4.63	18.58
Vallisneria	12.79	14.74	13.84	93.15	1.77	14.72
Cabbage	11.67	16.09	6.06	91.78	1.43	11.71
Pellets	34.48	17.00	10.78	7.80	2.97	2.30

NB: results expressed on wet (fresh) bases

* For the conversion of nitrogen content to protein content, the factor 6.25 was used

4.5.4 The amino acid profiles for the experimental diets

The amino acid content of the experimental diets was evaluated mainly because fish have a requirement for specific amino acids rather than the total protein content. In this study, the diets which gave the best growth performance also had the highest levels of all the essential amino acids. Fishmeal pellets had the highest levels followed by kikuyu grass, duckweed, cabbage then vallisneria. However, the sulphur containing amino acid methionine was the limiting amino acid in all the diets. Methionine was lowest (0.16%) in cabbage whilst fishmeal pellets had the highest (0.57%) level among the diets. Methionine levels in kikuyu, duckweed and cabbage were 0.45, 0.35 and 0.16% respectively (Table 4.3).

Lysine was also highest (1.69%) in fishmeal pellets, followed by kikuyu grass (1.42%), duckweed (1.2%), cabbage (0.7%) and vallisneria had the lowest levels of 0.69% (Table 4.3). Arginine was only limiting in cabbage (0.62%). Tyrosine was highest in pellets (0.88%), followed by kikuyu (0.80%), duckweed (0.66%), cabbage (0.26%) and vallisneria (0.21%) respectively. Tyrosine and histidine were limiting in all test diets. These amino acids were also highest in fishmeal pellets, followed by kikuyu grass, duckweed and cabbage respectively. Vallisneria had the lowest levels of both amino acids.

Threonine and iso-leucine were also limiting in all diets. Again fishmeal pellets had the highest levels. Kikuyu grass had the highest levels of both amino acids among the plant diets, followed by duckweed, vallisneria and cabbage respectively. Phenylalanine was not limiting in all diets with the exception of cabbage. The highest levels were found in fishmeal pellets (1.40%), followed by kikuyu grass (1.34%), duckweed (1.14%), vallisneria (0.63%) then cabbage (0.43%; Table 4.3).

Leucine was available in sufficient amounts in all experimental diets. Fishmeal pellets had the highest levels (2.75%), followed by kikuyu grass (2.33%), duckweed (1.86%), vallisneria (0.93%) and cabbage (0.75%) respectively. Valine was also available in sufficient amounts in all experimental diets. Fishmeal pellets had the highest levels (1.77%) of valine, followed by kikuyu grass (1.56%), duckweed (1.36%), cabbage (0.68%) and vallisneria (0.65%) respectively (Table 4.3).

Table 4.3: Amino acid profile of the experimental diets expressed as percent protein

	Duckweed	Kikuyu	Vallisneria	Cabbage	Pellets
Essential amino acids					
Arginine	2.34	1.44	0.73	0.61	2.17
Methionine	0.35	0.45	0.15	0.16	0.57
Tyrosine	0.66	0.80	0.21	0.26	0.88
Histidine	0.44	0.51	0.28	0.29	0.73
Threonine	0.87	0.93	0.41	0.39	1.19
Isoleucine	0.89	1.12	0.47	0.42	1.32
Leucine	1.86	2.33	0.93	0.75	2.75
Phenylalanine	1.14	1.34	0.62	0.43	1.40
Lysine	1.20	1.42	0.69	0.70	1.69
Valine	1.36	1.56	0.65	0.68	1.77
Non-essential amino acids					
Aspartic	3.69	2.23	1.61	1.05	3.01
Serine	0.81	0.95	0.54	0.43	1.38
Glutamine	2.31	2.65	1.15	1.64	4.89
Proline	1.00	1.35	0.56	0.52	2.04
Glycine	1.20	1.37	0.85	0.54	2.28
Alanine	1.89	2.00	0.69	0.82	2.17
Ammonia	2.07	2.20	1.06	0.97	1.81
Cysteine	0.66	0.43	0.64	0.43	0.45

4.5.5 Carcass composition of fish fed the experimental diets

Fish fed fishmeal pellets showed poor carcass qualities when compared to those fed any of the plant diets. Fish fed kikuyu grass had the highest protein (71.55%) in the fillet than those fed the other experimental diets. Duckweed fed fish had the second highest protein content (68.49%), then cabbage (64.61%). Fish fed fishmeal pellets had the lowest (62.02%) protein in the fillet. However, the energy content was highest (21.26 MJ/kg) in the carcass of fish fed fishmeal pellets, followed by fish fed cabbage (18.89 MJ/kg), and then those fed duckweed (17.21 MJ/kg). Fish fed kikuyu grass had the lowest (16.24 MJ/kg) energy retained in the carcass (Table 4.4).

Fish fed commercial fishmeal pellets had the highest fat content (19.24%), followed by fish fed cabbage (12.83%), and then fish fed duckweed (6.68%). Fish fed on kikuyu grass had the lowest fat content (3.28%) in the carcass (Table 4.4).

The ash content in the carcass of the experimental fish was highest (24.54%) in fish fed on kikuyu grass, followed by fish fed on duckweed (22.55%) and then those fed on cabbage (19.99%). Fish feeding on fishmeal pellets had the lowest ash content

(17.20%) in the carcass. The calcium levels in the fillets were also highest in fish fed kikuyu grass (6.76%), again followed by fish fed duckweed and cabbage respectively. Calcium levels were lowest (4.49%) in fish fed fishmeal pellets. The same trend was observed in the phosphorous levels in the carcass, kikuyu grass gave the highest phosphorous levels (3.72%) in the fillet, followed by duckweed (3.49%), cabbage (3.21%) and fishmeal pellets (2.65%; Table 4.4).

Table 4.4: Whole body proximate composition (% fresh weight basis) at the beginning and after feeding different experimental diets

Diet	Protein (%)	GE (MJ/kg)	Fat (%)	Moisture	Ash (%)	Ca (%)	P (%)
Initial fish	68.86	16.80	7.15	74.63	22.37	-	-
Fishmeal	62.02	21.26	19.24	70.00	17.20	4.49	2.65
Kikuyu	71.55	16.14	3.28	74.24	24.54	6.76	3.72
Duckweed	68.49	17.21	6.68	73.23	22.55	6.19	3.49
Cabbage	64.61	18.89	12.83	71.24	19.99	5.66	3.21

4.5.6 Fatty acid composition of the fish fillets

The results of this study show that all fish fed the plant diets had higher levels of the healthy omega-3 fatty acids (Table 4.5). Fish fed kikuyu grass had the highest amount of omega-3 fatty acids (25.13%) in the fillet, followed by fish fed cabbage (14.35%) while fish fed fishmeal pellets had the lowest amount of omega-3 fatty acids. The ratio of omega 3 to 6 fatty acids was also highest in kikuyu (2.16:1), followed by cabbage (1.70:1), duckweed (1.05:1), fish fed vallisneria and fishmeal had higher levels of omega 6 fatty acids with ratios of 0.58:1 and 0.28:1 respectively (Table 4.5).

Table 4.5: Percent fatty acids in the fillet of fish fed the different experimental diets

Common name	Lipid	Duckweed	Pellets	Vallisneria	Cabbage	Kikuyu
Myristic acid	C14:0	1.42	2.37	2.34	1.64	<0.10
Myristoleic acid	C14:1	0.24	0.14	0.32	0.33	0.51
Pentadecylic acid	C15:0	0.29	0.14	0.21	0.35	<0.10
Palmitic acid	C16:0	20.80	24.90	22.70	21.00	17.10
Palmitoleic acid	C16:1	5.88	7.14	6.79	5.91	0.68
Margaric acid	C17:0	0.63	0.21	0.40	0.98	1.18
Stearic acid	C18:0	9.32	7.60	10.00	10.40	12.50
Oleic acid	C18:1n9	24.80	34.60	24.20	16.60	6.42
Linoleic acid	C18:2n6	6.42	6.26	6.59	3.67	4.87
Gamma-linolenic	C18:3n6	0.79	0.44	0.62	0.66	1.78
Alpha -linolenic acid	C18:3n3	3.59	0.37	0.80	3.70	6.24
Arachidic acid	C20:0	0.76	0.19	0.28	0.42	<0.10
Gadoleic Acid	C20:1n9	1.70	2.11	1.65	1.11	<0.10
Eicosadienoic acid	C20:2	0.58	0.45	0.57	0.64	0.61
Dihomo-gamma-	C20:3n6	0.61	0.62	0.81	0.61	0.56
Eicosatrienic acid	C20:3n3	1.48	0.11	0.37	1.62	3.84
Arachidonic acid	C20:4n6	2.85	1.70	3.84	3.50	4.40
Eicosapentaenoic	C20:5n3	0.53	<0.10	0.36	0.60	1.15
Behenic acid	C22:0	0.21	<0.10	<0.10	<0.10	<0.10
Docosapentaenoic	C22:5n3	1.65	0.39	1.63	3.10	3.60
Lignoceric acid	C24:0	0.30	<0.10	0.24	0.32	0.52
Docosahexaenoic	C22:6n3	3.91	1.55	3.77	5.33	10.30
∑ Omega 3		11.16	2.52	6.93	14.35	25.13
∑ Omega 6		10.67	9.02	11.86	8.44	11.61
∑ Omega 9		26.50	36.71	25.85	17.71	6.52

4.5.7 Water quality

There were no significant differences ($P<0.05$, ANOVA) on the levels of ammonia, nitrate and nitrite in the water from the tanks where the experimental diets were given. Significant differences ($P<0.05$, ANOVA) were observed on the pH, bicarbonate alkalinity, total hardness and orthophosphate levels in the water. However, post-hoc tests (Tukey's) showed that there were no significant differences in the bicarbonate alkalinity of the water samples from tanks where kikuyu grass and vallisneria were fed (Table 4.6).

No significant differences ($P<0.05$, ANOVA) were observed in the total hardness in water samples collected from tanks where cabbage and kikuyu grass were fed. The orthophosphate levels showed significant differences, however post hoc test (Tukey's) showed no significant difference in the orthophosphate levels in tanks where cabbage, kikuyu grass, and vallisneria were fed. Orthophosphate levels did not differ significantly between water samples from tanks where duckweed and fishmeal pellets were fed (Table 4.6).

Table 4.6: Water quality parameters from the tanks where fish were fed the different test diets

	Cabbage	Duckweed	Kikuyu	Pellets	Vallisneria
pH	6.30^{a}	6.40^{b}	6.70^{c}	5.90^{d}	6.39^{e}
Bicarbonate alkalinity ($CaCO_3$, mg/l)	21.90^{a}	18.20^{b}	41.40^{c}	13.80^{d}	60.50^{c}
Total hardness ($CaCO_3$, mg/l)	32.26^{a}	51.59^{b}	33.87^{a}	59.16^{c}	66.82^{d}
Ammonia (NH_3-N, mg/l)	0.27^{a}	0.38^{a}	0.07^{a}	0.315^{a}	0.05^{a}
Nitrate (NO_3-N, mg/l)	7.65^{a}	13.30^{a}	4.20^{a}	13.60^{a}	8.30^{a}
Nitrite (NO_2-N, mg/l)	0.27^{a}	0.22^{a}	0.08^{a}	0.10^{a}	0.05^{a}
Orthophosphate (PO_4-P, mg/l)	1.70^{a}	4.53^{b}	1.60^{a}	5.47^{b}	1.86^{a}

NB: Figures in the same row with different superscripts are significantly different (ANOVA; $P<0.05$).

4.6 DISCUSSION

Growth performance indices

There are several dietary factors that affect the growth performance of fish; these include the protein, energy, lipid and fibre content as well as the presence of anti-nutritional factors. The growth performance indices (SGR, FCR, PER, yield and production), were all high in *T. rendalli* fed fishmeal pellets. Among the plant diets kikuyu grass gave the best growth performance, followed by duckweed and cabbage respectively. Fish given vallisneria lost weight and hence negative growth performance was observed in all the growth indices measured.

Effect of protein content

Protein is the major dietary nutrient affecting growth performance of fish (Lovell, 1989). It provides the essential and non-essential amino acids necessary for muscle formation and enzymatic function and in part provides energy for maintenance (Yang *et al.,* 2002). The protein requirement for adult tilapias ranges from 20 to 30% dietary protein for optimum performance (El-Sayed *et al.*, 2003). Diets with high protein content produce higher growth rates than those with lower protein content (El-Sayed, 2006; Bahnasawy, 2009; Musuka, *et al*., 2009).

The higher protein content and higher levels of the essential amino acids in fishmeal pellets may be the main reason for the high specific growth rates obtained in fish fed the pellets. Duckweed had the second highest level of protein after fishmeal pellets. It would be expected that fish fed on duckweed would have the second best specific growth rates after fishmeal pellets. However, duckweed failed to produce better specific growth rates when compared to kikuyu grass in spite of its higher protein content. Siddiqui *et al*. (1988) observed that fish do not have a specific requirement for crude protein; they rather need the right combination of essential amino acids. Nutritional values of proteins and protein sources vary as a function of amino acids profile and digestibility. Even though some feed ingredients have high crude protein (nitrogen) contents, a large proportion of this crude protein could be made of non-protein nitrogen (Altan and Korkut, 2011). These ingredients will thus not contribute enough amino acids to meet the nutritional requirements of fish. The results of this study support this theory, implying that the amino acid profile of the dietary protein is more important than the total protein value. Although duckweed had higher protein content than kikuyu grass, the grass had superior levels of the essential and limiting amino acids such as methionine and lysine. The nutritional value of proteins is based on the amino acid composition; especially the indispensible amino acids and the biological availability of the amino acids from the protein (Stickeny *et al*., 1983).

In the case of methionine, fish actually have a requirement for total sulphur containing amino acids, which can be met by methionine alone or a proper mixture of methionine and cysteine (Stickeny *et al*., 1983). The presence of dispensable amino acids in the diet is important. Although the fish can synthesise these amino acids, their presence represent a sparing effect by lowering the need for fish to synthesise them, for example, the conversion of methionine to cysteine and of phenlylalanine to tyrosine. The presence of these dispensable amino acids have been found to lower the dietary requirements of the indispensable amino acids, for example, cysteine was found to replace or spare up to 60% of dietary methionine and tyrosine cane spare up to 50% of total phenylalanine (Harding, *et al*., 1977). In the current study, cysteine was highest in duckweed, vallisneria, pellets respectively. Kikuyu grass and cabbage had equal amounts of cysteine. However, the sparing effect of cysteine on methionine was not immediately evident probably because methionine was limiting across all diets.

