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Incorporation of copra meal in the formulation of diet for milkfish (*Chanos chanos*), grey mullet (*Mugil cephalus*), red snapper (*Lutjanus campechanus*) and mangrove red snapper (*Lutjanus argentimaculatus*)

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Abstract

The study was conducted with an aim of formulating a cheap feed that can promote fish growth and survival. The milkfish, grey mullet (herbivorous), mangrove red snapper and red snapper (carnivorous) were cultured in cages. The culture diets formulated included: "sagana" (control-25% crude protein (CP)), 6%_s (6% sardine content) formulated (23% CP), 12%_s (12% sardine content) formulated (23% CP) and snapper formulated (12%_s) (25% CP). Milkfish's carcass lipid content increased with the increase in the amount of copra meal incorporated in fish diets. Milkfish's survival was high while grey mullet's was low when fed with "sagana", 6%_s formulated and 12%_s formulated diets. Survival of mangrove red and red snapper fed with "sagana" was higher than in the snapper formulated diet. There was insufficient fish growth in all the fish species cultured. Incorporation of copra meal aided in feed cost reduction by 3 times (6%_s formulated diet). The feeding level of 3% of the body weight once daily was low because it did not promote fish growth. The culture conditions were not at their optimum due to the low dietary protein, feeding level and feeding frequency. This led to low survival in grey mullet and insufficient fish growth. The incorporation of 12% fish meal could be appropriate for fish diets. The incorporation of copra meal in fish diets aids in feed cost reduction. The feeding level of 3% with the low crude protein diets was low and thus led to poor fish growth in the fish species cultured and low survival in grey mullet. Further investigation on incorporation of copra meal in fish diets and its impact on fish growth are recommended before full scale implementation. The incorporation of automatic feeders is recommended.

Key Words: Milkfish, grey mullet, red snapper, mangrove red snapper, copra meal

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Table of Contents

Abstract	i
Acknowledgements	iii
List of Figures	vii
List of Tables	ix
Abbreviations	x
1. Introduction	1
1.1. Fish culture and fish feeds	1
1.2. Problem statement	2
1.3. Justification	3
1.4. Objectives	3
1.5. Hypothesis	4
2. Literature review	5
2.1. Overview on mariculture	5
2.2. Enhancement of fish diets by incorporation various fish feed ingredients	6
2.3. Effect of dietary protein and feeding levels on fish growth	9
2.4. Formulation fish diets with the incorporation of copra meal	11
2.5. Investigation of the length-weight relationships in fish	12
2.6. Macrofouling and the use of fish to reduce it	13
3. Materials and methods	15
3.1. Approach	15
3.2. Study area	15
3.3. Experimental setup	16
3.3.1. Experimental fish	17
3.4. Proximate analysis procedure	18
3.4.1. Fish feed ingredients	18
3.4.2. Proximate analysis procedure	19
3.4.3. Preparation of fish diets	19
3.4.4. Proximate analysis of the fish feeds and milkfish carcass	21
3.5. Feeding	21
3.6. Sampling	21
3.6.1. Fish sampling	21
3.6.2. Water quality	21
3.7. Statistical analysis	22
3.7.1. Survival	22
3.7.2. Fulton's condition Factor (K)	22
3.7.3. Length-weight relationship	22
3.7.4. Feed cost test	23
3.7.5. Growth performance	23
3.8. Reducing macrofouling	23

4. Results	24
4.1. Proximate composition	24
4.1.1. Fish feed ingredients	24
4.1.2. Fish feeds	25
4.1.3. Milkfish carcass	26
4.2. Water quality	27
4.3. Multiple regression of fish length and water quality variables with the fish weight	28
4.4. Survival	30
4.5. Condition factor	32
4.6. Fish growth and the length-weight relationship	34
4.6.1. Fish weight at the start and end of the culture period	34
4.6.2. Length-weight relationship	35
4.7. Variation of weight with time	39
4.8. Growth performance of fish	43
4.9. Cost of feed	46
4.10. Reducing macrofouling	47
5. Discussion	48
5.1. Proximate composition of fish feed ingredients, fish feeds and milkfish carcass	48
5.2. Effect of fish length and water quality parameters on fish weight	49
5.3. Fish survival and condition factors	50
5.4. Fish growth	51
5.5. Length-weight relationship	52
5.6. Variation of fish weight with time	52
5.7. Fish growth performance	53
5.8. Feed cost test	54
5.9. Use of the grazers to control macrofouling in cages	55
5.10. Significance of the study	55
6. Conclusion	57
6.1. Recommendation	57
References	59
Appendices	65
Appendix A (ANOVA and Post Hoc results at 95% CI)	65
Appendix B (Multiple regression tables for the respective fish species)	70
Appendix C (Photographs showing the mangrove and red snapper cages before and after adding the grazers to the respective cages)	72

List of Figures

Figure 3.1	Map of Kenya showing the site where the experiment was conducted (Makongeni (Mombasa)) (Openstreetmap.org, 2015)	16
Figure 3.2	The 6% formulated, 12% formulated, snapper formulated and "sagana" diet and the various fish species to which they were fed (milkfish and grey mullet polyculture was done in three treatments while mangrove red snapper and red snapper polyculture was done in two treatments and all had three replicates) ((GM - Grey mullet), (MF - Milkfish), (MS - Mangrove red snapper) and (RS - Red snapper) and the number represents the number of fish stocked (15+15 means 15 fish were stocked at the start and 15 added later)).....	18
Figure 4.1	Survival of mangrove red snapper and red snapper fed with the snapper formulated diet and "sagana" diet (error bar represents standard error)	31
Figure 4.2	Survival of milkfish and grey mullet fed with 6% formulated diet, 12% formulated diet and "sagana" diet (error bar represents standard error)	32
Figure 4.3	Condition factor of mangrove red snapper and red snapper fed with the snapper formulated and "sagana" diet (error bar represents standard error)	33
Figure 4.4	Condition factor of milkfish and grey mullet fed with 6% formulated diet, 12% formulated diet and "sagana" diet (error bar represents standard error)	34
Figure 4.5	Length-weight relationship of milkfish fed with the "sagana" diet, 6% formulated diet and 12% formulated diet	36
Figure 4.6	Length-weight relationship for grey mullet fed with "sagana" diet, 6% formulated diet and 12% formulated diet	37
Figure 4.7	Length-weight relationship for mangrove red snapper fed with "sagana" diet and the snapper formulated diet	37
Figure 4.8	Length-weight relationship of red snapper fed with "sagana" diet and the snapper formulated diet.....	38
Figure 4.9	Variation of the milkfish weight with time in the 12% formulated diet, 6% formulated diet and "sagana" diet treatments (NOV=November, DEC=December and JAN=January) ..	39
Figure 4.10	Variation of the grey mullet weight with time in the 12% formulated diet, 6% formulated diet and "sagana" diet treatments (NOV=November, DEC=December and JAN=January).....	40
Figure 4.11	Variation of the mangrove red snapper weight with time in the "sagana" and snapper formulated diet treatments (NOV=November, DEC=December and JAN=January)	41
Figure 4.12	Variation of the red snapper weight with time in the "sagana" and snapper formulated diet treatments (NOV=November, DEC=December and JAN=January).....	42
Figure 4.13	Growth performance of milkfish fed with the respective diets (1("sagana" diet), 2 (6% formulated diet) and 3(12% formulated diet))	43
Figure 4.14	Growth performance of grey mullet fed with the respective diets (1("sagana" diet), 2 (6% formulated diet) and 3(12% formulated diet))	44
Figure 4.15	Growth performance of mangrove red snapper (fed with the respective diets 1 ("sagana" diet) and 2(snapper formulated diet))	45
Figure 4.16	Growth performance of red snapper fed with the respective diets (1 ("sagana" diet) and 2(snapper formulated diet))	45
Figure C.1	Cage 1 before adding the grazers to the stock	72
Figure C.2	Cage 1 one month after adding grazers to the stock	72
Figure C.3	Cage 2 before adding grazers to the stock	73
Figure C.4	Cage 2 one month after adding the grazers to the stock	73
Figure C.5	Cage 3 before adding the grazers to the stock	74
Figure C.6	Cage 3 one month after adding the grazers to the stock	74

Figure C.7	Cage 4 before adding grazers to the stock	75
Figure C.8	Cage 4 one month after adding grazers to the stock to the stock.....	75
Figure C.9	Cage 5 before adding grazers to the stock to the stock.....	76
Figure C.10	Cage 5 one month after adding the grazers to the stock	76
Figure C.11	Cage 6 before adding the grazers to the stock	77
Figure C.12	Cage 6 one month after adding grazers to the stock	77

List of Tables

Table 3.1	Formulation and composition of the diets and their crude protein content	20
Table 4.1	Proximate composition of fish feed ingredients	25
Table 4.2	Proximate composition of fish feeds	26
Table 4.3	Proximate composition of carcass composition of milkfish (<i>Chanos chanos</i>).....	27
Table 4.4	The pH, temperature, conductivity and dissolved oxygen at the experimental site.....	27
Table 4.5	Multiple regressions of fish length and water quality parameters with milkfish weight.....	28
Table 4.6	Multiple regressions of fish length and water quality parameters with grey mullet weight...	29
Table 4.7	Multiple regressions of fish length and water quality parameters with mangrove red snapper weight.....	29
Table 4.8	Multiple regressions of fish length and water quality parameters with red snapper weight...	30
Table 4.9	The average weight of fish at the start and end of culture period.....	35
Table 4.10	Fish feed ingredients and their costs per kilogram as per the market prices (October, 2014).....	46
Table 4.11	Cost of a kilogram of feed in the respective diets (October, 2014)	46
Table A.1	ANOVA result of the mangrove red snapper and red snapper condition factor.....	65
Table A.2	ANOVA result of the mangrove red snapper and red snapper survival	65
Table A.3	ANOVA result of the survival of milkfish and grey mullet	65
Table A.4	Bonferroni result of the survival of milkfish and grey mullet fed on "sagana" diet, 6% diet and 12% s diet	66
Table A.5	ANOVA result of the milkfish and grey mullet condition factor fed on 6% s diet and 12% s diet and "sagana" diet	66
Table A.6	Bonferroni result of the condition factor of milkfish and grey mullet fed on "sagana" diet, 6% s diet and 12% s diet.....	66
Table A.7	ANOVA results of proximate composition of milkfish.....	67
Table A.8	Bonferroni result of the lipid content of milkfish fed to the various diets.....	67
Table A.9	Bonferroni result of the ash content of milkfish fed to the various diets.....	68
Table A.10	T- test results of the difference in the weight of fish before and after the experiment	69
Table B.1	Multiple regressions of fish length and weights of milkfish fed with the "sagana" diet, 6% s formulated diet and 12% s formulated diet.....	70
Table B.2	Multiple regressions of fish length and weights of grey mullet fed with the "sagana" diet, 6% s formulated diet and 12% s formulated diet.....	70
Table B.3	Multiple regressions of fish length and weight of mangrove red snapper fed with "sagana" diet and snapper formulated diet	71
Table B.4	Multiple regressions of fish length and weight of red snapper fed with "sagana" diet and snapper formulated diet	71

Abbreviations

CF	Crude fibre
CP	Crude protein
DM	Dry matter
EE	Ether extracts (crude lipids)
FCR	Feed conversion ratio
K	Condition factor
PER	Protein efficiency ratio
SS	Sum of squares
DF	Mean difference
SE	Standard error
GM	Grey mullet
MS	Mangrove red snapper
MF	Milkfish
RS	Red snapper
MF before	Milkfish before the experiment
6%s	6% sardine content
12%s	12% sardine content
s	Sardine
LnL	Natural logarithm of Length
"Sagana" diet	Control diet in experiment (formulated by the Kenya Marine and Fisheries Research institute-Sagana station)
Omena	Dagaa (another local name)
Kg	Kilogram

CHAPTER 1

Introduction

1.1. Fish culture and fish feeds

Fish feeds account for about 40%-60% (Charo-Karisa *et al.*, 2013) of the fish production cost depending on the intensity of fish culture. The high cost of fish feeds is a constraint to the growing aquaculture ventures. The commercial fish feeds available are unaffordable for most rural sub-Saharan fish farmers (Charo-Karisa *et al.*, 2013). The price of commercial feeds is high due to increase in fish meal price. Its price is increasing due to increased demand and reduced supply from capture fisheries (Borski *et al.*, 2011). Fish meal has been used as the primary protein source and it constituted 20%-50% of the diet (Davis, Brown and Camilleri, 1997; Nguyen, 2008).

The financial viability of controlled culture of fish relies mainly on the cost of fish feed and principally protein: a costly constituent of artificial diet (Papaparaskeva-Papoutsoglou and Alexis, 1986). The cost of feed per unit of output for the cultured species depends primarily on: the feed conversion ratio and the unit price of the feed (Yung, 1995). The intensity of fish production depends mainly on: availability of feed that covers the nutritional needs of the cultured fish species (Abdel-Halim, 2009). The cost of production is reduced by formulation fish feeds using the locally available ingredients (Nguyen, 2008).

Mariculture is the rearing of aquatic organisms in brackish and saline water. Mariculture is a growing industry worldwide. This is due to increase in demand of marine products and the decrease in the catch from capture fisheries. The increase in mariculture in the tropical countries has led to a shift from extensive to intensive fish culture methods (Troell, 2009). This expansion of aquaculture and the production of teleost fish under the intensive culture

system necessitate provision of feed. Its success relies considerably on the availability of well balanced and cost- effective diet that meets the nutritional requirements of the cultured fish species (Davis, Brown and Camilleri, 1997; Borlongan, Eusebio and Welsh, 2003).

The rising cost of fish meal poses a setback in the formulation of cost-effective fish feeds. This has necessitated partial or complete replacement of fish meal by less costly plant protein sources (Seneriches and Chui, 1988). The plant protein ingredients sources can be raw or semi-processed agro-industrial by products (Charo-Karisa *et al.*, 2013). The replacement of fish meal partially or entirely by less expensive and locally available ingredients: aids in lowering the unit price of the feed (Yung, 1995). Various plant protein sources have been incorporated successfully in fish feed formulations (Borlongan, Eusebio and Welsh, 2003).

The crude protein content of the formulated feeds and amount of feed fed to fish can influence its growth (Sumagaysay and Borlongan, 1995). Fish growth is a result of the quantity and quality of feed the fish are fed. Therefore, the energy and protein a fish gains is as a result of the quantity of feed. The feed supplied should provide the fish's needs to be able to promote growth (Lupatsch, 2007)

Amongst the ingredients that can be incorporated in fish feed formulation is copra meal. This is a residue obtained after mechanical extraction of oil from coconut. It contains 7% oil and 20% - 25% DM of crude protein (Ohler, 1993). The main objective of the study was to formulate of cheap fish feed that can promote fish growth and survival by: (i) incorporation of copra meal in fish diet formulation, (ii) determining the appropriate level of fish meal that should be included in fish diets and (iii) examining if feeding at 3% body weight can promote fish growth.

1.2. Problem statement

Mariculture taps vast sources of bio-energy that can be exploited sustainably for the benefit of mankind (Nammalwar, 1997). There is extensive land, brackish and marine water areas available for aquaculture development. Its success relies significantly on a well balanced and cost effective diet that meets the nutritional requirements of the cultured species (Borlongan, Eusebio and Welsh, 2003).

Feed is among the major cost (Lupatsch, 2007). The cost of the feed can be reduced by incorporating the locally available ingredients in fish feed formulation as stated above. It is important to determine the lowest level of fish meal inclusion that can be able to support fish

growth and survival. Considerable research has been done on the replacement of fish meal with various locally available ingredients (Abbas *et al.*, 2005; Nguyen, 2008). However, there is need to continuously research on the locally available ingredients that can be incorporated in fish feeds. The feeding level is important and thus the need to determine if feeding at 3% body weight can promote fish growth.

1.3. Justification

The world's population is increasing and thus the demand for fish. Although, the catch from capture fisheries has been decreasing, mariculture industry has been growing worldwide (Troell, 2009). Marine fin culture is dependent on availability of feed that covers the nutritional needs of cultured species (Borlongan, Eusebio and Welsh, 2003). However, the commercial feeds available are costly. Mariculture feasibility lies in the need of more insight on potential of incorporating alternative plant sources of ingredients in aqua feeds. The appropriate level of fish meal that can be included, possibility of incorporating copra meal in fish feed and feeding at 3% level were investigated. These findings may contribute to aquaculture venture being affordable and sustainable for the Makongeni community.

1.4. Objectives

The overall objective was to formulate a cheap fish feed that can promote fish growth and survival. The study was carried out with the following being the specific objectives:

1. To compare the fish growth, condition factor, survival and growth performance in the fish fed with "sagana" diet, 6% formulated diet, 12% formulated diet and snapper formulated diet.
2. To compare the fish growth, condition factor, survival and growth performance between fish fed with 6% formulated diet and 12% formulated diet.
3. To establish the capacity of reducing feed cost by incorporating copra meal in fish feed.
4. To test the effect of copra meal on the milkfish carcass body composition before and after experimental feeding.
5. To investigate the effect of the fish length and water quality parameters on the fish weight.

