Effect of dietary protein level, feeding frequency and amount of food offered on growth and gastric evacuation of *Oreochromis mossambicus* fry.

by

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DECLARATION

I hereby declare that this thesis is the product of my own original work, and to the best of my knowledge, contains no material published previously, submitted for the award of any degree at another university. Any published information used was duly acknowledged.

12 November 2012

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Signature (Candidate) Date

12 November 2012

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Signature (Supervisor) Date

Dedication

This thesis is dedicated to:

- My child, Ndisalelani Rolivhuwa Raswiswi
- My parents (Mr Maluta and Mrs Eunice Luthada), my siblings (Ndivhuwo, Tshilidzi and Meshack) and Pfarelo Raswiswi for their endless love and support.

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ABSTRACT

Excess protein in fish diet may be wasteful and unnecessarily expensive. Furthermore, when fish are fed insufficiently or excessively, their growth or feed efficiency may decrease, resulting in increasing production costs and water quality deterioration. Therefore this study was conducted to determine the optimum dietary protein level, feeding frequency and feeding rate on growth, gastric and intestinal evacuation of *O. mossambicus* fry under hatchery conditions, in order to reduce the production costs while optimizing growth rate. Diets contained 20%, 30%, 35%, 40% and 45% protein levels, feeding frequency of once, twice, thrice, four times and five times per day and feeding rates of 10%, 15%, 20%, 25% and 30% body weight per day were tested in separated experiments conducted in a temperature and light cycle controlled environmental room. The results indicated that both dietary protein level and feeding frequency had a significant effect on weight gain, specific growth rate and gross food conversion ratio (ANOVA, $P<0.05$) but not survival rate (ANOVA, $P>0.05$). Feeding rate had a significant effect on weight gain and gross food conversion ratio (ANOVA, $P<0.05$) but not on specific growth rate and survival rate (ANOVA, $P>0.05$). A diet containing 30% protein level, feeding frequency of four times per day and 15% of the fry body weight per day were the optimal levels obtained from the growth experiments. Dietary protein level had a significant effect on gastric and intestinal evacuation (ANOVA, $P<0.05$); feeding frequency had a significant effect on intestinal evacuation rate and time (ANOVA, $p<0.05$) only but not on gastric evacuation rate and time (ANOVA, $P>0.05$), while feeding rate had no significant effect on both gastric and intestinal evacuation rate and time (ANOVA, $P>0.05$). The optimum levels obtained in gastric and intestinal evacuation are 40% dietary protein level, feeding frequency of twice per day and a feeding rate of 15% body weight per day.

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ABREVIATIONS

C	Cleavage
E	Embryonic
F	Free embryonic
J	Juvenile stages
Mt	Metric tons
g	Grams
G	Weight gain
W_1	Initial weight
W_2	Final weight
SGR	Specific growth rate
GFCR	Gross food conversion ratio
SR	Survival rate
t	Number of days in the feeding period
F	Weight of food fed
ANOVA	Analysis of variance
%	Percent
h	Hour
ºC	Degree Celsius
g/kg	Grams per kilograms
DF	Degree of freedom
P	Probability
S	Tukey HSD statistic
Ref	Reference
GER	Gastric evacuation rate
GET	Gastric evacuation time
IER	Intestinal evacuation rate
IET	Intestinal evacuation time
Yx	Mean geometric weight of food content at time x
A	A constant
R	Rate of evacuation

e	Natural logarithm

Chapter 1

General Introduction

1. GENERAL INTRODUCTION

1.1. Species introduction

Oreochromis mossambicus is a freshwater fish that belongs to the family Cichlidae, endemic to Southern Africa (El-Sayed 2006). *O. mossambicus* is regarded as a good candidate for aquaculture because of its rapid growth rates, high tolerance to low water quality, efficient food conversion, easy reproduction, resistance to diseases and good consumer acceptance (Balarin and Haller 1982; Rana 1988, and El-Sayed 2006). According to Goddard (1996), *O. mossambicus* can be raised in a variety of production systems from extensive, semi-intensive to highly intensive recirculation systems. Tilapia has been introduced and cultured in many countries including Taiwan, Thailand, North and South America, Europe, Asia, Australia and some Pacific island (Trewavas 1983; Popma & Lovshin 1996). The reasons for tilapia introduction into other countries are: farming as food fish, recreational fishing, aquatic weed control and research purposes (El-Sayed 2006).

Oreochromis mossambicus breed in summer, with females raising multiple broods every three to four weeks (Popma & Masser 1999). Prior to spawning, the male construct a nest on the bottom of a water body where the female lays the eggs and male fertilizes them. The female mouth broods the embryos and small fry until they are well developed and can shelter in the shallows (Popma and Masser 1999, and El-Sayed 2006). *O. mossambicus* fry are usually transparent with some pigment spots, which are used for species identification when they hatch (Iwai 1980).

Fry hatch from freshwater mouth brooder egg type, with mouth and anus closed, and gut lumen and pigmented retina present (Iwai 1980). Their rapid development of the digestive system enables them to take external foods before the yolk is exhausted. Development of taste buds occur before the commencement of feeding and the first appearance of immature taste buds has been observed in 1- day old fry of tilapia (Iwai 1980). The epithelium of 2-day fry is well differentiated with sensory cells, supporting cells and ciliated cells. The olfactory nerve is detected at the base of the epithelium. The sensory epithelium increases in size as fry grow. The nasal cavity is well developed by 6 days after hatching, but olfactory rosettes are not yet visible. Table 1.1.1, summaries the early ontogeny of *O. mossambicus* from directly after fertilization. The fry do not undergo any metamorphosis because they do not develop temporary larval structures (Holden and Bruton 1992, and Kruger 2008).

Table 1.1.1: Step summary of the early ontogeny of *O. mossambicus* (Adapted from Holden and Bruton 1992) C= Cleavage; E= Embryonic; F= Free Embryonic and J= Juvenile stages

Step	Age (days: hours)	Step summary
$C^1$1	00:00-00:01	Bipolar differentiation; perivitelline space formation; hardening of egg envelop
$C^2$2	00:01-00:22	Cleavage; germ ring formation; flattening of blastodisc
$C^3$3	00:22-01:11	Beginning of epiboly; formation of embryonic shield
$E^1$4	01:11-02:12	Neurulation; organogenesis; formation of fore-, mid- and hindbrain, optic vesicles, at least 17 pairs of somites, pericardial cavity and neuromeres; first pigmentation
$E^2$5	02:12-03:00	First cardiac and muscle contractions; initial blood flow; formation of eye lenses, otic capsules, and heart-tube; simple vascularization of the vitelline plexus-anterior vitelline veins, blood islands; hemispherical division of the brain; elongation of the separation of the tail
$E^3$6	03:00-03:20	Strong head and body circulation; branching of the posterior vitelline vein; twisting of the heart-tube; some haemoglobin; first eye pigmentation; simple median fanfold plexus; hatching begins
$E^4$7	03:20-04-16	Hepatic vitelline network and branchial arteries develop; arterial flow into the preanal plexus; pectoral anlagen; hatching of almost all individuals
$E^1$8	04:16-08:04	Formation of caudal fanfold network, intersegmental and pectoral fin vessels and heart chambers; blood vessels in gill filaments; maximization of median and vitelline plexii; anastomoses of profundal caudal vein for

		replacement of the inferior caudal vein; decline of median fanfold network; head free of yolksac; chondrification of skeletal structures; peristalsis; jaw and pectoral fin movements; differentiation of stomach, spleen, and gall-, urinary- and swimbladder; beginning fanfold differentiation; first iridocytes and retinal pigments
F^{2}9	08:04-11:00	Replacement of the inferior caudal vein by the profundal caudal vein; disappearance of fanfold respiratory network; marked decline in vitelline plexus; formation and vascularization of secondary gill lamellae; formation of dermal bones; fusion of neural and haemal arches; formation of caudal lepidotrichia and proximal pterygiophores of the median fins
F^{3}10	11:00-15:00	Mixed exogenous and endogenous feeding; ossification of chondriod bone, median, caudal and pectoral lepidotrichia; formation of vertebral rings and pelvic fin buds; filling of swimbladder; fanfold differentiation; enclosure of yolksac almost complete
J^{1}11	15: -?	Complete yolksac enclosure; ossification of vertebrae; vascularization of dorsal and anal fins; squamation

Definition of the term fry for the present study.

Coad and McAllister (2009), define fry, as any young fish from the start of exogenous feeding after the yolk is absorbed. According to Piper *et al*. (1983) fry is the stage in a fish's life from the time it hatches until it reaches 1 inch in length. In the present study, the fry were defined, as young fish from the start of exogenous feeding after the yolk is absorbed.

1.2. Problem statement and motivation

The production of *O. mossambicus* in South Africa was 45 metric tons (Mt) in 1998 (Hoffman *et al.* 2000), 130 Mt in 2000 (Brink 2003) and increased to 220 Mt in 2003 (Henrichsen and Brink 2004). However, the major limitations in the aquaculture production of tilapia in some regions of South Africa are their inability to tolerate low temperatures that results in poor growth and food conversion ratio and early sexual maturity that results in spawning before fish reach market size (Rouhani and Britz 2004). According to Charles *et al*. (1984) fish size and production in aquaculture determine the price, which in turn depends on growth. There are many factors affecting growth of cultured fish. Among the variables, food is considered as the most important factor affecting metabolism and growth. In aquaculture, feed that is not consumed in reasonable time represents an economic loss (Lovell 1989). Feed cost accounts approximately 40-60% of the operating costs in intensive culture systems (Goddard 1996 and Bahnasawy 2009).

According to Iwai (1980) and Thorpe and Hecht (1992), the availability of suitable food is essential for a high rearing success on a commercial scale and the transition from endogenous to exogenous feeding is one of the most important stages in freshwater fish culture. Optimization of feed management (which includes the diet, amount of food offered and frequency of feeding) is the only way of enhancing growth performance, improving the feed conversion efficiency and minimizing the cost of the feeds. The optimum feeding frequency and amount of food for maximum growth of fish is generally affected by fish size and culture conditions (De Silva and Anderson 1995).

Feeds formulated for fry, and juveniles generally contain 5-10% more protein than do grower diets formulated for older fish (Goddard 1996). These formulated feeds are expensive because expensive marine products such as fish meals and oils are used to provide sufficient quantities of essential nutrients and supplies of energy (Goddard 1996). Heavy mortality usually occurs early in the life history of fish (Iwai 1980). Therefore, for husbandry purposes, it is important to understand the fry's daily feeding pattern to match their appetite so that feeding time and amount can be set appropriately, for an increase survival rate, production of high quality fry that will be used as seed stock and minimum food wastage and production costs (Wang *et al*. 1998).

When fish are fed insufficiently or excessively, their growth or feed conversion efficiency may decrease, resulting in increasing fish production cost (Lovell 1989 and Mihelakakis *et al*. 2002), and deterioration of water quality (Ng *et al*. 2000) especially when fish are overfed. Information on gastric evacuation rate of fish is useful for assessing appetite return and allows one to estimate proper level of protein in diet, feeding frequency and the amount of food required (Lee *et al*. 2000). According to Goddard (1996), specific growth rate and food conversion ratio are the two most important factors indicating the effectiveness of feed management and economic performance in aquaculture.

In the present study, fry were reared under hatchery conditions at the Tilapia Hatchery of the University of Zululand, with operational hours between 07:30 h to 15:45 h during the week. The experiments were extended to Saturdays and Sundays when required. According to Jauncey and Ross (1982), fry under hatchery conditions have no access to natural feeds and dependent on formulated feeds due to the fact that the fry need a diet higher in protein, lipids, vitamins and minerals, and lower in carbohydrates as they are developing.

1.3. The objectives of the present study were:

1.To determine the optimum dietary protein level on growth, gastric and intestinal evacuation of *O. mossambicus* fry under local hatchery conditions.

2.To determine the optimum feeding frequency on growth, gastric and intestinal evacuation of *O. mossambicus* fry under local hatchery conditions.

3.To determine the optimum feeding rate on growth, gastric and intestinal evacuation of *O. mossambicus* fry that will minimize feeding costs while promoting growth under local hatchery conditions.

1.4. The following null hypotheses were tested:

1. There will be no difference in growth, gastric and intestinal evacuation of *O. mossambicus* fry among five dietary protein levels.

2. There will be no difference in growth, gastric and intestinal evacuation of *O. mossambicus* fry among five feeding frequency.

3.There will be no difference in growth, gastric and intestinal evacuation of *O. mossambicus* fry among five feeding rates.

1.5. Null hypotheses were tested to address the following specific questions grouped into four categories according to independent trials conducted:

A. Effects of dietary protein level on growth of *O. mossambicus* fry:

1.Will the fry fed different levels of protein have the same growth rate, food conversion ratio and survival rate?

2.Which protein level results in optimum growth rate, food conversion ratio and survival rate of the fry?

3.Will there be any relationship between dietary protein level and growth rate, food conversion ratio and survival rate of fry?

B. Effects of feeding frequency on growth of *O. mossambicus* fry:

1.Will fry fed the same amount of food at different feeding frequency have the same growth rate, food conversion ratio and survival rate?