Kikuyu grass had the highest levels of essential amino acids in relation to the other diets. This may be the reason for the higher specific growth rates obtained from fish fed this grass. Kikuyu grass also had the second highest protein efficiency ratio after fishmeal pellets. This further confirms the importance of the amino acid composition as opposed to the overall protein in the diet.

Fishmeal pellets gave the lowest feed conversion ratio, followed by kikuyu, duckweed and cabbage respectively. The feed conversion ratio of fish fed vallisneria was also negative. The results of the FCR are in agreement with the growth rates obtained when the test diets were fed. The low FCR values in fishmeal pellets and kikuyu can also be explained by the high protein and amino acid content of these diets.

Duckweed has been widely used in tilapia diets as a complete diet on its own. The popularity of duckweed is due to its relatively high protein content (26.3-45.5%) and favourable amino acid balance, with only tryptophan and methionine generally limiting (Mbagwu and Adeniji 1988). Several authors have demonstrated that duckweed can serve as an alternative dietary source for tilapia (El-Sayed, 1999; Fasakin *et al*., 1999).

The growth performance of fish fed exclusively on duckweed in intensive systems has yielded conflicting results. Gaigher *et al*. (1984) reported SGR of 0.67%/ day when hybrid tilapia was offered duckweed in tank culture. Similar results were reported by Cassani *et al*. (1982) and Hassan and Edwards (1992) who obtained SGR of 0.67 and 0.97%/day respectively. Although the SGR of 0.16%/day obtained in *T. rendalli* fed duckweed in this study is much lower than that reported in the preceding studies, it is consistent with that reported by El-Shafai *et al*. (2004) which ranged between 0.14-0.71%/day on *O. niloticus* fed duckweed. These discrepancies in the specific growth rates obtained from these studies may be a function of the protein content of the duckweed used, as this is affected by the nutritional status of the water where it is grown as well as genetic differences in the species used.

The specific growth rates (on wet weight basis) obtained in *T. rendalli* feeding on the plant diets in this study ranged from 0.13%/day in fish fed cabbage to 0.17%/day when kikuyu grass was fed. These results show that *T. rendalli* can effectively utilise plant based protein. The negative values obtained for SGR, FCR and PER in *T.* r*endalli* fed vallisneria may be due to its low nutritive value. Vallisneria is an aquatic macrophyte that has higher moisture content than the other plants. A large part of the feed weight was water. Furthermore, this macrophyte has low protein and energy content.

Effect of energy level

Fish are known to feed to satisfy their energy requirements, and if the diet does not contain sufficient energy levels, protein is used for energy rather than for growth (Cowey and Sargent, 1979). It is important to minimize the amount of protein used for energy, because protein is the most expensive nutrient in fish diets. Dietary energy is the second most important factor affecting the utilization of feeds by fish.

An inadequate dietary protein to energy ratio may result in lower growth as well as low protein and energy utilization in fish (Shiau and Lan, 1996; Samantaray and Mohanty, 1997; Ali *et al*., 2008). In diets with low protein to energy ratio, the use of dietary protein for growth and maintenance of body protein is maximized, while in diets with high protein to energy ratio, more protein is used for energy or stored as fat (Ali *et al*., 2008). According to (Van der Meer *et al*., 1997), the protein sparing effect of energy occurs only if the minimum protein requirements are met, including adequate amounts of amino acids.

Kikuyu grass had the highest energy levels (17.99%), followed by fishmeal pellets (17%), duckweed (16.43%), then cabbage (16.09%) and vallisneria had the lowest levels (14.74%). The high energy levels coupled with a good amino acid balance in kikuyu grass may have resulted in more protein spared for growth than in the other plant diets. The high protein and high energy levels in fishmeal pellets may result in excess protein stored as fat. Stickney *et al*. (1983) stated that the energy requirements for maintenance and voluntary activity must be satisfied before energy is available for growth. This may imply that the energy requirements were not met in fish fed vallisneria, resulting in the negative growth observed. The low dietary energy values may also explain the lower SGR, PER and higher FCR in *T. rendalli* fed duckweed and even poorer and negative results obtained for cabbage and vallisneria respectively.

The growth performance indices in this study confirm that diets with high energy levels produce higher SGR, PER, yield, production and lower FCR. This is in agreement with studies conducted by Soltan *et al*. (2010) who found that increasing dietary energy results in increase in all growth parameters in red tilapia. Similarly, Ali *et al*. (2008) reported poor FCR values in *O. niloticus* fed diets with low energy and protein content. These authors concluded that increasing dietary energy results in increased growth performance and reducing energy at the same protein level significantly reduced growth performance. In aquaculture, diets with sufficient energy levels are desired because fish eat to satisfy their energy demand and , for any aquaculture venture to be viable, the energy demand for the fish should be met by none protein sources.

Effect of the lipid content

The fat content of the experimental diets also plays an important role in the utilisation of fish diets. Dietary lipids may spare more protein for growth than carbohydrates (Teshima *et al*., 1985). It has been reported that tilapia can utilize carbohydrates and lipids as energy sources (El-Sayed and Garling, 1988).To maximise the protein utilisation, Jauncey (2000) suggested that the dietary fat should be 6-8% for fish more than 25 g. All diets used in this study had <5% fat. Kikuyu grass had the highest fat content (4.6%), followed by duckweed (4.3%), fishmeal pellets (2.9%), vallisneria (1.8%), while cabbage had the lowest (1.4%) fat content. The high fat content in kikuyu grass may have resulted in the better growth performance observed. Dietary lipids provide a major source of energy and facilitate the absorption of fat soluble vitamins (Jauncey, 2000). The lipid requirement for tilapia depends on lipid source, dietary protein, energy contents and tilapia size (El-Sayed, 2006). Studies on *T. zillii* (Teshima *et al*., 1978; El-Sayed and Garling, 1988) indicated that increasing diet oil up to 15% resulted in significant improvement in the protein efficiency ratio and protein production value. Similar results were reported by Teshima *et al*. (1985) on *O. niloticus.* Chou and Shiau (1996) fed tilapia hybrids diets with varying fat content and found that increasing the level of dietary lipid from 0-5% resulted in improved weight gain. However, an increase from 5-15% resulted in increased body lipid (which is not desirable) and did not result in improved weight gain in the fish fed the 5-15% lipid diets. Similar weight gains of the fish in these dietary groups were obtained suggesting that tilapia do not utilize the additional energy provided by the supplementary lipid (over 5% dietary lipid level) for growth. Similarly, Hanley (1991) also indicated that tilapia were able to store significant quantities of lipid in their carcass and viscera, but were not able to utilize this energy source to improve growth. The better growth performance of fish fed kikuyu grass in relation to the other plant diets may be a result of the protein sparing effect of fat in the grass.

Effect of the fibre content

Another important factor known to affect the utilisation of plant diets is the fibre content in the diet. A review of literature shows that fibre has never been satisfactory defined. It is known to include carbohydrates of varying degrees of complexity, which cannot be hydrolysed by higher vertebrate enzymes. These include mixtures of cellulose, hemicellulose, lignin, pentosans and other generally indigestible fractions of the feed. Lignin, although is classified among fibres, is a polyphenol, not a polysaccharide. A major reason why the feeding efficiency of herbivorous fish is usually low is that fish do not produce cellulase (Buddington, 1980; Hickling, 1996). However, fibrous compounds (except lignin) are subject to partial digestion by hydrochloric acid in the stomach especially in species with herbivorous tendencies such as *T. rendalli*. This was confirmed in this study, because kikuyu grass had the highest fibre content (18.58%), yet it was effectively utilised by *T. rendalli*. Vallisneria had a fibre content of 14.72%, followed by cabbage (11.71%), and duckweed had the lowest fibre content among the plant diets (10.37%).

Diets with high levels of fibre are not desirable in practical fish feeds as they hinder nutrient digestibility, resulting in poor utilisation of the feed and low growth rates (Anderson *et al*., 1984). However, studies have shown that some amount of fibre is required for optimal utilisation of the diet and overall growth. In experiments where the level of fibre in the diets was increased, the growth, protein efficiency ratio and survival were increased to a point and then declined (Dioundick and Stom, 1990). High levels of fibre in the diet also affect apparent digestibility and gastrointestinal transit rate in tilapias (Ribeiro *et al*., 2011). Plant food is less digestible than animal food because of the presence of cellulose which is difficult to break down. It was expected that kikuyu grass, having high fibre content would not be efficiently utilised by the fish. The results of this study however show better specific growth rates, PER and FCR in *T. rendalli* fed kikuyu grass when compared to the other plant diets. This suggests that *T. rendalli* is able to breakdown the fibre/cell wall and expose the cell contents and allow for assimilation of proteins by the fish.

Structural adaptations of *T. rendalli* play a major role in the utilisation of plant diets. The bi- and tricuspid teeth on its jaws cut and macerate the plant matter (De Silva and Anderson, 1995). Another important feature is the presence of pharyngeal teeth which aid in triturating the plant tissues and in breaking down the fibre making it more accessible to enzymes to act on it later in the gut. Coupled with these adaptations, is the ability of *T. rendalli* to produce hydrochloric acid in the stomach, resulting in pH levels <1 (Dempster *et al*., 1995). The low pH helps in the lyses of plant cell walls, and in facilitating the release of amino acids (Bowen, 1980). In addition, the long intestines provide plenty surface area for digestion and absorption. In this study, the amount of fibre in the diets appears not to hinder the growth performance in *T. rendalli*.

Effect of antinutritional factors

The presence of antinutritional factors in plant diets may also hinder their utilisation by fish. Most plant derived nutrient sources are known to contain a variety of antinutritional substances. These have been identified as substances which by themselves or through their metabolic products interfere with food utilisation and affect the health and production of fish. Antinutritional factors (such as protease inhibitors, tannins and lectins) affect protein utilisation and digestion, or mineral utilisation (phytates, gossypol pigments, oxalates, and glucosinolates). Other antinutritional factors include antivitamins, alkaloids, nitrate, saponins and photosensitizing agents (Francis *et al*., 2001).

It has been shown that kikuyu grass contains some indigestible material such as lignin, neutral detergent fibre, cellulose and hemicelluloce (Marais, 2001). The grass has also been associated with some phenolic compounds including *m*-coumaric acid, *p*-coumaric acid, vanillic acid, gallic acid and flavonoids (Chou *et al*., 1987). Anti-nutritional factors in duckweed include trypsin inhibitors, tannins, nitrite and nitrate (Ismail, 1998) cyanide, phytin (Fasakin *et al*., 1999). The inferior growth performance observed in fish fed the plant diets when compared to fish fed fishmeal pellets may also be attributed to the presence of antinutritional factors in the plant diets. Although the presence of antinutritional factors was not tested for in the current study, their possible presence and effect on utilisation cannot be ignored.

In conclusion, the amino acid composition of the diet may be regarded as the overriding factor influencing the growth performance of *T. rendalli*. Fishmeal pellets had the highest level of the essential amino acids, followed by kikuyu, duckweed, cabbage and vallisneria. This was confirmed by all the growth performance indices measured. The best growth performance was observed in *T. rendalli* fed fishmeal pellets, kikuyu grass, duckweed, cabbage and vallisneria respectively. *T. rendalli* can effectively utilise protein rich plant diets in spite of high fibre content and the possible presence of antinutritional factors.

Food consumption versus water temperature

The inability of *Tilapia rendalli* to feed at low temperatures is a serious constraint for commercial culture of this species in South Africa. Food consumption declined markedly when water temperature fell below 16°C in winter. For most tilapias, feeding ceases at temperatures below 15°C. It is important to note however, that fish fed the plant diets continued feeding throughout the winter period although at a lower rate. In chapter 3, it was also observed that *T. rendalli* at flag Boshielo Dam did not feed in winter. The reduced in feeding observed at Flag Boshielo Dam may have been due to the unavailability of marginal vegetation because of the drawdown in winter. In this experiment however, *T. rendalli* did not completely stop feeding on the plant diets even when the water temperature was below 16°C. Based on the regression of feed consumption and water temperature, *T. rendalli* stops feeding on fishmeal pellets at 13.58°C, but continues feeding on the plant diets at lower temperatures up to 5.79°C for duckweed. The ability of fish to continue feeding on the plant diets at low temperatures is explained by Fagbenro (1999) who hypothesised that the high lysine content in duckweed meal supported fish growth and utilisation at lower temperature in *Clarias gariepinus*. In the current study however, Fabenro (1999)'s hypothesis is not supported as fishmeal pellets had the highest lysine content than all the plant diets and yet feeding stops at a higher temperature.

It is important to note that these experiments were conducted in a green house where the temperature was regulated and the low winter temperature higher than that in earthen ponds. This implies that in practical pond culture the observed ability to continue feeding in winter may be reduced and the growth rate also reduced.

Water quality in static rearing systems

Orthophosphate levels in this study were high in static systems where fishmeal pellets were fed (5.47 PO_4^-P, mg/l), followed by duckweed (4.53 PO_4^-P, mg/l), vallisneria (1.86 PO_4^-P, mg/l) then cabbage (1.7 PO_4^-P, mg/l). Orthophosphate levels were lowest (1.6 PO_4^-P, mg/l) in tanks where kikuyu grass was fed. The target water range for orthophosphate in aquaculture is 0.1 PO_4^-P, mg/l (DWAF, 1996). Phosphate is an important plant nutrient, stimulating the growth of both algae and aquatic macrophytes. , high levels of phosphates in pond systems result in excessive growth of plants and algae leading to eutrophication and increased biological oxygen demand.

The target water quality range for ammonia ($NH_{3\text{-}}N$ mg/l) in aquaculture is 0-0.03 mg/l and the sub lethal range for tilapias is 0.3-0.8 mg/ (DWAF, 1996). When the recirculation system was stopped to simulate a pond system, tanks where vallisneria and kikuyu grass were fed had the lowest levels of ammonia 0.05 and 0.07 mg/l respectively. The highest ammonia levels were recorded in tanks where duckweed and fishmeal was fed. High ammonia levels result in the depletion of dissolved oxygen in fish ponds resulting in reduced feed intake and poor growth of fish. Although tilapias tolerate high levels of ammonia up to 2.4 mg/l, caution must be taken when feeding fishmeal pellets and duckweed in pond systems.