6. To examine if feeding the fish with the formulated diets at 3% of body weight was sufficient to promote fish growth.

1.5. Hypothesis

The study that was conducted to answer the questions stated above has the following hypothesis:

1. There is no difference in fish growth, condition factor, survival and growth performance among the fish fed with "sagana" diet, 6% formulated diet, 12% formulated diet and snapper formulated diet.
2. There is no difference in fish growth, condition factor, survival and growth performance between the fish fed with 6% formulated diet and 12% formulated diet.
3. The incorporation of copra meal does not aid in cost reduction.
4. Incorporation of copra meal in the fish diet has no effect on the milkfish carcass body composition before and after experimental feeding.
5. The fish length and the water quality parameters have no effect on the fish weight.
6. Feeding the fish at 3% of body weight cannot support fish growth.

CHAPTER 2

Literature review

Mariculture feasibility is dependent on the provision of artificial feed that meets the nutritional requirement of cultured fish species. The feed cost is about 50% of the production cost as stated in chapter one. An overview on mariculture will be outlined. The various fish diets that have been formulated by incorporation of various fish feed ingredients will also be reviewed. Various experiments that have been done and the formulation of the various dietary protein and feeding levels on fish growth will be looked into. The various trials to formulated fish diets with the incorporation of copra meal in fish feeds will also be examined. The length-weight relationship in fish will be outlined. Finally, macrofouling and the use of fish to reduce it will be reviewed.

2.1. Overview on mariculture

The culture of marine fin fish started about five centuries ago. The mangrove swamps were being converted to ponds. In the past, milkfish farmers in the Philippine could stock about 1500 to 3000 fish per hectare. This low stocking density was because they were relying on fertilizing ponds to increase pond productivity. Stocking of high densities could lead to undesired market prices for their harvest. Supplemental feeding was practised by few individuals and single ingredients e.g. rice bran, corn bran was utilized. This supplemental feeding lead to increased fish yield (Sumagaysay, Marquez and Chui-Chern, 1991).

In Kenya, mariculture has made progress through the development of simple innovative technologies. Tide fed ponds and cages (using the locally available materials e.g. mangrove trees and plastic drums) have been constructed. The less costly fish species e.g. milkfish

(*Chanos chanos*), grey mullet (*Mugil cephalus*) were cultured. Other attempts have been made in the culture of mangrove red snapper (*Lutjanus argentimaculatus*) (Mirera, 2011).

Milkfish is popularly cultured fish specie. Its global production has been increasing (Llameg and Serrano, 2014). It is euryhaline and herbivorous fish (Borlongan, Eusebio and Welsh, 2003; Luzzana *et al.*, 2005). The grey mullet is an ecologically important cultured fish species. It can survive in a widely range of dissolved oxygen and its omnivorous (Biswas *et al.*, 2012; Whitefield, Panfill and Durand, 2012). On the other hand, the mangrove red snapper and red snappers are commonly cultured species and of comMercial importance. They are hardy and thus are potential for mariculture (Catacutan and Pagador, 2004; Miller, Davis and Phelps, 2005).

Mariculture is increasingly developing due to demands from the growing population and the levelling of marine catch (Troell, 2009). Feasibility of marine fish farming lies on feed formulation that can lead to sufficient fish growth and survival at an affordable cost. This has lead to research initiatives on nutritive necessities of various fish species. Further, the nutritive value of various ingredients has been assessed. Simple feed technology in fish feed formulation has been developing. Various attempts have been made in formulating fish feeds for various marine fin fishes. Fish meal has been replaced partially or fully by locally available ingredients (Borlongan, Eusebio and Welsh, 2003).

2.2. Enhancement of fish diets by incorporation various fish feed ingredients

An experimental study was conducted in aquariums (Papaparaskeva-Papoutsoglous and Alexis, 1986). Five semi-purified diets with casein were fed to grey mullet fry. Defatted fish meal and corn starch base diet containing 12%-60% crude protein (CP) at an increment of 12% were tested on grey mullet (*Mugil capito*) fry. The protein consisted of a mixture of casein 50%, defatted fish meal 50% and amino acid supplement. Modification of protein content was by replacement of raw corn starch for the protein mixture and incorporation of 8% fat (mixture of corn oil and linseed oil). The grey mullet fry were fed 3 times daily, six days per week. The fish were weighed once a month. This interval between one sampling to another was too long. Unfortunately, it cannot aid in noting slight changes in fish weight. The grey mullet fry fed with dietary protein of 24% and 36% had the highest growth. However, the specific growth rate (SGR), percentage protein retention and protein efficiency level was

appreciably higher in fish fed with the 24% CP diet. The feed conversion ratio (FCR) decreased as protein content in the diet increased. Therefore, it's more economical to feed grey mullet with 24% CP diet (Papaparaskeva-Papoutsoglous and Alexis, 1986).

A study was carried out in an aquarium to establish the possibility of enhancing the nutritional value of milkfish fry (Seneriches and Chiu, 1988). This enhancement was by: partial substitution of corn gluten meal by white fish meal. In addition, the corn gluten meal diets were supplemented with either inorganic or lipid-soluble fractions from white fish meal. Five diets were formulated with corn gluten meal, with or without addition of fishmeal. The amino acids were supplemented to provide similar amino acid profiles for the diets. Diet 1 contained corn gluten meal as the only protein source. Diet 2 and 3 contained a combination of corn gluten meal and fish meal (15% and 30% respectively). Moreover, they were supplemented with amino acids (arginine, isoleucine, lysine and tryptophan). Diet 4 and 5 had a similar formulation to diet 2 and 3 respectively. Additionally, ash and lipid fractions were added from 30% of fish meal. All diets formulated contained 40% CP and an addition of soybean oil and cod liver oil at a ratio of 1:1. The milkfish fry were fed eight times during the daylight (between 05.40 and 18.20 hours) at 10% of their body weight for six weeks. The feed efficiency and survival did not differ in diets with 15% and 30% of fish meal. However, growth was highest in fish fed with diets with 30% fish meal. On the other hand, the diet that constituted of corn gluten as sole source of protein lead to poorer growth, survival and feed efficiency. Further supplementation with mineral and lipid fractions of white fish meal did lead to improved growth and survival. Besides that, the milkfish fry should not contain less than 15% fish meal to promote good growth and survival (Seneriches and Chiu, 1988).

Sumagaysay, Marquez and Chui-Chern, (1991) conducted a feeding test on milkfish in 800 m² tide fed ponds. Assessment of the outcome of feeding milkfish with rice bran (11.3% CP) and two pelleted diets of 22% and 27% CP. The 22% CP diet contained rice bran, Peruvian fish meal (4%), soybean meal and copra meal. The 27% diet consisted of: soybean meal, wheat polland, rice bran, copra meal and Peruvian and tuna fish meal (10%). There were four treatments: control (fish in a fertilized pond), fish fed with rice bran and the two pelleted diets. The treatments were replicated in four random pond compartments. The fish were fed at 08.00 and 16.00 hours at 2-5% of body weight. The fish growth was higher in the ponds with the pelleted diets. The highest fish growth was observed in the treatment with 27% CP and survival did not differ amongst the treatments. This result is similar to the findings of Sumagaysay and Borlongan, (1995). The dietary protein and the relative amount influence

fish growth, yield increases relative to the level of dietary protein. Fish fed with rice bran only had increase in growth. However, its inclusion in the fish diets lead to improved protein quality that promoted fish growth.

Assessment of the nutritional value of *Porphyra purpurea* meal was done for seventy days. Diets where this ingredient was incorporated were formulated for juveniles' thick lipped grey mullet. All the diets were isonitrogenous with respect to crude protein (45%), lipid and carbohydrates. The control diet contained a brown herring meal (65% CP) as a major component. Seaweed meal (25% CP) was incorporated at the level of 16.5% and 33% in the diets providing 9% and 18% CP respectively. Highly digestible carbohydrates and fish oil concentrate were also included. Optimal fish growth and specific growth rate was achieved in diets that did not contain seaweed. Increase in seaweed in the diet leads to reduced growth rate in fish. Consequently, the feed conversion efficiency, protein efficiency ratio (PER) and net protein utilization decreased with increase in the amount of seaweed in the diet. Therefore, seaweed meal may not be a suitable ingredient for grey mullet fish feed formulation (Davis, Brown and Camille, 1997).

Catacutan and Pagador, (2004) carried out an experiment where fish meal was replaced with soybean meal for mangrove red snapper diets. The survival, growth and feed efficiency response to diets was investigated. The experiment had five treatments and three replicates. The control diet had the following ingredients: Peruvian fish meal, squid meal, dextrin, rice bran, wheat flour, cod liver, vitamin mix and mineral mix. The four formulated diets had 12%, 24%, 36% and 48% CP. Fish meal was replaced by defatted soybean meal at 12.5%, 25%, 37.5% and 50% in the four diets respectively. Fish were fed at 9.30 and 16.00 hours at 12% of body weight for eight weeks and 5%-7% for the rest of eleven weeks. Proximate analysis of the fish carcass was done at the end of the experiment. The feeding frequency was less considering they were fry. The average weight, specific growth rate and feed conversion level did not differ among treatments. The fish feed on feed with 25% incorporation of defatted soybean meal had better growth, survival and feed conversion ratio. The protein content increased as body moisture decreased in all fish after the experiment compared to the initial values. The fish fed with the diet containing 50% defatted soybean meal had high NFE in the body composition. Therefore, soybean meal can be incorporated in fish feed. The fish diets with less defatted soybean meal were readily acceptable as compared to those with a high content (Catacutan and Pagador, 2004).

In a 8 week experiment, the effects of inclusion of soybean meal and torula protein sources on practical diets for grey mullet fingerling were evaluated. A control diet was formulated from: fish meal, spray-dried blood meal, soybean meal, wheat flour, wheat middling and wheat bran. The control diet contained 31.2% crude protein. The soybean based diet had 25% of fish meal and blood meal replaced with soybean meal (30% CP). The torula yeast-based diet had 25% of fish meal and blood meal replaced with torula yeast protein (30.2% CP). The fish were fed with these three diets twice a day at 4% of body weight. The growth, specific growth rate, carcass composition and protein efficiency ratio was not different between the treatments. The fish feed with torula had lower growth and feed efficiency. This finding is contrary to previous research that reported a positive feedback in mullet culture in Asia (Luzzana *et al.*, 2005).

2.3. Effect of dietary protein and feeding levels on fish growth

A study was conducted in brackish water ponds with milkfish. The most economical combination of dietary protein and feeding levels were investigated. The 12 units of 500 m² earthen ponds were fertilized one month before stocking of milkfish juveniles. The fish started being feed one month after stocking since that's when the natural food becomes inadequate. The fish feeds formulated had 24% and 31% CP and a well balanced amino acid profile. The fish were fed at 2% and 4% of their body weight at 08.00, 11.00 and 16.00 hours. The fish were weighed after two weeks and the feeding levels were adjusted accordingly. Growth increased in all treatments. However, the growth rate was significantly higher when the milkfish were fed at 4%. The feed conversion ratio was high at a higher feeding rate. The higher growth at the higher feeding level was attributed to the amino acids present for protein synthesis. Feeding milkfish with 24% protein diet with the right amino acid profile at 4% body weight is sustainable to promote growth (Sumagaysay and Borlongan, 1995).

Abbas *et al.*, (2005) did an experiment in recirculating water system. The effect of dietary protein on the growth and utilization of protein and energy of juveniles of mangrove red snapper was investigated. Six fish meal based diets were formulated from: fish meal, defatted soybean meal, shrimp meal, rice bran, wheat flour, whey, tapioca and dextrin. The diets formulated contained various percentages of protein (20%-45% at 5% increments). The fish were fed at 2% of the wet body weight thrice a day. The fish fed with 40% and 45% CP had

higher weight gains and specific growth rates than those fed with other diets. A condition factor (K) of 2.76 to 2.78 was attained for the fish fed with 40% to 45% protein respectively. The protein efficiency level decreased from 1.34 to 1.12 as dietary protein increased. The diets that contained 20%-35% protein had a significantly high PER of 1.30-1.34. On the contrary, the specific growth rate was low (2.49-2.51). This implied that the snappers could have efficiently utilized the low protein diet for protein synthesis. This utilization led to the increase of the PER and thus exhibiting a compensatory mechanism (Berger and Halver, 1987). The recommended dietary requirement of mangrove red snapper is 40% CP. A similar result was reported by Abbas and Siddiqui, (2003) who found comparable specific growth rate for mangrove red snapper. The fish were fed with fish meal diet (40% CP) and another formulated diet of 42% CP (fishmeal cum soy bean based diet). These findings concur with the findings of Abbas, Siddiqui and Jamil (2011) that carried out an experiment to find out dietary requirements of juvenile mangrove red snapper.

A research was carried out in a semi-closed recirculating system (Miller, Davis and Phelps, 2005). The effect of varying dietary protein level and the lipid on the body composition and growth of juvenile and sub-adult red snappers was investigated. Trial one contained crude protein varying from 32%-44% at 4% difference. These formulated feeds were fed to fish at 8% of mean weight and was reduced as the fish grew. Trial two had diets containing 44% CP and each contained 8%, 10%, 12% and 14% lipid content each. The fish were fed based on the percentage of mean body weight twice a day. Trial three had the diets with similar protein as trial one but contain 6%, 8%, 10% and 12% dietary lipid. The fish were fed at 3% of the mean body weight twice a day. Trial four had diet containing 32% crude protein and 6%, 8%, 10% and 12% dietary lipid each. The fish were fed at 2.5% of mean body weight divided into two (morning and the evening). The juveniles that were fed with 32% dietary protein exhibited high protein conversion efficiency compared to those fed with 44% CP. The juvenile that had 8% lipid included in diet had higher weight gain as opposed to 6% lipid inclusion. Based on the findings inclusion of 32% to 36% protein can support fish growth. Incorporation of 10% dietary lipid can sustainably support energetic demands of fish and it led to high crude protein content in the body composition. The fish that were fed on 32% to 36% CP retained a high percentage of dietary protein than those fish that were fed on 44% CP. There was no significant difference in growth amongst all the treatments. The dietary protein level of 32% to 36% is sufficient to support fish growth (Miller, Davis and Phelps, 2005).

Abbas and Siddiqui (2009) conducted an experiment on the feeding level of mangrove red snapper. A diet of 40% Cp was formulated using locally available ingredients. The feeding level of 1%-4.5% at 0.5% increment was tested for 75 days. The fish were fed thrice daily. The feeding level of 2.5% body weight per day lead to high fish growth and specific growth rate. The FCR and PER did not differ much among the treatments. However, they decreased when fish were fed at a feeding level of more than 3% body weight.

A four factorial design experiment was conducted on stripped mullet (*Mugil cephalus* fry). The effect of dietary protein and energy levels on growth performance, survival rate and feed utilization was determined. Eight practical diets were formulated using two levels of metabolizable energy and each of the diets had the four levels of dietary protein (16%, 20%, 24% and 28%). The proximate analysis of the fish body was conducted before and after the experiment. Each of the diets was fed twice daily for 10 weeks. The fish were weighed after every two weeks. The fish growth was affected by the level of dietary protein and the energy levels. The fish that were fed on the 28% CP had the best survival rate and feed conversion ratio. This diet was efficient to culture grey mullet since it lead to the maximum growth and feed efficiency. On the other hand, the fish that were fed on the 16% CP diet had the lowest survival rate. The fish moisture content was not different in the different treatments and the dietary protein did not influence the lipid content (El-Dahhar, Amer and EL-Tawal, 2011).

2.4. Formulation fish diets with the incorporation of copra meal

An experiment was done in the diet formulation for rohu (*Labeo rohita*) fingerling. The effect of partial substitution of fish meal with copra meal was investigated. In the experimental diets formulated: fish meal was substituted by copra meal at 30%, 40% and 50% level in diets D1, D2 and D3 respectively. Diets D4, D5, and D6 had the basic ingredient composition as diets D1, D2 and D3 but lysine was supplemented at 5.7% of dietary protein. Supplementation with methionine and cystine at 3.1% was done for diets D7, D8 and D9 while diets D10, D11 and D12 were supplemented with cystine, lysine and methionine. All the diets contained 35% crude protein and the fish were fed twice per day at 5% body weight. The highest attained fish weight was in fish fed with diets that had incorporation of 50% copra meal and supplemented with lysine, methionine and cystine (D12). The diets with 50% CP that had no amino supplementation had a low protein efficiency ratio. Lysine, cystine and

methionine (supplemental amino acids) were effective in improving the nutritive value of fermented deoiled copra incorporated in fish diets (Mukhopadhyay, 2000).