2.Which feeding frequency results in optimum growth rate, food conversion ratio and survival rate of the fry?

3.Will there be any relationship between feeding frequency and growth rate, food conversion ratio and survival rate of fry?

C. Effects of amount of food offered on growth of *O. mossambicus* fry:

1. Will the fry fed different amounts of food have the same growth rate?
2. Which amount of food offered results in optimum growth rate, food conversion ratio and survival rate of the fry?
3. Will there be any relationship between the amount of food offered and growth rate, food conversion ratio, and survival rate?

D. Effects of dietary protein level, feeding frequency and feeding rate on gastric evacuation of *O. mossambicus* fry:

1. Will the fry fed different protein level have the same gastric evacuation rate?
2. In which dietary protein level do the fry evacuate their stomach and intestinal contents faster?
3. Will the fry fed at different feeding frequency have the same evacuate rate?
4. In which feeding frequency do the fry evacuate their stomach and intestinal faster?
5. Will the fry fed different feeding rate have the same evacuation rate?
6. Which feeding rate will result in faster evacuation rate?

E. Last chapter is a synthesis.

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Chapter 2

Effect of dietary protein level on growth of *O. mossambicus* fry

2.1. INTRODUCTION

Proteins are the basic components of any animal tissues and are, therefore, an essential nutrient for both maintenance and growth (Hepher 1988). Proteins provide the essential and non-essential amino acids, which are necessary for muscle formation and enzymatic function, and in part provide energy for maintenance (Bahnasawy 2009).

According to Jauncey and Ross (1982), the amount of dietary protein required to produce maximum growth of fish is influenced by many factors including the fish's age, energy content of the diet, amino acid composition of the dietary protein and amino acid availability, physiological state of the animal and feeding habits, level of feeding and environmental factors such as temperature, salinity, etc. Generally, protein requirements decrease, as the fish grows older (De Silva and Anderson 1995). The protein requirements of tilapia differ with each life stage of the fish (Jauncey and Ross 1982). According to Jauncey and Ross (1982), fry and fingerlings require a diet high in protein, lipids, vitamins and minerals and lower in carbohydrates. Adult fish need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Table 2.1.1 shows the recommended percentage protein level of tilapia according to their sizes.

Table 2.1.1. General protein requirements of tilapia. Adapted from Jauncey and Ross (1982).

Stages	Protein (%)
First feeding fry	45-50
0.02 - 2.0 g	40
2.0 - 35 g	35
35 g - harvest	30-32

Protein is, however, the most expensive dietary source in intensive aquaculture. It represents about 50% of total feed costs (El-Sayed 1999). To reduce the costs of feeds, some researchers: Jauncey (1982), Siddiqui *et al.* (1988), Wee and Tuan (1988), El-Sayed and Teshima (1992) have used different protein sources to replace fishmeal, which is the most expensive protein source.

Protein sources are divided into animal protein sources, plant protein sources and single-cell proteins (El-Sayed 1999). According to El-Sayed (1999), animal protein sources are also divided into fishery and terrestrial by-products. Some examples of fishery by-products are shrimp meal, krill meal, and squid meal. Some examples of terrestrial by-products are blood meal, meat and bone meal. Plant protein sources are divided into oilseed plant, aquatic plants and grain legumes and plant protein concentrates. Examples are soybean meal, duckweed and cassava leaf meal respectively (El-Sayed 1999). Single cell proteins are group of microorganisms including algae, fungi, bacteria, cyanobacteria, and yeast (El-Sayed 1999). The fact that there are many sources of protein may contribute to differences in results between growth studies on tilapia. For the present study, a mixture of terrestrial by-product, oilseed plants together with fishmeal were used as source of protein. This was done to reduce the costs of the formulated diets as less fishmeal, the most expensive protein source was used.

Currently, there are no published studies conducted on effect of dietary protein level on growth of *O. mossambicus* fry in South Africa. *Oreochromis niloticus* has received more attention than *O. mossambicus* in other countries *(*Jauncey 1982*)*. Therefore, the aim of this study was to determine the optimum dietary protein level on growth of *O. mossambicus* fry under local hatchery conditions.

2.2. MATERIALS AND METHODS

2.2.1. Experimental fry and treatment allocation

Prior to the experiment, 12 broodstock of *O. mossambicus* weighing 200-250 g were stocked in a circular fiberglass tank of 2 m diameter, 0.5 m depth and 1500 litres to provide the experimental fry. Broodstock were stocked in a ratio of 2 females per male, and allowed to acclimatize for seven days. The fish were fed a diet containing 28% protein, at 2% of their body weight, three times per day (per day in the present study refers to the normal hatchery operational times, between 8:00 and 16:00 h).

Fry (c. 0.06 g) were randomly assigned to five treatments in twenty fiberglass tanks with a volume of 40 litres each in a Completely Randomized Design experiment. Treatments consisted of five diets of 25%, 30%, 35%, 40% and 45% dietary protein level with similar energy levels. The ingredients (Table 2.2.1) that were used in the preparation of the diets were adapted from Al-Hafedh (1999). Each treatment was replicated four times with ten fry per replicate. The fry were fed 10% of their body weight (Siraj *et al.* 1988), three times per day at 8:00, 12:00 and 16:00 hrs for 30 days. Ten percent of the water was siphoned to remove excreta and replaced after every two days. Daily rations were adjusted according to the body weight per tank after measuring the weight of the fry every week. The fry were starved during the day of measurements as recommended by Goddard (1996). Each fry was placed in a weighing boat, after blotting off excess water, and weighed on a Denver balance (Model SI 234). Rations were measured in a weighing boat on a Denver balance daily, prior to each feeding according to the weight and number of the fry per tank.

Water temperature was kept at 28±2ºC and light was set at a 12h light: 12h dark cycle. Dissolved oxygen and pH were measured daily before the fry were fed, using a Microprocessor Dissolved Oxygen meter (Model HI 9146), and

Microcomputer Ph Meter (Model HI 8424). Thirty milliliter water samples were taken from each tank for the determination of ammonia, nitrate, and nitrite concentrations every week. Samples were analysed using a Spectroquant Photometer (Model SQ 118) following the method described in Merck Spectroquant Photometer Manual (Merck Company). All water quality parameters results were within tolerance ranges published for tilapia species (El-Sayed, 2006). Mortality was monitored throughout the experimental period.

2.2.2. Analysis of experimental diets

Samples of five prepared diets were sent to Bioindustrials Services Laboratory for full nutritional analysis. The results are shown in Table 2.2.2.

Table 2.2.1. Composition of experimental diets (%). Adapted from Al- Hafedh (1999).

	Dietary protein level (%)				
Ingredients	25	30	35	40	45
Corn	44.44	29.90	19.30	8.60	-
Wheat flour	5.00	12.50	10.00	12.20	13.60
Wheat bran	9.70	3.00	2.50	-	-
Soya meal	21.10	34.51	47.97	55.34	57.11
Fish meal	10.00	10.00	10.00	10.00	16.70
Meat and bone meal	5.00	5.00	5.00	10.00	10.00
Fish oil	1.80	2.23	2.45	2.44	2.08
Lysine	0.15	-	-	-	-
Methionine	0.03	-	-	0.40	0.01
Limestone	2.58	2.36	2.28	0.88	-
Mineral and vitamin mix	0.50	0.50	0.50	0.50	0.50

Table 2.2.2. Chemical composition of experimental diets (%). Determined by Bioindustrials Services Laboratory.

	Protein level (%)				
Constituents	25	30	35	40	45
Protein	22.95	28.71	35.48	42.15	44.49
Crude fibre	2.14	2.13	2.36	3.86	2.71
Fat	3.69	4.28	4.15	4.43	4.32
Ash	7.25	6.83	7.95	7.45	7.74
Moisture	13.46	11.03	5.63	6.18	9.04
Metabolic energy(MJ/kg)	13.44	14.15	14.42	14.99	15.14
Total digestible nutrients	89.83	94.11	96.11	100.14	101.18

2.2.3. Growth measurements

In each tank, all the fry were individually weighed every week to obtain a mean wet weight for the fry per tank. This was done to determine the correct amount of food to add to experimental tanks per week as the fry grow. Weight gain, specific growth rate, gross food conversion ratio and survival rate were calculated using the formula described by Goddard (1996), Puvanendran *et al.* (2003) and Ajani *et al.* (2011) as follows:

Weight gain (G) = W_2-W_1

Specific growth rate (SGR) = (L_n (W_2) -L_n (W_1))/t x100

Gross food conversion ratio (GFCR) = F/G

Survival rate (SR) = Number of surviving fry/Number of fry initially stocked x 100

GFCR was calculated instead of food conversion ratio (FCR) reported by other authors because it was not possible to measure uneaten food. W_1 and W_2 are initial and final fish weight, respectively and t is the number of days in the feeding period. F is the weight of food fed and G is the weight gain of the fish during that duration.

2.2.4. Statistical analysis

Statistical analyses were done using the QED statistics program (Henderson and Seaby 2007). The Shapiro – Wilk test indicated no significant deviation from normality ($p > 0.05$) for replicate G, SGR, GFCR and SR values calculated for the different treatments. One–way analysis of variance was used to test for significant differences at a significance level of $\alpha = 0.05$ between the means of the treatments. The results were considered significantly different at a probability (p) < 0.05. Where there was a significant difference in means Tukey′s multiple comparison test was used to compare the variance among the means.

2.3. RESULTS

Weight gains and specific growth rates of *O. mossambicus* fry were significantly affected by dietary protein level (ANOVA, Table 2.3.1). Increasing trends in weight gain and specific growth rate with an increase in dietary protein level were evident (Table 2.3.2, Figures 2A and 2B). However, only the mean values for weight gain and specific growth rate of the fry that were fed 25% were significantly lower than those fed 45% (Tukey test, Table 2.3.3). There were no significant differences in weight gain or specific growth rate of the fry fed 25% and other treatments (Table 2.3.3 and Figures 2A and 2B).

The results of one-way analysis of variance indicated that there were no significant differences in gross food conversion ratio among most of the treatments (ANOVA, Table 2.3.1), except for fry fed 25% and 45% protein (Tukey test, Table 2.3.3). The best food conversion ratio of 1.81 was obtained at 30% protein level due to the fact that gross food conversion ratios were not significantly different between fry fed 30 - 45% protein level (Table 2.3.3 and Figure 2C).

Survival rates of *O. mossambicus* fry were not significantly affected by dietary protein level (ANOVA, Table 2.3.1). Survival rate was above 70% in all treatments (Figure 2D).

Table 2.3.1. ANOVA results for weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR), and survival rate (SR) for *O. mossambicus* fry fed 25%, 30%, 35%, 40%, and 45% protein level for 30 days. Degrees of freedom for all variables: between groups = 4, within groups = 15, F = F Statistic, and P = Probability.

Variables	F	P
G	4.37435	0.015279
SGR	3.76154	0.025971
GFCR	2.81422	0.063271
SR	0.432432	0.783113

Table 2.3.2. Mean weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR) and survival rate for *O. mossambicus* fry fed different protein levels for 30 days. Values are means (±SD) of four replicates for each treatment.

Variables	Dietary protein level (%)				
	25	30	35	40	45
Weight gain (g)	0.27±0.08	0.45±0.09	0.38±0.09	0.46±0.09	0.53±0.11
SGR (%/day)	5.78±0.87	7.08±0.49	6.74±0.75	7.31±0.84	7.71±0.75
GFCR	2.32±0.38	1.81±0.34	1.89±0.22	1.78±0.47	1.51±0.25
Survival rate (%)	82.5±9.57	77.5±9.57	82.5±12.58	72.5±20.62	79.5±12.58

Table 2.3.3. Tukey's multiple comparison test summary results for weight gain (G) and specific growth rate (SGR) and gross food conversion ratio (GFCR) for *O. mossambicus* fry fed 25%, 30%, 35%, 40% and 45% protein level for 30 days. S = Tukey HSD statistic, P = probability and * = significant difference at $P < 0.05$.

