GROWTH PERFORMANCE OF RABBITFISH (*Siganus sutor*) REARED IN BRACKISH WATER EARTHEN PONDS, OFFERED BLACK SOLDIER FLY (BSF) LARVAE (*Hermetia illucens*)

OKEMWA DOUGLAS MINYONGA

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A THESIS SUBMITTED TO SCHOOL OF NATURAL RESOURCES AND ENVIRONMENTAL STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE CONFERMENT OF THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL STUDIES,

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for award of a degree in any other University or for any other award

Signature: …………………………. Date: …………………………….

Okemwa Douglas Minyonga
(N301/1510P/19)
Department of Environmental Studies
Karatina University

Declaration by the supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors

Signature: ………………………… Date: ………………………….

Prof. Charles Ngugi, EBS
Department of Natural Resources
Karatina University

Signature: …………………………. Date: …………………….

Dr. David Mirera
Department of Mariculture
Kenya Marine and Fisheries Research Institute

DEDICATION

I dedicate this work to my family Mr. and Mrs. Okemwa, My siblings (Justine Marvalene and Wycliffe) for their daily prayer support. You will forever be my heroes.

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ABBREVIATIONS AND ACRONYMS

DDGS	Dried distiller's grains with soluble
DHA	Docosahexaenoic acid
EPA	Eicosatetraenoic acid
FAO	Food and Agricultural Program
UN	United Nations
W.H. O	World Health Organization
NEPAD	New Partnership for Africa's Development
USD	United States of America Dollar
AOAC	Association of Official Analytical Chemist
KMFRI	Kenya Marine and Fisheries Research Institute
ANOVA	Analysis of Variance
CAADP	Comprehensive Africa Agriculture Development Program
DGW	Daily Growth Rate
SGR	Standard Growth Rate
FCR	Feed Conversion Rate
L	Length
W	Width
DO	Dissolved Oxygen
Cl	Chlorine
NH_4	Ammonium
NO_3	Nitrate
NO_2	Nitrite
PO_4	Phosphorous
BSFL	Black Soldier Fly Larvae
DWR	Daily Growth Rate
T_1	Treatment 1
T_2	Treatment 2
T_3	Treatment 3

T_4	Treatment 4
KCSAP	Kenya Climate Smart Agriculture Project
KMFRI	Kenya Marine and Fisheries Research Institute
ADL	Average Daily Length
FAO	Food agricultural organization.
Chl-*a*	Chlorophyll- *a*
GDP	Gross Domestic Product
SPSS	Statical package for the social science

ABSTRACT

The trend in mariculture development in Kenya is rising as the population and the demand for food increases. Rabbitfish (*Siganus sutor)* is a suitable candidate for mariculture in Kenya since it can be raised in ponds, accepts formulated feeds, and has a better growth performance as compared to other marine finfishes. It is also acceptable to local and international consumers and thus will have no marketing problem. Currently, *S. sutor* is the most targeted species and heavily fished on the Kenyan coast and Mariculture would increase its availability and sustainability. This study presents a 90 day's first trial on the growth performance of *S.sutor* fed on black soldier fly meal in brackish water environment on the north coast in Kenya earthen ponds (hapa nets). The study had a complete block randomized design (CBRD) with four treatments, which were performed in triplicates and assigned randomly among twelve hapa net cages set in three rows (black nylon, sized (#1mm) and 1.5 x 1.0 x 1.2 meters). *S.sutor* were stocked at 15/fish/hapa and fed twice a day (0900HRS and 01600HRS) at 5% body weight throughout the study period. The cages were allocated treatments randomly as, T1 100% black soldier fly larvae, 0% fish meal, T2 25% fish meal replaced with 75% black soldier fly larvae), T3 50% fish meal was replaced with 50% BSFL and T4 was used as a control (40% commercial feed). Sampling was done monthly for total length (cm) and weight (g). The results showed that 100% BSFL feed had the highest initial mean weight of 36.06±0.99g, 40% commercial feed had the lowest at 33.76±0.68g. The highest final mean weight was observed in T1, (100% BSFL) at 68.36±0.42 and T2, (25% fish meal 75% BSFL) the lowest at 57.28±0.33g. Feed conversion ratio (FCR) in all diets and growth performance among the different treatments had no significant difference ($p<0.05$) at ($F=6.9122$, $df=3$, $p<0.05$). The water temperatures, pH, salinity, dissolved oxygen (DO), nitrogen (NH_3-N), Nitrites - nitrogen (NO_2-N) and total ammonia (NH3-N) values in all treatments were within the optimal limits for *S. sutor* growth. The study reports useful information on the growth performance and survival of *S.sutor* fed on black soldier fly larval diet. These findings, from this study revealed that *S. sutor* is suitable for culturing in brackish waters and the community can easily benefit from the species and act as food security and provide an alternative source of income for rural communities.

Keywords: Rabbitfish, growth performance, brackish water, mariculture, *Hermetia illucen*

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Globally there are over one billion people who consume fish (capture and culture), as one of the major sources of high-quality protein (Ahmad, Siddiqui, Nabi, Khan & Zamir, 2020; Khalid, Habib, Mohammad, Momin, & Abdel-Aziz, 2020). The drastic increase of the world population has led to food scarcity and protein deficiency in particular (Cochrane, Young, Soto & Bahri, 2009). Conventional food production systems such as agriculture, fishing and livestock rearing are unable to cope with the food scarcity demand (Foley *et al*., 2011, Mekonnen & Hoekstra, 2012). Efforts to boost productivity via the conventional methods not only generate large quantities of organic wastes but also results in loss of biodiversity due to increased utilization of natural resources and climate change (Mutafela, 2015). As we move towards 2050, the problem of food security, protein malnutrition and the quality of sanitation are expected to decline globally (FAO, 2013a)

World aquaculture production and growth vary, currently, Asia is the leading in aquaculture production at 59.9%, while Africa has fewer quantities of aquaculture to report on globally at 1.8% even though there is availability of natural resources in some regions (FAO, 2016c, 2018b). Aquaculture has been recognized as playing an important role in food security, good nutrition and social development (FAO, 2017c; FAO 2019 d; FAO, 2017f).

Most of aquaculture production (approximately 99%) in Africa comes from inland freshwater systems and is mostly dominated by the culture of indigenous and abundant species of tilapia and African catfish, while mariculture only contributes a meager 1% to the total quantity produced. Nevertheless, mariculture is an emerging and promising subsector (FAO 2016b, 2018c). Both capture fisheries and aquaculture are geared towards providing fish products to the increasing human population (Tidwell and Allan, 2001). However, capture from fisheries has been over-exploited, leading to massive fish yield reduction FAO (2014). This has led to a shift of focus to aquaculture to bridge the gap, as it's visioned as the fastest growing food sector at 5.6% (FAO, 2010).

On the other hand, fish importation has declined drastically from 36,000 metric tons in the year 2006 to 23,000 metric tons in 2017 (FAO, 2018) (Figure 1). In order to be able to feed the growing population as well as maintain the per capita consumption rate of (4.5/kg/year) this means that 270,000 metric tons of fish will be required by the year 2030. With a production of 120,000 metric tons per year in 2017, aquaculture need to be enhanced including fish feeds formulation to meet the 150,000 metric tons deficit by the year 2030 (FAO, 2018)

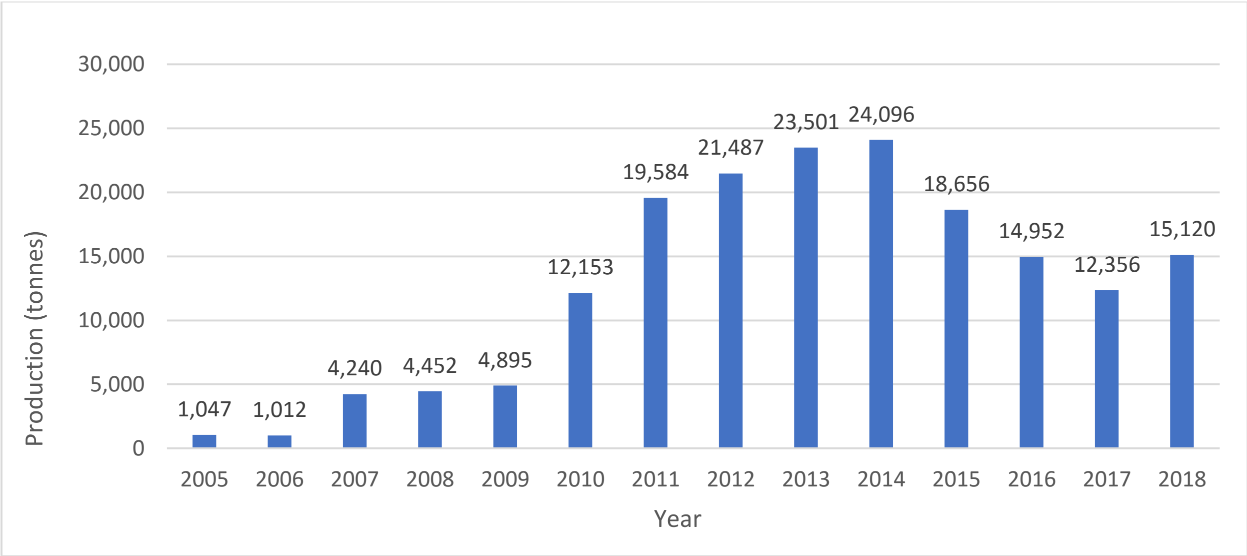

Figure 1: Global capture fisheries and aquaculture production (a) historic and projected production excluding aquatic plants 1980–2030 and (b) production including aquatic plants 1980–2018

Aquaculture production trend in Kenya (2005 to 2018). Source: (FAO, 2016a; KNBS, 2019).

Incase capture fisheries rejuvenated and becomes stable at the production of 120,000 metric tons, this would mean that aquaculture has to produce 150, 000 metric tons in order to meet the 270,000 tons of fish as per the demand by the year 2030 (FAO, 2018e) (Figure 2).

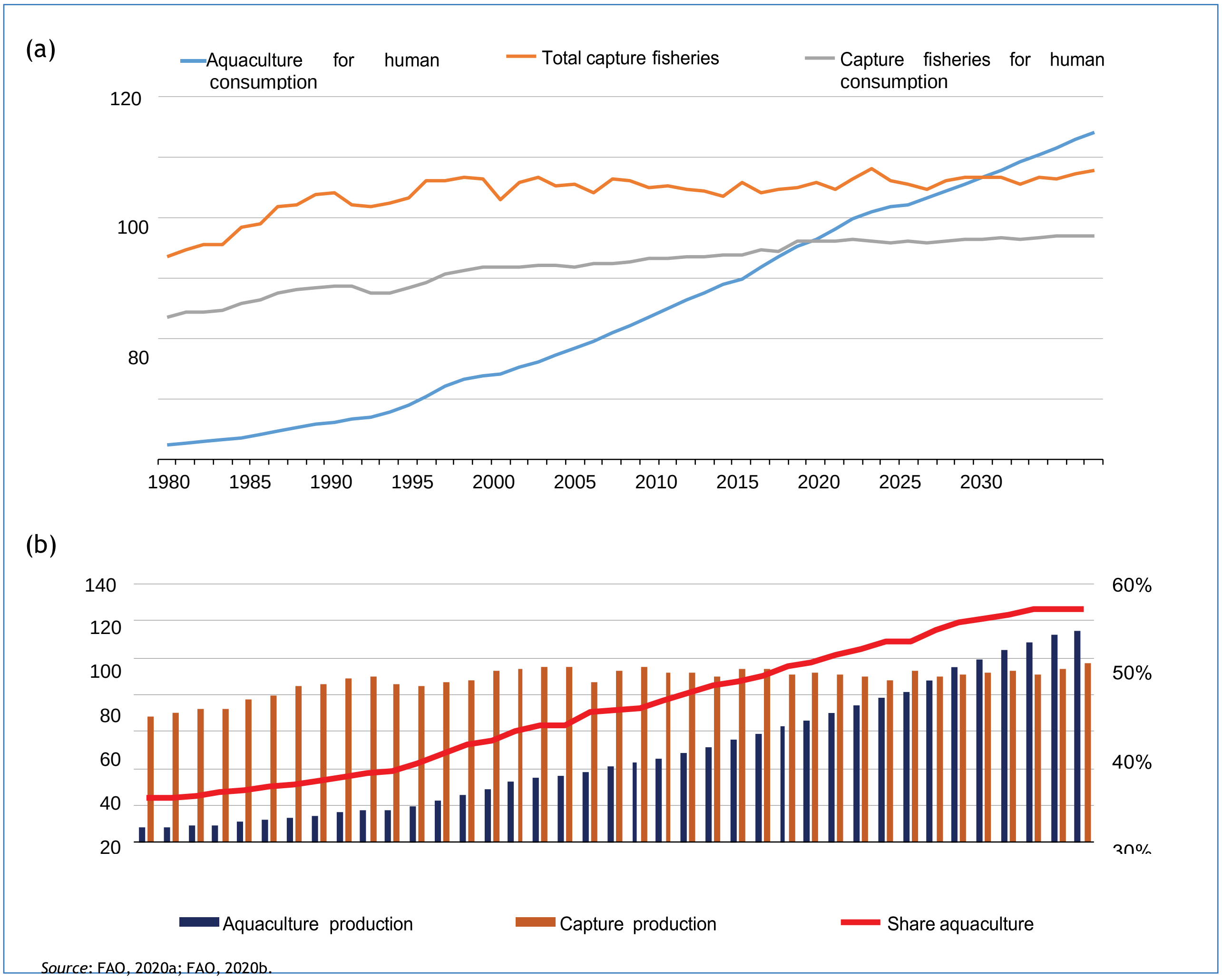

Figure 2:Global capture fisheries and aquaculture production (a) historic and projected productionexcluding aquatic plants 1980–2030 and (b) production including aquatic plants 1980–2018.

1.2 Status, Trends, Challenges and Future Outlook of Aquaculture in Kenya

According to Ngugi, Bowman and Omolo (2007) aquaculture in Kenya took root in the early 1900s through introduction of trout in rivers for sport fishing. Later, in the 1920s the sport fishing advanced to static pond culture which involved the culturing of tilapia, common carp and catfish species. Sagana and Kiganjo trout fish farms were the pioneers of small-scale rural fish farming. The farms focused on rearing the warm water and cold-water species respectively (Ngugi & Manyala, 2004; Ngugi, Bowman & Omolo, 2007). By the year 1960, aquaculture had become popular in most regions around the country with a dramatic growth of the sector being observed over the last decade. Currently, aquaculture is majorly practiced in Kakamega, Bungoma, Busia, Kisii, Meru, Nyeri, Kisumu, Muranga and Embu counties with less practice in Kitui, Lamu and Elgeyo Marakwet (Opiyo, Marijani, Muendo, Odede, Leschen & Charo-Karisa, 2018). Approximately 1.4 million hectares of land is suitable for aquaculture production in the country, however of this, only 0.014% is currently being utilized with 95% of the practice being small scale (Otieno, 2011).

In 2003, thanks to the "Eat More Fish Campaigns" championed by the government, the production rose from 1000 to 4000 Metric Tonnes (MT) (Opiyo *et al*., 2018). By 2007, approximately 722.4 hectares of land was being used by about 7500 farmers for production of fish from approximately 7,477 production units (Nyonje, Charo-Karisa, Macharia & Mbugua, 2011; Munguti, Kim & Ogello, 2014). From these units, the mean yield was estimated to be approximately 5.84 MT/year which accounted for only 3% of the total fish production (Munguti *et al.,* 2014).

In an attempt to alleviate poverty, curb malnutrition, enhance economic development, promote economic recovery and trigger regional development in Kenya (Nyonje *et al.*, 2011) the government channeled Ksh. 22 billion into key sectors, the major beneficiary being the aquaculture sector. From this, the ambitious Economic Stimulus Program-Fish

Farming Enterprise Productivity Program (ESP - FFEPP) was born in 2009. "The program aimed to increase production of farmed fish from 4,000 MT to over 20,000 MT in the medium term and to more than 100,000 MT in the long term" (Charo-Karisa & Maithya, 2010).

