Optimising Integrated Multitrophic Aquaculture (IMTA) on a South African Abalone Farm

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Abstract

The efficiency of fed nutrient utilisation in integrated multitrophic aquaculture (IMTA) system was evaluated on a South African abalone farm. On many commercial abalone farms in South Africa, *Haliotis midae* are fed a combination of pelleted feed and live macroalgae cultured downstream in abalone effluent. This production technique reduces the discharge of dissolved nutrients into the environment and improves farm productivity as unquantified proportion of the waste metabolites is captured as macroalgal biomass. However, the solid waste from abalone culture tanks remains unutilised and discharged to the coastal environments. Thus, there is scope to improve the dissolved nutrients removal efficiency of the macroalgae and to reduce the discharge of particulate nutrients using detritus waste extractive organisms. The present study aimed at the production and environmental performance of a shore-based abalone/macroalgae IMTA improving farm in South Africa by improving the nutrient utilisation efficiency of farmed abalone and seaweed and testing the waste solids removal potential of a sea cucumber species. Monoculture systems, where abalone and seaweed were cultured separately, both in fresh seawater (salinity: 35 g/L), were compared with an integrated culture system where the seaweed (*Ulva lacinulata*) was cultured downstream in the wastewater flowing from abalone tanks that were up-channel. Based on the findings from quantifying the performance of these production systems, methods to improve the nutrient utilisation and production efficiencies of the production systems were explored. These assessed methods included (1) the removal of abalone biodeposits by detritus extractive sea cucumber production, (2) evaluating the potential of farmed macroalgae as supplement in formulated diet, and (3) replacing mineral fertilisers with eco-friendly live microbial fertilisers for seaweed farming. Furthermore, the overall

environmental performance of the two farm systems was quantified using a life cycle analysis methodology.

Monitoring of the nutrient flows through the monoculture and IMTA systems revealed that the highest inputs of nutrients (nitrogen and phosphorus) into the abalone and seaweed culture tanks of the two production systems were abalone feed pellet (70-81%) and mineral fertilisers respectively (63-93%). About 48-51% of the nitrogen supplied from the feed was utilised by abalone in the IMTA and monoculture systems, while the remaining portion was lost as organic waste nitrogen on the production tank floor (20-30%) and as dissolved nitrogen in post-abalone tank effluent (30-36%). In the seaweed tanks receiving abalone effluent (IMTA), 69% of the dissolved nitrogen input was absorbed by cultured *Ulva* while 25% of the nitrogen was lost to the post-seaweed effluent which returned to the environment. However, in the monoculture system, 52% of the nitrogen from supplemented inorganic fertiliser was absorbed by cultured *Ulva* while ca. 46% of the nutrient was lost to coastal waters through the post-seaweed effluent. Moreover, while the feed accounted for ca. 74-78% input of the phosphorus in abalone of the two production systems, not more than 19% and 13% of this phosphorus was utilised by *H. midae* in the IMTA and monoculture systems respectively, while the largest portions were lost as organic waste in the sediment (34-45%) and dissolved waste phosphorus in the effluent (33-54%). In the seaweed tanks, a small portion (11-15%) of supplied phosphorus was removed by farmed *Ulva* while 77-89% was lost in the post-seaweed effluent discharged to coastal environment.

The substitution of 50% mineral fertilisers with live microbial fertilisers during seaweed production significantly reduced the discharge of dissolved nitrogen and phosphorus from

macroalgae raceways to coastal environment by 55 and 45% respectively, without impacting their growth, yield and nutrient compositions.

A life cycle analysis of the measured energy and nutrients utilisation efficiency of these production systems was compiled, and the impacts of the inputs and outputs from each production system on the environment was assessed. The electrical energy input to abalone and seaweed tanks constituted the highest contribution to all assessed environmental impact categories for the two production systems, followed by the contributions from the nutrients supplied to farmed abalone (formulated diet) and seaweed (mineral fertilisers). The impact of these inputs on the environment was most evident on marine aquatic ecotoxicity being 2.11E+03 kg 1.4-DB eq and 4.43E+03 kg 1.4-DB eq for IMTA and monoculture systems respectively. The measured impact of seaweed aquaculture on the environment was reduced by 50-52% when *Ulva* was cultured in abalone effluent (IMTA) compared to culture in fresh seawater (monoculture). However, the input of chemical fertilisers in the two systems of cultivation resulted in similar eutrophication potentials (8.09 - 8.41E-02 kg PO4--- eq).