Dietary protein level (%)	G (g)		SGR (%/d)		GFCR	
	S	P	S	P	S	P
25 vs 30	3.9075	0.0905	3.4572	0.1568	2.9101	0.2873
25 vs 35	2.4147	0.4585	2.5375	0.4119	2.4666	0.4384
25 vs 40	4.0969	0.0711	4.0483	0.7571	3.1239	0.2289
25 vs 45	5.5102	0.0106*	5.1284	0.0179*	4.6277	0.0354*
30 vs 35	1.4928	0.8258	0.9197	0.9639	0.4435	0.9977
30 vs 40	0.1894	0.9999	0.5911	0.9929	0.2137	0.9999
30 vs 45	1.6027	0.7870	1.6713	0.7614	1.7176	0.7435
35 vs 40	1.6822	0.7572	1.5108	0.8186	0.6573	0.9895
35 vs 45	3.6822	0.2361	2.5909	0.3924	2.1611	0.5611
40 vs 45	1.4133	0.8517	1.0801	0.9372	1.5039	0.8219

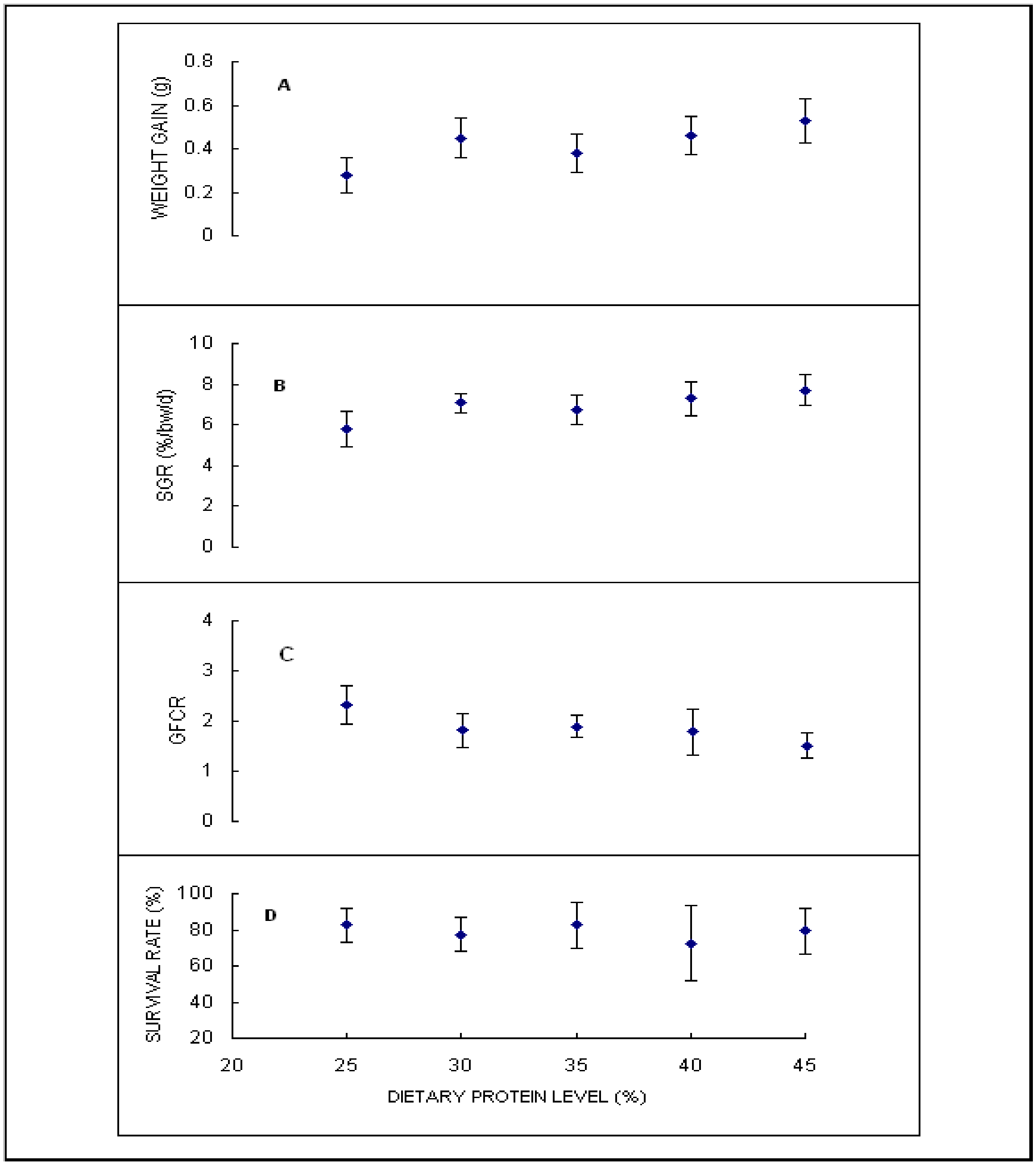

Figure 2. Average weight gain (A), SGR = specific growth rate (B), GFCR = gross food conversion ratio (C) and survival rate (D) of *O. mossambicus* fry fed different protein levels for 30 days. Values are means of four replicates for each treatment. Vertical bars indicate standard deviation.

2.4. DISCUSSION

The results of the present study indicated that the weight gain, specific growth rate and gross food conversion ratio of *O. mossambicus* fry were significantly affected by dietary protein level (Table 2.3.2). Significant differences were however, only between fry fed 25% and 45% protein levels. According to Jauncey (1982), El-Sayed and Teshma (1992), each fish size has a certain protein limit after which excess protein could not be utilized efficiently for growth because energy for growth is utilized for deamination and excretion of excess amino acids absorbed. Similar results, where growth performance increased with increasing dietary protein level up to a certain level (optimum level), were reported for *O. niloticus* (Siddiqui *et al*. 1988), juvenile *S. mossambicus* (Jauncey 1982), four young tilapia species *(O. mossambicus, O. niloticus, Oreochromis aureus and Tilapia zillii)* (De Silva *et al.* 1989), hybrid tilapia (*O. niloticus* x *O. aureus*) (Shiau and Huang 1989), hybrid Clarias catfish (Giri *et al*. 2003) and monosex Nile tilapia (Bahnasawy 2009). Furthermore, dietary protein requirements in many fish species including tilapia species in general, is known to decrease with increasing size and age (De Silva *et al.* 1989, Jauncey and Ross 1982).

Gross food conversion ratio, which is similar to feed conversion ratio determined by other authors, is generally known to decrease with increasing dietary protein level (Jauncy 1982, Siddiqui *et al.* 1988, Shiau and Huang 1989, Al-Hafedh 1999, Bahnasawy 2009). Results of the present study, with gross food conversion ratios ranging from 2.03 - 1.5, are in agreement with these studies. The gross food conversion ratio obtained at the 30% protein level was regarded as best because it was not significant different from those of 35 - 45%. Al-Hafedh (1999) and Al-Hafedh *et al.* (1999) reported food conversion ratios ranging from 2.5 to 1.6 for the fry of Nile tilapia (0.51g), a congeneric species to *O. mossambicus* used in the present study.

A number of studies have been carried out to determine the effect of dietary protein level for different tilapia species (Table 2.4.1). The difference in optimum protein levels maybe due to the variables such as species, initial size, water temperature and protein sources used. However, regardless of these variables, the results in the table emphasize that each specific size has its own optimum protein level. It is therefore important to determine optimum protein level for each size group.

Table 2.4.1. The optimum dietary protein levels selected by various authors for different fish species. ODPL = Optimum dietary protein level, IS = Initial size, WT = Water temperature, EP = Experimental period. Superscripts a=Mazid *et al*. 1979, b= Jauncey (1982) (*S. mossambicus* synonym of *O. mossambicus)*, c=Siddiqui *et al.* (1988), d=El-Sayed and Teshima (1992), e= Al-Hafedh (1999), f= Bahnasawy (2009), and g= Present study.

	Variables			
Species	ODPL(%)	IS(g)	WT(°C)	EP (days)[Ref]
T. zillii	35	1.65	25±1	18[a]
S. mossambicus	40	1.8	27±1	40[b]
O. niloticus	40	0.838	-	98[c]
O. niloticus	45	0.012	25-27	28[d]
O. niloticus	40-45	0.51	27±1	140[e]
O. niloticus	30	2.5	18-32	180[f]
O. mossambicus	30	0.06	28±2	30[g]

Survival rate of *O. mossambicus* fry was not significantly affected by dietary protein level, ranging from 72.5 to 82.5%. Similar results were obtained by Abdel-Tawwab *et al*. (2010) who reported that different protein levels did not significantly affect the survival rate of Nile tilapia. In the present study, mortality might have been due to stress while being measured every week in order for the amount of food to be adjusted.

In conclusion, *O. mossambicus* fry, fed different dietary protein levels, had significantly different growth rate and gross food conversion ratio. There was an increase in growth rate and decrease in gross food conversion ratio with increasing dietary protein levels up to 30%, and no significant increase above 30%. Given that there were no significant differences in weight gain, specific growth rate and gross food conversion ratios among the fry fed 30, 35, 40 and 45% protein level, the most economic dietary protein level for optimum growth of *O. mossambicus* fry would be 30%. Dietary protein level had no effect on the survival rate of *O. mossambicus* fry.

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Chapter 3

Effect of feeding frequency on growth of *O. mosssambicus* fry

3.1. INTRODUCTION

Thorpe and Hecht (1992) and Goddard (1996) reported that the optimum feeding frequency might vary with species, age, size, environmental factors, and food quality. Another factor that determines the most suitable feeding frequency is the interval between meals, because the intake of food is related to the capacity of the stomach and the rate of digestion and evacuation, and evacuation time is related to the feeding sequence and the size of the fish. De Silva and Anderson (1995) reported that feeding frequency is important to ensure maximal food conversion ratio and weight of cultured organism. Higher feeding frequencies decrease aggressive behavior in some fish species and these results in faster growth and uniformity in size. Yeoh *et al*. (2010) reported that adult tilapia fed at 2-3 hour intervals eat more food than their stomachs can hold and the extra food eaten passes through the stomach and is considered wasted while adult tilapia fed at 4-5 hours intervals eat nearly the same amount of food needed to refill their stomachs. Feeding requirements are different in the case of tilapia fry because the fry require frequent feeding for their rapid growth, high-energy requirements, and small stomachs (El-Sayed 2006 and Yeoh *et al*. 2010). The recommended feeding frequency of tilapia fry is 8-10 times a day in a recirculation system (Goddard 1996 and Yeoh *et al*. 2010).

The influence of feeding frequency on growth has been examined in several fish species: Siraj *et al.* (1988) conducted a study on effects of feeding frequency on growth, food conversion and survival of Red Tilapia (*O. mossambicus*/*O. niloticus*) hybrid fry. Charles *et al.* (1984) conducted a study on the effect of feeding frequency on growth and food conversion of *Cyprinus carpio* fry. Mihelakakis *et al.* (2001) conducted a study on the effect of feeding frequency on growth, feed efficiency and body composition in young common Pandora.

However, there is no published information on the effect of feeding frequency on growth of *O. mossambicus* fry. The present study aims to determine the optimum feeding frequency during normal local hatchery operational hours that will result in optimum growth and conversion ratio while minimizing the food wastage and production costs. According to Philippart and Ruwet (1982), *O. mossambicus* feed during the day. The current study investigated feeding frequency on growth of *O. mossambicus* fry when fed during day light hours between 8:00 h and 16:00 h, i.e. the normal operational time of the hatchery at the University of Zululand.

3.2. MATERIALS AND METHODS

3.2.1. Experimental fry and treatments allocation

Prior to the experiment, 12 broodstock of *O. mossambicus* weighing 200-250 g were stocked in a circular fiberglass tank of 2 m diameter, 0.5 m depth and 1500 litres to provide the experimental fry. Broodstock were stocked in a ratio of 2 females per male, and allowed to acclimatize for seven days. The broodstock fish were fed a diet containing 28% protein, at 2% of their body weight, three times per day (per day in the present study refers to the normal hatchery operational times between 8:00 and 16:00 h).

O. mossambicus fry (c. 0.06 g) were randomly assigned to five treatments in twenty fiberglass tanks with a volume of 40 litres each in a Completely Randomized Design. Treatments with regard to feeding frequencies were as follows: Once daily (08:00 h), twice daily (08:00 and 16:00 h), three times daily (08:00, 12:00 and 16:00 h), four times daily (08:00, 10:00, 12:00 and 16:00 h) and five times daily (08:00, 10:00, 12:00, 14:00, and 16:00 h). Each treatment (feeding pattern) was replicated four times with 10 fry per replicate. The fry were fed a commercial diet containing 41% crude protein (as recommended for first feeding fry by manufacturer) obtained from Aquanutro (pty) Ltd (tilapia starter granules of less than 2 mm, that was ground and sieved through a 900 micron sieve), at a daily feeding rate of 10% body weight (Siraj *et al.* 1988) for 30 days. The composition of the diet used in this experiment is shown in Table 3.2.1. Ten percent of the water was siphoned to remove excreta and replaced after every two days. Daily rations were adjusted according to the body weight of the fry per tank after measuring their weight every week. The fry were weighed as explained in chapter two. Water temperature was kept at 28±2ºC and light was set at a 12h light: 12h dark cycle. Water quality parameters were measured and tested as described for chapter two.

All water quality parameters results were within tolerance ranges published for tilapia species (El-Sayed, 2006). Mortality was monitored throughout the experimental period.

Table 3.2.1. Composition of experimental diet obtained from Aquanutro.

Analysed composition	Quantity (g/kg)
Protein	400
Moisture	120
Lipid	80
Fibre	40
Phosphorus	7
Calcium	30

3.2.2. Growth measurements

In each tank, all the fry were individually weighed every week to obtain a mean wet weight for the fry per tank. This was done to determine the correct amount of food per week as fry grow faster. Weight gain, Specific growth rate, Gross food conversion ratio and Survival rate were calculated using the formulas described by Goddard, (1996), Puvanendran *et al.* (2003), and Ajani *et al.* (2011) as follows:

Weight gain (G) = W_2-W_1

Specific growth rate (SGR) = (L_n (W_2) -L_n (W_1))/t x100

Gross food conversion ratio (GFCR) = F/G.