The first year of the program foresaw the construction of over 200 new fishponds. The fishpond area increased from 220 hectares in 2008 to 468 hectares in 2009 while the total gross land for aquaculture rose to 825 hectares (2009) from 728 hectares (2008) (Charo-Karisa & Maithya,2010; Opiyo *et al.*,2018). With these, the yields rose 6 from 4895 MT to 12,153MT/year (Nyonje et al., 2011; Opiyo *et al*., 2018) and placed Kenya 4th in the aquaculture production in Africa (Nyonje *et al*., 2011).

Positive growth in fish production in the country was seen following the ESP - FFEPP. In 2014 a peak production was experienced at 24,096MT. However, in 2015 the production dropped to 18,656MT and further to 14,952 MT in 2016 (Opiyo *et al*., 2018). The reduction in the number of operational fishponds from 69,194 (2013) to 60,277 (2015) and the shrinking of the operational area from 2,105 to 1,873 hectares in 2013 and in 2015 respectively was attributed to by various factors among them; poor extension services to the beneficiaries, inadequate capacity building, poor husbandry practices by the farmers, inadequate quality and quantity of fish farm inputs like feeds, poor marketing infrastructure and dependency syndrome on government and donor support. The major blow was felt when the aquaculture sector was devolved from the national government into the county government operations. Counties that lacked sufficient support programs for fish recorded a reduction in aquaculture practices (Opiyo *et al*., 2018).

The Government of Kenya through Vision 2030 recognizes aquaculture as a key driver of food security, employment creation and economic growth (Vision 2030, GoK, 2007.

In Kenya, the technology for culture of marine finfish, shellfish, and seaweeds is still developing and yet to be maximized to ensure increased production (Mirera, 2009; Mirera & Ngugi, 2009; Mirera, 2011a; Mirera, 2011b). Mariculture in Kenya is slowly taking shape, due to interventions from FAO, which has led to the farming of several marine species by organized community groups (Mirera, 2009). Since 2005, milkfish (*Chanos chanos*) is the most candidate cultured species along the Kenyan coast in intertidal earthen ponds (Mwaluma, 2003, Mirera and Ngugi 2009, Mirera 2011a). However, other species that are under considerations in pond culture are rabbitfish (*Siganus sutor*) and mullet (Sivalingam, 19; Mirera, 2007a).

There is increased interest in the culture of rabbitfish in ponds or cages in several areas (Duray, 1990), as it has high tolerances to environmental factors, rough handling and crowding and it can be stocked at very high density (Saoud *et al*., 2007a). In Kenyan coastal areas of Kilifi creek and Shimoni have rich and diverse ecosystems such as estuaries, mangroves, sea grass beds and coral reefs that are found there. These ecosystems are vital spawning areas, sheltering and feeding areas for a variety of fishes (Cullen-Unsworth & Unsworth, 2013; Mirera, Kairo, Kimani & Waweru, 2010)). The presence of spawning areas gives an indication of the availability of juveniles for mariculture. *Siganus sutor* is one of the main fish species which is predominantly caught in the Kenyan coast, Robinson (2011). Thus, a suitable candidate for food security enhancement for the coastal communities (Foale *et al*., 2012).

The cost of fish feed accounts for over 60% of the total production costs and the most expensive to venture and thus hindering the potential of aquaculture sector to bridge the gap between fish demand and supply (Gabriel, Akinrotimi, Bekibele, Onunkwo & Anyanwu, 2007), and it makes the product expensive and unaffordable (FAO, 2007). Locally, the main source of fish meal is from silver cyprinid (*Rastrineobola argentea)* locally known as “Omena” from Lake Victoria and the Indian Ocean waters (Bokea & Ikiara, 2000). The report shows that 50 and 65% of the silver cyprinid which comes from the Lake is used to produce fishmeal (FAO, 2007). And, is for this reason that fish meal supply is considered a major constraint facing the animal feed industry (Bokea & Ikiara, 2000).

Livestock, poultry and aquaculture farming requires use of fishmeal in feed diets (Merino *et al.*, 2012; Mutafela, 2015, Oonincx, Van Huis & Van Loon, 2015, FAO, 2016). Approximately 30% of the fish catch (55.86% wild catch and 44.14% aquaculture) is diverted from human consumption to make fishmeal, a source of protein for animal feeds (FAO, 2016a; 2016b). The competition between humans and animals has led to overexploitation of these fish species and consequently, the cost of feeds has become expensive and unaffordable to most farmers (Merino *et al.*, 2012; Thurstan & Roberts, 2014a; FAO, 2016c). This has necessitated the search for alternative feedstocks for aquaculture such as plant and edible insects, which in turn can allow more fish to be used for human consumption (Bondari & Sheppard, 1981, Bondari & Sheppard, 1987; Devic and Marquart, 2015; Karapanagiotidis, Karapanagiotidis, Daskalopoulou, Vogiatzis, Rumbos, Mente & Athanassiou, 2014).

There is therefore a need for an alternative cheap and easily available source of protein, which are not suitable for direct human consumption and meets the protein requirements (Kassahun, Waidbacher & Zollitsch, 2012). In the recent focus has shifted to insect-based protein sources Okemwa, Ngugi and Mirera (2022) which are considered to have additional benefits such; as usage of fewer resources like land and water than crop proteins; and are suitable for fish feeds unlike plant proteins which have ant nutritive factors (Bondari and Sheppard 1981; van Huis *et al.*, 2013; Nguyen, Tomberlin & Vanlaerhoven, 2015).

To curb these challenges, a comprehensive study was designed in an attempt to culture *S. sutor* in coastal earthen ponds brackish water using hapa nets, and the juveniles were sourced from the wild and fed on black soldier fly larvae meal. Thus, the main aim of this study was to assess the growth performance of *S. sutor* cultured in coastal brackish water earthen ponds, hapa net cages.

1.3 Statement of the Problem

African fisheries make vital contributions to food security for 200 million people and provides income for over 10 million engaged in ancillary services such as fishing, fish farming or processing (NEPAD, 2006). Rising fish prices as a result of; decline in capture fisheries by foreign vessels, climate change and lack of modernized farming technologies among others are increasingly threatening food security in many sub-Saharan countries (FAO, 2009). As a result of drastic decline in capture fisheries calls to attention (Hecht, 2006). Although freshwater aquaculture has progressed significantly over the last decade, the mariculture sector presents the biggest opportunity, as it is yet to be fully exploited. Along the coastal of Kenya, the most commonly farmed finfish species is milkfish, which accounts for 90 percent, of the total products (FAO, 2018).

Ninety percent of fish farming along the Kenyan Coast is accounted by finfish species called milkfish fish. Fish farming requires provision of quality fish feeds for over 60% of total operational costs in aquaculture (Munguti *et al*., 2012). Protein plays a vital role in fish feeds, and it is the most expensive ingredients of all the feeds (El-Sayed, 2014). For decades, fish meal has been used in fish feed formulation due to its high digestibility, acceptance, protein, essential amino acids, and fatty acid profile (Tacon & Metin, 2009).

Due to climate changes and high human population growth, studies have shown that fish meal stocks are in decline, leading to high prices of the ingredient consequently high fish production costs (Barroso, De Haro, Sánchez-Muros, Venegas, Martínez-Sánchez & Pérez-Bañón, 2014; Muin, Taufek, Kamarudin & Razak, 2017). Schiavone, De Marco, Martínez, Dabbou, Renna, Madrid and Gasco (2007) suggested the need to find a high quality, cost-effective, accessible and sustainable alternative to protein source for fish meal in fish feeds formulation, which in return will enable optimum growth and positive development of species being cultured to in order to improve the aquaculture industry.

1.4 Justification

The *Siganus sutor* are considered to be a high market value fish in most countries including Kenya. In some countries such as South Pacific, the fish is sold for USD 14-18/kg) (FA0, 2020). In Singapore 20USD/kg in Kenya it sold for 5 USD /kg. This shows that the market demand for rabbitfish can be developed not only for local consumptions but also for export. Through the World Population Prospect, 2017, Kenya’s population is expected to reach 67 million by the year 2030. The rate of fish consumption in Kenya is 4.5 kg/person per year

(Opiyo et., al 2018), yet capture from fisheries has been on a decline yielding 170,000 metric tons in 2014 to 120,000 metric tons in 2017 (FAO, 2018) which is much lower.

The fish has received attention in aquaculture due to its environmental resilience and ability to tolerate conditions of captivity. The fish has a remarkable tolerance to crowded conditions, readily accepts artificial diets and due to its low trophic levels, the fish requires less protein or inexpensive feeds (FAO, 2018). Rabbitfish can be cultured either in brackish water ponds or in marine floating cages (Rachmansyah & Triyanto, 1997)

Proper nutrition has long also been recognized as a critical factor in promoting normal growth and sustaining fish health. Prepared diets not only provide the essential nutrients that are required for normal growth physiological functioning but also serve as a medium by which fish receive other components that may affect their health (Gatlin, 2002).

Any effective fish farming requires the correct use of protein ratios in feeds. Despite numerous animal and plant-based proteins being available for rearing fish, fish meal is still the leading main protein source in feed formulation. Research has shown a drastic fishmeal availability leading to high market prices. Black soldier fly larvae meal (BSFL) is an insect-based meal with potential to replace fishmeal to its availability, accessibility, low cost of production and high protein content with balanced amino acid profile (Diclaro & Kaufaman 2009). Black soldier fly larvae have antifungal activity and antibacterial peptides that may increase the shelf-life of insect-containing feeds.

1.5 Research Questions

1. What is the growth performance and survival rate of Rabbitfish fed different levels of formulated diet with (BSFL) diet as a substitute for fishmeal?
2. What is the proximate composition of formulated diet with (BSFL) as compared to fish meal?

1.6 Hypotheses

1. A diet with black soldier fly larvae meal diet has no significant effects on growth performance and survival rate of Rabbitfish as a replacer of fish meal.
2. Proximate composition of a diet with black soldier fly larvae meal is superior to that of fish meal.

1.7 Objectives of the Study

1.7.1 The general Objective

To assess of growth performance of rabbitfish (*Siganus sutor)* fed on locally formulated black soldier fly larvae meal (*Hermetia illucens*) diet and reared in brackish water earthen ponds in Kenya.

1.7.2 Specific Objectives

The specific objectives of the study were:

1. To assess the growth performance of rabbitfish offered black soldier fly larvae meal and fish meal in brackish water.

2. To compare the nutritional composition of locally formulated black soldier fly larvae and fish meal used in the formulation of rabbitfish diets.

1.8 Significance of the Research

Aquaculture has the potential to meet the demand for fishery products, providing employment, and supplying products in high demand without increasing pressure on the fisheries and increasing competition for resources. The sector, is important for provision of protein diet worldwide as per FAO's recommendation per capita consumption of fish. Unfortunately, aquaculture would not grow as anticipated without proper provision of quality feeds. The little fishmeal available is expensive due to competition from livestock and human consumption. This research will therefore help fish farmers to produce their own protein ingredient for fish feeds, and thus bring down the production cost and improve fish growth performance results. Thus, making fish farming viable to venture in with positive predictable outcomes.

1.9 Scope of the study

The study was conducted at Kilifi, County, Kibokoni Self-help, that has 54 members (14 men and 40 women). The focus was to develop a climate smart feed using black soldier fly, as the alternative feed in aquaculture. *S. sutor* was used to determine the quality, availability, affordability and the effectiveness of BSFL feed as compared with other diets. This reflected the true technology and information to be disseminated to other stakeholders. The study also was an insight to the community to engage in *S. sutor* farming in intertidal earthen ponds for livelihood.

1.10 Limitations

Production of black soldier fly in quantities more so during rainy season was a challenge, elsewhere, the collection enough *S. sutor* for this study took time since the season was low and thus the fishermen demanded more payment in order to source for the fingerlings.

1.11 Assumptions of the study

It was anticipated to have mass production of black soldier fly larvae for feed formulation, as well as high expectations to get collaborations from the counties and private parties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rabbitfish (*Siganus sutor*)

Siganus sutor is a member of Siganidae, commonly known as rabbitfish, the family is widely distributed in the Indian Ocean region (Lam 1974; Randall, Allen & Steene, 1995) and 13 species occur in the western Indian Ocean (WIO) including the endemic *S. Sutor* (Woodland, 1990). The fish occurs in coastal waters to a depth of at least 40 m and is an important target species in many artisanal and commercial fisheries.

2.1.1 Biology of Rabbitfish

The *Siganus sutor* (Figure 3) is distinguished by a deep compressed body, a stout resembling that of a rabbit, 13 dorsal, seven anal and two strong ventral fin spines. They possess a leathery skin, smooth, small and closely adherent scales, and are frequently mistaken to be scale less. *Lo* comprises of five species, with extended snouts and prominent face stripes earning them name of "fox face fishes". Snout shapes, caudal fins, body depths and shapes have been useful in distinguishing the members of these two genera, (Wambiji, Aura, Maina & Manyala, 2016; Kamukuru, 2009).

Figure 3*:* Rabbitfish (*Siganus sutor)*

Siganids colours change with age, after death and during preservation period in specimens (Randall & Kulbicki, 2005). Study shows that a mixture of environmental factors such as; temperatures, salinity, dissolved oxygen, radiation, water depth and currents flow may induce morphological variations between fishes (Brraich & Akhter, 2015). Other factors such as; reproduction and gonad development also influence fish morphology (Fakunmoju Boboye & Ijimakinde, 2014; Kashefi, Bani & Ebrahimi, 2012).

2.1.2 *Siganus sutor* Preference

The *S. sutor* has a high demand and is preferred by customers due to its taste and flesh quality, the high market price, and high demand (Singh, 2000). *S. sutor*, are nutritionally important fish with economic value in several Indo-Pacific countries and Eastern Mediterranean nations (FAO, 2020). The fish is fleshly and its good taste makes it desirable to many in Indo-Pacific and Eastern Mediterranean as well East Africa. The existing demand and market potential for these fishes in the domestic market are growing along the

Kenyan coast. *Siganus sutor,* are nutritionally important fish with economic value in several Indo-Pacific countries and Eastern Mediterranean nations (FAO, 2020).

2.2 Studies on *Siganus sutor*

Most studies on *S. sutor* in Kenya have been on reproductive biology, estimated size frequency distribution of isolated oocytes, gonad maturity stages, breeding cycle, length-weight relationship, condition factor, lunar periodicity of spawning and size at maturity (Wambiji *et al*, 2008). Furthermore, there have been studies on assessing impacts of habitat alteration (Logez & Pont, 2011), estimates of habitat suitability for species re-introductions (Lek, Belaud, Baran, Dimopoulos & Delacoste, 1996), predicting the likelihood of species invasions on threatened or endangered species (Dauwalter & Rahel, 2008). Other studies have also shown that rabbitfish breeds throughout the year with peaks occurring between March, April and November.

The *S. sutor* are herbivorous feeding mainly on blue green algae, zooplanktons some browse individually among corals (Foster & Smith, 2000; Froese, Demirel & Coro, 2013). Further studies on Rabbitfishes have been to, determine the optimum dietary protein ratio for *Siganus* (Abou-Daoud, Ghanawi, Farran, Davis & Saoud, 2014), stomach contents and length-relationship of white spotted rabbitfish (Park, Lee, Choi, Shin & Park, 2009). Other studies include, life cycle and fecundity of rabbitfishes (El-Sayed, and Barry, 1994), some aspects of the life history of Siganidae (Hasse, Madraisau & Vey, 1977) and morphometric relationship and condition factor of *Siganus stellatus, S. canaliculatus and S. sutor* (Wambiji *et al.* 2008).

2.3 Habitat of *Siganus sutor*

The fish species are distributed in reefs around sea grass, mangroves and estuarine habitats and also in shallow lagoons of tropical and subtropical coastal environment (Hoey, Brandl & Bellwood, 2013) and live in small schools of up to around 10 individuals as adult (Borsa, Lemer, Aurelle, Borsa, Lemer, 2011). A few species of this group have been cultured because of their herbivorous food habits, rapid growth and economic value (Hoey et al., 2013). They are often seen in a school when foraging the seagrass beds and algal flats, whereas some pair-bonding individuals' species are often seen on coral reefs (Seal & Ellies, 2019).