The target water range for nitrite in aquaculture is 0-0.05 NO_2^-N, mg/l (DWAF, 1996). In the current study, the highest nitrite levels were recorded in tanks where cabbage (0.27 mg/l), duckweed (0.22 mg/l) and fishmeal pellets (0.1 mg/l) were fed respectively. The lowest levels were recorded in tanks where vallisneria and kikuyu grass were fed. High nitrite levels in aquaculture systems are usually a result of inefficient nitrification in systems with high nitrogen loading rates, in the form of feed protein, and high stocking rates. Toxic levels result in lower productivity, activity, growth and poor health (DWAF, 1996).

Nitrate (NO_3^-N) levels were highest (13.6 mg/l) in tanks where fishmeal pellets were fed, followed by tanks where duckweed, vallisneria, cabbage and kikuyu were fed respectively. Nitrate is the most stable positive oxidation state of nitrogen. Nitrate is required by aquatic plants and high concentrations may results in eutrophication. The levels recorded in this study were within the acceptable limits (<300 NO_3^-N mg/l; DWAF, 1996) for all diets.

The high levels of orthophosphate, ammonia, nitrite and nitrate recorded in duckweed and fishmeal tanks may be a result of the high protein levels in these diets. This is in agreement with the findings of Cho (1990) who inferred that high levels of crude protein as a result of non-protein nitrogen in fish diets will increase the production of ammonia and the rate of nitrogen excretion by the fish, lowering productivity and water quality of production systems.

Bicarbonate alkalinity in tanks with kikuyu grass, vallisneria and cabbage was within the target water quality range (20-100 $CaCO_3$ mg/l) for aquaculture. However, in tanks where fishmeal pellets and duckweed was fed, the levels were below those recommended for optimal production. Alkalinity levels <20 mg/l are associated with unstable water chemistry because of poor buffering capacity (DWAF, 1996).

It can be concluded that kikuyu grass, vallisneria and cabbage can be fed *ad libitum* in static water systems without affecting the water quality in the production of *T. rendalli*. The feeding of duckweed and fishmeal pellets however should be accompanied by close monitoring of the water quality parameters especially ammonia, nitrite and phosphorous.

Carcass composition

The effect of the experimental diets on the carcass composition was determined. Jobling (1998) stated that it is necessary to have information on body composition changes induced by any experimental treatment. This information is mandatory in studies where the aim is to examine the effects of different diets on fish intended for human consumption. In the present study, fish fed the plant diets had higher levels of protein in the muscle than fish fed the commercial pellets. Fish fed kikuyu grass had the highest (71%) protein in the muscle followed by those fed duckweed (68%), then cabbage (64%) and fishmeal gave the lowest (62%). Kikuyu grass improved the protein content in *T. rendalli* when compared to the protein content from the initial fish sample. The ash content was used as a measure of the overall mineral content. Fish fed fishmeal pellets had the lowest ash content in the carcass while kikuyu grass fed fish had the highest levels followed by fish fed duckweed and cabbage respectively.

Calcium and phosphorous are considered the two most essential and major minerals in fish. Fishmeal fed fish had lower levels of calcium and phosphorus when compared to fish fed the plant diets. Among the plant diets, kikuyu grass produced high levels of the minerals. Plants are primary producers and are able to absorb most nutrients from the soil. Fish fed on plant diets are better sources of these minerals compared to fishmeal fed fish.

The fat content of the fish fed the different experimental diets varied with diet. Fish fed fishmeal pellets had the highest fat content in the fillet, more than twice the amount in the fillet of fish before they were subjected to the experimental diets (initial sample). Fish fed on kikuyu grass had the lowest fat content, followed by those fed on duckweed. Fish fed cabbage had the highest fat content among fish fed plant diets. Protein and fat are the major nutrients in fish and their levels help define the nutritional status of the organism (Aberoumad and Pourshafi, 2010). The fillet of fish fed kikuyu had the lowest levels of total fat in the fillet, yet it had the highest levels of omega 3- fatty acids. On the contrary, fish fed on the commercial fishmeal pellets had the highest levels of total fat and lowest levels of omega 3 – fatty acids.

The findings of this study are in agreement with those of Perschbacher *et al*. (2010) who found that *Spirulina* fed *O. niloticus* contained significantly greater linolenic, docosapentaenoic, eicosatrienoic, and eicosatetraenoic (arachidonic) fatty acids than fish fed commercial fishmeal pellets. This author concluded that *Spirulina* can be effectively utilised by tilapias and this would result in reduced feed costs and a better product.

Human consumption of fish and fish products has increased in recent years mostly because of the high ratio of omega 3:omega 6 fatty acids which are associated with beneficial effects in the prevention of coronary heart disease. Nutritionally important omega 3 fatty acids include α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) all of which are polyunsaturated. Several studies indicate that a high ratio of omega 3:omega 6 fatty acids can reduce sudden death from ventricular fibrillation and tachycardia. These fatty acids also reduce tendency to thrombosis and thus help prevent myocardial infarction. EPA and DHA also have several actions that inhibit the development of atherosclerosis (Connor, 2001). While these fatty acids do not lower plasma cholesterol levels, they do have a substantial triglyceride – lowering effect and also raise levels of the high-density lipoprotein (HDL) a ‘good cholesterol’ (Connor, 2001). From this study it is fitting to conclude that the consumption of *T. rendalli* fed on plant diets especially kikuyu grass will have a beneficial effect in the prevention of coronary heart disease.

The human body cannot synthesize omega 3 fatty acids but has limited ability to form the long chain EPA and DHA from the short chain ALA. Studies by Usydus *et al*. (2011) have shown that consumption of low concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has no significant effect in the prevention of coronary heart disease. For effective disease control, consumers with symptoms of cardiovascular diseases should include fish with high concentrations of EPA and DHA in their diets. This may imply that *T. rendalli* fed on fishmeal pellets may not have substantial beneficial effect on the prevention of coronary heart disease. Fish fed on kikuyu grass had the highest levels of ALA, EPA and DHA, consumption of this fish may significantly prevent coronary artery disease and sudden death. *T. rendalli* fed on kikuyu grass may have immense public health significance for the control of coronary heart disease.

In this experiment kikuyu grass gave the best growth performance in *T. rendalli* compared to duckweed, cabbage and vallisneria. The good amino acid profile of kikuyu grass is cited as the main reason for the observed good performance. It is however important to look at other factors that may have influenced the growth performances reported in this chapter. It became important to determine if the digestibility of the different plant diets had any bearing on the observed growth performance. Furthermore, the gastric evacuation rates for these diets had to be investigated because the amount of time a particular feed item spends in the gastrointestinal tract will affect the amount of nutrients absorbed and consequently the growth realised. Preference or selection for a particular diet is another important factor that may affect utilisation and growth from a particular diet. It was important to determine if the plant diet that gave the best growth performance was also preferred by *T. rendalli*. These factors and their effect on the growth performance observed will be explored in the next chapter.

CHAPTER 5: AN INVESTIGATION INTO THE FACTORS AFFECTING FEEDING SELECTIVITY, GASTRIC EVACUATION AND PLANT DIGESTIBILITY IN *TILAPIA RENDALLI*

5.1 INTRODUCTION

Digestibility, gastric transit time and evacuation rate of *T. rendalli* grazing on the plant diets were evaluated in this chapter. These parameters were measured to determine if they have any effect on the growth performance observed in the previous chapter when the fresh plant diets were offered. There is paucity of information on the interactions between gastric evacuation rate and plant nutrients in *T. rendalli*. An understanding of these interactions is important in the development of a feeding strategy for *T. rendalli* when it is cultured in extensive low technology culture systems. The energy value of the plant diet, the rate at which it is consumed, the digestive efficiency of the fish for the plant and nutrient content (in particular protein) are factors generally considered when assessing food value (Horn, 1983) and in turn determine growth.

Herbivorous fish have been reported to feed selectively on available macrophytes, showing preference for some plants over others (Chifamba, 1990). Several factors have been linked to plant selection by fish and these include nutrient composition, accessibility, and ease of mastication, temperature and the coarseness of the plant (Hasan and Chakrabarti, 2009). In addition, secondary metabolites (Wylie and Paul, 1988; Paul and Hay, 1986) and relative availability (Horn and Neighbours, 1984) have also been shown to affect food selection by herbivorous fish.

Most plant preference or consumption studies are based on the fish’s ability to control aquatic weeds and rarely on utilisation and fish growth. In chapter 4, it was demonstrated that plant nutrient composition (especially the level of the essential amino acids) influenced the growth performance in *T. rendalli*. However, the preference of the different plant diets was not taken into account.

5.2 OBJECTIVES

i) To determine the digestibility of duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

ii) To determine the gastric transit time of commercial fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

iii) To determine the gastric evacuation rate of fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

iv) To determine the preference for duckweed, kikuyu, cabbage and vallisneria by *Tilapia rendalli.*

5.3 NULL HYPOTHESIS

i) There is no significant difference in the digestibility of duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

ii) There is no significant difference in the gastric transit time of commercial fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

iii) There is no significant difference in the gastric evacuation rate of fishmeal pellets, duckweed, kikuyu, cabbage and vallisneria in *Tilapia rendalli.*

iv) There is no significant difference in the preference for duckweed, kikuyu, cabbage and vallisneria by *Tilapia rendalli.*

5.4 MATERIALS AND METHODS

5.4.1 Digestibility of the experimental plant diets by *Tilapia rendalli*

From the growth experiments in chapter 4, it was evident that the different diets produced different growth performances in *T. rendalli*. The ability of the fish to digest or breakdown nutrients in the plant diets is likely to affect the utilisation of the particular diet. An experiment on the digestibility of the plant diets was carried out to determine if the digestive efficiency is related to dietary composition and if this may contribute to the observed differences in utilisation of the experimental diets. Furthermore, the digestibility studies were necessary to determine if the ability to digest and assimilate certain plants will affect choice.

Digestibility units used for these experiments were designed according to Cho *et al*. (1982) slight modifications were made to the original design (Figure 5.1). Each pair of 50 L fibre glass tanks was connected to a water reservoir and a pump was fitted in the reservoir to pump water into the tanks from the reservoir. Air was introduced into each tank and diffused through air stones and the water temperature was maintained at 28°C using submersible thermostatic glass heaters. Sub-adult *T. rendalli* with a mean weight of 40±5 g were stocked into the fibre glass tanks at six fish per tank. There were 8 tanks used, the four plant diets were randomly allocated to each pair of tanks. After stocking, the fish were allowed to acclimatize in experimental tanks for two weeks.

Figure 5.1: Digestibility tanks

The fish were fed *ad libitum* for these experiments, feeding was done each morning while the valves at the bottom of the tanks were closed and only opened at the end of the feeding period. Faeces were collected from the bottom of the collection tube into plastic bottles and frozen until enough samples were collected and then taken to the laboratory for analysis.

Cellulose is assumed to be indigestible, and its quality should be constant as it passes through the digestive tract. Cellulose was used as a marker and a reference to which all measurements were compared. Total and nutrient digestibility of the different plant diets was calculated according to the method used by Buddington (1979) to determine the digestibility of fresh plants:

Total digestibility (%) = 100 – 100 (% plant cellulose / % faecal cellulose)
Nutrient digestibility (%) = 100 – 100 (% plant cellulose / % faecal cellulose) x
(faecal nutrient / plant nutrient)

5.4.2 Gastric transit time

Gastric transit and evacuation studies are an important component in feed management in aquaculture. These can be used in modelling feed intake and estimation of the return of appetite. Furthermore, these affect the amount of feed consumed and consequently the growth rates obtained from feeding the particular diet. It became necessary to determine the gastric transit and evacuation rate of the test diets in order to evaluate if these may have had an effect on the growth performance or the digestibility of a particular plant.

The gastric transit time of the different experimental diets (fishmeal pellets, kikuyu grass, vallisneria, cabbage and duckweed) was determined in *T. rendalli*. Fifteen fish (30±5 g) were stocked in five 20 L glass tanks. The fish were individually weighed; three fish per tank were stocked and acclimatised for one week. During the acclimatisation period fish were fed fishmeal pellets at 5% of their body weight once daily. The time for appearance of first faeces was recorded. Water temperature was maintained at 28°C using thermostatic emission heaters.

5.4.3 Gastric evacuation rate

The gastric evacuation rate of the different experimental diets (fishmeal pellets, kikuyu grass, vallisneria, cabbage and duckweed) was determined in *T. rendalli*. Fish (50±5 g) were stocked in 1 m^3 fibre glass tanks at 40 fish per tank. This was duplicated for each diet. Fish were allowed to acclimatise in the experimental environment for 1 week. During this period, fish were offered the experimental diets *ad libitum*.

Fish were fasted for 12 hours prior to the start of the experiment to ensure that the stomach is empty. The method used was the serial slaughter technique (Windell, 1971). This method involves sacrificing fish at regular time intervals after feeding and measuring the amount of feed remaining in the stomach.

Two fish were sampled from each tank at the beginning of the serial slaughter trial to ensure that the stomach was empty. All remaining fish were fed their allocated diet to apparent satiation for 1 hour. After the 1 hour feeding period, all uneaten feed was removed from all tanks. Two fish were sampled from each tank at 4 hour intervals for analysis. Fish were killed by placing on ice and then dissected to remove the stomach.

The stomach content (all material between the posterior end of the oesophagus and the anterior end of the small intestine) was removed and placed in pre-weighed oven-proof containers, the stomach content in wet mass was recorded, then the content was dried to constant weight at 90°C for 24 hours and dry mass measured.

The gastric evacuation rate for the different plant diets was expressed by the exponential equation:

$S_t = S_o \, . \, e^{-bt}$

where: S_t = weight of stomach content

S_o = weight of meal eaten

t = time in hours

b = constant

5.4.4 Establishing the feeding preferences for different plant diets

In chapter 3 it was established that *T. rendalli* feeds on marginal vegetation. The rationale for carrying out preference experiments was to determine if *T. rendalli* selects any particular plant. This information is important in the development of a feed management strategy if *T. rendalli* is to be cultured in ponds.

Preference for the plant diets by *T. rendalli* was tested under laboratory conditions independent of the growth experiments. *Tilapia rendalli* of a mean weight 35±5 g were randomly stocked into twelve fibre glass tanks (1 m^3), in a recirculating system (three tanks for each plant diet). Ten fish were stocked in each tank and acclimatised for a period of one week, during which they were fed fish meal pellets at 5% body weight. Tanks were supplied with air diffused through air stones.

