APPLICATION OF AN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM IN ENHANCING PRODUCTION AND PROFITABILITY IN SMALL SCALE MARICULTURE ENTERPRISES IN KENYA

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented in any other University or any other Award.

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DEDICATION

This thesis is dedicated to:

Alpha, Erica and Eliel (My three lovely children)

And

Jecinta Njeri Magondu (My loving mother and prayer partner)

And

Josphat Gikonyo Wachira (My partner of a long journey)

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ABSTRACT

This study presents the first application of an integrated multi-trophic aquaculture (IMTA) system in pond culture in Kenya using local species. The overall goal was to identify and recruit new marine species for diversification of mariculture and increased income through adoption of environmentally friendly culture systems. The study was conducted in two trials: the **first trial** compared a monoculture of Indian white shrimp (*Penaeus indicus*) with an IMTA of Indian white shrimp as the fed component, and introduced sea cucumber (*Holothuria scabra*) and cockles (*Anadara antiquata*) as the extractive components. The trial was conducted in six brackish water ponds of 120 m^2 and 1 m depth over a 135 days culture period. The monoculture treatment (T1) ponds were stocked with *P. indicus* juveniles at a density of 5 ind/m^2. The IMTA treatment (T2) stocked *H. scabra*, *P. Indicus* and *A. antiquata* at 1.2 ind/m^2, 5 ind/m^2, and 3.5 ind/m^2, respectively. Results showed that shrimps in monoculture treatment T1 attained a weight gain of 13.2± 0.75 g while in the IMTA treatment shrimps had a weight gain of 13.2 ± 0.57 g, sea cucumber gained 175.03 ± 27.84 g and cockles gained 44 ± 0.97 g. Survival was highest for *P. indicus* in IMTA at 80.1% and monoculture at 72.8% and lowest for *H. scabra* at 56.32%. The inorganic nutrient parameter of water significantly improved in IMTA system ($p < 0.05$) as compared to monoculture system. Of the five (5) phytoplankton classes recorded in water and plankton samples Bacillariophyceae dominated with 13.04 ± 20.62 cells/m^3 in IMTA and 6.57 ± 17.84 cells/m^3 in monoculture.

The **second trial** used Nile tilapia (*Oreochromis niloticus*) acclimatized in marine water as the fed component in IMTA system with different species combinations. The study design had a monoculture of Nile tilapia as the control (C), a combination of *O. niloticus* and *H. scabra* as treatment 1 (T1), a combination of *O. niloticus*, *H. scabra* and oysters *Sacostrea cucullata* as T2, and T3 had a combination of *O. niloticus* and *S. cucullata*.

Stocking in replicate ponds was at 2 ind./m^2, 1.9 ind./m^2 and 2.1 ind./m^2 for Nile tilapia, sea cucumber and oysters respectively and in all treatments. Results showed that all organisms recorded weight gain over the 150 days study period; with *O. niloticus* recording a significantly higher ($p < 0.05$) average body weight (ABW) in T2. Treatments that had *H. scabra* as one of the extractive species recorded low survival rates i.e. 53 % in T1 and 45 % in T2. Production was highest in T2 where all three species were integrated. Net income was US$ 77.5 in IMTA compared to US$ (209) for the control while CBR was ≈1.0 for IMTA and ≈0.4 for the control. Collectively, the findings of these experimental studies demonstrated the potential of IMTA as a more profitable mariculture practice compared to monoculture; and is therefore recommended for adoption to promote production in earthen pond mariculture systems in coastal Kenya.

Key words: IMTA, Monoculture, Sea cucumber, Cockles, Tilapia, Shrimps, Oysters, Economics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
AWWA	American Water Works Association
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis Of Variance
AFCR	Apparent Feed Conversion Ratio
CBR	Cost Benefit Ratio
CITES	Convention on International Trade on Endangered Species
EA	Elemental Analyser
DAP	Di-ammonium Phosphate
DOM	Dissolved Organic Matter
DGR	Daily Growth Rate
DWG	Daily Weight Gain
EAA	Ecosystem Approach to Aquaculture
FARM	Farm Aquaculture Resources Management
GLM	Generalized Linear Model
HSD	Honestly Significant difference
IMTA	Integrated Multitrophic Aquaculture
IRMS	Isotopic Ratio Mass Spectrometry
KMFRI	Kenya Marine and Fisheries Research Institute
KSCAP	Kenya Climate Smart Agriculture Productivity Project
NWG	Net Weight Gain
NCST	National Council for Science and Technology
NACOSTI	National Commission for Science Technology and Innovation

POM	Particulate Organic Matter
SPM	Suspended Particulate Matter
SGR	Specific Growth Rate
UN-SDG	United Nations Sustainable Development Goals
TDS	Total Dissolved Solids
TAN	Total Ammonium Nitrogen
USD	United States Dollar
WPCF	Water pollution Control Federation
WIO	Western Indian Ocean

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

World aquaculture production continues to be the fastest growing animal-food producing sector which tremendously increased from 32.4 MT in 2000 to 66.6 MT in 2012, and subsequently to a record 114.5 MT in 2018 (FAO, 2020). Aquaculture products have proved to be an important source of animal protein, essential fatty acids, and macro nutrients like vitamin A, iron, zinc and selenium that is crucial for human nutrition and health (FAO, 2016). Adeogun and Alimi (2014) noted that fisheries has been depended upon by millions of people globally, and the continued increasing consumer demand for aquatic foods creating an impetus to expand fish production through aquaculture. Pond farming takes the largest share of aquaculture production facilities as indicated in FAO (2014) with more that 80% production of fish and 98% for shrimps globally, being produced in ponds. In addition, there has been a dramatic increase in population globally bringing about more food requirements; which raises concern since the existing traditional extensive fish production systems cannot satisfy the present and future market needs (FAO, 2020).

Elsewhere it has been noted that such background provides a unique opportunity for trial of other fish culture technologies for increased production and economic gains. For instance, Troell (2009) noted that promotion of integrated culture systems in the tropics has been on the rise; with focus shifting to modern integrated aquaculture approaches such as Integrated Multitrophic Aquaculture (IMTA) that are being used in developed countries like Canada, China, and Israel among others (Chopin *et al*., 2001; Troell *et al*., 2006; Neori *et al*., 2007). The IMTA approach has been defined as the farming of aquaculture species of different trophic levels and with complementary ecosystem functions in a way that allows one species uneaten feed, nutrients and wastes to be recaptured and converted into fertilizer, feed, and energy by co-cultured organisms; hence take advantage of the synergistic interactions between the cultured species (Chopin *et al.,* 2008; Neori *et al*., 2004). The IMTA approach aims at increasing long-term sustainability and profitability per cultivation unit and not per species in isolation as in monoculture. The organisms cultured in an IMTA can, in turn, be harvested, and marketed as healthy seafood. The potential benefits of IMTA as reported by Chopin (2013) include; bio-mitigation to the ecosystem, improved economic farm output, lowered production costs, product diversification, risk reduction, disease control in culture facilities and job creation for coastal rural communities.

Community-based aquaculture has been adopted in the Western Indian Ocean (WIO) region and has played a great role towards growth of mariculture; enhancing food security and strengthening the resilience of the coastal communities to climate change effects and environmental challenges (Bene *et al.,* 2016; Gentry *et al*., 2017). In Kenya, marine aquaculture is practiced at small scale level and its production potential has been estimated at 100 Mt/year of finfish, shellfish, and seaweeds (Munguti *et al.,* 2017). However, Ateweberhan *et al*. (2018) and Opiyo *et al*. (2018) noted that Kenya's mariculture sector has great potential. To increase production and diversify aquaculture products, fish farmers in Kenya have hitherto used the integrated aquaculture concept which basically entails the use of an output from one farming system as an input for another subsystem. Integrated aquaculture has mostly been done in freshwater paddies, ponds and aquaponics for the production of fish-rice, fish–livestock, and fish-vegetable combinations (Ngugi *et al*., 2007; Soto, 2009; Knaus and Palm, 2017). 'In the marine aquaculture sector, milkfish-shrimp, milkfish-mullet, and mullet species combinations under earthen pond polyculture systems have been tried in the intertidal areas' (Mirera, 2011; Dayal *et al*., 2017).

Wild caught fisheries has levelled off with most of the main fishing areas globally collapsing (FAO, 2020). Concurrently, the demand for sea food is rising with increase in per- capita fish consumption related to growing world population. Therefore, the growing gap between demand and supply for aquatic food products can only be met through aquaculture. In Kenya, fresh water aquaculture has shown promising production statistics with adoption of different culture systems like cage farming in Lake Victoria and pond farming in different parts of the country like Western and Central Kenya (Munguti *et al*., 2017; Orina *et al*., 2021). Marine aquaculture production on the other hand has not been very promising due to relatively lower numbers of fish farmers; it is undertaken by few small scale organized farmer groups for instance the Umoja self-help mariculture group in Kilifi County. The sector is faced with challenges of seasonality of seed availability and culture management practices employed. Clearly there is need for interventions to enable fish farmers in the coastal region to overcome the challenges hence improve production from the mariculture sector.

Development of the mariculture sector along the Kenyan coast has largely been due to the continued increase in demand for seafood products against decreasing production from the marine capture fisheries; and search for alternative livelihoods for the coastal communities (FAO, 2020). Mariculture in Kenya and the Western Indian Ocean (WIO) region has been practiced in two ways; at private farmer level or organized into community-based groups which have been mainly centered on the production of different marine organisms such as; finfish mainly milkfish (*Chanos chanos*, Forsskal, 1775), Nile tilapia (*Oreochromis niloticus* L. 1758) Mirera and Okemwa (2022, *under review)*, seaweeds (*Eucheuma denticulutum* (N. L Burman) Collins and Hervey 1917, shellfish mainly Mud crab (*Sycella serrata* Forsskal, 1775) and two species of marine shrimps (*Penaues monodon*, Fabrious 1798 and *Penaues indicus*, H. Milne Edwards 1837). However, even with this existing diversity of culture species, production from the sector still remains low which has been attributed to several factors such as; low education levels, lack of skills, poverty, and lack of formal rights of the practitioners to the resource they depend on (Anderson and Saidi 2011; Cinner *et al*., 2012; Ateweberhan *et al*., 2018). This has been compounded by habitat degradation and reliance on the already exploited fisheries resources (Forster *et al*., 2014).

An IMTA system has five (5) main components namely: the fed component, the organic substrate extractive component, the organic particulate suspended matter extractive component, the inorganic substrate extractive component and finally the microbial component (Chopin, 2013; Dong *et al*., 2018). In this study, marine shrimps (*Penaeus indicus*) and Nile tilapia were used as fed components in the first and second IMTA trial respectively. Extractive components included microalgae which removed inorganic nutrients from the IMTA system; filter feeders such cockles and oysters which utilized suspended particulate matter, and sea cucumbers - deposit feeders which fed on deposited organic substrates. The overall goal was to achieve improved output, lower costs, product diversification, risk reduction, and job creation Neori *et al*. (2004) for the local communities who engage in mariculture along the Kenya coast.

1.2 Problem Statement

The main fresh water aquaculture species farmed in Kenya includes farming of Nile tilapia *(oreochromis niloticus*) and African catfish (*Clarius gariepinus*). A high propotion of farmers produce tilapia and catfish in monoculture systems Opiyo *et al*., (2018) due to lack of knowledge of polyculture and other integrated culture systems by farmers. In the mariculture sector poor production has been experienced with monoculture of fin fish species like mullets and milkfish which are slow growing and do not attract good market especially milkfish due to its boney nature. Culture of shellfishes such as marine shrimps and mud crabs is also been done in monoculture systems where production levels are still low. The practices are not sustainable due to the high cost of production in terms of inputs such as fertilizers and feeds. Further monoculture has been associated with degradation of the ecosystem due to discharge of nutrient rich culture water to the natural systems. The aforementioned challenges show the need to diversify and introduce the integration concept that has interventions including adoption of technologies and farming systems such as IMTA that promote increased production and diversification of income streams for long-term benefits and sustainability to support livelihoods of the coastal marine fish farmers.

1.3 Justification of the study

Monoculture entails farming of a single species in a system while in polyculture more than one species are farmed together. Both practices have been conducted widely in fish farming both in marine and fresh water systems. Culture of the three Indian major carps (Catla, Rohu and Mrigal) and the exotic carps (Silver carp, Grass carp and Common carp) have contributed to over 70% of inland production (Das *et al.*, 2022). Brackish water aquaculture is dominated by single species mostly shrimps which has shown indications of overburdening the ecosystem through release of untreated pond effluents resulting to environmental degradation. Polyculture involving shrimps, mullets and milkfish has been practiced in brackish water ponds (Biswas *et al*., 2012), though profitable, the systems need improvement to achieve environmental sustainability.

Moving towards system diversification has proved to provide great benefit to fish farmers in terms of economic returns and mitigation of risks of loss during fish culture. As pointed out by several workers (Chopin *et al*., 2001; 2006; Neori *et al*., 2007), adoption of IMTA has additional benefits of lowering cost of feed inputs and suitable water quality conditions lowering chances of disease attacks. In addition, as an ecosystem approach in marine aquaculture, IMTA has the potential to solve pollution problems that come along with fish farming. Application of this concept makes use of commercially viable species such as marine shrimps, bivalves, and marine plants that are also environmentally sustainable. Uneaten feed and metabolic excretion of the fed species are useful for the growth of the other species providing a natural self-cleansing mechanism (Chopin *et al*., 2001; Troell *et al.*, 2003; Neori *et al*., 2004). According to Avinmelech and Ritvo (2003) an average of 15 % of nutrients arising from inputs supplied in aquaculture farms are normally retained by fish while the rest goes to water and pond sediment. Removal of these nutrients accumulated overtime is currently a major concern towards the sustainability and health of existing natural aquatic ecosystems. Therefore, diversification of culture species and improvement of culture systems being used are key elements for consideration as a way of creating more economic benefits. It is against this background that trial applications of IMTA system for the culture of different species combinations such as marine shrimps, Nile tilapia, cockles, oysters, and sea cucumbers were experimented in the present study so as to enhance production and diversify income streams from small scale pond culture enterprises by the Umoja Self-help Group at Kibokoni, Kilifi County.

1.4 Research Hypothesis

H_0: An (IMTA) system would enhance overall production and profitability in small-scale mariculture in Kenya

H$_A$: An (IMTA) system would not enhance overall production and profitability in small-scale mariculture in Kenya

1.5 Objectives of the study

1.5.1 Main objective of the study

The main objective was to introduce an Integrated Multitropic Aquaculture (IMTA) system for the culture of marine organisms to enhance overall production and profitability in small-scale mariculture in Kenya.

1.5.2 Specific objectives

The specific objectives of the study were to:

i. determine the viability of integrating an IMTA of sea cucumber, cockles and marine shrimps in established earthen ponds.

ii. determine the viability of integrating an IMTA of sea cucumber, oysters and Nile tilapia in earthen ponds.

iii. assess the potential profitability of IMTA systems in comparison with monoculture systems.

iv. assess the environmental and economic benefits of IMTA in pond systems.

1.6 Description of the study

This study applied Integrated Multitrophic Aquaculture system (IMTA) in intertidal earthen ponds owned by the Umoja Self Help Group at Kibokoni along the Kilifi creek in Kenya. The study recruited several new organisms and used them in different species combinations to understand the IMTA effect and interactions between the biotic and abiotic factors in the system so as to determine combinations with highest production and economic returns. Two trials were conducted; the first trial compared marine shrimp monoculture with an IMTA of sea cucumbers, cockles, and marine shrimps. In order to determine whether IMTA can be profitable and good alternative to semi intensive production systems in earthen ponds, a second trial with three treatments of different functional groups was used consisting of a complete and partial IMTA arrangement and further compared with Nile tilapia monoculture as a control. The species combinations used were Nile tilapia, mangrove oyster, and sea cucumbers. Periodic sampling was conducted and experimental data collected to define the performance of the system according to rearing conditions and biological functions like growth, water quality management, and nutrient cycling.

1.7 Significance and Scope of the Study

The present study investigated the viability of IMTA system in an earthen ponds mariculture set up along the Kenya coast so as to understand the chemical, biological, and nutritional processes that take place within the system. The study used different combinations of organisms feeding at different trophic levels, some of the organisms have not been cultured locally before such as cockles and sea cucumber, and hence prospects for their culture enrich the mariculture sector. On the other hand, shrimps, Nile tilapia, and oysters have been tried at commercial and experimental levels. Results of the economic analysis done in this study give an indication of the profitability of the IMTA culture system versus the traditional monoculture system. This study also highlights the environmental benefits derived from integration promoting system productivity.

The study focused on application of IMTA technology in an intertidal earthen pond culture system to evaluate feasibility of cultivation and profitability gained from species combinations and potential for nutrient remediation. This was based on data collected in two field trials at the Umoja Self Help Group earthen ponds at Kibokoni along the Kilifi creek and laboratory analysis of samples from the ponds done at the Kenya marine and Fisheries Research Institute laboratories.

1.8 Structure of the thesis

This thesis comprises seven chapters, each of which focuses on a different aspect of the study. General aspects are presented in Chapter 1: general introduction, Chapter 2: literature review Chapter 3: general materials and methods. Chapter 4 focuses on the first IMTA trial that involved integration of sea cucumber and cockles into marine shrimp earthen ponds, whereas Chapter 5 presents the second IMTA trial designed to test different species combinations of sea cucumbers, oysters, and Nile tilapia in earthen ponds. In both trials growth and yield parameters were determined to assess pond production. Water quality of the culture environment was assessed using data of parameters taken insitu and laboratory analysis of samples. Chapter 6 presents findings of economic analysis of both experiments focused on net income, cost benefit analysis, and return on investment to determine profitability. Chapter 7 summarizes general discussion of the overall findings from the two trials, and presents conclusions and recommendations of future research directions for development of integrated multitrophic aquaculture in Kenya. Subsequent to the list of references of sources cited; summaries of data, evidence of ethical review and approval of the study, research permits, evidence of two published papers, and working titles of two other manuscripts under preparation for peer reviewed publication are included as appendices to this thesis.

CHAPTER TWO

LITERATURE REVIEW

2.1 IMTA concept: Description, global and regional review of its application

Integrated multi-trophic aquaculture (IMTA) has been described as the farming in proximity, of different species combinations from different trophic levels and with complementary ecosystem functions in a way that allows one species' uneaten feed and wastes, available nutrients and by-products to be recaptured and converted into fertilizer, feed and energy for other crops, and to take advantage of the synergistic interactions among the cultured species while bio mitigation takes place (Chopin, 2013). The concept has been identified as the most preferred approach to address concerns that limit aquaculture nutrients and organic outputs through bio-mitigation enhancing economic and environmental sustainability (Ridler *et al*., 2007b; Chopin *et al*., 2012; Chang *et al*., 2020).

The world population has continued to grow over the years, one of the effects is that the annual consumption of seafood has been rising and has doubled over the last three decades (FAO, 2016). Production from capture fisheries on the other hand, have continued to diminish threatening a resource that has continued to be relied on by the fisher communities (Cinner, 2011). Aquaculture has been identified as an alternative solution for providing the growing population with protein-rich food and alternative livelihood sources (Gonzales *et al*., 2006; Allison, 2011; Beveridge *et al*., 2013). To promote growth in the aquaculture sector and create positive impacts, there is a need to develop better management practices, environmentally sustainable, profitable technologies, and practices. These should be ecologically efficient, naturally benign, product-diversified, and societally acceptable and beneficial (Chopin, 2013; Cunha *et al*., 2019). One way to enhance aquaculture production is adoption of IMTA instead of relying on either monoculture or polyculture. IMTA improves production methods by making them more sustainable through combinations of appropriate proportions and scales of management in species cultivation (Chopin, 2021). IMTA also contributes to the United Nations' Sustainable Development Goals (SDG) Number 14 that recognizes life under water (Chopin, 2021).

The concept of integration in IMTA refers to the more intensive cultivation of the different species in proximity of each other, connected by vertical nutrient and energy transfers through water movements (Sukhdhane *et al*., 2018). This allows one species' uneaten feed, faecal waste, nutrients, and by-products arising from the culture system to be recaptured and converted into fertilizer, feed, and energy for other co-cultured species (Chopin *et al*., 2001). The fed organisms in an IMTA such as finfish species or shrimps are usually combined with extractive organisms such as filter feeders and marine plants to take up excess nutrients and wastes from fed organism (Chopin, 2013).

'IMTA is a flexible concept that has been developed in different parts of the world according to prevailing economic, societal, environmental, biological, and physical conditions' (Chopin, 2013). The concept can be applied in open water or pond systems, marine or freshwater, and both temperate and tropical environments. Different organisms have been integrated in various IMTA in line with the objective of the system. For example, seaweed (*Glacilaria lemaneiformis*) integrated in Atlantic salmon (*Salmo salar*) farms in Chile, demonstrated dual roles as a nutrient scrubber and commercial crop for agar production (Buschmann *et al.*, 2001; Neori and Shpigel 1999). This not only increased profits but also reduced the environmental costs of waste discharge (Troell *et al*., 1997; Chopin *et al*, 2001).

In the Philippines, Largo *et al.* (2016) developed an open sea cages IMTA by testing the Donkey's ear abalone (*Haliotis asinina*) as the fed species and mixed seaweeds (*Gracilaria heteroclada*, *Caulerpa lentillifera* and, *Eucheuma denticulatum*) as the extractive components. The findings of the study showed possibilities of developing a successful IMTA concept emphasizing the use of local high-value species for adoption and sustainability. Donkey's ear abalone grew to a body weight of 37.8 g while seaweeds' natural filtering capacity maintained a balanced ecosystem by countering accumulation of organic compounds that could accumulate over time during the farming season (Largo *et al*., 2016).

Culture trials using sea cucumbers (*Holothuria scabra*) as organic extractive organisms in combination with seaweeds; *Eucheuma denticulatum (*Namukose *et al*., 2016) and *Kappaphycus striatum* (Beltran-Gutierrez *et al*., 2014) as inorganic extractive organisms formed an IMTA co-culture in Zanzibar. The integration of sea cucumbers with seaweeds resulted in mutual benefits to the cultured organisms (Namukose *et al.*, 2016). Significant growth rates of both culture organisms contributed to the overall increase in production. Seaweeds assimilated the organic matter and nutrients that were re-suspended by sea cucumbers, therefore, cleaning the environment (Namukose *et al.*, 2016). These studies demonstrated the potential viability of the co-culture of commercial species which could offer marked benefits to farmers and allow increased production from mariculture.

In India, open water, brackish water and land based IMTA systems have been tried with shrimps (*Penaeus indicus*) and finfish (*Chanos chanos, Etroplus suratensis, Mugil cephalus*) as fed species and oysters (*Crassostrea madrasensis*) as non-fed species (Balasubramanian *et al*., 2018; Biswas *et al.*, 2019). The studies reported significantly high production and better culture environment in IMTA as compared to conventional polyculture and monoculture systems (Biswas *et al*., 2019). The seaweed species used in India has no commercial value and thus alternative plants such as water spinach (*Ipomoe aquatica)* which have shown some economic value and hence being used in IMTA systems for environmental remediation by absorbing dissolved inorganic particles (Hu *et al*., 2008; Jampeetong *et al*., 2012).

China has a rich history of successful aquaculture innovations. A review of the literature indicates that the IMTA concept has been practiced in Sanggou Bay since late 1980s as shown by (Fang *et al.,* 1996, 2016). Some examples of species combinations include co-culture of abalone and kelp Tang *et al*. (2013) resulting to benefits of food and waste uptake. In addition, there has been successful integration of finfish, bivalves, and kelp in the Bay as well as benthic culture of abalone, sea cucumber, clam and seaweed (Tang *et al*., 2013). IMTA has thus resulted to improved environmental and economic benefits, job creation, and new aquaculture innovations in Sanggou bay (Fang and Zhang, 2015).

Using IMTA as a recycling concept, organisms at higher trophic level provided an input energy to the lower trophic organisms such as bivalves (Irisarri *et al.,* 2015). The IMTA concept has been tested in Indonesia using a combination of three fed organisms (Milkfish, Tilapia, and Vanamei shrimp as fed organisms and green mussle and Gracilaria seaweed as extractive species (Rejeki *et al*., 2016). In the study, the characteristics of shrimp, milkfish, and seaweed made the application of the concept possible as the species biologically complement each other. The fed organisms provide organic matter through uneaten feeds and faecal deposits. On the other hand Glacilaria seaweed provides dissolved oxygen through photosynthetic activity, mussles being filter feeders consume planktons by filtering the water (Neori *et al.,* 2000; Chopin *et al*., 2004; Lander *et al*., 2004). The integration led to good water quality and availability of natural food that supported good production.

Integrated mariculture systems in Israel have developed through research efforts that led to traditional multitrophic culture systems consisting of green water aquaculture ponds and polyculture as reported by Hepher (1985) and Kolkovsky *et al*. (2003). In those studies pumped sea water into ponds was used for rearing fish and shrimps fed with pelleted diets. The effluent water was then stripped of nutrients in ponds containing micro and macro-algae (Noeri *et al.*, 2017). Most IMTA trials have used finfish species, oysters, seaweeds of ulva species and abalone and sea urchins (Xu *et al*., 2011). Other organisms in IMTA trials in Israel include seaweed of *Glacilaria* species and grey mullet (Shpigel and Neori, 2007; Neori *et al.*, 2017). Further, Levy *et al*. (2017) reported that in Israel there has been application of IMTA using finfish with periphyton biofilters (combination of algal and bacteria) that remove organic and inorganic nutrients from water through their metabolic activities.

In South Africa, seaweeds such as kelp and ulva are grown in IMTA systems where they are fed to abalone (*Haliotis midae L*) with fertilizers serving as exogenous sources of nutrients (Nobre *et al.,* 2010). South Africa has been recorded as the third largest abalone producer (Gordon and Cook, 2004). Abalone aquaculture has benefitted from adoption of IMTA with marine seaweeds (Robertson-Andersson *et al*., 2008). The IMTA approach has been embraced because it addresses environmental concerns such as nutrient loading into the environment and productivity (Abreu *et al*., 2009; Buschmann *et al*., 2009). Seahorses (*Hippocampus reidi)* have also been studied in IMTA systems in South Africa without addition of exogenous food and fertilizers (Fonseca *et al*., 2015). The set up involves culture of seahorses in cages installed inside shrimp ponds and hence depend on natural food generated in the system. XU and MU (2017) reported success while integrating seahorses (*Hippocampus kuda*) with seaweed (*Gracilaria lichevoides*) without external feed supply. A recent study by Cohen and Valenti (2019), in Brazil looked at the opportunities and challenges of developing low-cost aquaculture of seahorses in mangrove estuaries. The study used *Hippocampus reidi* one of the largest seahorse species. The results were successful and confirmed feasibility of seahorse cage culture in mangroves using more sustainable IMTA approach.

Coastal bays such as Bay of Fundy in Canada has also been widely used as study sites for trials of application of IMTA approach as a way of mitigating environmental pressures (Troell *et al*., 2009; Chopin, 2013). Atlantic salmon (*Salmo salar*), kelp (*Alaria esculenta*), and blue mussel (*Mytilus edulis*) were reared together in an IMTA in the Bay of Fundy, Canada (Chopin, 2013). The study demonstrated higher growth rates of 50% average than the control sites which indicates an increase in nutrients and food available for kelps and mussels from the fish culture cages. Studies by Chopin (2013) showed that maintaining production from IMTA enhances sustainability from an environmental, economic, social, and technical perspectives. This has been brought about by the enhanced awareness of more and more demanding consumers of seafood products on issues regarding quality, traceability, and production conditions (Neori *et al*., 2007). Clearly, IMTA has the potential to play a role in reaching these needs by cultivating fed species such as finfish or shrimp in combination with extractive species such macro and micro algae and plants which utilize the inorganic nutrients (Chang *et al*., 2020). Shellfish filter feeders and invertebrate deposit feeders utilize suspended and deposited organic nutrients from aquaculture for their growth (Chopin, 2021). Figure 1 shows an illustration of the different components in an IMTA as developed by Chopin (2013).

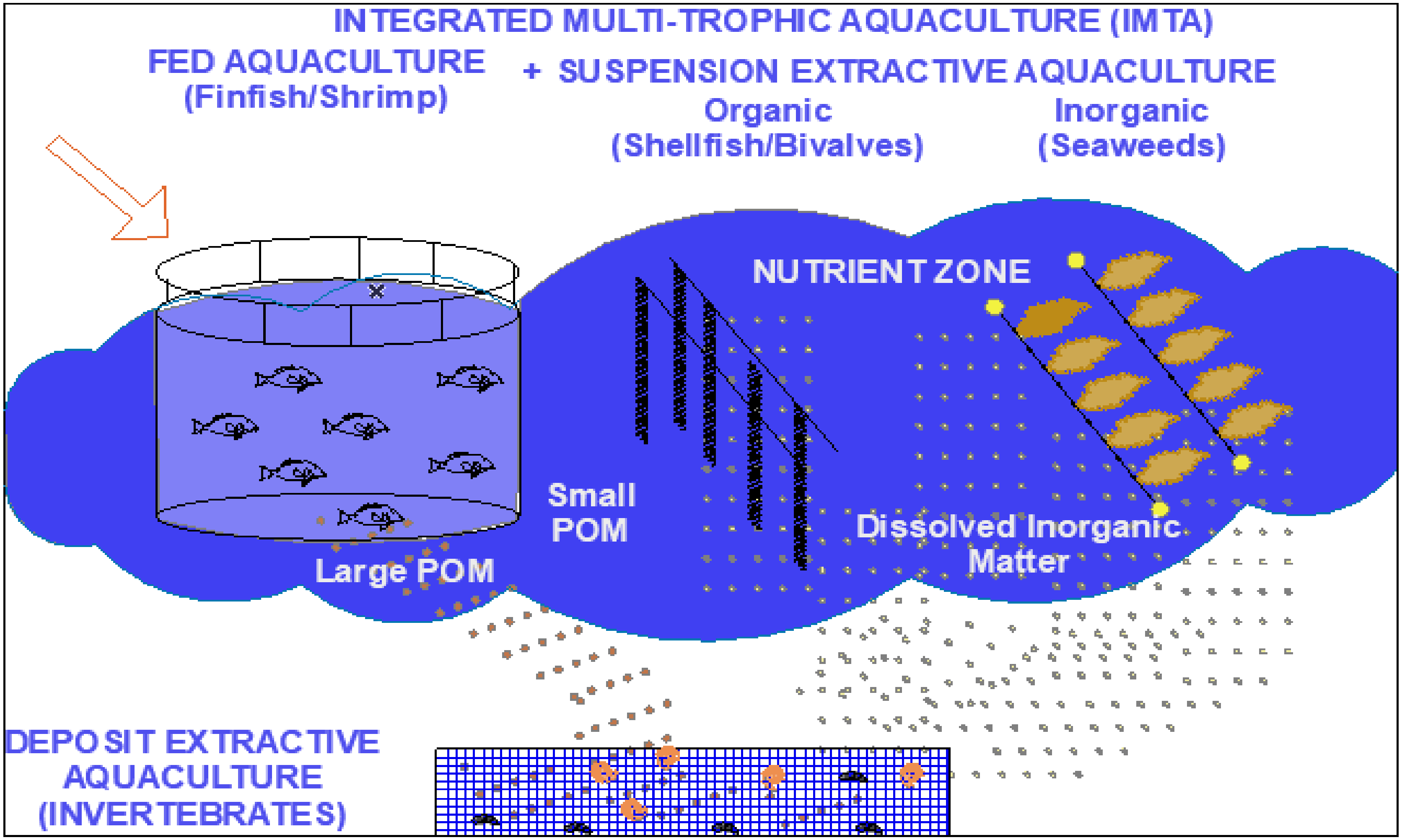

Figure 1 . Components of an IMTA (source: Chopin, 2013)

IMTA interventions have been undertaken in the marine environment with main aspects of concern being; increasing production, species diversification, environmental degradation, and emission of greenhouse gases, resource usage, economic and social aspects, and financial viability (Neori *et al*., 2004). Studies have shown that IMTA may offer opportunities for the efficient usage of water, utilization of nutrients, increased productivity and profits, providing in a single package, practical and creative solutions to most problems of waste management and pollution (Neori *et al*., 2004). IMTA creates a win-win situation that ensures large portions of nitrogen and phosphorous from the feeds administered to the culture organisms are assimilated by fish or shrimps and uneaten feeds and waste filtered by the bivalves which can all be sold contributing to more profits.