2.3.1 Feeding habits of *Siganus sutor*

Rabbitfishes graze on algae, seaweeds and sea grasses and are important to reef ecosystems because of their grazing prevent corals from being smothered by mats of filamentous and leafy algae. Siganids show lunar synchronized spawning activity, similar to other reef fish species (Harahap, Takemura, Nakamura, Rahman & Takano, 2001; Robinson & Samoilys, 2013) also they feed on the bottom or benthic seaweed (Nanami, 2018).

2.4 Black soldier fly (*Hermetia illucens,* Linnaeus, 1758) Diptera: *Stratiomyidae)*

Black soldier fly is native to South America, found mainly in warm temperate climates (Kalova & Borkovcova, 2013). The insect has been reported to occur in 80% of the worldwide including Africa (Robinson & Samoilys, 2013). In Africa, the insect is commonly found in Ghana and South Africa. In Kenya the insect is found in small scale in cultured systems with few researches being carried out on fresh aquaculture on tilapia

(*Oreochromis niloticus*). Black soldier fly (BSFL) *Hermetia illucens* is considered an important species for animal and aquatic feeds manufacturer (Cammack & Tomberlin, 2017; Van Huis, 2013). This is because BSFL contains high protein content as well as balanced profile of essential amino acids (AA), (Henry, Gasco, Piccolo & Fountoulaki, 2015; Wang & Shelomi, 2019).

2.4.1 Lifecycle of black soldier fly

Black soldier fly as an insect it undergoes a complete life cycle (Figure 4), that comprises of four stages such as; egg, larvae, pupa and adult (Li *et al*., 2011). The eggs hatch into larvae within 3-4 days after being laid (Diclaro & Kaufaman 2009). With favorable conditions (food, temperatures, humidity) larvae mature into prepupa within two weeks. Prepupa within two weeks it changes into pupa in a process known as pupation. Prepupa changes into pupa when they find a dry medium to burrow within.

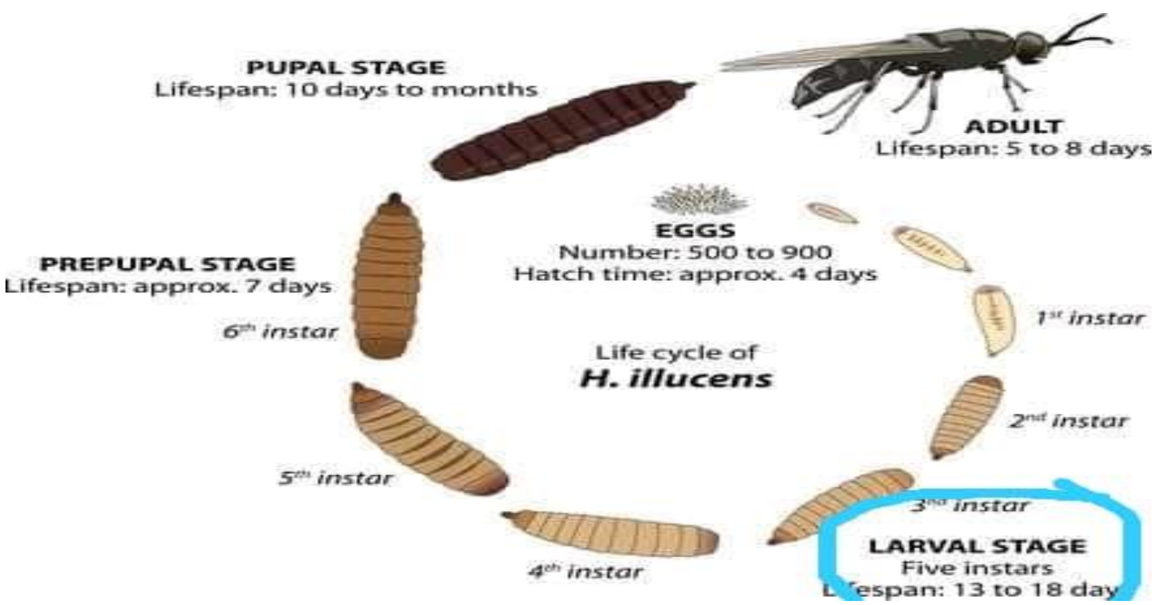

Figure *4:* Life cycle of black soldierly (*Hermetia illucens)*

Pupa go into sleeping mode in a dry medium for two weeks, in this time the embryo further develops within their exoskeletal casing, which later it breaks up at the tip when it develops to release an adult fly in the process known as emergence (Sheppard, 2002). The newly undeveloped fly has folded wings which slowly unfold within 2-3 hours

The adult has a life span of 5-12 days during which they mate and lay eggs (Diclaro & Kaufman, 2009). The adult lays 500-1200 eggs, which depends on fertility of female, diet and rearing conditions (Tomberlin, Adler & Myers, 2009). Study have shown the life cycle of black soldier fly to be 40-43 days under optimum rearing conditions even to six months, as larval and pupal stages recording the longest life cycle (Popa and Green, 2012). Larva stage in the entire lifecycle is the most economic and significance to human (Mutafela, 2015).

2.4.2 Morphology of black soldier fly

Adult black soldier fly (Figure 5) is black in colour, measuring 15-20mm long with black wings. The body is divided into three distinct parts namely; head, Thorax and abdomen (Mutafela, 2015), the first segments of the abdomen having two characteristics translucent areas, and the terminal segment elongated which give it an elbowed appearance. The legs are black with white coloration, (Sheppard, 2002).

The adult fly exhibits dimorphism in where females are larger than males and have a reddish abdominal tip whereas males have bronze-silverly tip (Caruso, Devic, Subamia, Talamond & Baras, 2014). The females lay eggs near or on moist and putrescent organic waste and in the absence of such a medium, mated females cannot lay eggs. The adult males do not go near rotting organic wastes thus are found resting on nearby vegetations to mate with a passing female (Caruso *et al*., 2014). The adults fly are weak fliers found in terrestrial and mostly found resting or basking within a heavily shaded vegetation of plants (Bonso, 2013).

Figure 5: Image of adult black soldier fly

2.5 Waste Management by Use of Insects

Nature has many ways of providing solutions to manage waste produced by organisms. One of the ways nature manages wastes is through decomposition by bacteria, fungi, protozoa and insects (Veldkamp, Van Duinkerken, Van Huis, Lakemond, Ottevanger, Bosch & Van Boekel, 2012). This gives a good indicator that insects can be used to manage waste and produce useful protein sources which h belongs to the orders of Diptera (Veldkamp *et al*., 2012).

The use of fruits and vegetables substrates has received endorsement from the European Food and Safety Authority (EFSA) as having the highest potential for use as feed for insect production due to low risk of transmitting zoonotic diseases to humans as compared to substrates such as; manure, catering waste or waste containing meat and fish which are not allowed since insects are considered as farmed animals (Committee, 2015). Fruits and vegetables commodities have a high proportion of post-harvest losses and several byproducts of fruit and vegetables processing industries, which are therefore a sustainable insect rearing substrate (FAO, 2011, Nguyen *et al*. 2015, Paz, Carrejo & Rodríguez, 2015).

2.6 Food Security

Food security is when there is accessibility of safe and nutritious food in order to maintain and have good health (WHO, 2010). FAO (2002), described food security as a state when all people have physical, social and economic access to sufficient, safe and nutritious food which meets their healthy status. The aim of sustainable development goals has been to reduce the number of people suffering from hunger (UN, 2010). Thus, improved food security is key in reducing hunger and poverty. Nevertheless, with continued population, the consumption has increased leading to raise in global demand for food. In developing countries including Kenya, it's estimated that over 800 million people are affected by hunger and the number are increasing, more so in Africa and Southern Asia (FAO, 2006). Children are malnourished and the most affected is due to lack of quality food (UN, 2010).

2.7 Importance of fish and aquaculture to alleviate poverty and malnutrition

The fisheries sector and fish play a vital resource role towards poverty alleviation and food security for most poor households (Cinner, 2009). The fishing industry supports over 170 million people with earning an income. According to (FAO, 2009) a total of 520 million people worldwide directly rely on income from fisheries. Fisheries contributes greatly in achieving food security (Simon, 2009). Globally, fish contributes to 15-16% of the total animal protein consumed by about 2.5 billion people (FAO, 2009). Fish has always been a major source of high-quality dietary protein, essential vitamins, minerals and other micronutrients for over one billion people, in the rural communities of developing and low-income countries of income earners (FAO, 2003).

Fish oil is important source of essential fatty acids such as, Eicosapentaenoic acid (EPA) and Decosahexaenoic acid (DHA) which are vital for brain development in unborn and infant children (Bonham, 2009). Studies have shown that fish oil prevents cardiovascular diseases (Lemieux & Wuon, 2010). According to Stephen (2010), more than 200 million Africans eat fish regularly besides fish and fish products are a good source of iodine (Fenton, Bonham, Alvarez & Pedrera, 2010).

In the poorest countries the percentage of fish protein consumption can exceed 50% of the animal protein more so in places where other sources of animal protein are scarce or expensive, and that fish provides about 180 calories per capital per day (John, 2009). Fish consumed at any substance level, are able to bring change between adequate and inadequate nutrition, recovered health and prolonged illness or between food security and starvation. Additionally, fish is easily accessible and affordable source of protein for many poor and developing countries. Fish products contributes significantly to local and international economic sustainability through trade and exports. Fish and fish products contributes directly to nutritional food security in countries where staple crops are of poor and low protein (Smith & Barnes, 2015). Claire (2000) found out that even with small quantity of fish being consumed its effect on human health are huge.

2.8 Availability and utilization of fish feed in Kenya

Fish meal is the main ingredient for feeds formulation, as human population increasing and balanced diet needed, so does the high consumption and competition, thus leading to shortage. Lack of fish diets has led to majority of fish farmers using locally available materials such as, rice bran, wheat bran, cassava and corn meal for fish feeding. Some ingredients such as brans are deficient in macro and micro nutrients while the high crude content of crude fiber in some brans reduce digestibility and palatability leading to low fish yields (Munguti *et al*., 2012)

The most commonly used feed ingredients include; Ochonga (*Caridinanilotica*) Omena (*Rastrineobolaargentea),* wheat bran, rice bran, sunflower or cotton seedcake, and cassava or bread floor as a binder (Munguti *et al.,* 2012). Fish feeds are normally formulated by mixing several raw ingredients to make a balanced diet. The ingredients are ground separately, mixed into ratios with water before being pelletized to produce semi-floating pellets, the pellets are sun-dried and stored in gunny bags (Liti, Mugo, Munguti & Waidbacher, 2005).

2.9 Fish Nutrient Requirement

Energy, and dietary requirements for all aquaculture species can be considered in five different nutrient categories such as proteins, lipids, carbohydrates, vitamins, and minerals (FAO, 2013). Protein is the highest expensive ingredient in fish feed formulation for a balanced diet. This is because protein is an important component for human and animal consumption. Fish meal is one of the ingredients, which has been used for decades as a source of protein in aqua feed. Due to its unrivaled properties of high biological value digestible energy and an array of amino acid components when in comparison with other commercially available protein sources (EL sayed, 1998). Nevertheless, fish meal is scarce and the most expensive single ingredient in the market, thus increasing demand and competition for human and aqua feeds intake. Raising the cost of production and reduced profit margin and fish consumers are faced with high prices per kilo (Eyo, 2003).

Fishmeal is produced from small fishes caught in deep-sea and lake waters, increasing the coast of production. Worldwide capture fish are not consumed directly by humans but are reduced to fishmeal and fish oil and also consumed in feeds by farm-raised animals such as chicken, pigs, and fish (Lang & Heasman, 2015). High cost and scarcity have raised concerns as the demand for fishmeal and fish oil in the aquaculture industry will lead to further price increase heavy pressure on wild fisheries to produce fish for feeds (Delgado, Wada, Rosegrant, Meijer & Ahmed, 2003). In addition, is therefore urgent to find alternative good quality, renewable sources of protein that can partially or wholly fishmeal.

2.9.1 Fishmeal Alternatives

Several protein sources have been studied and shows the potential of replace fishmeal in aquaculture without affecting fish growth performance of fish (Tacon & Melian, 2009). Studies have shown evidence of possible partial or total replacement of fishmeal with protein from other sources. Alternative protein sources include plant protein concentrates and novel proteins such as algae, yeast, dried distillers' grains with soluble (DDGS), insect meal, and animal protein from slaughter houses. Complete replacement of fishmeal with plant-based protein is possible without loss of growth performance (Webster *et al.,* 1992), however, plant-based protein from lacks the amino acid profile as required by fish (Hardy, 2007). For example, Methionine is the limiting amino acid in soybean meal (SBM) lacks the amino acid Methionine while corn gluten is deficient in lysine and lysine and arginine are limiting in Wheat gluten meal lacks two amino acids, lysine and arginine.

Novel protein is any type of food that does not come from the source that have dominated our diets for generations such as; lamb, chicken and poultry products. Novel protein is obtained from single cell organisms and invertebrates such as algae and yeast. The protein is costly due to increasing fishmeal prices and consumption. However, as the prices of fishmeal are increasing the use of novel protein has been under considerations, and researchers have started evaluating the economic feasibility and optimum usage of these novel proteins as fishmeal substitutes.

2.9.2 Demand for insect proteins

The demand for insect proteins varies from one region to another. In Asia and South America, the demand is from the human food industry. This is especially in countries such as Thailand, Laos's Peoples' Republic and Mexico where crickets and mealworms are produced and consumed (Hanboonsong, Jamjanya & Durst, 2013). In Africa, the demand for insect protein is primarily from the poultry and fish industries (Leek, 2017, Schönfeldt and Hall, 2012). However, production is still at infancy despite the huge potential of the region in terms of climatic conditions and supply of agricultural wastes as raw materials. In Europeand North America, strict legislations have restricted the use of insects to the aqua feed andpet food markets with future prospects for livestock and poultry sectors (Leek, 2017).

2.9.3 Challenges in the use of insects as a food and feed resource

Operations for the production of BSF have been faced with different challenges which mostly relate to the use of the larvae as feed rather than as an agent of waste management (Mutafaela, 2015). To start with, Black Soldier Fly production requires a warm environment. This requirement has proved difficult and energy consuming to sustain in the temperate climates and during winter periods (Holmes, Vanlaerhoven & Tomberlin, 2012). Use of greenhouses to ensure continued production during the cold seasons within the tropics and equatorial climates has made the enterprise expensive (Holmes *et al.,* 2012).

The duration of the life cycle ranges between several weeks to several months depending on temperatures, quality and quantity of the diet. This makes prediction of production a challenge (Veldkamp *et al.,* 2012). The continued lack of legal framework and specific legislations on the use of insects discourages investment in the sector (Leek, 2017). For example, within the European Union (EU), strict sanitary regulations, a lack of guidelines on the mass rearing of insects, lack of clarity on which insect types are authorized for the market, and prohibition of some common types of substrates for insect production have also hindered progress in the acceptance and establishment of the insect market (van Huis, 2013). This is in contrast to countries in Africa where there is virtually no restriction on the kind of substrates used (Leek, 2017).

Issues of feed quality due to the potential of BSF to bio-accumulate toxins and heavy metals from pesticides, chemical fertilizers, herbicides and other chemicals sprayed on production substrates and genetic engineering technologies presents another challenge (Diener, Solano, Gutiérrez, Zurbrügg & Tockner, 2009). High sodium levels in processed food stuffs have also proved problematic. Most of these accumulate in ecosystems and in larva, and at higher concentrations may be toxic both to the larvae and the consuming animals along the food chain (van Huis *et al.*, 2013). This therefore limits the potential sources of suitable substrates. Another concern involves acceptance and perception of insects. Insects that fall under the category of 'flies' are commonly perceived as filthy and unsanitary (van Huis *et al.,* 2013; Mutafaela, 2015). This is perhaps because society associates them with houseflies which are a known health risk. This is the basis for the EU restrictions on the use of insects as feed ingredients of animals destined for human consumption (Leek, 2017).

The generalization is affecting even harmless flies like BSF and is largely due to lack of awareness. Lack of collaboration among experts in the field to make necessary explanations to the naïve public and create awareness on potential of insects as a food and feed resource has contributed to poor acceptance and persistence of the wrong perceptions (Smith & Barnes, 2015). However, the perceived benefits of insects such as sustainable production, lowered dependence on imported protein sources and lower environmental impact are mitigating for improved change of attitude towards broad acceptance and are considered more important than the perceived risks such as microbiological contamination, chemical residues in the food chain and lower consumer acceptance of animal products (Verbeke, 2015).