Survival rate (SR) = Number of surviving fry/Number of fry initially stocked x 100

GFCR was used to calculate food conversion ratio (FCR) because it was not possible to measure uneaten food. W_1 and W_2 are initial and final fish weight, respectively and t is the number of days in the feeding period. F is the weight of food fed and G is the weight gain during that period.

3.2.3. Statistical analysis

Statistical analyses were done using the QED statistics program (Henderson and Seaby 2007). The Shapiro – Wilk test indicated no significant deviation from normality ($p > 0.05$) for replicate G, SGR, GFCR and SR values calculated for the different treatments. One–way analysis of variance was used to test for significant differences at a significance level of $\alpha = 0.05$ between the means of the treatments. The results were considered significantly different at a probability (p) < 0.05. Where there was a significant difference in means Tukey′s multiple comparison test was used to compare the variance among the means.

3.3. RESULTS

Feeding frequency had a significant effect on weight gain, specific growth rate and gross food conversion ratio (ANOVA, Table 3.3.1) but not survival rate (ANOVA, Table 3.3.1). Both weight gain and specific growth rate increased with increasing feeding frequency (Table 3.3.2). However, there were no significant differences in weight gain and specific growth rate of the fry fed three, four and five times per day, nor between once, twice an three times per day (Tukey test, Table 3.3.3 and Figures 3A and 3B). Gross food conversion ratios decreased with increasing feeding frequency to feeding frequency of four times per day, and then slightly increased to feeding frequency of five times per day (Figure 3C). However, there was no significant difference in gross food conversion ratio of fry fed three, four and five times nor between those fed once, twice or three times. There was no clear statistical difference for feeding frequency between three, four and five times per day, feeding frequency of four times per day was the optimum in this study. Survival rate of *O. mossambicus* fry were not significantly affected by the feeding frequency (Table 3.3.1). The survival rate was high in all feeding frequency (Figure 3D).

Table 3.3.1. ANOVA results for weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR), and survival rate (SR) for *O. mossambicus* fry fed once, twice, three times, four times, and five times for 30 days. Degrees of freedom for all variables: between groups = 4, within groups = 15, F = F Statistic, and P = Probability

Variables	F	P
G	6.51116	0.003031
SGR	8.8631	0.000702
GFCR	4.38118	0.015192
SR	2.13636	0.12652

Table 3.3.2. Mean weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR) and survival rate of *O. mossambicus* fry fed once, twice, three times, four times and five times per day for 30 days. Values are means (±SD) of four replicates for each treatment. The results were significantly different at $p<0.05$.

	Feeding frequency (per day)				
Parameters	Once	Twice	Three times	Four times	Five times
Weight gain (g)	0.04±0.01	0.06±0.01	0.09±0.05	0.11±0.02	0.12±0.03
SGR (%/d)	2.11±0.24	2.85±0.29	3.48±1.09	4.26±0.38	4.41±0.77
GFCR	6.70±0.87	5.07±0.92	5.13±2.39	3.54±0.51	3.61±0.79
Survival rate (%)	82.5±5.00	85.0±5.77	95.0±5.77	92.5±9.57	92.5±9.57

Table 3.3.3. Tukey′s multiple comparison test summary results of weight gain (G), specific growth rate (SGR) and gross food conversion ratio (GFCR) of *O. mossambicus* fry fed once, twice, three times, four times and five times per day for 30 days. S= Tukey HSD statistic, P= probability and * = significant difference at $P<0.05$.

Feeding frequency (per day)	G (g)		SGR (%/d)		GFCR	
	S	P	S	P	S	P
Once vs twice	1.4015	0.8553	2.2575	0.5213	2.6250	0.3802
Once vs three times	3.6783	0.1203	4.2347	0.0595	2.5208	0.4179
Once vs four times	5.1061	0.0185*	6.6465	0.0023*	5.0763	0.0193*
Once vs five times	6.1549	0.0045*	7.0603	0.0014*	4.9622	0.0225*
Twice vs three times	2.2767	0.5135	1.9772	0.6381	0.1043	0.9999
Twice vs four times	3.7046	0.1165	4.3889	0.0486*	2.4512	0.4443
Twice vs five times	4.7534	0.0299	4.8028	0.0279*	2.3372	0.4891
Three vs four times	1.4279	0.8471	2.4118	0.4596	2.5555	0.4053
Three vs five times	2.4767	0.4346	2.8256	0.3131	2.4415	0.4053
Four vs five times	1.0488	0.9431	0.4138	0.9983	0.1141	0.9999

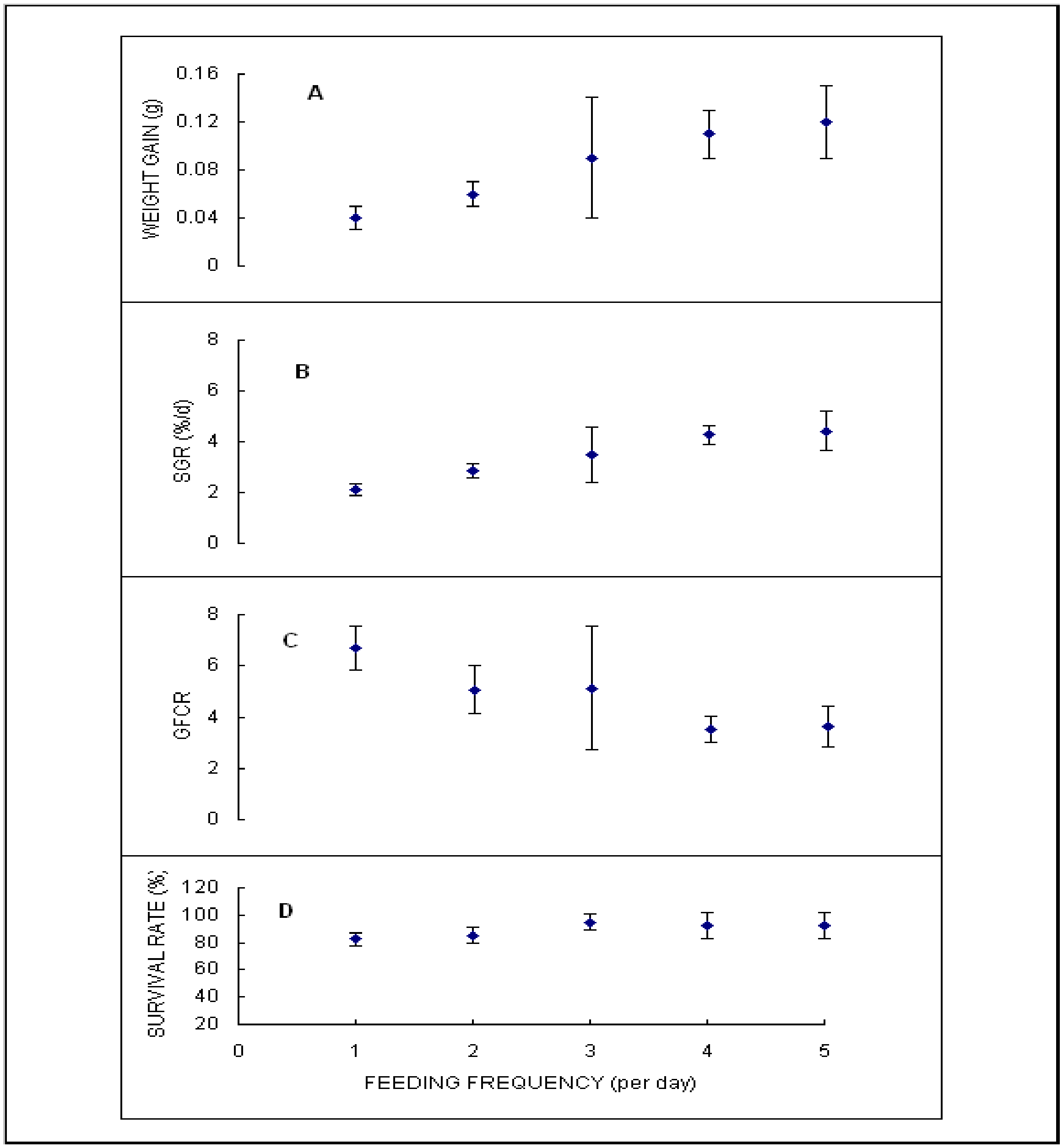

Figure 3. Average weight gain (A), SGR = specific growth rate (B), GFCR = gross food conversion ratio (C) and survival rate (D) of *O. mossambicus* fry fed different feeding frequencies for 30 days. Values are means of four replicates for each treatment. Vertical bars indicate standard deviation.

3.4. DISCUSSION

Feeding frequency had a significant effect on the weight gain and specific growth rate of *O. mossambicus* fry in the present study. Fry that were fed at higher feeding frequencies (four and five times per day) had gained significantly more weight and higher growth rate. This gain in weight and growth rate was higher compared with those fed less frequently (once and twice daily). The results for feeding frequency of three times per day were not significantly different to those of fry fed once and twice or four and five times. High weight gain and specific growth rate at higher feeding frequencies have also been reported for red tilapia hybrid fry (Siraj *et al.* 1988) and juvenile *O. niloticus* (Riche *et al*. 2004).

Food conversion ratio, which is similar to gross food conversion ratio determined in the current study, is generally known to improve with increasing feeding frequency (Goddard 1996). The results of the present study are in agreement to this statement. The gross food conversion ratio was significantly poorer for the fry that were fed once daily and improved with increasing feeding frequency to be better at feeding frequency of four times per day in the present study. The results were indirectly compared to those reported by Siraj *et al.* (1988) for red tilapia hybrid fry by looking at the trend and the trend was similar to the present study. According to Biswas *et al.* (2010), reducing feeding frequency to once or twice times per day, significantly decreased growth and increase food conversion in sea bass fry due to the fact that the less frequently fed fishes could not meet up the nutrient and energy requirements for their maintenance and somatic development. This is in agreement to the findings of the present study because the ones that were fed four and five times, utilized the feed more efficiently than those fed once per day.

Although mean survival rate was highest for the fry fed three times per day and lowest for those fed once per day, the difference was not statistically significant. Similar results where there were no statistically significant differences in survival rate for different feeding frequencies were reported for red tilapia hybrid fry (Siraj *et al*. 1988); juvenile flounder (Lee *et al*. 2000); juvenile sunshine bass (Webster *et al*. 2001) and *Barbus luteus* fry (Gokcek *et al*. 2008). The survival rates in the present study ranged from 82.5% to 95%; from 92.0% to 96.7% for red tilapia hybrid fry (Siraj *et al.* 1988); 62.3% to 74.7% for juvenile sunshine bass (Webster *et al*., 2001) and 96.67% to 97.77% for *B. luteus* fry (Gokcek *et al*. 2008).

In light of the lack of published information on the subject and importance of optimizing feeding in order to reduce feed costs, the objective of the present study was to determine the optimum feeding frequency on the growth of *O. mossambicus* fry under local hatchery conditions. Different studies (Siraj *et al.* 1988, Wang *et al.* 1998, and Lee *et al.* 2000) have been conducted and different optimum feeding frequency (two times, three times and three times respectively) attained because optimum feeding frequency varies depending on species, size, environmental factors such as water temperature, husbandry and feed quality (Goddard 1996). In a recirculation system, the recommended feeding frequency of tilapia fry in general is 8-10 times a day (Goddard 1996 and Yeoh *et al*. 2010) because of their small stomach. While there was no significant difference in performance of feeding frequencies of three, four and five times per day, unfavourable economic considerations like a large variations in FCR and weight gain for treatment three, combined with an additional cost of feeding five times per day, suggest that feeding frequency of four times per day should be favoured.

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Chapter 4

Effect of feeding rate on growth of *O. mossambicus* fry.

4.1. INTRODUCTION

Feeding rate (the amount of feed made available to the cultured organisms over a specific time period) is considered as an important factor that varies with the fish size and water temperature and any restriction to feeding rate results in a lower metabolic rate (De Silva and Anderson 1995). De Silva and Anderson (1995) Izquierdo *et al*. (2001), Craig and Helfrich (2002), reported that underfeeding results in poor growth and production, whereas overfeeding results in wastage and water quality deterioration, low dissolved oxygen levels, increased biological oxygen demand and increased bacterial loads. It was also found that a higher energy ratio with low feeding rate is better than feeding large amounts of low nutrient diet. According to Riche *et al.* (2004) fish eat available food depending on stomach fullness and at intervals determined by the time it takes to empty the stomach. Therefore amount of food offered to fish is also regarded as another factor that determines how fast the stomach is emptied.

Feeding rate needs to be modified according to the size and water temperature of the cultured organism as shown (e.g.) in Table 4.1.1 for tilapia in general.

Table 4.1.1. Recommended feeding rate for tilapia in general at 28ºC. Adapted from Jauncey and Ross (1982) and Lovell (1989).