Aquatic plants, seaweeds and mangrove forests connected to IMTA systems do provide ecosystem services that can be used to offset carbon and carbon trading taxes which makes IMTA operations more competitive (Chopin, 2021). Notwithstanding the aforementioned benefits, IMTA is not a panacea to everything but a possible improvement option. For example, it has been noted that the IMTA concept has some challenges which include; high initial investment especially if the technology is being applied in the open sea where technological and engineering knowledge is needed (Troell *et al*., 2009).

Experiences of difficult coordination have been mentioned where different operators working independently on different organisms contributing to the IMTA in an open system, for instance, fish farmers and mussel farmers (Chopin, 2013). Stakeholder conflicts on resource use have also been mentioned with concerns about pollution and impacts on wild populations through escapees where an expansion of IMTA practices has been challenged (Froehlich *et al*., 2017). Open water IMTA has shown difficulties in implementation without leasing policies where decisions on site selection are sometimes subjected to political pressures (Krause *et al*., 2003). In addition, Troell *et al*. (2017) and Oyinlola *et al*. (2018) noted that future effects of climate change affecting water temperature and chemistry could lead to a reduction of suitable farming areas. Chopin (2021) has shown that the IMTA concept broad, flexible and keeps on evolving with time. Its main applications has been in open-water, land based systems, marine and fresh water ponds and aquaponics and in both temperate and tropical climates.

2.2 Application of IMTA in marine pond aquaculture

Marine pond aquaculture, which has often also been referred to as brackish water aquaculture, is usually dominated by single species (Biswas *et al*., 2020). Farming activities in marine ponds have mostly been done with shrimp intensive culture which has been blamed for overburdening the ecosystem through accumulation of organic matter at the pond bottom (Peterson and Daniels, 1992). Polyculture involving shrimps, mullets, and milkfish has also been tried in brackish water ponds and has shown to be more sustainable (Mirera, 2011; Biswas *et al*., 2012) as it requires fewer inputs as compared to the intensive monoculture.

Studies have shown that IMTA in earthen ponds is aimed at achieving ecosystem approach to aquaculture (EAA) where aquaculture is integrated to the wider ecosystem aimed at contributing to sustainable development (Soto *et al*., 2008). To achieve more economic gains and improve on environmental sustainability, trials of IMTA in ponds have been undertaken and their performance reported by several workers. Fourooghifard *et al*. (2017) used pond IMTA of seaweed and shrimp integration to improve pond water quality and income for the farmers in situations of limited water exchange. Studies conducted in a culture of *Litopenaeus vannamei* shrimp indicated that combination with seaweed increased growth by promoting utilization of artificial feeds as well as acting as a feed supplement (Cruz-Suarez *et al*., 2010). The integration of *P. monondon* with *Glacilaria verrucosa* resulted in higher growth and survival than controls (Izzati, 2011).

Pond IMTA systems have been conducted in India with shrimp (*P. indicus*) and finfish (*Chanos chanos*, *Mugil cephalus*, *Entoplus suratensis*) as fed species and backwater oyster (*Crassostrea madrasensis*) as the extractive species (Balasubramanian *et al*., 2018). After 110 days culture duration for this study an economic analysis evaluation revealed higher net income and cost benefit ratio in IMTA ponds compared to shrimp monoculture ponds. In addition, environmental benefits were realized where an accumulation of organic matter was found to be low in IMTA ponds compared to monoculture ponds (Balasubramanian *et al*., 2018). Polyculture of shrimps, mud crabs and seaweeds has been common in Vietnam as described by (Brzeski and Newkirk, 1997). However, due to technological advancements and move towards more production IMTA has been adopted to replace these traditional practices that address issues of mitigation while at the same time enhancing nutrient use efficiency and promoting system sustainability (Kumar *et al*., 2000). IMTA studies in brackish water ponds using mullets, shrimps, oysters, and seaweeds conducted by Biswas *et al.* (2019) achieved higher production and better water quality compared to conventional polyculture. IMTA model has proved to be productive and economically viable with environmental bio-remediation effects (Huo *et al*., 2012; Irisarri *et al*., 2015). Besides environmental benefits, IMTA farms offer social benefits to producers which includes the ability to green label their harvested products which lowers public concerns over ecological and environmental impacts at the culture sites (Ridler *et al*, 2007 a and b; Barrington *et al.*, 2008).

Sea cucumber culture has been done in ponds where studies have shown that survival has been much higher (50-85%) in ponds than at sea (Bell *et al*., 2007; Agudo, 2012; Gamboa *et al*., 2012). Sea cucumbers feed on organic matter in pond bottoms and bioturbate the sediments Purcell (2004) extracting beneficial nutrients for growth. Integration of sea cucumber with other organisms like sea bass (*Lates calcarifer*) was successful while trials with milkfish (*Chanos chanos*) and silver pompano (*Trachinotus blochii*) showed promising results (Purcell *et al.,* 2012).

Integrated systems employing the IMTA concept have made use of net cages set in open ponds. For instance a study by Rejeki *et al*. (2016) used cages stocked with milkfish, tilapia and vanamae shrimp as fed organisms. The results showed that the cultivated organisms grew well with a specific growth rate of 7.9 % for shrimp and 6.8 % for milkfish suggesting that milkfish and shrimps can be cultivated together in an IMTA farming systems a finding that is also supported by studies conducted by Nurdin (2006) and Mangampa (2014). In a study by Sallih (2005) lines of racilaria seaweed and green mussle were located in the netting of abraded dykes on the brackish water ponds. In this IMTA set up the gracilaria curtailed blooming of harmful dinoflagellata plankton. Green mussels fed on phytoplankton, macrophytes and re-suspended detritus. IMTA in earthen ponds usually combines natural and pond aquaculture with carbon and oxygen transformation cycles depending on energy from the sun (Egna and Boyd, 1997). In integrated systems the source of energy is from both solar and external feeds fed to fish (Waite *et al.,* 2014). Cunha *et al*. (2019) studying fish, oyster and macro algae integrated production in ponds, found it a more improved way in comparison to the usual semi-intensive fish production system. The results indicated that water quality in the systems increased that led to better fish performance, higher biomass production, and reduction in daily energy consumption that resulted to higher profits.

Innovations in pond aquaculture have contributed to diversification where phytoplankton species have been cultured and monitored (Milhazes-Cunha and Otero, 2017). Tried systems involve a series of cascading ponds where effluent from fish ponds is passed through phytoplankton ponds and finally to ponds having filter feeders such as bivalves. Such IMTA systems have high nutrient removal efficiencies of greater than 90% (Shpigel *et al.* 1993).

2.3 A review of literature on experimental organisms tested in the present study

Chopin (2006) reported that co-cultured organisms in an IMTA should be more than just biofilters; in addition, they should also be harvestable species of commercial value. A working IMTA should result in greater productivity for the overall system, based on mutual benefits within the co-cultured species, and improved ecosystem health (Neori *et al.*, 2004). The IMTA concept has continued to be applied in different environments and using different approaches and also test organisms. The principles have thus continually evolved and more innovations being tried (Chopin, 2013; Jansen *et al.*, 2015; Fang *et al*., 2016; Noeri *et al*, 2019). Examples of organisms that have been cultured in IMTA systems elsewhere include; kelps, mussels, and salmon (Chopin *et al.*, 2012), seaweed and sea cucumbers (Beltran-Gutierrez *et al*., 2014), seahorse and seaweeds (Xu and Mu, 2017) shrimps, oysters and finfish (Balasubramanian *et al*., 2018) and many other species combinations. A shrimp and sea cucumber integration was tried in China (Zuo and Zhang, 2014). The nutrient rich feacal waste and uneaten feed provided good nutrition for the bottom feeding sea cucumbers (Zuo and Zhang, 2014). Studies by He *et al*. (2008) showed that high production, total revenue and profits were achieved from shrimp and sea cucumber integration.

Fish and sea cucumber integration has been practiced and found promising since some species have been found to feed and grow on debris from aquaculture farms (Zamora *et al*., 2018). *H.scabra* has shown potential as an IMTA species 'due to its capacity to prevent sediments from being anoxic as a result of high nutrient loads' (Lee *et al*., 2017). In IMTA systems algae play a vital role of autotrophic metaboliosm. 'Algae absorb and recycle waste nutrients from the feeding animals and from microbial metabolism a biological mechanism that reduces pond eutrophication from nutrient enrichment from culture water' (Xu *et al.,* 2011; Huang *et al*., 2013; Ge *et al.,* 2016). In addition macro algae are powerful biological purifiers for aquaculture effluents due to their high aborption efficiency of dissolved nitrogen compounds (de Oliveira *et al.,* 2016). Further, algae have shown potential to eliminate aquaculture pathogens (Natrah *et al*., 2014; Tsutsui *et al.,* 2015).

Bivalves are suspension feeders and through their feeding activity, they affect the plankton structure and thus enhancing sediment nutrient cycling through coupling of the benthic and pelagic organisms (Newell 2004; Smyth *et al*., 2013). Bivalves have been found to excrete nutrients into water column which supports phytoplankton production. Figure 2 shows the organisms that were used in IMTA trials in this study, and whose literature is reviewed in subsequent subsections.

Figure 2. Organisms used in IMTA trials in the current study; (A) Marine shrimps, (B) Cockles, (C) Nile tilapia, (D) Sea cucumbers, and (E) Oysters.

2.3.1 Marine shrimps *(Penaeus indicus* H. Milne Edwards 1837)

The development of shellfish aquaculture is vital for sustainable food supply and food security (FAO, 2020). Shrimp farming has been identified as the most valuable mariculture activity in the Western Indian Ocean (WIO) region contributing significantly to the total regional mariculture revenues (Hetch *et al*., 2019). Production in the Western

Indian Ocean region is dominated by *Penaeus monondon* mainly from Madagascar and small portions of *Penaeus indicus* and is managed by small community based mariculture groups in Kenya and Tanzania (Hetch *et al*., 2019). *Penaeus indicus* is also produced in small quantities in South East Asia. According to Primavera (1997) the shrimp farming venture has, without any doubt, been a major source of foreign exchange earnings for the developing countries through exports. In addition shrimp farming has created jobs for the coastal communities including wild seed collectors, processors, and those in the hotel industry (Hetch *et al*., 2019). The needs for investment in mariculture research for high value species like shrimps in order to ensure development of the sector for food security and income generation have been identified (Mirera, 2011). Production of shrimps in Kenya has been mainly done on a semi-intensive or extensive culture basis. The farming operations are dominated by Indian white shrimps (*Penaeus indicus*) mainly used in the food industry in processed or unprocessed form Black tiger shrimps (*Penaeus monodon*) is also available but in lesser proportions (Mirera, 2011). Many shrimp farms constitute of uneaten feeds, Sandifer and Hopkins (1996) that are normally released into the natural environment leading to eutrophication that gradually leads to toxicity in the body of aquatic organisms (Neori *et al.,* 2000; Troell *et al*., 1999). Consideration of waste water exchange and treatment of waste water as part of the whole shrimp culture system is important (Hopkins *et al*., 1995). Integration of seaweeds and shrimp culture has been tried for nutrient absorption (Neori *et al.,* 1991; Seema and Jayasankar, 2005). Studies by Troell *et al*. (2003) showed that macro algae have potential to transform occurring nutrients such as nitrogen and phosphorous into compounds necessary for their nourishment and improvement of water quality for cultivated organisms.

IMTA has been identified as a suitable approach to address environmental, social and implementation of good management practices related to aquaculture in China (Farquhar *et al*., 2017). The approach limits aquaculture nutrients and organic matter outputs through bio mitigation and has been applied globally (Neori *et al.*, 2004; Chopin *et al.*, 2008; Browdy *et al*., 2012; Samocha *et al*., 2015; Chang *et al.*, 2020). Shrimp based IMTA has been practiced in China with great success with currently being regarded as the direction to sustainable growth of marine pond aquaculture, the main advantages being; biosecurity and product safety, ecological efficiency, environmental friendly and economically viable (Tang, 2017). In shrimp pond IMTA systems nutrient waste from the shrimp is taken by the extractive organisms in the system such as plankton and filter feeders such as cockles, oysters, clams among others that are then eaten by other organisms (Samocha *et al.*, 2015; Chang *et al*., 2020). Prey organisms such as amphipods are known to naturally develop in shrimp IMTA ponds Han *et al.* (2012a) where they provide extra nourishment to the shrimps together with other crustaceans. In addition movement of organisms such as fish integrated in shrimp pond IMTA systems, contribute to stirring the sediment which enhances microbial metabolism and recycling of sediment trapped nutrients (Tian *et al.*, 1999). Therefore, properly cultured IMTA has potential of turning pollutant nutrients into commercial crops increasing farm profits (Tian *et al.*, 1999; Neori *et al*; 2004, Li, 2007; Han *et al.*, 2012b).

2.3.2 Nile Tilapia (*Oreochromis niloticus* Linnaeus 1758*)*

Tilapiine are among the most popular and important warm water fishes farmed globally (Charo-Karisa *et al.*, 2006). Nile tilapia (*Oreochromis niloticus*) has for many years been farmed in freshwater environment in several parts of Kenya contributing up to 80% of aquaculture production (Mbugua, 2008; Opiyo *et al*., 2018). Production of Nile tilapia entails use of different culture technologies such as ponds, raceways, integrated aquaculture, recirculating aquaculture systems, cages, finger ponds among others (Denny *et al*., 2006, Ngugi *et al.*, 2007; Shoko *et al* 2011).

However, shortage of freshwater in many countries and competition with agriculture and other urban activities has triggered the desire to continue diversifying aquaculture in marine environment. Nile tilapia has proved to be an excellent candidate for brackish water aquaculture due to its ability to tolerate wide salinity ranges (Al-harbit and Uddin, 2005). Studies have shown that tilapiine are less euryhaline with ability to tolerate salinities ranging from 20 to 30 ppt, with most able to grow, and reproduce at 0-29 ppt depending on species (Abdel-fattah, 2006, Tseng and Hwang, 2008). Nile tilapia is a high value fish with ready consumer acceptability and market demand. In the marine environment Nile tilapia has been introduced and previous research through pond culture by Mirera *et al.* (2019) in the intertidal ponds. Tilapia has advantages of being easily bred, it has efficient conversion for low protein diets, and has shown resistance to handling stress and diseases (Liti *et al*., 2005; El-Greisy and El-Gamal, 2012). Nile tilapia was picked for trials in IMTA for this study after preliminary studies at community level by Magondu *et al*. (2019) showed that the species could acclimatize and perform well in marine water in cages. Development towards culture of marine species like milkfish, rabbit fish, Nile tilapia and shrimps requires first, the identification of quality seed either from wild or from marine hatcheries.

In pond systems cultured tilapia fishes have been observed to recycle nutrients through consumption of live and dead microalgae while providing the existing live algae with inorganic carbon required for their growth (Gilles *et al*., 2013). Tilapia has also been reported to recycle nutrients in form of faecal matter or uneaten feed through excretion promoting phytoplankton production (Elser *et al*., 1990). Nile tilapia are generally column feeders, Turker *et al*. (2003) showed that fish grazing cannot be able to control phytoplankton explosion resulting from nutrients from feed and fish waste. Further studies by Kucuk *et al*. (2013) reported that fish in marine or freshwater environments use energy to hold ions in or out of their bodies through osmoregulation which makes salinity a vital water quality parameter for fish growth.

Water quality monitoring in the culture environment is therefore of necessity and is normally done by use of mechanical and biological filters, periodic water exchange and technologies like biofloc where microbial development in the culture water environment of fish ponds is stimulated through addition of carbohydrates such as molasses, tapioca and maize floor (Avnimelech, 2012; Magondu *et al*., 2015; Luo *et al*., 2017). Nile tilapia has also shown good results through integrated culture in brackish water (Gilles *et al*., 2013). 'IMTA has been used as a management strategy for improving yield per unit area of production by rearing organisms possessing complementary ecosystem functions' (Chopin *et al*., 2012). IMTA improves interaction between culture and the natural environment thereby promoting biosecurity from that of monoculture (Pietrak *et al*., 2012; Martinez-Espineira *et al*., 2015). Examples of IMTA studies that have used Nile tilapia as a target fed species include works by (Trenasti *et al*., 2019; David *et al*., 2021).

2.3.3 Cockles *(Anadara antiquata* Linnaeus 1758)

Cockles have been described as burrowing bivalves with a common occurrence in sandy and muddy intertidal zones of the Western Indian Ocean (Richmond, 2002). Cockles have the potential of breaking down organic matter in the sediment and filtering particulate matter and benthic algae from the water for their sustenance (Pronker *et al*., 2015). Cockles are consumed because of their taste and nutritional benefits with their meat being rich in vitamins, minerals, omega 3 and low in saturated fats (Murray, 2011; Heid, 2018). European countries such as UK, Spain, Portugal, The Netherlands and Denmark have a rich history of harvesting cockles as shown by Montgomery *et al*, (2013) with production ranging between 14,000 to 26,000 tonnes for the years 2014 to 2017 (Carss *et al.*, 2020). Cockles build their own bodies mainly the shell and body flesh; while degrading suspended organic particles like uneaten feed, feaces, and plankton, which they assimilate from the water (Ren *et al*., 2014). In bivalves, measurements of shell linear increment determines growth which is then converted to somatic growth (Siahainenia *et al.*, 2018), a conversion normally done using the relationship of tissue weight and shell size (Hughes, 1986). Several studies on *A. antiquata* have shown allometric growth Selanno (2006) and Khow (2009) with shell width and shell height being associated with the shell length (Siahainenia *et al*., 2018). Shell production from bivalves plays a key role in the carbon cycle through carbon fixation and has a contribution to mitigation of climate change thorough carbon sequestration (Peterson and Lipcius, 2003; Hickey, 2009).

Shellfishes have been shown to be an important component of coastal biodiversity by playing important roles in delivering ecosystem services such as food production and bio mitigation of the aquatic system (Smaal *et al.*, 2019; van der Schatte Oliver *et al*., 2018). Nonfood ecosystem services provided by shellfishes include use of collected shells for ornaments and construction (Kelly, 2009; Morris *et al*., 2018). 'Cockles have been classified as surface bio diffusers attributed to their function of sediment reworking and bio-irrigation within the surface of the sediment column' (Norkko and Shumway, 2011; Kristensen *et al*., 2012). Cockles burrow and move displacing water and solute exchanges across the sediment water interface which induces a continuous mixing of particulate matter enhancing sediment stability (Mermillod-Blondin *et al*., 2005; Anderson *et al*., 2010). Studies by Tolhurst *et a*l. (2002) and Meadows *et al.* (2012) showed that cockles stimulate the secretion of exopolymeric substances that create bonds in between particles, a process that enhances cohesion and sediment stability. Cockles are thus important and effective in promoting primary productivity of soft bottomed intertidal ecosystems and structure of macrobenthic community (Donadi *et al.,* 2015; Magondu *et al.,* 2022).

Bivalves, have different ecosystem functions which include serving as an important source of nutrients for many organisms, providing shelter and creating space for other associated organisms thus enhancing species diversity (Sukhdhane *et al*., 2018). Jacobsen and Esherick (2007) noted that cockles have primarily been collected by women along the East African Coast and continue to be an important food source as well as a commodity with an attached commercial value. The culture of cockles could be a potential opportunity for contributing to income and food security as well as the creation of structural diversity within their habitat ecosystems (Jacobsen and Esherick, 2007). In addition using detritivores has proved a novel option for pond-based IMTA (Cunha *et al.,* 2019). 'Cockles and sea cucumbers do assimilate the waste into their bodies which saves on water treatment costs while adding on valuable products that do not require purchased feeds for their culture' (Shipgel, 2013).

2.3.4 Sea cucumbers *(Holothuria scabra* Jaeger, 1833*)*

Sea cucumbers *(Holothuria Scabra)*, a species of commercial value, has shown potential for integration in culture with an aim of increasing production, economic returns per production unit and decreasing the much fishing pressure being applied to the natural stocks (Conand and Muthiga, 2007; Purcell *et al*., 2018). Sea cucumber culture trials have been previously done in different environments such as tanks, open sea cages and pens, and ponds (Purcell *et al.,* 2012; Trenasti *et al*., 2019; Cutajar *et al*., 2022). Survival of *H. scabra* juveniles to adult stage has been observed to be much higher in pond environment than in the sea at about over 50 % (Duy, 2012; Gamboa *et al.*, 2012). Among other commercial sea cucumber species, *H. scabra* has been naturally adapted to silty habitats and hence responsive to pond conditions where they consume organic waste matter and bioturbate pond sediments (Purcell, 2004; Raison, 2008). Battaglene *et al*. (1999) indicated that 'co-culture of sea cucumbers with other organisms is more attractive over monoculture' an argument that was supported by Purcell *et al.* (2012) giving options of co-culture with fish, shellfish and algae.

Sea cucumbers are deposit feeders and thus excellent in the recycling of nutrients arising as a result of co-culture and in natural benthic ecosystems (Tian *et al*., 1999; Uthicke, 2001). Intensive marine aquaculture has been blamed for loss of benthic diversity, pollution and eutrophication due to waste deposition (Kalantzi and Karakassis, 2006; Tomassetti *et al*., 2016). A well designed IMTA with appropriate species combinations can reduce the excess accumulation of organic nutrients in sediments (Cubillo *et al*., 2016). Further, a working IMTA should turn pollutant nutrients into beneficial aquaculture products significantly contribute to farms' profitability (Tian *et al*., 1999; Troell *et al*., 2003; Neori *et al*., 2004). Sea cucumbers have the ability to stir and reduce sediment organic contents being added by other aquaculture species through bacterial action (Feng *et al*., 2014). Besides waste bio mitigation potential of sea cucumbers, their use in IMTA is also due to their economic value (Toral-Granda *et al*., 2008). In addition, sea cucumbers bioturbate pond sediments and utilize the available particulate organic matter from the culture environment Purcell (2004) thereby contributing to the health of the environment. The benefits of IMTA systems have been shown through reduced nutrient and waste output, lower feeding requirements, higher yields per unit area of production, more efficient resource use, and synergy (Lin *et al*., 1992 and Hu *et al*., 1995).

Studies have shown that fishery stocks of high value sea cucumbers have been fully exploited in some hot spot areas that regulatory measures and enforcement cannot achieve much in restoring them back to their natural state (Pakoa *et al*., 2009; Purcell *et al*., 2009; Friedman *et al*., 2011). *Holothuria scabra* is the most valuable and has been sold in Hong Kong at prices ranging from USD 115-640 Kg^{-1} dried (Purcell *et al*., 2012). Different options for farming tropical sea cucumbers, in co-culture with fish, shellfish, or algae have been recommended (Purcell *et al*., 2012). 'Integration of commercially valuable sea cucumbers into existing aquaculture can increase economic yields and reduce environmental impacts' Beltran-Gutierrez (2014). Other examples of *Holothurian spp* in the Mediterranean region in particular *Holothuria pori* and *Holothuria tubulosa* have been exploited for their commercial value. Currently they are being considered as potential candidates for mariculture (Gonzalez-Wanguemert *et al*., 2018; Rakaj *et al*., 2019). Studies have shown that sea cucumbers are able to show good growth responses and survival while being fed on organic waste from fish and shrimp culture (MacDonald *et al*., 2013; Yokoyama, 2013; Cutajar *et al.*, 2022)

2.3.5 Mangrove oysters (*Saccostrea cucullata* Born 1778)

Oysters are filter feeders that are known to feed mainly on plankton Cunha *et al*. (2019), bacteria and microalgae Moore, (1982), as well as diatoms (Coutteau and Sorgeloos, 1992; Dupuy *et al*., 2000). *Saccostrea cucullata* is an edible oyster found in the upper littoral zone of the Indian Ocean, Eastern Atlantic, and the Mediterranean (Poutiers, 1998). It attaches on trunks and stilt roots of mangrove plants and rocky substrata in brackish marine environment. Oysters are classified among bivalve mollusks that are cultivated. Estimation of growth in oysters involves measuring the shell dimensions and volume of the animal according to (Deval, 2001). During the lifecycle of oysters they have competitors, predators, and environmental stressors which are the major causes of mortality. Recruitment however involves steps such as reproduction, larval dispersal, settlement and survival of spat (Tenjing, 2020). Interaction of temperature with other environmental attributes like salinity and food availability usually defines the growth and age of *S. cucullata* (Tenjing, 2020).

Studies have shown that incorporation of filter feeders like oysters (*Crassostrea cutackensis*) in an IMTA resulted to increased income, higher productivity with good feed conversion rates, improved biosecurity and better water quality as compared to conventional polyculture (Waite *et al*., 2014; Cunha *et al*., 2019). In other studies, oysters have been used to filter feed suspended particulate matter from culture environment, Pacific oyster, *Crassostrea gigas* was observed to reduce 56% waste content from fish-farm that had effluent comprised mainly of feacal waste from European bass (*Dicentrarchus labrax*). 'Filter feeding oyster, *Crassostrea cuttackensis* suspended in IMTA ponds efficiently utilized particulate organic matter which reduced inorganic nutrient content significantly' (Biswas *et al*. 2020). In this study *S. cucullata* was used as a filter feeder for uptake of suspended particles from the culture water.

Oysters contribute to both ecological and economic impacts. They are considered a high value sea food with many nutritional benefits in form of vitamins, minerals, and organic compounds (Joaquim *et al*., 2011; Noel *et al*., 2012). In addition, in the natural environment, oysters grow in dense mats that impact on the surrounding ecosystem with their ability to change areas of soft substrate like mud and silt into hard substrate hence more stable (Ruesink *et al*., 2005, 2006). Due to their ability to filter feed, they consume plankton and organic matter hence improving the clarity of water as observed by Cunha *et al*. (2019).

2.3.6 Planktonic algae

Phytoplankton are microscopic organisms (microalgae) often characterized by a drifting lifestyle as opposed to macro-algae or seaweeds which are normally attached to substratum. Macro and micro algae in culture systems play a key function of purifying the water thus generating the oxygen required by the farmed fish (Neori *et al*., 2004; Hargreaves, 2006).

In the ecosystem algae function by making use of sunlight to build their biomass while assimilating dissolved inorganic substances in form of nitrogen and phosphorus extracted from the water (Neori *et al*. 2004; Qiao *et al*., 2020). Through this process pond eutrophication and nutrient enrichment is controlled thus providing suitable water quality for the organism under culture (Burford *et al*., 2002; Abreu *et al*., 2011). They have the ability to respond to available anthropogenic nutrients such as nitrogen and phosphorus making them efficient instruments for bioremediation (Sukhdhane *et al*., 2018). Marine plants have internal mechanisms that assist in transforming the available nutrients such as nitrogen and phosphorous into composites necessary for their growth and development. Consequently, the water quality is improved and nutrients in the environment also reduced (Troell *et al*., 2003). In an ideal IMTA system, the biological and chemical processes should balance (Troell *et al*., 2009) which is brought about by the action of the culture species combinations in place.

2.3.7 Review of literature on economic analysis in IMTA systems

Sustainable aquaculture requires the employed practices to be environmentally friendly and also be economically feasible. In IMTA social acceptability to human mind is key for consumers to be willing to purchase the farmed products according to (Gempesaw *et al*., 1995). On the other hand the level of profit depends on the inputs that the farmer contributes to yield output. Maximum profit is achieved when each input is fully utilized (Henderson and Quandt, 1980). A review by Knowler *et al*. (2020) stated that 'there are a few studies that have undertaken complete analysis of an IMTA production system while taking into consideration the external costs and benefits' (Chopin *et al*., 2001; Rose *et al*., 2014; Kambey and Chung, 2016). In these studies valuation of waste removal in form of nitrogen by a mix of potential land-based IMTA systems while considering a different species such as abalone, finfish, sea cucumbers, and seaweeds was done.

In order to assess the net income based on estimated returns on sales and costs, different setups have been investigated. Goada *et al.* (2015) researched on economic potential aquaponics systems in Egypt using integrated recirculating aquaculture and hydroponics to grow finfish, crustaceans, molluscs and greenhouse vegetables. In another study, Bunting and Shpigel (2009) assessed the financial potential of adopting 'horizontally integrated land-based marine aquaculture' by making use of bio-economic modelling approach. Knowler *et al*. (2020) showed that economics of IMTA has focused on three areas: economics of the environment, financial analysis addressing profitability and market analysis looking at consumer perceptions on acceptability of IMTA products. In competitive markets prices of products have been determined by interaction between supply and demand (Shang, 1981; Jolly and Clonts 1993). Therefore, when determining the price of aquaculture products, farmers need to cover the costs of production, aim to generate profit and set a price that consumers are willing to pay (Musa *et al*., 2021). Aquaculture enterprises have used cost benefit ratio analysis widely for rational and systematic decision making as shown by Molinos-Senante *et al.* (2010). The concept has also been used to assess environmental impacts of projects on economic perspective and effects spreading over time (Zheng *et al*., 2009). Ecological-economic assessment of aquaculture can therefore be assessed using cost benefit analysis (Nobre *et al*., 2010).

From the foregoing review, it is clear that the potential of IMTA can be realized by satisfying key stakeholders especially the farmers and environmentalists and realized benefits up-scaled to commercial level (Alexander *et al*., 2016). Some of the potential benefits include; increased economic value of farm operations through product diversification, economic growth through direct or indirect employment in farm operations, product processing, or distribution, improved ecosystem health where waste nutrients are converted to valuable resource and reused by the cultured organisms reducing cost of feeding and increasing the overall profits.