Healthy risks from a variety of pathogens, parasites and diseases are a major challenge in insect production systems (Leek, 2017). Knowledge of disease and health management in intensive insect rearing is still limited and population crashes sometimes involving the whole colony do occur (Leek, 2017). For instance, in Georgia, a parasitoid wasp of the

Trichopria genus has been reported to infect 21-32% of Black Soldier Fly pupae (Mutafaela, 2015).

Current mitigation measures involve minimizing the health risks by ensuring bio-security in a breeding colony, use of very 'clean' substrates and separate housing of the different stages of the breeding stock to avoid cross infection between the different stages (Leek, 2017). In addition, predators such as rats, mongooses and lizards do feed on larva and adults and can therefore significantly contribute to diminishing of populations and returns.

The quantity of insect currently produced is a significant obstacle for the use of insects in animal feed as it does not guarantee a constant supply. Consequently, the prices for insects and insect meals are presently very high, and cannot compete with other protein sources in this respect. This makes insect protein too expensive compared with common sources of proteins used in poultry diets (Leek, 2017). At current prices, BSF is at par with fishmeal which over recent years has all but disappeared from most livestock diets. However, with increase in campaigns to increase adoption of insect rearing technologies, supply is expected to rise and with it, reduction of price (Rumpold & Schlüter, 2013).

2.9.4 Insect Meal as a protein Ingredient

Currently insects are considered a new protein source for animal feed (Shanthi, Premalatha & Anantharaman, 2011). The global insect diversity is expected to be 80 million, however, only about 1 million of them are known to mankind (Erwin, 2004). In using insects as food source for feeding fishes (Table 1), the natural feeding habits of cultivable fish species need to be considered (Bell et al., 1994).

Table 1: Summary findings from different authors on BSF larvae fed on various fish species

Fish species	Suggestions on growth performance on different species	BSFL inclusion levels (%)	Author (s)
Jiancarp *(Cyprinus carpio)*	The study suggests a possibility of substituting up to 100% Fish meal by black soldier fly larvae in diets for Jian carp without negative effect on growth and fee utilization efficiencies	**100**	Li *et al*., (2017)
Mearger (*Argyrosomus regius)* juvenile	When a 10% of *Hermetia illucens* were included in Meagre diets, no adverse effects observed on growth performance and feed utilization. An increase of BSF larvae in diets, a negative growth was observed.	**10**	Guerreiro *et al.* (2020)
Nile tilapia *(Oreochromis niloticus)*	Soya bean protein concentrate was 50% replaced with defatted BSF larvae. No negative growth effect was observed	**50**	Dietz and Liebert (2018)
Siberian sturgeon (*Acipenser baerri*)	The study showed possibility of replacing up to 25% fish meal with BSF larvae in diets for Siberian sturgeons with no effect on growth performance	**25**	Caimi *et al.* (2020)
Rainbow trout (*Oreochromis mykiss*)	The study recommends an inclusion of 13% BSF larvae for rainbow trout diets, any increase in diet composition led to growth decline	**13.2**	Dumas *et al*., (2018)
Nile tilapia *(Oreochromis niloticus)*	The study shows that there are possibilities of substituting 100 % fish meal with black soldier fly larvae without any effects in terms of growth performance, feed utilization and body composition	**100**	Muin *et al* (2017)
European bass *(Dicentrachus labrax)*	Black soldier fly larvae can substitute fish meal at 50% without any effect on growth performance.	**50**	Abdel-Tawwab *et al.* (2020)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Geographical location

This study was carried out in Kilifi County (Figure 7), Umoja self-help group farmer intertidal mariculture farm earthen ponds (Figure 8). The mariculture farm is located at Kibokoni along Kilifi creek about 10 km West of Kilifi Township at Longitude 039 50^0 32^{00}E and Latitude 03 36^0 12^0S (Figure 6). The fishponds are located within the brackish water zone inhabited by *Rhizophora mucronata* and *Avicennia marina* mangroves species on either side of the sea and land respectively (Mirera, 2009).

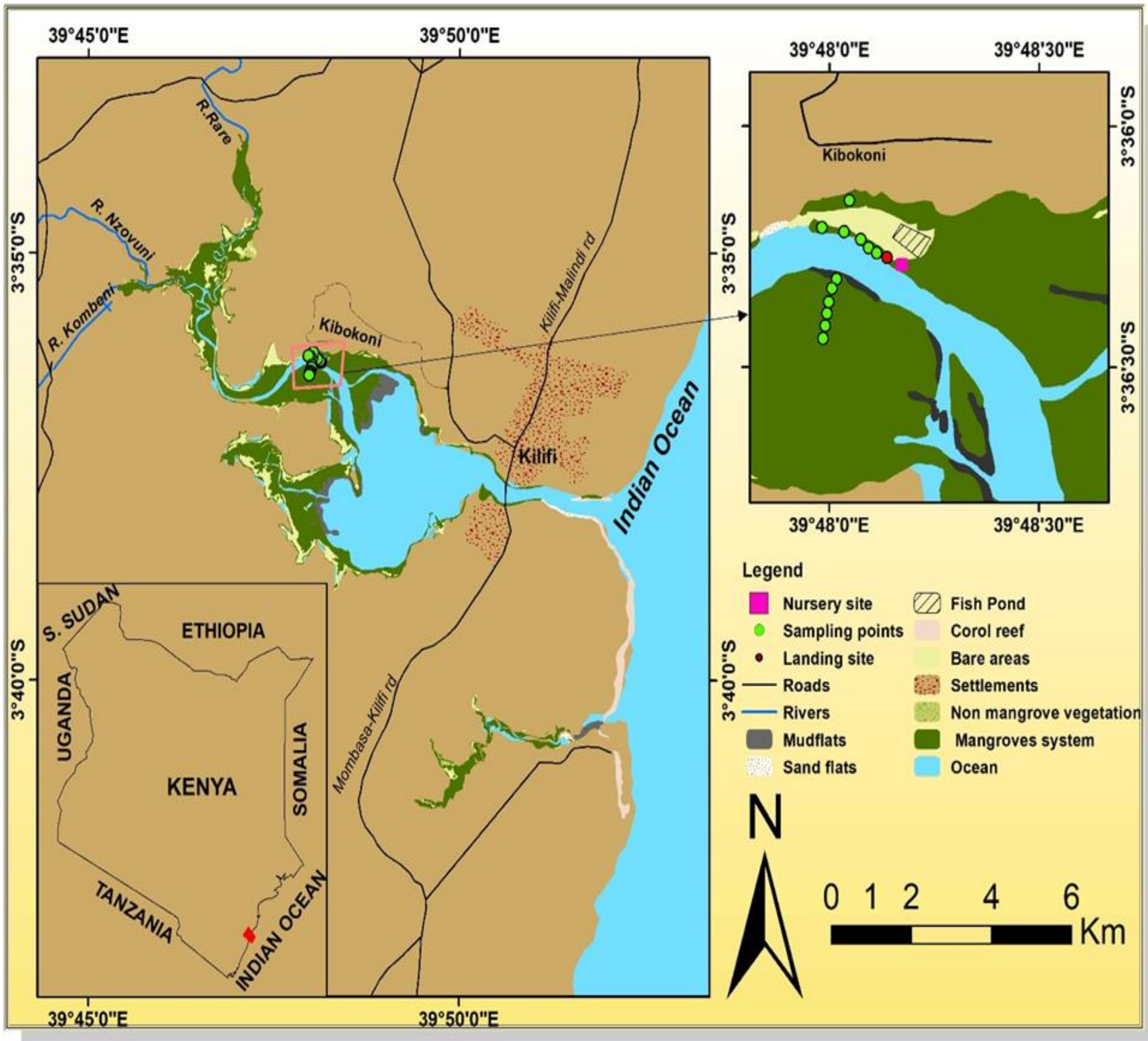

Figure 6: Map showing site locations for Umoja Self-Help group Mariculture ponds in Kibokoni Kilifi County, Kenya

The ponds are designed to enable a free exchange of ocean water to the ponds during spring tides with minimal or no exchange of water during neap tides

Figure 7: Umoja Self-Help Group study site.

3.2 Climate

3.2.1 Temperature and rainfall

The coastal belt of Kenya experiences a tropical monsoon climate dominated by two seasons, the southeast monsoon (SEM) prevailing from April to October and the northeast monsoon (NEM) from November to March. The two seasons are characterized by distinct differences in physical and chemical conditions of the coastal waters (Lutjeharms, 2006). The area is characterized by generally high temperatures with a mean minimum of 24.7^{0}C, maximum 30.2^{0}C and high humidity (76%). The area receives annual rainfall amount that ranges from 88mm per annum in two rainy seasons (Kenya Meteorological Department, 2012)**.** The SEM is associated with strong winds, low air and water temperatures, low solar radiation, and heavy rains. During the NEM, these conditions are reversed (Trenberth, Stepaniak & Caron, 2000). The tides are mixed semidiurnal, with tidal ranges of about 4.0m.

3.3 Study design

The study had complete block randomized design (CBRD) with four treatments (Figure 9) which were performed in triplicates and assigned randomly among twelve hapa net cages in one pond measuring 20m x 40m x 1m (800m^2). The hapa nets cage were made of black nylon with 1 mm mesh and sized 1.5 x 1.0 x 1.2 m. The twelve hapa cages were set in three rows and hanged on a wooden raft in the fishpond using a mangrove pole to hold the hapas at a depth of 80 cm.

The *S. sutor* were stocked at 15fish/hapa and fed twice a day (0900HRS and 01600HRS) at 5% body weight throughout the experimental period. A total number of 180 fish (average mean weight, 11.64±0.967-12.017±0.2133g and mean length 9.1167±0.13614-9.8967±0.99949cm /fish) were used in this study. The fish fingerlings for this study were sourced along Kilifi creek by fishermen.

Figure 8: Brackish earthen pond stacked with hapas of different treatments

The hapa net cages were allocated treatments randomly as, T1 (100% BSFL with 0% fish meal), T2 (25% fish meal replaced with 75% BSFL), T3 (50% fish meal replaced with 50% BSFL) and T4 used as a control (40% KMFRI artificial feed). A second bigger sized net-cage sized (#5mm) was installed outside the hapas nets along the dykes to prevent any foreign materials from entering the study site hapas such as (crabs). The crabs have a tendency of cutting fish nets which may lead to fish escape. Sampling was carried out monthly for total length (cm) and weight (g). The hapa-cage were cleaned monthly during sampling period to prevent clogging and enhance good circulation of fresh water.

3.4 Pond preparation and hapa nets installation

The already built community pond (Figure 10) measuring 20m x 40m x 1m ($800m^2$) length, width and height respectively was repaired prior to commencement of the study. The pond was drained and quick limed to help maintain pH and pond hygiene. The pond was allowed to dry for one week in order to eliminate parasites and other unwanted organism. The pond was fertilized after fortnightly with cowdung (5 g/m^2), urea (60 g/m^2) and triple super phosphate (90 g/m^2), to facilitate planktons' growth (Lubambula, 1997; Liti *et al*., 2006).

The inlets were open to let water in during the high tides and the levels were maintained at 1m depth during high spring tide for compensating vaporization and seepage as well as to maintain the right salinity and temperature. The transparency of water was judged using a wooden measuring a ruler (150 cm) on weekly basis to help in determining manuring period. The soil was blackish which indicates poor alkalinity, in order to keep the levels within the optimal range, calcium carbonate was applied at a rate of $250g/m^2$.

Figure 9: A sketch plan of the Kibokoni showing brackish water ponds where the study was carried out (Drawing not to scale).

3.5 Rabbitfish fingerlings source

Fish taxonomist (Mr. Kadhengi) and taxonomy book was used to guide in identification of *S. sutor* species since there are several species of rabbitfishes, fingerlings resemble the adult. The species were identified mainly by means of colour and morphological characteristics such as snout shape, color patterns, body depth and caudal fin shape as described by (Anam & Mostrada, 2012).

Fish fingerlings were sourced from the Indian Ocean wild stock along the creek; in Kilifi County by fishermen using ring net. The net was rapidly lowered into ocean waters once a school of rabbitfish was sighted and the trapped fish were encircled by closing the bottom of the net using a threaded footrope through metal rings. The rapid lowering of the net was aided by use of gunny bags filled by sand to act as sinkers, and helping to minimize escape of fish below the net. To reduce damage, the net was kept off the bottom by underwater divers to reduce net damages. The surface of the net was pulled to bring the net together. *S. sutor* fingerlings were then sorted according to size and species and held in clean seawater bail with portable aerators in readiness for packaging and transportation. Bigger fish (less than 10g in weight) and unwanted species were released back to ocean water.

The selected fingerlings were packaged in plastic transportation bags and transportation was done in late evenings when temperatures were low. Three black tanks sized 500L was used for transportation. The tanks were filled with three quarter clean ocean water, portable aerators were fixed and 2kgs of ice cubes added to lower the water temperature and reduce fish activities in each tank. Once the fish reached the experimental site, they were acclimatized in a large holding hapas net measuring 5m^2 set in a pond from the study ponds.

The plastic bags were floated in the pond water for 30 minutes, to equalize the water temperature in the bag and that of pond water. The fish were released slowly from the plastic bags to large holding hapa net for acclimatization for a period of 14 days before stocking.

3.6 Black soldier fly production

Pre-pupae colony samples were sourced from Vicky farm in Karen End, Nairobi County. The farm is located about 50 km Southeast of Nairobi County, a mainly cold and highly populated area. The waste substrate collection was conducted within five households along Mtwapa town Kilifi County, Kenya (3° 57’ 0 S, 39° 45’ 0 E). The Distance from one household to another was approximately 100 meters. Two containers (150 L) each were allocated to a household for sorting and waste collection. The households were to put all food waste in the container such as; all kitchen waste (bread, vegetables, rice, meat and fruits). The sorted and collected waste was weighed after every two days and transported to the BSF larvae breeding site. Market waste substrate (MWS) was sourced from Mtwapa market center in Mtwapa, Kilifi County. The waste substrate of fruits such as; mangoes, oranges, bananas, and vegetables were collected by gathering along the market and later transported to the experimental site. The waste substrate was chopped into small pieces using a knife and passed through a hollow tray to remove water. The chosen substrates were majorly used in households and market waste form within the selected study site. The six study trays (90 x 60 x 90 cm) were selected and clearly labelled according to the intended respective feeding treatment.

3.6.1 Culturing of black soldier fly (BSF) larvae

Considering the biological behavior of the BSF, a calm and quiet area was selected to avoid the disturbance. The production site was surrounded by different types of trees which made the environment natural and ideal. One greenhouse structure was constructed measuring 6 x 4 x 4 m, and was used to construct a love cage (inside green house) where a nylon white cage net of 1.8 x 2 x 1.5 m was placed to enable flies to mate and lay eggs. Pre- colony were placed in three basins measuring 60 x 30 x 15cm. A sawdust was put in a basin where 5 kg pre-pupal colony was put in for metamorphosis process to take place. A dark cage house measuring 6 x 4 x 4 m was used for hatching eggs and larvae growth.

3.6.2 Production of black soldier fly (BSF) larvae

3.6.2.1 Love cage

A pre-pupal amount of 5 kg was placed in each of three different rectangular basins (60 x 30 x 15 cm) containing sawdust inside a nylon white cage net (1.8m x 2m x 1.5m) as mentioned by Xu, Zhang, Wu, Liu and Wang *(*2011) to contain the BSF in one position for easy eggs laying and collection as shown in (Figure 11).

A bunch of corrugated sheets 2-3 inches long was tied together and hung over the waste substrates in (a bin) for laying eggs as BSF does not lay eggs directly on the waste inside love cage (Sheppard, 2002). After 5-8 days the adult emerges from the pupal and are ready for mating and lay eggs. The adults BSF does not feed but only drink sugary water that is provided inside the nylon cage net. Wet fish and goat offal were placed inside a container (30 x 40 cm) inside the love cage to produce a putrid odor once rotten, to act as an attractant for female BSF to enter the bins and lay eggs in the corrugated sheets hanging over the waste substrate. Mating was observed between the adults, where they were in contact, cream eggs were observed hanging on corrugated sheets after 4-5 days. The eggs were transferred to a dark cage for hatching and growth at humidity greater than 50-70%% as following (Sheppard, 2002).