Weight (grams)	Feeding rate (% Body Weight /Day)
2 day old to 1	30-10
1 - 5	10 - 6
5 - 20	6- 4
20 – 100	4-3
Larger than 100	3- 1.5

Even though feed costs are high in South Africa, studies on the effect of feeding rate on growth and gastric evacuation of *O. mossambicus* fry especially, because they require a higher feeding rate than adults, are not available. The present study aims to determine the optimum feeding rate (which is defined as a ration that gives the best growth and food conversion ratio, De Silva and Anderson 1995) for fry, which will results in faster growth rate and efficient food conversion ratio. This will be useful in aquaculture to reduce overfeeding.

4.2. MATERIALS AND METHODS

4.2.1. Fry breeding and experimental setup

Prior to the experiment, 12 broodstock of *O. mossambicus* weighing 200-250 g were stocked in a circular fiberglass tank of 2 m diameter, a depth of 0.5 m, with a capacity of 1500 liters to accommodate the experimental batch of fry. Broodstock were stocked in a ratio of 2 females per male, and allowed to acclimatize for seven days. The fish were fed a diet containing 28% protein, at 2% of body weight, three times per day ('Per day' in the present study refers to the normal hatchery operational times between 8:00 and 16:00 h).

The experimental fry (c. 0.06 g) were randomly assigned to five dietary treatments in twenty fiberglass tanks with a volume of 40 liters each in a completely randomized design. The five dietary treatments were feed amounts offered at 10%, 15% 20%, 25% and 30% of fish body weight per day. Each treatment was replicated four times. Therefore, there were 40 fry in each treatment and 10 fry in each replication. The fry were fed a commercial diet containing 41% crude protein (as recommended for first feeding fry by manufacturer) obtained from Aquanutro (pty) Ltd (an agglomerated tilapia starter of less than 2 mm, that was ground and sieved through a 900 micron sieve), three times per day at 8:00, 12:00 and 16:00 hrs for 30 days. The composition of the diet used in this experiment is shown in Table 4.2.1. Ten percent of the water was siphoned to remove excreta and replaced after every two days. Daily rations were adjusted according to the body weight of the fry per tank after measuring their weight every week. The fry were weighed as explained in chapter two. Water temperature was kept at 28 ± 2ºC and light was set at a 12 h light: 12 h dark cycle. Water quality parameters were measured and tested as described for chapter two. All water quality parameters were within tolerance ranges published for tilapia species (El-Sayed, 2006). Mortality was monitored throughout the experimental period.

Table 4.2.1. Composition of experimental diet obtained from Aquanutro.

Analysed composition	Quantity (g/kg)
Protein	400
Moisture	120
Lipid	80
Fibre	40
Phosphorus	7
Calcium	30

4.2.2. Growth measurements

In each tank, all the fry were individually weighed every week to obtain a mean weight for the fry per tank. Weight gain, Specific growth rate and Gross food conversion ratio were calculated using the formulas described by Goddard (1996), Puvanendran *et al.* (2003) and Ajani *et al.* (2011) as follows:

Weight gain (G) = W_2-W_1

Specific growth rate (SGR) = (L_n (W_2) -L_n (W_1))/t x100

Gross food conversion ratio (GFCR) = F/G

Survival rate (SR) = Number of surviving fry/Number of fry initially stocked x 100

GFCR was calculated instead of food conversion ratio (FCR) reported by other authors because it was not possible to measure uneaten food. W_1 and W_2 are initial and final fish weight, respectively and t is the number of days in the feeding period. F is the weight of food fed and G is the weight gain of the fish during that duration.

4.2.3. Statistical analysis

Statistical analyses were done using the QED statistics program (Henderson and Seaby 2007). The Shapiro – Wilk test indicated no significant deviation from normality ($p > 0.05$) for replicate G, SGR, GFCR and SR values calculated for the different treatments. One–way analysis of variance was used to test for significant differences at a significance level of $\alpha = 0.05$ between the means of the treatments. The results were considered significantly different at a probability $(p) < 0.05$. Where there was a significant difference in means Tukey′s multiple comparison test was used to compare the variance among the means.

4.3. RESULTS

Feeding rate had a significant effect on the weight gain and gross food conversion ratio (ANOVA, Table 4.3.1) but not the specific growth rate and survival rate (ANOVA, Table 4.3.1). It was only the weight gain of the fry fed 10% of their body weight per day that was significantly lower than the weight gain of the fry fed 15%, 20% and 30% of their body weight per day (Tukey test, Table 4.3.3, and Figure 4A). Specific growth rates have increased with increasing feeding rate up to feeding rate of 20% body weight per day and then start to decrease slightly with further increased feeding rate (Figure 4B). There were no significant differences in gross food conversion ratio of the fry fed 10%, 15% and 20% of their body weight per day (Tukey test, Table 4.3.3 and Figure 4C). Gross food conversion ratio of the fry fed 25% and 30% of their body weight was significantly higher compared to the food conversion ratio of the fry fed 10%, 15% and 20% (Tukey test, Table 4.3.3). There were no significant differences in gross food conversion ratio between the fry fed 20% and 25% of their body weight per day (Tukey test, Table 4.3.3). The survival rate ranged from 82.5%-92.5% (Figure 4D). This shows that the rate of survival was high in all feeding rates.

Table 4.3.1. ANOVA results of weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR), and survival rate (SR) for *O. mossambicus* fry fed 10%, 15%, 20%, 25%, and 30% for 30 days. Degrees of freedom for all variables: between groups = 4, within groups = 15, F = F Statistic, and P = Probability

Variables	F	P
G	6.78617	0.002517
SGR	1.90894	0.161248
GFCR	24.0331	2.26E-06
SR	0.256098	0.901413

Table: 4.3.2. Mean weight gain, specific growth rate, gross food conversion ratio (GFCR) and survival rate for *O. mossambicus* fry fed 10%, 15%, 20%, 25% and 30% of their body weight per day for 30 days. Values are means (± SD) of four replicates for each treatment.

	Amounts of food offered (% body weight per day)				
Parameters	10	15	20	25	30
Weight gain (g)	0.56±0.04	0.89±0.14	0.89±0.26	0.78±0.06	1.09±0.15
Specific growth rate (%/d)	7.84±0.29	9.52±0.55	9.77±0.85	9.55±0.68	9.48±0.15
GFCR	1.79±0.17	2.34±0.18	3.01±0.38	4.31±0.36	5.62±1.24
Survival rate (%)	90.0±8.16	92.5±5.00	87.5±5.00	90.0±11.50	87.5±9.57

Table 4.3.3. Tukey's multiple comparison test summary results for weight gain (G) and gross food conversion ratio (GFCR) of *O. mossambicus* fry fed 10%, 15%, 20%, 25% and 30% of their body weight per day for 30 days. S = Tukey HSD statistic, P = probability and * = significant difference at $P < 0.05$.

Feeding rates (% bw/d)	G (g)		GFCR	
	S	P	S	P
10 vs 15	4.4081	0.0474*	1.3078	0.8830
10 vs 20	4.5251	0.0405*	3.4438	0.1593
10 vs 25	2.9077	0.2880	7.6508	0.0007*
10 vs 30	7.1186	0.0013*	11.9382	0.0001*
15 vs 20	0.1170	0.9999	2.1360	0.5715
15 vs 25	1.5003	0.8232	6.3431	0.0035*
15 vs 30	2.7106	0.3506	10.6304	0.0001*
20 vs 25	1.6174	0.7816	4.2071	0.0617
20 vs 30	2.5935	0.3915	8.4944	0.0003*
25 vs 30	4.2109	0.0614	4.2873	0.0556

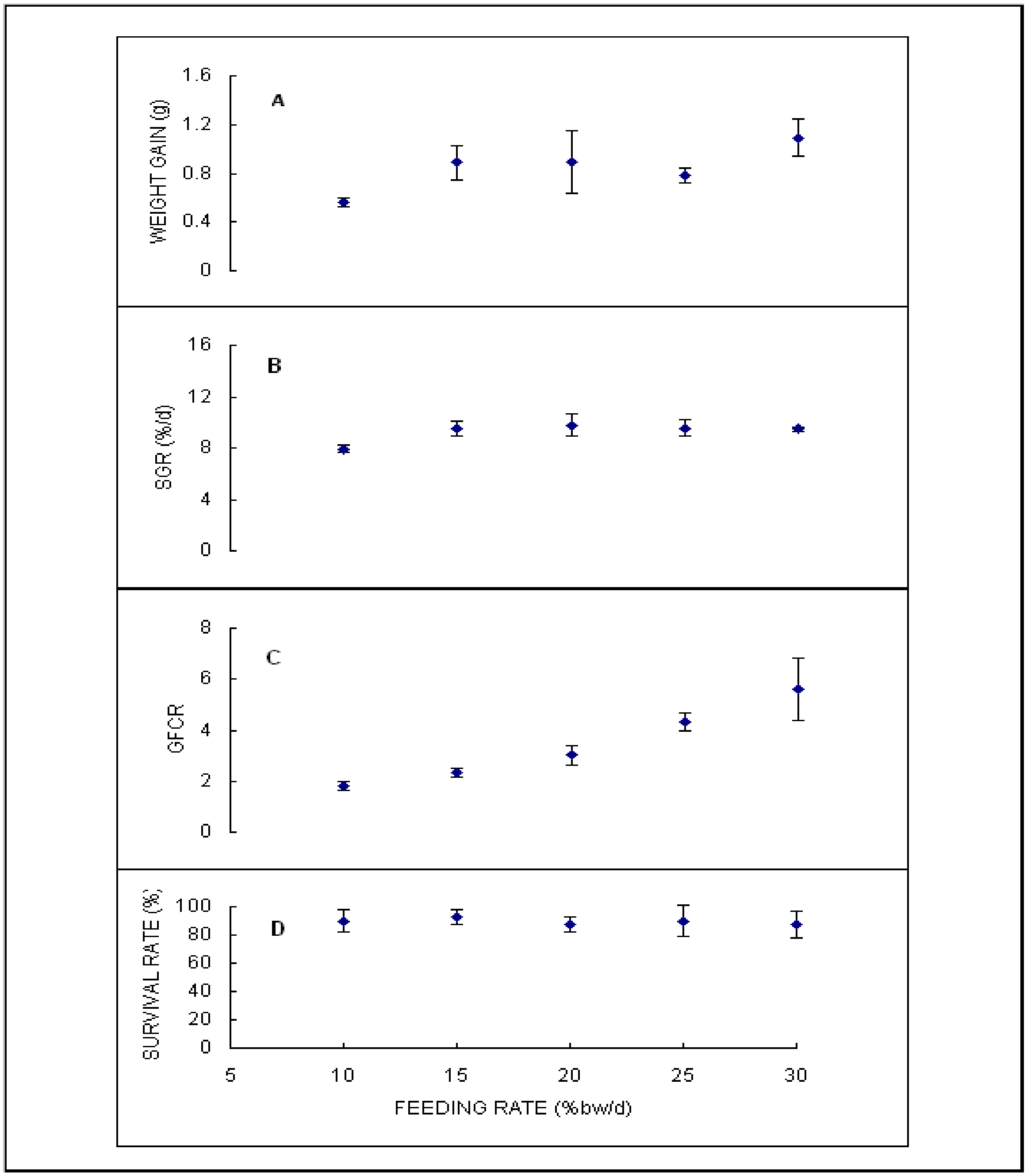

Figure 4. Average weight gain (A), SGR = specific growth rate (B), GFCR = gross food conversion ratio (C) and survival rate (D) of *O. mossambicus* fry fed different amount of food for 30 days. Values are means of four replicates for each treatment. Vertical bars indicate standard deviation.

4.4. DISCUSSION

Weight gains of *O. mossambicus* fry were significantly affected by the feeding rate, i.e. fry fed the highest percentage of their body weight per day (30%) had gained significantly more weight than those fed the lowest percentage of their body weight per day (10%). The same trends were obtained for polycultured Nile tilapia, Common carp and Silver carp (Abdelghany and Ahmad 2002), Olive flounder (Cho *et al*. 2006) and Cuneate drum (Wang *et al.* 2007). Determination of weight gain is important due to the fact that it is used together with the amount of food offered to determine the feed conversion ratio (Goddard 1996). Maximum weight gain resulting from the highest feeding rate, as in the present study, does not necessarily mean that this feeding rate is the optimum ration, especially if it is accompanied by a high feed conversion ratio value. This generally indicates that food was wasted through overfeeding (De Silva and Anderson 1995).

Unlike the weight gain, specific growth rate was not significantly affected by the feeding rate. This may be due to the fact that the formula used to calculate specific growth rate is based on the natural logarithm of the body weight (Goddard 1996). According to FNRL Group (1999-2006), this underestimates the weight gain between the initial and the final body weights. Furthermore specific growth rate is dependent on the initial body weight and is the main reason for the discrepancy in the results from different studies. Puvanendran *et al.* (2003) has reported similar results of specific growth rate that was not affected by the feeding rate in 0^+ yellowtail flounder. However, this is contrary to the findings of Abdelghany *et al.* (2002), and Cho, *et al.* (2006), who reported significant effect of feeding rate on specific growth rate for polycultured Nile tilapia, Common carp, and Silver carp and juvenile Olive flounder respectively.