A feed experiment was conducted in hapa nets suspended in an outdoor concrete tank at an experimental site (Olude, Alegbeleye and Obasa, 2008). The nutritive value of soaked copra meal as dietary protein supplement for soybean in Nile tilapia diet was evaluated. Four isonitrogenous (30% CP) were formulated with soaked copra meal substituting soybean by 0%, 15%, 30%, and 40%. The feeds were further fortified with methionine, lysine and vitamins. Fish were fed at 10.00 and 16.00 hours at 3% of wet body weight for 70 days. The fish were weighed weekly and the ratio of quantity of feed adjusted accordingly. The fish carcass proximate analysis was done in the laboratory. The fish that were fed on diets that had 15% and 30% copra meal replacement had increased fish growth. Their fish growth was comparable to that of fish fed on the base diet of: fish meal, soybean, ground nut cake and com meal. Therefore, copra meal can be incorporated in diets of Nile tilapia up to 30% without deleterious effect on fish growth. This result is comparable to the findings of Mukhopadhyay, (2000) that the inclusion of 30-40% of copra meal in fish feed formulation is sustainable. Its incorporation in fish feeds aids in reducing of cost of production in areas where it can be obtained locally. Crude protein increased as copra meal was reduced in the diet. The moisture content was similar and among the treatments. The lipid content was no significant different in all treatments. However, in this experiment the inclusion of copra meal at 40% lead to decreased fish growth. On the other hand, the increase in copra meal leads to decreased digestibility. This is contrary to Mukhopadhyay, (2000) findings that 50% replacement by copra meal leads to increased digestibility (Olude, Alegbeleye and Obasa, 2008).

2.5. Investigation of the length-weight relationships in fish

Milkfish was culture in earthen ponds using inland saline ground water for two years. The length and weight relationship exhibited a curvilinear relationship. The b value was <3. This indicates that the fish did not maintain a constant body shape at the different growth intervals. This is associated to the effect of environmental factors and the feeding regimes on fish growth. There was a high correlation between the length and weight. The condition factor was 0.9-1 thus the fish grew normally in saline ground water (Raizada *et al.*, 2005). A similar investigation was done for grey mullet in the Amassoma flood plain for six months. The

correlation coefficient obtained was 0.85, indicating a strong association between the length and weight. The regression coefficient was >3 . The fish exhibited a positive allometric growth. The condition factor was 0.995 thus the fish were in a good condition (Ezekiel and Abowei, 2014).

Biswas *et al.*, 2011 conducted an experiment in the culture of milkfish (*Chanos chanos*) in tide fed ponds. The length and weight relationship and the monthly Fulton's condition factor were investigated. The length-weight relationship showed a curvilinear growth pattern. The fish exhibited an isometric growth ($b=3$). The fish maintained a constant body shape with the length and weight increasing proportionally. The Fulton's condition factor ranged between 0.43 and 1.43 with the juvenile fish exhibiting a high value and vice versa. Small fish grow better than big fish.

2.6. Macrofouling and the use of fish to reduce it

Macrofouling is one of the major challenges in cage culture. The attachment of marine bio-fouling on submerged net cages releases substances for other bio-fouling to live and grow e.g. diatoms, fungi and macroalgae sperm. The attachment first starts with colonization by bacteria and diatoms. Later, the second colony of macroalgae, protozoans and fungi forms. This interferes with water circulation and disposal of fish wastes thus reducing oxygen supply. The macroalgae can be advantageous for marine fish cage culture since this is fed by other fish species thus creating room for polyculture (Rejeski, Susilowati and Aryati, 2010).

A study was conducted at a 600m section of sub-tidal windward reef crest in Hawaii (Hixon and Brostoff, 1996). The benthic assemblages were two: those that were inside and outside the territory. Those that were outside the territory were exposed to grazing schools e.g. parrot fishes and they were dominated by prostate algae and low lying crustose. Those inside the territories were exposed to grazing by resident fish e.g. damselfish. This area was dominated by erect filamentous algae. The experiment was done by attaching and sampling on the settling surfaces installed for colonization. The control was settling surfaces in cages. The schooling fish e.g. parrot fishes lead to deflection of succession thus lowering the biomass and biodiversity. The areas where damselfishes grazed had decelerated succession. On the other hand, inside the cages there was succession within a year. First, the simple brown and green filaments and midsuccession of thin and finely branched filamentous algae. The later stage was dominated by bladed and coarse branched thick filaments. Consequently,

herbivory has an effect on the rate of succession in both species composition, abundance and their change over time. Herbivory helps in decreasing succession and thus causing the community to follow a different trajectory (Hixon and Brostoff, 1996).

Based on the literature reviewed various researchers have incorporated various locally available ingredients in formulating fish feeds for milkfish, grey mullet and mangrove red snapper and red snapper. The incorporation of various fish feed ingredients in fish feed led to improved fish growth. Few studies have carried out with feeds that have incorporated copra meal. Less investigation has been done on the appropriate level of animal protein that should be included in fish feeds.

CHAPTER 3

Materials and methods

3.1. Approach

Based on the literature review undertaken, few studies have been done on fish feeds where copra meal was incorporated and the appropriate percentage of animal protein that should be incorporated. Thus, there was need for this study to be carried out to contribute to the knowledge gaps. The study area is shown in Figure 3.1. Thereafter, the experimental setup and proximate analysis procedure are described. The fish feeds were prepared and fish were fed at 3% of their body weight once daily. Fish and water quality were sampled once every two weeks. The statistical analysis done for the data obtained are stated. The use of parrot and tongue fishes to reduce macrofouling in the cages was denoted.

3.2. Study area

The study was conducted in floating cages that are located in a mangrove section at the Indian Ocean, Makongeni (Mombasa, Kenya) (latitude (-4°24'7"-19.5"N) and longitude (39°30'56")). The water depth is about one and half meters at low tide and 6 meters at high tide. The milkfish and grey mullet were collected from the mangrove wetlands using seine nets and stocked in ponds near the cages. They were later transferred using buckets and stocked in cages. The mangrove red snappers and red snappers were collected using traps that were set in water near the cages. They were then collected using buckets and stocked in the

cages. A motor boat or canoe was used by individuals taking the traps and collecting the stock.



Figure 3.1 Map of Kenya showing the site where the experiment was conducted (Makongeni (Mombasa)) (Openstreetmap.org, 2015)

3.3. Experimental setup

The experimental setup described under is summarised in Figure 3.2. The following fish species were cultured: milkfish, grey mullet, mangrove red snapper and red snapper. Polyculture of milkfish and grey mullet (herbivorous) was done in 1m by 1m by 1m cage. The polyculture of mangrove red snappers and red snappers (carnivorous) was carried out in 1m by 1m by 3m cage. The cage structure was made using hardwood and joined using bolts.

Plastic drums were used to give buoyancy to the cages and sewn nylon nets were inserted in the cages and tied using nylon ropes.

The first treatment (control) for milkfish (MF), grey mullet (GM), red snapper (RS) and mangrove red snapper (MS) were fed with the "sagana" diet (25% CP). The second treatment for the red snapper and mangrove red snapper were fed with the snapper formulated diet (12% s) (25% CP). The polyculture of mangrove red snapper and red snapper was done in two treatments and three replicates (6 cages).

Milkfish and grey mullet's second treatment and third treatment were fed with 6% s (6% sardine content) formulated diet (23% CP) and 12% s (12% sardine content) formulated diet (23% CP) respectively. The two diets were fed to the herbivorous fish. All the treatments had three replicates randomly situated in the cage structures. The experiment was done in three treatments and three replicates in the polyculture of grey mullet and milkfish (9 cages).

3.3.1. Experimental fish

The milkfish stocked were juvenile fish while grey mullet's stock was a mixture of juvenile and fry fish. The mangrove red snappers stocked were juvenile fish while the red snapper's stock was a mixture of juvenile and adult fish. Each of the experimental cages had thirty milkfish and thirty grey mullet in the polyculture of milkfish and grey mullet. The Polyculture of mangrove red snappers and red snappers had a varied number of fish. At the start of the experiment around 10 red snappers were stocked and 5 fish were added later. The mangrove red snapper had 15 stocked at the start of the experiment and 15 fish were added later. The fish were replaced when mortality occurred.

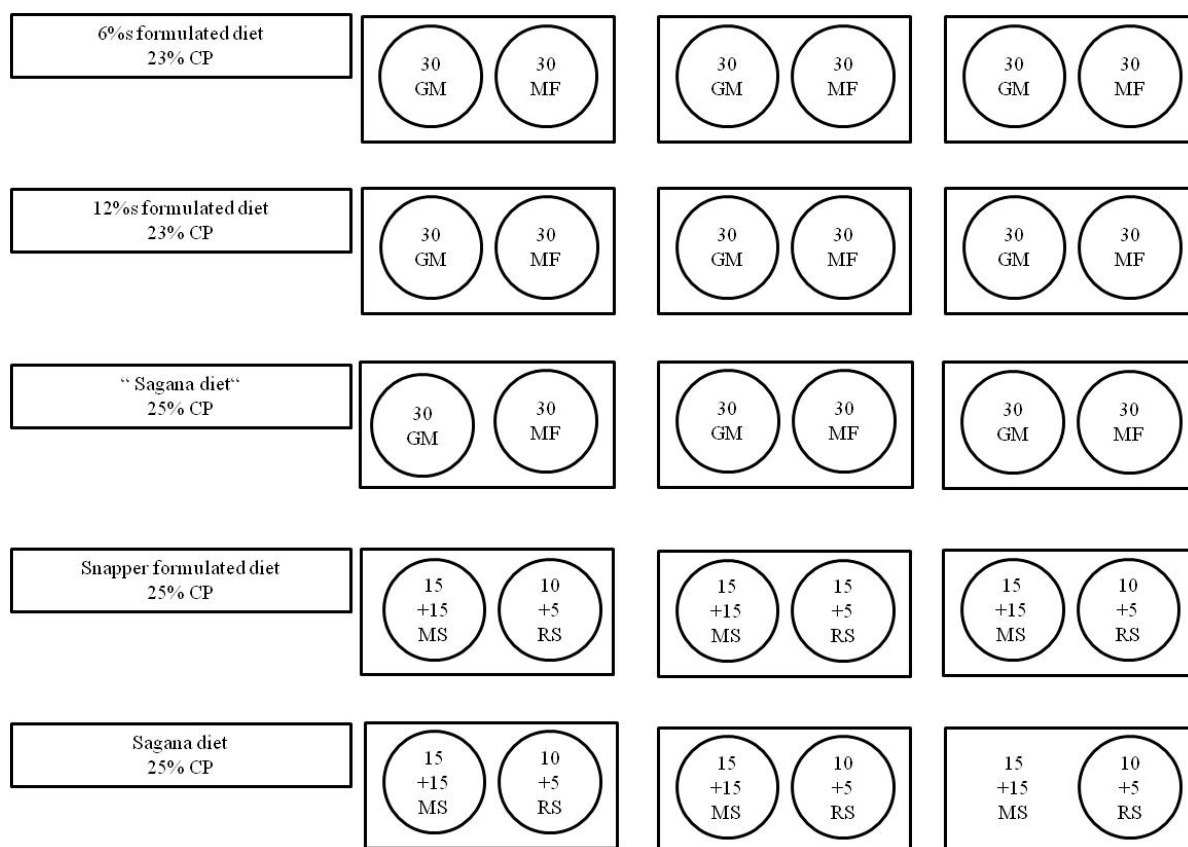


Figure 3.2 The 6% formulated, 12% formulated, snapper formulated and "sagana" diet and the various fish species to which they were fed (milkfish and grey mullet polyculture was done in three treatments while mangrove red snapper and red snapper polyculture was done in two treatments and all had three replicates) ((GM - Grey mullet), (MF - Milkfish), (MS - Mangrove red snapper) and (RS - Red snapper) and the number represents the number of fish stocked (15+15 means 15 fish were stocked at the start and 15 added later))

3.4. Proximate analysis procedure

3.4.1. Fish feed ingredients

The following fish feed ingredients were collected: (Omena (*Rastrineobola argentea*), sardine (*Sardina pilchardus*), wheat bran, cottonseed cake and copra meal. Omena was bought from a local market (Ukunda, Mombasa) and it's a product of Lake Victoria fisheries (Owaga, Onyango and Njoroge, 2010). Sardine was bought from the local market in Mombasa and it's a by catch in marine fishery. Cottonseed cake was unavailable in the nearby local market and was bought in Eldoret. The wheat bran was bought in local market (Ukunda, Mombasa). Copra meal was bought in a coconut oil producing factor near the site (Mvindeni, Mombasa).

3.4.2. Proximate analysis procedure

The proximate analysis was done in University of Eldoret laboratory (latitude ($0^{\circ}34'46.83''\text{N}$) and longitude ($35^{\circ}18'20.09''$)). Analysis of crude protein (CP), crude fibre (CF), ether extracts (EE), ash and moisture content were done in triplicates. The procedure of Association of Official Analytical Chemists (AOAC) was followed. The fish feed ingredients were sun dried and ground to fine powder using an electric grinder. The dry matter (DM) was determined by: drying 5 grams of the sample for 6 hours at 105°C . Later, it was cooled in the desiccator and weighed using an analytical balance (citizen balance). An amount of 5 grams of the sample was ashed in muffle furnace for 6 hours at 550°C to determine the ash content. After ashing it was cooled in a desiccator and weighed.

Crude protein analysis was done using 0.4 grams of the sample that was digested using Behroset InKje digestion apparatus. Prior to titration, the digested sample was distilled in the distillation unit. The distillate containing ammonia was trapped in 4% boric acid and titrated against 0.1HCL. The crude protein was determined by multiplying the nitrogen content by a factor of 6.25. The ether extracts (crude lipids) were analysed using 5 grams of the sample in a soxhlet apparatus. The extractor used was petroleum Ether (boiling point $40\text{-}60^{\circ}\text{C}$). The flasks were weighed before and after the extraction and the difference in weight were used to calculate the lipid content.

The crude fibre was determined by boiling 1g of the sample in 3.13% H_2SO_4 for 10 minutes. The sample used is fat free (after crude lipid extraction). The residue was rinsed in hot water followed by boiling in 3.13% NaOH for 10 minutes. The sample was rinsed repeatedly with hot water and later by Ethanol. The concentration of the sample during rinsing was done in a centrifuge. The residue was dried in the oven at 105°C , cooled in a desiccator and weighed. Thereafter, it was ashed at 550°C for 6 hours in the muffle furnace. The crude fibre was quantified in terms of loss in weight as a percentage of the sample. The nitrogen free extracts were estimated by difference ($100 - (\text{DM} + \text{CP} + \text{EE} + \text{CF} + \text{Ash})$).

3.4.3. Preparation of fish diets

The fish feed ingredients were obtained from the local market and their prices were recorded. The information obtained from the proximate analysis aided in fish feed formulation. Table 3.1 shows the fish feed ingredients and the amounts in the formulated diets. All diet had three ingredients. The "sagana" diet (control for all species) had the

following ingredients: omena (*Rastrineobola argentea*), cottonseed cake and wheat bran. Cottonseed cake was high in percentage in this diet. The 6%*s* (6% sardine content) formulated diet, 12%*s* (12% sardine content) formulated diet and snapper formulated diet (12% sardine content) were composed of the same ingredients. The ingredients were sardine (*Sardina pilchardus*), copra meal and wheat bran. In these diets cottonseed cake was replaced by copra meal which formed a higher percentage in these diets.

The "sagana" diet (control), 6%*s* formulated diet and 12%*s* formulated diet were fed to milkfish and grey mullet. The 6%*s* formulated diet contained 6% fish meal while 12%*s* formulated diet consisted of 12% fish meal incorporated in the fish diets. Mangrove red snapper and red snapper diets were "sagana" diet and snapper formulated diet. The fish feed ingredients for the respective diets were mixed with water to make them moist. A meat mincer was improvised to pellet the fish feeds. The fish feeds were aired in the sun using nylon papers to dry them.

Table 3.1 Formulation and composition of the diets and their crude protein content

Diet	Ingredients	Amount (% weight)	Crude protein (%)
"sagana" diet (control)	Omena (<i>Rastrineobola argentea</i>) Cottonseed cake Wheat bran	12 64 24	25
6% <i>s</i> formulated diet	Sardine (<i>Sardina pilchardus</i>) Copra meal Wheat bran	6 92 2	23
12% <i>s</i> formulated diet	Sardine (<i>Sardina pilchardus</i>) Copra meal Wheat bran	12 60 28	23
Snapper formulated diet	Sardine (<i>Sardina pilchardus</i>) Copra meal Wheat bran	12 85 3	25

3.4.4. Proximate analysis of the fish feeds and milkfish carcass

After preparing the diets, proximate analysis of all the diets was done using the procedure stated above. The milkfish carcass before and after the experiment in the respective diets was collected and proximate analysis was also done.