In this study, an IMTA system that involves culture species that belong to different trophic levels in the aquatic food chain was applied. Micro algae acted as bio-filters by utilizing inorganic by-products from the cultured shrimps and finfish. Cockles and oysters filtered the suspended organic particulates while the sea cucumber fed on the deposited organic substrates. Suitability of the species combinations to be used in an IMTA setup has to be considered to achieve economic value from the farm. Examples of species combinations that have been tried elsewhere include; fish/seaweed/shellfish, fish/shrimp, seaweed/shrimp (Troell *et al*., 2003). The current study applied the IMTA system with the ultimate twin goals of (i) diversifying the cultured species and (ii) enhancing aquaculture production and profitability from the earthen ponds of small scale farmers of the Umoja Self-help Group at Kibokoni village along the Kilifi creek.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted in earthen ponds at Kibokoni, a coastal village along the Kilifi creek, and about 10 Km West of Kilifi town (Figure 3). The ponds (Figure 4 and 5) were constructed by Umoja self-help mariculture group and were hired for use during the study. The study site is located at Longitude 039° 50′ 32′′ E and Latitude 03° 36′ 12′′ S. The Umoja self-help group has a total of 17 ponds; (9) production ponds and (8) nursey ponds. The ponds were constructed through support from different projects and agencies like National Council for Science and Technology (NCST), Kenya Coastal Development Project (KCDP), Pwani University and Kenya Marine and Fisheries Research Institute (KMFRI). The big ponds are mainly used for production and do measure 1200 m^2 (30M x 40M) while the small ponds used for research measure 120 m^2 (10M x 12M). The organisms cultured by the group include Nile tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*), marine shrimps (*Peaneaus indicus* and *Penaeus monondon),* and sometimes mud crab (*Scylla serrata*) fattening. The spinefootrabbit fish (*Siganus sutor*) is on culture trials. The production history indicates increase over time with a production of 100 kg from four ponds during the experimental phase which translated to Ksh. 15,000 at Ksh.120 per kg of fish and Ksh. 400 per kg of prawns. Following several different interventions overtime subsequent harvests have provided an average of Ksh. 20,000/pond per production cycle of six months (Mwaluma et al., 2014). Market for the produce is mainly at the farm gate with excess being channeled to markets and hotels in Kilifi and Malindi. Figure 4 shows a layout of the ponds at the study site and Figure 5 shows a section of the ponds used in the present study.

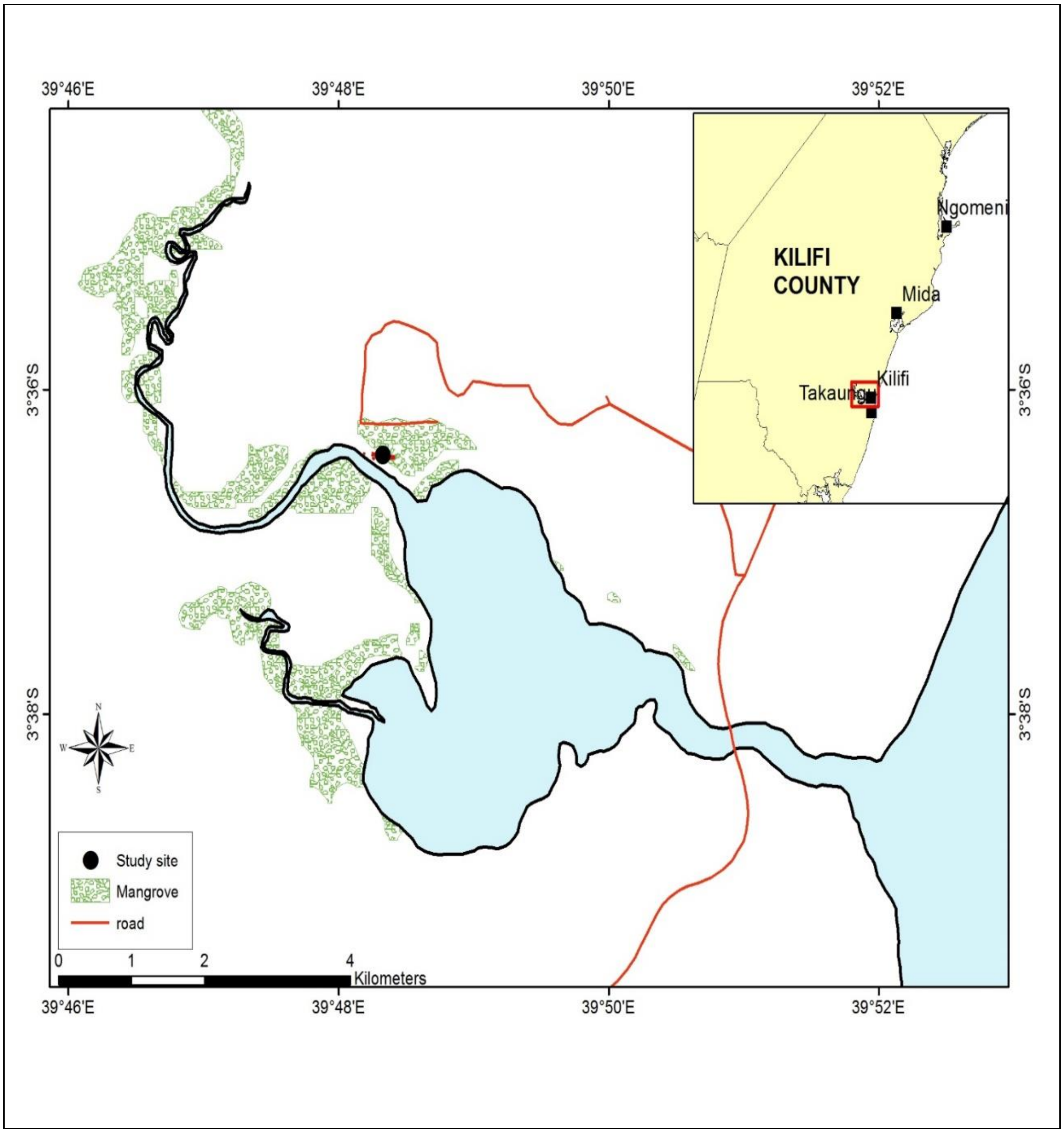

Figure 3. Site location for Umoja self-help group in Kibokoni, Kilifi County

Figure 4. Layout of the ponds at Kibokoni; (a) production ponds and (b) research ponds

Figure 5. Experimental ponds being used for the IMTA study (a) farmers assisting in putting the predator control net and (b) stocking of cucumbers into the ponds,

3.2 Experimental design and sampling

3.2.1 Experimental design

In this study, IMTA was applied for sustainable and improved production of marine shrimps (*Penaeus indicus*), in co-culture with cockle (*Anadara antiquata*) and sea cucumber *(Holothuria scabra*) for the first trial. The second trial entailed use of Nile tilapia (*Oreochromis niloticus)* in co-culture with oysters *(Saccostrea cucullata)* and sea cucumber (*Holothuria scabra).* The study was designed to allow for species diversification to increase profit in addition to what the farmers have been relying on. Currently the farmers are raising marine shrimp and Nile tilapia monoculture fed and harvested after four and six months respectively. The design of the integrated system entailed sand treatment of the pond bottom to provide the experimental organisms mainly sea cucumbers and cockles with an environment that simulate their natural habitat while ensuring maximum exposure to organic and inorganic waste from the culture system. The synergy between the species ensured environmentally sound utilization of the pond improved productivity and profitability.

Six ponds of 120 m^2 each were used for the **first trial** that involved testing of two culture systems which formed the treatments namely IMTA treatment and marine shrimp monoculture treatment. Before the experiment acclimatization of the test organisms was done to provide for adaptability into the culture environment. The IMTA treatment consisted of the three different study organisms cultured in replicate ponds at different densities. The other treatment was a marine shrimp monoculture set up which acted as a control experiment and was also conducted in replicate ponds stocked with *P. indicus*. Each treatment had three randomly assigned replicate ponds. Of the study species in the IMTA treatment, *P. indicus* was the fed species whereas A. *antiquata,* and *H. scabra* were the extractive organisms in the system. Under the **second trial** the design had a monoculture of marine water tilapia referred to as control (C) and three IMTA treatment models with different species combinations; T1 had tilapia and sea cucumbers, T2 had tilapia, sea cucumbers, oysters, and T3 had tilapia and oysters. Each treatment had replicates.

3.2.2 Sampling

Growth data of all organisms were collected after every 25 days throughout the experimental period from a random sample of at least 10% of the stocked quantity so as to monitor feed response by the fed organisms and efficiency of the system to the extractive organisms. Organisms were measured for total length (cm), and weighed (body weight (g)) for computation of growth in length and weight gain; and counted for computation of percent survival (%). Length and weight measurements of oyster juveniles under culture in second trial were monitored on monthly basis.

At monthly intervals, in situ measurement of water quality parameters were taken and pond water samples were collected for laboratory analysis of nutrients. Standard methods by APHA (2005) were applied during analysis. The content of particulate organic matter in the water was also determined monthly using protocols by Irisarri *et al*. (2013). Water samples for phytoplankton and zooplankton analysis were collected fortnightly by pooling 20 L samples of water for phytoplankton analysis and 1000 L for zooplankton analysis from randomly selected locations in each pond. The concentrated samples were then passed through a 20-µm net for phytoplankton collection and 200-µm net for zooplankton collection. Each concentrated phytoplankton sample was shifted to a 250 ml measuring bottle and fixed with 0.5ml of acidified lugols solution. Concentrated zooplankton samples were diluted to 100 ml with formalin and distilled water to obtain a 5% buffered formalin solution. Qualitative and quantitative estimations of phytoplankton and zooplankton numbers was carried out using a Sedgewick–Rafter (S–R) cell (Asaduzzaman *et al*., 2010). Identification keys were later used to identify the organisms up to genus level (Karlson *et al*., 2010).

Sediment samples were collected on monthly basis from three locations of each pond using a corer of 6 cm diameter and 10 cm in sampling depth. Samples were dried, ground and sieved with a 2 mm sieve, soil pH was determined by directly reading the pH meter with a soil water ratio of 1:2.5 (Mclean, 1982). Organic matter of sediment was determined by ignition method (Page *et al*., 1989). Total nitrogen of sediment was determined by micro-kjedahl digestion method following Page *et al*. (1989). Total phosphorus of sediment samples was determined by acid digestion method (Jones and Case, 1990; Watson and Isaac, 1990).

Benthic macroinvertebrates were determined by collecting sediment samples from three different locations using an Ekman grab, which were later combined into a composite sample. Benthic macro-invertebrates were collected after sieving sediments through a 250 µm mesh sieve and stored in vials containing 10% buffered formalin. Identification keys from Richmond (2002) were used to identify macro-invertebrates.

3.2.3 Assessment of profitability in IMTA in comparison with Monoculture system

This study explored the effects of marine shrimp and tilapia integration with micro algae, filter feeders and the deposit feeders to growth, water quality, and sediment quality of the entire system so as to increase income and promote ecosystem sustainability. In addition, the potential profitability of the introduced IMTA system was assessed in comparison to the existing marine shrimp monoculture systems by evaluating the production status of the two culture systems.

In order to assess the profitability status of the IMTA and monoculture pracises, an economic analysis was carried out based on the developed IMTA system in comparison to the shrimp monoculture system in Trial 1 and IMTA combinations in Trial 2. Project costs comprised of material input cost including the cost of the juveniles of all the test organisms, feeds administered, labour, fertilizers, lime, netting material, hire of experimental ponds and any other operational costs like purchase of tools for pond maintenance, payment of security hire and pond attendant. The cost of production was estimated based on local market value price in Ksh and the current United States Dollar (USD) equivalence (1 USD =Ksh 110 in 2020 and 1 USD =Ksh 105 in 2021) being the period under which the two trials were carried out. Income was based on the difference between benefits achieved from the sale of the produce and the costs incurred.

3.2.4 Assessment of environmental and economic benefits in IMTA and pond systems

In order to draw justifiable conclusions on the application of the IMTA system, an analysis of the environmental and economic benefits from the overall productivity of the applied farming systems was done.

As production from aquaculture increases, demand of inputs such feeds, fertilizers, and antibiotics to treat and control occurrence of diseases goes high. Studies have shown that approximately 80% of nitrogen and phosphorus from these inputs is accumulated in pond sediment with the remainder being removed by the cultured organisms (Avnimelech, 1998). Wastes in form of feacal material, uneaten feeds and inorganic compounds of nitrogen and phosphorous have resulted into environmental degradation of water bodies (Haque *et al*., 2016). In this study, environmental benefits will be assessed by studying the water quality of the culture environment in both IMTA and monoculture, looking at both the nutrients in form of phosphates, ammonia, nitrates and nitrites and the insitu parameters such as dissolved oxygen, temperature, pH, salinity, and transparency. Studies on the sediment quality mainly the total organic carbon, alkalinity, presence of nitrogenous substances and phosphates were studied and their relation to the overlying water with influx and exchanges taking place.

Sustainability of the environment is a major consideration in IMTA practices as it guides on species selection and combinations for suitability in the culture units. Application of IMTA has demonstrated processes like bioremediation that minimize environmental impacts whereby extractive organisms take up excess organic and inorganic wastes from the system (Ridler *et al*., 2007).

3.2.5 Ethics Statement

Before undertaking this study, the proposal was subjected to review and approval by the Ethics Review Committee of Pwani University and a research permit was granted by the Kenya Wildlife Service which allowed collection of proposed organisms; specifically *H. scabra* which has been listed in the Convention on International Trade on Endangered Species (CITES) list of endangered species. The permit was granted under reference number KWS/BRP/5001 (Appendix 4). Further, a research license from the National Commission for Science, Technology, and Innovation was applied for and granted under License No. NACOSTI/P/21/8321 (Appendix 5). Copies of these documents are attached to the appendices of this thesis. All the culture organisms were humanly handled by trained persons during stocking, sampling and at harvest.

CHAPTER FOUR

INTEGRATING SEA CUCUMBERS AND COCKLES INTO MARINE SHRIMP EARTHEN PONDS

Abstract

This study presents the first trial application of an integrated multi-trophic aquaculture (IMTA) system in pond culture in Kenya using a combination of locally available species. Sea cucumber, *Holothuria scabra*, and cockles, *Anadara antiquata* were obtained from the wild to be used for culture trials with Indian white shrimp, *Penaeus indicus* in an IMTA set up for comparison with monoculture of Indian white shrimps in intertidal earthen ponds. The monoculture treatment (T1) ponds were stocked with *P. indicus* juveniles at a stocking density of (5 ind/m^2 ; 12.9 g/m^2) while the IMTA treatment (T2) had a combination of *H. scabra*, *P. Indicus* and *A. antiquata* stocked at (1.2 ind./m^2 ; 105.78 g/m^2, 5 ind./m^2; 12.9 g/m^2 and 3.5 ind./m^2 ; 142.48 g/m^2) respectively. During the culture period of 135 days, the harvest weight gain (mean ±SE) for Indian white shrimps in T1 was 13.17 ± 0.75 g while the organisms in T2 combination had a weight gain of 13.19 ± 0.57 g for Indian white shrimps, 175.03 ± 27.84 g for sea cucumber and 44 ± 0.97 g for cockles was achieved. Analysis of plankton samples collected from the two treatments revealed occurrence of 5 phytoplankton classes in both treatments and 10 and 11 zooplankton classes for IMTA and monoculture respectively. Bacillariophyceae was the dominating class consisting of 13.04 ± 20.62 cells/m ^{3} in T2 and 6.57 ± 17.84 cells/m ^{3} in T1. Among the zooplankton, class Maxillopoda had the highest abundance of zooplanktonic organisms at 930 ind./m ^{3} for T2 and 390 ind./m ^{3} for T1. The Naididae family was the most dominant among benthic macroinvertebrates having an abundance of 8.9 ind./m^3 for T2 and 3.8 ind./m^3 for T1. Sediment quality evaluated the environmental conditions of both systems where pH values determined were 7.57 ± 0.13 for T1 and 6.6

± 0.13 for T2. There were lower phosphorous (p) levels of 0.38 ± 0.03 mg l^{-1} in T2 as compared to 0.71 ± 0.03 mg l^{-1} in T1. Total organic carbon and total nitrogen also showed a similar trend with IMTA treatment having lower levels as compared to monoculture treatment. This results indicated that IMTA may lead to better utilization of pond communities and further improve pond productivity. The findings of this study further provide a basis for integration of *H. scabra* and *A. antiquata* into Kenya's coastal mariculture through application of pond IMTA technology'.

4.1 Introduction

The exploitation of the sea cucumber and cockles in capture fisheries has raised worldwide conservation concerns (Purcell *et al.*, 2012). The heavy exploitation of these species has been related to the high prices they fetch in the international market and increasing demand. Although these organisms are cultured in other parts of the world, available culture techniques have not been introduced in Kenya. This study reports results of culture trials of these organisms in existing earthen pond culture facilities using integrated multi-trophic aquaculture (IMTA) culture technology for income diversification, increase in production and improvement of livelihoods of the farmers depending on marine aquaculture enterprises. The study was designed to evaluate the potential of introducing organisms feeding at different trophic levels in the earthen pond culture system. The IMTA set up used deposit feeding sea cucumbers and filter feeding cockles as extractive organisms while marine shrimp formed the fed component of the system.

The trial used the sandfish *Holothuria scabra* Jaeger 1883, the most commercially valuable sea cucumber species commonly found in the tropic and sub-tropic countries (Conand and Muthiga, 2007). Depletion of wild stock and increased demand for the species triggered interest to develop culture techniques for *H. scabra* aimed at meeting the increasing demand, and reducing fishing pressure on the wild stocks while providing alternative sources of income in different countries across the globe (Kumara and Dissanayake, 2017). Some countries like Vietnam, Madagascar, Tanzania and the Philippines have initiated restocking, sea ranching and successful sea cucumber and shrimp integration (Purcell *et al.*, 2012; Eriksson *et al.*, 2014; Watanabe *et al*., 2014; Kunzmann *et al*., 2018; Senff *et al.*, 2020) for conservation purposes

The increasing demand for seafood products and search for alternative livelihoods for the coastal communities led to promotion of integrated aquaculture (Troell, 2009; Chopin *et al*, 2012; Chang *et al*, 2020) and subsequently focus shifted to modern integrated aquaculture approaches such as IMTA. The IMTA approach is widely used in developed countries like Japan, China and South Korea where sea cucumber *Apostichopus japonicas* has shown great potential for integration with other organisms such as oysters and shrimps (Chopin *et al*., 2001; Neori *et al*., 2007; Chopin *et al.*, 2008; Yokoyama, 2013; Yu *et al.*, 2014; Zamora *et al*., 2018). As a culture system, IMTA has been defined as the farming in proximity of aquaculture species of different trophic levels and with complementary ecosystem functions (Chopin *et al*, 2001; Neori *et al*., 2004). The main advantage of the IMTA approach is that several species are cultured together in a way that allows one species uneaten feed, nutrients and wastes to be recaptured and converted into fertilizer, feed, and energy for the co-cultured organisms; and to take advantage of the synergistic interactions between the cultured species as they feed at different trophic levels (Chopin *et al*, 2001; Neori *et al*., 2004). Alexander et *al*. (2016) and Sara *et al.* (2021) recognized IMTA for increased long-term sustainability and profitability per cultivation unit and not per species in isolation as is the case in monoculture system. Thus adoption of novel culture technologies like the IMTA offers opportunities for increased production and economic gains as compared to existing monoculture practices being conducted in most pond systems. In the present study, cockle *Anadara antiquata*, an edible bivalve species that feeds through filter feedings and sea cucumber *Holothuria scabra* which scavenges for organic detritus in the sediment, were introduced to utilize the waste from the co-cultured organisms. This was largely informed by previous reports of successful integrations of sea cucumber with shrimps (Purcell *et al*., 2006), and sea cucumber with mollusks (Slater and Carton, 2007) in countries such as Tanzania and Madagascar.

Although integrated culture in earthen ponds is widely practiced in various parts of Kenya, (Ogello and Opiyo, 2011, Ogello *et al*. 2013) there has so far been no research effort on IMTA to assess its potential and economic viability in coastal mariculture systems that would boost the development of the Blue Economy. Aquaculture production and species diversification in Kenya has been promoted through among other technologies the adoption of integrated aquaculture where output from one agriculture system is used as in input for another subsystem (Ngugi *et al*., 2007). According to Ngugi *et al.* (2007) ‘Integrated aquaculture is majorly practiced in freshwater paddies and ponds for the production of fish-rice, fish–livestock, and fish-vegetable combinations’. In the marine aquaculture sector, milkfish-shrimp, milkfish-mullet combinations under pond polyculture systems have been tried in the intertidal areas (Mirera, 2011) where promising results were reported with milkfish growth rate being significantly different between the wet (0.52±0.18 g/day) and dry (1.21±1.0 g/day) seasons ($P < 0.05$), as was that of mullet (0.15±0.04 and 0.29±0.15 g/day, respectively; $P < 0.05$). Wanjiru (2008) did a study on integrated aquaculture of marine shrimps and milkfish in mangrove areas which showed high potential for marine shrimp farming. Marine aquaculture production estimates in Kenya are over 100 Mt/year of finfish, shellfish, and seaweeds all produced at a small-scale level with more than 90% of the farms being managed by organized community groups (Munguti *et al*., 2017). The main contributing factors for this are; overfishing of local populations of the culture species, limited farming space, and pollution from aquaculture. The aim of the present study was therefore to assess the potential of integration of sea cucumber and cockles with marine shrimps raised in intertidal earthen ponds based on data on growth, survival, water quality, and economic performance. The trials recruited sea cucumber and cockles from the wild which usually has a disadvantage of being scarce and seasonal in addition to other critical aspects such as seed quality, uniformity, and low survival rates. The Juveniles were grown under Kenya's coastal mariculture thereby diversifying culture species and income streams from small scale earthen pond shrimp farms.

4.2 Materials and methods

4.2.1 Experimental design

The study used a complete randomized experimental design with two treatments each in triplicate and assigned randomly among six (10 x 12 m) ponds of 1m depth. The study compared shrimps raised in monoculture system as practiced by Umoja Self Help Group with those raised as a fed component in an IMTA system being tried for the first time in Kenya. The trial was conducted over a period of 135 days, which is the usual period of a culture cycle of shrimps (Damodaran *et al*., 2019). The IMTA system co-cultured sea cucumber and cockles as an extractive component.

4.2.2 Preparation of experimental ponds

Experimental ponds were drained off completely and renovated by raising and compacting the dykes. A 0.5 m high mesh netting was also put around the ponds to prevent mud crabs from the channels from crawling into the ponds and also to prevent loss of experimental organisms during occurrence of extreme tides. Before starting the experiment the ponds were left to sun dry for a period of one week so as to eradicate of all unwanted fishes and other organisms. Lime, ($CaCO_3$) was applied to all the ponds at the rate of 300 g/m^2 and allowed to dry for 2 weeks (Ngugi *et al*., 2007). To create a muddy sandy substrate in IMTA treatment, sand was collected from the surrounding open area next to the culture ponds, community members were mobilized to collect the sand and ferry into the culture ponds using buckets. A layer of sand of approximately 1 cm was spread at the bottom and on the lower section of the dyke slopes near the bottom to cater for the crawling sea cucumbers and cockles which prefer such conditions in nature (Watanabe *et al*., 2012; Soisson *et al*., 2019). Ponds were then filled with water from a nearby channel during high tide. All the ponds were then fertilized with recommended amounts of urea (3 g/m^2) and diammonium phosphate (2 g/m^2). After fertilization, the ponds were left for 10 days to allow for growth of natural food in the water column. The water level in all experimental ponds was topped up and maintained at a depth of 1 m over the study period.

4.2.3 Collection and transportation of study organisms

All the organisms used in the experiment were collected from the wild. A total of 600 juveniles of sea cucumbers were collected from Vanga (4°39´S, 39°13´E) South Coast, Kenya on 22nd July 2020. The collection activity was done by engaging fishermen experienced in sea cucumber fishery who hand-picked the organisms during low tide and at night time when they are known to be active. The juveniles were sorted to remove weak ones and transferred into inert polyethylene containers with sea water for transportation to a holding tank at Kenya Marine and Fisheries Research Institute (KMFRI) Mombasa. A total of 1400 cockle spats were collected from Gazi bay (4°42´S, 39°51´E) South Coast, Kenya on 25th July 2020. Women fishers experienced in cockle collection were engaged in this activity. Collected cockle spats were placed in open basins filled with sea water and provided with aeration for transportation to a holding tank at KMFRI. A total of 3,800 juveniles of marine shrimps were collected from Kilifi creek in the mangrove channels near the project site (3°36´S, 39°50E) North Coast, Kenya. Juvenile marine shrimps were collected by some members of the Umoja Self Help Group at the project site were engaged from 27th to 29th July 2020. The collected marine shrimp juveniles were held in two nursery ponds owned by Umoja Self Help Group at Kibokoni.

Juvenile sea cucumbers and cockles spats held in holding tanks at KMFRI awaiting stocking were assessed and healthy individuals selected for stocking. A total of 450 sea cucumber and 1280 cockles were selected and measured as described in section 4.2.5, and transported to the experimental site for stocking. A total of 3,630 shrimps were also selected and transferred for stocking in the experimental ponds. For each species allowance of 5 extra individuals above the stocking density for each pond were added as precaution for unforeseen mortality during handling and transportation (Magondu *et al*., 2019).

4.2.4 Stocking and pond management

Selection and stocking of the organisms in the experimental ponds was done on 8th August 2020. In the first treatment, marine shrimp *P. indicus* were stocked in replicate ponds and raised as monoculture system which served as the control. The second treatment was the IMTA system in which the three study organisms; marine shrimp, cockles, and sea cucumber were stocked in replicate ponds at different densities. Of the study organisms in the IMTA treatment, *P. indicus* was the fed species whereas *A. antiquata*, and *H. scabra* were the extractive organisms in the system. Juveniles of *P. indicus* (2.05 ± 0.02 g) were stocked in the 6 ponds of both treatment 1 and 2 at a density of 5 inds./m^{-2} (Zaki *et al*., 2004). In the IMTA treatment, wild sourced and acclimatized specimen of *H. scabra* and *A. antiquata* weighing an average of (88.15 ± 0.05 g) and (40.71 ± 0.02 g) respectively were stocked at a density of 1.2 inds. /m^{-2} for *H. scabra* and 3.5 inds. /m^{-2} for *A. antiquata.* The selection of these species and densities was informed by findings reported by Troell *et al*. (2011); Beltran-Gutierrez *et al*. (2014) and Namukose *et al*. (2016). Differences in total length and body weight of these juveniles were analyzed with a non-parametric analysis of variance (Kruskal–Wallis test, 1952). No significant differences were found in the initial total length and body weight of the organisms stocked in the different experimental ponds (Kruskal–Wallis; H = 2.02, P = 0.36).

Feeding of the cultured organisms was done twice daily at 9:00 am and 3:00 pm by casting the feed over the pond surface to achieve even distribution. A daily feeding rate of 5% body weight per day for the first two months of the experiment was applied, it was changed gradually to 3% body weight per day during the last 2 months of the culture period. However, after every sampling adjustment of feeding quantity was done based on the biomass achieved from the shrimps. An assumption of 85% survival of the stock in each pond was applied due to an avoidable mortality during sampling and undetected predation that might have occurred. Predator control was done by putting net screens at the pond inlets and outlets and also having manilla ropes across the ponds to prevent birds predating on shrimps. To maintain pond productivity and optimum alkalinity in the ponds lime and fertilizer was applied on a biweekly basis using recommended rates as shown in section 4.2.2. Pond water level was maintained at 1 m depth during high spring tide after compensating for evaporation and seepage.

4.2.5 Sampling and measurement of cultured organisms

Determination of length and weight of cultured organisms was done during sampling which took place after every 25 days subsequent to the next high tide. The weight of 60 number of shrimps from each replicate were measured using a digital scale (Aslor model) to the nearest 0.01g. The shrimp length measurements were taken as the distance between the tip of the rostrum to the tip of the teslon using a ruler scale to the nearest 0.01 cm (Lalramchhani *et al*., 2020). A sample of 40 sea cucumbers from each replicate were picked and measured for body length to the nearest 0.01 cm using a meter rule and body weight to the nearest 0.01 g using a digital balance (Watanabe *et al*., 2014). Sampling of cockles involved hand picking of 40 pieces from each replicate pond and placing them in a basin with clean water. Shell length from the anterior to the posterior side of the cockle body was determined using a vernier caliper to the nearest 0.1 mm and later converted to cm during calculations. Individual weight of the cockles was also determined using a digital weighing balance to the nearest 0.01 g (Karnisa *et al*., 2019).

4.2.6 Harvesting of cultured organisms

At the end of the experiment all organisms were harvested; weighed and measured for total length as described in section 4.2.5. Each experimental pond was first drained to 0.25 m level by opening the outlets to allow the water out while retaining the screens to prevent the organisms from escaping. Shrimps in monoculture ponds were first partially harvested using a drag net. A scoop net was then used to harvest the remaining ones. In the IMTA ponds, sea cucumber and cockles were harvested by handpicking from the pond bottom and placing them in separate labeled basins for later measurement and weighing. Shrimps in the IMTA ponds were harvested using a drag net and scoop net and placed in basins with water for subsequent weighing and measurement. All harvested organisms were counted and recorded.

4.2.7 Determination of water quality parameters

Physicochemical parameters to establish the water quality profile of the treatment ponds were taken once every two weeks, on a day before the tide water exchange. Water samples were collected using a horizontal water sampler from three sampling points in each pond for both IMTA and monoculture treatments and from the creek channels that brought in water to the ponds. Salinity, pH, dissolved oxygen, water temperature, conductivity, and total dissolved solids (TDS) were determined in situ using a multi-parameter meter kit Hanna instruments model. Light penetration as a measure of water transparency from each sampling station was determined using a Secchi disk. Water samples for chemical analysis to determine Nitrite-nitrogen (NO_2-N), Total ammonium nitrogen (TAN), nitrate-nitrogen (NO_3-N), and Phosphate-phosphorous (PO_4-P) were ice-chilled and taken to KMFRI Mombasa center laboratory for preservation in preparation for analysis according to (Valderama, 1995). Before nutrient analysis, water samples were filtered through microfiber glass filter paper (Whatman GF/C) using a vacuum pressure air pump (Shemer *et al*., 2017). The samples were then subjected to an auto analyser for analysis using the Continuous Flow Analysis technique. All the analysis were done according to standard methods described in American Public Health Association (APHA), American Water Works Association (AWWA) and Water pollution Control Federation (WPCF) 2005.