Feeding rate had significantly affected the gross food conversion ratio in the present study. Fry that were fed at the lowest feeding rate (10% bw/day) showed the best gross food conversion ratio; however, taking into consideration the weight gain, specific growth rate together with gross food conversion ratio (which showed no significant differences for the fry fed 10%, 15% and 20%), the results support 15% as the optimum rate for *O. mossambicus* fry. This ration (15%) was selected as the optimum for O *mossambicus* fry over 20%, because less feed was used, there was a reduction of food wastage, which led to a significant beneficial impact on feed costs and quality fingerlings (same size) were produced. These results are in agreement with Qin & Fast (1996), and Eroldogan *et al.* (2004), who reported that the fish that are fed at low feeding rates tend to optimize their digestion to extract more nutrients efficiently. The fry that were fed 30% of their body weight per day had significantly poor gross food conversion ratio. Although the remaining feed was not measured, it was always observed in the tanks of fry fed 30% of their body weight per day. Meaning that the fry were fed above their appetite, and this resulted in loss of nutrients and food wastage as fish took longer time to consume food. Similar trend in gross food conversion ratio was observed in *Cirrhinus mrigala* fed at ration size higher than optimum (Khan *et al*. 2004).

The survival rates of *O. mossambicus* fry were not significantly affected by the feeding rate. Survival rates were more than 87% for all feeding rates. These results are similar to those reported for *O. niloticus,* European sea bass, juvenile Gilthead sea bream, 0^+ yellowtail flounder, juvenile Olive flounder and Cuneate drum respectively (Pouomogne and Mbongblang 1993, Mihelakakis *et al*. 2002, Puvanendran *et al*. 2003, Eroldogan *et al*. 2004, Cho *et al*. 2006, Wang *et al*. 2007;). However, some authors (Santiago *et al*. 1987, Abdelghany and Ahmad 2002) have reported significantly lower survival rates for lowest feeding rates in studies on *O. niloticus* fry and polycultured Nile tilapia, Common carp and Silver carp respectively.

Lovell (1989) recommended that feeding rates for tilapia fry should decrease from 30% body weight for 2 days old to 6% body weight on reaching a size of 5 grams. Taking into considerations weight gain, specific growth rate and gross food conversion ratio, the results support a feeding rate of 15% body weight per day as optimum. This is also within the range recommended by Lovell (1989) and De Silva and Anderson (1995). However, it is important to determine the optimum feeding rate for each size group because the recommended feeding rates are commonly generalized rates supplied by feed manufacturing companies. Goddard (1996) has emphasized that their tables provide only a general guide to feed intake and do not take into account either short or long-term appetite fluctuations in response to numerous physiological and environmental factors.

In conclusion, growth rate and gross food conversion ratio of the fry fed different amount of food were significantly affected by the amount of food offered. Feeding the fry 15% of their body weight was supported by the results as optimum ration in this study due to the fact that better growth rate and gross food conversion were obtained for the specific conditions and size group used in the present study.

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Chapter 5

Effect of dietary protein level, feeding frequency and feeding rate on gastric and intestinal evacuation of *O. mossambicus* fry.

5.1. INTRODUCTION

Gastric evacuation rate is defined as the rate at which food passes through the stomach and digestion is considered to be complete when the stomach becomes empty of all measurable remains (Hossain *et al.* 1998). Evacuation rate is affected by many factors including meal size, temperature, meal quality, fish size and methods of feeding (Karjalainen *et al.* 1991). Understanding the rate of digestion and its relationship to gastric evacuation rate can allow one to predict the return of appetite under a given set of conditions and diets. Information on gastric evacuation is needed to determine the frequency of feeding and for calculating food ration. According to De Silva and Anderson (1995), appetite is the desire for food. Complexes of mechanisms, including metabolic, neurophysiological, and hormonal, are involved in the regulation of appetite and feeding behavior in fish (Goddard 1996). Appetite is known to be affected by stomach fullness and rate of gastric evacuation, temperature, fish size, respiratory rate, circulating metabolites, glucose metabolism and dietary energy content (Jobling 1980, and Simon and Jeffs 2008).

The following methods are used to determine gastric evacuation rate in fish:

1. Sacrifice method

In this method, fish that have been fed a predetermined quantity of food are sacrificed at predetermined intervals and the amount of food remaining in the stomach is then estimated. The amount remaining in the stomach can be estimated as a percentage of the volume, weight (dry or wet), and ash-free dry weight or in calorific value of the amount ingested (De Silva and Owoyemi 1983).

2. X-radiography method

In this method, appropriate markers such as barium sulphate and iron particles are added to the food and tracked after feeding using X-rays. The advantage of this method is that there is no need to sacrifice any fish as live fish can be used repeatedly for this purpose. This is a quantitative method that can be used to measure food consumption, gut evacuation rates and assimilation efficiency (Talbot and Higgins 1983).

3. Use of dyes

Dyes, artemia shells and rubber pieces are incorporated into diets, and the time at which the dye, artemia shells or rubber pieces appears first in the faeces is determined (Riche *et. al*. 2004).

4. Direct observation

Diets are labeled in quantum and detected in faeces. One of disadvantage of this method is that, it is difficult to separate non-consumed food from faeces (Wuenschel and Werner 2004). This method is often recommended for larval stages when the gut and its contents are visible in transparent larvae.

In the present study sacrifice method was used rather than direct observations because the experimental fry used were very small and accurate direct observation not practical.

Currently, there are no studies published on effect of dietary protein level, feeding frequency and feeding rate or meal size on gastric evacuation of *O. mossambicus* fry. Some published gastric evacuation studies were those by Sveier *et al.* (1999), who investigated growth, feed and nutrients utilization and gastrointestinal evacuation time in Atlantic salmon, De Silva and Owoyemi (1983), who investigated the effect of dietary quality using *O. niloticus* and Flowerdew and Grove (1979), did some observation on the effects of body weight, temperature, meal size and quality on gastric emptying time in turbot. To address this paucity the present study aims to determine the effect of dietary protein level, feeding frequency and feeding rate on gastric evacuation rate of *O. mossambicus* fry.

5.2. MATERIALS AND METHODS

Three trials were conducted separately in a temperature and light cycle controlled environmental room at the University of Zululand. Procedures for all three trials conducted in this chapter are the same. Therefore experimental fry, data collection, calculations and statistical analysis for trials 2 and 3 referred to trial 1, to avoid repetitions.

5.2.1. Trial 1: Effect of dietary protein level on gastric and intestinal evacuation of *O. mossambicus* fry.

5.2.1.1. Experimental fry and treatment allocation

Fry were fed until a minimum size of c. 0.06 g. Thereafter, fry were stocked in twenty fiberglass tanks with a volume of 40 litres each and fed for five days, using the same treatments (diets of 25%, 30%, 35%, 40%, and 45% protein level) that were used for the growth experiment (Chapter two) before starting the experiment on gastric evacuation. Each treatment had four replicates with 15 fry each. After five days, the tanks were cleaned, and the fry were starved for sixteen hours after their last feed, (De Silva and Owoyemi 1983) because, according to Charles *et al.* (1984), fish that were fed after 12 h starvation increased its meal consumption to 83.6% of the maximum meal consumption. Further increase in the deprivation time to 24, 48 and 72 h decreased the meal consumption to 64%, 47% and 58%, respectively. After sixteen hours starvation, samples of eight fry per treatment were randomly selected for the determination of empty stomach and intestines. The remaining fry were fed 25%, 30%, 35%, 40% or 45% protein level at a daily feeding rate of 10% body weight, three times per day at 8:00, 12:00 and 16:00 h. Fifteen minutes after the last feed, the tanks were cleaned and samples of fry were selected as explained in data collection.

5.2.1.2. Data collection

Eight fry (two per replicate) were randomly selected from each treatment in intervals of 30 min, 1 hour, 2 hours, 3 hours, 4 hours and 5 hours after feeding. The fry were sacrificed in 1:2500 Benzocaine solutions and kept frozen at -15ºC until analysed (Gomes *et al*., 2001). Frozen fry were dissected. The stomach and intestines were weighed separately with their contents to determine the wet weight using an electronic balance. Wet weight was determined because the amount of food material that remained in the stomach and intestines was small and errors could have been introduced by trying to dry the contents (De Silva and Owoyeni 1983).

5.2.1.3. Calculations and statistical analysis

From the raw data of the wet weight of stomach and intestine with their contents, and the weight of empty stomach and intestine, the weight of food contents in the stomach and intestines were calculated. One–way analysis of variance was used to analyze the data. Where there were significant differences in means, Tukey multiple comparisons test was used. Evacuation rates were computed using the exponential model (De Silva and Owoyemi 1983) and expressed by the equation:
$Yx=Ae^{-RX,}$
where Yx = mean geometric weight of food contents at time X, A = a constant, R = rate of evacuation, and e = the natural logarithm. Evacuation times (GET and IET) were computed by using the linear form of the above equation as:
$\log_e Yx = \log_e A^{-RX}$

5.2.2. Trial 2: Effect of feeding frequency on gastric and intestinal evacuation of *O. mossambicus* fry.

5.2.2.1. Experimental fry and treatment allocation

Size of the fry and methods used were similar to trail one (as described in 5.2.1.1), however protein level treatments were replaced by feeding frequency treatments of once, twice, three, four and five times per day.

5.2.2.2. Data collection
Similar to trial 1 (see 5.2.1.2).

5.2.2.3. Calculations and statistical analysis
Similar to trial 1 (see 5.2.1.3).

5.2.3. Trial 3: Effect of feeding rate on gastric and intestinal evacuation of *O. mossambicus* fry.

5.2.3.1. Experimental fry and treatment allocation

Size of the fry and methods used were similar to trail one (see 5.2.1.1), however treatments were feeding amounts of 10%, 15%, 20%, 25% or 30% of the fry body weight per day.

5.2.3.2. Data collection
Similar to trial 1 (see 5.2.1.2.).

5.2.3.3. Calculations and statistical analysis
Similar to trial 1 (see 5.2.1.3.).

5.3. RESULTS

5.3.1. Dietary protein level on gastric and intestinal evacuation of *O. mossambicus* fry.

Although the fry had fed the same percentage of their body weight (10%), the results of the current study indicated that both stomach and intestinal contents decreased with time and varied from treatment to treatment at the first time of sampling (Figures 5A and 5B). Dietary protein level had a significant effect (ANOVA, Table 5.3.1.1) on gastric and intestinal evacuation rate of *O. mossambicus* fry. The rate of gastric evacuation for the fry that were fed 25% was significantly slower compared to those fed 35%, 40% and 45% protein level (Tukey test, Table 5.3.1.2). The rate of gastric evacuation for the fry that were fed 30% protein level was significantly slower than those fed 40% protein level (Tukey test, Table 5.3.1.2), gastric evacuation rate increased with increased dietary protein level (Table 5.3.1.3).

The intestinal evacuation rate also increased with dietary protein level up to 40% protein level and then starts to decrease slightly at 45% protein level (Table 5.3.1.4). Intestinal rate of the fry that were fed 25% protein level was significantly slower than those fed 40% protein level (Tukey test, Table 5.3.1.2).

Both gastric and intestinal evacuation time decreased with increased dietary protein level up to 40% and then slightly increased at 45%. Furthermore, the fry that were fed 40% protein level had used less time to evacuate their gastric and intestinal contents (Tables 5.3.1.3. and 5.3.1.4).

Table 5.3.1.1. ANOVA results of stomach and intestines food contents of *O. mossambicus* fry fed 25%, 30%, 35%, 40% and 45% protein levels for five days. Degrees of freedom for all variables: between groups = 4, within = 25, F = F Statistic, and P = Probability

	F	P
Stomach	9.44931	8.5235E-005
Intestines	3.08033	0.0342478

Table 5.3.1.2. Tukey′s multiple comparison test summary results of gastric and intestinal evacuation rate of *O. mossambicus* fry fed 25%, 30%, 35%, 40% and 45% protein level for five days. S= Tukey HSD statistic, P= probability value and * = significant difference at P<0.05.

Dietary protein level (%)	Stomach		Intestines	
	S	P	S	P
25 vs 30	3.0673	0.2240	3.2953	0.1688
25 vs 35	4.4696	0.0305*	2.9723	0.2507
25 vs 40	7.5369	0.0002*	4.7815	0.0184*
25 vs 45	7.0111	0.0005*	3.3599	0.1553
30 vs 35	1.4022	0.8567	0.3231	0.9994
30 vs 40	4.4696	0.0305*	1.4861	0.8293
30 vs 45	3.9437	0.0685	0.0646	0.9999
35 vs 40	3.0673	0.2240	1.8082	0.7059
35 vs 45	2.5415	0.3971	0.3877	0.9987
40 vs 45	0.5258	0.9957	1.4215	0.8506

Table 5.3.1.3. Regression equations (RE), Gastric evacuation rate (GER) and Gastric evacuation times (GET) of *O. mossambicus* fry fed different dietary protein levels for five days.