3.5. Feeding

The fish were fed at 3% of the body weight once a day for three months. The amount of feed was weighed using a sensitive balance (beurer) and put in labeled feed containers for the respective cages. After each sampling, new feed weights were determined and adjusted according to the fish weight.

3.6. Sampling

3.6.1. Fish sampling

The fish were sampled once every two weeks using a seine net. Thus, sampling was done twice a month throughout the experimental period. All the fish's length and weight were measured. The fish weight was measured using a sensitive balance with an accuracy of 1g (beurer). The length was measured using a ruler to the nearest centimetre. All the fish were sampled at each sampling time.

3.6.2. Water quality

Water quality variables were measured at the cage site. The measurement was done at one point since the cages were floating on water and the area has efficient exchange of water by tides. The water quality parameters measured included: dissolved oxygen, temperature, conductivity and pH. These parameters were measured once when the fish were being sampled. The sampling was always done at low tide. The measurements were taken on-site using multiparameter meter (HACH).

3.7. Statistical analysis

Data analysis was done using Microsoft excel, Statistical Package for Social Scientists (SPSS) and statgraphics. All the tests were done at 95% Confidence interval. The numerical values were analysed using analysis of variance (ANOVA) and t-test. The regression of the fish length and weight was done in Microsoft excel. The multiple regressions of the fish weight with fish length and water quality parameters were done in statgraphics ("sagana" diet). The following are the formulas used to calculate the survival, condition factor and the length-weight relationship.

3.7.1. Survival

This is the percentage of fish that survived throughout the experiment.

$$\text{Survival} = 100 * ((\text{stocked fish} - \text{mortality}) / \text{stock})$$

3.7.2. Fulton's condition Factor (K)

This equation below was used to find the condition factor of each fish.

$$K = 100 \left(\frac{W}{L^3} \right) \quad (3.1)$$

Where: K was the condition factor, W (fish weight (g)) and L (total body length (cm)) (Biswas *et al.*, 2011).

3.7.3. Length-weight relationship

The length and weight relationship was estimated using the equation below

$$W = aL^b \quad (3.2)$$

Where: a was intercept, b was the slope, W (fish weight (g)) and L (total body length (cm)) (Abbas *et al*, 2005).

3.7.4. Feed cost test

The cost each feed was calculated. This was done by multiplying the cost of the ingredient and the relative proportion used in the formulation of the diet. All the costs of all ingredients were added. This was the cost of that particular feed.

3.7.5. Growth performance

The natural logarithm for all the fish length and weight was calculated in Microsoft excel. The multiple regressions were done in statgraphics and the slope values obtained were used to plot the graphs in Microsoft excel.

3.8. Reducing macrofouling

Two months after the start of the experiment macrofouling occurred in the mangrove red snapper and red snapper cages. Due to this macrofouling in the cages: parrot fishes and tongue fishes were added to the red snapper and mangrove snapper stock. Cage 1 to cage 3 had only 2-3 tongue fishes added to the stock. One parrot and tongue fish were added to the stock in cage 4 - 6. The possibility of using tongue fish and parrot fish to graze on the macrofouling was evaluated. This was done by taking photographs before and one month after adding the tongue and parrot fishes. The data was collected using a camera.

CHAPTER 4

Results

Proximate analysis was done in the laboratory. The water quality, fish length and weight were measured after every two weeks. The proximate composition of the fish feed ingredients, fish feeds and milkfish carcass was outlaid in tables. The water quality variables were stated in Table 4.4. The multiple regressions of the fish length and the water quality parameters with fish weight were presented. Fish survival and the condition factors of all the fish species in the respective diets were exhibited. The fish growth and the length-weight relationships were illustrated. Variation of the fish weight throughout the entire culture period and the growth performance of the fish also were depicted. Finally, the cost of the fish feed ingredients and feed cost were added.

4.1. Proximate composition

4.1.1. Fish feed ingredients

Copra meal had the highest lipid content while wheat bran had the lowest (Table 4.1). Sardine had the highest moisture content whereas copra meal had the lowest. *Rastrineobola argentea* had a higher protein content compared to *sardina pilchardus*. Wheat bran had the lowest crude protein content and the highest nitrogen free extracts. Cottonseed cake had the highest ash content and crude fibre while copra meal's was lower.

Table 4.1 Proximate composition of fish feed ingredients

Ingredient Proximate composition	Copra meal	Cottonseed cake	Wheat bran	Sardine (<i>Sardina pilchardus</i>)	Omena (<i>Rastrineobola argentea</i>)
Crude lipids (%)	11.53±1.62	5.17±0.18	3.32±0.11	9.37±0.13	6.85±1.07
Moisture (%)	4.87±0.13	5.33±0.13	5.27±0.067	7.67±0.067	6.73±0.8
DM (%)	95.13±0.13	94.67±0.13	94.73±0.067	92.33±0.067	93.27±0.8
Ash (%)	2.26±0.067	23.13±0.067	5.53±0.067	15.53±0.44	12.6±0.12
Crude proteins (%)	22.02±3.10	34.78±0.36	13.13±0.75	46.16±1.45	57.42±3
Crude fibre (%)	10.84±2.63	13.64±2.24	12.58±0.57	-	-
Nitrogen free extracts (%)	18.64	17.93	60.17	-	-

4.1.2. Fish feeds

The "sagana" diet and the snapper formulated diet had a higher percentage of protein compared to 6% formulated and 12% formulated diet (Table 4.2). Snapper formulated diet, 6% formulated diet and 12% formulated diet had a higher moisture content compared to "sagana" diet. "Sagana" diet had a lower content of the crude lipids and high ash content.

Table 4.2 Proximate composition of fish feeds

Proximate composition Sample	Crude lipids (%)	Moisture (%)	DM (%)	Ash (%)	Crude proteins (%)
"sagana" diet (Control)	3.84±0.25	6.53±0.18	93.47	13.2±0.23	24.94±0.45
Snapper formulated diet	8.34±0.11	13.4±0.12	86.6	6.49±0.007	25.52±0.77
6% _s formulated diet	10.42±0.11	13.4±0.23	86.6	5.93±0.47	23.47±0.64
12% _s formulated diet	7.3±0.7	12.85±0.4	89.2	9.85±0.44	22.89±0.84

4.1.3. Milkfish carcass

The ash and protein content were not different ($P>0.05$). The lipid and moisture content ($P<0.05$) were different before the experiment and in the respective treatments (Table A.7). There was a strong decrease in the lipid content when comparing milkfish before the experiment with milkfish in the respective treatments after the experiment (Table 4.3).

Table 4.3 Proximate composition of carcass composition of milkfish (*Chanos chanos*)

Proximate Composition Sample	Crude lipids (%)	Moisture (%)	Ash (%)	Crude proteins (%)
Milkfish (before the experiment)	14.14±07	76.6±0.8	15.59±0.1	26.03±1.66
Milkfish (12% <i>s</i> Formulated diet)	5.28±0.14	79.72±0.45	14.38±0.59	24.72±1.46
Milkfish ("sagana" diet- control)	5.94±0.26	76.3±0.09	13.72±0.77	25.08±0.53
Milkfish (6% <i>s</i> formulated diet)	5.72±0.28	78.83±1.13	15.78±1.05	26.76±0.7

4.2. Water quality

The Table 4.4 shows pH, temperature, conductivity and dissolved oxygen of the water at the study site throughout the culture period. All the water quality parameters measured did not have much fluctuation throughout the 3 months. There was low dissolved oxygen observed.

Table 4.4 The pH, temperature, conductivity and dissolved oxygen at the experimental site

Parameters	Mean
pH	6.47±0.048
Temperature (° C)	29.15±0.5
Conductivity (mS/cm)	51.32±1.24
Dissolved oxygen (mg/l)	4.34±0.78

4.3. Multiple regression of fish length and water quality variables with the fish weight

The fish length, pH, Temperature, conductivity and dissolved oxygen had an effect on fish weight ($P < 0.05$) (Table 4.5). The fish length and temperature had a positive effect while pH, Conductivity and dissolved oxygen had a negative effect on milkfish weight.

Table 4.5 Multiple regressions of fish length and water quality parameters with milkfish weight

Parameter	Estimates	Standard error	T-statistic	P-value
Constant	-0.0073	0.0098	-0.74	0.46
LnL	1	0.01	98.08	0
pH	-0.054	0.012	-4.46	0
Temperature (° C)	0.082	0.012	7.06	0
Conductivity (mS/cm)	-0.047	0.015	-3.09	0.0021
Dissolved oxygen (mg/l)	-0.047	0.012	-3.77	0.002

The fish length, pH, conductivity and dissolved oxygen had an effect on the fish weight ($P < 0.05$) (Table 4.6). The dissolved oxygen had a negative effect while the fish length, pH and conductivity had a positive effect on the grey mullet weight.

Table 4.6 Multiple regressions of fish length and water quality parameters with grey mullet weight

Parameter	Estimates	Standard error	T-statistic	P-value
Constant	0.000000000018	0.018	0.000000000096	1
LnL	0.93	0.018	50.91	0
pH	0.13	0.021	6.25	0
Temperature (° C)	0.074	0.022	0.34	0.74
Conductivity (mS/cm)	0.091	0.027	3.31	0.001
Dissolved oxygen (mg/l)	-0.061	0.024	-2.48	0.014

The dissolved oxygen had a negative effect while the fish length, pH, temperature and conductivity had positive effect on the mangrove red snapper weight ($P < 0.05$) (Table 4.7).

Table 4.7 Multiple regressions of fish length and water quality parameters with mangrove red snapper weight

Parameter	Estimates	Standard error	T-statistic	P-value
Constant	0.0024	0.0013	1.85	0.065
LnL	2.00052	0.02	98.27	0
pH	0.0093	0.0017	5.55	0
Temperature (° C)	0.0039	0.0018	2.22	0.027
Conductivity (mS/cm)	0.007	0.002	3.44	0.0007
Dissolved oxygen (mg/l)	-0.054	0.0016	-3.34	0.0009

The temperature and the fish length had a positive impact while the dissolved oxygen had a significant negative effect on the red snapper weight ($P < 0.05$) (Table 4.8).

Table 4.8 Multiple regressions of fish length and water quality parameters with red snapper weight

Parameter	Estimates	Standard error	T-statistic	P-value
Constant	-0.48	0.02	2.4	0.19
LnL	1.0021	0.024	42.63	0
pH	-0.0055	0.025	-0.22	0.83
Temperature (° C)	0.12	0.024	4.83	0
Conductivity (mS/cm)	-0.045	0.033	-1.37	0.17
Dissolved oxygen (mg/l)	-0.07	0.027	-2.65	0.0096

4.4. Survival

The mangrove red snapper and red snapper survival were different between the different treatments ($P < 0.05$) (Table A.2). The mangrove red snapper and red snapper fed with the "sagana" diet had a higher survival (76% and 87.18% respectively) (Figure 4.1). On the other hand, to those fed with snapper formulated diet had a survival of 49.55% and 59.31% for the mangrove red snapper and red snapper respectively.

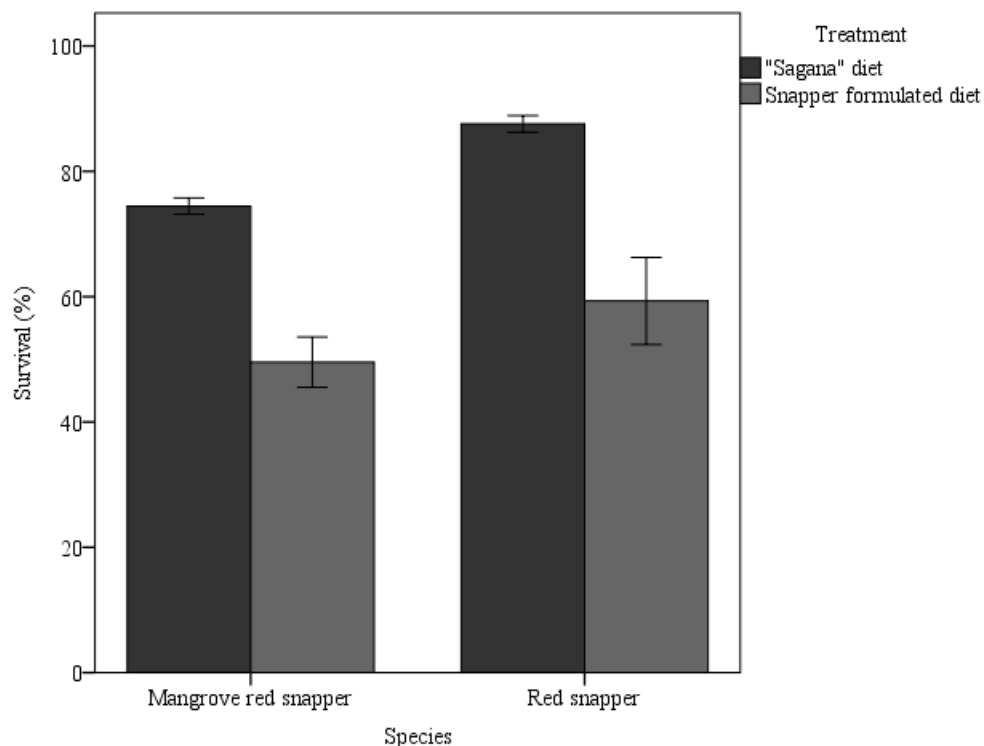


Figure 4.1 Survival of mangrove red snapper and red snapper fed with the snapper formulated diet and "sagana" diet (error bar represents standard error)

The survival of grey mullet and milkfish were different among the different treatments ($P < 0.05$) (Table A.3). The differences among the treatments are very small. The significant difference among the treatments may be due to the large sample size. Milkfish had a higher survival compared to grey mullet (Figure 4.2). Milkfish fed with 12% formulated diet had a survival of 86.24% and 6% formulated diet had 80.63% survival. Those fed to "sagana" had (81.17%) survival. The grey mullet fed with "sagana" diet had 13.95%, 6% formulated diet had 18.48% and the 12% formulated diet had 17.36% survival.

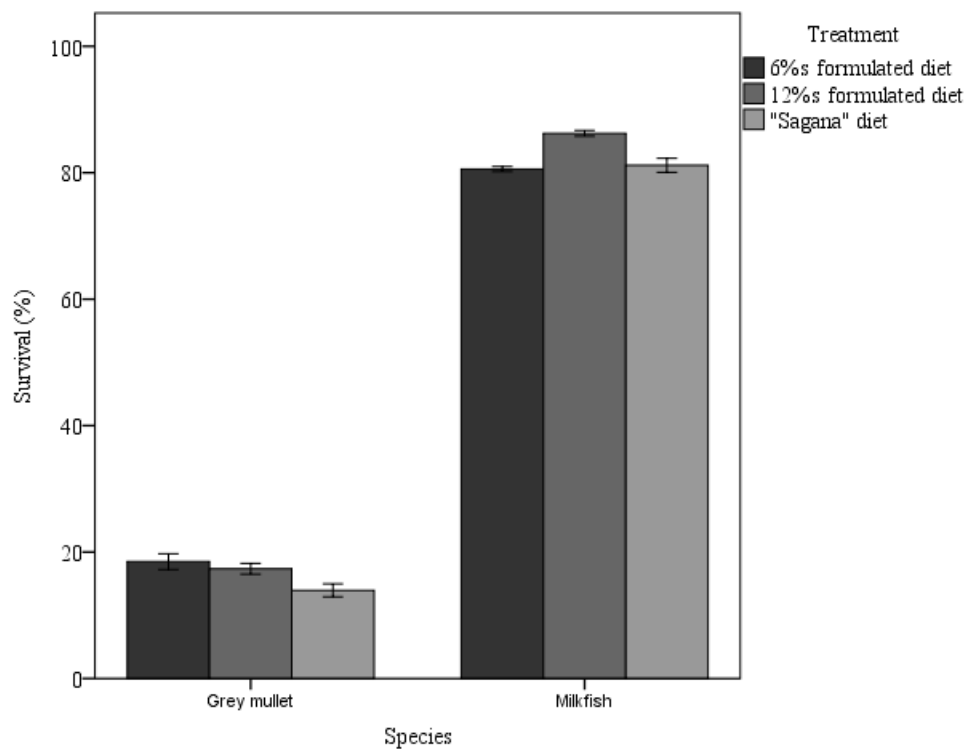


Figure 4.2 Survival of milkfish and grey mullet fed with 6% formulated diet, 12% formulated diet and "sagana" diet (error bar represents standard error)

4.5. Condition factor

The condition factor of mangrove red snapper and red snapper was different between the treatments ($P < 0.05$) (Table A.1). The mangrove red snapper fed with "sagana" diet had a condition factor of 1.48 while those fed with the snapper formulated diet had 1.37 (Figure 4.3). On the other hand the red snapper fed with snapper formulated diet had a higher condition factor (1.48) compared to those fed with "sagana" diet (1.43).