4.2.8 Collection of plankton samples

Phytoplankton samples were collected from two randomly selected stations in every pond, in order to get adequate cells, a 20 litre water bucket was used to draw water from the pond sampling position and then passed through a 20 micron phytoplankton net (Kalson *et al*., 2010). Sampling containers were pre-labeled suitably for each pond. The collected residue was passed into the pre-labelled sample bottles and fixed with 0.5 ml of acidified lugol's solution. The process was repeated to get a replicate sample for each point in the pond and for all of the other ponds. Sampling net and buckets were rinsed thoroughly after each collection to avoid transfer of cells from one sampling station to another. Sampling bottles were transported to KMFRI biological laboratory at KMFRI and stored in dark containers covered with foil paper to prevent exposure to sunlight. In the laboratory sample qualification and quantification was done for identification of species. Identification was done using a normal slide preparation on a compound microscope. Quantification was done by standardizing the collected samples to known volumes (Karlson *et al*., 2010). A sedge wick–rafter counting cell was then used with a compound microscope where a standard volume of the sample was filled into the counting cell. A standard volume x100 magnification was used in each preparation.

Zooplankton samples were collected from randomly selected locations in each pond. A 1000 L of sample water was passed through a 200 µm net for zooplankton collection (Evans *et al*., 1982). Concentrated zooplankton samples were diluted to 100 ml with formalin and distilled water to obtain a 5% buffered formalin solution. Pre-labelled containers were used for sample collection which was done in replicate. After sample collection they were transported and stored at the biological laboratory at KMFRI awaiting analysis. Sample preparation for counting was done by filtration method according to De Bernardi (1984) and at low pressure to avoid damaging fragile organisms. Rotifers and nauplii were counted using a sedge wick-rafter counting cell under an inverted compound microscope. Following Edmondson *et al*. (1971), a standard volume (1 ml) of the sample was filled into the counting cell and a standard x100 magnification was used for identification and count of organisms. Crustaceans were counted using a counting chamber 5-10 ml that had grooves to confine the subsamples to tracks of a constant width as described in McCauley (1984).

4.2.9 Collection and processing of benthic macroinvertebrates

Benthic macro-invertebrate samples from experimental ponds were collected monthly using a 225 cm^2 Ekman grab. One grab sample was collected from each of different locations on the bottom of each experimental pond; the inlet, middle and at the outlet. The three grab samples from each pond were then combined into a composite sample which was then washed through a 250 µm mesh sieve to obtain the benthic macroinvertebrates. Benthic macro-invertebrates from each composite sample were hand-picked using tweezers from the sieve and preserved in labelled plastic vials containing 10 % buffered formalin for later sorting, counting, and identification. In the laboratory, samples were washed in 0.5 mm mesh sieve to remove formalin. Under x10 magnification on a dissecting microscope, the macroinvertebrates were sorted, counted, and identified to at least the family level using identification keys as described by Richmond (2002) and APHA (2005).

4.2.10 Determination of sediment quality

Sediment samples were collected monthly from three locations at the shallow end, central point, and deep end point of each pond using a core tube of 6 cm diameter and 10 cm in sampling depth. Measurement of soil pH was done for determination of soil acidity. It was measured in each new core by pushing the electrode into the sediment through the side holes in the core tube until the pH meter reading stabilized (Mclean, 1982).

Sediment composition was assumed to be homogenous in each pond at the start of the experiment. Samples were ground and sieved with a 2 mm sieve and then dried at 50 °C to a constant weight. Organic matter in sediment was determined by ignition method (Page *et al.*, 1989). Total nitrogen of sediment was determined by micro-Kjedahl digestion method following Page *et al*. (1989). Total phosphorus of sediment samples was determined by acid digestion methods described by Jones and Case (1990) and Watson and Isaac (1990).

4.2.11 Formulation and proximate analysis of the experimental feed

Pearson's square method of feed formulation (Wagner *et al.*, 2006) was used to prepare a low cost pellet floating feed containing 40% protein formulated from locally sourced ingredients (soya bean meal, fish meal, wheat pollard, rice bran, seaweed powder, and cassava binder) which was used for the trial. Before use in the experimental trial, the feed was subjected to proximate analysis to determine its proximate composition of crude protein, crude fibre, lipids, ash, organic matter, and moisture content following the standard procedure described in AOAC (2000).

A sample of the formulated experimental feed was taken to the Kenya Marine and Fisheries Research Institute (KMFRI) laboratory for proximate analysis. The feed was ground into finer particles using an electric grinder fitted with a 1 mm sieve (Thomas-Wiley intermediate mill, 3348-L10 series, USA) and dried in an oven to a constant weight at 60 °C. Triplicate samples of the resultant dry feed were analyzed for crude protein, crude fibre, ether extracts, ash and moisture content. Crude protein was quantified by the standard micro-Kjeldahl Nitrogen method AOAC (2000) using a sample size of 0.5 g, a Behroset InKje M digestion apparatus and a Behr S 1 steam distillation apparatus (both:

Labor-Technik GmbH, Düsseldorf, Germany). The distillate containing ammonia was trapped in 4 % boric acid solution prior to titration with 0.1N Hydrochloric acid (HCl). The crude protein content in the feed was estimated by multiplying the nitrogen content with a factor of 6.25. Ether extracts were analyzed using a sample size of 2 g in a soxhlet extractor with petroleum ether (boiling point 40–60 °C). Crude fiber (CF) was determined by boiling 2 g of sample in a standard solution of 3.13 % sulphiric acid (H_2SO4) for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % Sodium hydroxide (NaOH) for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with hot water followed by acetone. The residue was oven dried at 60° C for 4 hours, cooled in a desiccator and weighed. The residue was ashed at 550 °C in a muffle furnace overnight. CF was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample. Dry matter (DM) was determined by drying 5 grams of sample in an oven for six hours to constant weight at 105 °C. Nitrogen Free Extracts were estimated by difference of (100-(CP % + EE % + CF % +Ash %). The organic matter in the feed was estimated by getting the difference of (100-Ash %). The results of the proximate composition are presented as shown in Table 1.

Table 1. Proximate composition of feed used in shrimp monoculture and IMTA systems.

Feed composition	**%Dry matter**
Crude protein	40.12
Crude lipid	5.12
Crude fibre	24.23
Ash	20.65
Nitrogen Free Extract (NFE)[a]	9.88
Organic matter[b]	79.35

[a] Nitrogen free extract = 100-(Crude protein%+ Lipid%+Crude fibre% +Ash%)

[b] Organic matter=100-Ash%

4.2.12 Data analysis

Mean size and growth of culture organisms

Raw data collected during sampling were recorded in a field notebook and later entered in an Excel version 2013 spreadsheet. The descriptive statistical tool in Excel was used to compute means (±SE). Graphical plots of mean weight (±SE) against time were used to study arithmetic growth of the experimental organisms. Data on mean weights were used to compute growth metrics.

- Average daily growth rate (ADGR) was computed as the difference of mean final weight and mean initial weight divided by the culture days (Ricker, 1979).
- Net weight gain (NWG, g) was the difference between mean final body weight and mean initial body weight of the culture organism over a period of time (WF-Wi) where WF is final weight and Wi is initial weight (Bagenal, 1978)
- Daily weight gain (DWG, g day^{-1}) was calculated as WF-Wi/t, where Wf is the final weight at harvest, Wi is the initial weight at stocking and t is the duration of culture (Bagenal, 1978)
- Specific growth rate (SGR % day^{-1}) refers to the instantaneous change in fish weight expressed as the percentage increase in body weight per day over any given time interval and is determined by taking the natural logarithms of body weight, and express growth as % per day (Ricker, 1979). In this study specific growth rate was calculated using the formula, $SGR\ (\ln WT_F - \ln WT_I)*\ 100/T$ where WT_F=average final fish weight (g), WT_I=average initial fish weight (g), ln is the natural logarithm, T=duration of the experiment (days).

Apparent Food conversion ratio (AFCR)

Feed conversion ratio is the ratio of the quantity of food distributed (g) to the weight gain of fish (g), over the culture period and serves as a measure of diet's efficiency. It was calculated by dividing the total amount of feed used (dry matter basis) and then dividing by the weight gain of the shrimps (Castell and Tiews, 1980). In the current study, AFCR was calculated for the shrimps only as they were the fed organism in the system.

FCR = Total feed fed / Live weight gained by fish.

Survival of culture organisms

Survival rate in fish culture is a parameter used to indicate the number of organisms in a stock that make it to from stocking to harvesting (Ricker, 1975). For both monoculture and IMTA ponds, the percent survival was determined by calculating the difference of initial number stocked and the final number harvested against the initial stocking multiplied by 100 (Ricker, 1975):

$$\text{Survival (\%)} = \frac{\text{Initial number stocked} - \text{Final number harvested}}{\text{Initial number stocked}} \times 100$$

Abundance of plankton

Generated plankton microscopy data was transferred to an excel sheet for appropriate data processing. Sample identification of the organisms to the genus level was done using recognized reference materials by (Carmelo, 1997; Cronberg and Annadoffer, 2006; Karlson *et al.,* 2010). Plankton abundance was calculated according to APHA (2005) using the formula:

N= 1/Vd X Vt/Vs X n; where N= plankton abundance (cells/m^3); Vd= Volume of filtered water sample (m^3); Vt = volume of filtered sample (ml); Vs= concentrate volume of Sedgewick Rafter Cell (ml); n= number of observed plankton.

Abundance of benthic macro invertebrates

Benthic macro-invertebrate abundance was calculated using the formula by Stazner, (1981).

N=Y x 10 000/3A; where N=the number of benthic organisms per m^2, Y= total number of benthic organisms counted in three samples; A= area of dredge in cm^2

Statistical analyses

Comparisons of the different treatments; IMTA against monoculture were analyzed by calculating the mean values of length and weights and expressing the results in table and graphical formats. The independent t test was used to test equality of means. Water quality data was analyzed by repeated measures ANOVA with treatment as the main factor and time as a sub factor (Zar, 1996) this was to take care of time and treatment effects during the study period.

Sediment quality, count data on abundance of benthic organisms were analyzed using independent T-test to compare differences between the two treatments. Plankton abundance in densities were determined by calculating the mean values of the identified organisms from the two treatments and further expressing the results as ± SEM. Data were checked for normality and homogeneity of variance using Shapiro-wilk's and Levene's tests respectively. Statistical analyses was done using Excel version 2013 and SPSS statistical software IBM version 22. In all statistical testing, differences were considered to be statistically significant at $p < 0.05$.

4.3 Results

4.3.1 General observations

The cultured organisms in both treatments were healthy with no incidence of disease occurring during the experimental period. However, during the first month of the experiment some sea cucumbers (≈ 20) in one experimental pond were observed to have open wounds which were likely inflicted by mud crab predators from an adjacent pond. A few dead organism were encountered over the study period which may be attributed to pond environment dynamics as the organisms adapted to culture conditions. Incidences of mild algal blooms were observed in all the ponds especially when weather conditions were hotter as was the case in December 2020. Summary data on numbers of individuals of each cultured species stocked into and harvested from experimental ponds are presented in Appendix 1 and Appendix 2 for monoculture and IMTA treatments respectively.

4.3.2 Growth performance of the cultured organisms

Mean sizes (± SE) of *P. indicus* raised under monoculture at each sampling are presented in Table 2. Results show initial slow growth then faster growth at end of the culture period. T.test showed there were no differences in mean size at stocking ($t=-0.88$, $p=0.46$). Graphical plots of the results are shown in Figure 6.

Table 2. Mean body weight in grams (± SE) of *P. indicus* per sampling in monoculture treatment during the experimental period.

Pond No	Organism	Sampling					
		Stocking	1st	2nd	3rd	4th	5th
Pond 1	***P. indicus***	2.12 ± 0.03	4.81 ± 0.4	5.18 ± 0.57	5.67 ± 0.44	10.05 ± 0.57	16.23 ± 0.89
Pond 5		3.52 ± 0.1	4.25 ± 0.28	5.46 ± 0.22	6.93 ± 0.31	12.92 ± 0.67	15.21 ± 0.84
Pond 8		2.12 ± 0.14	4.65 ± 0.35	5.88 ± 0.13	7.66 ± 0.44	12.33 ± 0.78	15.85 ± 0.76

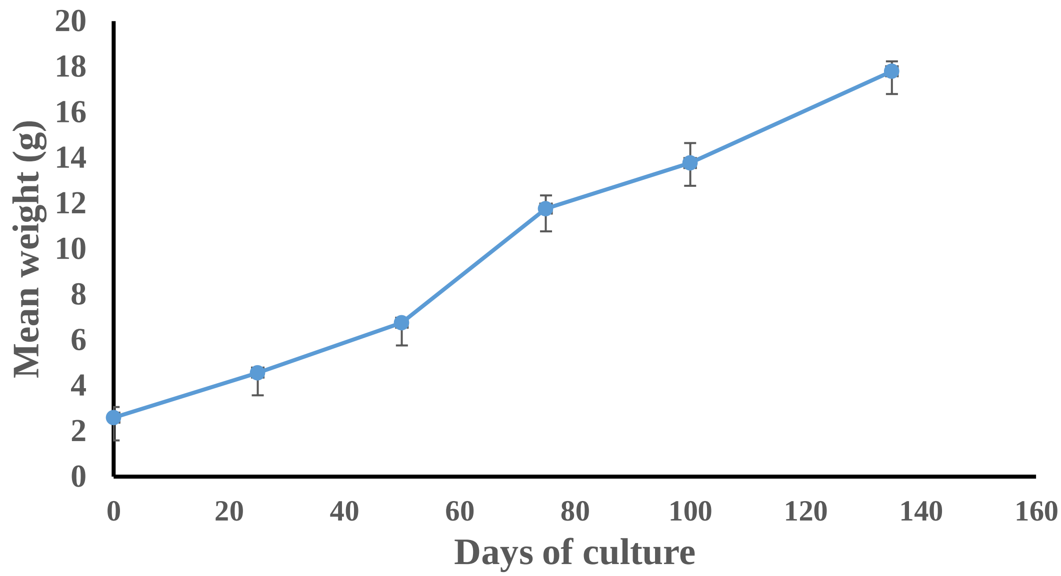

Figure 6. Mean weight of *Penaeus indicus* in monoculture ponds. Values are means (± SEM) of pooled data of individual shrimps in three replicate ponds.

Mean sizes (± SE**)** of the culture organisms under IMTA treatment are presented Table 3. One sample t. tests showed there were no differences in mean sizes at stocking (t=-4.12, p =0.06; t=-0.31, p=0.78; t=-3.16, p=0.08) for *P. indicus, H. scabra and A. antiquata* respectively. Figure 7 shows the growth trend over the culture period.

Table 3. Mean body weight in grams (± SE) of *P. indicus*, *H. scabra* and *A. antiquata* per sampling in IMTA treatment during the experimental period

Pond No	Organism	Sampling					
		Stocking	1st	2nd	3rd	4th	5th
3	***P. indicus***	2.33 ± 0.12	4.67 ± 0.32	5.98 ± 0.33	8.28 ± 0.41	10.86 ± 0.55	14.72 ± 0.71
6		2.73 ± 0.39	3.98 ± 0.33	4.96 ± 0.13	7.71 ± 0.38	10.44 ± 0.39	16.12 ± 1.24
7		2.51 ± 0.58	5.84 ± 0.27	7.02 ± 0.35	7.78 ± 0.31	10.2 ± 0.63	16.45 ± 1.09
3	***H. scabra***	78.87 ± 1.55	93.1 ± 6.43	99.78 ± 7.06	131.51 ± 8.74	139.88 ± 9.78	200.8 ± 11.84
6		86.4 ± 2.36	166.82 ± 6.37	173.6 ± 8.26	210.61 ± 10.15	281.93 ± 11.14	302.53 ± 14.49
7		99.18 ± 2.62	171.72 ± 6.06	180.75 ± 10.36	231.32 ± 13.7	251.4 ± 14.87	286.22 ± 16.71
3	***A.antiquata***	40.14 ± 0.82	57.35 ± 4.62	64.47 ± 4.25	70.68 ± 4.35	78.58 ± 2.84	82.44 ± 4.34
6		41.5 ± 0.65	51.33 ± 3.63	70.72 ± 5.2	84.01 ± 5.51	87.23 ± 4.08	86.39 ± 2.34
7		40.5 ± 1.44	58.16 ± 3.94	65.16 ± 3.32	69.98 ± 3.73	79.98 ± 3.84	85.95 ± 2.98

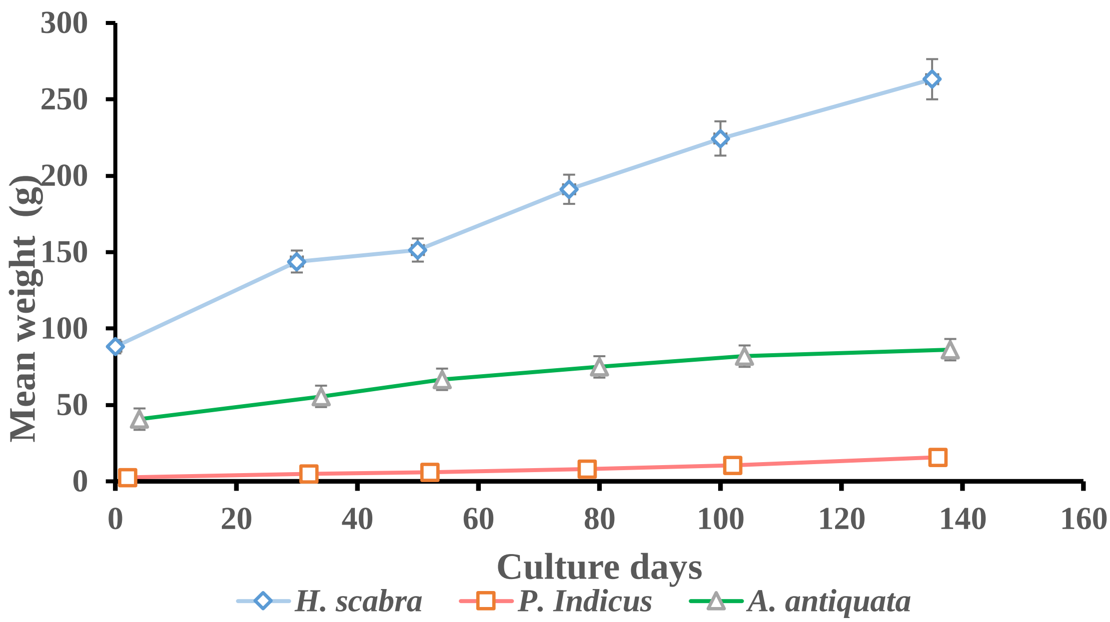

Figure 7. Mean weight of *Penaeus indicus, Holothuria scabra*, and *Anadara antiquata* in IMTA ponds during the experimental period. Values are means (± SEM) of pooled data of three replicates in each pond per sampling.

Table 4 presents the numbers and mean sizes at stocking and harvesting for *P. indicus* in monoculture ponds. The numbers reduced at harvesting while the mean sizes in the different replicate ponds increased. The total *P. indicus* stocked were 1,800 while the harvested individuals were 1,311.

Table 4. Numbers and mean size in grams (+SE) of culture organisms at stocking and harvesting in the various experimental ponds in monoculture treatment

Pond	Organism	No. Stocked	Mean size	No. harvested	Mean size
1	*P. indicus*	600	2.12 ± 0.03	455	16.23 ± 0.89
5		600	3.52 ± 0.1	432	15.21 ± 0.84
8		600	2.12 ± 0.14	424	15.85 ± 0.76
	Total	1,800		1,311	

Table 5 presents the numbers and mean sizes of the culture organisms at stocking and harvesting for different experimental ponds in IMTA treatment. For all the organisms the numbers decreased at harvesting while the mean sizes increased at harvesting.

Table 5. Numbers and mean size in grams (+SE) of culture organisms at stocking and harvesting in the various experimental ponds in IMTA treatment.

Pond	Organism	No. Stocked	Mean size	No. harvested	Mean Size
3	*P. indicus*	600	2.33 ± 0.12	410	14.72 ± 0.71
6		600	2.73 ± 0.39	512	16.12 ± 1.24
7		600	2.51 ± 0.58	520	16.45 ± 1.09
3	*H. scabra*	145	78.87 ± 1.55	60	200.8 ± 11.84
6		145	86.4 ± 2.36	90	302.53 ± 14.49
7		145	99.18 ± 2.62	95	286.22 ± 16.71
3	*A. antiquata*	420	40.14 ± 0.82	256	82.44 ± 4.34
6		420	41.5 ± 0.65	320	86.39 ± 2.34
7		420	40.5 ± 1.44	358	85.95 ± 2.98

At the end of the study *P. indicus* in both treatment 1 and 2 attained final body weight of 15.76 ± 2.29 g and 15.76 ± 0.53 g respectively. Statistical testing using one way ANOVA revealed no significant differences ($p > 0.05$). Among the organisms cultured in the IMTA treatment *H. scabra* attained the highest final average body weight (ABW) of 263.18 ± 54.63 g followed by *A. antiquata* at 84.92 ±2.16 g. NWG, DWG, and SGR did not show any difference for *P. indicus* in both treatments. *H. scabra* had a high weight gain of 175.03 ± 27.84 g, a daily growth rate of 1.29 % and a SGR of 4.1%. Cockles co-cultured in the IMTA had a NWG of 44 ± 0.97 g, a DGR of 0.32 % and a SGR of 3.25 %. The growth performance data recorded during the experimental period and calculations done at the end of the experiment are presented in Table 6.

Table 6. Results of analysis for the two treatments and data (Mean, ±SEM) for initial and final weights and lengths calculated on pooled data of individual *P. indicus*, *H. scabra* and *A. antiquata*.

Growth and yield parameters	T1 Monoculture of *P. indicus*	T2 IMTA of *H.scabra*	*A. antiquata*	*P indicus*
Stocking density (Ind/m2)	5	1.2	3.5	5
Total Stock (n)	600	144	420	600
Individual stocking weight (g)	2.58±0.46	88.15 ± 5.92	40.71 ± 0.41	2.58±0.46
Individual harvest weight (g)	15.76±2.29	263.18±31.53	84.92±1.24	15.76±0.53
Individual stocking length (cm)	6.4±0.6	10.5±0.1	6.5±0.03	6.4±0.6
Individual harvest length (cm)	13.11±0.69	21.74±0.79	9.33±0.12	13.09±0.67
Individual net weight gain (g)	13.17±0.75	175.03±27.84	44±0.97	13.19±0.57
Daily growth rate (g day^{-1})	0.96±0.03	1.29±0.21	0.32±0.01	0.96±0.01
Specific growth rate (% day^{-1})	1.99±0.02	4.1±0.09	3.25±0.01	1.99±0.02
% Survival	72.8	56.3	74.1	80.1

The results show that highest percent survival (80.1% was recorded among *P. indicus* in IMTA treatment whereas those in monoculture treatment had a survival of 72.8%. Of the three organisms raised in the IMTA treatment sea cucumbers had the lowest percent survival. In this study, apparent food conversion ratio (AFCR) for both treatments was not significant at ($t = -0.28$, $p = 0.8$ and $t = -0.109$, $p = 0.92$) for monoculture and IMTA treatments respectively. However, monoculture treatment recorded a lower AFCR value of 0.88 as compared to IMTA treatment at a value of 0.97 as shown in Table 7.

Table 7. Apparent feed conversion ratio for monoculture and fed component of IMTA.

	Apparent feed conversion ratio	
Organism	**Monoculture**	**IMTA**
P. indicus	0.88 ± 0.13	0.97 ± 0.24
P. Value	0.8	0.92
T.Statistic	-0.28	-0.109

4.3.3 Water quality parameters

Physico-chemical parameters of the culture water in experimental ponds are as presented in Table 8. Transparency in the IMTA treatment was lower than in monoculture treatment. However, temperature, pH, salinity, and dissolved oxygen did not show marked variations in water quality between the treatments during the culture period. All the nutrient parameters had significant differences between IMTA and monoculture treatments ($p < 0.05$). Among the monitored parameters in the culture environment, temperature in the ponds varied between 25.6°C and 34.5°C and salinity ranged from 35 ppt to 43 ppt for both treatment 1 and treatment 2 during the experimental period. Mean values of inorganic nitrogenous (NO_2-N, NO_3-N and TAN) and phosphate-phosphorous (PO_4-P) concentrations were lower in T2 as compared to T1. The repeated measures analysis portrayed a time and treatment effect on temperature, salinity, pH and phosphate phosphorous with significant differences ($p < 0.05$) being observed during the third and fourth month of the experimental period.

Table 8. Physico-chemical characteristics of experimental pond water collected from marine shrimp monoculture (T1) and IMTA (T2) ponds. Mean values followed by different superscripts indicate significant difference at $p < 0.05$.

Water quality parameters	Means Tukey test					
	Treatments		**Sampling time**			
	Monoculture	**IMTA**	**10/8/2020**	**1/9/2020**	**22/9/2020**	**13/10/2020**
Dissolved oxygen (mgl^{-1})	3.69 ± 0.39	3.65 ± 0.39	3.5 ± 0.35	3.2 ± 0.24	3.9 ± 0.34	3.96 ± 0.33
Temperature (°C)	30.78 ± 0.41	30.5 ± 0.41	27.15 ± 0.34^{c}	30.67 ± 0.06^{b}	32.54 ± 0.52^{a}	32.2 ± 0.54
Transparency	32.08 ± 0.14^{a}	30.17 ± 0.14^{b}	30.88 ± 0.23	31.16 ± 0.085	31.3 ± 0.25	31.17 ± 0.05
Salinity (ppt)	38.58 ± 0.51	38.3 ± 0.51	36.6 ± 0.14^{b}	41.35 ± 0.81^{a}	38.72 ± 0.41	37.04 ± 0.51
pH	8.37 ± 0.08	8.32 ± 0.08	8.36 ± 0.16	8.74 ± 0.25	7.72 ± 0.074^{a}	8.56 ± 0.13
Phosphate phosphorous (mgl^{-1})	0.103 ± 0.007^{a}	0.06 ± 0.007^{b}	0.083 ± 0.004	0.085 ± 0.06	0.078 ± 0.004^{c}	0.08 ± 0.007
Nitrate-nitrogen (mgl^{-1})	0.062 ± 0.003^{a}	0.023 ± 0.003^{b}	0.048 ± 0.004	0.038 ± 0.005	0.04 ± 0.003	0.044 ± 0.004
Ammonia nitrogen (mgl^{-1})	0.061 ± 0.004^{a}	0.029 ± 0.004^{b}	0.042 ± 0.002	0.052 ± 0.006	0.041 ± 0.004	0.044 ± 0.003
Nitrite nitogen (mgl^{-1})	0.05 ± 0.008	0.03 ± 0.008	0.03 ± 0.005	0.045 ± 0.01	0.041 ± 0.008	0.043 ± 0.004

4.3.4 Sediment quality

The sediment quality parameters for the two treatments are summarized in Table 9. The measured parameters; total organic carbon, pH, total phosphorous and total nitrogen showed lower levels in IMTA treatment as compared to monoculture treatment. The pH values determined were 7.57 ± 0.13 for T1 and 6.6 ± 0.13 for T2 which indicated an alkaline environment for T1 and an acidic environment for T2. There were lower phosphorous (p) levels of 0.38 mg l^{-1} in T2 as compared to 0.71mg l^{-1} in T1. Total organic carbon and total nitrogen also showed a similar trend with IMTA treatment having lower levels as compared to monoculture treatment.

Table 9. Sediment quality characteristics of experimental pond water collected from marine shrimp monoculture (T1) and IMTA (T2) ponds. Mean values followed by different superscript letter indicate significant difference at $p < 0.05$.

Parameter	**Treatment**	
	Monoculture	**IMTA**
pH	7.57 ± 0.13^{a}	6.603 ± 0.13^{b}
Total Organic carbon (%)	2.66 ± 0.15^{a}	1.26 ± 0.15^{b}
Total Nitrogen (%)	3.37 ± 0.38^{a}	2.37 ± 0.38^{b}
Total Phosphorous (mgl^{-1})	0.71 ± 0.032^{a}	0.38 ± 0.032^{b}

4.3.5 Benthic macro invertebrates

The benthic macroinvertebrates identified belonged to ten (10) families: Syllidae, Hesionidae, Nereidae, Eunicidae, Capitellidae, Spionidae, Rhabditidae, Balanidae, Gammaridae, and Naididae. Naididae was the most dominant family with abundance of between 8.9 and 3.8 ind./m^2 for IMTA and monoculture treatment respectively. The other groups had an abundance of less than 5 ind./m^2 while unidentified group had the least representation. Table 10 shows the difference in abundance between the IMTA and monoculture treatment and also the temporal dynamics during the experimental period. The results show that abundance of Syllidae, Hesionidae, Capitellidae, Spionidae, Rhabditidae and Naididae were significantly higher in IMTA treatment ($p < 0.05$) and increased with time during the experimental period. However there were no differences noticed between Nereidae and Eunicidae in the treatments but their abundance was significantly higher during the third sampling. Among the unidentified groups there were no differences observed between the treatments and during the study period. Results on abundance of benthic macroinvertebrates are presented in Figure 8.

Table 10. Abundance (Ind./m^2) of different groups of benthic macroinvertebrates in ponds based on repeated measures ANOVA. Mean values followed by different superscript letter in each factor indicate significant difference at $p < 0.05$.