Eggs were collected by unfolding a bunch of corrugated card board, and scrapped off using a dry clean knife into a dry clean mesh cloth. The bunches were folded and returned to their respective bins.

Figure 10: (A) Flies laying eggs (B) eggs collection (C) collected eggs

3.6.2.2 Dark cage

A small dark cage house (6 x 4 x 4 m) was constructed for the hatching of eggs and growth of BSF larvae. The dark cage house had one rack (3 x 2 x 2 m) with the stands inserted in a container containing oil to prevent ants and other insects from entering the BSF house.

The dark cage was covered entirely to enable eggs to hatch and grow as well as the newly emerged larvae to grow as well to the provided effectively conserve the waste substrate provided.

3.6.2.3 Inoculation and hatching inside dark cage

The harvested eggs were placed over an open hatchling container (3 x 2 x 2 m) with a high-quality food source (wheat bran 70% and sugary water). The light creamed colored larvae hatched from the eggs after 4-5 days in optimum temperature of 26-30^0 C and humidity 60%-70% as reported by (Zheng & Liu, 2012a). Larvae, which fell from the eggs into the hatchling container they began feeding immediately in the same hatchling containers for five days.

Using a weighing balance, 2000 larvae of the same size and age were weighed and transferred into each of the six larger containers in the dark cage. The larvae in the dark cage were separately and continuously fed with both market and household waste (50g) on a daily basis until they transformed into prepupae within two weeks. The prepupae (Figure 12) began to leave the food source after two weeks in search for a more suitable dry location to pupate.

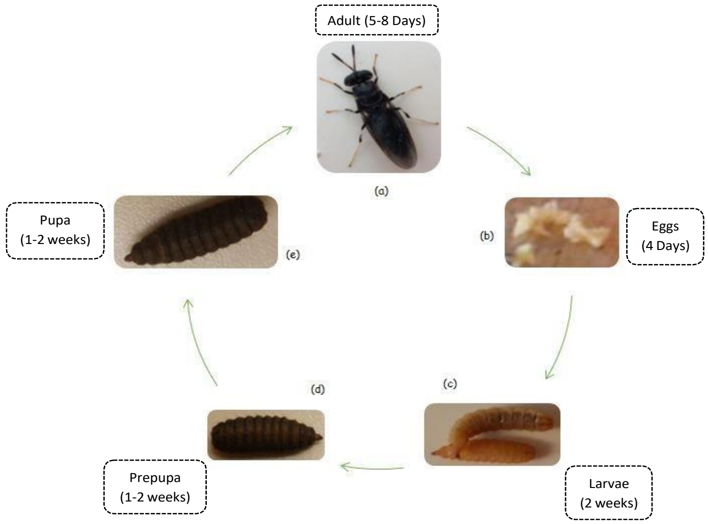

Figure 11: Metamorphosis of black soldier fly larvae

3.6.2.4 Collection of Pre-pupae

The larvae attained the pupa stage (Figure 13), they stopped feeding and emptied their digestive tracts (Banks, 2014; Dortmans, Diener, Bart & Zurbrügg, 2017). The pupa used their hook-shaped mouthparts to emerge from the food source in the nursery container and reach a dry, dark and protected location to pupate into an adult fly (Diener, 2010).

Figure 12: Handpicking of pre-pupae black soldier fly larvae

Some collected pupae were returned to love cage for continuous laying of eggs. However, 5kgs of the pupa collected from both household and market waste were taken for proximate nutrient analysis (Table 4) and 25kgs for feed formulation.

3.6.3 Breeding of BSF larvae conditions

3.6.3.1 Feed Preparations

The wet BSFL were dried in an oven at 70^0C for five days (Figure 14). Other ingredients such as fishmeal (Figure 14), wheat bran, maize bran, and cassava (binder) were obtained from the nearby Kongowea market. Each, the ingredients were ground separately into fine powder. The ground ingredients were then sieved using a no. 40 sieve and weighed using a digital weighing balance, in the preparations of the feeds, the amount of ingredients that were incorporated in this experimental diet was computed using the Pearson Square Method (Banks, 2014). In the formulation of feeds using different ratios of 100% BSFL, 25% fish and 75% BSFL, 50% fish meal replaced with 50% BSFL and commercial feed 40% was used as a control.

All the dry ingredients (fishmeal, BSFL, cassava, maize bran and wheat bran) were mixed thoroughly and vitamin mix was incorporated into the dry mixture. The binder (cassava powdered) was added (50g cassava binder in 200 ml water for 1 kg of feed) and then mixed until a stiff dough was formed. The formed dough was squeezed through a pelletizing machine. The size of the pellets was about 20-30% of the fish's mouth in order to facilitate ingestion and avoid any loss.

Figure 13: Ingredients used for feed formulation (A) dry BSF larvae (B) omena (*Rastrineobolaargentea)* and wet BSF larvae

Each pellet diet was sun-dried to achieve a moisture content of 20% (Figure 15). The pelleted diets were packaged in airtight plastic containers and stored in a cool, dry place. A sample (50g) was taken from each treatment for proximate analysis.

Figure 14: Formulated diets, fishmeal **(L)** and BSFL **(R)**

3.6.4 Stocking and feeding regime

A total number of 180 rabbitfish (average mean weight, 11.51±0.96 g and mean length 9.12±0.14 fish) were used in this study from October 2021 to January 2022. Fish were randomly allocated into 12 hapas nets at a density of 15 fish per m^2 in each hapa. Each hapa was then randomly assigned to one of three replicates for the four dietary treatments. The amount feed consumed in each treatment was calculated after every sampling and adjusted according to new weight. All fish were weighed and measured individually at the beginning, every month and the end of the experiment period. The fish were physically examined for any signs of diseases or bruises that may cause diseases and the weak were isolated.

There were four different diets used in this experiment were T1 (100% BSFL with 0% fish meal), T2 (25% fish meal replaced with 75% BSFL), T3 (50% fish meal replaced with 50% BSFL) and T4 used as a control (40% KMFRI artificial feed). The fish were fed on 5%

body weight and feeding was adjusted according to the body biomass of fish (Jena *et al*. 2001) during the monthly sampling.

Feeding was done twice a day at 0900HRS and 01600HRS. The hapas were being cleaned weekly to prevent clogging, and water quality parameters such as temperatures, salinity, and pH and dissolved oxygen measured monthly.

3.6.5 Proximate analysis

All the diets were analyzed for proximate analyses to determine their nutritional composition (Table 3). Dry matter (DM) was determined by drying 5 grams of sample placed in crucibles in an oven for six hours to constant weight at 105^{o}C. The samples were cooled in a desiccator at room temperature and weighed.

Ash was determined by heating the samples in a muffle furnace set at 600^{0}C for three hours.

DM (%) = [(weight of initial sample (g) - weight of dried sample (g) / initial sample weight] × 100 CP was quantified by the standard micro-Kjeldahl Nitrogen method as described in (AOAC, 1995). Using a sample size of 0.4 g, a Behroset InKje M digestion apparatus and a Behr S 1 steam distillation apparatus (both: Labor-Technik GmbH, Düsseldorf, Germany). The ammonia distillate was trapped in 4 % boric acid solution prior to titration with 0.1N HCl.

Crude protein was estimated by: % Nitrogen = [(ml standard acid - ml blank) x N of acid x 1.4007] / weight of sample in grams. CP = (% total nitrogen x 6.25) Ether extracts were analyzed using a sample size of 2 grams in a Soxhlet extractor with petroleum ether at a boiling point of 40 – 60^{o}C. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with water followed by acetone. The residue was then oven dried at 60^{o}C for 4 hours, cooled in a desiccator and weighed. The residue was a shed at 550^{o}C in a muffle furnace overnight.

3.6.6 Water quality monitoring

The water quality parameters were monitored every spring tide and neap tides (Figure 16). Physic-chemical parameters such as, temperature, dissolved oxygen, pH, total ammonium nitrogen (NH_3-N) and nitrites-nitrogen (NO_2-N) were determined for the duration of the experiment. Temperature (°C), dissolved oxygen (mg L^{-1}) and pH, were measured *in situ* using a multi-parameter water quality meter model number H19828 (Hanna Instruments Ltd., Chicago, USA). Readings were recorded monthly morning hours (00800HRS).

Water samples for Chlorophyll-*a*(chl-*a*), total ammonium nitrogen ((NH_3-N) (mg L^{-1}) and nitrites-nitrogen (NO_2-N) (mg L^{-1}) were collected monthly using standard methods by Boyd and Tucker (1998) at each treatment hapa in a plastic cylinder, mixed and then sub-sampled to obtain a representative sample for laboratory analysis. The samples were stored in a cooler box with ice and transported to Kenya Marine and Fisheries Research Institute (KMFRI) nutrient laboratory, where within three hours' analysis was done by titration.

Figure 15: Laboratory technician collecting water quality parameters

3.6.6.1 Plankton Sampling

Plankton samples were collected by pooling 10L of water from different locations within the hapas cages in the pond. The water was passed through a 45 µm mesh plankton net. The concentrated samples were preserved in small plastic bottles (300ml), with 5% buffered formalin, and subsequently filled up to 100ml with distilled water. Quantitative and qualitative estimations of plankton were carried out using a Sedgewick -Rafter (S-R) cell containing 1000 1-mm^3 cells. 1 ml sample was put in the (S-R) cell and left for 15 minutes undisturbed to allow plankton to settle. The plankton in 10 randomly selected cells were identified up to genus level and counted under a compound microscope (Swift, M-400). Planktons were identified using keys by Belcher and Swale (1976) and Bellinger (1992). Plankton abundance was calculated using the following formula: N= (PxCX100)/L. Where N=the number of planktonic organisms per liter of original pond water; P=the number of planktonic organisms counted in the fields; C=the volume of the plastic bottle holding the samples (100ml) and L=the volume of the pond water sample (10L)

3.6.6.2 Identification of benthic macro-invertebrates

The benthic macro invertebrates' samples were collected before stocking and during harvesting with an Ekman grab (area 225cm^2). In each treatment, bottom mud samples were collected from 4 treatments locations, which were then combined into a composite sample. Benthic macroinvertebrates were then collected after filtering sediments through a 250 µm mesh sieve and preserved in a plastic vial containing 10% buffered formalin. The identification key by Brinkhurst (1971) and Pinder and Reiss (1983) were used. Benthic macro invertebrates' density was calculated using the formula:

N=Yx10000/3A

Where N=the number of benthic organisms per m^2 Y= total number of benthic organisms counted in 3 samples; A=Area of the dredge in cm^2. The bio volumes of plankton and benthic macro invertebrates were calculated using Rahman, Hussain, Azad, Kamruzzaman, Rasid, Rahman and Hasan (2006). Zooplankton were identified using key by Mc Cauley (1984). In some cases, bio volume approximation was made using the values of species of similar shape. For benthic macro invertebrates, the bio volumes were calculated according to Riera et al. (1991).

3.7 Fish Gutting

Fish gutting was carried out to check for acceptability of the diets (Figure 17). One fish per hapa per treatments was removed and gutting took place by dissecting the fish, and checked the intestinal organs for any traces of pelleted diets. From the dissection process some traces of pellets were seen which showed the acceptability of the diet and the fish consumed the diet.

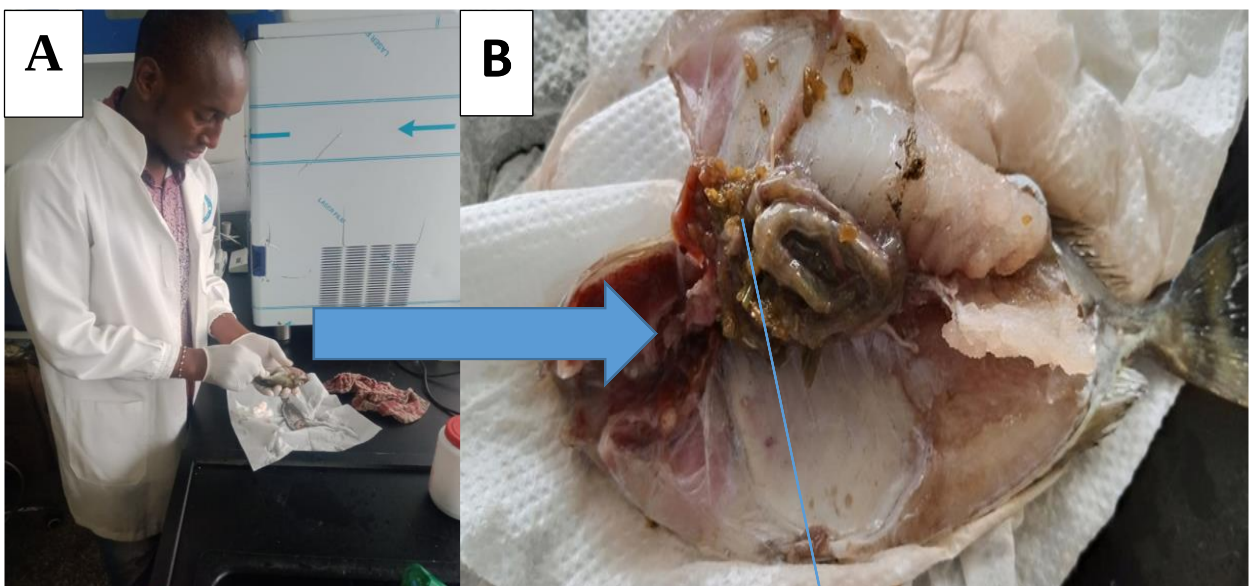

Figure 16: Laboratory analysis, gutting S. sutor (A) and (B) gutted S. sutor to monitor diet acceptance

3.8 Fish Sampling

A total number of 15 fish were collected from each hapa net for individual weight and length measurements (Figure 18). To avoid stress fish were handled under mangrove shade in a plastic basin halfway filled with pond clean ocean water. Further caution in fish handling was ensured by aerating the water using 12 Volt aquarium aerators to ensure adequate dissolved oxygen concentrations.

Fish were weighed on a digital balance (0.01g) (model KERN 572-33, Germany) and total length was measured on a measuring board (0.10 cm) as described by Caspers and Richer (1969). Fish were returned to their respective hapa cages after measurements.

Fish growth performance under different treatments was evaluated in terms of: total length (cm), wet weight (g), daily weight gain (DWG, g day^{-1}), specific growth rate (SGR, % day^{-1}), percentage survival (%) and feed conversion ratio (FCR).

Figure 17: (**A**) hapa net lifting for sampling, (**B**) fish measurement

3.9 Data analysis

All the data were recorded in field notebook then entered in Microsoft Excel 2000 version 7.0. Fish growth performance was evaluated in terms of: wet weight (g), daily weight gain (DWG, g day^{-1}), standard growth rate (SGR, % day^{-1}), percentage survival (%) and feed conversion ratio (FCR) under the following formulas:

SGR (%) = (Ln final weight–ln initial weight)/rearing days*100

Weight gain = Mean final weight–mean initial weight

DWG = Mean final weight–mean initial weight/culture days

DGR = Mean final weight–mean initial weight/culture days

FCR = Feed given (g) (dry weight)/live weight gain (g)

Weight gain (g) = Final weight -initial weight

Survival (%) = Final number harvested/initial number stocked*100

Weight gain (%) = final weight-initial weight/ Initial weight*100

ADL= final length-initial length/number culture days

ADG= final weight-initial weight/number culture days

The experiment comprised of four treatments with three replicates. Data was subjected to descriptive analysis then to a one-way analysis of variance (ANOVA), followed by a comparison of means (Tukey's HSD test) to test the effects of the growth performance on fish. At $P<0.05$ was regarded as statistically significant. All statistics were performed using the Statical package for the social science (SPSS) package (Version 23.0). Growth performance curves were generated using Excel and Isometric growth (b=3) was performed for log length weight relationship.