All fish were starved for 48 hours, to allow for the clearing of the digestive tract. After the starvation period, fish were offered equal amounts of all the test diets. All plants (with the exception of duckweed) were chopped into 1 cm pieces. The pieces were counted, and offered at the start of the feeding period. Equal proportions (100 pieces) of cabbage, duckweed, vallisneria and kikuyu were introduced into the three tanks. The amount of uneaten pieces was counted every 20 minutes. The feeding trial lasted for 3 hours. Manly's alpha (Manly, 1974) was used to measure food preference by *T. rendalli* under laboratory conditions. The formula for estimating Manly's alpha when prey numbers are declining is:

$$\alpha_i = \frac{\log \mathrm{P_i}}{\sum \log P_j}$$

where: α_i = Manly's alpha

$\mathrm{P_i}$ = proportion of feed item *i* remaining

$\mathrm{P_j}$ = total number of feed items

A Manly's alpha value of 0 is obtained when there is no selection or preference for the particular diet and a value of 1 indicates exclusive preference for that diet. A Manly's alpha value of >0.125 indicates preference (Fields *et al*., 2003).

5.4.5 Statistical analysis

To determine if the different plant diets had any significant ($P<0.05$) effect on gastric transit time, one way analysis of variance (ANOVA) on SPSS 17.0 was used. Means that were significantly different ($P< 0.05$) were separated by Tukey’s post-hoc test. Analysis of covariance (ANCOVA) was used to determine if there were any significant ($P<0.05$) differences in the gastric evacuation rate of *T. rendalli* fed the experimental diets. Manly’s alpha was used to determine if there was preference for any plant diet by *T. rendalli* under laboratory conditions.

5.5 RESULTS

5.5.1 Digestion of plant diets by *Tilapia rendalli*

Cellulose is presumed to be indigestible in fish. Its concentration in the faeces was expected to be higher than in the fresh plants. In this study however, the amount of both cellulose and fibre in the faeces was lower than in the fresh plants fed (Table 5.1).

Table 5.1: Proximate composition (%) of the plant diets and faecal samples collected from the digestibility experiments

	Ash	*Protein	Fibre	Energy (MJ/kg)	NDF	ADF	**Cellulose
Kikuyu	1.42	3.27	2.73	2.29	6.77	2.93	3.84
Vallisneria	0.81	0.76	0.74	0.60	1.65	1.14	0.51
Cabbage	0.60	1.20	0.72	1.35	1.05	1.13	-0.08
Duckweed	0.64	1.09	0.54	0.65	1.35	0.80	0.55
Faeces kikuyu	0.25	0.40	1.71	0.72	3.09	1.73	1.36
Faeces vallisneria	0.32	0.37	0.91	0.43	1.57	1.46	0.11
Faeces cabbage	0.33	0.38	0.75	0.36	1.08	1.20	-0.12
Faeces duckweed	0.54	0.24	0.57	0.31	1.05	0.66	0.39

NB: Results expressed on dry matter bases

* For the conversion of nitrogen content to protein content, the factor 6.25 was used

** Cellulose was calculated as: neutral detergent fibre (NDF) – acid detergent fibre (ADF).

Total digestibility values were negative for all the diets except for cabbage because the cabbage cellulose values were negative in both the diet and the faecal samples (Table 5.2). The protein and energy digestibility values were higher for cabbage followed by those of duckweed and kikuyu respectively. However, protein and energy digestibility values remained negative for vallisneria (Table 5.2).

Table 5.2: Digestibility coefficients of the plant diets fed to *Tilapia rendalli*

Plant diet	Total Digestibility	Protein digestibility	Energy digestibility
Kikuyu	- 182.35	65.46	11.23
Duckweed	- 41.03	68.95	32.74
Cabbage	33.33	78.89	82.22
Vallisneria	-363.64	-125.72	-232.27

5.5.2 Gastric transit time and time for complete evacuation

Digestibility of the plant diets could not be accurately determined because of the ability of *T. rendalli* to breakdown cellulose (which was used as a marker), resulting in negative digestibility values. The times taken by the different test diets in the gastrointestinal tract of *T. rendalli* were used as a measure of the ability of the fish to breakdown and digest the diets. The gastric transit time of the experimental diets ingested by *T. rendalli* varied significantly (ANOVA, $P<0.05$). The diets that gave the best growth performance took longer periods in the gastrointestinal tract of *T. rendalli* than those that gave poorer performance. Fishmeal pellets were in the gastrointestinal tract for the longest period (3 hours and 51 minutes). Vallisneria was in the gastrointestinal tract for the shortest period, with first faeces appearing after 2 hours and 21 minutes. The gastric transit time of vallisneria was followed by that of cabbage, duckweed and kikuyu respectively. Post-hoc test showed that the gastric transit time was not significantly different ($P>0.05$; Tukey) between fish fed kikuyu grass and duckweed, but significantly different ($P<0.05$) for the other diets (Table 5.3).

Time for complete evacuation was shortest in fish fed vallisneria (34 hours), followed by those fed cabbage (36 hours; Table 5.3). Fishmeal pellets took the longest time (48 hours) to be completely evacuated from the gastro-intestinal tract. There was no statistical significance in the time for complete evacuation between fish fed vallisneria or cabbage (Tukey>0.05). Similarly, no significant differences were found in the time for complete evacuation between fish fed kikuyu grass or duckweed.

Table 5.3: Gastric transit time (GTT) and time for complete evacuation (±SE) in *Tilapia rendalli* fed the experimental diets

Fish diet	GTT (hrs)	Time for complete evacuation (hrs)
Pellets	3.51 ± 0.05^{a}	48 ± 0.12^{a}
Kikuyu	3.35 ± 0.15^{b}	43 ± 0.21^{b}
Duckweed	3.28 ± 0.07^{b}	40 ± 0.26^{b}
Cabbage	2.45 ± 0.09^{c}	36 ± 0.32^{c}
Vallisneria	2.21 ± 0.68^{d}	34 ± 0.15^{c}

NB: Figures in the same column with different superscripts are significantly different ($P<0.05$).

5.5.3 Effect of the experimental diets on gastric evacuation rate

The gastric evacuation rates of the different experimental diets in relation to the amount of feed remaining in the stomach of *T. rendalli* was determined. The gastric evacuation rates of different plant diets followed the same trend as the gastric transit time. The diets that were in the digestive tract for longer periods were also evacuated much slower. These results show an inverse relationship between dietary nutrient content and consumption rates in *T. rendalli* (Fig. 5.2). Fishmeal pellets had the highest protein content (34.5%) and better amino acid profile and was consumed in less amounts. Vallisneria and cabbage on the other hand had low protein contents and were consumed in large quantities. There were significant differences ($P > 0.05$; ANCOVA) in the gastric evacuation rates of different test diets in *T. rendalli*. Vallisneria was evacuated faster, followed by cabbage, duckweed, kikuyu grass and fish fed fishmeal pellets were evacuated more slowly. The depletion of stomach contents with time after feeding is shown in Fig. 5.2 below.

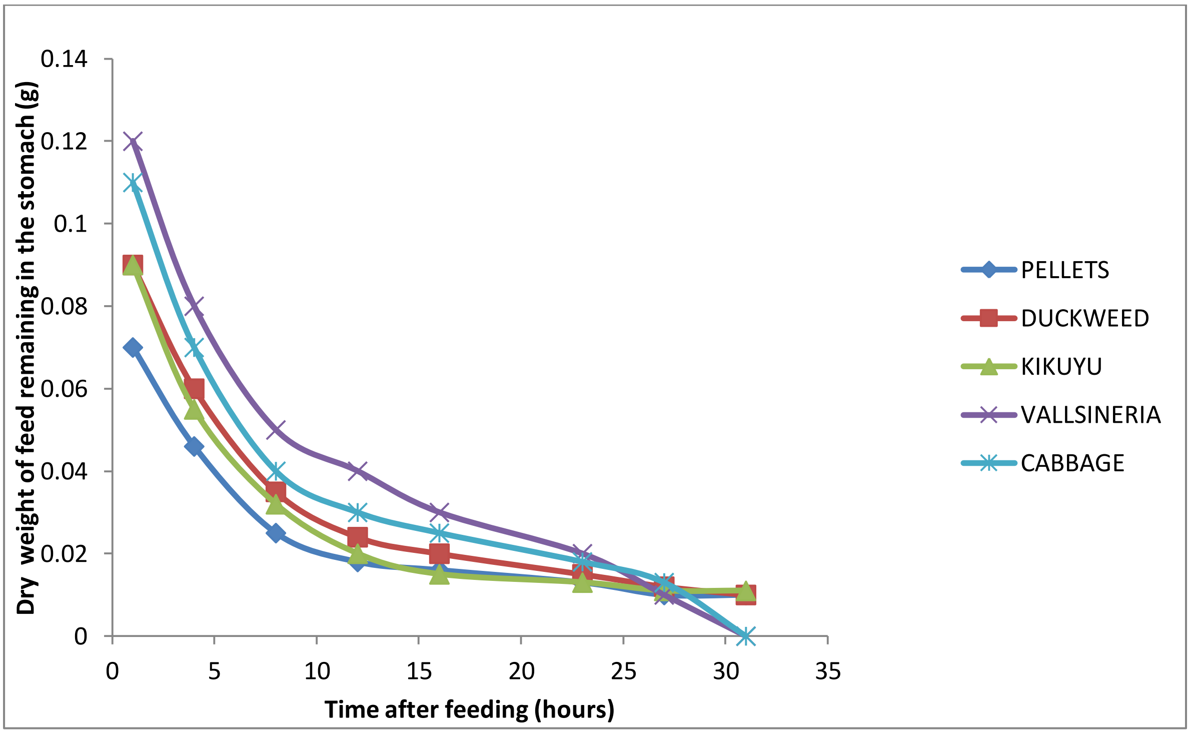


Figure 5.2: Gastric evacuation rate of *Tilapia rendalli* fed the experimental diets.

The depletion of stomach contents after feeding fitted an exponential model. The exponential curve (Fig. 5.3) represents gastric evacuation rate of pellets in *T. rendalli*. This curve is represented by the equation: $St = 0.07 * \exp^{-0.005t}$.

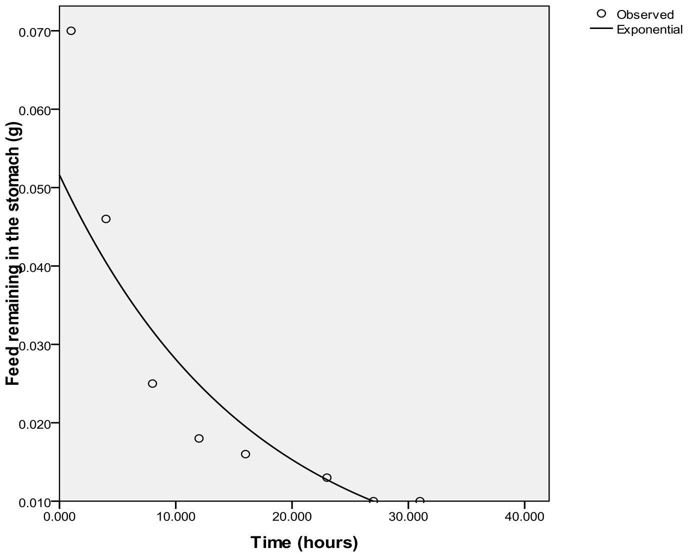


Figure 5.3: Exponential curve representing gastric evacuation rate of pellets in *Tilapia rendalli*

The equation St = 0.09 * exp $^{-0.063t}$ represents the gastric evacuation rate of kikuyu grass in *T. rendalli*. The exponential evacuation model is given in Fig. 5.4.

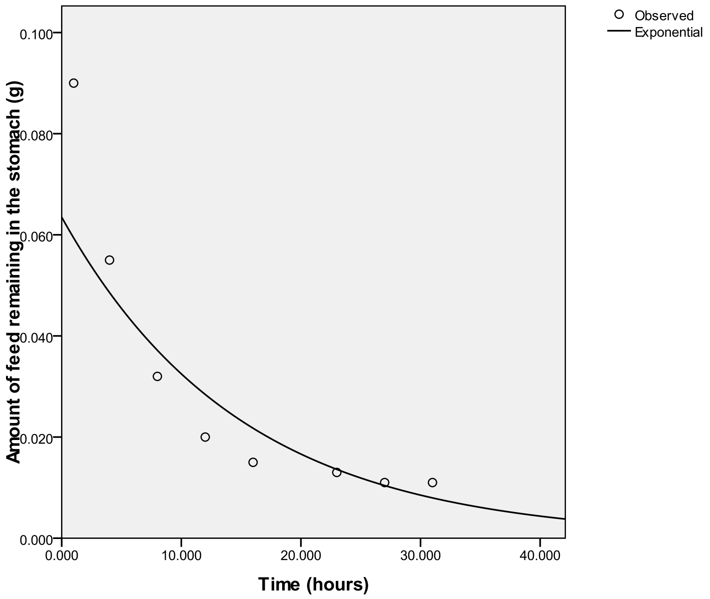


Figure 5.4: Exponential curve representing gastric evacuation rate of kikuyu in *Tilapia rendalli*

The gastric evacuation rate of duckweed in *T. rendalli* is represented by the following equation: St = 0.09 * exp $^{-0.072t}$. Fig. 5.5 shows the exponential curve modelling the evacuation rate of duckweed in the stomach of *T. rendalli*.

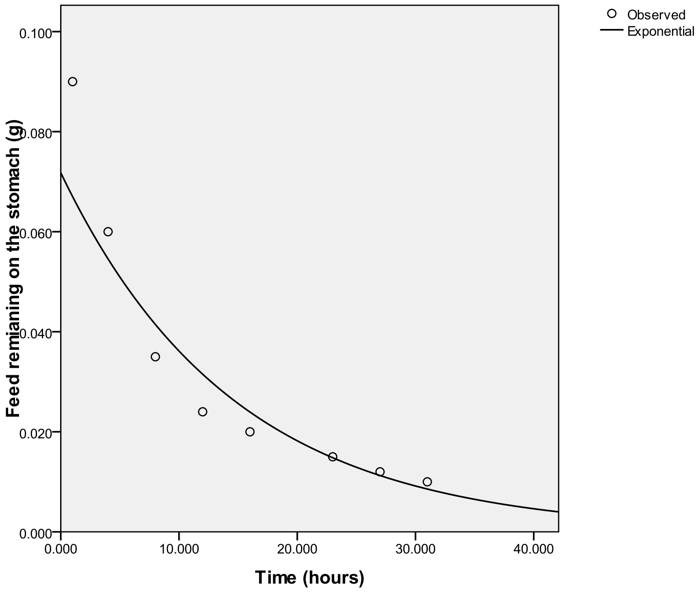


Figure 5.5: Exponential curve representing gastric evacuation rate of duckweed in *Tilapia rendalli*

The modelled exponential curve for the evacuation of cabbage from the stomach of *T. rendalli* is shown in Fig. 5.6 and is represented by the equation:

St = 0.11 * exp $^{-0.091t}$.