To reduce the solid waste discharge from abalone tanks, and create an additional high-value crop, an endemic sea cucumber species (*Neostichopus grammatus*) was introduced on the floor of the abalone culture tanks in a pilot abalone/detritivore/macroalgae IMTA system. The sea cucumber utilised the biodeposits in abalone tanks as food which reduced organic solid discharge to the environment by 11%. However, the sea cucumbers displayed poor nutrient utilisation, a negative growth rate (- 0.59% day^{-1}) and 49% weight loss by the end of the trial that was probably due to sub-optimal habitat conditions (lack of a sand substrate).

The potential of including farmed *Ulva* (IMTA and monoculture) meal in pelleted feed for *H. midae* was evaluated as a means of improving farming efficiency and reducing the levels of

fishmeal and soya in the pellet. In an initial trial, *Ulva* was included at 12% dry weight in commercial diet and fed to farmed *H. midae* for 244 d. The 12% inclusion of IMTA and monoculture *Ulva* resulted in poor feed conversion and nutrient utilisation by *H. midae*. In a follow-up trial which tested graded inclusion levels of *Ulva* meal (0.75, 1.50, 3.00, 6.00 and 12.00%), the growth rate and feed utilisation of *H. midae* was enhanced at a 0.75-6.00% inclusion level of the seaweed in the diet, while at a 6.00-12.00% inclusion level growth rates and feed conversion efficiencies decreased. Therefore, it is recommended that for sub-adult South African abalone, up to 6.00% IMTA *Ulva* meal can be included in the diet formulation without impacting their growth performance and nutrient utilisation efficiency negatively.

This present study contributes to the understanding of the nutrient utilisation dynamics on integrated abalone farms in South Africa. The evidence from the different trials suggests the IMTA techniques tested could be adopted to improve the production performance and reduce the impact of abalone farming on the environment.

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ANOVA	Analysis of variance
BW	Body weight
CF	Condition factor
DO	Dissolved oxygen
DISL	Daily increase in shell length
DGBW	Daily growth in body weight
DW	Dry weight
EER	Energy efficiency ratio
FCR	Feed conversion ratio
H	Hour
ISO	International Organization for Standardization
IMTA	Integrated multitrophic aquaculture
KVA	kilovolt-ampere
LCA	Life cycle analysis
MAP	Mono ammonium phosphate
MKP	Monopotassium phosphate
MM	Millimeters
MIN	Minutes
Mg	Milligram
PER	Protein efficiency ratio
RM	Repeated measure
SL	Shell length
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TOC	Total organic carbon
TSS	Total suspended solid
WCA	Wild Coast Abalone
WW	Wet weight

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Chapter 1: Introduction

Aquaculture continues to be the fastest developing animal food producing sector, expanding by approximately 6.6% annually (Granada *et al.* 2016; Ragasa *et al.* 2018). However, there are growing concerns related to the negative impact of farming activities on the environment. For example, the trend towards intensification using fed monoculture systems can affect the environment through habitat alteration and destruction, diseases and alien species introductions, waste release from farming sites, soil acidification, eutrophication, nitrification of effluent receiving ecosystems and the interbreeding of wild species and selected strains (Dosdat 2009; Little *et al*. 2016; Henriksson *et al.* 2018). Aquaculture is therefore under increasing societal, economic, and environmental pressure to become more sustainable in terms of natural resources use, waste discharge, and continuous fish production from the same number of resources. Several options that can improve production efficiency and minimise impacts on the environment are available. These include moving away from single-species farming to the adoption of Integrated multitrophic aquaculture (IMTA) systems where the supplied nutrients are more efficiently used by culturing organisms with complementary ecosystem functions (Knowler *et al.* 2020).

Integrated Multitrophic Aquaculture is a farming technique which allows the combination of multiple aquaculture species from different trophic levels to be included in a production system such that the by-products of one cultured species serve as nutrient inputs for other species in the production system (Chopin *et al*. 2012). This means that the low trophic species can be cultured on the biodeposits of the fed component of the production system, resulting in more efficient nutrient utilisation and enhanced total production yield. Low trophic species include plants that can utilise dissolved waste nutrients and solids feeders that feed on

detritus and finer particulates. The integration of benthic detritivores such as sea cucumbers under suspended primary production systems allows for more efficient utilisation of space with lesser impact of farming activities on the environment (Fang *et al.* 2009; Chopin *et al.* 2012; Zamora *et al.* 2018).