	Means Tukey test				
	Treatments		Sample time		
Taxa	**Monoculture**	**IMTA**	**1**	**2**	**3**
Syllidae	1.39 ± 0.34^b	4.23 ± 0.34^a	2.50 ± 0.25	2.43 ± 0.31^b	3.49 ± 0.26^a
Hesionidae	2.01 ± 0.48^b	4.42 ± 0.4^a	2.78 ± 0.4	3.05 ± 0.33^b	3.82 ± 0.37^a
Nereidae	3.22 ± 0.12	3.49 ± 0.11	2.59 ± 0.14^b	3.11 ± 0.19	4.37 ± 0.19^a
Eunicidae	2.92 ± 0.2	3.21 ± 0.24	2.72 ± 0.46	2.96 ± 0.13^b	3.53 ± 0.15^a
Capitellidae	0.15 ± 0.56^b	1.46 ± 0.5^a	0.53 ± 0.19	0.63 ± 0.11^b	1.26 ± 0.11^a
Spionidae	0.35 ± 0.13^b	1.68 ± 0.14^a	0.62 ± 0.11^c	0.97 ± 0.13^b	1.45 ± 0.92^a
Naididae	3.81 ± 0.39^b	8.97 ± 0.38^a	5.71 ± 0.47	6.45 ± 0.22	7.02 ± 0.21
Rhabditidae	3.43 ± 0.36^b	8.3 ± 0.35^a	5.31 ± 0.33^c	5.84 ± 0.25^b	6.45 ± 0.23^a
Balanidae	0	2.16 ± 0.85^a	0.82 ± 0.04	1.13 ± 0.06	1.3 ± 0.16
Gammridae	0	2.61 ± 0.052^a	1.27 ± 0.07	1.27 ± 0.03	1.35 ± 0.02
Unidentified group	0.318 ± 0.11	0.66 ± 012	0.357 ± 0.74	0.49 ± 0.14	0.62 ± 0.09

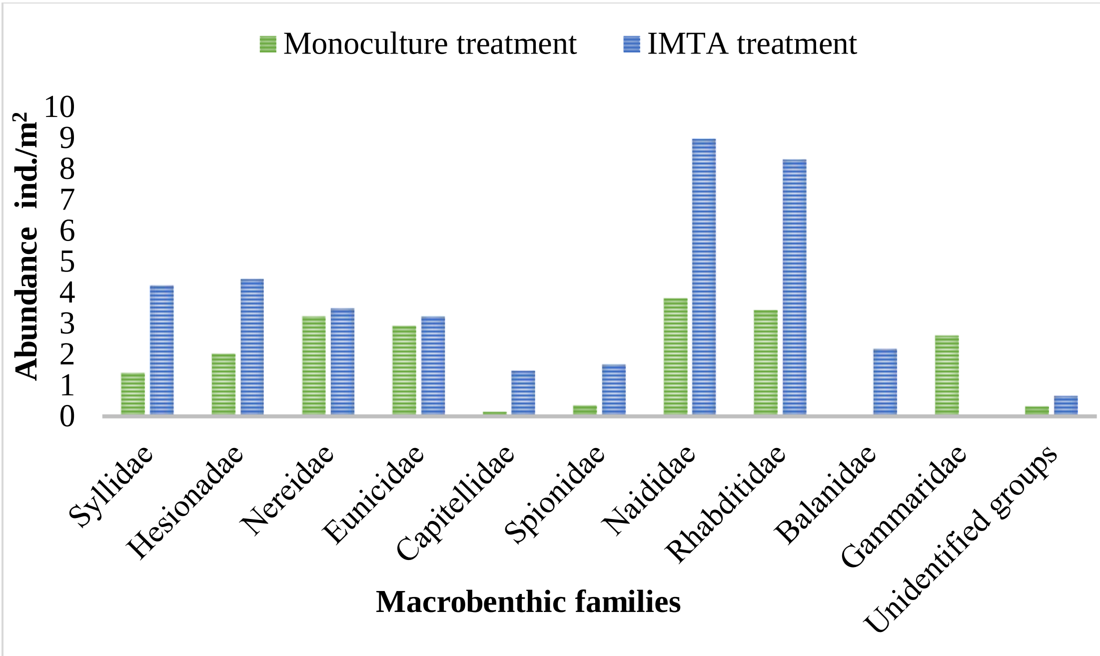

Figure 8. Abundance of different groups of benthic macro-invertebrates (Ind./m^2) in experimental ponds.

4.3.6 Phytoplankton

In the studied systems, phytoplankton analysis revealed a total of thirty four genera belonging to five classes; Bacillariophycea (eighteen genera), Dinophyceae (six genera), Cyanophyceae (six genera), Chlorophyceae (two genera) and Euglenophyceae (two genera) as shown in Table 11. The two treatments were dominated by Bacillariophycea with T2 consisting of 13.04 ± 20.62 cells/m^3 which was twice as much that in the T1 at 6.57 ± 17.84 cells/m^3. Dinophyceae and Cyanophyceae had almost similar abundance in T2 of 9.42 and 9.44 cells/m^3 respectively which was significantly less ($p< 0.05$) less than T1 which had a density of 5.5 ± 16.03 cells/m^3 for Dinophyceae and 6.54 ± 3.6 cells/m^3 for cyanophyceae this is presented in Figure 9 a and b. Other less representative algae were in the class of Chlorophyceae and Euglenophyceae with an abundance of less than 1 cell/m^3. Total abundance in the different genera is as shown in Table 12.

Table 11. Mean densities (± SEM) of different algal groups (cells x10^{3}ml^{-1}) in experimental ponds using water from monoculture and IMTA treatments. Values in parentheses are the genus within each algal group

Algal class	**Phytoplankton density (cells m^{-3})**	
	Monoculture	**IMTA**
Bacillariophyceae	6.57 ± 17.84^{b}(18)	13.04 ± 20.62^{a} (18)
Dinophyceae	5.5 ± 16.03$^{\mathbf{b}}$(6)	9.42 ± 11.6^{a} (6)
Cyanophyceae	6.54 ± 3.6^{b} (6)	9.44 ± 3.6^{a} (6)
Chlorophyceae	0.014 ± 0.8 (2)	0.016 ± 2.04 (2)
Euglenophyceae	0.208 ± 1.2 (2)	0.29 ± 1.96 (2)

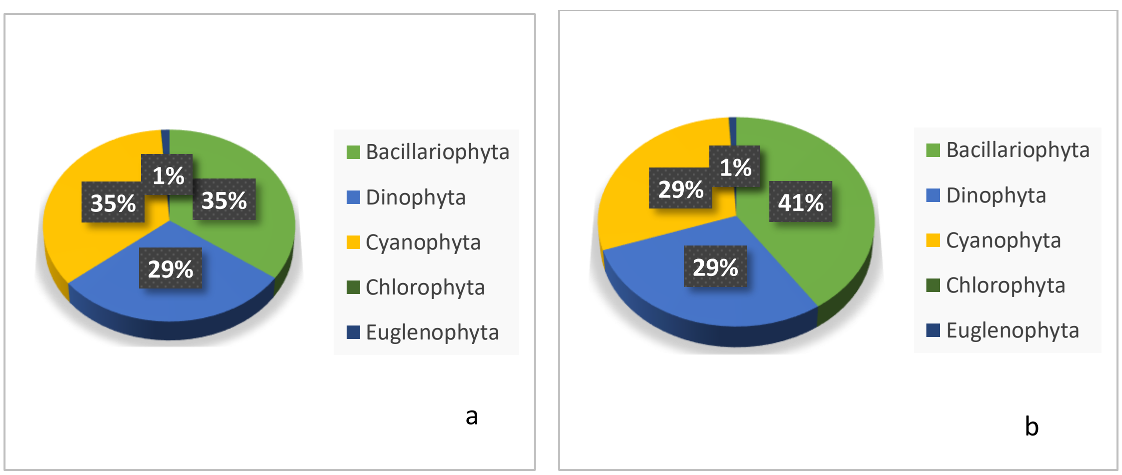

Figure 9. Percentage distribution of Plankton density in (a) monoculture and (b) IMTA treatments.

Table 12. Phytoplankton classes and genera with total abundance calculated from the samples collected from both monoculture and IMTA treatments.

Phytoplankton class	Genera	Monoculture cell/m^3	IMTA cells/m^3
Bacillariophyceae (diatoms)	*Pleurosigma*	1051	2250
	Bacillaria	825	700
	Navicula	521	1265
	Entomonies	751	1875
	Peridinium	1230	1302
	Nitzschia	1120	1235
	Nitzschia sigma	250	900
	Amphora	25	0
	Chymatopleura	125	1235
	Coscinodiscus	100	250
	Cyclotella	25	75
	Thalassionema	25	25
	Fragilaria	200	50
	Thalassiophysa	50	50
	Surirella	272	1850
	Gyrosigma	0	50
	Akashiwo	0	10
	Trichodesmum	0	18
Total Bacillariophyceae		6570	13040
Dinophyceae (dinoflagellates)	*Protoperidinium*	2765	4021
	Scrippsiella	1356	2436
	Alexandrium	854	2375
	Ceratium furca	475	550
	Gymnodinium	0	25
	Prorocent micans	50	13
Total Dinophyceae		5500	9420
Cyanophyceae (Cyanobacteria)	*Oscillatoria*	925	4560
	Chroococcus	250	330
	Trichodesmium	3000	1440
	Spirulina	1206	2750
	Lyngbya	509	250
	Anabaena	650	320
Total Cyanophyceae		6540	9440
Chlorophyceae	*Coelastrum*	2	4
	Staurodesmus	9	7
Total Chlorophyceae		3	5
		14	16
Euglenaceae (Euglena)	*Euglena*	75	250
	Trachelomonas	133	40
Total Euglenophyceae		208	290

4.3.7 Zooplankton

The zooplankton were represented by 11 classes for Monoculture treatment and 10 classes IMTA treatment as shown in Table 13. The class Maxillopoda had the highest abundance of the identified organisms; 390 ind. /m^3 for the monoculture and 930. ind /m^3 for the IMTA treatment as shown in Figure 10. Other classes like Malacostraca and Gastropoda had less than 250 ind. /m^3. Classes Branchiopoda, Arachinida, Cephalocarida, Ostracoda, Sagittoidea, Insecta, and Polychaeta had < 100 ind. /m^3.

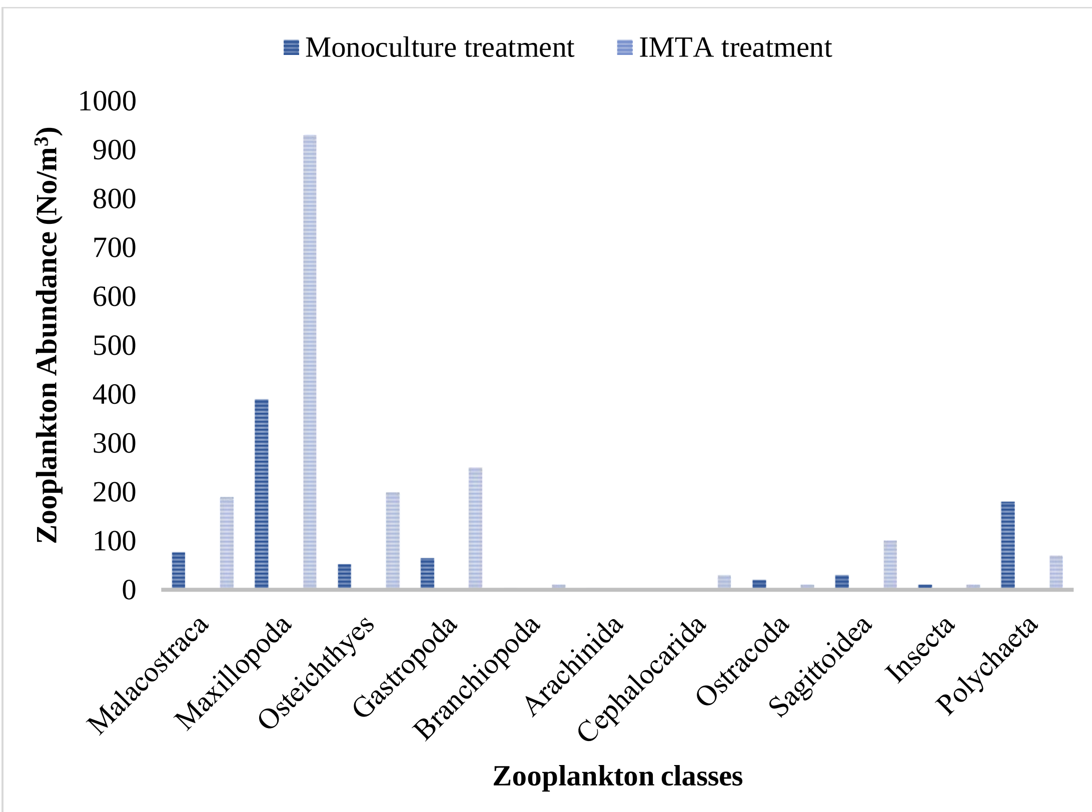

Figure 10 Zooplankton abundance for both monoculture (T1) and IMTA (T2) treatments

Table 13. Zooplankton classification and abundance calculated from the samples collected for both monoculture and IMTA treatments.

Zooplankton claassification		Monoculture	IMTA
Class	**Infraorder/genus**	**Zooplankton abundance (No/m^3)**	
Malacostraca	*Caridea*	62	180
	Brachyura	2	0
	Lucifer	10	10
	Isopod	2	0
Maxillopoda	*Acartia*	132	410
	Copepodite nauplii	136	400
	Oncaea	2	0
	Labidocera	0	60
	Harpaticoida	100	20
	Eucalanus	20	10
Osteichthyes	*Fish larvae*	52	200
Gastropoda	*Bivalvia*	64	250
Brachiopoda		2	10
Cephalocarida		2	0
Arachnida		0	30
Ostracoda		20	0
Sagittoidea	*Bathybelos*	30	100
Insecta		10	10
Polychaeta	*polychaeta larvae*	180	70

4.4 Discussion

The findings indicate the IMTA approach is viable with a number of potential benefits to farmers which includes; (i) improved water quality in terms of nutrient concentrations; (ii) improved growth and performance of culture species; and (iii) increased efficiency of the culture system through integration.

4.4.1 Pond IMTA

The goal of an IMTA system is to decrease dependence on external inputs such as fertilizer applications and feeds in culture systems and increase system efficiency by optimizing use of nutrients in the production loop as well as energy. In return there is decreased waste from the system through recirculation which is used to feed other crops within the system. IMTA provides for a diversity of farm products generating more robust income sources as compared to conventional monoculture systems. Studies have shown that in monoculture ponds the efficiency of nutrient utilization is relatively low with feed and fertilizer accounting for over 72 % of the nitrogen input with only an average of 25% transformed to shrimp biomass at harvest (Thakur and lin, 2003; Chang *et al* 2020). In shrimp fed pond IMTA system, nutrients are generated from uneaten feeds and waste which are then assimilated by extractive microscopic plants such as algae and planktons, which are then fed on by other organisms like filter feeders and deposit feeders like sea cucumber (Samocha *et al*., 2015). The algae growing in the system normally use sunlight to build their biomass through autotropism while taking up the dissolved inorganic nutrients in form of nitrogen and phosphorous from the pond water (Neori *et al.,* 2004). Bivalves used in IMTA like cockles build their bodies by filtering suspended particulate matter in form of wasted feeds, feacal waste, and plankton (Ren *et al.,* 2014). The process of shell production plays a key role in the carbon cycle through carbon fixation and has a contribution to mitigation of climate change through sequestering carbon in form of calcium carbonate (Hickey, 2009; Beamount *et al.,* 2014).

Other environmental benefits derived in an IMTA pond system are generated from the movement action of co-cultured fish or deposit feeders in shrimp ponds that stir up the sediment enhancing 'microbial metabolism and recycling of sediment trapped nutrients' (Tian *et al*., 1999). This shows that a properly designed IMTA can convert pollutant nutrients into commercial crops contributing significantly to the production of the farm and the profit earned (Neori *et al*., 2004; Han *et al.*, 2012).

4.4.2 Improved growth through integration

A comparative system performance analysis between the IMTA treatment and monoculture conducted in this study portrayed the potential of farming shrimps, bivalves and sandfish together in low fed brackish ecosystem. The IMTA model performed better in terms of organism growth performance, environmental health, and ecological benefits as compared to the monoculture system, an outcome that concurs with recent studies by Tang (2017) and Chang *et al*. (2020). Growth performance of Indian white shrimp in this study gave similar results of an average final body weight of 15.76 ± 2.29 g and a SGR of 1.99% for both monoculture and IMTA trials, an indication that the conditions for growth and species combinations were optimum for both treatments which also enhanced the recorded good survival rates of 72.8% and 80% for monoculture and IMTA respectively. Studies by Mai *et al*. (2010) and Khoi and Fotender (2011) reported similar growth and survival of shrimp in IMTA to that in monoculture. This concurs with the findings of this study that inclusion of extractive crop may not affect growth and survival of shrimp.

There was no potential effect on lack of extra sand in monoculture ponds. Sea cucumbers in the IMTA treatment grew bigger and faster at a SGR of 4.1% and a net weight gain of 175 g in 135 days of culture exhibiting a growth of 38 g/ month which could have been contributed by *H. scabra* intrinsic high growth potential and presence of rich organic feed in the pond bottom mainly from uneaten feed and faecal waste of the fed species in the system. This is comparable to studies by Agudo (2012) and Purcell (2004) which showed that growth of sandfish in earthen ponds was 20-72 g month^{-1}. Shelley (1985) estimated that in the natural *H. scabra* can grow at 14 g /month. Growth of sea cucumber in pens has shown to be slower than the earthen ponds with likely reason being that the natural sediments are not enriched with faecal waste and uneaten feeds and also the fact that there are more competitors and predators in pen culture (Purcell *et al*., 2012). In this study survival of sea cucumbers was 56.3 % which was the lowest as compared to the other co-cultured organisms. This could have been attributed to predation from mud crabs that are commonly found at the experimental site of this study. Several studies have shown that survival of *H. Scabra* is normally range between 50-85% in ponds (Agudo, 2012; Duy, 2012; Gamboa *et al.*, 2012) as compared to the open sea pens where there has been variation in survivorship averaging at 48% (Robinson and Pascal, 2012).

Bivalves have been used in IMTA systems based on their capacity to directly capture suspended particulate organic matter from the culture site and also nutrient extraction from the system (Chopin, 2013). In this study cockles, *A. antiquata* which are bivalves were used as filter feeders from the system consuming small particulate matter in the water column including both living and non-living organisms (Carss, *et al.,* 2020). Through filtration bivalves in IMTA improve water quality by increasing transparency and removing nutrients (van der Schatte Oliver *et al.*, 2018; Mcleod *et al.,* 2019). Cockles also offer potential benefits for sustainable food production as shown by Yip *et al*. (2017). The growth performance exhibited by the cockles was satisfactory with a net weight gain of 44 g, a SGR of 3.25 % and a survival rate of 74.1 %. Population studies by Mzighani (2005) showed that *A.antiquata* in the natural environment exhibits an isometric growth with a minimum shell length of 5 cm and a maximum of 72 cm and a corresponding whole live weight of 5 g to 60 g. Differences in size composition happens to be a function of fishing pressure and environmental factors. In the current study, cockles were found to be feasible in the co-culture IMTA pond system with the Indian white shrimp by improving the culture environment and increasing production in shrimps and sea cucumbers integration.

4.4.3 Water quality in integrated culture systems

The recorded physico-chemical parameters were within the required optimum range for shrimp culture in brackish environment (Chakraborti *et al*., 2002). The tidal water exchange, initial application of lime and monthly fertilizer application resulted to the optimal water quality in the culture systems. The inorganic nutrient parameter of water improved in the IMTA system in comparison to the monoculture with more effective elimination of both inorganic nitrogenous and phosphate-phosphorous concentrations from the culture water. In the IMTA treatment inorganic nutrient removal might have been facilitated by algal biomass, presence of cockles and sea cucumbers as extractive organisms which might have also enabled uptake of particulate organic matter through filter feeding and consumption of organic matter from the pond bottom as demonstrated by Chang *et al*. (2020). Studies have shown that water quality parameters like temperature, dissolved oxygen, and salinity are capable of influencing growth of cultured organisms (Tsuzuki *et al*., 2000). However in this study there were no significant variations in insitu water quality parameters among the two treatments an indication that the different results achieved in organism weight gain and production could be attributed to variations in species combinations. In both systems natural growth of microscopic aquatic plants provided a possible mechanism for extraction of inorganic nutrients from the culture water (Cunha et *al*., 2019). In the IMTA system, cockles were used in extraction of the suspended particulate matter in the system through filter feeding (Shpigel *et al*., 1993). The effective role of bioremediation was played by the sea cucumbers which fed on organic matter deposited on the pond bottom which further improved nutrient recirculation through bioturbation (Purcell, 2004).

4.4.4 Influence of sediment quality parameters on pond productivity

Among the organisms which had been cultured in the pond systems, IMTA treatment had *Holothuria scabra* which contributes to bioturbation affecting transport of nutrients mainly phosphorous and nitrogen through the sediment-water interface (Brönmark *et al*., 2005). As a result, mineralisation of nutrients takes place causing flux of nutrients from underlying sediment to overlying water which could have contributed to the lower values shown under IMTA. On the other hand, nitrogen fumigates from ponds during natural processes of nitrification of N-NO_3 and denitrification to N_2 retaining only a small amount of nitrogen compounds in sediment (Knosche *et al*., 2000). Presence of benthic macroinvertebrates like those belonging to the family Balanidae also plays a key role in bioturbation processing large quantities of sediment to extract organic matter which eventually influences the carbon and nitrogen contents in the system (Risnoveanu *et al*., 2004; Zhang *et al*., 2010). The sediment that settles in pond bottoms is usually a contribution of faecal waste, wasted feeds, dead organisms among the cultured ones and detritus from sinking phytoplankton and pond macrophytes (Ren *et al*., 2012). The amount of sediment carbon and nitrogen present in benthic compartment is therefore as a result of the rate of sedimentation and remineralization of the present sources (Ren *et al*., 2012). Pond management practices like liming, fertilization and supplemental feeding could have significantly affected the available phosphorus (P) in sediment according to Schlumberger (2002) who indicated that 90% of P is adsorbed by the sediment with a possibility of release to the pond water when a deficit occurs. The observed changes in sediment pH and availability of total organic carbon and total phosphorous might have contributed to total pond productivity in this study.

4.4.5 Contribution of benthic macroinvertebrates to pond productivity

There was an observed increase in counts of benthic macroinvertebrates during the culture period for both IMTA and monoculture treatments. However, for the monoculture treatment absence of predation by the cultured species could have been the primary cause of presence of the shown benthic organisms Figure 8. ‘Presence of sea cucumbers in IMTA treatment in combination with benthic macroinvertebrates could have enhanced influx of ammonia and food materials across the sediment water interface through burrowing’ (McCall *et al*., 1979; Mactavish *et al*., 2012). The exchange of sediment materials through fluid transport, diffusion of dissolved substances, and excretion of metabolites showed creation of hot spots where bacterial activity was concentrated (Henriksen *et al*., 1983, Aller and Aller, 1986; Kristensen, 2000, Marinelli *et al*., 2002). This could have contributed to better culture environment and pond productivity (Magondu *et al.,* 2022). Benthic macroinvertebrates naturally live on or burrowed inside the deposit at the bottom of a water body (Gosselin and Hare, 2003). They do form an integral part of the aquatic ecosystem playing an important role in the circulation and recirculation of nutrients within the culture system (Hossain, 2011). In addition they provide a connection between the unavailable nutrients (Badiou *et al*., 2011) in detritus while also serving as food for wide range of fishes (Idowu and Ugwamba, 2005). Macroinvertebrates biomass estimations by Gamito *et al*. (2020) working on pond IMTA experiments found similar invertebrate fauna to the results of the present study.

4.4.6 Influence of phytoplankton composition and abundance to pond productivity

Phytoplanktonic algae in this study consisted of different classes such as Bacilariophyceae, Chlorophyceae, Euglenophyceae, Cyanophyceae, and Dinophyceae. Bacillariophyceae were found dominant in IMTA environment among the phytoplankton community structure which concur with Cunha *et al. (*2019 and Qiao *et al. (*2020). In the current study, microscopic organisms in IMTA treatment may have assimilated inorganic waste streams originating from culture system, while in turn serving as a valuable food source for the shrimps, cockles and sea cucumbers which showed good production as shown by Magondu *et al*. (2021) and further agreeing with Lefebvre *et al*. (2000), Jones *et al*. (2001), Milhazes-Cunha and Otero (2017).

Phytoplankton results show high abundance of genera among the five classes that were present which are important as they supply natural food organisms which supplement manufactured feed and with a more important function of feeding juveniles of fish and shrimps after stocking. Phytoplankton supply dissolved oxygen and biomass into the system through autotrophic metabolism (Neori *et al*., 2004). In addition, they play an important role in pond ecology and causes influence on quality of culture water in rearing facilities. The abundance of phytoplankton in a pond is usually controlled by concentration of inorganic nutrients from nitrogen or phosphate compounds that are supplied from additions of fertilizer and feeds to the culture systems (Azim and Little, 2006).

In this study, faecal waste and nutrients in the residue feeds that were fed to shrimps in both treatments might have accumulated constantly with time in the pond water that led to flourishing of phytoplankton and hence higher phytoplankton abundance for

Dinophyceae, Bacillariophyceae, and Cyanophyceae in both treatments which may not have been exploited by the filter feeders. These observations agree with studies by Cloern *et al.* (2014) and Dalu *et al.* (2016). The dominance and succession of different phytoplanktonic algae could have been regulated by availability of light, nutrients and favorable temperature in the culture ponds (Yusoff *et al*., 2002). Comparable results were reported by Rodriguez and Paez-Osuna (2003) studying shrimp ponds in Mexico found out that Bacillariophyceae, Cyanophyceae, Chlorophyceae and Euglenophyceae were the dominant groups in coastal water shrimp farms.

4.4.7 Role of zooplankton composition and abundance towards pond productivity

Zooplankton are useful water quality indicators and an excellent measure of secondary productivity in shrimp culture farms. The results of zooplankton analysis showed that the maxillopoda class maintained high populations in both treatments because they are swift and powerful swimmers and could therefore not be predated on easily during the different stages of the culture season which concurs with studies by Geiger and Turner (1990). Conversely, most of the other zooplankton classes presented low populations due to consumption by the cultured organisms. In addition larvae of polychaeta, insect larvae, mollusks and other crustaceans are considered an important food for shrimps. In this study the zooplankton abundance for the maxillopoda class in both IMTA and monoculture treatments was significantly high at 930 ind./m^3 and 390 ind./m^3 respectively. However among the other zooplankton classes the abundance in IMTA treatment was higher than in monoculture treatment. These results indicate that the species combinations in the IMTA treatment had less preference for the zooplankton. Studies by Attayde and Hansson (1999) showed that zooplankton represent the trophic link between primary producers and fish production and also serves as an important element in nutrient recycling chain in culture water. Specifically for pond culture zooplanktons are essential food resource for fish larvae, juveniles and adults (Nunn *et al*., 2012).

4.5 Conclusion and recommendation

Pond integration is an improved system compared to the usual monoculture shrimp production. In the present study, higher production was reached by integration and cultivation of extractive species which assimilated excess nutrients maintaining a balance in the system. The results showed that there was high abundance of plankton and benthic macroinvertebrates in IMTA ponds as compared to the monoculture ponds. This supported the higher production of cultured species raised in the IMTA treatment in which marine shrimps, sea cucumbers and cockles were integrated under culture. Primary production through increase in plankton abundance was realized in the IMTA treatment which played a crucial role of feeding the bivalves in addition to regulating the dissolved oxygen levels and assimilation of excess nutrients. Sediment quality improved through optimum pH levels, lower levels of total nitrogen, organic carbon, and total phosphorous which might have created a suitable environment for primary productivity. Therefore, the findings of this study exhibited that IMTA involving marine shrimps as fed species with cockles and sea cucumbers as extractive organisms is appropriate towards sustainable development of mariculture in earthen ponds as a low intensive culture system that can be adopted by the coastal farmers in the region. Further research on longer culture period and by using different species combinations of commercial value need to be explored to assess suitability for culture and application in IMTA systems.

CHAPTER FIVE

INTEGRATING SEA CUCUMBER, OYSTERS AND NILE TILAPIA IN EARTHEN PONDS

Abstract

This study compared the growth performance, production, and quality of culture environment of integrated multitrophic aquaculture (IMTA) systems with different species combinations in earthen ponds. The objective of this study was to assess the viability of IMTA system using different species combinations with tilapia as the fed component. The control (C) had a monoculture of Nile tilapia (*Oreochromis niloticus*), T1 had a combination of *Oreochromis niloticus* and *Holothuria scabra,* T2 had a combination of *Oreochromis niloticus, Holothuria scabra* and *Sacostrea cucullata* and T3 had a combination of *Oreochromis niloticus* and *Sacostrea cucullata.* Stocking was 2 ind./m^2, 1.9 ind./m^2 and 2.1 ind./m^2 for tilapia, sea cucumber and oysters respectively and replicated in all the treatments. During the 150 days culture period, *S. cucullata* attained growth weight gain of 23.74 ± 2.6 g and 26.55 ± 0.26 g in T2 and T3 respectively. Weight gain of *H. scabra* in T1 and T2 was 146.7 ± 6 g and 153.39 ± 2.04 g respectively. The final average body weight (ABW) of the fed species *O. niloticus* was significantly higher ($p < 0.05$) in T2 which had all the three species. The fed species in TI and T3 attained higher final ABW of 198.85 ± 2.5 g and 186.12 ± 7.9 g respectively than the 160.75 ± 3.75 g realized by those in the control. Survival rates were low for *H. scabra* in T2 (45%) and T1 (53 %). Production was highest in T2 which had an integration of all the three species. The findings of the study show that the IMTA that had a combination of all three species outperformed those that had two species as well as the control. However, the performance of Nile tilapia as a fed component in IMTA was somewhat

compromised by observation of wounds on some sea cucumber attributable to likely predatory attack by this species.

5.1 Introduction

Fish consumption globally has been on the increase at an annual rate of 3.1 % which is nearly twice that of yearly world population growth of 1.6 % and more than all other animal protein food like meat and milk which have registered an increase of 2.1 % per year (FAO, 2020). This has been as a result of world aquaculture production which has constantly been on the increase reaching 46 % in the year 2018 up from 25.7 % in the year 2000 (FAO, 2020). However, the rapid expansion in aquaculture has raised several concerns mainly on environmental and biosecurity issues resulting from accumulation of nutrients and pathogens in aquatic ecosystems as a result of external inputs in form of fertilizers, feeds and effluents directed to open culture systems (Skriptsova and Miroshnikova, 2011; Dong *et al.*, 2018). A possible remedy is *insitu* removal of organic and inorganic nutrients which could be attained through biological means by introduction of micro algae, filter feeders and deposit feeders (Neori *et al*., 2004, Samocha *et al*., 2015, Irisarri *et al*., 2015). This approach comprises the concept of integrated multi trophic aquaculture (IMTA) (Neori *et al*., 2004). In IMTA systems the waste of the main cultured species (the fed component) is utilized by the introduced extractive species which depend on it for energy and growth while at the same time cleaning the culture environment (Chopin, 2013). In addition to supporting sustainable and resilient aquaculture, Integration has resulted in benefits like higher productivity, improved resource–use efficiency, and reduced environmental impacts (FAO, 2020).

In Kenya, marine aquaculture has mainly been practiced as a single species (monoculture) venture in most of the farms with most farmers growing either shrimps (*Penaeus monondon or Penaeus indicus*), milkfish (*Chanos chanos*), mud crab (*Sylla seratta*) or recently Nile tilapia (*Oreochromis niloticus*) in marine environment (Mirera *et al*., 2019). There have also been trials of polyculture of shrimp and milkfish in intertidal ponds (Roonback *et al.*, 2002; Mirera, 2011). The main difference between polyculture and IMTA is that whereas in polyculture the cultured organisms are both fed species sharing the same chemical and biological processes, in IMTA system the cultured organisms usually feed at different trophic levels provided by the system. The IMTA system thus, in addition, has gains of higher nutrient utilization efficiency and environmental mitigation as compared to polyculture system (Biswas *et al*., 2020).

Practice of IMTA has advantages of boosting production and achieving environmental sustainability, moreover the introduction of other species enhances diversification of culture species which is key towards resilience of marine aquaculture sector. Previous studies by Largo *et al*. (2016); Cunha *et al*. (2019) and Chang *et al*. (2020) have demonstrated viability of IMTA systems established within the intertidal zone and as well as those within sheltered bays. The studies used different species combinations of fed and extractive species. A successful IMTA system therefore requires identification of suitable combinations of species which will yield better returns on investment and mitigate some negative environmental impacts of aquaculture.