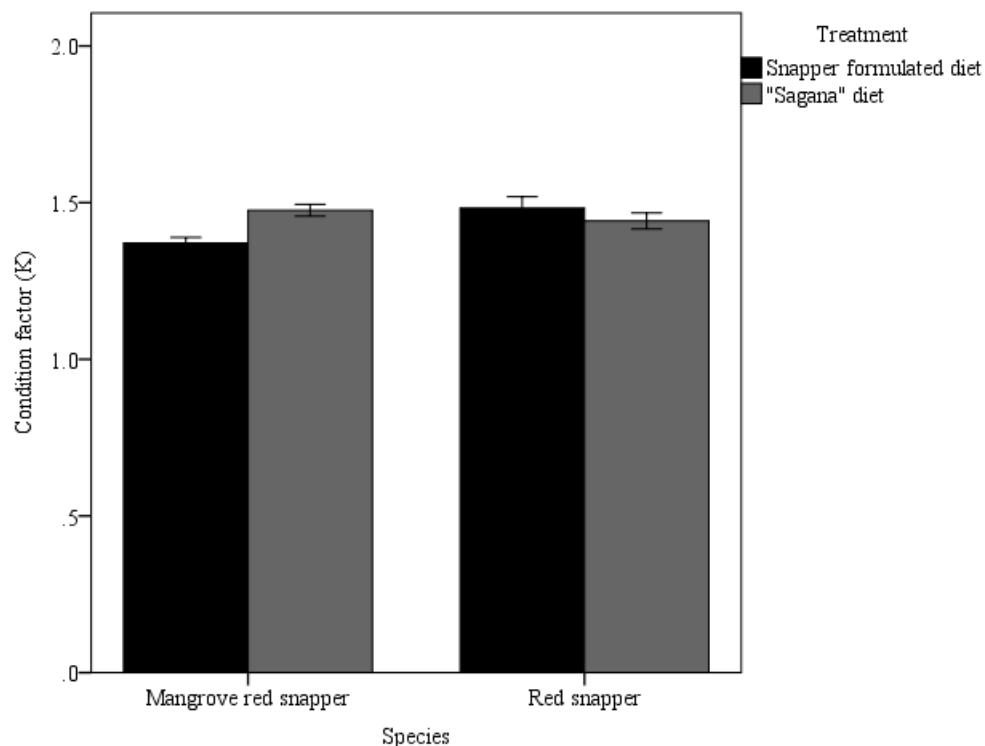


Figure 4.3 Condition factor of mangrove red snapper and red snapper fed with the snapper formulated and "sagana" diet (error bar represents standard error)

The condition factor of grey mullet and milkfish were different among the different treatments ($P < 0.05$) (Table A.5). The differences among the treatments are very small. The significant difference among the treatments may be due to the large sample size. The grey mullet had a higher condition factor compared to milkfish (Figure 4.4). The grey mullet fed with "sagana" diet had a condition factor (1.58) while 6% formulated diet had 1.49. Those fed with 12% formulated diet had 1.46. The milkfish's condition factor ranged from 0.63-0.68.

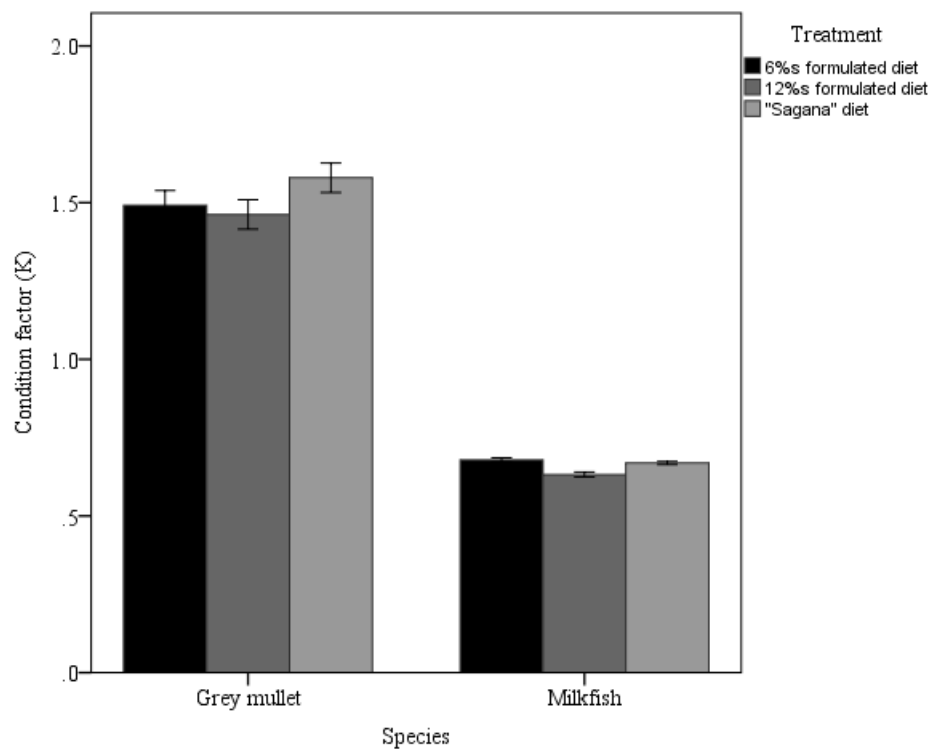


Figure 4.4 Condition factor of milkfish and grey mullet fed with 6% formulated diet, 12% formulated diet and "sagana" diet (error bar represents standard error)

4.6. Fish growth and the length-weight relationship

4.6.1. Fish weight at the start and end of the culture period

There was slight fish growth observed in red snapper, mangrove red snapper ("sagana" diet) (Table 4.9). Although, the t-test shows that there was no difference in the fish weight before and after the experiment (Table A.10). This slight growth is so small compared to the three months' culture period. There was a decrease in the weight of milkfish fed with the 12% formulated diet ($P < 0.05$). Although, the difference is very small and this may be associated with the large sample size.

Table 4.9 The average weight of fish at the start and end of culture period

Diet	Species	Weight at the start (g)	Weight at the end (g)
"Sagana" diet (Control)	Red snapper	104.53±8.5	114.88±10.61
	Mangrove red snapper	38.74±5.52	40.49±2.24
	Milkfish	33.93±1.57	32.86±1.67
	Grey mullet	7.6±1.19	5.45±0.1.18
6% s formulated diet	Milkfish	33.62±1.36	30.41±1.24
	Grey mullet	12.37±1.12	5.70±0.98
12% s formulated diet	Milkfish	34.85±1.29	29.00±1.12
	Grey mullet	9.31±1.96	5.94±0.66
Snapper formulated diet	Red snapper	111.83.38±3.97	102.37±15.10
	Mangrove red snapper	37.93±4.75	34.73±2.29

4.6.2. Length-weight relationship

The regression lines followed a curvilinear growth pattern and the fish had allometric growth ($b < 3$) (Figure 4.5). The regression coefficient was high thus indicating a strong correlation between the fish length and weight (0.91 ("sagana" diet and 6% s formulated diet)) and 0.86 (12% s formulated diet).

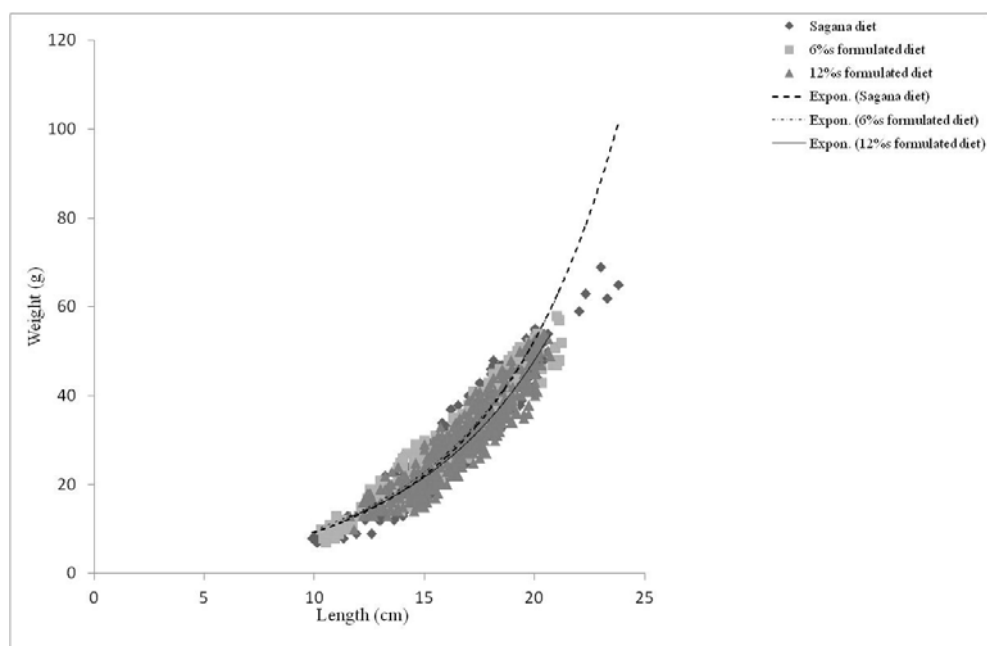


Figure 4.5 Length-weight relationship of milkfish fed with the "sagana" diet, 6% formulated diet and 12% formulated diet

The regression lines showed a curvilinear growth pattern and the fish exhibited allometric growth ($b < 3$) (Figure 4.6). There was a good correlation between the fish length and weight (0.68 ("sagana" diet)). A high correlation was observed in fish fed with 6% formulated diet (0.82) and 12% formulated diet (0.89).

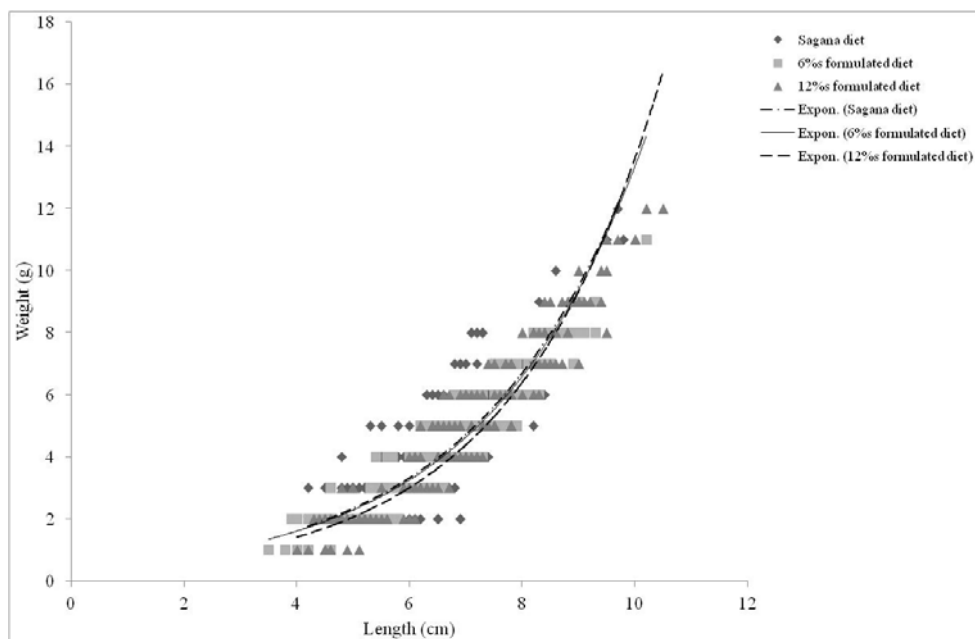


Figure 4.6 Length-weight relationship for grey mullet fed with "sagana" diet, 6% formulated diet and 12% formulated diet

The regression lines followed a curvilinear growth pattern and the fish did not grow isometrically ($b < 3$) (Figure 4.7). There was a strong correlation between the fish length and weight (0.94 ("sagana" diet) and 0.91 (snapper formulated diet).

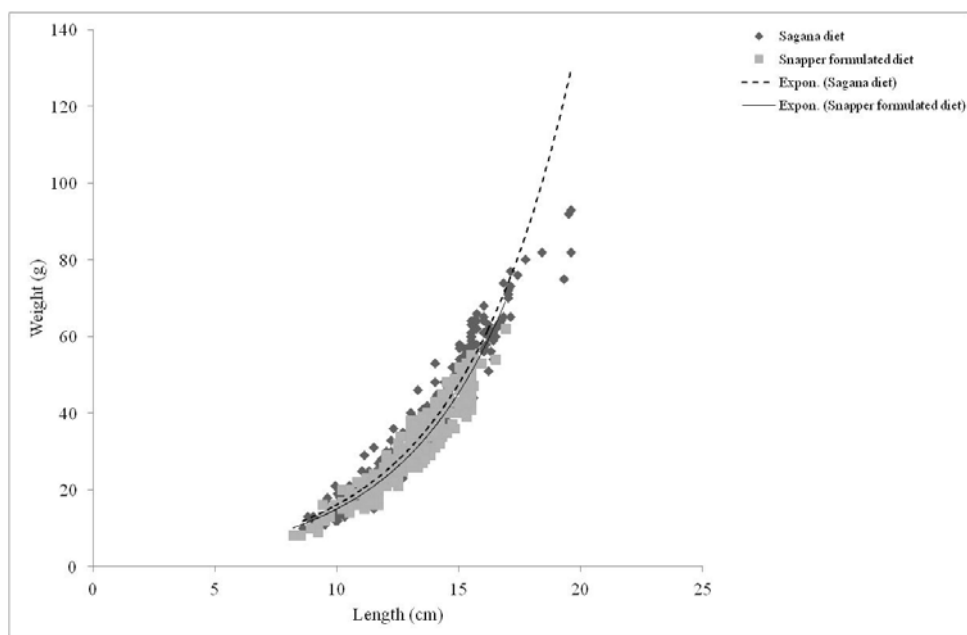


Figure 4.7 Length-weight relationship for mangrove red snapper fed with "sagana" diet and the snapper formulated diet

Red snapper's length-weight relationships showed a curvilinear growth pattern and the fish showed allometric growth ($b < 3$) (Figure 4.8). There was a strong correlation between the fish length and weight (0.91 ("sagana" diet)) and 0.82 (snapper formulated diet).

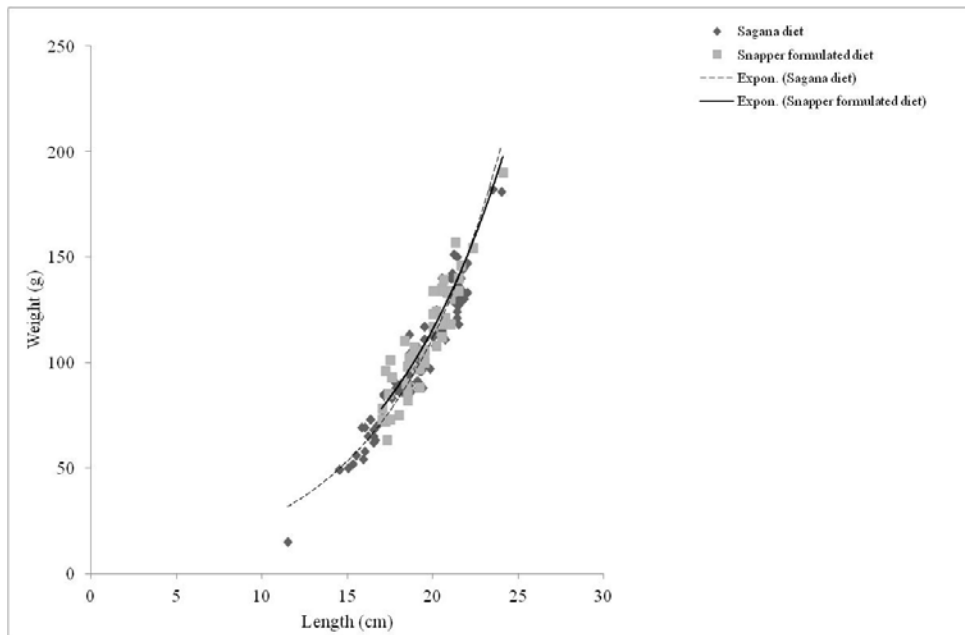


Figure 4.8 Length-weight relationship of red snapper fed with "sagana" diet and the snapper formulated diet

4.7. Variation of weight with time

Milkfish weight did not increase with time (Figure 4.9). There was a decreasing trend at the start of the experiment and later a slight increase. There was no difference in the weight before and after the experiment in fish fed with the "sagana" and 6%*s* formulated. The milkfish fed with 12%*s* formulated diet had a decrease in weight ($p < 0.05$) (Table A.10). Although, the difference was very small and this may be associated with the large sample size.