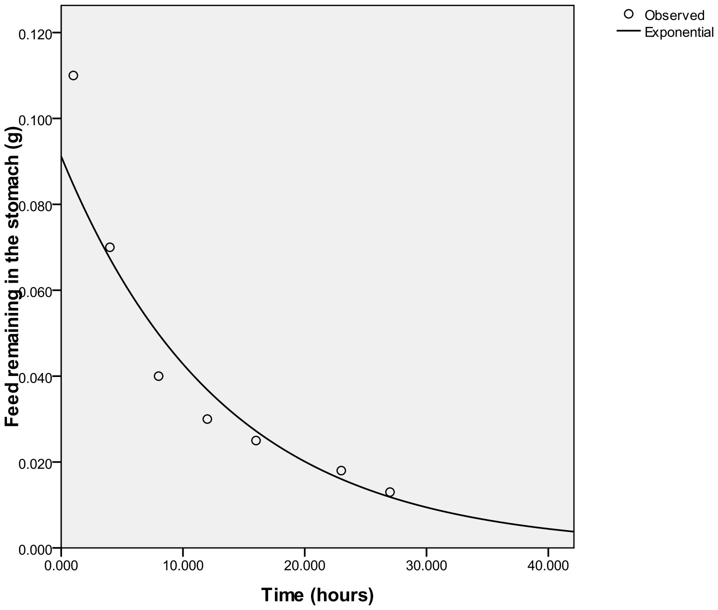


Figure 5.6: Exponential curve representing gastric evacuation rate of cabbage in *Tilapia rendalli*

The evacuation of vallisneria from the stomach of *T. rendalli* was also represented by an exponential as shown in Fig. 5.7. $St = 0.12 * \exp^{-0.116t}$ represents the equation of the evacuation rate.

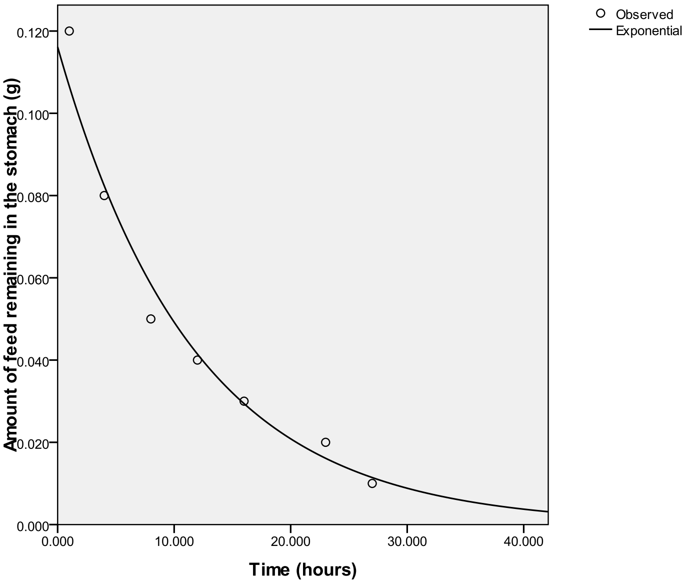


Figure 5.7: Exponential curve representing gastric evacuation rate of vallisneria in *Tilapia rendalli*

The above Figures (5.3-5.7) present the gastric evacuation rate in relation to food remaining in the stomach. The vallisneria required 9 hours to evacuate half of the initial food content, followed by cabbage with 9.6 hours, then kikuyu grass with 10.4 hours followed by duckweed with 11.2 hours and still longer the control diet (commercial fishmeal pellets) with 12.5 hours. In practical terms, the return of appetite will be faster in fish fed vallisneria and slowest in fish fed the control diet.

5.5.4 Preference experiments

One of the most commonly encountered difficulties, when alternative feed sources are used is acceptability of the diets fed to fish. In these experiments however, *T. rendalli* readily consumed all the plants offered (duckweed, vallisneria, kikuyu and cabbage). When the plants were introduced into a tank, the fish immediately approached them and fed readily.

Manly's alpha indicated that *T. rendalli* showed preference for duckweed, followed by kikuyu then vallisneria from the first 15 minutes (min) of feeding, whilst cabbage was selected against. This order of preference continued until the end of the feeding period. Manly's alpha at the end of the feeding period was: 0.53 for duckweed the highly preferred plant, followed by 0.28 for kikuyu grass. These values indicate that *T. rendalli* did not show preference for vallisneria (0.11) and cabbage (0.08) in the presence of duckweed and kikuyu grass under laboratory conditions (Table 5.4). These results show that the plant diets that were preferred by *T. rendalli* were those that gave the best growth performance and were not quickly evacuated by the fish.

Table 5.4: Preference by *Tilapia rendalli* when given equal proportions of the experimental plant diets

	Duckweed	Vallisneria	Kikuyu	Cabbage
No. Stocked	100	100	100	100
No. after 15 min	65	80	72	95
α_i	0.417	0.216	0.318	0.050
No. after 30 min	50	74	66	90
α_i	0.457	0.199	0.274	0.070
No. after 45 min	46	70	58	83
α_i	0.417	0.191	0.292	0.099
No. after 60 min	21	58	41	70
α_i	0.465	0.162	0.266	0.106
No. after 75 min	15	50	33	65
α_i	0.459	0.168	0.268	0.104
No. after 90 min	10	48	27	61
α_i	0.475	0.152	0.271	0.102
No. after 105 min	4	42	20	60
α_i	0.519	0.14	0.259	0.082
No. after 120 min	2	43	13	56
α_i	0.530	0.114	0.277	0.079

5.6 DISCUSSION

Digestibility of plant diets

Total digestive efficiency which is the ability of the fish to digest and absorb nutrients in a diet, was negative for the fresh plant diets fed to *T. rendalli.* The amount of both fibre and cellulose in the faeces was lower than in the fresh plants. This suggests that *T. rendalli* may be able to breakdown cellulose, making it an inefficient reference/marker. The results of the present study contradict those obtained by Buddington (1979), who fed *Najas sp* to *T. zillii* and the amount of cellulose in the faeces was higher than that in the fresh plant. This author reported *Najas* had a total digestibility of 29.3%, using cellulose as a reference. Furthermore, Van Dyke and Sutton (1977) reported digestibility value of 60% in grass carp fed *Lemna* using cellulose as a marker.

Stickney and Shumway (1974); Van Dyke and Sutton (1977) reported that fish are not capable of producing enzymes which can hydrolyse cellulose. Moreover, fish do not maintain a specialised cellulolytic gut flora (Prejs and Blaszczyk, 1977). Nonetheless, the combination of low stomach pH and the presence of microorganisms may have facilitated the breakdown of cellulose and fibre by *T. rendalli*. This contradicts suggestions by Van Dyke and Sutton (1977) that plant cell walls and intact cells of higher plants are indigestible by alimentary canal secretions of fish. These authors however, were working with grass carp which do not have pharyngeal teeth and is not able to truncate and breakdown the plant cell walls before exposure to digestive juices. Furthermore, Buddington (1979) reported that microscopic observation of faeces from *T. zillii* showed that many *Najas* cells were ruptured and the cell contents digested. This author concluded that *T. zillii* depends on mastication to allow utilisation of higher plants.

Mechanical rupture of plant cells is necessary for digestion of cell contents. The well-developed pharyngeal mill in *T. rendalli* triturates plant leaves and exposes the cell contents to digestive enzymes. The ability of *T. rendalli* to breakdown fibre and cellulose explains its efficiency in utilising the plant diets. This explains the ability of *T. rendalli* to effectively utilise kikuyu, in spite of the high fibre and cellulose content, allowing for the effective utilisation of the high protein content in the grass.

Gastric transit time and gastric evacuation rate

Gastric transit time is the time interval between the ingestion of the feedstuff to the appearance of first faeces. It gives an indication of the time it takes for the feedstuff to be digested. Gastric transit time was shortest in fish fed vallisneria followed by cabbage, duckweed and kikuyu respectively. The longest gastric transit time was recorded in *T. rendalli* fed fishmeal pellets.

The nutritional composition of the different plant feeds had an effect on the gastric evacuation rate in *T. rendalli*. Pellets and kikuyu grass had higher energy levels and had slow evacuation rates. The evacuation rate increased with decreasing energy content in the feed. Similarly, vallisneria with the lowest energy content had the fastest evacuation rate. The findings of this study support results reported by Temming and Herrmann (2003) who reported that differences in evacuation rates between different diets could be largely explained by differences in energy; diets with higher energy content are evacuated slower than diets with lower energy. This was also reported in earlier studies (Flowerdew and Grove, 1979; Jobling, 1980). Similarly, De Silva and Anderson (1995) indicated that gastric evacuation is regulated by the energy content of the food; the greater the energy content, the slower the evacuation rate. The rate of digestion and its relationship to gastric evacuation rate can allow one to predict the return of appetite (Booth *et al*., 2008) because gastric evacuation rate controls the return of appetite under a given set of conditions and diets (Lee *et al*., 2000; Riche *et al*., 2004).

The gastrointestinal transit time and evacuation rate of a feed can also be affected by the presence of high fibre which alters the digestibility of other nutrients (Eusebio *et al*., 2004). However, Zhou *et al*. (2004) inferred that low dietary fibre (3-5%) may have beneficial effects on digestibility and fish growth, and high fibre (>8%) may decrease dry matter digestibility and reduce nutrient availability. In the present study, the fibre content probably did not play an important role on gastric transit time and evacuation rate in *T. rendalli*. This may be because of the ability of this fish to secrete hydrochloric acid in the stomach and partially breakdown fibre.

In the present study, plant diets that gave the better growth performance in *T*. *rendalli* had longer gut transit time and slower gastric evacuation rates. It can be concluded that gastric transit time, time for complete evacuation and evacuation rate in *T. rendalli* fed higher energy diets was slower than in low energy diets. These results as well as work on digestive enzymes in herbivorous fish (Zemke-White *et al*., 2000; Moran and Clements, 2002) suggest that digestive processes are important in determining the food choice in these fish. However, this is contrary to the findings of Pérez-Casanova *et al*. (2009) who reported that food consumption was not influenced by diet composition.

Preference of experimental diets by *Tilapia rendalli*

Tilapia rendalli showed preference for duckweed, followed by kikuyu grass, then vallisneria and cabbage was the least preferred plant. Several factors are known to affect preference/selection of different plants by herbivorous fish. These include; the nutritional quality (energy, protein, ash and lipid content) presence of deterrent secondary metabolites and Thallus toughness (Peters *et al*., 2002). In this study, *T. rendalli* showed preference for duckweed then kikuyu grass. The Manly's alpha values for vallisneria and cabbage indicated that these two diets were not preferred by *T. rendalli* (<0.125).

The preference for duckweed and kikuyu grass may be explained by these plants' higher nutritive value compared to vallisneria and cabbage. Duckweed and kikuyu grass had higher protein, energy, and fat content than cabbage and vallisneria. Although the energy content in kikuyu grass was high compared to that of duckweed, duckweed was more preferred (α_i = 0.53) than kikuyu grass (α_i = 0.28). Another factor that may have influenced this selection is probably the higher fibre content in kikuyu and stronger 'toughness' compared to duckweed. The high ash content in duckweed may have also contributed to the preference of this plant by *T. rendalli*. These results are in agreement with those of Setlikova and Adamek (2004) who found that the Nile tilapia preferred a plant diet that had the highest mineral content. Leonard *et al*. (1998) also reported that *O. aureus* preferred leaves to roots of *Azolla filiculoides* (Lam.) which had higher contents of P, K, ash and crude protein.

After kikuyu grass, vallisneria was more preferred than cabbage. This preference is also likely to be influenced by the higher protein content in vallisneria. Again, the energy content of the plants did not have a major influence on preference as cabbage had higher (16.09 MJ/kg) energy values than vallisneria (14.74 MJ/kg). The results of this study support the findings of Mattson (1980) who concluded that plants with high energy and protein content are favoured. Although cabbage had a lower fibre content compared to vallisneria, it was not preferred over this macrophyte probably because of its stronger ‘toughness’ compared to the softer submerged macrophyte.

The fat content of the plant is also known to influence preference. In this study however, it did not seem to have affected selection because kikuyu grass had the highest fat content amongst the diets, duckweed was still the most preferred diet.

The selective feeding displayed by *T. rendalli* in the current study is in agreement with the findings of several authors (Schwartz and Maughan, 1984; Bonar *et al.*, 1990; Chifamba, 1990; Leonard *et al*., 1998) who reported that tilapias are selective feeders when given a variety of plant diets. Schwartz and Maughan (1984) observed that tilapias preferred macrophytes that are easily broken down and digestible. Begon *et al.* (1997) found a relationship between plant selection and plant nutrient content, reporting that tilapia fed *Myriophyllum spicatum* ate the only young leaves which contain the higher concentrations of proteins (nitrogen) and minerals. This is in agreement with the optimal foraging theory which assumes that organisms feed in a way that maximises their net rate of energy intake per unit time (Pyke *et al*., 1977). An organism may either maximise its daily energy intake or minimise the time spent feeding in order to meet minimum requirements. Herbivores are energy maximisers (Belovsky, 1986) and accomplish this maximising behaviour by choosing food that is of high quality and has low search and low handling time (Pyke *et al*., 1977). These results also support the findings of Legner and Murray (1981) who concluded that tilapias ingest plants with high food (protein) value rather than those with low food value. However, the findings of this study are not in agreement with Wiley *et al.* (1986) who found no correlation between preference and plant characteristics, such as protein, caloric content and crude fibre, and they suggested fish eat first those plants that they can consume more easily.

Other factors such as structural material and polyphenolic compounds, ‘Thallus toughness’, the presence of deterrent secondary metabolites (anti-nutritional factors) and the abundance of the particular plant are also known to affect selection. The effect of these was not determined in this study. It is appreciated nonetheless that they may have contributed to selection most likely at a lower extent.