The benefits derived from integrated multitrophic aquaculture systems are both ecological and economic in nature Chopin (2021) and involves recovery and reuse of wastes through trophic interactions by growing valuable complementary species (Barrington *et al*., 2009). The farmer must therefore understand the ecological dynamics of the culture system and the organisms under culture for better performance of the enterprise. In Kenya, there has not been any studies on semi-intensive mariculture in earthen ponds where IMTA technologies have been applied. In this context, this study was designed to compare the performance of different species combinations; a herbivorous fish, a filter feeder, a deposit feeder, and naturally occurring algae in earthen ponds in an IMTA setup.

5.2 Materials and methods

5.2.1 Experimental design and study organisms

To assess the performance of species that feed at different trophic levels, three IMTA treatments were setup and growth of cultured species in different combination investigated on for a period of 150 days, from 12th February 2021 to 12th July 2021. Eight rectangular earthen ponds each measuring 12 x 10 m and 1m depth were hired from Umoja self-help group for use in this study. The study employed an IMTA design with three species combinations and two species combinations constituted four treatments referred to as C (control), T1, T2, and T3. Where (i) Control (C) had a monoculture of Nile tilapia (*Oreochromis niloticus*), (ii) T1 had a combination of *Oreochromis niloticus* and *Holothuria scabra, (*iii) T2 had a combination of *Oreochromis niloticus, Holothuria scabra* and *Sacostrea cucullata; and (*iv) T3 had a combination of *Oreochromis niloticus* and *Sacostrea cucullata.* Each treatment had two randomly assigned replicate ponds.

5.2.2 Preparation of experimental ponds

The experimental ponds were drained completely and sundried for a period of two weeks to eradicate predators and other living organisms. Sand treatment was done on the pond bottom of the treatments that were to have sea cucumber (T1 and T2) so as to simulate a close to natural habitat environment for the sea cucumbers as described in section 4.2.2.

Lime ($CaCo_3$) was then applied to each pond bottom at 300 g/m^2 and the ponds allowed dry for 7 days. The ponds were then filled with water during high tide coming in from the creek through the dug in channels. Three days after filling ponds with water, they were fertilized using a combination of Urea and Diammonium Phosphate fertilizers at 3 and 2 g/m^2 respectively. The ponds were left for 7 days to allow growth of natural food organisms.

5.2.3 Collection and transportation of study organisms

Sea cucumbers and oysters used in this study were collected from the wild while Nile tilapia were obtained from the hatchery facility at KMFRI, Mombasa. A total of 1000 juveniles of sea cucumbers were collected from Vanga (4°39´S, 39°13´E) South Coast, Kenya on 2nd of February 2021. The collection activity was done by engaging fishermen who hand-picked the organisms during low tide, transferring them into inert polythene bags and transporting them to a holding tank at KMFRI, Mombasa. A total of 1050 oyster spats were collected on 11th of February 2021 during low tide from Kilifi creek (3°36´S, 39°50E) North Coast, Kenya, where they settled on mangrove plant trunks. Fishers experienced in oyster collection were engaged in this activity. Collected oyster spats were placed in open basins filled with sea water for transportation to the culture baskets mounted in the experimental ponds. A total of 1950 juveniles of Nile tilapia raised at KMFRI Mombasa together with the acclimatized sea cucumbers were transported to the experimental site for stocking into the culture ponds on 12th February 2021.

5.2.4 Stocking and management of ponds

Each pond was stocked with the culture organisms according to the stocking numbers as illustrated in Table 14 and according to the species combinations in the four treatments. Among the stocked species, Nile tilapia (*O. niloticus*) was the fed species, whereas oyster (*S. cucullata*) and sea cucumber (*H. scabra*) were the extractive species. Oysters were put in perforated plastic baskets used for shell fish culture and suspended in water column from horizontally fixed poles in respective experimental ponds following Higgins *et al*. (2011).

A low cost feed of 30% crude protein formulated as described in section 4.2.11 was given as supplementary to the Nile tilapia which were the fed species in the IMTA setup for this study. Fish were fed at 5% body weight of the formulated feed twice daily at 9:00 am and 3:00 pm by casting the feed over the pond surface to achieve even distribution. The quantity of feed was adjusted at 20 days interval based on the body weight of fish calculated from periodic sampling. An assumption of 90 % survival was made during the culture period, which might have occurred due to unavoidable mortality during sampling and undetected predation. Predator control was done by putting net screens at the pond inlets and outlets and strings on top across the ponds to prevent bird predation. Desirable alkalinity levels, water quality, and primary productivity were maintained by monthly application of lime and inorganic fertilizer at 300 g/m^2 and 2 g/m^2 respectively on monthly basis. Pond water level was maintained at 1 m depth during high spring tide after compensating for evaporation and seepage.

Table 14. Number of individuals of the cultured species stocked in control (C) and IMTA treatments (T1, T2 and T3

Species	C	T1	T2	T3
Oreochromis niloticus	240	240	240	240
Holothuria scabra		228	228	
Sacostrea cucullata			252	252

5.2.5 Sampling and measurement of cultured organisms

Determination of length and weight of cultured organisms was done during sampling which took place after every 20 days subsequent to the next high tide. A 20 % sample size of the stocking numbers of the different organisms was measured. The weight of 50 individuals of Nile tilapia from each replicate were measured using a digital scale (Aslor model) to the nearest 0.01g. The Nile tilapia total length measurements were taken using a ruler scale to the nearest 0.01 cm. A sample of 45 sea cucumbers from each replicate were handpicked and measured for body length (to the nearest 0.01 cm) using a meter ruler and weighed (to the nearest 0.01 g) using a digital balance (Watanabe *et al*., 2014). Sampling of oysters involved hand picking of 50 pieces from each replicate pond and placing them in a basin with clean water. Shell length of the oyster was determined using a Vernier caliper to the nearest 0.1 mm and later converted to cm during calculations. Individual weight of the oysters was also determined on a digital weighing balance to the nearest 0.01 g (Comeau, 2013).

5.2.6 Harvesting of cultured organisms

At the end of the experiment all organisms were harvested; weighed and measured for total length as described in section 5.2.5. Each experimental pond was first drained to

0.25 m level by opening the outlets to allow the water out while retaining the screens to prevent the organisms from escaping. Nile tilapia in the control were first partially harvested using a drag net. A scoop net was then used to harvest the remaining ones. In the IMTA ponds, harvesting of the oysters was first conducted by emptying the oyster culture baskets and placing them in labeled basins with water, Sea cucumber were then harvested by handpicking from the pond bottom and placing them in separate labeled basins for later measurement and weighing. All harvested organisms were counted and recorded.

5.2.7 Determination of water quality parameters

Sampling for water quality parameters took place on monthly basis preceding the next high or low tide. The main parameters monitored were; salinity, water temperature, transparency, pH, dissolved oxygen, total dissolved solids which were done in-situ using a multi-parameter meter kit Hanna instruments model. Light penetration as a measure of water transparency from each sampling station was determined using a white Secchi disk. Measurements for nutrients in form of Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N), Nitrogen-ammonia (NH_3-N) (TAN) and phosphate-phosphorous (PO_4-P) were done from pond water by collecting water samples using a horizontal water sampler from three sampling points in each pond and preserving samples at 10:00hrs during the sampling day. Before nutrient analysis, water samples were filtered through microfiber glass filter paper (Whatman GF/C) using a vacuum pressure air pump (Shemer *et al*., 2017). The samples were then subjected to an auto analyser for analysis using the Continuous Flow Analysis technique and following standard methods (APHA, 2005).

5.2.8 Data analysis

Mean size and growth of culture organisms

Raw data collected during sampling were recorded in a field notebook and later entered in an Excel version 2013 spreadsheet. The descriptive statistical tool in Excel was used to compute means (±SE). Graphical plots of mean weight (±SE) against time were used to study arithmetic growth of the experimental organisms. Calculations of Apparent Food Conversion ratio, survival and growth metrics was done following procedure described in section 4.2.12.

Statistical analyses

Comparisons of the different IMTA treatments and the control were analyzed by calculating the mean and standard error of the mean of length and weights of replicated biomass using the descriptive statistics tool in Microsoft Excel 2013. Comparisons for performance of the fed organism (fish) and water quality parameters among the four treatments was done by one-way ANOVA and repeated measures ANOVA respectively followed by post hoc Tukey HSD test (Zar, 1998). Data were checked for normality and homogeneity of variance using Shapiro-wilk's and Levene's tests respectively. Statistical analyses was done using Excel version 2013 and SPSS statistical software IBM version 22. In all statistical testing, differences were considered to be statistically significant at $p < 0.05$.

5.3 Results

5.3.1 Observations during the experimental period

Nile tilapia in all the experimental ponds grew well during the entire experimental period apart from minimal mortality of 15 % that was experienced after sampling which could be attributed to stress during handling. Broken oyster shells were found inside the culture baskets during extreme hot weather which was an indication of mortality. Body wounds were observed on some sea cucumbers which were likely inflicted by Nile tilapia while searching for food at pond bottom. Algal blooms were evident in all the experimental ponds especially during hot weather in the months of March and June.

5.3.2 Growth performance of the cultured organisms among the different treatments

The final average body weight (ABW) of the fed species (*O. niloticus*) was significantly higher ($p < 0.05$) in T2 which had a full integration of both fed and extractive species. The other two IMTA treatments (TI and T3) showed higher final ABW of the fed species as compared to the control which had a mean of 160.75 ± 3.75 g (Table 15). There were no significant differences in final body weight among the extractive species *H. scabra* and *S. cucullatta* (t=-0.47, p=0.54 and t=-1.46, p=0.38) respectively. During the 150 days culture period, there was a weight gain of *S. cucullata* of 23.74 ± 2.6 g and 26.55 ± 0.26 g in T2 and T3 respectively. Over the same period *H. scabra* in T1 and T2 had respective weight gain of 146.7 ± 6 g and 153.39 ± 2.04 g. Results in the graphical plot indicate that a harvesting weight gain of the fed species (*O. niloticus*) was highest in T2 and lowest in the control (Figure 11).

Table 15. Initial and final average body weight in grams of test organisms under control and IMTA treatments T1, T2, and T3. Values are mean ± SEM; means with different superscripts in a row differ significantly $p < 0.05$.

		C	T1	T2	T3
Test Species	**Initial ABW (g)**	**Final ABW (g)**	**Final ABW (g)**	**Final ABW (g)**	**Final ABW (g)**
O. niloticus	22.62 ± 0.97	160.75 ± 3.75^{c}	198.9 ± 2.6^{b}	218.82 ± 1.55^{a}	186 ± 7.97
H. scabra	53.5 ±2.5		199.85 ± 3.65	206.87 ± 3.02	
S. cucullata	42.5 ± 1.5			68.4 ± 0.05	65.93 ± 0.84

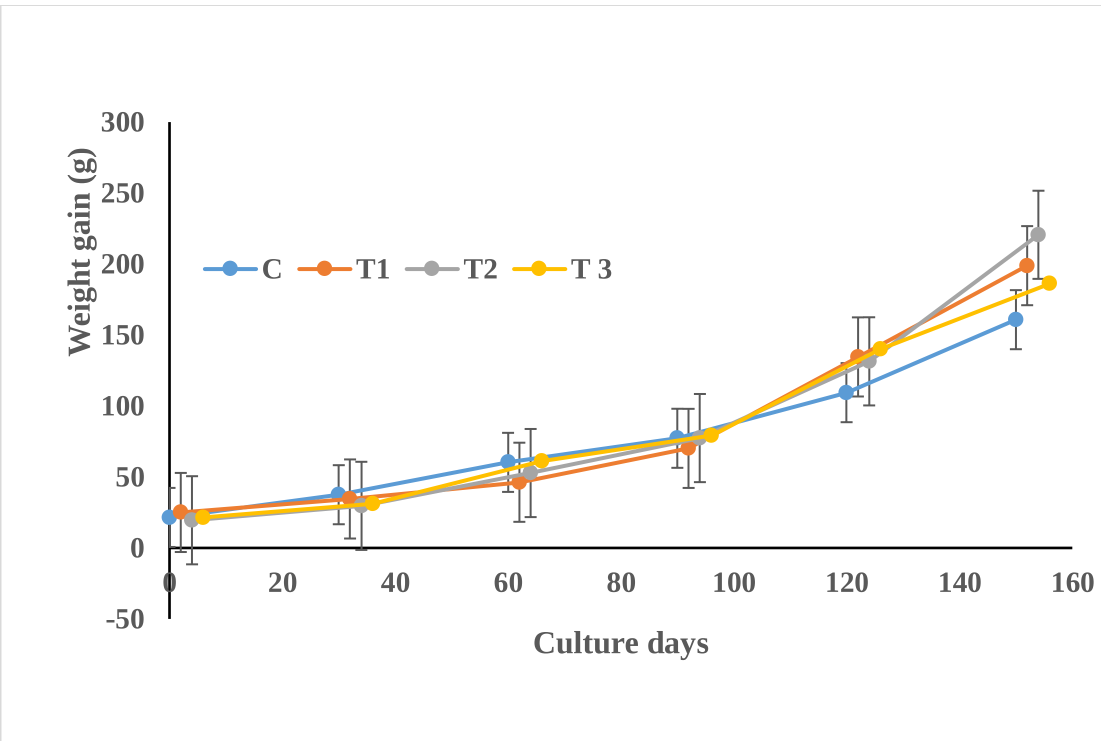

Figure 11. Weight gain in (g) of the fed species (*O. niloticus*) in the four treatments. Values are means (± SEM) of pooled data of replicates in each pond per sampling.

The highest survival was recorded among the *O. niloticus* in T2 at 92.9 % compared to those in other treatment which ranged from79.16 % in T3 to 85.40 % in the control (Table 16). Survival for *the S. cucullata* was 87.5 % and 79.2 % for the T2 and T3 treatments respectively. Lower survival was recorded for *H. scabra* at 53.2 % and 45.6 % for T1 and T2 treatments respectively.

Table 16. Percent survival of the cultured organisms in both control and IMTA treatments.

			Survival % day	
Organism	**Control**	**Treatment 1**	**Treatment 2**	**Treatment 3**
O. niloticus	85.4	81.25	92.91	79.16
H. scabra		53.26	45.6	
S. cucullata			87.5	79.2

Food conversion ratio determined for the fed organisms (*O. niloticus)* was lowest in T2 at 1.78 ± 0.04 and highest in T3 at 2.32 ± 0.3 (Table 17). Mean food conversion ratio was significantly different (t= -13.67, p=0.046) for *O. niloticus* in the control but not for those in the other treatments (Table 17).

Table 17. Apparent feed conversion ratio for control and fed component of IMTA treatments

		Apparent feed conversion ratio		
Organism	Control	Treatment 1	Treatment 2	Treatment 3
O. niloticus	2.09 ± 0.3	1.97 ± 0.09	1.78 ± 0.04	2.32 ± 0.3
T.statistic	-13.67	-0.36	-11.00	-0.89
P. value	0.046	0.778	0.058	0.535

Results in Table 18 show that growth of experimental organisms in the different treatments was highest in IMTA treatment T2 which had a combination of *O. niloticus*, *H. scabra* and *S. cucullata*. The fed component, *O. niloticus* in T2 had a weight gain of 182.11 ± 16.6 g, which was followed by T1 that had 173.85 ± 2.05 g and then T3 that had 164.62 ± 6.9 g. The fed species (*O. niloticus)* in the control had the lowest mean weight gain of 136.5 ± 2.8 g. Among the extractive organisms; H. *scabra* in T2 attained higher mean weight gain of 153.39 ± 2.04 g than those in T1 which had 146.7 ± 6 g. *S. cucullata* in T3 had slightly higher weight gain of 26.55 ± 0.26 g as compared to T2 that had a weight gain of 23.74 ± 2.6 g. The daily growth rate was high for *H. scabra* and *O. niloticus* in T2 as compared to those in T1 and control. Specific growth rate (SGR) was lowest for oysters, 4.1 % in both T2 and T3 (Table 18).

Table 18.Results of analysis of mean length and weight (±SEM), weight gain, DGR and SGR of *O. niloticus*, H. scabra and *S. cucullata* in the control (C) and the IMTA treatments (T1, T2, and T3).

Treatments	**C**	**T1**		**T2**			**T3**	
Experimental organisms	***O.niloticus***	***O.niloticus***	***H.scabra***	***O.niloticus***	***H.scabra***	***S.cucullata***	***O.niloticus***	***S.cucullata***
Growth and yield parameters								
Stocking density (Ind/m2)	2	2	1.9	2	1.9	2.1	2	2.1
Total Stock (n)	240	240	228	240	228	252	240	252
Initial ABW (g)	21.5 ± 1	25±0.5	53.5 ± 2.5	19.5 ± 1	53.5 ± 1	43.5 ± 1.5	21.5 ± 1	41.5 ± 1
Final ABW (g)	160.8 ± 3.8	198.85 ± 2.5	199.7 ± 3.5	218.81 ± 1.54	206.86 ± 3.04	68.4 ± 0.07	186.12 ±7.9	65.96 ±0.75
Initial stocking length (cm)	13.97 ± 1.5	12.66 ± 0.85	14.41 ± 1.66	13.08 ± 0.63	7.93 ± 0.42	8.94 ± 0.11	8.26 ± 0.41	7.69 ± 0.15
Final stocking Length (cm)	21.07 ± 0.48	20.35 ± 0.14	21.22 ± 1.01	22.19 ± 0.72	15.44 ± 0.78	14.55 ± 0.13	14.32 ± 0.35	14.07 ± 0.11
Individual net weight gain (g)	136.5 ± 2.8	173.85 ± 2.05	146.7 ± 6	182.11 ± 16.6	153.39 ± 2.04	23.74 ± 2.6	164.62 ± 6.9	26.55 ±0.26
Daily growth rate (g day-1)	0.91 ± 0.18	1.15 ± 0.01	0.97 ± 0.04	1.21 ± 0.11	1.02 ± 0.01	0.15 ± 0.01	1.09 ± 0.05	0.17 ±0.001
Specific growth rate (% day-1)	5.04 ± 0.23	5.28 ± 0.012	5.28 ±0.017	5.29 ± 0.78	5.31 ± 0.01	4.17 ±0.02	5.21 ± 0.043	4.18 ±0.011

Figure 12 shows the growth of the organisms in treatment 2 from stocking to harvesting that had full IMTA combination. *O.niloticus and H. scabra* performed better as compared to *S.cucullata.* Figure 13 shows samples of the different organisms harvested from the IMTA ponds at harvest.

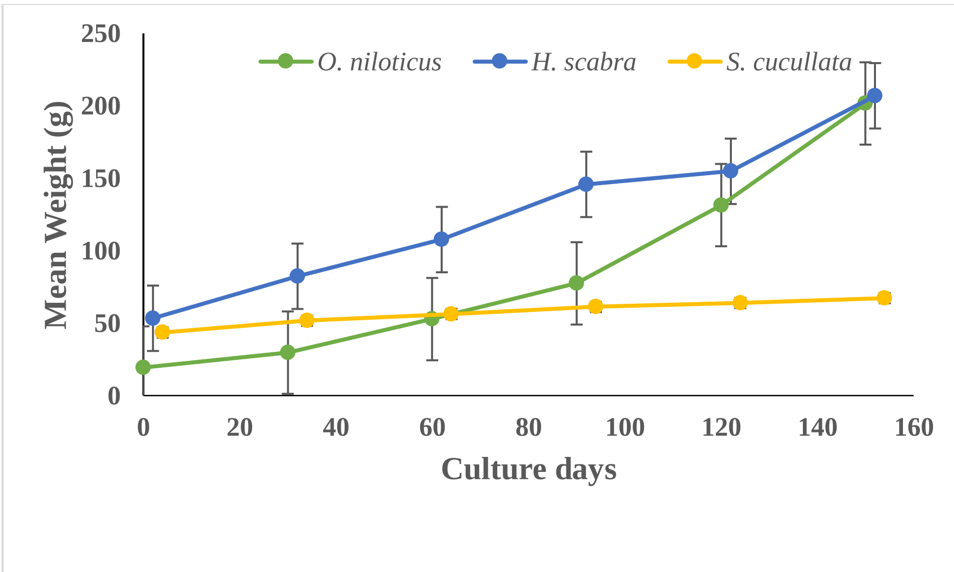

Figure 12. Growth of organisms in Full IMTA treatment (T2) from stocking to harvesting.

Figure 13. Harvested organisms from the IMTA system in second trial. A. Nile tilapia, B. Sea cucumbers, and C is oysters.

Some sea cucumbers were observed to have physical injuries in IMTA treatment T1 and T2, both of which had Nile tilapia as a co-cultured species. As reported earlier results in Table 16 also show sea cucumber had the lowest survival of three species cultured in the IMTA trial.

Dorsal and ventral injuries were observed among injured individuals (Figure 14)

Figure 14. Sea cucumber (*Holothuria scabra)* attacked by Nile tilapia (*Oreochromis niloticus*)

5.3.3 Water quality parameters

Among the physico-chemical parameters of culture water from the experimental ponds, transparency differed significantly ($p < 0.05$) between treatments being higher (32.72 ± 0.25) in T2 than the control that had a transparency of 24.84 ± 0.2 (Table 19). Over the 150 days study period, water temperature in the culture facilities varied between 21.4 and 32.6 °C, while salinity ranged from 28.5 to 40.5 ppt throughout the experimental period.

Dissolved oxygen levels were within suitable ranges for culture and was significantly higher ($p < 0.05$) in the IMTA treatments as compared to the control. T2 had the highest dissolved oxygen at 3.901 ± 0.01 mg/l^{-1} whereas the control had the lowest at 3.25 ± 0.01 mg/l^{-1}. The pH values did not vary much between the treatments, ranging from 7.6 in T2 to 8.0 in T3. Both T1 and the control had of pH 7.9.

The lowest value of TAN (ammonia nitrogen) was observed in T2 0.024 ± 0.007 mg/l^{-1} which was significantly different ($p < 0.05$) from that of T3 0.076 ± 0.007 mg/l^{-1} but not from T1 and control ($p > 0.05$). The Nitrate–nitrogen differed significantly ($p < 0.05$) being lower in the IMTA treatment T2 (0.025 ± 0.007) mg/l^{-1} which had the three species than in the control (0.057 ± 0.007 mg/l^{-1}) that had tilapia monoculture. Mean value of nitrite- nitrogen was lowest in T2 (0.023 ± 0.004 mg/l^{-1}). There were no any significant differences on phosphate phosphorous concentrations among the treatments.

Table 19. Mean (±SEM) values of various water quality parameters of pond water collected at 30 day interval from the monoculture (C) and IMTA treatment ponds (T1, T2, and T3). Means with different superscripts in a row differ significantly ($p < 0.05$).

Water quality parameters

Parameter	**Treatments**			
	C	**T1**	**T2**	**T3**
Dissolved oxygen (mgl^{-1})	3.25± 0.011	3.32 ± 0.01^{c}	3.901 ± 0.01^{a}	3.51± 0.01^{b}
Temperature (°C)	29.37 ± 0.309	28.28 ± 0.3	29.22 ± 0.31	28.91 ± 0.31
Turbidity (NTU)	28.2 ± 1.7^{a}	28.4 ± 1.78^{a}	22.3 ±1.7^{b}	17.9 ± 1.78^{c}
Salinity (ppt)	36.95 ± 0.3	35.81 ± 0.31	36.57 ± 0.32	34.81 ± 0.3
pH	7.9 ± 0.12	7.93 ± 0.1	7.76 ± 0.13	8.06 ± 0.12
Transparency	24.84 ± 0.2	25.34 ± 0.25^{b}	32.71 ± 0.25^{a}	30.56 ± 0.2
Phosphate phosphorous (mgl^{-1})	0.157 ± 0.04	0.142 ± 0.047	0.053 ± 0.05	0.057 ± 0.04
Nitrate-nitrogen (mgl^{-1})	0.057 ± 0.007^{a}	0.052 ± 0.007	0.025 ± 0.007^{b}	0.048 ± 0.007
Ammonia nitrogen (mgl^{-1})	0.067 ± 0.007	0.043 ± 0.007	0.024 ± 0.007^{b}	0.076 ± 0.007^{a}
Nitrite nitogen (mgl^{-1})	0.041 ± 0.004	0.036 ± 0.004	0.023 ± 0.004^{a}	0.034 ± 0.004

Parameter	**Sampling time**				
	2/20/2021	**3/26/2021**	**4/25/2021**	**5/24/2021**	**6/25/2021**
Dissolved oxygen (mgl^{-1})	3.53 ± 0.01	3.26 ± 0.25^{a}	3.32 ± 0.11	3.59 ± 0.067	3.38 ± 0.06
Temperature (°C)	27.32 ± 0.27^{c}	29.22 ± 0.16^{b}	32.15 ± 0.56^{a}	32.62 ± 0.23^{a}	21.39 ± 0.34^{d}
Turbidity (NTU)	20.11 ± 1.14	20.55 ± 1.21	21.91 ± 0.58	22.73 ± 0.76^{b}	23.23 ± 0.95 ^{a}
Salinity (ppt)	36.53 ± 0.55	40.47 ± 0.49^{a}	37.86 ± 0.51	36.8 ± 0.27	28.51 ± 0.45 ^{b}
pH	8.02 ± 0.26^{a}	7.6 ± 0.034^{b}	7.64 ±0.16^{b}	8.2 ± 0.11^{a}	8.1 ± 0.75^{a}
Transparency	22.35 ± 0.39	29.78 ± 0.79^{a}	25.49 ± 0.58	25.49 ± 0.58	25.2 ± 0.36
Phosphate phosphorous (mgl^{-1})	0.076 ± 0.005	0.277 ± 0.15	0.057 ± 0.016	0.056 ± 0.013	0.045 ± 0.013
Nitrate-nitrogen (mgl^{-1})	0.059 ± 0.005^{a}	0.051 ± 0.003	0.042 ± 0.004	0.045 ± 0.01	0.031 ±0.005^{b}
Ammonia nitrogen (mgl^{-1})	0.052 ± 0.002	0.06 ± 0.005	0.053 ± 0.004	0.048 ± 0.004	0.05 ± 0.007
Nitrite nitogen (mgl^{-1})	0.029 ± 0.006^{b}	0.028 ± 0.004	0.037 ± 0.003^{a}	0.041 ± 0.003	0.033 ± 0.002

5.4 Discussions

5.4.1 Growth performance of the cultured organisms

The comparison of IMTA system and the control made in this study tested the feasibility of culturing fin fish, oysters, and sea cucumbers together in an intertidal earthen pond ecosystem. The results showed that organisms cultured in the IMTA set up realized better growth and production than those in the control monoculture system. Individual weight gain and total production differed among cultured species in the different treatments which concurs with studies by Cunha *et al*, (2019). Nile tilapia which was the fed component of the IMTA set up in the study showed good performance during the 5 months culture period as compared to the co-cultured extractive species; sea cucumbers and oysters.

The low production of sea cucumber observed among the different treatments could be attributed to predation from the Nile tilapia, the fed component in the IMTA set up. Sea cucumbers are deposit feeders that consume mud particles Tresnati *et al*. (2019), a feeding habit that helps reduce the organic load from the bottom of earthen ponds while being integrated with other organisms (Funge-Smith and Briggs, 1998). This attribute, coupled with the species high market value makes sea cucumber a good candidate for multitrophic aquaculture systems (Magondu *et al*., 2021).

In the present study, the fed component of the IMTA T2 showed significantly ($p > 0.05$) high individual body weight of Nile tilapia at 218.82 ± 1.6 g, which was followed by T1 and T3 that had an integration of tilapia and sea cucumber at 198.9 ± 2.6 g and tilapia and oysters at 186 ± 7.9 g respectively. Nile tilapia in the control achieved relatively lower individual weight of 160.75 ± 3.8 g. The better growth of Nile tilapia in IMTA could be attributed to efficient removal of nutrient wastes from the culture environment as nutrient waste, feacal waste and uneaten feeds were extracted from the system and utilized by the extractive organisms such as algae, filter feeders and deposit feeders (Samocha *et al*., 2015).

The specific growth rate of cultured organims ranged from 4.18 % day^{-1} among oysters to 5.3 % day^{-1} among sea cucumbers. Given the water quality parameters were within optimal range, these findings indicate the conditions for growth and species combinations tested the three IMTA treatments in this trial were favorable. In aquaculture, feed conversion ratio (FCR) is used to determine efficiency of utilization of fed feed that is converted to flesh. Nile tilapia in IMTA treatment T2 in this study had an FCR of 1.78 ± 0.04 compared to T1, T3 and the control which had slightly higher levels Table 18. This might have been as a result of better feed utilization by the cultured organisms. Waite *et al*. (2014) showed that FCR is related to environmental performance as it provides indications of nutrients loss to the environment and any undesirable output. A lower FCR shows an efficient system (Hasan and Soto, 2017). Similar studies showed that 'species combination in an IMTA reduced FCR by 12-15% compared to monoculture' (Shpigel *et al*., 2016).

Bivalves have been used in IMTA systems based on their potential to directly filter organic particulates from the culture site and also nutrient extraction from the system (Chopin, 2013). Being suspension feeders, bivalves alter the plankton structure which promotes nutrient cycling in the culture environment (Smyth *et al*., 2013; Murphy *et al.*, 2016). In this study oysters were used as filter feeders and contributed to increased biomass and efficiency of the system. In the current study, integration of *S. cucullata* was found to be feasible in the co-culture IMTA pond system with Nile tilapia through improving water quality and increasing production in tilapia and sea cucumbers integration. Several studies have shown that extractive species in IMTA systems grow faster than in monoculture which implies that IMTA farms can provide greater economic gains and environmental benefits (Whitmarsh, *et al*., 2006; Sara *et al* .,2009; Sanderson *et al*., 2012).

5.4.2 Survival of culture organisms in different treatments

IMTA treatment (T2) achieved the highest survival for the fed species at 92.2%. Results of water quality and the culture environment in T2 were suitable for culture of the different organisms. Integration led to pond ecological balance that ensured high survival of tilapia as the fed organisms and sufficient nutrients that were captured by the oyster filter feeders which also showed high survival rates of 87.5 % and 79.2 % in T2 and T3 respectively. Sea cucumber was used as one of the extractive organisms due to its bio-mitigation ability and potential to reduce excess accumulation of waste from the pond bottom (Purcell, 2015; Cubillo *et al*., 2016). In this study survival of sea cucumber was lowest in treatment T2 that had 45.6 % and T1 that had 53.26 %. Some sea cucumbers were observed with injuries which were likely caused by predatory attacks by Nile tilapia, and it is possible that severe wounds may as well explain the higher overall mortality of this species reported in this study. Incidences of predator attack on *H. scabra* has earlier been reported on studies by Lavitra *et al*. (2009) and Tresnati *et al*. (2019). In sea cucumber farming, challenges related to biotic and abiotic parameters are normally experienced (Purcell, 2004; Wang *et al.*, 2004). These include; salinity drops against the optimum of 28 to 31 ppt, Xilin (2004) that occurs in seasons of heavy rainfall Chen (2004) and especially during low tides a situation that makes the organisms to burrow underground even when they are supposed to be active and foraging for food. Abnormal occurrence of high numbers of parasites such as Isopods in ponds during hot season has been a cause for sea cucumber mortality (Pitt and Duy 2004). In addition, abundance of crabs near the culture environments has been blamed for attack on both juveniles and adult sea cucumbers and cause of high mortality (Magondu *et al.*, 2021). Skin ulcerations were also evident which were followed by mucus secretions on the body, skin discoloration and behavior changes as reported by Purcell and Eeckhaut (2005).