There is thus a growing global interest in adopting IMTA systems as an effective and reliable food production technique of the future (Chopin 2017). Integrated farming of fish and rice has been in practice in different Asian countries for several decades and the technique is now applied over vast areas with strong government support enjoyed at all levels (Chopin 2017). In the West, there has been an increased awareness of the IMTA over the last two decades with initiatives to convert existing fish monoculture sites into pre-commercial IMTA farms with invertebrates and seaweeds as the secondary species (Chopin 2017). Examples of commercial IMTA systems exist in Canada, South Africa, Chile and Israel (Hossain *et al.* 2022; Barrington *et al.* 2009).

The global adoption of Integrated aquaculture system faces significant environmental, economic, technical, regulatory and social challenges. Several regions around the world are not suitable for IMTA practice due to specific environmental requirements for different species (Soto 2009). The viability of IMTA system is affected by complex ecosystem and climate change impact on water temperature and quality (Chopin 2013; Ahmed and Thompson 2019). Additionally, the implementation of IMTA systems requires an initial capital investment for infrastructure, technology, species stock and market development for cultured species (Barrington *et al*. 2009; Troell *et al*. 2009). Integrated multitrophic aquaculture also require the complex management of muti-species system as identification of compatible and beneficial species can be difficult (Chopin *et al*. 2012; Hughes & Black, 2016).

The South African aquaculture industry is dominated by land-based monoculture systems for abalone aquaculture where indigenous perlemoen abalone, *Haliotis midae* is cultured in continuous flow-through concrete and fibre-reinforced tanks (Britz and Venter 2016). This production technique is based on high stocking densities and the external input of nutrients in the form of formulated feed pellets and wild harvested or cultured fresh macroalgae. The single pass systems generate high volumes of nutrient-laden dissolved and solid waste which are released to coastal environment. Additionally, the pump-ashore technique requires a very high input of direct energy to move seawater into land-based tanks. These factors have led majority of South African abalone farmers to search for ways of improving production efficiencies and reducing their environmental footprint.

One strategy that has been implemented to improve production efficiency is to convert from the monoculture system of abalone farming to an integrated production technique where the wastewater from abalone tanks is used for the production of other low trophic species of economic and environmental importance, such as algae, filter feeders and detritivores. This production technique is environmentally beneficial as it reduces the discharge of waste from farms to the environment and generate revenue for farms in form of additional cultured products (Yearsley *et al.* 2011). The primary downstream cultured crop is *Ulva species* which is fed to the abalone, and there is scope for culturing invertebrate detritivores species such as sea cucumbers, sea urchins and polychaete worms (Troell *et al.* 2006; Yearsley *et al.* 2011). For example, the culture of the bloodworm (*Arenicola loveni loveni*) in nutrient-enriched abalone effluent significantly reduced the discharge of organic particulate from production tanks to coastal environment (Yearsley *et al.* 2011).

This current research formed a part of the European Union H2020 AquaVitae project which was aimed at developing culture technology for low trophic species in IMTA systems. The present study quantified the production efficiency of an existing abalone-seaweed IMTA system on a commercial Abalone farm in South Africa, the Wild Coast Abalone Pty Ltd (WCA) and tested methods to enhance IMTA production on the farm. To achieve this, the following research questions were formulated:

i. What quantity and quality of dissolved and particulate waste is produced at WCA?

ii. What factors contribute to the rate at which this waste nutrient is generated?

iii. What time of the day would be best to sample abalone effluent for dissolved nutrient compositions at Wild Coast Abalone?

iv. Using a nutrient mass balance and life cycle analysis (LCA) approach, which of the two operating systems (IMTA and monoculture) is more efficient, contributing positively towards a better environmental performance?

v. What components of the farming system contribute significantly to the overall impact of the farm on the environment?

vi. How best can abalone production efficiency be improved with less impact on the environment using IMTA techniques?

vii. Can farmed macroalgae meal partly replace conventional feedstuff in abalone formulated feed pellet without impairing the growth and nutrient utilisation efficiency of *H. midae*?

viii. Will integrating sea cucumber into abalone and seaweed IMTA system improve nutrient utilisation and farm's productivity?

ix. Will substituting mineral fertilisers with live microbial fertiliser improve seaweed production performance in terms of the growth, yield, and nutrient compositions?

1.1 Literature review

This literature review provides an overview of the South African abalone industry and the adoption of integrated multitrophic aquaculture on many abalone farms in South Africa. The processes, advantages and challenges associated with land-based farming of abalone and seaweed are described to motivate the research approach and objectives to improve the efficiency of the farming systems using IMTA techniques.