T. rendalli in the current study showed preference for plant diets with higher nutritive value, particularly, protein, energy and fat. The fibre content of the plants played a lesser role in food selection.

CHAPTER 6: EVALUATION OF KIKUYU GRASS AS A REPLACEMENT FOR FISHMEAL IN PRACTICAL DIETS FOR *TILAPIA RENDALLI*

6.1 INTRODUCTION

Protein is not only the most expensive ingredient in fish diets, but is also the main ingredient used. The development of commercial aquaculture feeds has been traditionally based on fishmeal as the main protein source because of its high protein content, well balanced amino acid profile, fatty acid, vitamin and mineral composition (Nguyen *et al*., 2009). However, the availability of fishmeal in future can no longer be guaranteed because the ocean stocks have been depleted. In addition, the price of fish meal is continuously rising, thereby affecting the profitability of aquaculture enterprises (Sintayehu *et al*., 1996).

In recent years, researchers have been vigorously searching for alternative protein ingredients for use in aquaculture feeds. Plants with high protein levels are preferred because dietary protein affects the growth performance in tilapia (Musuka *et al*., 2009). Research efforts have focused on the replacement of fishmeal with less expensive locally available sources (Enami, 2011). These include poultry by-product meal (Soltan, 2009); terrestrial plant seeds such as; soybean (Nyirenda *et al*., 2000; Goda, 2007; Effiong *et al*., 2009; Nguyen *et al*., 2009; Lin and Luo, 2011), a combination of soybean and cottonseed (El-Saidy and Saad, 2011), a combination of soybean, sunflower, and cottonseed (Kang'ombe and Brown, 2008; Chebbaki *et al*., 2010), or soybean meal forfeited with phytase (Hassan *et al*., 2009) and aquatic plants such as duckweed (Skillicorn *et al*., 1993; Fasakin *et al*., 1999 Chowdhury *et al*., 2008) and *Azolla* (El-Sayed, 1999; Fiogbé *et al*., 2004).

Only a few studies have been done on the use of green leaves as dietary protein sources for fish, these include the use of leucaena leaf meal (Lim and Dominy, 1991; Osman *et al*., 1996) cassava leaf meal (Ng and Wee, 1989) and leaf protein concentrates such as rye grass and alfalfa (Olvera-Novoa *et al.,* 1990). However, the results from a majority of these studies are contradictory; this contradiction may be due to a number of factors, including the protein concentration in the plants used, amino acid profile, apparent digestibility, phosphorous content, palatability and the presence of antinutritional factors (Ogunji, 2004).

Soybean meal (SBM) is currently the most commonly used plant protein source as a cost-effective feed ingredient in fish feeds and comprises 50% of the diet of freshwater omnivorous fish species (El-Sayed, 1999; Yue and Zhou, 2008). Soybean meal is regarded as one of the most nutritious of all plant protein sources (Lovell, 1988) because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price and steady supply (Storebakken *et al*., 2000).

However, soybean meal is deficient in sulphur-containing amino acids (methionine, lysine, and cysteine) and contains endogenous anti-nutrients, including protease (trypsin) inhibitor, and anti-vitamins (El-Sayed, 2006). Moreover, soybean may not be suitable in some countries where it would have to be imported to meet demand, thereby increasing the production costs. Thus there is need to evaluate more readily available and locally produced sources of protein.

Duckweed has also been widely used as a fishmeal replacer in tilapia diets. It is known for its high nutritive value with as much as 40% crude protein depending on the culture system (Hassan and Edwards, 1992; Ahmad *et al*., 2003). It has a better array of essential amino acids than most other vegetable proteins and more closely resembles animal protein (Hillman and Culley, 1978).

Other plant protein sources for inclusion in tilapia feeds have to be explored to further reduce feed costs. Successful fishmeal replacement with cheaper and readily available plant sources will make tilapia culture profitable and sustainable in small scale semi-intensive systems. Including other less expensive and readily available high protein plant sources such as kikuyu grass in *T. rendalli* diets would be beneficial in reducing feed costs.

Kikuyu grass has relatively high protein (26.4%) content (Chapter 4). This grass had a better array of essential amino acids, higher energy and fat content than duckweed. In the previous chapter, *T. rendalli* fed kikuyu grass showed better growth performance and carcass composition than those fed duckweed, cabbage or vallisneria.

The effect replacing fishmeal with dried kikuyu grass meal as a fishmeal substitute in tilapia feeds has not been evaluated before. It is in light of the better performance of *T. rendalli* fed fresh kikuyu grass that a subsequent study was undertaken to determine the effect of using kikuyu grass as a fishmeal replacer in the diets of *T. rendalli*. Kikuyu grass has several attributes which make it a suitable fishmeal replacer in *T. rendalli* diets. This grass is widely available, used mainly as a lawn grass, and is of little or no financial value. It is easy to cultivate and maintain and is available all year round in most parts of South Africa.

The search for a solution to lower costs in aquaculture should not only focus on cheaper feeds sources, but also on the culture of a suitable fish species with a low demand for animal based protein in its diet and is capable of efficiently converting plant protein into animal tissue. *T. rendalli* is pre-adapted to effectively utilise plant protein with its adults feeding almost exclusively on a plant diet in nature (Chapter 3). Moreover, the use of expensive animal protein for feeding herbivorous fish such as tilapia is unnecessary (Olvera-Novoa *et al*., 2002). The main focus in this chapter is to evaluate the extent to which dried kikuyu grass can replace fishmeal in practical diets of *T. rendalli*.

6.2 OBJECTIVES

1. To determine the effect of partial and total replacement of fishmeal with dried kikuyu grass meal on the specific growth rate of *Tilapia rendalli*.
2. To determine the effect of partial and total replacement of fishmeal with dried kikuyu grass meal on the feed conversion ratio of *Tilapia rendalli*.
3. To determine the effect of partial and total replacement of fishmeal with dried kikuyu grass meal on the relative growth rate of *Tilapia rendalli*.
4. To determine the effect of partial and total replacement of fishmeal with dried kikuyu grass meal on the average weight gain of *Tilapia rendalli*.
5. To determine the effect of partial and total replacement of fishmeal with dried kikuyu grass meal on the average daily gain of *Tilapia rendalli*.

NULL HYPOTHESIS

1. There are no significant differences in the specific growth rate of *Tilapia rendalli* fed diets containing different levels of dried kikuyu grass meal.
2. There are no significant differences in the feed conversion ratio of *Tilapia rendalli* fed diets containing different levels of dried kikuyu grass meal.
3. There are no significant differences in the relative growth rate of *Tilapia rendalli* fed diets containing different levels of dried kikuyu grass meal.
4. There are no significant differences in the average weight gain of *Tilapia rendalli* fed diets containing different levels of dried kikuyu grass meal.
5. There are no significant differences in the average daily gain of *Tilapia rendalli* fed diets containing different levels of dried kikuyu grass meal.

6.4 MATERIALS AND METHODS

6.4.1 Experimental fish and rearing conditions

Tilapia rendalli were collected from Flag Boshielo Dam and transported in an aerated plastic container to the University of Limpopo. The study was carried out at the Aquaculture Research Unit of the university. Captured fish were transferred to 1000 L aqua dams for acclimatisation 1 month prior to the start of the experiment. During the acclimatisation period fish were fed a commercial tilapia diet.

6.4.2 Formulation of the experimental diets

Fresh kikuyu grass was harvested from the University of Limpopo's Aquaculture Research Unit grounds. The fresh grass was shade dried and milled with a hammer mill before being passed through a 250 µm sieve. Five practical diets were formulated using commercially available ingredients (Table 6.1). The diets were formulated to be isonitrogenous and isocaloric with crude protein content of 16.7% and gross energy of 15.20 MJ/kg.

The test diets were formulated by substituting fishmeal with kikuyu meal at levels of 80% (diet 1), 60% (diet 2), 40% (diet 3), 20% (diet 4) and 0% (diet 5). Commercial pellets (diet 6) were used as a control. The crude protein and energy content of the different test diets were equalized by adjusting the level of maize meal, brewer's dry grain, sunflower meal, soybean meal, fishmeal and fish oil (Table 6.1). DL-methionine was added to meet the required dietary amino acids level (Portz and Cyrino, 2004). Supplementation with the DL-methionine decreased as the inclusion level of the grass increased because the grass had sufficient levels of the amino acid when compared with the amino acid in the fillet of the original fish from the initial stock. Diets were formulated using a WinFeed 2; EFG Software program.

Table 6.1: Ingredients used in the formulation of practical diets for *Tilapia rendalli* using kikuyu meal as a fishmeal substitute

	Inclusion level (%) of kikuyu meal				
	80	**60**	**40**	**20**	**0**
Diet	**1**	**2**	**3**	**4**	**5**
DL- methionine	0.00	0.49	0.99	1.48	1.97
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Fishmeal	0.00	1.63	3.27	4.90	6.53
Brewer’s dry grain	0.00	0.25	0.50	0.75	1.00
Maize	0.00	10.50	21.00	31.50	42.00
Soybean	0.00	4.53	9.05	13.58	18.10
Sunflower	0.00	1.00	2.00	3.00	4.00
Fish oil	2.00	2.13	2.25	2.38	2.50
Kikuyu meal	80.00	60.00	40.00	20.00	0.00
Potato starch	15.00	14.25	13.50	12.75	12.00
Benzonite	0.00	2.22	4.45	6.67	8.90

6.4.3 Preparation of the experimental diets

The dry ingredients were passed through a 250 µm sieve, weighed, placed into a bowl and thoroughly mixed by hand for approximately 10 minutes. Fish oil and water were added to the mixture to attain a consistency appropriate for passing the mixture through an extruder. The paste was homogenised by kneading for an additional 10 minutes. A hand extruder was used to produce pellets of about 2 mm diameter. The extruded strands were oven dried at 30°C for 48 hours, thereafter broken into approximately 5 mm lengths and stored in plastic bags at room temperature.

6.4.4 Experimental design

Eighteen 1 m^3 fibre glass tanks housed in a greenhouse were used. These were connected to a recirculating system; water was re-circulated through a bio-filter before being reintroduced to the experimental tanks. Air was continuously supplied using a side channel blower and diffused through air stones in each tank.

Sub-adult *T. rendalli* of similar sizes (36±2 g) were selected and randomly distributed in the eighteen tanks, at 10 fish per tank. Three replicate groups of fish were used for testing each diet. Fish were hand fed their allocated diet at 5% body mass three times daily at 0900, 1300 and 1700 hours. All fish from each treatment were weighed once every 30 days to assess growth performance. The feeding trial lasted for 60 days from the 1st of February 2011 to the 2nd of April 2011.

6.4.5 Growth performance and feed utilisation parameters

Specific growth rate (SGR), feed conversion ratio (FCR), relative growth rate (RGR), average weight gain (AWG) and average daily gain (ADG), of *T. rendalli* were calculated for all treatments as follows:

Specific growth rate (SGR %)

SGR was calculated according Winberg (1956);

$$\text{SGR}=\frac{\ln W_t - \ln W_0}{t}\times 100$$

where: W_t = final body weight (g)
W_0 = initial body weight (g)
t = time feeding period (days)
ln = natural Logarithm $(\log)^{-10}$

Feed conversion ratio (FCR):

$$\text{Feed conversion ratio (FCR)} = \frac{\text{food consumed (g)}}{\text{mass gained (g)}}$$

Relative growth rate (RGR):

$$\text{Relative growth rate} = \frac{W_2 - W_1}{W_1}\times 100$$

where: W_1 = initial weight
W_2 = final weight

Average weight gain (AWG):

$$\text{Average weight gain} = \frac{W_2 - W_1}{\text{number of fish}}$$

where: W_1 = initial weight (g)
W_2 = final weight (g)

Average daily gain (ADG):

Average daily gain (ADG) was estimated according to the following formula:

$$\text{Average daily gain} = \frac{W_2 - W_1}{T}$$

where: W_1 = initial weight (g)

W_2 = final weight (g)

T = experimental period (days)

6.4.6 Proximate composition of experimental diets

All diets used in this study were analysed for crude protein, lipid, crude fibre, energy and ash, following the procedures stipulated by the Association of Official Analytical Chemists (AOAC International; 2003). Dry matter was determined by freeze-drying each sample for 72 hours. Nitrogen content of the dry matter of the feed was determined using a LECO FP2000 Nitrogen Analyser using the Dumas combustion with protein content calculated as % nitrogen x 6.25. Lipid content was assessed by Soxhlet extraction of the freeze-dried samples with petroleum ether at 50°C. Ash was determined by burning the samples in a muffle furnace at 550°C for 4 hours. Gross energy was established with a DDS isothermal CP 500 bomb calorimeter.

6.4.7 Water quality monitoring

Daily measurements were taken for temperature (°C), dissolved oxygen (mg/l) and pH, using a handheld YSI (556 MPS) multi-meter. Air was continuously supplied using a cyclone blower and diffused through air stones into each tank.

6.4.8 Statistical analysis

One-way analysis of variance (ANOVA) on the Statistical Package and Service Solutions (SPSS version 17.0) was used to determine if there were any significant differences ($P < 0.05$) in the specific growth rate, feed conversion ratio, relative growth rate, average weight gain and average daily gain of *T. rendalli* fed the experimental diets.

6.5 RESULTS

6.5.1 Growth performance indices

The specific growth rate (SGR) showed a decreasing trend with increasing levels of kikuyu grass in the diet (Table 6.2). The highest SGR was recorded in fish fed the control diet (diet 6/commercial pellets) followed by fish fed diet 5 (0% kikuyu), diet 4 (20% kikuyu), diet 3 (40% kikuyu), diet 2 (60% kikuyu) and fish fed diet 1 (80% kikuyu) had the lowest specific growth rate. However, there was no statistical significant difference (P>0.05, ANOVA) in the SGR of *T. rendalli* fed the different experimental diets.

The lowest food conversion ratio (FCR) was recorded for fish fed diet 6, followed by those fed diet 5, diet 4, diet 3 and diet 2, and fish fed diet 1 gave the highest FCR. The FCR decreased with decreasing levels of kikuyu in the diet (80-0%). There was no significant difference (P>0.05, ANOVA) in the food conversion ratio of *T. rendalli* fed the experimental diets (Table 6.2).

Relative growth rate (RGR) increased with decreasing level of kikuyu in the diet. The RGR was highest in *T. rendalli* fed diet 6, followed by fish fed diet 5, 4, 3, 2 with the lowest RGR observed in fish fed diet 1. RGR between treatments was not significantly different (P> 0.05, ANOVA; Table 6.2).