CHAPTER FOUR

RESULTS

4.1 Water Quality Parameters

Water quality parameters are as shown in (Table 2). The dissolved Oxygen (DO) values ranged between 6.5-6.9mg/L (mean =6.30±0.64 mg/L). Water temperature ranged from 27.8-29.91^{0}C (mean =29.46±0.58^{0}C). The pH values ranged from 7.57-7.88 (means=7.78±0.088). Salinity levels were relatively stable and ranged from 39.27-41.87 mg/L (means=41.56±1.23mg/L). During the dry season (December) some water quality parameters differed significantly as there was no incoming fresh water for refreshing. For instance, the values temperatures ranged from 28.8^{0}C morning hours and 30.2 ^{0}C evenings. Salinities were high ranging from 43.7 ^{0}C -52.3^{0}C due to lack of water exchange. During rainy season (October and November) the temperatures were relatively stable at 26.7-29.5^{0}C, the salinities drastically dropped at a range of 24.3-24.7ppt. Ammonia (2.23mg/L), Phosphate (0.223mg/L), Nitrate (0.976mg/L) respectively.

The water quality parameters were generally within the recommended values of *S. sutor* culture in brackish environment. Also, Nitrate were at 1.543 mg/L, Nitrite 2.293 mg/L, Phosphate at 0.449. The ammonia levels were at 3.725mg/L and Chlorophyll-*a* concentration (means =0.11mg^{-3}). Phosphates (PO4 mg/L) 0.449

Table 2: Mean (±SD) of water quality parameters recorded in hapa cage in coastal earthen ponds.

Parameters	Mean and StDev	Min	Max
Temperature(^{0}C)	29.45±0.58	27.8	29.9
DO (mg/L)	0.63±0.03	0.08	2.32
Transparency (cm)	31.50±0.12	30.23	44.2
PH	7.78±0.08	7.57	7.88
Salinity(mg/L)	41.56±1.23	39.27	44.67
PO4(mg/L)	0.44±0.01	0.45	0.45
NO2(mg/L)	2.19±0.03	1.20	2.20
NO3(mg/L)	1.50±0.01	0.93	1.50
NH3(mg/L)	3.72±0.05	2.12	3.73
Chl-*a* (mg m-3)	0.11±0.00	0.09	0.11

4.2 Proximate Composition of Formulated Fish Feed (% moisture basis)

Table 3 shows analyzed summary of proximate analysis, the moisture content was high in T3 (10.23%) and lowest in T1 (8.55%). Lipid content was high in T1 14.4% and lowest in T3 (6.1%), Crude protein observed high in T4 24.87% and T1 17.38% low. Ash was high in T3 8.64% while T1 had the lowest 4.67% in BSFL. From the analysis the carbohydrates content was high on T1 54.99% and low in T2 47.06%.

Table 3: Proximate analysis of formulated diets

Treatments	**T1(100% BSFL)**	**T2(25% FM, 75% BSFL)**	**T3(50% FM, 50% BSFL)**	**T4(40%Commercial diet)**
Moisture (%)	8.55 ±0.23	9.96 ±0.46	10.23±0.43	9.47 ±0.23
Lipid (%)	14.41±0.22	10.39±0.12	6.1 ±0.31	6.03±0.34
Crude protein (%)	27.38 ±0.11	24.59 ±0.09	25.28 ±0.15	24.87 ±0.01
Ash (%)	4.67±0.22	8.0 ±0.32	8.64±0.40	8.2 ±0.11
Crude fiber (%)	18.43±0.24	16.77 ±0.21	14.27 ± 0.21	18.30 ± 0.12
Carbohydrates (%)	36.56 ±0.34	35.48 ±0.09	35.48 ±0.21	33.13 ±0.10

Note: Data are mean ± StDEV for 90-day study period.

During the 90-day study period, the fish grew from an average the initial mean weight of 11.51± 2.89g and average final mean weight 64.59±0.42g. The final weight among the treatments ranged from 64.59±0.42g (T1) to 59.17±0.33. The mean BWG of all fish was 50.3±23g and ranged between 53.08±4.05 (T1) and 47.66±3.89 (T2) among the treatments. The average SGR of the fish was 3.74±0.06% and ranged between 3.88±0.08% (T2) and 3.65±0.02 % (T1) among the treatments. Concerning the feed utilization parameters, FCR ranged from 5.66±0.2 (T2) to 4.61±0.3 (T1) among the treatments. The average ADL was 0.05±0.02 and ranged between 0.068±0.016 (T1) to 0.045±0.002 (T2). The average mean survival rate (%) was 83.86 and ranged between 93.3±4.7 % (T2) and 71.1±9.1% (T1) (Figure 20). Specific growth rate indicates that fish put up weight faster during the initial stages (Figure 19).

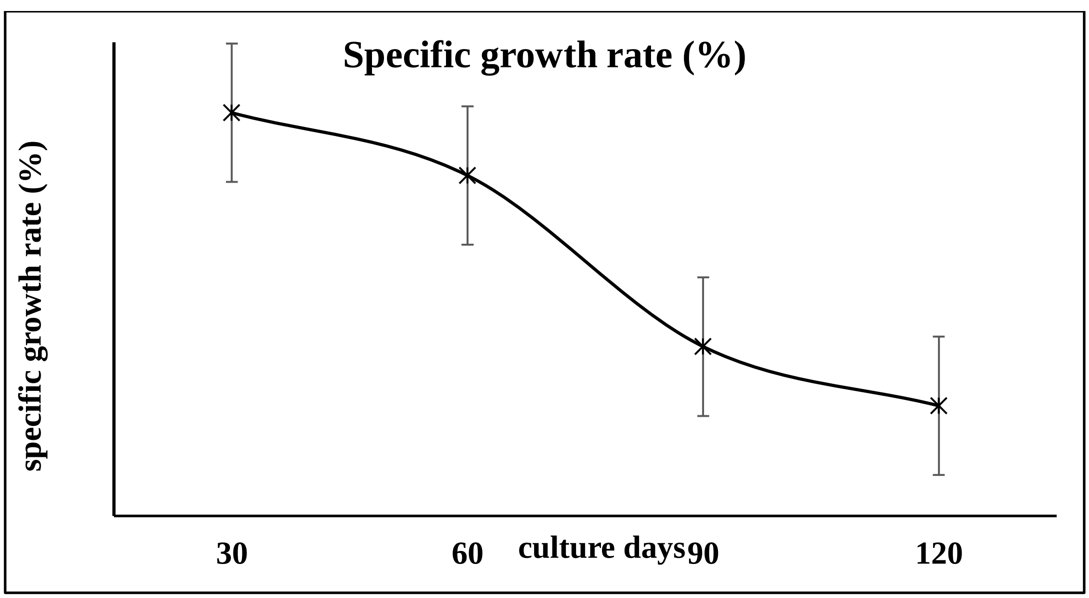

Figure 18: S. sutor mean Specific Growth Rate during growth period (90 days)

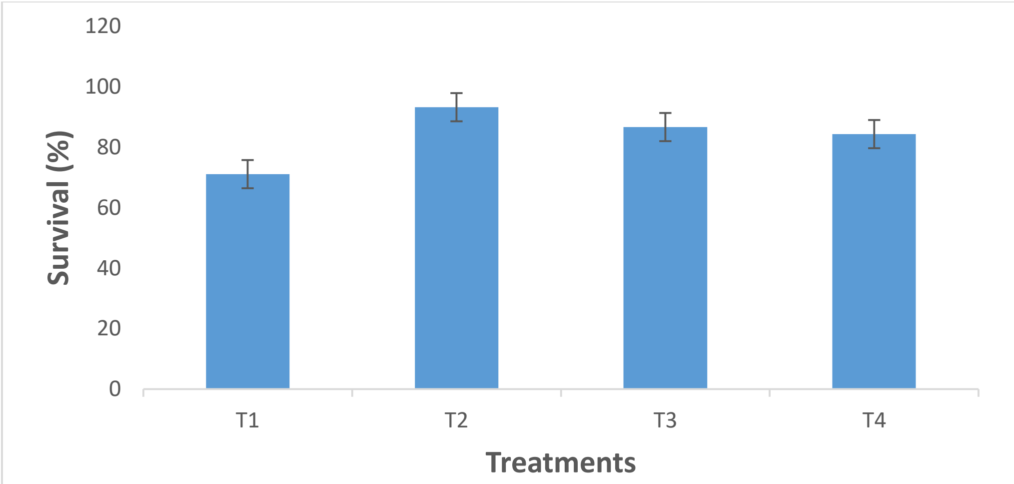

Figure 19: Percentage survival of fish in different treatments

There was significance difference There was significant difference ($p>0.05$) in terms of SGR between the treatments. However, pairwise Tukey's HSD test was used to check on the difference within the means in the groups (T1 vs T2, T2 vs T3, T1 vs T3), only diet of T1 and T3 were significantly (Figure 21)

Table 4: Mean growth performance of *S. sutor* on brackish water in under treatment

Parameter	**T1 100% BSFL** *(n=180)*	**T 2 75% BSFL** *(n=180)*	**T3 50% BSFL** *(n=180)*	**T4 40% Control** *(n=180)*
Initial BW(g)	11.5± 0.99	11.5±0.78	11.5±0.78	11.5 ± 0.68
Final BW (g)	64.59±0.42	59.17±0.33	61.02±0.42	62.47±0.41
Initial length (cm)	8.7± 0.13	8.7 ± 0.16	8.7 ± 0.07	8.7 ± 0.21
Final Length (cm)	27.3±0.15	28.4±0.24	28.7±0.19	28.6±0.89
BWG (g)	53.08±4.05	47.66±3.89	49.51±1.81	50.96±0.14
DWG	0.58±0.09	0.53±0.03	0.53±0.03	0.57±0.05
ADL	0.07±0.02	0.05±0.02	0.05±0.05	0.05±0.05
ADG	0.359±0.05	0.253±0.03	0.293±0.02	0.321±0.04
SGR	3.68±0.09	3.88±0.08	3.74±0.09	3.65±0.02
FCR	1.48±0.24	3.41±0.22	0.91±0.30	2.98±0.31

Note: Data are mean ± StDEV for 90-day study period.

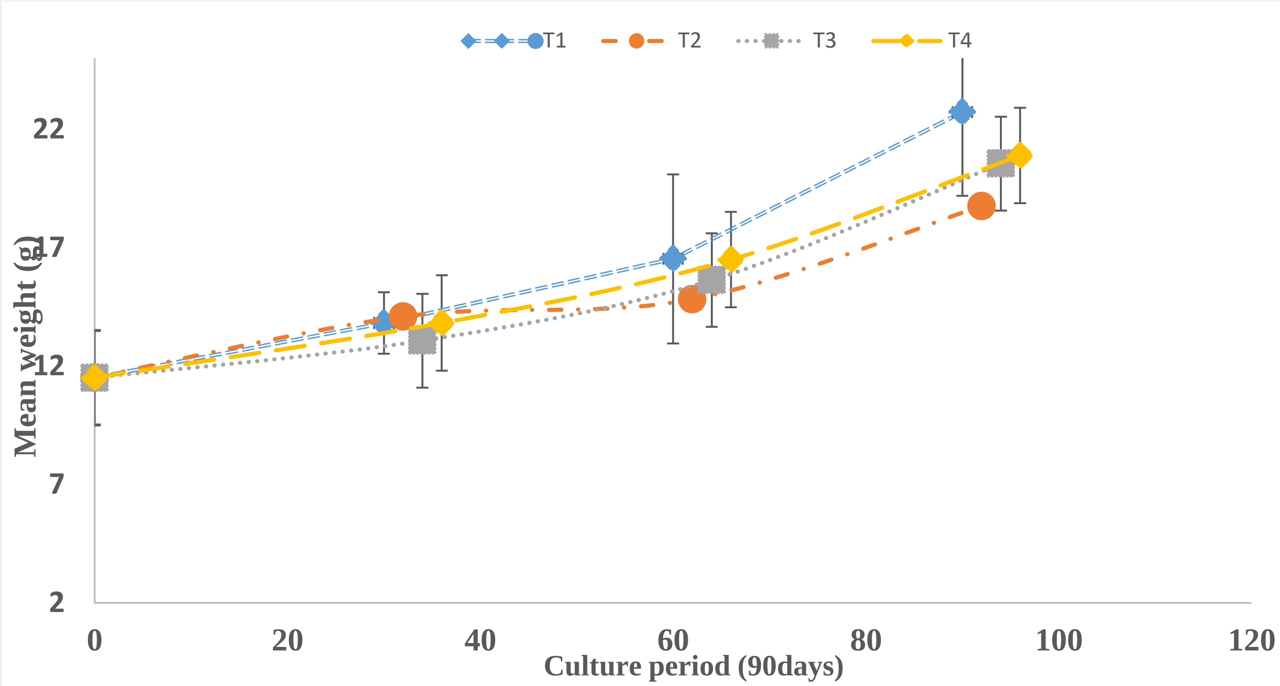

Figure 20: Growth performance curves for *S. sutor* reared on brackish water and fed on formulated diets with varying amount of BSFL, (values are means ±StDEV)

A graph represents weight of fish against time in days (Figure 20), the graph shows the growth trend of fish fed on different diet composition with BSFL (100%) having a significant difference ($p > 0.05$). All fish showed a steady increase in weight after exposure to their respective diets throughout the 90-day of the study period. During the first 30 days, all the curves displayed some overlap (T1 and T2, T3 and T4), by the middle of 50th day T2 overlapped T4 and T3. During the 60th day separation of the curves was observed between the diets and T2 overlapped T3, at the 90th day T3 and T4 overlapped. At the end of experimental period (90th day), the growth curves in diet T1 registered the highest weight followed by diet in T4 which had an overlap with T3, while T2 had the lowest growth weight. However, T1 and T3 showed some significance difference ($p<0.05$).

Elsewhere, T1 (100% BSFL) recorded the lowest r^2 (0.5386) (Figure 22), as T4 (40% commercial feed) had highest r^2 (0.85) (Figure 26), and it ranged between (0.53 to 0.85).

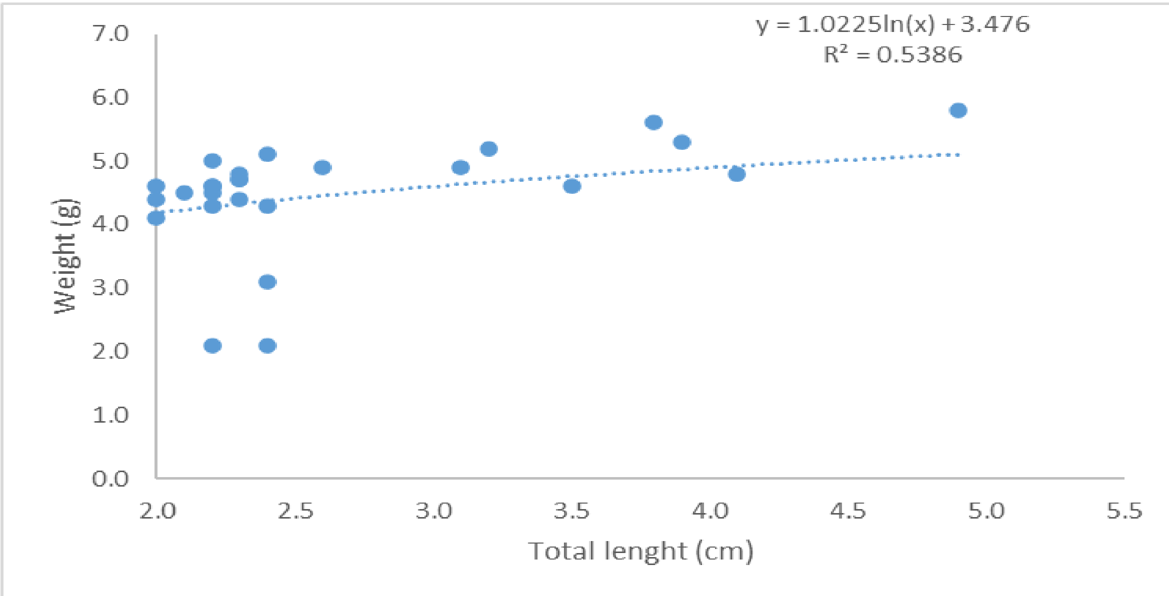

Figure 21: T1 logarithmic relationship between length and weight of *S. sutor*.