The observed mortality of sea cucumber in T1 and T2 could be attributed to the presence of Nile tilapia. These attacks were more serious during foraging for feeds from the pond environment. Nile Tilapia has been categorized as herbivorous fishes Getachew and Fernando (1989), which mainly feed on algae, while other workers have classified them as omnivores with food composition dominated by zooplanktons like copepods and rotifers (Bwanika *et al*., 2004). Such feeding habits may also have contributed to the observed attacks on sea cucumber as the tilapia forage for food at the pond bottom where the sea cucumbers dwell. Dorsal and ventral injuries and skin ulcerations were observed on most injured sea cucumbers (Figure 13). Other possible predators were mud crabs and mangrove snails which were found to be present in some of the experimental ponds concurring with findings by Tresnati *et al.* (2019). However these were removed physically during the pond preparation before stocking and by use of lime to kill any predator eggs and larvae present. Another plausible explanation for the low number of sea cucumber harvested is that some individuals burrowed into the substrate to avoid predator attack by tilapia (Pitt and Duy, 2005). Such burrowing caused substrate disturbance (bioturbation) leading to better nutrient recycling and utilization benefiting the overall culture environment.

5.4.3 Water quality parameters of culture environment

The water quality parameters in this study showed better results in the IMTA system as compared to the control monoculture. The parameters were stable throughout the experimental period and within acceptable range of finfish mariculture (Biswas *et al*., 2019). The dissolved oxygen levels shown in the three IMTA treatments could have been attributed to the species combinations that filtered the culture water by consuming the algae available making the culture water less turbid. Presence of fish, oyster, sea cucumber, and phytoplankton contributed to pond ecological balance due to the synergies created. The enhanced water quality led to better fish performance and higher biomass production. Further, less amount of energy was needed to maintain pond oxygen levels which improved the system efficiency. The photosynthetic activity within the systems was dependent on the type of treatment whereby ponds without oysters (T1) and (C) showed significantly lower dissolved oxygen production in comparison with the treatments that had oysters (T2 and T3). The treatments without oysters had lower Secchi disk readings which implied higher turbidity due to suspended particulate matter in the water column which prevented light penetration that hampered photosynthetic activity and consequently feed accessibility. In other studies, poor water quality has often been blamed for lack of sustainability and profitability in aquaculture enterprises (Biao, 2007; Cao *et al*., 2007). Studies by Newell (1988) showed that oysters filter water at a rate of $0.12m^3g^{-1}$ dry weight per day which has shown potential to extract large amounts of organic particles from the culture water.

5.5 Conclusion

The IMTA set up involves maintaining a clean culture environment at reduced costs of inputs such as feed and fertilizers to facilitate improved efficiency while supporting sustainable production. Growth performance of Nile tilapia (the fed species) was better in the IMTA treatments than in the monoculture (control). The co-cultured species sea cucumber and oysters also recorded good growth in the IMTA ponds. In the present study, Nile tilapia as the fed component of the system contributed to the overall production of the system in addition to providing food and energy that the co-cultured organisms depended upon. Sea cucumbers experienced predation attacks from the tilapia leading to their low survival rates. This finding thus recommends further analysis of the Nile tilapia stomach contents. Therefore, the findings of this study revealed that use of Nile tilapia as fed species in an IMTA system with sea cucumbers as an extractive component is not viable due to predation attacks. Possibilities of using other fed species for IMTA studies need to be explored and provision of appropriate substrates that the sea cucumbers can burrow in to escape predation.

CHAPTER SIX.

ECONOMIC ANALYSIS OF TESTED IMTA AND MONOCULTURE SYSTEMS IN EARTHEN PONDS

Abstract

Integrated multi-trophic aquaculture (IMTA) entails raising of species belonging to different trophic levels in the same culture system whereby the organic and inorganic wastes generated are recycled to provide nutritional requirements for the others. The benefits derived from IMTA range from reducing ecological impacts of aquaculture operations, provision of financial benefits through diversification of products, risk reduction, and disease control. In this study, economic potential of IMTA was exhibited through integration of extractive species with existing fed-monoculture operations that aimed at boosting farm profits. Two trials were conducted; trial 1 had integration of marine shrimps with cockles and sea cucumbers. Economic analysis was performed for **Trial 1** to estimate the net income, cost benefit ratio (CBR) and return on investment (ROI) between the shrimp monoculture ponds and the IMTA ponds. There was 84.7 %, 41.8 %, and 5.2 % increase in net income, CBR and ROI respectively in the IMTA than the monoculture treatment. A CBR of 1.77 higher than the monoculture treatment was achieved. **Trial 2** had an integration of Nile tilapia, oysters, and sea cucumbers. Net income, CBR and ROI were significantly better ($p < 0.05$) in IMTA experiments. CBR was higher in IMTA than the control ($p < 0.05$). Net income was highest in the IMTA with a value of USD 77.5 while monoculture did not break even and had a value of USD (209). The calculated CBR was 1 for IMTA and 0.4 for the control. The calculated ROI 8.6 % and 17.6 % for IMTA treatment 1 and 2 respectively. Results of economic analysis using data from the two trials in this study demonstrated that IMTA yields better

economic returns compared to monoculture, and is therefore recommended for adoption in earthen pond mariculture systems along the Kenya coast.

6.1 Introduction

The economic importance of fish farming results from the value associated with the cultured species taking into consideration the external costs and benefits, the differences in profitability between the adopted practices such as IMTA or monoculture and also the startup costs (Nobre *et al*., 2010; Knowler *et al*., 2020). There has been an increasing demand on some of the species farmed in IMTA set ups such as sea cucumbers, oysters, finfish, and shell fish (Chopin 2021). This has opened up new markets for high value sea cucumbers in Asian markets, oysters for the tourist markets and food fish for the local and international market. IMTA is increasingly being recognized as a high yielding investment with high estimated annual returns, for instance an integrated shrimp, oyster IMTA yielded an Internal rate of return of 131.1 % (Fonseca *et al*., 2015). IMTA is therefore deemed suitable for coastal communities because besides requiring larger capital, it is less labour intensive due to production of more than one crop in a particular unit (Magondu *et al.*, 2021). Despite the socio-economic importance of IMTA in developing countries, Barrington *et al*., (2010) noted that the economics of fish farming enterprises applying IMTA are poorly known. A number of studies have investigated the economics of IMTA (Bunting and Shpigel, 2009; Kitchen and Knowler, 2013; Yip *et al*., 2017). Generally the results these studies indicate that costs and revenues of IMTA set ups vary according to the species combinations employed and the environmental conditions either pond or open sea (Carras *et al*., 2019).

Expansion of aquaculture is on the rise globally (FAO, 2020). This situation therefore necessitates adoption of technologies that minimize damage to the environment such as

IMTA which is growing interest as part of 'Blue transformation programme' that reduce nutrients release into the environment while enhancing overall productivity and economic gains for a sustainable aquaculture industry (FAO, 2022). Aquaculture has further been regarded as an alternative to reducing the widening gap between fish demand and supply (Flores and Pedroza Filho, 2019). Therefore encouraging creation of conducive economic circumstances, that allow technological advancement aimed at increasing production (Cardia and Lovatelli, 2015). The studied intensive monoculture systems have not satisfied the market demands besides being an available alternative for sustainability (Flores and Pedroza Filho, 2019). Reasons being the focus on few and expensive species that require heavy investment in terms of resources and production of undesirable wastes that deteriorate the environment. One option to improve sustainability in aquaculture is the application of IMTA technology since it raises the assimilative capacity of the farm Knowler *et al*. (2020) hence reducing the environmental cost of aquaculture. Further, integration of extractive species into the farm operations can contribute to increased farm profitability levels and provide new investment opportunities under the 'Blue Economy' concept.

IMTA is a sustainable aquaculture technology with economic benefits that have been investigated in a number of countries including; China, Japan and Canada (Ridler *et al* 2007). In IMTA, organisms from different trophic levels are cultured in a single system with organic and inorganic wastes from the fed organisms serving as nutritional inputs for others (Chopin et *al*., 2001). Use of IMTA technology in aquaculture has a number of benefits that contribute to the overall productivity and economic gains of a farm enterprise such as sediment quality (Magondu *et al.*, 2022) which has contributed to reduction in benthic ecological impacts that would otherwise impact negatively on the environment (Ridler and Ridler, 2011). IMTA further contributes to financial benefits through product diversification and fast production cycles as shown by Whitmarsh *et al*. (2006) and Shi (2013). A discounted flow analysis was used by Ridler *et al*. (2007) to assess IMTA profitability against salmon monoculture in Canada, the results showed that the IMTA set up resulted in higher financial returns as compared to monoculture.

In recent years, IMTA technology has increasingly become established with both sea based (open water and sheltered bays) and land based (ponds and tanks) applications being tried in different parts of the world and with different species combinations (Cranford *et al*., 2013; Largo *et al*., 2016; Balasubramanian *et al*., 2018; Zamora *et al*., 2018; Gamito *et al*., 2020;). However, environmental, production yields and economic benefits of IMTA are still not clear (Knowler *et al.*, 2020). In order to increase economic gains and contribute to environmental conservation, IMTA has proved to be one of the promising routes as it contributes to assimilation of expensive nitrogenous wastes converting them into valuable products that increases farmer profits, reduces wastage on fish feeds, improves the food conversion ratio, growth rates of culture organisms, creates opportunities for more jobs and above all reduction of environmental pollution (Magondu *et al*., 2021; 2022).

Different approaches are being applied to assess the sustainability of aquaculture practices. Nobre *et al*. (2010) compared the ecological and economic benefits between monoculture and IMTA in South Africa by applying the differential drivers-pressure-state-impact-response approach. Simulation method was used by Ridler *et al.* (2007a) to evaluate IMTA in Canada. Farm Aquaculture Resources Management (FARM) model was used by Ferreira *et al*. (2012) to evaluate production and environmental effects of gilthead bream farming in monoculture and IMTA setups. These studies have shown that IMTA is a sustainable culture model which is in consistent with our study applying the cost-benefit analysis to evaluate production in IMTA and monoculture setups.

The current state of capture fisheries resources shows that most of them have stagnated or are on the verge of collapse due to overfishing (FAO, 2018). On the other hand the global population is on the increase which poses a challenge to food security (FAO, 2020). Therefore, aquaculture being a fast growing animal production sector in the world could offer possible solutions through production of affordable protein using viable technological innovations. The performance demonstrated in this study is attractive to fishermen and farmers within the coastal rural communities as an opportunity of improving their socio economic welfare.

The aim of an aquaculture farmer is to maximize profits by cutting down the cost of production. The total profit received by the farmer is calculated by the quantity produced and sold multiplied by price per unit Wakibia *et al.* (2011) less the total cost of production. Economic viability is then determined at the point where the farmer breaks even and makes a profit. Application of IMTA in ponds in Kenya has shown positive ecological results which through synergies created, contributes to increased production, and hence profits. Using costs and revenues data from the trials, a Cost benefit Ratio (CBR) analysis was used to evaluate the profitability of IMTA versus monoculture as mariculture practices in intertidal earthen ponds along the Kenya coast.

6.2 Materials and methods

6.2.1 Collection of data used for economic analysis

Two trials were conducted in this study with experimental designs as described in sections 4.2.1 and 5.2.1. The size of the culture facilities used was uniform having measurements 12 x 10 m and 1 m depth for all the ponds used. Data on production costs and projected sales revenues were gathered so that the cost of production and revenue from the 120 m^2 experimental ponds could be estimated. The economic analysis data were collected by taking into consideration the initial costs at the beginning of the experiment which consisted of purchase of pond repair tools, hire of the culture ponds and purchase of juveniles of all the experimental organisms (*H. scabra, P. indicus, O. niloticus, A. Antiquata and S. cucullata).* Operational costs included, purchase inputs such as lime used for regulating the pH of the ponds, fertilizers mainly DAP and UREA used for promoting primary productivity of the culture water, fish feeds were purchased for the fed organisms in the different treatments. Other costs were incurred in payment of security services for securing the organisms from theft by human and other predators such as birds.

At the start of the experiment all the ponds were repaired before stocking with costs incurred being purchase of nets, spades, and labour cost. During the experimental duration, periodic repairs were conducted to maintain the culture facilities that were periodically damaged by tides by enhancing the dykes, replacing worn out screens and the predator nets. The total investments requirements were expressed in terms of capital assets and operating costs where the capital assets included; pipes, predator nets, spades, and compactors used for pond repair. While determining the costs and revenue, straight line method of annual depreciation was used for capital assets and were assumed to have no residual value at the end of their useful life (Shang, 1990).

6.2.2 Economic analysis

The revenue from sale of harvested organisms was based on the market price and average yield obtained in the culture ponds during the experimental period. Different indicators were used to determine the economic performance and profitability analysis including: net income (NI), total income (TI), total expenditure (TE), cost benefit ratio (CBR), and return on investment (ROI) adopted from Biswas *et al*. (2012) and Yuan *et al*. (2017) as follows:

NI=TI – TE

ROI=100*(Net income/Total investment)

CBR=TI/TE

The costs of equipment, materials, supplies and other inputs were based on prevailing market prices in the region. Return on investment (ROI) was the standard economic indicator used to evaluate investment feasibility in the present study according to Shang (1990). The ROI was calculated by dividing the annual net income from the ponds by the total investment cost. Economic sensitivity analysis was also performed to determine ROI on both IMTA and monoculture treatments as shown by Hurtado and Agabayani (2002).

An economic analysis was therefore carried out based on the developed IMTA system in comparison to the shrimp monoculture system in Trial 1 and IMTA combinations in Trial 2. Expenditure comprised of material input cost including the cost of the juveniles of all the test organisms, feeds administered, labour, fertilizers, lime, netting material, and any other operational costs like purchase of tools for pond maintenance, payment of security hire and pond attendant. The cost of production was estimated based on local market value price in Ksh and the current United States Dollar (USD) equivalence (1 USD =Ksh 110 in 2020 and 1 USD =Ksh 105 in 2021).

Produced harvest of shrimps and Nile tilapia was sold at the local market outlet and hotel while the sea cucumbers were sold to a local dealer for export. Cockles and oysters were harvested for sale at the farm gate while taking into consideration their market price. Total income from the crop produced was estimated by price of organisms sold. Net income was estimated by subtracting the total production cost from the total return. The costing did not include the initial pond excavations but rather pond renovations before start of the experiment and the routine farm operations. The outcome of the economic analysis was used to determine the viability of the culture systems.

6.2.3 Statistical analysis

Data used for economic analysis between the monoculture and IMTA treatment was done by independent sample T test for Trial 1 to test the equality of means for net income and CBR. One-way ANOVA with the General Linear Model (GLM) followed by post hoc Tukey HSD test were used for comparison of net income, CBR and ROI among the IMTA replicates under Trial 2. Statistical analyses was done using Excel version 2013 and SPSS statistical software IBM version 22. In all statistical testing, differences were considered to be statistically significant at $p < 0.05$.

6.3 Results

6.3.1 Production

The production values of the monoculture and IMTA treatment for trial 1 are presented in Table 20. Production of 20.57 ± 2.25 kg from *P. indicus* was obtained in monoculture treatment which was not significantly different from 22.84 ± 2.21 kg obtained in IMTA. Among the IMTA combination, organisms had a higher overall weight of 66.46 ± 4.24 kg and 97.51 ± 3.25 kg for the *H. scabra* and *A. antiquata* respectively over the 135 days culture period.

Table 20. Production of the cultured organisms in monoculture and IMTA treatment.

Production Kg / Treatment 135 days^{-1}		
Organism	**Monoculture**	**IMTA**
P. indicus	20.57 ± 2.25	22.84 ± 2.21
H. scabra		66.46 ± 4.24
A. antiquata		79.51 ± 3.25

Table 21 shows the production values for trial 2 that had a control and IMTA treatments. Treatment 2 that had a complete IMTA combination had the highest production from the fed species, *O. niloticus* at 45.03 ±4.5 kg. The production of *H. scabra* from IMTA treatment 1 was 24.47 ± 0.9 kg while IMTA 2 had 21.75 ± 2.3 kg which showed no significant difference P > 0.05. Production from *S. cucullata* was the lowest at 14.63 ± 1.08 kg and 13.44 ±0.32 for both IMTA treatments 2 and 3 respectively.

Table 21. Production of the cultured organisms in control and IMTA treatments in trial 2.

Treatment	Test organism	Production (Kg/treatment 150 days^{-1})
Control	*O. niloticus*	33.78 ± 1.6
Treatment 1	*O. niloticus*	38.80 ±2.4
	H. scabra	24.47 ± 0.9
Treatment 2	*O. niloticus*	45.03 ±4.5
	H. scabra	21.75 ± 2.3
	S. cucullata	14.63 ± 1.08
Treatment 3	*O. niloticus*	35.40 ± 2.4
	S. cucullata	13.44 ± 0.32

6.3.2 Economic comparisons for Trial 1

Costs and revenues

The total initial investment for the monoculture and IMTA treatment was estimated at USD 536 and USD 793 respectively (Table 22). This amount covered the capital assets and initial operating costs needed for production. The annual depreciation was USD 55 computed by a straight line method based on the estimated economic lives of the supplies and materials (Wakibia *et al.*, 2011)

Table 22. Initial investment and annual depreciation for one monoculture and IMTA pond in Kenya (Kshs 110=US$ 1 in 2020).

Item	Quantity	Unit cost (USD)	Total cost (USD)	Economic life (years)	Annual depreciation
Capital Assests					
Spades	10	2.7	27	2	14
Pipes	5	4.09	20	5	4
Predator nets	1	50	50	2	25
Compactors	5	5	25	2	13
Sub total			**123**		**55**
Operating cost monoculture pond			413		
Operating cost IMTA pond			670		
Total initial investment Monoculture pond			**536**		
Total initial investment IMTA pond			**793**		

The incurred costs and gained revenues for the monoculture and IMTA treatment in trial 1 are presented in Table 23. A partial enterprise budget for IMTA and monoculture treatments in Trial 1 is shown in table 24.The operating costs was USD 413 and USD 670 for Monoculture and IMTA treatments respectively. It was based on cash and non-cash expenses based on averages obtained from one culture cycle of the fed species (marine shrimp). Labour was the highest operating cost that contributed to over 40% of the production cost for both monoculture and IMTA treatments at USD 181.82. Labour costs included the initial pond dyke renovations, repair of pipes and installation of predator nets, hired labour during sampling, pond maintenance, and security hire.

Economic analysis was performed to evaluate the net income, cost benefit ratio and return on investment between the shrimp monoculture ponds and the IMTA ponds according to Biswas *et al*. (2012). The analysis showed that net income, CBR and ROI were higher in IMTA ponds as shown in Table 24. There was 84.7 %, 41.9 %, and 5.2 % increase in net income, CBR and ROI respectively in the IMTA than the monoculture treatment. This was as a result of increased production and higher selling price of the harvested organisms contributed by higher profitability in IMTA system. The monoculture treatment did not break even and had a value of USD (300.63) while the net income achieved from the IMTA treatment was USD 514.63. The calculated CBR was 1.77 and 0.27 from the IMTA and monoculture treatment respectively. This was as a result of increased production that attracted good prices. Return on investment for the IMTA was 76.9 % while the monoculture treatment showed a negative value of (72.6 %).

Table 23. Expenditure and economic returns in monoculture (T1) Versus IMTA (T2) systems. Values for total income, net income, and cost benefit ratio represent means of three replicates over the 135 days culture period.

		T1					T2			
		Expenditure		**Income**			**Expenditure**		**Income**	
Items			**USD**	**Items**	**USD**			**USD**	**Items**	**USD**
stocking biomass	*P. indicus*	1800 x 0.05 =	82	20.6x 5.45 =	112.36		1800 x 0.05 =	82	22.8x 5.45	124.26
	H. scabra	-	-	-	-		435 x 0.36 =	157	46.48x18.18	845
	A. antiquata	-	-	-	-		1260 x0.09 =	113	80 x 2.72	217.6
Labour			181.82					181.82		
Lime			38.63					38.63		
Fertilizer			31.81					22.72		
Feeds			33.45					29.81		
Others			45.45					45.45		
Total expenditure (TE)			413.16	Total income (TI)	112.36	Total expenditure (TE)		670.43	Total Income (TI)	1186.86
Net income		TI-TE			-300.8					516.43
Cost Benefit Ratio (CBR)		TI/TE			0.27195					1.770297
Return on Investment (%)		NI/TE*100			-72.6					76.9

Calculation for 135 days culture period in US Dollar (1USD=110 Kenya currency)

Table 24. Enterprise partial budget for monoculture and IMTA treatment. Costs and prices are given in US$. Calculation for 135 days culture period.

BENEFITS		**Value (USD)**	
		Monoculture	**IMTA**
Income from	*P. indicus*	112.36	124.26
production	*H. scabra*		845
	A. antiquata		217.16
Total income		112.36	1186.86
COSTS			
Purchase of juveniles		82	352
Labour		181.82	181.82
Lime		38.63	38.63
Fertilizer		31.81	22.72
Feeds		33.45	29.81
Others		45.45	45.45
Total Costs		413.16	670.43
Net benefits		**-300.8**	**516.43**

6.3.2 Economic comparison for Trial 2

An economic analysis was performed to evaluate the net returns and cost benefit ratio between the control ponds with Nile tilapia and the IMTA ponds that had different treatment combinations. The analysis showed that, net income, and CBR were significantly better ($p < 0.05$) in T2, T1, and C as shown in Table 24. T1 and T2 had similar CBR which was higher than the control ($p < 0.05$). Net income was highest in the IMTA T2 with a value of USD 77.5 followed by IMTA T1 that had a value of USD 33.9. This was as a result of increased production and higher selling price of the harvested organisms in T2 and T1 that contributed by higher profit in IMTA system. T3 and control had the lowest income which did not breakeven with USD values of (193) and (209) respectively. The calculated CBR was 1 for both IMTA T1 and T2, a zero value for T3 and 0.4 for the control. The determined return on investment was highest in T2 at 17.6 % while IMTA T1 had 8.6 %. IMTA T3 and the control showed negative rates.

Table 25. Operational costs and economic returns among monoculture (C) and IMTA treatments (T1, T2, and T3). Calculations were for 150 days experimental duration. Currency indicated in USD (1USD=Ksh 105).

Items	Quantity	Price Rate (USD)	C	T1	T2	T3
Operational costs						
Oreochromis niloticus seed	240	0.19	45.7	45.7	45.7	45.7
Holothuria scabra seed	250	0.28		71.4	71.4	
Sacostrea cucullata seed	230	0.14			32.8	32.8
Lime ($CaCo_3$)	120kg	0.21	25.1	25.1	25.1	25.1
Fertilizer	DAP 4.8 kg	0.62	3.01	3	3	3
	UREA 7.2 kg	0.51	3.7	3.7	3.7	3.7
Feeds for different treatments	117.5 kg,88.8 kg, 106 kg,107kg respectively	1	118	88.8	106	107
Pond hire	2	28.5	57	57	57	57
Casual labour	5	19.04	95	95	65.2	95
Total operational Cost			347.4	390	440	370
Economic returns						
O. niloticus advanced fingerling sale	300,220,180,200 fingerlings respectively	0.19	57	42	34.2	38
Sale of harvested *O. niloticus* from different treatment	34kg,38.8kg,45kg,35.4kg respectively	2.4	81	93	107.1	83.3
H. scabra sale	24.4kg, 21.7 kg respectively	19.04		289	325	
S. cucullata sale	13.4kg,14.6kg respectively	3.8			51.04	55.6
Gross return			138[d]	460[b]	481.6[a]	177[c]
Net income (TI-TE)			-209	33.9[b]	77.5[a]	-193
Cost Benefit Ratio (CBR) TI/TE			0.4[b]	1[a]	1[a]	0
Return on Investment (%)	NI/TE*100		-60.2	8.6	17.6	-52.1

6.4 Discussion

The studies reported in this thesis are the first to report on pond integrated culture of *H. scabra*, with *P. indicus* and *A. antiquata* and also the combination of *H. scabra, O. niloticus* and *S. cucullata*. The approach employed is viable with a number of potential benefits to farmers which include; (i) improved yield and income through production of sea cucumbers, cockles and oysters that benefit from the nutrients generated in the culture system (ii) decreased reliance on external inputs such as nutrients from fertilizer applications and feeds hence lowering down the cost of production from aquaculture.

For Trial 1, shrimps in the IMTA treatment showed slightly higher production (22.84 ± 2.21 kg) compared to those in monoculture treatment (20.57± 2.25 kg). This could be attributed to efficient nutrient utilization where nutrient waste, feacal waste and uneaten feeds were extracted from the system by the extractive organisms such as algae, filter feeders and deposit feeders (Samocha *et al*., 2015). Under Trial 2, the full IMTA set up treatment 2 showed higher production weight of Nile tilapia, sea cucumbers, and oysters at 218.82 ± 1.55 g, 206.87 ±3.02 g and 68.4 ± 0.05 g respectively.

6.4.1 Economic performance

The current study found that IMTA had a higher net income, cost benefit ratio and return on investment than either of the monoculture operations in both trial 1 and 2. Despite these encouraging results, IMTA is likely to be justifiable for investors only if there is additional profitability (Whitmarsh *et al*., 2006; Ridler and Ridler, 2011; Ridler *et al*., 2011). Whitmarsh *et al.* (2006), Ridler *et al.* (2007), and Shi *et al.* (2013) all suggested that higher profitability is possible with IMTA than with monoculture aquaculture farms, ascribing it to higher growth rates of co-cultured extractive IMTA species, the ability to spread some of the IMTA's administrative and operational expenses over a wider range of products (e.g. marketing and sales costs, salaries and wages, utilities), and access to additional income streams.

Clearly, economic sustainability is a key objective to be considered before venturing into commercial aquaculture practices. Hishamunda *et al*. (2014) showed that without economic viability, aquaculture ventures can only continue if subsidized. Higher profitability in IMTA farms has contributed to its more preference to monoculture practices by farmers attributing it to higher growth rates of the co-cultured extractive species, possibilities of spreading costs over a wide range of products in addition to access to different income streams (Whitmarsh *et al*., 2006; Ridler *et al*., 2007b; Shi *et al*., 2013). Presence of co-cultured items in an aquaculture enterprise can contribute to water quality improvement, additional productivity, and profitability of the farm operation (Fantini-Hoag *et al*., 2022).

6.4.2 Economic performance for Trial 1

The total economic benefit and yield of a fish production system depends on the choice of fish or crop selected (Wang *et al*., 1999). In trial 1 of this study a marine shrimp monoculture was compared with an IMTA model using marine shrimps, cockles and sea cucumber which are detritivore in nature. The choice of the species combination was key as they assimilate waste into their bodies hence generating a significant saving in waste treatment cost and in addition serving as valuable products which do not require much feed intake during their culture. Results of economic analysis portrayed viability of the IMTA system. On the other hand, most of the mariculture farmers along the Kenyan coast use their own resources such as seed collected from the wild, land, labour, organic fertilizers thus the actual input cost would be lower and net returns higher in farms that use on-farm resources (Uddin *et al*., 2009; Biswas *et al*., 2012).

6.4.3 Economic performance for Trial 2

A comparative economic performance analysis between three IMTA treatments (TI, T2, T3) and a control monoculture (C) made in the second trial study indicated the possibilities of farming a fin fish, oysters and sea cucumber together in brackish water intertidal ponds. One complete (T2) and two partial IMTA treatments (T1 and T3) were tested in this study in comparison to fin fish monoculture. The full IMTA (T2) performed better in terms of production and economic returns as compared to the other treatments. Gross returns, net income, and cost benefit ratio (CBR) showed significant differences with highest values in T2 followed by T1. This was as a result of increased total production and higher selling price of harvested fin fish and sea cucumbers that had better weight gain in T2. In addition the estimated market price of oysters increased the net income further for T3. The results of economic analysis show the viability of the IMTA system as a suitable farming option. Under the two scenarios, the analysis revealed that the IMTA system was more profitable, looking at the benefits of product diversification, reduction of production risk and reduced cost of inputs. Review studies by Knowler *et al*. (2020) showed that economics of IMTA has been mainly focusing on financial analysis on profitability, economics of environmental externalities and market analysis on consumer perceptions and acceptability of IMTA systems and products.

6.5 Conclusion

The production costs, net incomes, CBR and ROI were higher in IMTA set ups. The species combination and pond environment were important factors for increasing production suggesting more attention should be paid to these parameters for more economic gains. The main production challenges identified were: predation and limited suppliers of seeds which caused price hike. In this study we used the cost benefit analysis to assess the sustainability of monoculture and IMTA at a community mariculture farm in Kibokoni Kilifi creek of Kenya. The CBR was high for IMTA T2 and T3 that indicated IMTA production system is a profitable business. IMTA showed sustainability from both environmental and economic aspects than the monoculture set up tested during the study period.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS, AND FUTURE DIRECTIONS

7.1 IMTA concept

The IMTA concept works to achieve sustainable aquaculture development whereby targeted species are cultivated with others having different trophic levels which ensures recycling of aquaculture wastes and food resources (Neori *et al*., 2017). IMTA ultimately aims at balancing production with environmental sustainability, it promotes economic stability through product diversification and risk reduction and further encourages social acceptability through better management practices (Troell *et al*., 2003; Chopin, 2006). In IMTA, the wastes generated by the target species like fish or shrimp becomes food for other species for instance suspended organic substrates in the water and from the sediment are used by the filter feeders which are mostly the bivalves. Large particles of organic matter that sink at bottom of the system are consumed by the deposit feeders which are mainly sea cucumbers and polychaeta worms in most IMTA applications. Plants and algae in the system consume dissolved inorganic substances released by the cultured organisms or released from the sediment through mineralization and exchange of culture water. Zhang *et al*. (2019) indicated that bio mitigation in IMTA contributes to assimilation of wastes and possible pollutants which could cause adverse effects to the system.