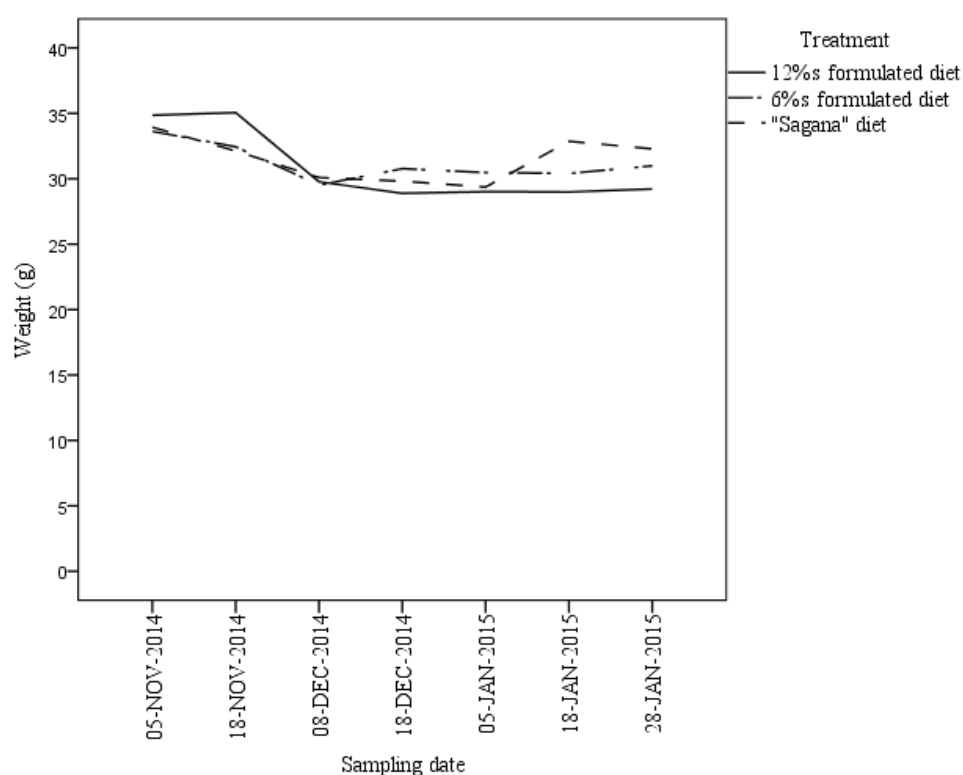


Figure 4.9 Variation of the milkfish weight with time in the 12%*s* formulated diet, 6%*s* formulated diet and "sagana" diet treatments (NOV=November, DEC=December and JAN=January)

The grey mullet fed with the "sagana" diet maintained the weight at the start of the experiment (Figure 4.10). Thereafter, a decreasing trend was observed. Grey mullets fed with 6% formulated diet and 12% formulated diets had a decrease in weight from the start of the experiment. There was no difference in weight before and after the experiment (Table A.10). The slight increments observed were due to grey mullet replacement of the mortality.

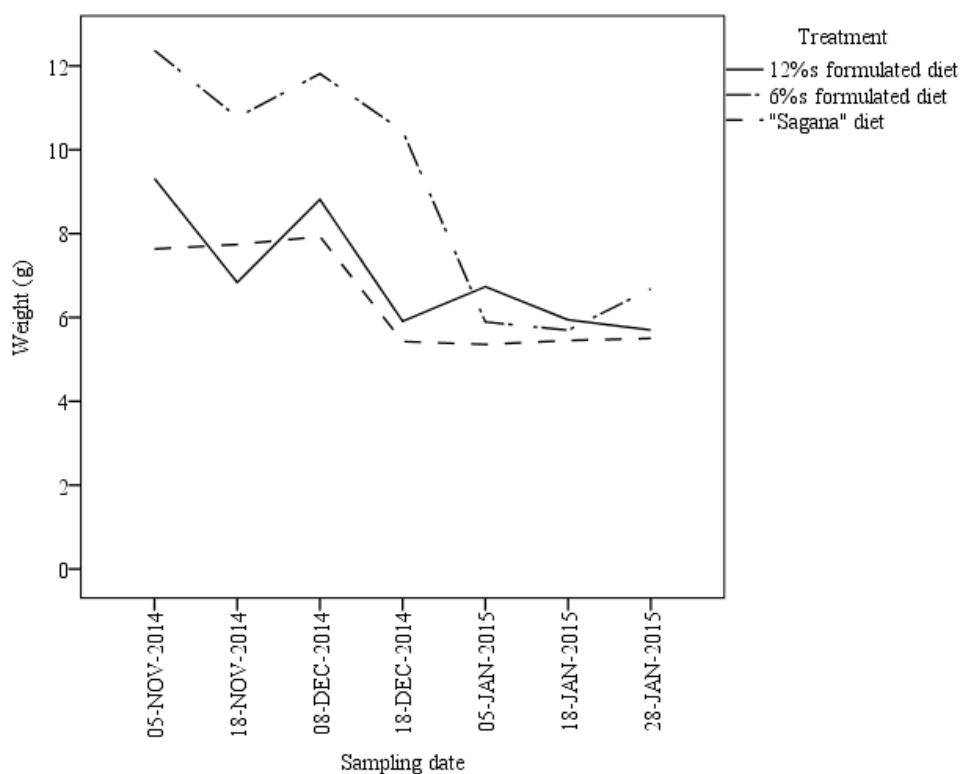


Figure 4.10 Variation of the grey mullet weight with time in the 12% formulated diet, 6% formulated diet and "sagana" diet treatments (NOV=November, DEC=December and JAN=January)

The mangrove red snapper had a decrease of the weight at the start of the experiment for both treatments (Figure 4.11). There were slight increments in weight and subsequently a decrease. There was no difference in weight before and after the experiment (Table A.10) ($P>0.05$).

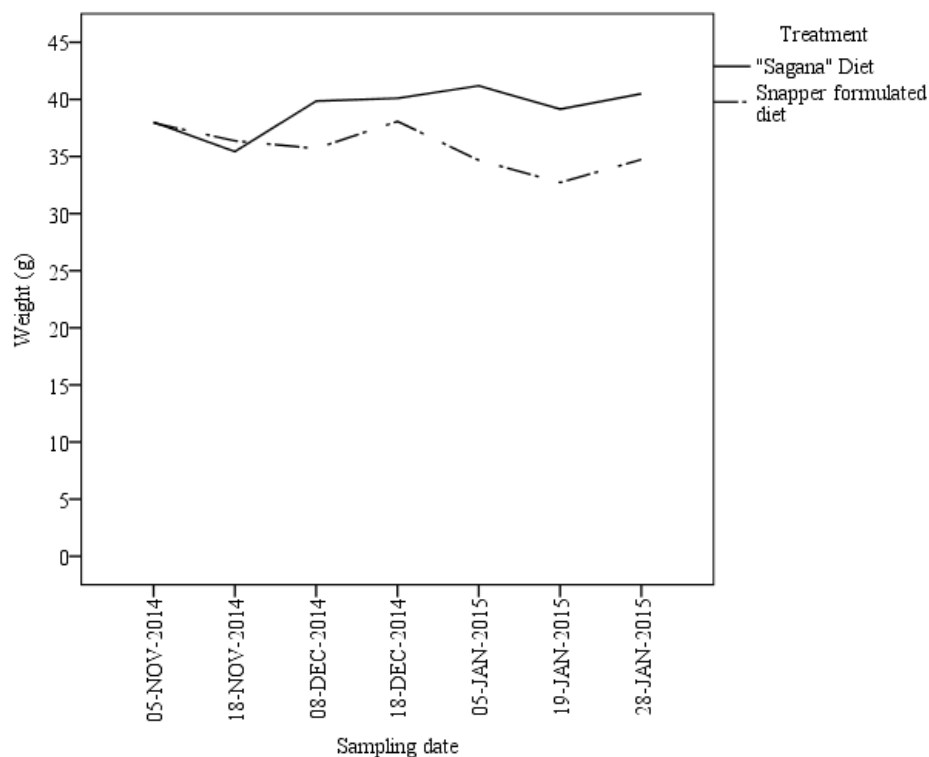


Figure 4.11 Variation of the mangrove red snapper weight with time in the "sagana" and snapper formulated diet treatments (NOV=November, DEC=December and JAN=January)

The red snapper had an increase of weight in both treatments at the beginning of the experiment (Figure 4.12). Thereafter, a decrease in weight was observed. There was no difference in weight before and after the experiment (Table A.10).

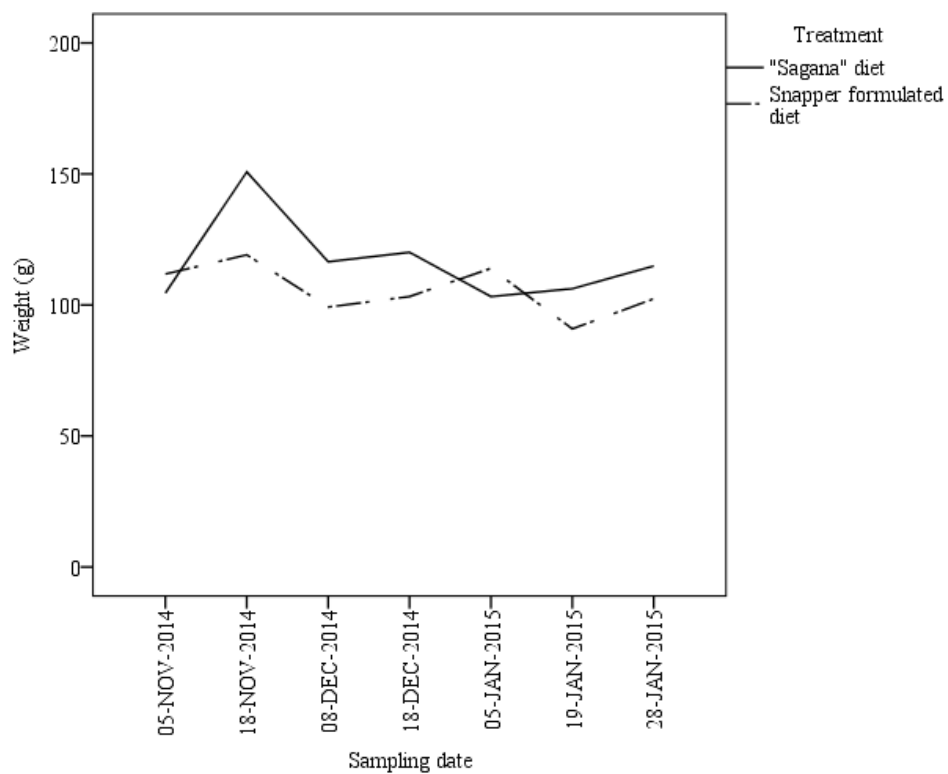


Figure 4.12 Variation of the red snapper weight with time in the "sagana" and snapper formulated diet treatments (NOV=November, DEC=December and JAN=January)

4.8. Growth performance of fish

The growth performance of milkfish fed with "sagana" diet was similar to milkfish fed with 12% formulated diet ($P>0.05$) (Table B.1). The fish fed with these diets had a higher growth performance compared to those fed with 6% formulated diet (Figure 4.13).

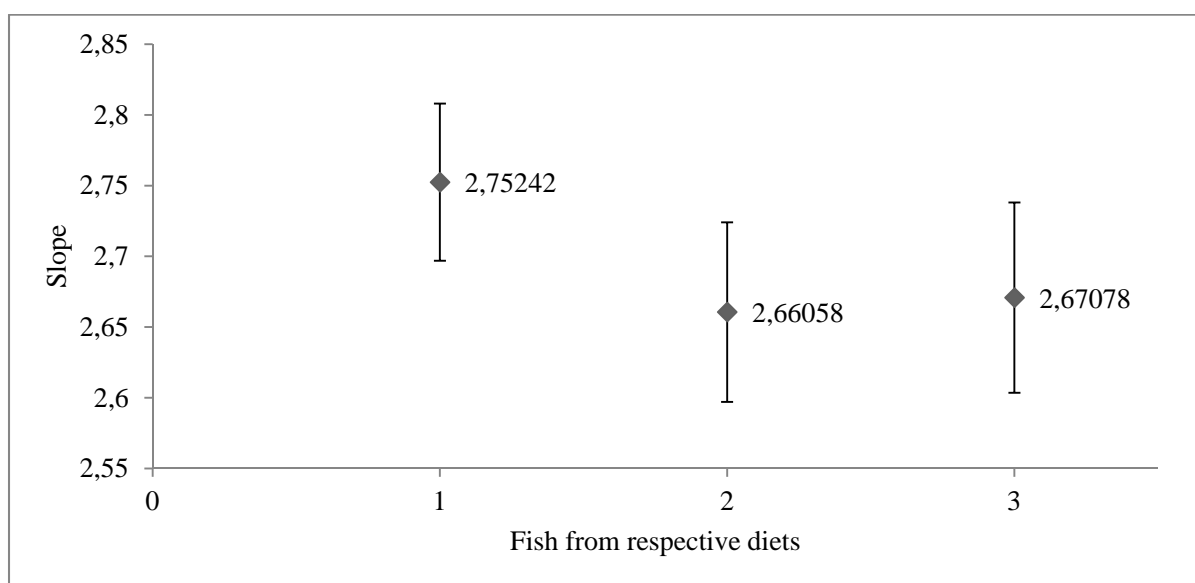


Figure 4.13 Growth performance of milkfish fed with the respective diets (1("sagana" diet), 2 (6% formulated diet) and 3(12% formulated diet))

The grey mullet fed with "sagana" diet and 6% formulated diet had similar growth performance ($P>0.05$) (Table B.2). Whereas, fish fed with 12% formulated diet had the highest growth performance (Figure 4.14).

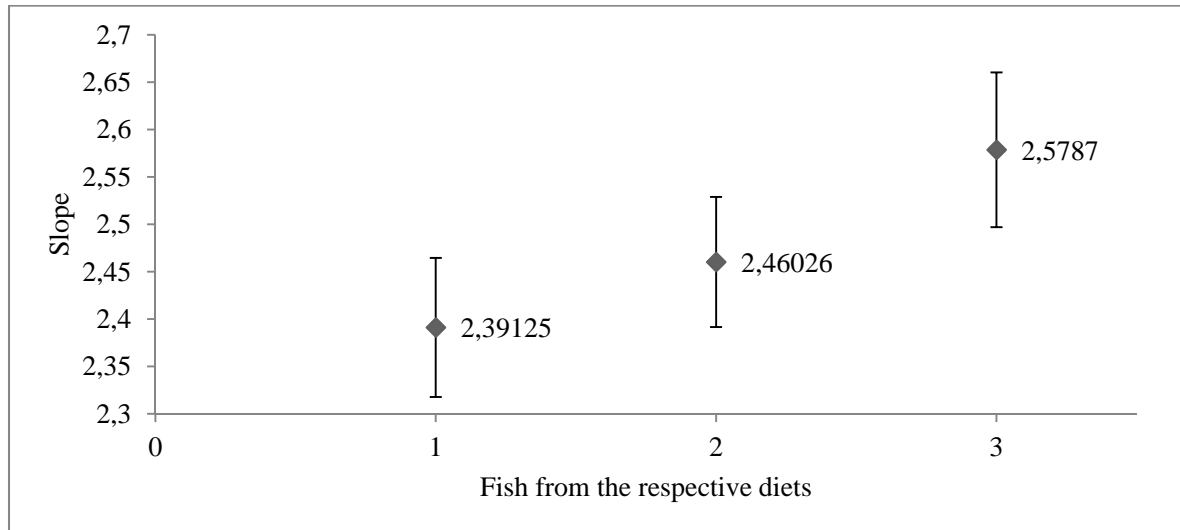


Figure 4.14 Growth performance of grey mullet fed with the respective diets (1("sagana" diet), 2 (6% formulated diet) and 3(12% formulated diet))

The growth performance of the mangrove snappers fed with "sagana" diet and snapper formulated diet were not different ($P>0.05$) (Table B.3) (Figure 4.15).