Similarly, weight gain (AWG) and average daily gain (ADG) were highest in fish fed diet 6. As the amount of kikuyu in the diet decreased, the AWG and ADG increased. The highest AWG and ADG were recorded in fish fed diet 6 and the lowest values recorded for fish fed diet 1. Analysis of variance showed that there were no significant differences (P>0.05, ANOVA) in the AWG and ADG of *T. rendalli* fed the different diets (Table 6.2).

Table 6.2: Growth performance indices (±SE) measured in *Tilapia rendalli* fed kikuyu based diets

Diet	1 (80%)	2 (60%)	3 (40%)	4(20%)	5(0%)	6 (control)
Initial weight	368.73±10	385.90±12	386.27±11	343.69±21	338.17±13	387.30±20
Final weight	800.40±16	860.57±18	878.77±20	881.33±18	880.86±17	1080.45±12
SGR	1.29±0.12	1.33±0.89	1.37±0.92	1.53±0.20	1.60±0.05	1.71±0.02
FCR	2.56±0.03	2.48±0.23	2.26±0.18	2.10±0.38	1.86 ±0.10	1.67±0.38
RGR	117.03±1.60	122.79±1.97	127.50±3.53	154.66±3.69	161.48±2.06	179.15±2.40
AWG	43.17±2.59	47.47±5.06	49.25±5.45	53.76±4.52	54.27±1.79	69.32±3.29
ADG	7.19±0.43	7.91±0.84	8.21±0.91	8.96±2.15	9.04±0.30	11.55±0.55

6.5.2 Proximate composition of experimental diets

The amino acid analysis of the formulated experimental diets showed that as more kikuyu was added the level of the methionine and arginine decreased. However, lysine content remained relatively equal across all the diets (Table 6.3).

Table 6.3: Amino acid and proximate composition (%) of formulated diets

	Inclusion level (%) of kikuyu meal					
Diet	1 (80)	2 (60)	3 (40)	4 (20)	5 (0)	6(*C.P)
Essential amino acids						
Arginine	0.69	0.75	0.87	0.98	1.04	2.17
Methionine	0.30	0.54	1.11	1.64	1.69	0.57
Tyrosine	0.67	0.26	0.66	0.72	0.64	0.88
Histidine	0.34	0.28	0.33	0.81	0.41	0.73
Threonine	0.48	0.45	0.52	0.55	0.52	1.19
Isoleucine	0.48	0.48	0.56	0.56	0.59	1.32
Phenylalanine	0.57	0.60	0.62	0.63	0.62	1.40
Lysine	0.85	0.85	0.90	0.98	0.88	1.69
Valine	0.59	0.58	0.62	0.65	0.63	1.77
Leucine	0.95	0.92	1.01	1.06	1.00	2.75
Non-essential amino acids						
Serine	0.57	0.56	0.62	0.78	0.64	1.38
Aspatic acid	1.15	0.43	1.22	1.24	1.20	3.01
Glutamic acid	1.24	0.79	1.75	1.91	2.13	4.89
Glycine	0.52	0.58	0.65	0.74	0.74	2.28
Alanine	0.90	0.70	0.90	0.92	0.80	2.17
Proline	0.60	0.61	0.68	0.69	0.72	2.04
HO-Proline	0.05	0.05	0.06	0.06	0.05	-
Proximate composition						
**Crude protein (%)	15.72	16.24	16.87	17.11	17.57	34.48
Lipid (%)	1.37	2.56	3.22	3.57	4.40	2.97
Crude fibre (%)	17.38	13.89	10.72	6.77	2.50	2.30
Gross energy	15.31	15.06	15.46	15.31	14.87	17.00
Ash	11.26	12.75	12.35	12.41	11.94	10.78

*C.P=commercial pellets; **for the conversion of nitrogen content to crude protein, the factor 6.25 was used.

6.5.3 Water quality monitoring

Water temperature ranged from 25.0°C to 28.0°C (average 26.1±2.2°C). The pH values in all the experimental tanks varied within a range of 7.2-7.7. Dissolved oxygen ranged between 6.24-7.10 mg/l. Ammonia ranged between 0.62-0.87 ppm. All the water quality parameters were within the acceptable limits for *T. rendalli*.

6.6 DISCUSSION

Growth performance indices

There was no significant difference in the growth performance of *T. rendalli* when kikuyu grass meal was used to replace fishmeal. This implies that kikuyu may be a suitable replacement for fishmeal in *T. rendalli* diets. No adverse effects were observed in the fish health and the fish readily accepted all the diets. However, there was an inverse relationship between the growth of *T. rendalli* and dietary kikuyu inclusion. The best growth performance was attained at the lowest kikuyu grass inclusion level (20%) and the highest inclusion level (80%) yielded the poorest growth rates.

The potential of an alternative ingredient to be used as a fishmeal replacer in fish diets can be evaluated on the basis of its acceptability by the fish; its proximate chemical composition, which comprises the moisture content, crude protein, gross energy, crude lipid, total ash, crude fibre, as well as its digestibility. When alternative sources of feedstuff such as plant protein are used in fish diets, one of the common problems is the acceptability by fish and this has to do with the palatability of the diet (Rodriguez *et al*., 1996). In the present study, all the experimental diets were accepted by *T. rendalli*, indicating that all the levels of incorporation of kikuyu grass leaf meal did not adversely affect the palatability of the diets.

The high protein and well balanced amino acid levels in the grass resulted in the good growth performance of *T. rendalli* fed kikuyu grass based diets. The highest inclusion level (80%) of kikuyu grass meal in the diet gave poorest SGR of 1.29%/day and the best SGR was 1.71%/day recorded for fish fed the lowest inclusion level 20% of the grass. These results suggest that kikuyu grass may be a better fishmeal replacement in *T. rendalli* feeds than most studied leaf meal replacements. Adewolu (2008) replaced fishmeal with sweet potato leaf meal (23.57%, CP) in *T. zillii* reported SGRs of 1.56 %/day when the leaf meal replaced 5% of fishmeal and a SGR of 0.01%/day when 20% fishmeal was replaced. This author concluded that sweet potato leaf meal can only replace up to 15% fishmeal in *T. zillii* diets and that diets containing 20% resulted in poor growth. Richter *et al*. (2003) evaluated the suitability of freeze-dried *Moringa* leaf meal, as an alternative protein source for Nile tilapia in isonitrogenous (35% CP) and isoenergetic (20 kJ/g) diets. The leaf meal was included at 10, 20 and 30% of the total dietary protein and the control included only fish meal and wheat meal as protein sources. This author found that diets with higher inclusion levels of *Moringa* leaves 20-30 % significantly depressed growth performance of the fish (SGR: 2.0% in both groups) compared to the control and 10% inclusion having SGR of 2.7% and 2.4%, respectively. The protein levels of the formulated diets in the above studies were much higher than the average of 16.7% used in the present study. This may explain the lower SGR obtained in this study. However, it is not possible to accurately compare the results obtained in this study with previous work as there has never been any published work on the utilisation of kikuyu grass in tilapia feeds. Furthermore, the variations in growth performance of fish among different studies might be due to different experimental conditions such as feed formulation and diet content, stocking density, age and sex of fish. Moreover, most of these studies were based on the Nile tilapia which is a plankton feeder and not necessarily adapted for feeding on higher plants.

The growth rates of *T. rendalli* observed in this study were slightly lower than those of other tilapias reported in other studies. This may be because most of these studies are done on the Nile or hybrid tilapias which genetically have higher growth rates than *T. rendalli* evidenced by the growth performance indices reported by Pauly *et al*. (1988). Another factor may be the difference in the size of fish used. Most studies were based on juvenile fish which generally have a higher growth rate than the sub-adults (36±2 g) used in this study. The production system also affects the growth performance of fish. For example, Kang'ombe and Brown (2008) reported SGR ranging from 2.1 to 3.6%/day in *T. rendalli* fed different plant based diets, these results were better than those obtained from the current study (1.29-1.71). Firstly these authors used juvenile (mean, 4.7 g) fish, secondly these experiments were conducted in fertilised ponds where natural food is abundant and thirdly, the protein content of the formulated diets was much higher than that used in this study. In another study however, Musuka *et al*. (2009) reported SGRs lower than those obtained in this study (0.65-1.15%) and FCR values ranging from 2.74-4.82 when *T. rendalli* (9.25 g) were fed diets containing 30-40% protein.

In these studies, the level to which the alternative leaf meals could be used in tilapia diets was lower than that obtained in this study. The main reason may be the lower protein content of these plant meals used. These various workers however, support the findings of this study that leaf meal protein can be used as fishmeal replacement in tilapia diets at low inclusion levels without reduction in fish growth performance.

Effect of dietary fibre content

The depression in growth performance at high levels of kikuyu could be attributed to several factors, including the fibre content in the diet. The fibre content of the experimental diets increased with increasing amount of kikuyu grass meal in the diets. The higher fibre content in the diets with high levels of kikuyu grass meal might have been the cause of reduced growth performance attained when these diets were fed. The higher fibre content in diets with high kikuyu grass meal may have resulted in reduced digestibility and consequently poor nutrient utilisation, resulting in the observed reduction in growth performance. Anderson *et al*. (1984) reported a drastic reduction in growth, PER, FCE and whole body fat in Nile tilapia when more than 10% α-cellulose was included in the diet. Fasakin *et al*. (1999) reported that an increase in dietary duckweed (*Spirodela polyrrhiza*) inclusion resulted in progressively reduced growth performance and nutrients utilization of fish.

Reduced growth performance in *T. rendalli* with increasing kikuyu levels is in agreement with findings by a number of authors who used plant based protein to replace fishmeal in tilapia diets. For example, Garduño-Lugo and Olvera-Novoa (2008) evaluated the effects of replacing fishmeal with peanut (*Arachis hypogaea*) leaf meal in practical diets for all male *O. niloticus*, and found no statistical difference among fish fed the control and those fed 20% peanut meal. These authors however, found that higher inclusion levels of peanut leaf meal (30%) produced significantly lower growth performance. Similarly, Lin and Luo (2011) reported reduced growth rates when the level of soybean meal was increased in the diet of *O. niloticus.* In another study, Olvera-Novoa, *et al*. (1990) indicated that diets containing 35% alfalfa leaf protein gave the better growth rates than those obtained with a fish meal based diet and that increasing the level over 35% resulted in decreased growth rate. In all these studies, the fibre content in these plant based diets increased as the inclusion levels of the plants were increased. The increase in the fibre content may have resulted in the observed decline in growth performance at higher inclusions of the plant diets.

Effect of antinutritional factors

Although antinutritional factors were not identified, their concentration in the diet is expected to increase with increasing levels of kikuyu grass meal. The higher concentration of antinutritional factors in the diets with high kikuyu grass meal may have contributed to the decreased growth performance. Antinutritional factors include cell wall constituents (NDF and ADF), high levels of saponins, phenolics and phytic acid. Saponins are the main factors causing growth retardation when plant based protein sources are used. These are found in a number of potential alternative plant-derived feed sources, and are considered to have a detrimental effect on fish.

The presence of antinutritional factors in plant based protein sources restricts the level at which these plants can be included in tilapia feeds. Kikuyu grass is associated with a number of anti-nutritional factors including some phenolic compounds (*m*-coumaric acid, *p*-coumaric acid, vanillic acid, gallic acid and flavonoids) as reported by Chou *et al*. (1987) as well as some indigestible material such as lignin, neutral detergent fibre, cellulose and hemicelluloce (Marais, 2001). Antinutritional factors cause reduced feed intake and some bind with other nutrients (protein), resulting in reduced digestibility and utilisation of these nutrients.

In this study, no measures were taken to destroy any anti-nutrients that may have been present in kikuyu grass. It is hypothesised that processing of the grass before formulating the diets may reduce antinutritional factors and increase the growth performance of *T. rendalli*. It has been reported that common processing techniques such as different cooking methods, soaking, drying, wet heating and adding feed supplements, reduces the concentration of anti-nutritional factors in plant feeds and improve the feed intake. These processing techniques have been shown to improve growth performance. Thus the quality of plant protein sources depends on the initial processing method used. For example, Richter *et al*. (2003) reported that *Moringa oleifera* leaf meal could only replace 10% fish meal in tilapia diets without causing reduced growth rates. Whilst Afuang *et al*. (2003) reported that solvent extracted *Moringa* leaf meal could replace up to 30% of fish meal in *O. niloticus* diets with no reduction in growth when compared with the control. In another study, Wassef *et al*. (1988) working with soybean found that germinating and defatting of soybean meal reduced the activity of protease inhibitors and consequently improved growth performance.

The findings of this research suggest that kikuyu grass meal may prove to be a suitable replacement for fish meal protein in *T. rendalli* diets. In view of the favourable amino acid profile of the grass and its wide availability throughout the tropics and subtropics, kikuyu can be considered as a potential feed component with high nutritive value for fish. However, more investigations must be done on a larger scale for longer periods of time and for different size groups of *T. rendalli*.

CHAPTER 7: GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

The warm water aquaculture industry in South Africa has failed to reach sustainable levels. Despite efforts from the government and the donor community, warm water aquaculture remains low. However, continued population increase puts pressure on the government and its stake holders to find cheaper high value protein sources to meet the population demands.

Aquaculture continues to be one of the fastest growing industries in the world, providing high quality protein and much needed sustainable income in the developing world especially in Asia. In South Africa however, the aquaculture industry is beset with a number of challenges. Firstly, the high production costs that are associated with intensive culture system have resulted in losses and abandonment of the donor funded projects. Secondly, the South African climate is not conducive for warm water aquaculture because of the short duration of the warm season.

Any sustainable aquaculture venture has to consider different methods to address the current aquaculture situation. In this study the aquaculture potential of the herbivorous macrophagous feeder, *T. rendalli* was evaluated. Two main factors were considered in determining this potential. These are its feeding habits and its growth rate. *T. rendalli* feeds on submerged, emergent or floating macrophytes, even on marginal vegetation in nature. This species was proved to be a potentially good aquaculture candidate because of its feeding habits and because it is native to South African river systems.

Feeding habits of T. rendalli

The feeding habits of *T. rendalli* in its natural environment were determined in order to establish the time when it is able to utilise plant diets. The findings of this study show that *T. rendalli* starts feeding on marginal vegetation at 2-5 cm (SL). This implies that less expensive plant based diets can be introduced in *T. rendalli* diets at an early age, thereby substantially reducing feed costs. It is important to note that the introduction of plant based diets at an early stage may result in slower growth rates, however, this slow growth can be compensated for by the low feed costs.