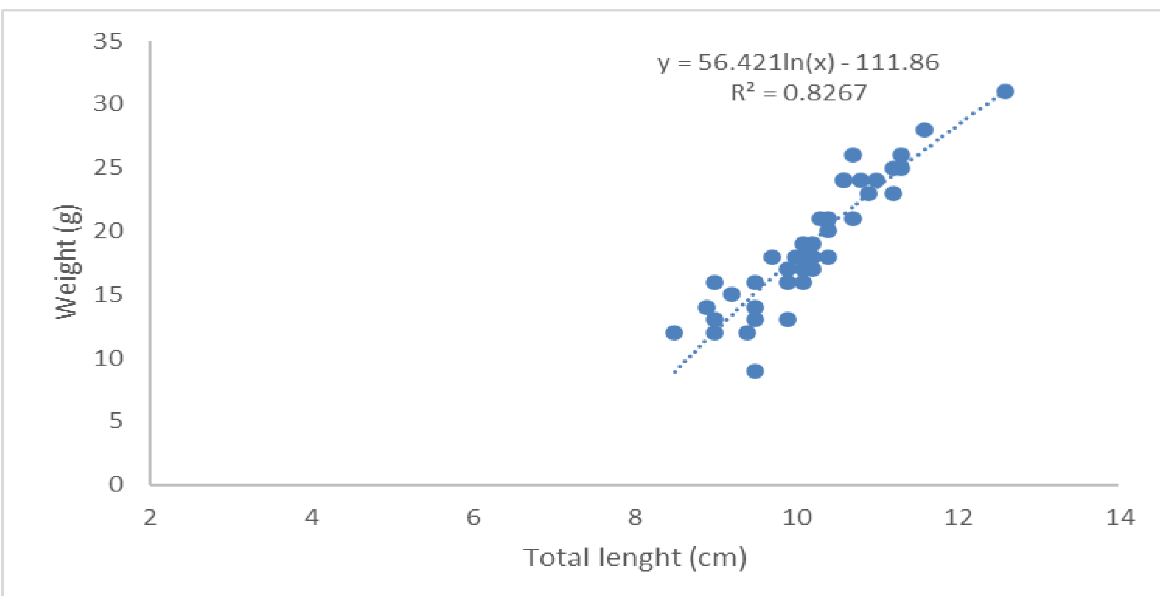

Figure 22: T2, Logarithmic relationship between length and weight of *S. sutor*

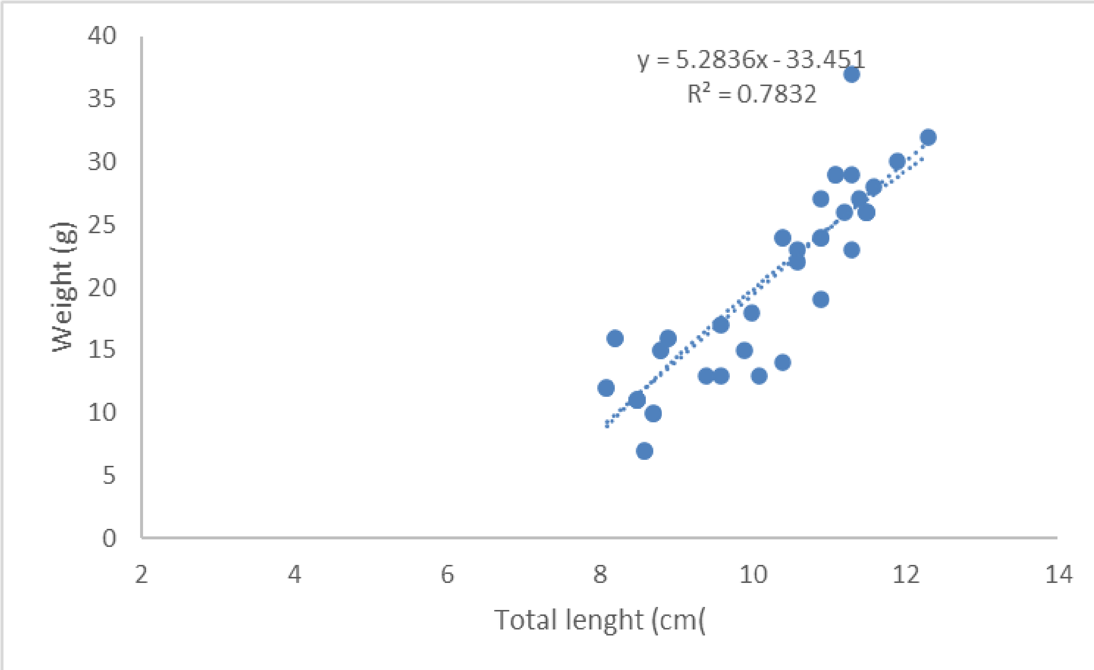

Figure 23: Logarithmic relationship between length and weight of *S. sutor*

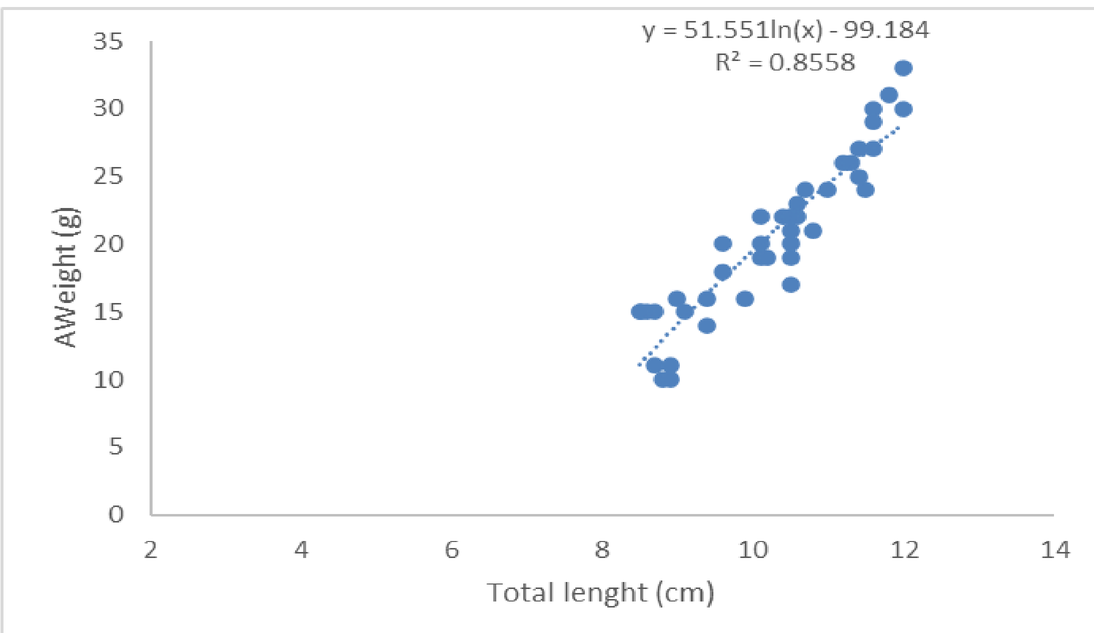

Figure 24: Logarithmic relationship between length and weight of *S. sutor*

4.3 Gutting

S. sutor are herbivorous fish and mainly graze on seaweeds, however, they can also feed on formulated feed or trash fishes when introductory (Figure 17). Gutting process showed traces of pellets which signals acceptance and intake of formulated diets.

4.4 Breeding BSF larvae conditions

The optimal breeding conditions that were monitored include temperature, humidity, light and diet, (Table 5). Eggs performed better at temperatures of 28^0C-30^0C and humidity of >76% with no diets and dark with0-50% daily light, juvenile larvae had lowest temperatures 23-33^0C, with no diet, and in dark area. The adults performed better during morning sunlight with sugary water and humidity of > 60%. Larvae over 4 days old had better growth when subjected to special diet at the dark and temperature of 24-33^0C with humidity of 65-70%. Prepupal on the other hand needed no diet, with a temperature of 24-33^0C and humidity 60-70% and they performed better on darkness.

Table 5: Optimal breeding conditions for black soldier fly larvae

Lifecycle stage	**Temp**	**Humidity**	**Light**	**Diet**
Eggs	28^0C-30^0C	>76%	Dark with 0-50% daily light	Diet
Juvenile larvae (4-6) days	23-33^0C	65-70%	Dark	None
Larvae over 4 days old	24-33^0C	65-70%	Dark	Species diet
Prepupae/pupae	24-33^0C	60-70%	Dark	None
Adults	25-32^0C	>60%	Morning sunlight	Watery sugary

In continuous feeding market waste substrate (MWS) had better prepupal yield 3154.9±0.219g as compared to household waste (HWS) 2950.9± 0.045g. Market waste recorded high prepupal weight 0.2357± 0.219 g while household waste had better prepupal length of 2.283±0.362 cm as compared to market waste 2.030 ± 0.448 (Table 6). Market waste substrate was significantly high (P<0.05) as compared to household waste.

Table 6: Summary of weight, yield, mean length and crude protein content of BSF prepupa fed on the different waste substrate (means ±standard deviation*)*

Waste substrate	Prepupal weight(g)	Prepupal yield(g)	Prepupal length (cm)
Household (HWS)	0.1560 ± 0.045	2950.9 ± 0.045	2.283 ±0.362[a]
Market (MWS)	0.2357 ± 0.219	3154.9 ± 0.219	2.030 ± 0.448[a]

4.5 Effects of waste

Effects of litter based on the priority of concern was analyzed on a scale of 1-5 (1 being lowest, 2 slightly low, 3 medium, 4 slightly high and 5 being the highest). Littering and looks bad had the highest priority concern (45) followed by environment (40), human health (36) and causes diseases (35). Ocean pollution had the lowest priority of concern (28).

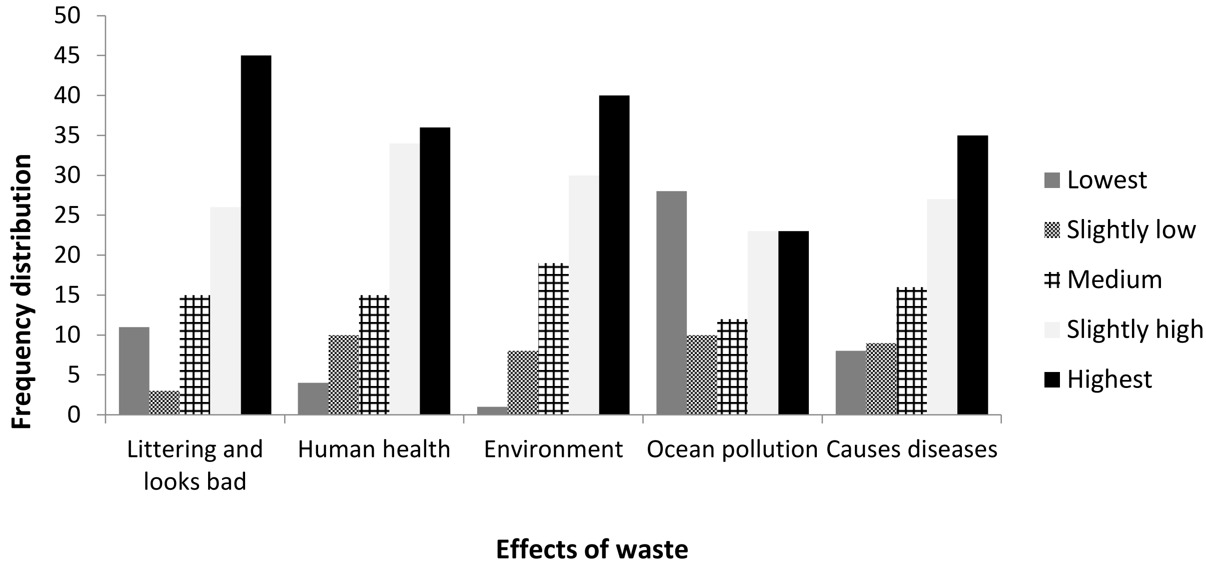

Figure 25:Effects of waste

4.6 Problems with Waste Management

From figure 28, odor (29%) was one of the major problems with the current waste management system closely followed by waste lying around (22%). Other problems as identified included flies (18%), diseases (18%) among others.

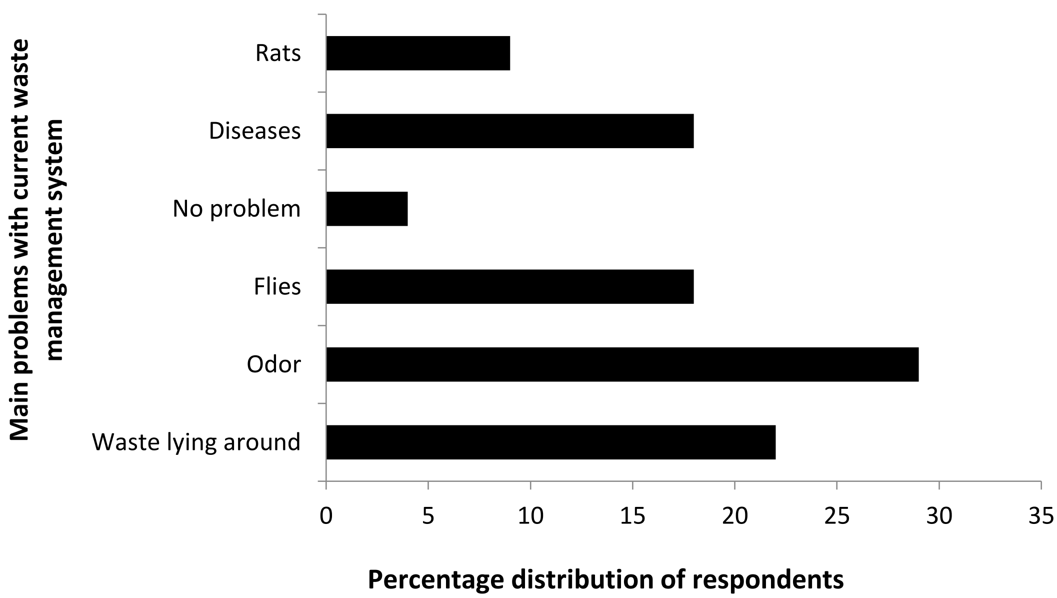

Figure 26: Main problems with current waste management system

4.7 Causes of Environmental Degradation

Causes of environmental degradation was analyzed on a scale of 1-5 (1 being lowest, 3 medium and 5 highest). Air pollution and water pollution had medium rate of causing environmental degradation, waste pollution had the highest rate of causing environmental degradation, noise pollution and damage to scenic beauty had the lowest rate (figure 27).

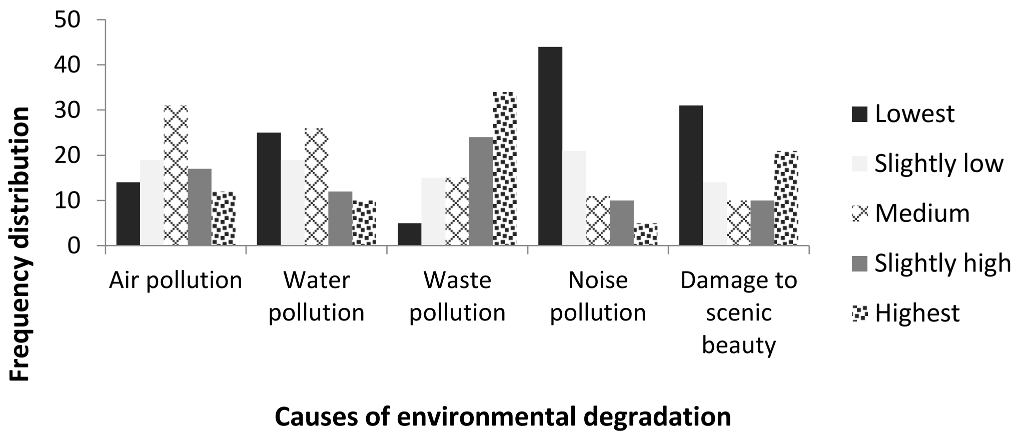

Figure 27: Causes of environmental distribution

4.8 Phytoplankton

Planktons provide excellent indicators of environmental quality conditions and health within the cultural facilities (Maristela et al., 2008). Primavera (1998) noted that planktons respond to high nutrient levels, abundance or shortage of food, low oxygen levels, contamination, and predation. The prevailing state of a functional pond can be assessed by looking at plankton indicators, mainly the abundance, biomass and diversity (Burford, 1997; Primavera, 1998). There were 34 genera belonging to five classes Bacillariophyceae (18 genera), Dinophyceae (6 genera), Cyanophyceae (6 genera), Chlorophyceae (2 genera) and Euglenophyceae (2 genera) and Bacillariophyceae was dominant.