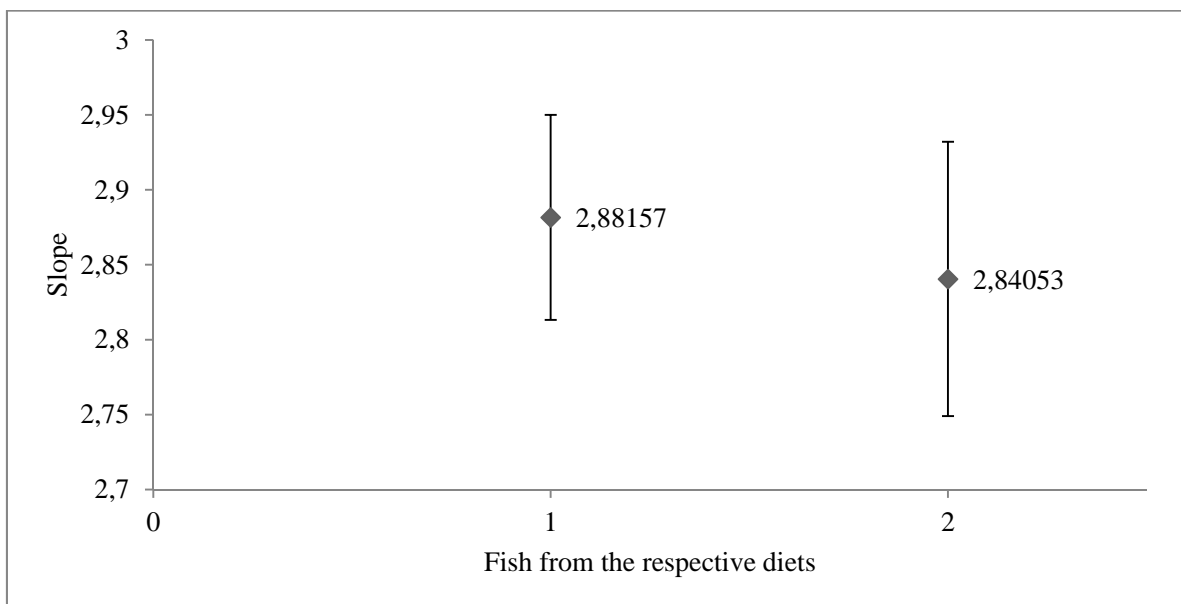


Figure 4.15 Growth performance of mangrove red snapper (fed with the respective diets 1 ("sagana" diet) and 2(snapper formulated diet))

The red snapper's growth performances were similar in the fish fed with "sagana" diet and snapper formulated diet $P > 0.05$) (Table B.4) (Figure 4.16).

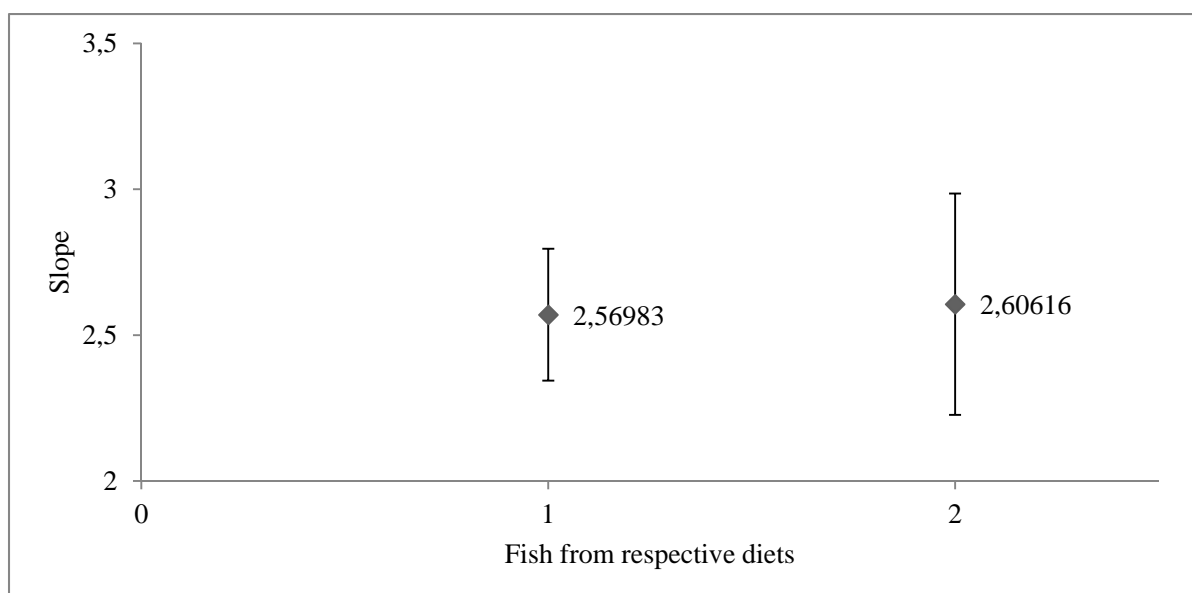


Figure 4.16 Growth performance of red snapper fed with the respective diets (1 ("sagana" diet) and 2(snapper formulated diet))

4.9. Cost of feed

Omena (*Rastrineobola argentea*) had a higher price compared to the sardine (*Sardina pilchardus*) (Table 4.10). These two ingredients (fish meal) were the more costly. Cottonseed cake was three times higher in price compared to copra meal and wheat bran (plant proteins).

Table 4.10 Fish feed ingredients and their costs per kilogram as per the market prices (October, 2014)

Fish feed ingredients	Cost @ Kg in KSH
Cottonseed cake	50
Copra meal	15
Wheat bran	18
Omena (<i>Rastrineobola argentea</i>)	450
Sardine (<i>Sardina pilchardus</i>)	330

The cheapest feed was the 6%*s* formulated diet while the highest cost incurred was in the ("sagana" diet) (Table 4.11). The snapper formulated diet had a similar cost as 12%*s* formulated diet. The snapper formulated diet and 12%*s* formulated diet was 1.5 times higher compared to the 6%*s* formulated diet. Incorporation of copra meal aided in feed cost reduction by 3 times (6%*s* formulated diet).

Table 4.11 Cost of a kilogram of feed in the respective diets (October, 2014)

Diet	Cost @Kg in KSH
"Sagana" diet	90.32
Snapper formulated diet	52.89
6% <i>s</i> formulated diet	35.04
12% <i>s</i> formulated diet	53.64

4.10.Reducing macrofouling

The photographs of the cages before and after the adding of the tongue and parrot fish are attached in appendix C. All the cages had an early dominance of the green (Figure C.1 and C.3) and brown filaments (Figure C.5, C.7, C.9 and C.11). There is a reduction of the fouling one month after adding the tongue and parrot fish to the cages (Figure C.2, C.4, C.6, C.8, C.10, and C.12).

CHAPTER 5

Discussion

The proximate composition of the fish feed ingredients, fish feeds and milkfish carcass will be looked into. Water quality and the fish length effect on fish weight will be expounded on. The survival and the condition factors for the respective fish species in the various treatments will be discussed. Fish's growth, length-weight relationship and the variation of fish weight with time will be expounded on. Subsequently, the growth performance in the respective diets will be addressed in detail. The feed cost test will be expounded and finally, the use of grazers to reduce macrofouling in the cages will be elaborated.

5.1. Proximate composition of fish feed ingredients, fish feeds and milkfish carcass

The sardine (*Sardina pilchardus*) and omena (*Rastrineobola argentea*) had the highest amount of crude protein. The *Rastrineobola argentea* crude protein obtained was similar to that attained in a previous experiment (Maina *et al.*, 2007). The *Sardina pilchardus* crude protein was lower compared to those attained in a previous experiment where 65% CP was attained (Table 4.1). The proximate composition varies in various places and this variations may be due to postharvest handling (Elhag and Elkhanjari, 1992; Owaga, Onyango and Njoroge, 2010).

Copra meal's crude lipid content was higher than that attained in a previous experiment (3.95%) (FAO, 2015). However, the results concur with other findings. Copra's crude lipid content may vary from 5%-15% (Ohler, 1993). The proximate composition of the fish feed ingredients may influence that of the fish feed. For instance: the snapper formulated diet, 6% formulated diet and 12% formulated diet had a high lipid content compared to "sagana" diet

(Table 4.2). The 6% formulated diet had a high percentage of crude lipids since it had 92% copra meal (Table 3.1). This lipid content may have been influenced by the lipid content of the 6% formulated diet (highest lipid content). Therefore, the content of the diets was influenced by the ingredients.

Cottonseed cake had a high amount of ash and crude fibre. However, the ash content previously attained was 2.7 times higher than the findings in this experiment (Addass *et al.*, 2011). The ash content is reflected on the "sagana" diet (had the highest ash content). Wheat bran had a high nitrogen free extracts and is concurring with the previous findings. Although, its crude protein content was lower than that attained previously (17.50%) (Yaseen *et al.*, 2012) The proximate composition of fish feed ingredients from different areas may vary.

The lipid content decreased in all treatments compared to milkfish's carcass before the experiment (Table 4.3). However, milkfish fed with the 6% formulated diet had higher lipid content compared to that fed with 12% formulated diet. The 6% formulated diet had 92% of the diet composed of copra meal (Table 3.2). The level of copra inclusion in the diets influences the lipid content of the carcass. The decrease in the lipid content may be probably due to the fish using lipids as a source of energy (Amoah, 2012). The dietary protein fed to the fish may have affected the proximate composition of the milkfish carcass (Abdel-Tawwab *et al.*, 2010).

The dietary protein was low to supply the fish's needs and allow deposition of protein and lipids (Lupatsch, 2007). The protein content did not increase and the lipid decreased due to the low feeding level and frequency. A similar result was attained in the culture of the juvenile mangrove snapper. The protein and lipid content decreased in the feeding level of 1% and 1.5% body weight. The dietary protein was 40% CP and the fish were fed thrice daily. This may be probably due to the food being a limiting factor as the biomass increased: protein and energy were the first limiting factors (Abbas and Siddiqui, 2009).

5.2. Effect of fish length and water quality parameters on fish weight

At the site where the cages were located: the pH, temperature, conductivity and dissolved oxygen were within the range of the milkfish, grey mullet, red snapper and mangrove red snapper (Table 4.4) (Ramkumar, Khandagale and Sujith, 2014). The low dissolved oxygen was due to an error in multiparameter caused by the oxygen membrane.

The fish length and temperature had a positive effect on the milkfish weight. On the other hand, the pH, Conductivity and Dissolved oxygen had a negative effect on milkfish weight (Table 4.5). The Mangrove red snapper and grey mullet's length, pH, conductivity and temperature had a positive effect on their fish weights. The dissolved oxygen had a negative effect (Table 4.6 and 4.7). Red snapper's fish length and temperature had a positive effect while dissolved oxygen had a negative effect on the fish weight (Table 4.8). The fish growth can be influenced by the water quality and fish length. The fish length was strongly correlated with the fish weight ($\text{LnL} \geq 1$ (estimates)). The water quality variable effect (whether positive or negative) was weak. The increase of all the variables that had a positive effect may lead to an increase in fish weight and vice versa (Jana, Garg and Patra, 2006).

5.3. Fish survival and condition factors

The mangrove red snapper and red snapper survival was high those fed with "sagana" diet. The fish had low acceptability of the snapper formulated diet and may have probably led to low survival. However, all the survival was lower than 100% survival attained previously (Figure 4.1) This may also be attributed to the low dietary protein content since they are carnivorous fish (Catacutan and Pagador, 2004; Abbas *et al.*, 2005). However, cannibalism was observed during the experiment. The similar size of fish should be cultured.

The grey mullet had a low survival (Figure 4.2) as compared to previous findings. The fish fed to 24% CP had 70% survival although they were fed twice daily in that experiment. The low survival in grey mullet is probably due to the low feeding level and frequency. However, the "sagana" diet had higher protein content and yet it had a low survival (El-Dahhar, Amer and EL-Tawal, 2011). The low survival of grey mullet could probably be associated with handling stress since fish died after being weighed. The low feeding frequency was due to the inaccessibility of the site due to transport logistics.

The Mangrove red snapper and red snapper in both treatments had a lower condition factors compared to those attained in previous experiments (Figure 4.3). The condition factors of 2.72 and 2.74 were attained in 20%-35% CP at 5% increment experiment where the effect of dietary protein on growth was tested (Abbas *et al.*, 2005). The fish may not have been in

the optimum culture conditions. However, the red snapper stock was mostly adult and the number stocked in the experiment was low.

Generally, grey mullet had a high condition factor (Figure 4.4). This condition factor exhibited a healthy and robust condition in relation to compatibility with the culture conditions (Biswas *et al.*, 2011). This is contrary to the expectation that this culture conditions could promote a high survival (Zubia *et al.*, 2014). The condition factor was approximately 1.5 which is higher than those obtained in a previous experiment. Ezekiel and Abowei, 2014 attained a condition factor of 1 in their experiment on grey mullet. This high condition factor (1.5) may be probably due to replacement of fish after mortality.

Milkfish's condition factor was similar to that attained in earlier experiments (Figure 4.4) (Barman, Garg and Bhatnagar, 2012). Nevertheless, this condition factor was attained in large grown fish (Biswas *et al.*, 2011). Contrary to that, a higher condition factor of 0.9-1 was attained in another previous experiment (Raizada *et al.*, 2005). The low value of milkfish condition factor may be due to lack of growth (Zubia *et al.*, 2014). Therefore, probably the culture conditions were not at the optimum: the amount of feed given may not have been sufficient to support growth.

5.4. Fish growth

There was slight growth observed in mangrove red snapper and red snapper fed with "sagana" diet. In spite of the fact that, this change in fish weight was small compared the culture period. There was no growth observed in all the other species and treatments. This could probably be attributed to the low feeding level at a low feeding frequency (Olude, Alegbeleye and Obasa, 2008).

The milkfish and grey mullet fed with "sagana" diet had a no growth although the dietary protein level was higher compared to 6% formulated diet and the 12% formulated diet. This is probably due to the presence of gossypol, phytic acid, phyto estrogens, antivitamin and cyclopropenoic in cottonseed cake. The presence of these antinutritional factors may interfere with feed utilization in fish. These contents can vary substantially in the batches of the different production. Consequently, this may probably cause growth reduction due to the toxicity of this antinutritional factor (George, Makkar and Becker, 2001).

On the other hand, the incorporation of a higher percentage of copra meal in fish feeds for milkfish, grey mullet, mangrove red snapper and red snapper may have probably contributed

(Table 3.2). The incorporation of high copra meal may lead to decreased fish growth (Mukhopadhyay, 2000; Olude, Alegbeleye and Obasa, 2008). Copra meal lacks lysine, methionine and sulphur amino acids (Ohler, 1993). This is analogous with an experiment done on milkfish where poor growth and survival was observed in amino acid deficient diets (Borlongan and Coloso, 1993; Mukhopadhyay, 2000)

Feeding trial with this lower dietary protein contents at a higher feeding level and frequency have been successful in promoting fish growth. In a previous experiment milkfish fed with 24% CP diet at 4% body weight supported fish growth (Sumagaysay and Borlongan, 1995). The low crude protein of the diets may not have been sufficient to support the growth of the carnivorous fishes (mangrove red snapper and red snapper). This finding is similar to an experiment on varying dietary protein levels of mangrove red snapper. The fish fed to 28%, 33% and 38% CP had lower growth compared to those fed with 43% CP diet (Abbas and Siddiqui, 2013).

5.5. Length-weight relationship

All the fish exhibited negative allometric growth $b < 3$ (Biswas *et al.*, 2011; Zubia *et al.*, 2014)). The body became slender as the fish length increases. This may be probably due to the low protein content fed to fish at a low feeding level (feeding regime). Inadequate protein content may probably lead to reduction in growth due to withdrawal of the protein from the less vital tissues to maintain the body (Amoah, 2012). The fish growth was probably affected by the dietary protein (Abdel-Tawas *et al.*, 2010).

The length-weight relationship determines the condition of the fish. The b values may on the other hand vary within the same species due to amount of food available, maturity stage, growth rate, difference in age, time sampling was done and the sample size. The environmental condition in which the fish is cultured may also affects fish growth (Biswas *et al.*, 2014).

5.6. Variation of fish weight with time

The variation of the grey mullet weight with time showed a decreasing trend (Figure 4.10). The slight increments may be due to replacement of fish mortality. The milkfish and mangrove red snapper's weight decreased slightly and later increased although there was no

difference in the weight (Figure 4.9, 4.11). The slight decrease in fish weight at the start of the experiment may have been due to low acceptability of the feed. The red snappers on the other hand had a slight increase in fish weight and thereafter decreasing (Figure 4.12). Lack of fish growth may probably be attributed to the 3% feeding level once daily being low. This low level of feeding was probably not able to supply the energy requirements of the cultured fish species (Sumagaysay and Borlongan, 1995).

Experiments have been done to investigate the most economical combination of dietary protein and the lowest feeding levels. The feeding level of 2% and 4% was investigated. The 4% feeding level demonstrated high energy assimilation by milkfish and thus leading to fish growth. Therefore, the feeding level may influence fish growth since it affects the amount of energy assimilated (Sumagaysay and Borlongan, 1995). The feeding level of 3% has been successful in other experiments. Despite, the fish were fed twice a day and the crude protein content of those diets was high (30%) (Olude, Alegbeleye and Obasa, 2008).