The diet of adult *T. rendalli* at Flag Boshielo Dam was dominated by marginal vegetation particularly *Cyperus sexangulasris* and *Panicum schinzi* as there were no macrophytes. All size groups of *T. rendalli* remained opportunistic in their feeding habits when resources were scarce. The euryphagous feeding nature of *T. rendalli* makes it an ideal candidate for aquaculture. Euryphagous fish like *T. rendalli* feed on a wide variety of resources making their culture easier than stenophagous fish which have a restricted diet. This ability to feed on a wide variety of feed resources is important in aquaculture.

T. rendalli's voracious appetite for vegetation was demonstrated as marginal vegetation was its major food resource throughout the different seasons. *T. rendalli* showed to be an opportunistic feeder, taking advantage of whatever food is available. It is evident from the observed feeding habits that *T. rendalli* is pre-adapted to efficiently utilise plant protein. *T. rendalli*'s appetite for plant diets was also evident when it was offered different plant diets under culture conditions. The fish were fed on readily available fresh plant diets, namely: kikuyu grass, vallisneria, duckweed and cabbage. *T. rendalli* showed positive growth when fed on all the diets except when fed vallisneria. The negative growth observed in fish fed vallisneria was attributed to low protein and amino acid levels as well as poor digestibility of the submerged macrophyte. This was evidenced by the shorter gastric evacuation rate in *T. rendalli* fed vallisneria. Fish fed kikuyu grass performed better than those fed the other plant diets. The higher growth performance was attributed to the high protein levels and good amino acid profile of this grass.

The ability of *T. rendalli* to effectively utilise plant diets was further demonstrated when this fish was able to effectively break down both cellulose and hemicellulose in the fresh plants. This allows *T. rendalli* to get maximum nutrition even from diets with high structural components by releasing nutrients that may be bound to the fibre/cellulose. This is a valuable trait in extensive aquaculture systems as farmers do not need to rely on high cost processed diets to achieve good growth performance. In these systems, rural poor farmers who have no access to commercial pellets may still be able to culture *T. rendalli* on plant diets for subsistence use. The growth performance of *T. rendalli* fed on fresh plant diets in semi-intensive or extensive pond systems can be improved by encouraging the growth of natural food. *T. rendalli* is not only euryphagous, but feeds low in the food chain. Enrichment of pond water with fertilisers/manure will lead to increased production of plankton, this will in turn increase fish production. The availability of zooplankton and insects would increase the total protein available to the fish. Fertilisation with chicken manure which contains high amounts phosphorous and nitrogen (Kang'ombe and Brown, 2008) the most limiting nutrients for the growth of algae, is not only feasible, but also cost effective and should be encouraged in extensive culture of *T. rendalli*.

Fresh kikuyu grass gave better growth performance in *T. rendalli* and good carcass composition with higher omega 3 fatty acids than the other plant diets used. The percentage of omega 3 fatty acid in *T. rendalli* fed kikuyu grass (25.13) was comparable to values found in both wild and farmed salmon (17-26%) reported by Hamilton *et al*. (2005). This grass had a good amino acid composition and relatively high protein content. The performance of fish fed this grass can be enhanced by improving the protein content of the grass. Application of organic or inorganic fertilisers to the cultivated grass will improve its nitrogen, phosphorous and potassium content. This will lead to higher protein content and better growth rates.

These experiments confirm that *T. rendalli* is pre-adapted to feeding on higher plants since it feeds on macrophytes and marginal vegetation in nature. This highly versatile, herbivorous-macrophagous feeder is well suited to low technology farming systems in Africa. The inferior growth performance observed in *T. rendalli* fed the plant diets compared to when fed with commercial fishmeal pellets can be compensated for by the lower cost incurred when plant diets are used.

Kikuyu grass was further evaluated as a potential substitute for fishmeal in practical diets of *T. rendalli.* A fishmeal substitute must possess certain characteristics, including nutritional suitability, ready availability, ease of handling and must be affordable (Naylor *et al*., 2009). Kikuyu grass meets these requirements. Results from the replacement diets (Chapter 6), indicate that *T. rendalli* does not rely on expensive sources of protein to achieve good growth. Replacing fishmeal with kikuyu grass can be a major step towards the production of low cost diets. The low production cost of the kikuyu diets show that this lawn grass has great potential for use in *T. rendalli* diets. In South Africa, kikuyu grass grows well in hot areas that are also suitable for the culture of *T. rendalli*. The hot dry air also makes these areas suitable for sun-drying the grass, a factor that would also reduce costs. At present, large quantities of kikuyu grass cut from lawns have no financial value and are unused and could play an important role in lowering the production costs of *T. rendalli*.

In the preference experiments, *T. rendalli* showed preference for duckweed over the other plant diets. Several studies have been done on partially replacing fishmeal with duckweed in practical diets for tilapia. For example, Tavares *et al*. (2008) concluded that duckweed can replace up to 50% fishmeal without any negative effect on the growth of *O. niloticus*. It is therefore important to highlight the possibility of using duckweed in *T. rendalli* practical diets.

The growth performance of T. rendalli

The ability to feed on a wide range of feed items allows *T. rendalli* to attain good growth performance even in systems that do not have its preferred diet (macrophytes) like Flag Boshielo Dam. The growth performance of *T. rendalli* in this dam was good and comparable to that reported in other systems where macrophytes were abundant.

T. rendalli was also able to produce good growth rates under culture conditions. The growth observed in *T. rendalli* fed the commercial fishmeal pellets was good and comparable to that obtained by other researches (Nguyeni *et al.* 2009) in the widely cultured *O. mossambicus*. This implies that *T. rendalli* can attain growth results as good as those of O*. mossambicus* when fed on the commercial fishmeal pellets.

Furthermore, kikuyu based diets also produced good growth rates in *T. rendalli* which were not significantly different to those obtained from feeding fishmeal pellets. The good growth observed contribute to the reasons why *T. rendalli* is a good species that should be explored in South African aquaculture. The ability of

T. rendalli to effectively utilise kikuyu based diets makes it a species of choice in aquaculture.

The culture of *T. rendalli* has not been fully explored and tilapia production in Africa is dominated by *O. niloticus* and in South Africa, *O. mossambicus* is the most widely cultured tilapia. *T. rendalli* may be the most suitable tilapia for culture on plant based diets because of its ability to feed on higher plants even of terrestrial origin. Furthermore, the culture of *O. niloticus* is banned in South Africa because of interbreeding with indigenous fish species. Tilapias of the genus *Oreochromis* are microphagous and feed on phytoplankton and periphyton (El-Sayed, 2006). In experiments where tilapias of the *Oreochromis* genus are fed on plant diets the growth performance is often poor. For example, feeding *O. niloticus* on *Spirodela polyrhiza and Myriophyllum spicatum* resulted in negative growth performance (Setlikova and Adamek, 2004). Okeyo and Montgomery, (1992) also reported that *O. aureus* also lost weight when fed three aquatic macrophytes (*Elodea canadensis, Myriophyllum spicatum* and *Potamogeton gramineous*).

However, there is paucity of information on direct comparison of the growth performance of *T. rendalli* and the widely cultured tilapia species (*O. mossumbiciss* and *O. niloticus*). Further comparative studies on the growth performance of these species fed on kikuyu based diets are recommended.

How can the growth of *T. rendalli* be further enhanced?

Enhancing the growth performance of *T. rendalli* on kikuyu grass based diets may be the key to increasing the level at which the grass can be incorporated in their diets without causing adverse effect on the growth. Growth may be enhanced in several ways including; the destruction of antinutritional factors, incorporating enzymes in the diet and by the inclusion of effective microbes for fibre degradation.

Destroying antinutritional factors

The presence of antinutritional substances is a critical factor that limits the utilisation of plant diets. The growth performance of *T. rendalli* fed on kikuyu based diets can be improved by destroying antinutritional factors in the grass. However, in literature little is known about the effects of specific antinutrients in *T. rendalli*. Most of the work shows that antinutrients, at levels currently found in plant based fish feed do not lead to mortality, but could produce adverse effects on the growth and decrease productivity. At present, it is difficult to make firm conclusions regarding specific plant secondary metabolites causing deleterious effects and their threshold levels in fish diets. The nutritional and physiological effects of the antinutrients on *T. rendalli* need to be explored. It is highly recommended that the antinutritional factors in kikuyu grass be identified and their effects on *T. rendalli* determined. This information is critical in the formulation of *T. rendalli* practical diets from kikuyu grass as appropriate threshold levels will be set to maximise productivity.

Several techniques have been used to destroy antinutritional factors in plant diets. The common processing techniques, like dry and wet heating, extracting with water, and addition of feed additives have been widely and successfully used to reduce the concentration of antinutrients in plant feeds. However, caution needs to be exercised when selecting the treatment method because they may sometimes cause unintended adverse effects on the nutritional quality of the feed material. For example, heat treatment reportedly alters the chemical nature and decreases the nutritional quality of proteins and carbohydrates. The tolerance limits of *T. rendalli* to the presence of antinutrients also need to be considered before deciding on treatment procedures to reduce their levels. Feeding experiments using purified individual antinutrients are needed to determine the threshold limits that will not adversely affect the productivity of *T. rendalli* fed kikuyu based diets. Another important factor to be considered is the interactions between various antinutritional factors in a particular substance as these interactions in some instances lead to a decrease or even increase in the toxic effect of the interacting antinutrients. A more detailed study of such interactions would be particularly useful in potential fishmeal replacers such as kikuyu grass which contain more than one of the antinutrients. Further studies are needed to expose the effects of mixtures of antinutrients in proportions similar to those present in kikuyu grass.

Incorporation of enzymes

Enzymes can inactivate anti-nutritional factors and enhance the nutritional value of plant-based protein in feeds. They provide a natural way to transform complex feed components into absorbable nutrients. The addition of enzymes in kikuyu based diets given to *T. rendalli* can improve nutrient utilization. Exogenous enzymes such as protease, cellulose, pentanose, α-galactose and amylase can be used for several purposes in the culture of *T. rendalli*.

Phytic acid is one of the most powerful anti-nutritional factors in plant ingredients. The anti-nutritional activity of phytic acid in plant based diets can be eliminated by the addition of relevant enzymes, such as phytase. The phytic acid or phytate found in plant diets is bound with phosphorus, calcium, magnesium, trace elements like iron and zinc, protein and amino acids; this leaves these nutrients to pass undigested. This implies that high proportions of valuable nutrients from the plant diets used in this study may have not been utilised by the fish and were wasted as excreta. The incorporation of the feed enzyme (phytase) in *T. rendalli* would not only facilitate the release of phosphorus from the phytate but also in the release of other minerals and amino acids that are also bound thus paving the way for maximum utilization of nutrients and improving protein and amino acid digestion in *T. rendalli*. At present, no work has been done on the inclusion of these enzymes in *T. rendalli* diets. It is hypothesised that their inclusion in kikuyu grass based diets will improve the growth performance of *T. rendalli*. Phytase added diets have been shown to have a higher feed intake, growth and better food conversion efficiency than control diets in Channel catfish, as well as reduced phosphorus load in their faecal matter (Jackson *et al*., 1996). A feeding trial conducted with tilapia *O. niloticus* fingerlings showed that fish fed commercial phytase enzyme “Natuphas” showed higher weight gain and a better food conversion ratio (Feord, 1996). Similarly, high growth rates were reported in tilapia (*Oreochromis* sp) fed diets based on Malaysian palm, which was treated with an enzyme complex (protease, cellulose, pentanose, α-galactose and amylase) Ng *et al*. (2002).

Furthermore, enzyme treatment can reduce eutrophication. Excessive phosphorus is an important factor in the eutrophication of aquaculture systems and the discharge of nutrients into the surrounding ecosystems. Phosphorus bound in phytate may be unavailable to the fish but it will still ultimately be released into the environment as microbial action breaks down the fish waste. The addition of phytase in *T. rendalli* diets may reduce the release of nutrients into the environment by making the bound phosphorus available to the fish for growth so that it is incorporated into the fish's body instead.

Use of Effective Microorganisms Technology

Tilapia rendalli is pre-adapted to breakdown and effectively utilise plant diets. *T. rendalli*'s utilisation of plant diets can be further enhanced by incorporating effective microbes (EMs) in the diets. EMs are a combination of specially selected microorganisms including predominant populations of lactic acid bacteria, yeasts, actinomycetes and other types of organisms. All these are mutually compatible with one another and can coexist in a liquid culture. EMs can quickly decompose organic matter, metabolize antioxidant substances and inhibit the proliferation of harmful microorganisms (Li and Liu, 2001).

The application of EM technology in aquaculture has been done on several fish species and results show that fish fed on EM-mixed feeds grow quickly. In general, the weight gain increased by more than 30% (Zhou *et al*., 2009). Furthermore, the addition of EMs allows for the stocking density be increased by up to 30% (Zhou *et al*., 2009). The benefits of using EMs in pond culture of *T. rendalli* also include good water quality. Chen (1997) reported that pond water treated with EMs appears bloomy and clear, because EMs prohibit harmful bacteria from proliferating effectively, eliminating ammonia, raising DO level, and increasing plankton growth for the fish to consume.

There is paucity of information regarding the efficacy and beneficial effects of EM in treating the meal itself, especially plant based diets in the culture of *T. rendalli*. There is a need to further explore the use of EM technology in breaking down fibre in plant (kikuyu) based diets, to increase nutrient availability in *T. rendalli*.

The effects of supplementing kikuyu based diets with EM as feed additives on the growth performance, feed utilization, and body composition of *T. rendalli* need further investigation. Furthermore, the economic benefits in terms of feed costs on using these methods (destruction of antinutritional factors, incorporation of enzymes and use of EMs) in *T. rendalli* culture need to be assessed.

In conclusion, the macrophagous feeding nature of *T. rendalli* makes it an ideal aquaculture candidate because it can be raised on cheap plant based diets, thus addressing the problem of high feed costs facing the aquaculture industry. *T. rendalli* appears to be a promising species for aquaculture due to its good growth rates, high survival, ease of handling (no mortalities observed) and ready acceptance of a wide variety of diets and readily receives pelleted supplementary diets. Furthermore, promoting the culture of *T. rendalli* using high quality plant based diets will be a positive step towards addressing the prevailing food security problem by providing high quality protein at low cost.

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*Not seen in original.

APPENDIX A

APPENDIX B

APPENDIX C