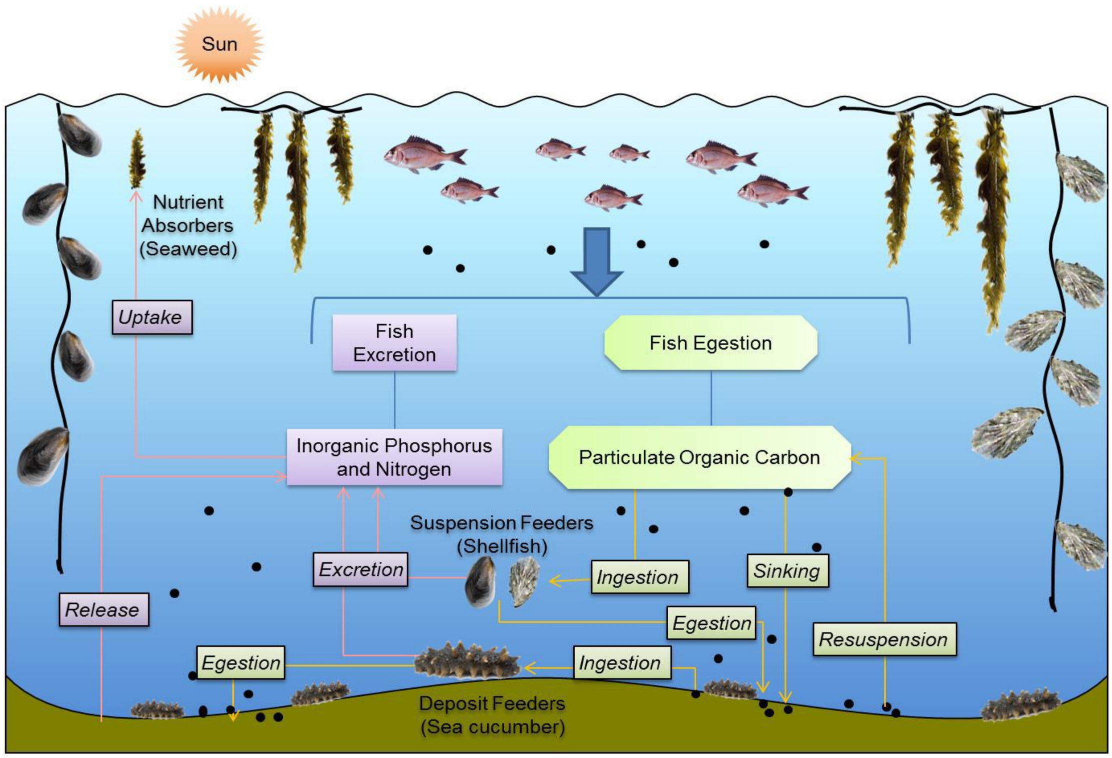

Figure 15. IMTA concept (Source*; Zhang et al., 2019*)

Figure 15 shows particles of suspended waste sinking to the bottom of the culture environment where they are consumed by deposit feeders which eliminates anoxic conditions in the pond bottoms and also through bioturbation by sea cucumbers the nutrients are recycled efficiently. Suspension feeders which are mainly shell fishes utilize free floating substances in the system. While the plants absorb nutrients in form of nitrogen and phosphorus from the system.

7.2. Contribution of IMTA to growth and yield of cultured organisms

The analysis of growth and yield parameters for the conducted trials showed feasibility of integrating shrimps, bivalves and sea cucumber under trial one. Marine shrimps which were used as the fed species achieved a growth of 15.76 g and a specific growth rate (SGR) of 1.99%. Sea cucumbers had a weight gain of 175 g and a SGR of 4.1% while cockles had a net weight gain of 44 g and a SGR of 3.25%. The growth achieved contributed to increase in net yield in the IMTA set up and better economic gains. The higher yield showed that the organisms cultured could efficiently utilize the nutrients extracted from the system (Samocha *et al.*, 2015).

In the second IMTA trial that used tilapia as fed component, an equally good growth was achieved with tilapia reared in IMTA treatment having a significantly high final body weight as compared to the control monoculture experiment. Sea cucumber reared in the integration had an equally higher body weight but survival was low due to significant predatory attacks from tilapia that caused mortality to sea cucumbers. The severity of the attacks showed that use of tilapia for integration in IMTA was not viable which agreed with studies by Tresnati *et al*. (2019). Oysters (*S. cucullata*) were used as filter feeders in the IMTA systems in trial 2, in this study they did not show any significant growth but contributed to the biomass and profitability of the system.

Application of IMTA influenced pond productivity, the performance of the fed species was better in IMTA ponds as compared to monoculture ponds. The production realized from both tilapia and shrimps being the fed species in both trials was high which was contributed by good nutrient utilization that boosted efficiency in the ponds. Blackish water ponds dominate the coastal fish and shrimp aquaculture in the region with high practical application at the community level. It was therefore less complicated to understand and introduce the concept of integration at the experimental site for this study.

7.3 Contribution of IMTA to water quality and plankton availability

The physico-chemical parameters studied in both IMTA trials were within the required optimum range for shrimp and tilapia culture in brackish water environment (Chakraborti *et al*., 2002; Biswas *et al*., 2019). Recycling of nutrients by the cultured organisms positively influenced water quality (Milstein *et al*., 2003). The inorganic nutrient variable of water improved in the IMTA system in comparison to the monoculture with more effective removal of both inorganic nitrogenous and phosphate-phosphorous concentrations from the culture water (Martinez-Cordova and Martinez-Pochas, 2006; Biswas *et al*., 2019). The species combinations in both studies contributed to pond ecological balance which enhanced nutrient circulation. In pond culture systems, phytoplankton are regarded as most important component for fixing energy and fueling the food web (Asaduzzaman *et al*., 2010). Productivity of phytoplankton was positively correlated to nutrient concentrations in ponds according to research by Boyd (1990). In this study, phytoplankton abundance was generally higher in IMTA treatments as compared to monoculture.

Summary comparison of results of IMTA and monoculture

i. The IMTA model performed better in terms of production, economic returns, water quality, and ecological benefits as compared to the monoculture system.
ii. Sea cucumbers in the IMTA treatment under trial 1 grew bigger and faster at a SGR of 4.1% and NWG of 175 g in 135 days of culture.
iii. Phytoplankton community structure in this study concur with Qiao *et al*., 2020; Cunha et al., 2019, where Bacillariophyceae were found dominant in IMTA environments as compared to monoculture.
iv. Macroinvertebrates biomass estimations by Gamito *et al.*, 2020 working on pond IMTA experiments found similar invertebrate fauna to the results of the present study.
v. Zooplankton density in IMTA ponds were found to have higher densities which was comparable to studies by Biswas *et al*., 2019 as compared to monoculture that had low densities.

vi. Benthos play key role in sediment processes influencing the C and N contents in the systems agrees with (Zhang et al, 2010). They were found to be higher in IMTA as compared to monoculture.

vii. In the second trial, IMTA T2 showed high production for all culture species due to efficient nutrient utilization; agreeing with studies by (Samocha *et al.*, 2015).

viii. Tilapia showed good performance in the IMTA treatments of trial 2 as compared to other species while *H. scabra* production was low due to predation attacks.

ix. Water quality parameters were better in IMTA experiments as compared to monoculture which was comparable to (Biswas *et al* 2019).

x. Gross return, net return, and benefit cost ratio (BCR) were significantly different with highest values in IMTA as compared to the monoculture for both Trial 1 and Trial 2.

7.4 Contribution of IMTA to sediment quality and benthic community structure

Sediment quality parameters investigated in the current study were total organic carbon, pH, total phosphorous and total nitrogen. The composition of these parameters showed lower levels in IMTA treatment as compared to monoculture treatment**.** Sediment in aquatic systems acts as a sink for nutrients, organic compounds, and solutes that result from accumulation of organic matter (Woodruff *et al*., 1999). The nutrients in sediment results from a contribution of faecal waste, wasted feeds, dead organisms among the cultured ones and detritus from sinking phytoplankton and pond macrophytes. Adamek and Marsalek (2013), indicated that movement of nutrients in form of carbon, phosphorous and nitrogen from the sediment to overlying water as a result of decaying and mineralisation process occurs often in aquatic environments. The amount of sediment carbon and nitrogen present in benthic compartment is therefore as a result of the rate of sedimentation and remineralization of the present sources (Ren *et al*., 2012). In the present study, the mineralization could have contributed to the lower total organic carbon and total nitrogen values shown under IMTA treatment which had *Holothuria scabra* that contributes to bioturbation affecting transport of nutrients mainly phosphorous and nitrogen through the sediment–water interface (Brönmark *et al*., 2005). Benthic organisms such as chironomids, oligochaetes, and crustaceans among many others also cause sediment bioturbation during their mechanical activities which also affect exchange of dissolved substances across sediment water interface (Zhang *et al.,* 2010). Certain species like the chironomid also contribute to aiding nutrient cycling (Jiang *et al*., 2010) while feeding at the surface and excreting at the bottom causing transport of nutrients in form of carbon, nitrogen, and phosphorus.

7.5 Nutrient retention efficiency in IMTA system

In order to achieve a circular economy in food production, systems are striving to minimize energy and nutrient losses and maximizing on resource use efficiency by closing the nutrient loop (Commission, 2015). Thus therefore contributing to the idea behind the IMTA approach where recycling of wastes which are nutrients in the system results in less nutrients being released to the environment while in return contributing to the productivity of the system (SAPEA, 2017). Nutrient removal efficiency depends on the farming techniques used, the quality of the wastes generated, the nutrient determination methods and the organisms under culture (Nederlof *et al*., 2021). Study by Schneider *et al.* (2005) reported nutrient removal efficiency varying between 2 to 100% for extractive species. In this study, pond systems were used thus causing partial environmental influences. In practical scenarios closed systems like recirculating aquaculture systems (RAS) and open systems like sea cages, ‘the environmental influence can be fully controlled for the former and very limited control can be done to the latter' (Nederlof *et al.*, 2021). To estimate nutrient retention efficiency in a pond, quantification of the wastes generated is done in comparison to the responses of extractive species which take up different quantities of wastes released by the fed species. Retention efficiencies reported for marine fishes range between 14% and 38% for C, 13% and 43% for N and 18% and 36% for P (Nederlof *et al*., 2021). The nutrients not retained by the fed organisms become input for the extractive component that consists of filter feeders which take up particulate matter suspensions, deposit feeders which consume organic substrates at the pond bottom while algae take up inorganic nutrients. Figure 16 shows a schematic representation of nutrient fluxes within a pond-IMTA system including tilapia or shrimp as fed organisms, cockles or oysters, and sea cucumbers as extractive species.

Studies by Nederlof *et al*., (2021) showed that feacal material and uneaten feeds make up the particulate organic matter (POM) waste in culture systems whereby 6-44% C, 5-45% N and 42-47% P in feeds are released as POM. In the culture system when POM breaks it results to dissolved organic matter (DOM). POM is normally divided into two forms; suspended solids with large particles that settle at pond bottom and suspended particulate matter (SPM) which is usually free floating in the culture water (Olsen and Olsen, 2008).

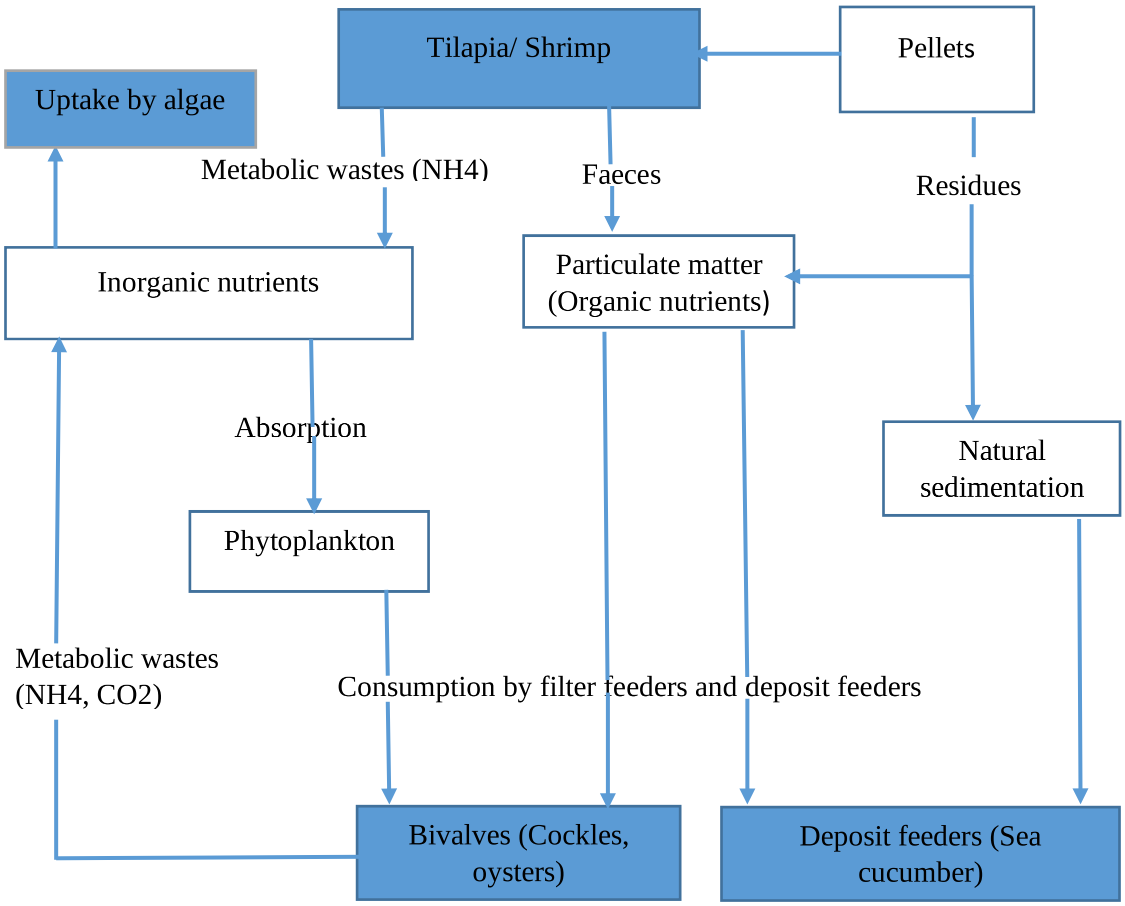

Figure 16. Schematic representation of nutrient fluxes within a pond-IMTA system showing the fed and extractive components.

Bioremediation potential of extractive species has been determined by i) measuring the nutrient removal rate such as feeding and assimilation efficiency (Yu *et al*., 2014 and Fang *et al*., 2017), ii) nutrient retention in biomass measured in IMTA systems and their controls (Jiang *et al*., 2012; Yu *et al*., 2014; Tolon *et al*., 2017) and iii) use of balance method where water flows and nutrient concentrations in sediments are determined (Marques *et al*., 2017).

Bivalves capture POM from the culture system through two different routes; either directly wastes in form of feacal material and uneaten feed particles and indirectly by filter feeding on plankton grown from inorganic waste (Lefebvre *et al*., 2000). In the current study cockles and oysters were used as filter organisms for the different trials (Chapter 4 and Chapter 5 respectively). Both retention and balance method of showing bioremediation potential of the bivalves used in the IMTA trials were applied. In the first trial the balance method was applied by determining quality of water and sediments in the IMTA set up in comparison with the control. The analyzed results gave an indication of good quality sediments in terms of organic carbon, pH, total nitrogen, and total phosphorous and water quality nutrients such as phosphates, nitrate nitrogen, ammonia nitrogen and nitrite nitrogen.

In many IMTA systems sea cucumbers and polychaeta have been used as deposit feeders meant to take up the settled particulate organic matter in the system. Sea cucumbers are deposit feeding organisms and have shown potential to consume and control accumulation of organic nutrients in sediments, they contribute to recycling of nutrients through bioturbation where they bury into the sediment. Purcell *et al*., 2012; Cubillo *et al*., 2016; Zamora *et al*., 2016 and Li *et al.* (2019) showed that deposit feeders have the potential to mitigate organic pollution by their capacity to rework 10,590 kg dry sediment m^2 /year while selectively feeding on enriched sediments. Studies by Yuan *et al*. (2006) and Zamora and Jeffs (2011) showed waste removal rate by sea cucumbers through increased consumption rate. In the current study, sediment quality showed lower organic carbon in IMTA which could have resulted due to decreased organic matter from the substrate. The recorded weight gain from sea cucumber was also an indication that the organisms fed on the substrates or organic matter in pond sediment. However, the composition of gut content was not done in this study and could be necessary to give a clear indication on what the sea cucumbers fed on. Other benefit achieved from the deposit feeders is bioturbation which facilitates decomposition and further nutrient recycling (Mactavish *et al*., 2012). Sea cucumbers have reported an assimilation efficiency rate of 14% to 88% (Nederlof *et al*. 2021) which takes place in integrated systems. Besides waste bio-mitigation potential by sea cucumbers, their high economic value contributes to recommendations for use in IMTA, (Purcell 2015) because the organisms fetch good market prices in Asia.

7.6 Environmental benefits of IMTA

Aquaculture intensification requires high levels of inputs in terms of feeds and fertilizers into the culture systems. Studies by Edwards (1993) and Avnimelech (1998) showed that approximately 80% of nitrogen and phosphorus from these inputs is accumulated in pond sediment with the remainder being removed by the cultured organisms. Cumulative deposition of the nutrients has shown to cause stress and diseases to the farmed organisms (Anka *et al*., 2013). In addition, wastes in form of feacal material and uneaten feeds and inorganic compounds of nitrogen and phosphorous have resulted into environmental degradation of water bodies (Haque *et al*., 2016). Competition for space has also been a limiting factor in coastal areas necessitating the need for optimization through innovations that are sustainable and have less environmental impact. Sustainability of the environment is a major consideration in IMTA practices as it guides on species selection and combinations for suitability in the culture units**.**

Application of IMTA has demonstrated processes like bioremediation that minimize environmental impacts whereby extractive organisms take up excess organic and inorganic wastes from the system (Ridler *et al*., 2007). In addition through IMTA, fish farm waste is taken up and converted into marketable biomass by fed species co-cultured with extractive organisms. This maximizes production and at the same time reduces the environmental impact of intensive farming (Chopin *et al*., 2012).

In the present study, recorded physico-chemical parameters under trail 1 were within the required optimum range for shrimp culture in brackish environment. The tidal water exchange, initial application of lime and monthly fertilizer application resulted to the optimal water quality in the culture systems. In instances of extreme weather, lime acted as a buffer to regulate the alkalinity levels in the culture environment. The analyzed nutrients within the culture environment improved in the IMTA system in comparison to the monoculture with more effective elimination of both inorganic nitrogenous and phosphate-phosphorous concentrations from the culture water. The presence of extractive organisms might have also enabled uptake of particulate organic matter through filter feeding and consumption of organic matter from the pond bottom as demonstrated by Chang *et al*. (2020). In both systems natural growth of microscopic aquatic plants provided a possible mechanism for extraction of inorganic nutrients from the culture environment. The effective role of bioremediation was played by the sea cucumbers which fed on organic matter deposited on the pond bottom which further improved nutrient recirculation through bioturbation (Purcell, 2004). The study deduced that mineralisation of nutrients takes place causing flux of nutrients from underlying sediment to overlying water which could have contributed to the lower values shown under IMTA. On the other hand, nitrogen fumigates from ponds during natural processes of nitrification of $N\text{-}NO_3$ and denitrification to N_2 retaining only a small amount of nitrogen compounds in sediment (Knosche *et al*., 2000)

7.7 Economic benefits of pond IMTA

Intensification in aquaculture has shown negative environmental implications. However, through application of IMTA it has led to increased productivity through the synergies created and the potential to grow diversified aquaculture products leading to increased productivity. Studies have shown that protein is the most costly component in aquaculture of which 75 % ends up as waste (Neori *et al*., 2019). Algae recaptures the waste nutrients in form of nitrogen, carbon, phosphates, and micronutrients and recycles them back into useful protein biomass (Azim *et al.,* 2008). It is therefore easy to deduce that the synergy created during waste treatment and algae production improves the profitability of an integrated farm that consists of two or more culture species combinations like shrimps or fish algae, bivalves and deposit feeders. The profitability and cost effectiveness of IMTA technology is therefore as a result of (i) Pond productivity which other organisms in the culture environment depend upon (Chapter 4) (ii)Sustainability of IMTA with low trophic level fish that is commercially acceptable (Chapter 5) (iii)Nutrient utilization and recycling of waste provided by the IMTA technology (This thesis).

7. 8 Conclusion and recommendations

The findings of this study exhibited that IMTA system benefited monoculture marine shrimp farming by achieving; (1) high profitability and improved economic output through integration and cultivation of extractive species which assimilated excess nutrients maintaining a balance in the system, (2) reduced risks because of species diversification, (3) bio-mitigation of the ecosystem through absorption of organic wastes by the extractive species, (4) lowered production costs due to decrease in use of feeds and fertilizers.

Under integration different species combinations were involved to play specific trophic roles. IMTA involves maintaining a clean culture environment at reduced cost to facilitate improved efficiency while supporting sustainable production. The first IMTA trial involving marine shrimps as fed species with cockles and sea cucumbers as extractive organisms proved an appropriate low intensive culture system toward sustainable development of mariculture in earthen ponds. Second IMTA trial, Nile tilapia was used as the fed component of the system contributed to the overall production of the system in addition to providing food and energy that the co-cultured oysters and sea cucumbers depended upon. However, sea cucumbers experienced predation attacks from the tilapia that led to low survival rates nevertheless, the specific growth rates and weight gain achieved from sea cucumber suggests possible contribution from consumption of organic matter from the pond bottom. Therefore, the findings of this study exhibited that IMTA involving Nile tilapia as fed species with oysters and sea cucumbers as extractive organisms is not viable due to predation attacks.

The adoption of IMTA using a combination of the above species, to achieve the objective of producing protein food sources in the form of fed species and other marine products from the extractive species, will have a positive impact in the way aquaculture is being done in Kenya. The choice of fed component species is driven more by its low maintenance cost, high consumer demand, availability of seed and high market value than most fish currently in culture such as milkfish. Anecdotally, aquaculture production has intensified though causing several negative impacts such as deterioration of water and sediment quality of ponds, stress, and poor growth of fish, a low profit margin, and overall environmental degradation. Against this backdrop, IMTA has shown a potential for species diversity, with a traditional food fish (tilapia), shrimps, bivalves and sea cucumbers harvested from the same pond. The diversity of production increase pond productivity greatly, keeping the environment optimum according to IMTA principles. This suggests that IMTA could be promoted through action research with potential pond farmers towards sustaining food security of the people.

In this study we used the cost benefit analysis to assess the sustainability of monoculture and IMTA at a community mariculture farm in Kibokoni Kilifi creek of Kenya. IMTA showed significantly higher sustainability from both environmental and economic aspects than the monoculture set up tested during the study period. This study supports successful application of IMTA in earthen ponds in Kenya. Though IMTA is certainly not a panacea for mariculture development at the coast region, but it needs to be looked into as possible option among others that can facilitate Blue Economy development.

7.9 Future research direction

This pioneer IMTA study has laid the foundation for developing IMTA systems in Kenya, based on the positive results that were achieved in a pond system. Moving forward the next steps towards implementing IMTA in Kenya would be:

1) Addressing the challenge of seed supply to farmers who currently entirely rely on wild collected seeds like marine shrimps from the mangrove channels through efforts towards hatchery production of seeds of species to be cultured in IMTA set ups such as sea cucumbers, oysters and Nile tilapia.
2) Identifying suitable sites for IMTA field trials using data generated from this study as baseline information and further considering important aspects that are vital in aquaculture like the impacts of the IMTA system to the environment, spatial management especially when considering offshore IMTA systems.
3) Commercialization of IMTA produced organisms by conducting further research on the nutritional benefits, yield quality and further identifying new potential uses, markets and the value of integration.

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APPENDICES

Appendix 1. Summary data on individuals stocked and harvested in monoculture treatment in trial 1 during the 135 days culture period

Pond	Organism	No. Stocked 8.8.2020	No. harvested 11.12.2020	Mortality	Survival%
1	***P. indicus***	600	455	145	75.83
5		600	432	168	72.00
8		600	424	176	70.67

Appendix 2. Summary data on indivduals stocked and harvested in IMTA treatment in trial 1 during the 135 days culture period.

Pond	Organism	No. Stocked 8.8.2020	No. harvested 11.12.2020	Mortality	Survival%
3	***P. indicus***	600	410	190	68.33
6		600	512	88	85.33
7		600	520	80	86.67
3	***H. scabra***	145	60	85	41.38
6		145	90	55	62.07
7		145	95	50	65.52
3	***A. antiquata***	420	256	164	60.95
6		420	320	100	76.19
7		420	358	62	85.24

Appendix 3. Confirmation and approval of PhD research proposal

Pwani UNIVERSITY

OFFICE OF THE DEAN SCHOOL OF GRADUATE STUDIES

Internal Memo

TO: Esther W. Magondu
Reg. No: SG85/PU/36068/19

FROM: Dean, School of Graduate Studies

REF: PU/ST/EWM/36068/4

DATE: 1st October 2020

RE: CONFIRMATION & APPROVAL OF PhD RESEARCH PROPOSAL

We are pleased to inform you that your PhD research proposal has been reviewed and approved by the Ethics Review Committee. You are therefore advised to proceed with your research. Attached find the copy of the ERC approval and the certificate.

Please note, that you are expected to file progress report of your work every four months. The progress report form can be downloaded from the PU – Website.

You can collect your original certificate of approval from the Chairman of Ethics Review Committee office. (Room 367)

Best wishes in your research.

Thank you.

PWANI UNIVERSITY DEAN 06 OCT 2020 SCHOOL OF GRADUATE STUDIES P.O. Box 195-80108, KILIFI

Prof. Mlewa C. Mwatete, PhD
DEAN: SCHOOL OF GRADUATE STUDIES

Cc -Dean, School of Pure & Applied Sciences
-Chair, Department of Biological Sciences

Appendix 4. Research Permit from KWS

KENYA WILDLIFE SERVICE

KWS/BRP/5001

17 November 2020

Esther Wairimu **MAGONDU**
Pwani University
Department of Biological Sciences
P. O. Box 195 - 80108
KILIFI, KENYA
Email: estherwairimu82@gmail.com

Dear Esther

PERMISSION TO CONDUCT RESEARCH ON THE PROJECT "*APPLICATION OF AN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM IN ENHANCING PRODUCTION AND PROFITABILITY IN SMALL SCALE MARICULTURE ENTERPRISES IN KENYA*"

We acknowledge receipt of your application requesting permission to conduct your PhD Project on ***"Application of an Integrated Multitrophic Aquaculture System in Enhancing Production and Profitability in Small Scale Mariculture Enterprises in Kenya".*** It is considered that, the study will generate important information for the country on the culture of marine shrimps, cockles, sea cucumbers, and seaweeds to enhance overall production and profitability in small-scale mariculture in Kenya.

You have been granted permission to conduct your research for the period **December 2020 - November 2021** as agreed upon under the Prior Informed Consent (PIC). However, this approval is subject to meeting the following terms and conditions:

That you will pay to KWS, Research fees of **KShs. 12,000** (PhD. study);
That you will discuss your research project with the KWS Principal Scientist in-charge of Coast Conservation Area before embarking on your field work;
That you will abide by the set KWS regulations regarding the carrying out of research in and outside Wildlife Protected Areas and
That you will submit to the Director, Biodiversity, Research and Planning, annual reports on your findings and at the end of your research, a copy of your thesis.

Yours

PATRICK OMONDI, *PhD, OGW*
DIRECTOR
BIODIVERSITY RESEARCH &PLANNING

Copy to:

Principal Scientist, Coast Conservation Area
AD – Coast Conservation Area

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Appendix 5. Research license from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 257298

Date of Issue: 14/January/2021

RESEARCH LICENSE

This is to Certify that Ms.. Esther Wairimu Magondu of Pwani University, has been licensed to conduct research in Kilifi on the topic: APPLICATION OF AN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM IN ENHANCING PRODUCTION AND PROFITABILITY IN SMALL SCALE MARICULTURE ENTERPRISES IN KENYA. for the period ending : 14/January/2022.

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Appendix 6. Publications

1st publication


Check for updates

Received: 31 March 2021 | Revised: 27 September 2021 | Accepted: 29 September 2021

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Journal of the World Aquaculture Society | WILEY

APPLIED STUDIES

Toward integration of sea cucumber and cockles with culture of shrimps in earthen ponds in Kenya

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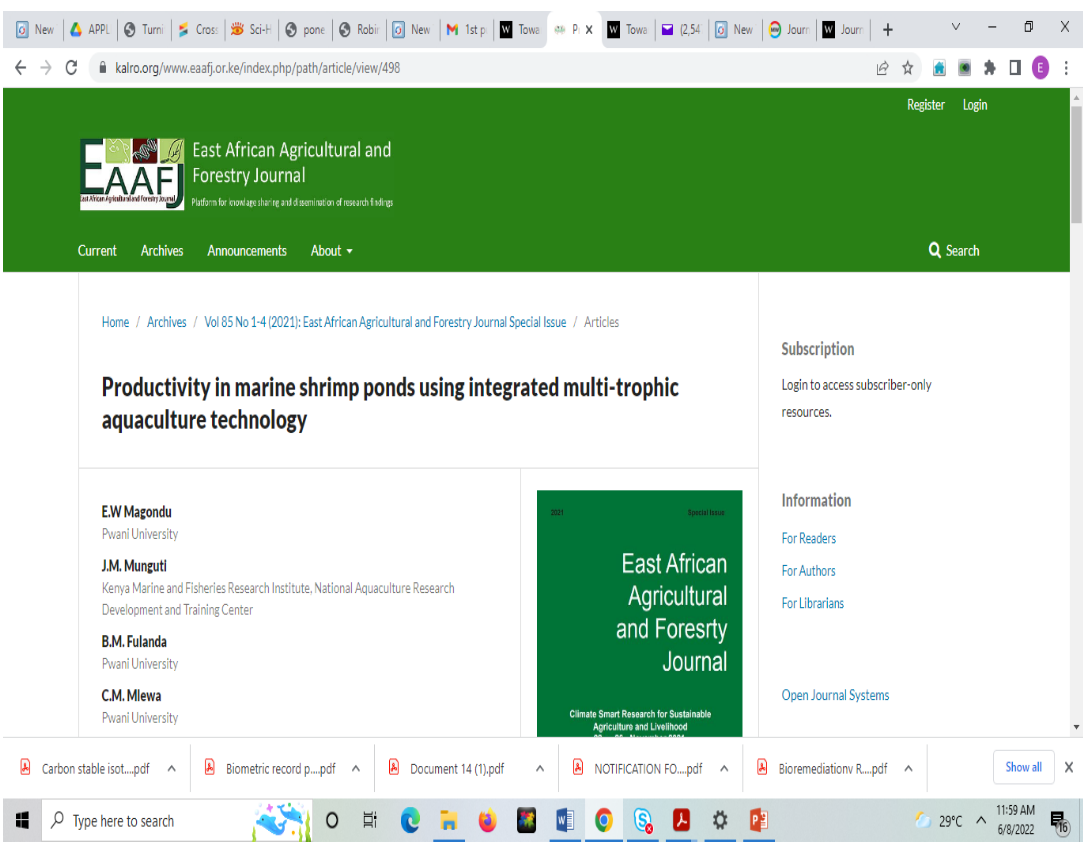
Abstract

This study presents the first trial application of an integrated multi-trophic aquaculture (IMTA) system in pond culture in

2nd publication



Manuscript 3.

Integrated Multitrophic Aquaculture of Nile tilapia, sea cucumber and oysters in earthen ponds.

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Manuscript

Title: Nutrient assimilation capacity of oysters in Integrated Multitrophic Aquauculture system.

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