Fish growth means deposition of lipids and proteins in the fish body. This material for building the new tissue and the energy to deposit it is supplied by fish feed. Additionally, the fish need the energy for maintenance to be supplied. Therefore, the protein and energy requirement of a particular fish is the sum of fish's needs and growth. The amount of energy and protein gained by the fish is a function of: amount of feed eaten by fish, amount of energy and protein content of the feed minus the energy that is spent via metabolism. Sufficient amount of feed has to be supplied for the fish to grow. The feeds formulated may be suitable for fish that feed low in the trophic level (milkfish and grey mullet). Consequently, they may be unsuitable for carnivorous fish (mangrove red snapper and red snapper). They require feed of higher protein content than those formulated in the experiment (Lupatsch, 2007).

5.7. Fish growth performance

The milkfish fed with "sagana" diet had a similar growth performance with those fed with 12% formulated diet (Figure 4.15). Grey mullet fed with 6% formulated diet had a homogenous growth performance with those fed with "sagana" diet. The grey mullet fed with 12% formulated diet had the highest growth performance (Figure 4.16). The 6% and 12% formulated diets had a lower dietary protein content compared to "sagana" diet (Table 4.2). This shows that the "sagana" diet had a lower growth performance. This is because it had

a higher protein content and thus high amount of energy to be assimilated. Hence, inclusion of cottonseed cake may have probably contributed to decrease in growth performance. Moreover, the inclusion of copra meal in fish feed formulation may lead to better growth performance (Mukhopadhyay, 2000; Meric *et al.*, 2011).

The mangrove red snapper and red snapper had similar growth performances. The "sagana" diet and snapper formulated diet had similar crude protein content. The dietary protein may affect the growth performance (Abdel-Tawwab, 2010). The 12% formulated diet in grey mullet had the highest growth performance. Incorporation of 12% fish meal was better compared to 6% fish meal in the diets. Incorporation of 12% fish meal could be appropriate for fish diets. This was because fish meal has a balance in the amino acid profile which promotes growth (Miles and Chapman, 2005).

The fish growth performance may be affected by the feeding frequency. This is visible in the lipid content of the milkfish carcass. There was a strong decrease in the lipid content in the milkfish carcass after the experiment in the respective treatments as discussed earlier. The low feeding frequency may have contributed to insufficient growth since there was no increase in the protein content. In an experiment on the red snapper the fish gained weight when fed at 3% body weight twice a day although the crude protein content in the diets was higher. Thus the feeding frequency may affect the growth performance (Miller, Davis and Phelps, 2005).

5.8. Feed cost test

Rastrineobola argentea is more costly than sardine (*Sardina pilchardus*). The sardine is a by catch in marine fisheries. This ingredient was locally available at the Kenyan coast (where the experiment was conducted). *Rastrineobola argentea* is a by catch in Lake Victoria fisheries (Charo-Karisa *et al.*, 2013). The prices of these fish meals were probably high due to increase in demand from aquaculture and other animal feed industries. Consequently, fish meal supply has decreased due to the decrease in capture fisheries (Borski *et al.*, 2011; Charo-Karisa *et al.*, 2014). In the plant proteins cottonseed cake was the most costly ingredient (Table 4.10). The cottonseed cake is rarely available at the Kenyan coast. Copra meal is easily available since it's a by-product of oil extraction from coconuts (available in Kenyan coast) (Ohler, 1993). Thus, this experiment was performed to replace cottonseed cake with copra meal.

The cottonseed cake was the most costly plant protein and had to be purchased upcountry. The cost of cottonseed cake is reflected in the "sagana" diet. The incorporation of copra meal for instance in the 6% formulaed diet aided in reducing the price by almost three times. The incorporation of copra meal in the fish feed formulation led to the decrease in the cost of the feed (Table 4.11). This concurs with Charo-Karisa *et al.*, 2013 who stated that incorporation of the locally available ingredients in the fish feed formulation aids in reducing the cost of feed. The incorporation of copra meal aids in reducing the cost of feed where it's locally available (Mukhophyay, 2000).

The amount of fish meal incorporated in fish diets influences the cost of the feeds. The "sagana" diet, snapper formulated diet and 12% formulated diet had 12% fish meal incorporated in the diets (Table 3.1). The cost of fish meal was high. The amount of fish meal incorporated in the diet may also influence the cost of the feed (Table 4.6). The 6% formulated diet had the lowest cost since it had 6% fish meal incorporation (Catacutan and Pagador, 2004; Borski *et al.*, 2008).

5.9. Use of the grazers to control macrofouling in cages

The growth of macroalgae hinders effective water exchange in the cages. The parrot and tongue fishes added to our experimental fish stock are herbivorous. The two species helped in deflecting succession which was at the first stage of brown (Figure C.5, C.7, C.9 and C.11) and green filaments (Figure C.1 and C.3). This two species' grazing effect decreased the biomass of the macroalgae in the cages (Figure C.2, C.4, C.6, C.8, C.10, and C.12). The grazing effects of the Parrot and tongue fish were successful in deflection of the succession of the macroalgae. Thus, herbivory has an effect on the rate of succession and may cause the macroalgae to follow a different trajectory. The macroalgae biomass and biodiversity decreased. Other than that: macrofouling could encourage polyculture since this macroalgae can be fed by other fish species (Hixon and Brostoff, 1996; Rejeski, Susilowati and Aryati, 2010). The tongue fish when stocked separately was effective in reducing macro algae as when stocked with parrot fish.

5.10. Significance of the study

This study was undertaken to determine: the possibility of incorporating copra meal in fish feeds, appropriate level of fish meal inclusion in diets and if feeding at 3% could promote fish growth and survival. Copra meal could be incorporated in fish feed diets. However, if incorporated in large quantities there should be amino acid supplementation. Incorporation of 12% fish meal may be appropriate for fish diets. The feeding level of 3% is sustainable at a high dietary protein and feeding frequency.

CHAPTER 6

Conclusion

The incorporation of copra meal affected the lipid content of milkfish carcass. The culture conditions were not at their optimum due to the low dietary protein, feeding level and feeding frequency. This led to low survival (grey mullet) and insufficient fish growth. The incorporation of 12% fish meal could be appropriate for fish diets. The incorporation of copra meal in fish feed contributes to the reduction of the cost of feed. The feeding level of 3% body weight once daily with the low crude protein diets is low. This led to poor growth in the fish species cultured and low survival in grey mullet. To feed the fish at a higher feeding frequency: a constant transport logistic is needed to support cage plots placed in mangroves. This is not easy for communities to manage and thus automatic feeder incorporation to the setup is recommended.

6.1. Recommendation

1. The feeding levels should be increased by incorporating an automatic feeder due to the tide schedule. The site location could only be accessed once a day due to the tide schedule.
2. The fish feeds with a high amount of copra meal incorporation should have amino acid supplementation. This is because copra meal is deficient of lysine, methionine and sulphur amino acids.
3. A similar size of fish should be cultured for the carnivorous fish species since cannibalism was observed.
4. Further research should be conducted before recommending the high amount of copra meal incorporation in fish feeds.

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Appendices

Appendix A (ANOVA and Post Hoc results at 95% CI)

Table A.1 ANOVA result of the mangrove red snapper and red snapper condition factor

	SS	DF	MS	F	P
Between groups	1.14	1	1.14	41.81	0.00
Within groups	22.51	827	0.27		
Total	23.65	828			

Table A.2 ANOVA result of the mangrove red snapper and red snapper survival

	SS	DF	MS	F	P
Between groups	77079.53	1	77079.53	136.9	0.00
Within groups	465626.77	827	563.031		
Total	542706.3	828			

Table A.3 ANOVA result of the survival of milkfish and grey mullet

F		SS	DF	MS	F	P
Species	Between groups	3563.57	2	1781.79	21.14	0.00
		71802.23	852	84.28		
	Within groups	75365.80	854			
	Total					
Treatment	Between groups	15404.21	2	7702.1	40.80	0.00
		515329.24	2730	188.77		
	Within groups	530733.44	2732			
	Total					

Table A.4 Bonferroni result of the survival of milkfish and grey mullet fed on "sagana" diet, 6%_s diet and 12%_s diet

(I)Treatment	(J) Treatment	MD(I-J)	SE	P	Lower bound	Upper bound
6% _s formulated diet	12% _s diet	-6.73	0.58	0.00	-8.13	-.5.34
	"sagana" diet	-1.78	0.52	0.00	-3.02	-0.54
12% _s formulated diet	6% _s diet	6.73	0.58	0.00	5.34	8.13
	"sagana" diet	4.95	0.52	0.00	3.71	6.2
"sagana" diet	12% _s diet	1.78	0.52	0.02	0.54	3.02
	6% _s diet	-4.95	0.52	0.00	-6,2	-3.71

Table A.5 ANOVA result of the milkfish and grey mullet condition factor fed on 6%_s diet and 12%_s diet and "sagana" diet

		SS	DF	MS	F	P
Species	Between	615.31	983	0.063	341.81	0.00
	groups	3.88	2116	0.002		
	Within groups	6.88	3099			
	Total					
Treatment	Between	1619.19	983	1.66	2.17	0.00
	groups	1635.29	2118	0.77		
	Within groups	3260.78	3101			
	Total					

Table A.6 Bonferroni result of the condition factor of milkfish and grey mullet fed on "sagana" diet, 6%_s diet and 12%_s diet

(I)Treatment	(J) Treatment	MD (I-J)	SE	P	Lower bound	Upper bound
12% _s formulated diet	6% _s formulated diet	0.80	0.11	0.00	0.053	0.108
	"sagana" diet	0.38	0.01	0.00	0.014	0.062

6% s formulated diet	12% s formulated diet "sagana" diet	-0.8 -0.42	0.11 0.11	0.00 0.00	-0.108 -0.066	-0.053 -0.018
"sagana" diet	12% s formulated diet 6% s formulated diet	-0.038 0.042	0.01 0.01	0.00 0.00	-0.062 0.018	-0.014 0.066

Table A.7 ANOVA results of proximate composition of milkfish

Component		SS	DF	MS	F	P
Protein	Between groups	7.19	3	2.40	0.378	0.77
	Within groups	50.75	8	6.34		
	Total	57.93	11			
Lipid	Between groups	167.85	3	55.95	163.83	0.00
	Within groups	2.73	8	0.342		
	Total	170.58	11			
Ash	Between groups	8.698	3	2.899	1.89	0.21
	Within groups	20.974	8	1.534		
	Total	12.276	11			
Moisture	Between groups	17.700	3	5.900	3.58	0.006
	Within groups	30.888	8	1.649		
	Total	13.189	11			

Table A.8 Bonferroni result of the lipid content of milkfish fed to the various diets

(I)Species	(J) Species	MD (I-J)	SE	P	Lower bound	Upper bound
MF 6% s formulated diet	MF 12% s formulated diet	8.28	0.48	0.00	6.62	9.94
	MF Before	8.69	0.48	0.00	7.03	10.35

	MF "sagana"	8.90	0.48	0.00	7.24	10.55
MF 12% formulated diet	MF 6% formulated diet	-8.28	0.48	0.00	-9.94	-6.62
	MF Before	-0.40	0.48	1.00	-1.26	2.06
	MF "sagana"	0.61	0.48	1.00	-1.05	2.27
MF Before	MF 6% formulated diet	-8.69	0.48	0.00	-10.35	-7.03
	MF 12% formulated diet	-0.40	0.48	1.00	-2.06	1.26
	MF "sagana"	0.21	0.48	1.00	-1.45	1.87
MF "sagana"	MF 6% formulated diet	-8.9	0.48	0.00	-10.55	-7.24
	MF 12% formulated diet	-0.61	0.48	1.00	-2.27	1.05
	MF Before	-0.21	0.48	1.00	-1.87	1.45

Table A.9 Bonferroni result of the ash content of milkfish fed to the various diets

(I)Species	(J) Species	MD(I-J)	SE	P	Lower bound	Upper bound
MF 6% formulated diet	MF 12% formulated diet	1.86	1.011	0.62	-1.66	5.38
	MF Before	-0.20	1.011	1.00	-3.72	3.32
	MF "sagana"	1.20	1.011	1.00	-2.32	4.72
MF 12% formulated diet	MF 6% formulated diet	-0.66	1.011	0.62	-5.38	1.66
	MF Before	-2.060	1.011	0.46	-5.58	1.46
	MF "sagana"	-1.86	1.011	1.00	-4.18	3.72

MF Before	MF 6% s formulated diet	0.20	1.011	1.00	-3.32	4.92
	MF 12% s formulated diet	2.06	1.011	0.46	-1.46	5.58
	MF "sagana"	1.40	1.011	1.00	-2.12	3.72
MF "sagana"	MF 6% s formulated diet	-1.204	1.011	1.00	-4.92	2.32
	MF 12% s formulated diet	0.066	1.011	1.00	-2.86	2.12
	MF Before	-1.402	1.011	1.00	-4.72	4.18

Table A.10 T- test results of the difference in the weight of fish before and after the experiment

Diet	Species	Mean	SD	SE	Lower bound	Upper bound	t	df	Sig (2 tailed test)
"sagana" diet	Red snapper	0.15	38.22	10.6	-22.94	23.25	0.015	12	0.99
	Mangrove red snapper	-0.26	46.67	8.38	-17.38	16.86	-0.031	30	0.98
	Milkfish	2.56	19.39	2.12	-1.65	6.77	1.21	83	0.23
	Grey mullet	3.81	13.77	1.68	0.45	7.17	2.26	66	0.27
6% s formulated diet	Milkfish	2.57	15.75	1.72	0.85	6.00	1.50	83	0.14
	Grey mullet	0.41	7.44	1.59	-2.89	3.71	0.26	21	0.80
12% s formulated diet	Milkfish	5.06	16.48	1.75	1.58	8.53	2.89	88	0.005
	Grey mullet	0.05	6.49	1.45	-3.00	3.085	0.034	19	0.97
Snapper formulated diet	Red snapper	16.38	43.98	15.55	-20.39	53.14	1.053	7	0.33
	Mangrove red snapper	2.76	28.54	5.3	-8.10	13.61	0.52	28	0.61

Appendix B (Multiple regression tables for the respective fish species)

Table B.1 Multiple regressions of fish length and weights of milkfish fed with the "sagana" diet, 6% formulated diet and 12% formulated diet.

Parameter	Estimate	SE	T-statistic	P
Constant	-4.32	0.079	-54.76	0.00
LnL	2.75	0.028	97.15	0.00
Code=2	0.26	0.12	2.18	0.029
Code=3	0.18	0.13	1.42	0.16
LnL*Code 2	-0.092	0.43	-2.14	- 0.033
LnL*Code 3	-0.082	0.045	-1.83	0.067

Where code= 2 is 6% formulated diet and code=3 is 12% formulated diet.

Table B.2 Multiple regressions of fish length and weights of grey mullet fed with the "sagana" diet, 6% formulated diet and 12% formulated diet.

Parameter	Estimate	SE	T-statistic	P
Constant	-3.02	0.075	-40.27	0.00
LnL	2.39	0.039	61.91	0.00
Code=2	-0.16	0.1	-1.59	0.11
Code=3	-0.44	0.11	-3.94	0.0001
LnL*Code 2	0.067	0.052	1.28	0.20
LnL*Code 3	0.19	0.057	3.23	0.0012

Where code= 2 is 6% s formulated diet and code=3 is 12% s formulated diet.

Table B.3 Multiple regressions of fish length and weight of mangrove red snapper fed with "sagana" diet and snapper formulated diet

Parameter	Estimate	SE	T-statistic	P
Constant	-3.88	0.12	-32.19	0.00
LnL	2.84	0.047	60.85	0.00
Code=2	-0.037	0.15	-0.25	0.81
LnL*Code 2	0.041	0.058	0.70	0.48

Where code=2 is the snapper formulated diet

Table B.4 Multiple regressions of fish length and weight of red snapper fed with "sagana" diet and snapper formulated diet

Parameter	Estimate	SE	T-statistic	P
Constant	-3	0.34	-8.86	0.00
LnL	2.57	0.11	22.45	0.00
Code=2	-0.11	0.66	-0.17	0.86
LnL*Code 2	0.036	0.22	0.16	0.87

Where code=2 is the snapper formulated diet

Appendix C (Photographs showing the mangrove and red snapper cages before and after adding the grazers to the respective cages)



Figure C.1 Cage 1 before adding the grazers to the stock



Figure C.2 Cage 1 one month after adding grazers to the stock



Figure C.3 Cage 2 before adding grazers to the stock



Figure C.4 Cage 2 one month after adding the grazers to the stock



Figure C.5 Cage 3 before adding the grazers to the stock



Figure C.6 Cage 3 one month after adding the grazers to the stock



Figure C.7 Cage 4 before adding grazers to the stock



Figure C.8 Cage 4 one month after adding grazers to the stock to the stock



Figure C.9 Cage 5 before adding grazers to the stock to the stock



Figure C.10 Cage 5 one month after adding the grazers to the stock



Figure C.11 Cage 6 before adding the grazers to the stock



Figure C.12 Cage 6 one month after adding grazers to the stock