Dietary protein level (%)	RE	GER	GET (min) at (y=0)
25	$y= 2.2275e^{-0.0013x}$	-0.0013	1713.5
30	$y=2.0764e^{-0.0038x}$	-0.0038	546.4
35	$y= 1.8544e^{-0.0047x}$	-0.0047	394.6
40	$y=0.944e^{-0.0071x}$	-0.0071	132.9
45	$y=1.4532e^{-0.0091x}$	-0.0091	159.7

Table 5.3.1.4. Regression equations (RE), Intestinal evacuation rate (IER), and Intestinal evacuation time (IET) of *O. mossambicus* fry fed different dietary protein levels for five days.

Dietary protein level (%)	RE	IER	IET (min) at (y=0)
25	$y=3.2953e^{-0.003x}$	-0.003	1098.4
30	$y= 1.9757e^{-0.0031x}$	-0.0031	637.3
35	$y= 2.344e^{-0.0039x}$	-0.0039	601.0
40	$y=2.1648e^{-0.0068x}$	-0.0068	318.4
45	$y= 2.4171e^{-0.0051x}$	-0.0051	473.9

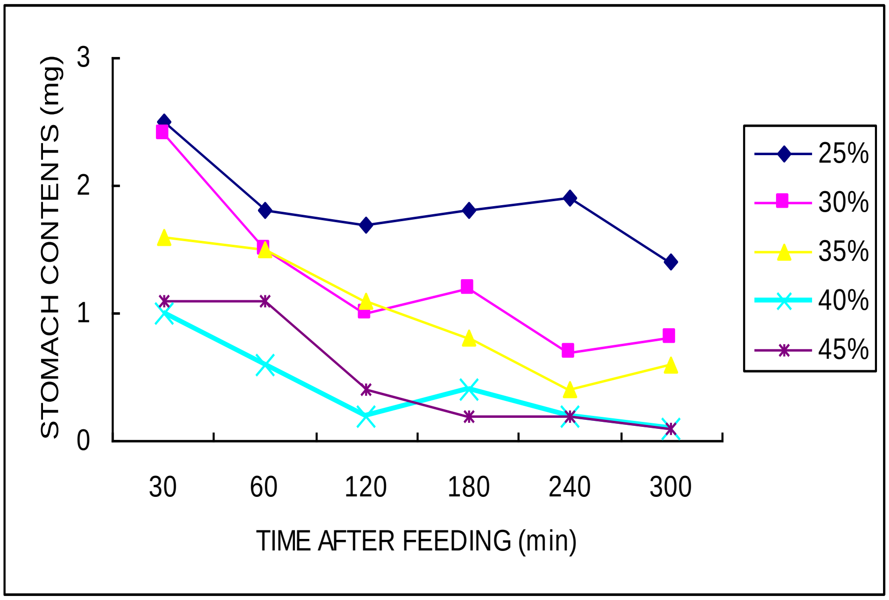

Figure 5A. The changes in mean stomach contents with time after feeding *O. mossambicus* fry different dietary protein levels

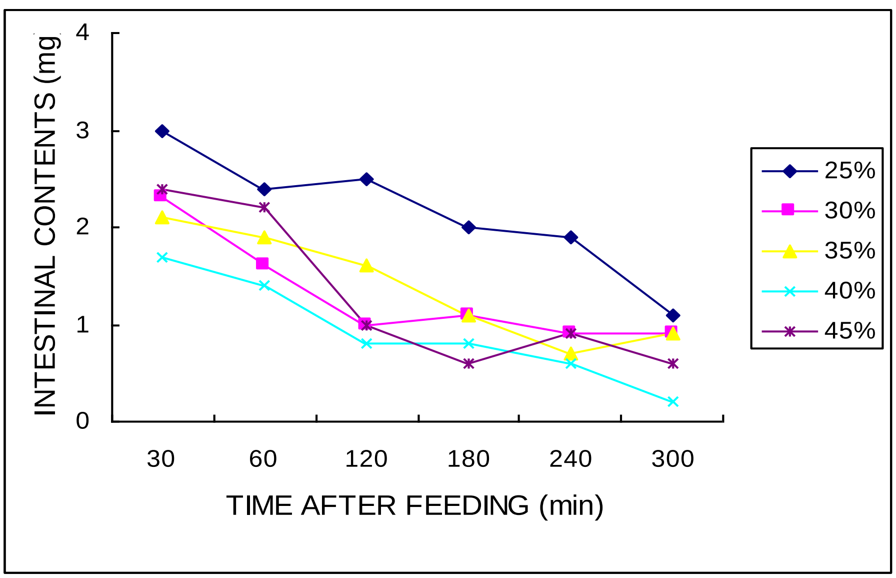

Figure 5B. The changes in mean intestinal contents with time after feeding *O. mossambicus* fry different dietary protein levels

5.3.2. Feeding frequency on gastric and intestinal evacuation of *O. mossambicus* fry

The results in figures 5C and 5D indicate that *O. mossambicus* fry fed at different frequencies same quantity of food, decreased their stomach and intestinal contents with time for all treatments. Feeding frequency had no significant effect (ANOVA, Table 5.3.2.1) on gastric evacuation rate and time of *O. mossambicus* fry. The rate of gastric evacuation increased with increased feeding frequency up to frequency of four times and then slightly decreased with further increased feeding frequency, while gastric evacuation time increased with increasing feeding frequency except for the fry fed once per day (Table 5.3.2.3).

Intestinal evacuation rate was affected by feeding frequency (ANOVA, Table 5.3.2.1). The fry that were fed once daily had significantly faster rate of evacuation compared to those fed five times daily (Tukey, Table 5.3.2.2 and Figure 5D). Except for the fry that were fed once daily, rate of intestinal evacuation had decreased with increasing feeding frequency while evacuation time increased with increasing feeding frequency (Table 5.3.2.4).

Table 5.3.2.1. ANOVA results of stomach and intestines food contents of *O. mossambicus* fry fed once, twice, three times, four times or five times per day for five days. Degrees of freedom for all variables: between groups = 4, within = 25, F = F Statistic, and P = Probability

	F	P
Stomach	2.24648	0.0926797
Intestines	3.86542	0.0140565

Table 5.3.2.2. Tukey′s multiple comparison test summary results of intestinal evacuation rate of *O. mossambicus* fry fed once, twice, three times, four times and five times per day for five days. S= Tukey HSD statistic, P= probability value and * = significant difference at $P<0.05$.

Feeding frequency (per day)	Intestines	
	S	P
Once vs twice	1.8101	0.7055
Once vs three times	3.7848	0.0857
Once vs four times	3.7848	0.0768
Once vs five times	4.9366	0.0142*
Twice vs three times	1.9747	0.6356
Twice vs four times	2.0569	0.5999
Twice vs five times	3.1265	0.2086
Three times vs four times	0.0823	0.9999
Three times vs five times	1.1519	0.9237
Four times vs five times	1.0696	0.9407

Table 5.3.2.3: Regression equations (RE), Gastric evacuation rate (GER), and Gastric evacuation time (GET) of *O. mossambicus* fry fed different times per day for five days.

Feeding frequency (per day)	RE	GER	GET (min) at (y=0)
Once	$y=0.4405e^{-0.0943x}$	-0.0943	4.67
Twice	$y=1.1094e^{-0.3382x}$	-0.3382	3.28
Three times	$y=2.6453e^{-0.5253x}$	-0.5253	5.04
Four times	$y=3.4629e^{-0.5158x}$	-0.5158	6.71
Five times	$y=2.5534e^{-0.2673x}$	-0.2673	9.55

Table 5.3.2.4: Regression equations (RE), Intestinal evacuation rate (IER), and Intestinal evacuation time (IET) of *O. mossambicus* fry fed different times per day for five days.

Feeding frequency (per day)	RE	IER	IET (min) at (y=0)
Once	$y=1.1446e^{-0.0007x}$	-0.0007	1635.14
Twice	$y=2.9664e^{-0.0053x}$	-0.0053	559.69
Three times	$y=2.6772e^{-0.0024x}$	-0.0024	1115.50
Four times	$y=2.5483e^{-0.002x}$	-0.002	1274.15
Five times	$y=2.2648e^{-0.0004x}$	-0.0004	5662.00

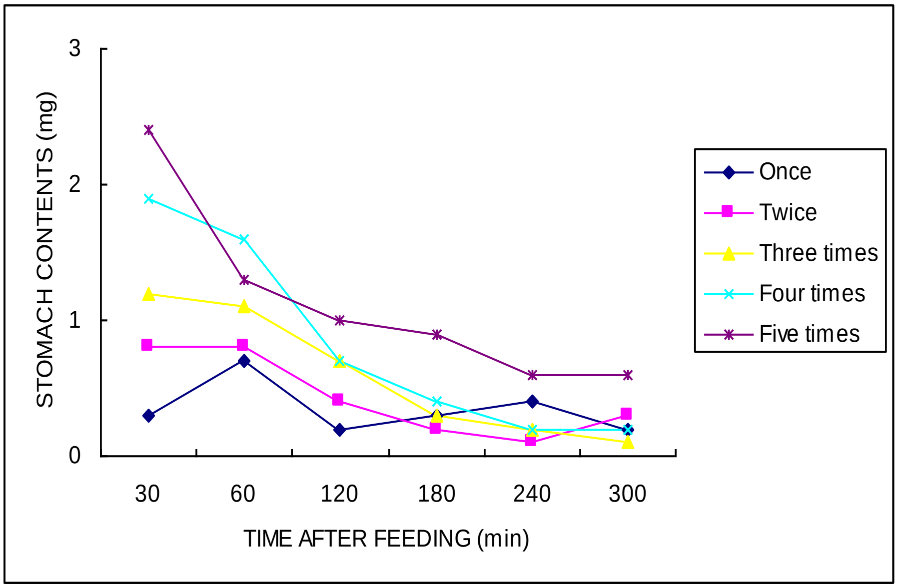

Figure 5C. The changes in mean stomach contents with time after feeding *O. mossambicus* fry different feeding frequency.

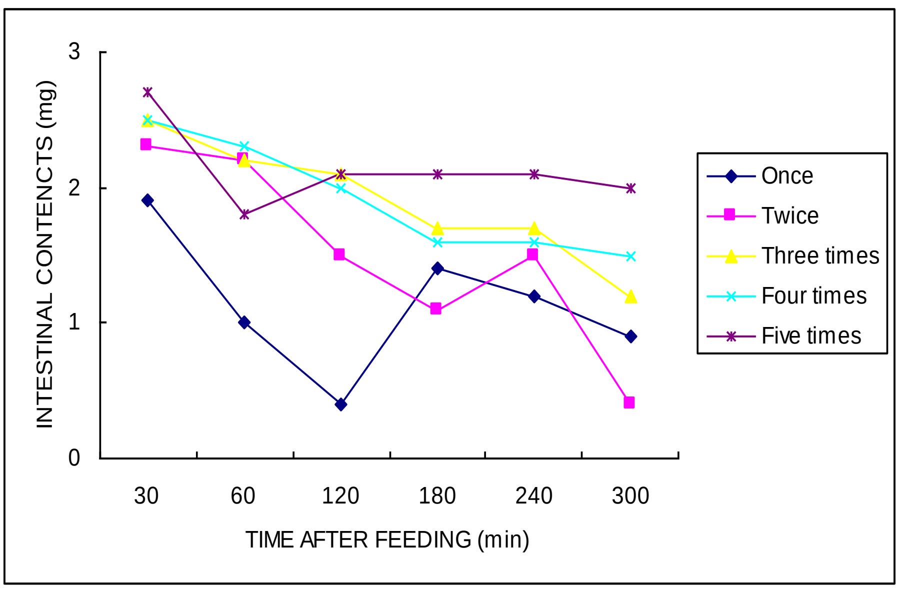

Figure 5D. The changes in mean intestinal contents with time after feeding *O. mossambicus* fry different feeding frequency.

5.3.3. Feeding rate on gastric and intestinal evacuation of *O. mossambicus* fry.

The results of the current study indicated that stomach and intestinal contents of *O. mossambicus* fry fed different amount of food decreased with time (Figures 5E and 5F). Feeding rate had no significant effect (ANOVA, Table 5.3.3.1) on gastric and intestinal evacuation rate of *O. mossambicus* fry. Except for the fry that were fed 10% of their body weight, gastric and intestinal evacuation rate increased with increased feeding rate (Tables 5.3.3.2 and 5.3.3.3). Like in gastric evacuation rate, gastric evacuation time increased with increasing feeding rate up to 25% body weight per day and then slightly decreased at 30% body weight per day (Table 5.3.3.2). Intestinal evacuation time decreased with increased feeding rate up to 15%, increased at 20%, decreased at 25% and then increased again at 30% body weight per day (Table 5.3.3.3). However, the fry that were fed 15% of their body weight per day had used the shortest time to evacuate their stomach and intestinal contents (Tables 5.3.3.2. and 5.3.3.3).