CHAPTER FIVE

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Growth performance

Research on *S. sutor* in the Western Indian Ocean region has centered on its biology, capture and spawning aggregation, and little effort has been made in farming species (Mbaru, Mlewa & Kimani, 2010; Akinyi, Sigana, Ondu, Wambiji, Ong'anda & Orembo, 2018). Therefore, the culture of *S. Sutor* in intertidal earthen pond cages is a new aspect in addition to feeding the species with BSFL formulated feed. The species is herbivorous (feeds on benthic algae, seaweeds, and seagrass) and can easily be weaned to formulated feeds in captivity (Li *et al*., 2018). We observed that all experimental fish in cages were of good healthy throughout the experimental period. The study established that the wild collected *S. sutor* seed responded well to all experimental diets, fact supported by Adewolu, Ikenweiwe and Mulero (2010). According to Li *et al*. (2018), *S. sutor* readily accept artificial feeds when introduced to the diets, thus making them suitable for commercial aquaculture.

Being herbivorous, *S. sutor* have a low protein requirement in the diet that makes them attractive for both small- and large-scale aquaculture (*Li et al*., 2018). The current findings show a possibility of substituting up to 100% fish meal with BSFL diet without any much effect. There was an observed decrease in BWG and SGR when the fish meal was substituted with BSFL meal of >50%, which is in agreement with the findings of Muin *et al*. (2015). The decrease in growth was comparable with different fish species, in our study there was increase in BWG. Devic, Leschen, Murray & Little (2018) reported a growth factor of two on fish fed on diets containing varying proportions of (0, 30, 50, and 80%) of BSF larvae as a fish meal replacer. Regarding growth parameters, we found convergence with previous studies on *O. niloticus* and Japanese rainbow trout where partial dietary replacement of fish meal with BSFL did not show any difference in BWG and SGR (Toriz-Roldan, Ruiz-Vega, García-Ulloa, Hernández-Llamas, Fonseca, Madrigal & Rodríguez-González, 2019; Devic, Leschen, Murray & Little, 2018; Belghit *et al*., 2019; Wang & Shelomi, 2019).

It's argued that a combination of two or three animal-based protein sources in the formulation of fish diets is a good way of promoting a balanced amino acid profile as well as compensating for EE in the larvae (Phonekhampheng, 2008). We established that a high SGR in T1 (100% BSFL) and in T4 (40% commercial diet) respectively, therefore supporting the argument by Phonekhampheng (2008) whose study on bass Japanese and rainbow trout did not show any difference in SGR. However, there is a need of further studies to establish the actual proportions of fish meal and BSFL replacement for a nutritionally balanced and cost-effective diet for *S. sutor* culture without compromising growth performance (Kroeckel, Harjes, Roth, Katz, Wuertz, Susenbeth, 2012).

Additionally, combining two or three animal-based protein sources has been documented to promote a balanced amino acid profile and also compensating for the high EE in the larvae (Phonekhampheng, 2008). But this was disqualified in this study, where some BSFL were combined with fish meal T2 (25% BSFL and 75% fish meal) and T3 (50% BSFL and 50% fish meal) and the results showed a single protein T1 (100% BSFL) performing well as compared to combined protein. Kroeckel *et al*., (2012) working on rearing whose

juvenile turbot on diets containing different propositions of BSFL found that, substitution levels of fish meal by BSFL led to a decrease in the growth performance parameters.
We also underscore the significance of managing water quality to acceptable levels in culture systems for a maximized growth performance of *S. sutor*. The study experienced high salinity levels during neap tides coinciding with seasonal dry periods. Extremes in water quality parameters may impact the growth performance and survival of *S. sutor*. Salinities of more than 30ppt have been observed to cause growth depression with reduced digestibility and feed conversion efficiency in milkfish (Jana, Ayyappan, Aravindakshan & Muduli, 2006).

In view of the economic side, the fishmeal and fish oil remain the main protein and lipid sources of feed for marine fish, and the development and application of formulated feeds are restricted by the high cost of these ingredients. Therefore, efforts should focus on producing commercial feeds for *S. sutor* at low cost and high efficiency (Xu et al., 2011).

5.1.2 Length-weight Relationship (LWR)

The LWR has been globally accepted as a measure of isometric growth in fish with the “b” values suggesting the consistency of density and form in an ideal fish. An ideal fish exhibiting isometric growth has an estimated “*b*” value of 3 (Allen,1938; Beverton and Holt, 1957) but could range between 2.5 and 3.5 as per (Carlander, 1982; Froese, 2006). The current study had exponential “*b”* values ranging from 3.78 - 4.09, which is above the anticipated 3 (cube law), but no significant difference was noted between treatments. Cube law changes could be associated to deviations in the physiological conditions of the fish, environmental parameters, sex, taxonomic differences, or reproductive traits (Le Cren, 1951; Ricker, 1973).

Akinyi *et al*., (2018) in her study on relatively larger sizes of *S. sutor* from the wild at fish landing sites in the northern part of Kenya, established that the fish had a "b" value of 2.82 suggesting that the fish were neither heavier nor lighter in relation to their individual lengths. Similarly, Mbaru *et al.,* (2010) working on *S. sutor* in the southern part of Kenya recorded a "b" value of 3.29, indicating that environmental factors, seasonality, habitat conditions and geographical location could play a role in determining the LWR of *Siganus sutor* (Kimmerer et al., 2005; Froese, 2006; Akinyi *et al*., 2018).

The current study experienced relatively high salinity 39.27-41.87ppt which could have influenced the form and shape, thus leading to the observed deviations in the cube law observed. In addition, since the fish were reared in ponds with availability of food from plankton and supplemental feeding, the fish could have become heavier in weight compared to the length increment as observed by Mommsen (1998).

5.1.3 Feed utilizations and Proximate composition

The feed conversion ratio (FCR) is an important indicator in fish farming since it provides information on the quality of fish feeds, how the best the nutrients are converted into flesh, and how efficiently the feeds are utilised to reduce wastage (Opiyo *et al*., 2020). In the current study, a feed conversion ratio of between 4.61 and 5.66 was obtained for the four treatments, which could be associated to the abundance of planktonic food that is equivalent to an increase in feeding frequency (Abdel-Aziz *et al.,* 2016), the fish showed significantly higher ($P<0.05$). The lower FCR in T3 (4.61±0.3) can be due to overloading of fish stomach and intestine, leading to decrease in digestive efficiency of fish and reduction in feed utilization (Liti *et al*., 2006)

The obtained FCR are similar to those obtained by (Liti *et al*., 2006) for freshwater tilapia that ranged between 3.4 and 4.0 and slightly varied with Opiyo *et al.* (2020) that ranged between 2.33 and 3.26. In the culture of tilapia in marine waters, Malik *et al*., (2018) recorded a FCR of 0.55 across all salinity levels, which was in agreement with other tilapia studies in the marine environment (Daudpota *et al*., 2014; Rahim, Rahman & Hussain, 2017a & b). Although Mapenzi and Mmochi (2016) obtained a FCR of between 1.01 and 2.85 in farming marine tilapia, while Jana et al. (2006) established that FCR worsened with a decrease in salinity in milkfish farming.

According to Abdel-Aziz and Ragab (2017), farming of *Siganus rivulatus* attained a FCR of 7.74 - 11.22 when fresh algae were given to the fish compared to formulated feeds (1.45 – 1.58). Even though there was no significant different in the FCR obtained in the different treatments, there was an observed trend where the percentage inclusion of BSFL enhanced the FCR, i.e., 25%, 50% and 100%. The control feed was only better in FCR than the 25% BSFL inclusion.

The 50% BSFL and 50% fish meal treatment provided the formulation with the lowest preferable FCR. This was an indication that proportional fish meal and BSF larvae mixture was well utilized for optimal growth of rabbitfish. This could be a result of increased palatability, availability of balanced essential amino acids, easy digestibility, and minerals for support of fish growth (Tran *et al.*, 2015).

Instances of poor FCR values can be attributed to variation in feed utilization efficiency within a single species and prevailing environmental conditions (Ellis *et al.*, 2002).

Therefore, the variations of FCR's among different studies might be explained by the different experimental conditions like feed formulation, diet content, stocking density, age, and sex of fish (Jauncey, 1982).

In addition, Ridha and Azad (2004) argues that the impact of FCR is more significant during the grow-out stage compared to the fry stage due to the increase in food consumption. Furthermore, it has been observed that processing of BSFL might reduce the nutrient availability and digestibility and the fact that the diet could be new to cultured fish like *S. sutor* (Sheppard *et al*., 2008).

5.1.4 Water quality parameters

The range of mean values of temperature, DO, pH, and other parameters in all treatments in this study remained within the acceptable and tolerable levels for both growth and survival of *S. sutor* as reported by Abdel-Aziz and Ragab (2017). Studies have indicated that *S. Sutor* grows well at difference ranges of salinity and can tolerate varied water quality parameters (Tidwell & Allan, 2001). In the current study, the averages of water temperature, pH, salinity, dissolved oxygen and nitrite (N-NO2), nitrate (N-NO3), and total ammonia N -NH3) values in all treatments were within the acceptable limits for *S. sutor* growth in brackish water (ANZECC, 2000, EPA, 2003, Saoud et al., 2007b and Saoud *et al*., 2008).

The good water quality in the ponds can be associated with the free flushing of water during spring high tides (Mirera, 2011a). However, the flushing effect is reduced during neap tides and in the dry season when pond water temperature and salinity were observed to increase, which could have impacted negatively on the fish. Frequency of water renewal has a significant effect on the growth performance and nutrient utilization of fish (Absalom and Omenaihe, 2000; Okomoda *et al*., 2016).

5.1.5 Percentage Survival

Fish survival is an important aspect in fish farming and is influenced by a number of factors ranging from environmental, feeding, water quality, and culture systems. The current study attained fish survival ranging between 71.1 and 93.3% for all treatments. Treatment with 100% fish meal replacement with BSFL had the lowest survival, which gives great significance to the influence of feed composition in fish survival.

The high mean survival 93.3% attained is in agreement with previous studies showing a survival rate of > 98% when using BSFL as a fish meal replacer in testing diets for yellow catfish which is also an indicator of feed acceptability (Muinet *et al*., 2015; Xiao *et al*., 2019). Abdel-Aziz and Ragab (2017) found that *Siganus rivulatus* fries fed with only seaweed as a replacement for artificial feed produced low survival (25%) compared to those fed on artificial feed (75%). In the study, treatment with a 50% mix (seaweed and artificial feed) attracted a moderate survival, situation observed in the current experiment where there was a 50% mix of BSFL and fish meal.

Furthermore, Abdel-Aziz *et al*. (2016) established that daily feeding frequency significantly influenced survival in rabbit fish, *Siganus rivulatus*. Frequent feeding provides enough energy required by the growing fish and provides nutrients required to develop disease resistance.

5.1.6 Waste Management

Generally, respondents are moderately satisfied with the attitude of waste collectors during waste collection. Improvements are recommended in terms of safe driving, friendliness, cleanliness of service areas, and proper placing of garbage carts and bins after collection (Okemwa *at el*, 2022). As far as customer service is concerned, more training should be given to the staffs who handle customer service. It appears that generally, the respondents are not satisfied with customer service.

Among the various ways by which waste can be minimized, reusing and recycling have been practiced by about one-third of the respondents. The figure is still not promising; more and continuous programs and campaigns, especially by the government and concessionaires, to educate and discipline the society are very much needed, in addition to providing recycling bins at various strategic locations. The campaigns should be able to instill the feeling of guilt among the society if they do not recycle their household waste.

The social media, mass media, schools and colleges, as well as places of worship should act as channels in educating the public about the importance of recycling. Another method of waste of minimization that can be practiced is composting. Although not many respondents compost their household waste, a majority of the respondents are keen in doing it if demonstrated, and are very supportive of the idea of centralized composting. Again, educating the public on the know-how is an important element of waste minimization.

Referring to the previous studies, it has been shown that household waste separation before disposal is at poor level. According to the results of the present study, considerable fraction of the households (48%) does not separate waste before disposal.

5.1.7 Sustainability of BSFL Production on Tested Substrates

All the substrates used in the current study are easily available. For instance, largeamounts of food wastes are generated by various producers along the food production chain such as farms, groceries, warehouses, learning institutions, municipal markets and eateries (FAO, 2013). The producers of the waste have few options and end up disposing them in dumping places and landfills (Everest *et al*.,2012)

The use of food waste in BSFL farming is economically feasible and sustainable, due to its widespread availability and low-cost implication which only relates to the collection cost.However, BSF substrate faces competition from the livestock feed industry in areas where zero grazing is practiced. The use of fruits (bananas, watermelons, pineapples and avocados) and vegetables as BSFL production substrates is feasible especially during seasons of overproduction, this is due to their short shelf life (Ahvenainen, 1996).

5.2 Conclusion

Based on the growth performance and survival findings, farming of *S. sutor* in cages installed in intertidal earthen ponds is feasible. Rabbitfish respondent to BSFL formulated diets comparable to commercial fish meal diets. However, more research is needed in open intertidal ponds and sea cages to assess the viability of the intervention. Production of BSFL using domestic and market wastes could be an asset for the aqua feed industry as a possible protein source, and harvested at market sized fish that are acceptable and benefit riparian communities.

5.3 Recommendations

Growing of *Siganus sutor* should be encouraged in brackish water while feeding them on formulated diets. Local farmers can now easily adopt this method of rearing rabbitfish with promising returns.

Production of BSFL using domestic and market wastes could be an asset for the aqua feed industry as a possible protein source.

Based on the growth performance and survival findings, farming of rabbit fish (*Sigunus sutor*) in cages installed in intertidal earthen ponds is feasible.

More research is needed in open intertidal ponds and sea cages to assess the viability of the intervention.

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APPENDICES

Appendix 1: Research License permit

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This is to Certify that Mr.. DOUGLAS Okemwa OKEMWA of Karatina University, has been licensed to conduct research in Kilifi on the topic: Growth performance of Rabbitfish on brackishwater coastal earthen ponds for the period ending : 15/September/2023.

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Appendix 2: Publications

E. Afri. Agri. For. J (2021,Volume 85, 1-4, Pg. 325-335)

GROWTH, NUTRITIVE VALUE AND BIOCONVERSION EFFICIENCY OF PRE-PUPAL BLACK SOLDIER FLY FED ON URBAN HOUSEHOLD AND MARKET WASTE

D. M. Okemwa[1,2#], C. C. Ngugi[2], and D. O. Mirera[1]

[1]Kenya Marine and Fisheries Research Institute, English Point, Mkomani, P.O Box 81651–80100 Mombasa, Kenya, [2]School of Natural Resource and Environmental Studies, Karatina University, P.O. Box 1957-10101 Karatina, Kenya

Appendix 3: Manuscript

Assess the perception of household waste management and minimization along the Kenyan coast: Awareness, issues and practices

Okemwa[1], M. D., Ngugi[1], C. and Mirera[2], O. D*

[1]The school of Natural Resource and Environmental studies Karatina University, P.O. Box 1957-10101 Karatina, Kenya;

[2] Kenya Marine and Fisheries Research Institute, P.O. Box 81651 80100 Mombasa, Kenya

Corresponding author: Email: douglasokemwa3@gmail.com

Appendix 4: Manuscripts

Growth performance of Rabbit fish (*Siganus sutor*) reared in intertidal brackish water earthen ponds using hapa nets

Okemwa[1,2], M. D., Mirera[2], O. D and Ngugi[1], C.*

[1]The school of Natural Resource and Environmental studies Karatina University, P.O. Box 1957-10101 Karatina, Kenya;

[2] Kenya Marine and Fisheries Research Institute, P.O. Box 81651 80100 Mombasa, Kenya

Corresponding author: Douglas Okemwa: douglasokemwa3@gmail.com