Table 5.3.3.1. ANOVA results of stomach and intestines food contents of *O. mossambicus* fry fed 10%, 15%, 20%, 25%, and 30% of their body weight per day for five days. Degrees of freedom for all variables: between groups = 4, within = 25, F = F Statistic, and P = Probability

	F	P
Stomach	0.523192	0.719562
Intestines	0.622538	0.650703

Table 5.3.3.2: Regression equations (RE), Gastric evacuation rate (GER), and Gastric evacuation time (GET) of *O. mossambicus* fry fed 10%, 15%, 20%, 25%, and 30% of their body weight per day for five days.

Feeding rate (%bw/d)	RE	GER	GET (min) at (y=0)
10	$y=11.81e^{-0.0128x}$	-0.0128	922.7
15	$y=1.2188e^{-0.0035x}$	-0.0035	348.2
20	$y=4.3605e^{-0.0066x}$	-0.0066	660.7
25	$y=6.0538e^{-0.009x}$	-0.009	672.6
30	$y=6.1703e^{-0.0098x}$	-0.0098	629.6

Table 5.3.3.3: Regression equations (RE), Intestinal evacuation rate (IER), and Intestinal evacuation time (IET) of *O. mossambicus* fry fed 10%, 15%, 20%, 25%, and 30% of their body weight per day for five days.

Feeding rate (%bw/d)	RE	IER	IET (min) at (y=0)
10	$y=19.98e^{-0.011x}$	-0.011	1816.4
15	$y=1.6941e^{0.0011x}$	0.0011	-1540.1
20	$y=7.0635e^{-0.006x}$	-0.006	1177.3
25	$y=11.22e^{-0.0104x}$	-0.0104	1078.8
30	$y=20.342e^{-0.0126x}$	-0.0126	1614.4

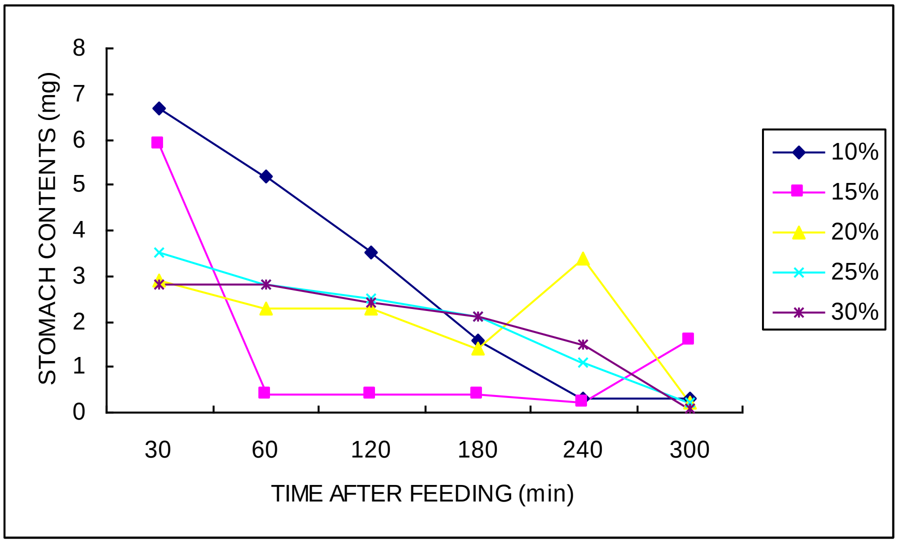

Figure 5E. The changes in mean stomach contents with time after feeding *O. mossambicus* fry different ration size.

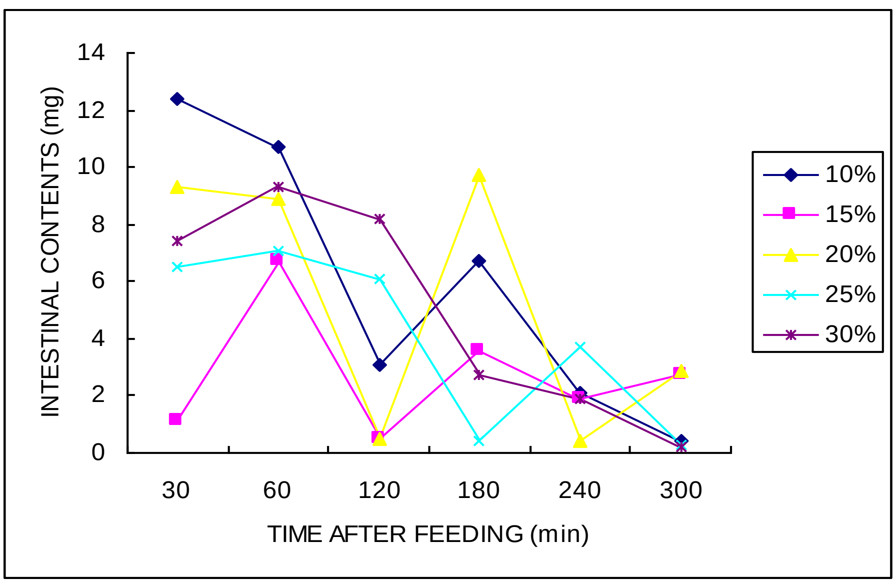

Figure 5F. The changes in mean intestinal contents with time after feeding *O. mossambicus* fry different ration size.

5.4. DISCUSSION

5.4.1. Effect of dietary protein level on gastric and intestinal evacuation of *O. mossambicus* fry

According to De Silva and Owoyemi (1983), sacrifice method had one disadvantage which is the difficulty to ensure that the initial amount ingested remain the same. This explains the variation in stomach and intestinal contents among the treatments during the first sampling time and there were no samples taken at time zero after feeding. The results of the current study indicated that dietary protein level had a significant effect on both gastric and intestinal evacuation rate. The fry that were fed lower protein level had slower rate of evacuation, while those fed higher protein level had a faster evacuation rate. This is similar to the findings for other animals, e.g. Shi *et al.* (1997) who reported significantly faster gastric emptying for rats fed higher protein diets and delayed emptying rate for rats fed lower protein diets. However, the results of the current study are contrary to the finding of Sveier *et al.* (1999), who reported that protein concentration had no effect on the time of evacuation in Atlantic salmon. According to Tekinay *et al.* (2003a) dietary protein level is among the most important factors affecting both gastric evacuation and appetite revival. However, most studies conducted on effect of dietary protein level were only focusing in growth rate and did not include its effect on gastric evacuation and appetite revival. This makes it difficult to find results from other studies to compare with the results of the current study. The fry that were fed a diet contained 40% protein level had used the shortest time to evacuate both gastric and intestinal contents. Therefore, 40% is regarded as the optimum level for gastric evacuation of *O. mossambicus* fry.

5.4.2. Effect of feeding frequency on gastric and intestinal evacuation of *O. mossambicus* fry

Variations in stomach and intestinal contents at first sampling time may be due to the fact that samples of fry were only selected 30 minutes after feeding and unequal initial amount were ingested. The results of the current study indicated that feeding frequency had no significant effect on gastric evacuation of *O. mossambicus* fry. These results are in agreement with the finding of Riche *et al.* (2004) who reported no significantly differences in instantaneous evacuation rates and suggested that gastric evacuation does not depend on feeding frequency. However, the results contradict the findings of Tekinay *et al.* (2003b), who reported a faster evacuation rate for Rainbow trout fed a single meal compared to those fed two or three times per day, Garcia-Galano *et al.* (2003), who reported a diminished gastric evacuation time as the number of meals per day increased in juvenile snook and Lee *et al.* (2000), who reported a close relationship between gastric evacuation rate and feeding frequency for juvenile Korean rockfish.

According to Booth *et al.* (2008) feeding too frequently can result in poorer feed conversion ratio due to increase gastric evacuation ratios or gastrointestinal overload. Where the intake of the next meal occurs before the previous bolus has been subjected to adequate gastric digestion. The fry that were fed twice per day had used the shortest time to evacuate their gastric and intestinal contents. Therefore, twice per day is the optimum feeding frequency for gastric evacuation of *O. mossambicus* fry. Different studies (Tekinay *et al.* 2003b, Garcia-Galano *et al.* 2003 and Lee *et. al.* 2000) have been conducted and different optimum feeding frequencies (once, three times, and once, respectively) attained because the optimum feeding frequency varies with species, size, environmental factors and food quality (Goddard 1996).

5.4.3. Effect of feeding rate on gastric and intestinal evacuation of *O. mossambicus* fry

The same reasons explained in dietary protein level and feeding frequency are the causes of variations in stomach and intestinal contents at first sampling time. In the present study, feeding rate had no significant effect on gastric and intestinal evacuation rate and time. The results are in agreement with Elliott and Persson (1978) who stated that the exponential rate of gastric evacuation is apparently not affected by fish size, meal size and frequency of feeding. Only the absolute rate (i.e. the quantity of food evacuated per unit time) is affected by these factors. Furthermore, there were no significant differences in gastric and intestinal evacuation rates (GER and IER) and times (GET and IET) for fry fed different amount of food. However the results of present study indicated that GER, GET, IER and IET increased with increased feeding rate (except for the fry fed 10% of their body weight). These results are in agreement with Garber (1983) who stated that larger meals are generally known to have increased gastric evacuation rate and Jobling and Davies (1979) who reported increased gastric evacuation time with increasing meal size, when plaice of a given weight were fed different sized meals. Other studies conducted on effect of meal size (feeding rate in current study) reported different results, e.g. Elliot (1972) reported non significant differences on rate of gastric evacuation for brown trout fed different sized meals. However, Persson (1981) reported a constant instantaneous rate of gastric evacuation for a large number of different meal sizes for perch, *Perca fluviatis*, while Paakkonen (1999) reported a decreased instantaneous rate with increases in meal size in burbot, *Lota lota*. Flowerdew and Grove (1979) found gastric evacuation rate to be proportional to meal size for turbot.

The discrepancy in results obtained from current study and other studies maybe due to the fact that feeding rate depend on fish size and species used. The amount of 15% body weight per day is regarded as the optimum level for gastric evacuation of *O. mossambicus* fry because fry fed at this level took the shortest time to evacuate both gastric and intestinal contents.

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Chapter 6

Synthesis

6.1. Synthesis

Available literature (Lovell 1989, Jauncey and Ross 1982, Thorpe and Hecht 1992 and Goddard 1996) had indicated that dietary protein level, feeding frequency and feeding rate depend on the size of the fish cultured and culture conditions. Although *O. mossambicus* is cultured worldwide, there is no published information available on effect and optimum dietary protein level, feeding frequency and feeding rate on growth, gastric and intestinal evacuation under hatchery conditions, especially for fry. However, lack of information does not necessarily mean that this species is less important in aquaculture. According to Hoffman, Swart and Brink (2000), Brink (2003) and Henrichsen and Brink (2004), the production of *O. mossambicus* in South Africa increased from 45 Mt in 1998 , 130 Mt in 2000 to 220 Mt in 2003 respectively. This increased production means that this species is important for aquaculture. Furthermore, Nature Conservation controls Tilapia species to be cultured, and South African aquaculturists and/or farmers are restricted to culture *O. mossambicus* as *O. niloticus* is an exotic species.

According to Bahnasawy (2009), excess protein in fish diet may be wasteful and cause diets to be unnecessarily expensive. Furthermore, when fish are fed insufficiently or excessively, their growth or feed efficiency may decrease, resulting in increasing production cost, and water quality deterioration (Lovell 1989). Therefore the overall objectives of this study were:

1. To determine the optimum dietary protein level on growth, gastric and intestinal evacuation of *O. mossambicus* fry under local hatchery conditions.
2. To determine the optimum feeding frequency on growth, gastric and intestinal evacuation of *O mossambicus* fry under local hatchery conditions.
3. To determine the optimum feeding rate on growth, gastric and intestinal evacuation of *O. mossambicus* fry that will minimize feeding costs while promoting growth under local hatchery conditions.

From the current study, the effect and optimum dietary protein level, feeding frequency and feeding rate on growth, gastric and intestinal evacuation of *O. mossambicus* were determined, and the results indicated that both dietary protein level and feeding frequency had a significant effect on weight gain, specific growth rate and gross food conversion ration but not survival rate. While feeding rate had a significant effect on weight gain and gross food conversion ratio but not in specific growth rate and survival rate. A diet containing 30% protein level, feeding frequency of four times per day and 15% of the fry body weight per day were the optimal levels obtained from the growth experiments. Furthermore, dietary protein level had a significant effect on gastric evacuation; feeding frequency had a significant effect on intestinal evacuation rate and time only but not on gastric evacuation rate and time, while feeding rate had no significant effect on both gastric and intestinal evacuation rate and time.

The optimum levels obtained in gastric and intestinal evacuation (chapter 5) are 40% dietary protein level, feeding frequency of twice per day and a feeding rate of 15% body weight per day. As different levels were tested, it is clear that without determining the optimum levels for each specific size group, fish may be fed insufficiently or excessively in terms of protein level, feeding frequency and feeding rate. That will result in unnecessary production costs increases and water quality deterioration, especially when fish are overfed.

Future studies should investigate interdependence between these factors on growth, gastric and intestinal evacuation using different size groups under hatchery conditions.

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