1.1.1 The South African alone industry

The aquaculture of *Haliotis midae* emerged in South Africa in the early 1990s around the same time other species of economic importance were domesticated in Asia, the USA, Chile, New Zealand and Australia (Sales & Britz 2000). The development of South African abalone aquaculture industry was spurred by the depletion of wild stock of *H. midae* caused by poaching activities due to the high market-demand for this product in Asia (Sales & Britz 2001; Troell *et al.* 2006; Hauck 2009; Cook 2019). Over the years, the combination of technology transfer and local innovation between South African research institutions and abalone industries established a robust commercial technology for the culture of *H. midae* (Sales and Britz 2001).

The South African abalone industry has grown steadily to meet the Asian demand for *H. midae* from an initial 181 tonnes in 2000 (DAFF 2017) to 2354 tonnes per annum in 2021 (Department Forestry, Fisheries and Environment statistics). Abalone is now the most

profitable cultured species in South Africa contributing around 75% of the total worth of this local aquaculture industry (Bachoo 2021).

Land-based abalone farming in South Africa is characterised by a single-pass flowthrough monoculture technique where fresh seawater is continuously pumped into abalone production tanks and the effluent is released directly to the sea (Troell *et al.* 2006). This production system allows high stocking density of abalone and the input of nutrients from formulated feed. However, the flowthrough technique requires large inputs of electrical and fossil fuel energy and the discharge large volumes of dilute wastewater into coastal environment (Fornshell *et al.* 2008). To improve nutrient utilisation and farming efficiency, abalone farms have begun to investigate ways to reduce dependence on grid electrical energy, as well as to utilise the farm organic waste to produce other culturable organisms with economic and ecological relevance using integrated multitrophic aquaculture systems (IMTA).

1.1.2 *IMTA principles and its application in aquaculture.*

Origin of the IMTA.

IMTA is an innovative and sustainable technique in which the culture of species from various trophic levels takes place within the same production system (Neori *et al.,* 2004; Reid *et al.* 2009; Thomas 2010; Chopin 2010). The term integrated multitrophic aquaculture was coined in 2004 by biologists Dr Thierry Chopin and Jack Taylor of the University of New Brunswick and Fisheries Ocean Canada, respectively. However, the practice of integrated farming can be traced to the beginnings of aquaculture. Integrated aquaculture was adopted in Asian countries such as Japan and China for decades to co-culture rice and fish through freshwater land-based operations, which later expanded to integrated marine systems (Neori *et al.,*

2004; Yang *et al.* 2000; Chopin 2013). For example, in 220-2100 B. C., a Chinese scholar, *You Hou Bin*, meticulously described the integrated culture of fish with aquatic plants and vegetables (Chopin 2013). Likewise, in ancient Egypt, an integrated agriculture and aquaculture pond system with floating plants and fruit trees, was illustrated on the tomb of the pharaoh Thebaine which was built in the era of the New Kingdom in Egypt (Chopin 2103). More recently (400 years ago) in Europe, royal IMTA was practised at the Château de Fontainebleau palace in the Southeast of Paris, France, as attested by the construction of the *Etang aux Carpes* (carp pond), which is still in operation till date (Chopin 2103). Likewise, extensive traditional forms of IMTA and polyculture systems of rice and shrimps are still functioning across Asia in countries such as China, Taiwan, Thailand, Japan, Vietnam. These traditional farming techniques are inherently more sustainable and environmentally friendly than the current intensive monoculture operations, as they utilise few input resources with relatively low stocking biomass, thereby minimising the quantity of waste released to the environment (Shpigel *et al*. 2013).

The Interest in integrated aquaculture was rekindled in 1975 through the work of John Ryther in his classic book "*Integrated waste recycling in marine polyculture systems*" (Ryther *et al.* 1975). An IMTA system is an aquaculture technique where organisms with complementary trophic niches are combined to resemble or mimic the natural ecosystem, with each component serving essential nutrient recycling and bio-mitigation services (Neori *et al.* 2004; Barrington *et al.* 2009; Chopin 2006; 2010). In an IMTA system, cultured or 'fed' species such as fish or shrimp are combined with organic nutrient extractive species (e.g., bivalves) and inorganic nutrient extractive aquaculture species (e.g., micro- and macroalgae) to produce an environmentally sustainable, economically balanced, and socially acceptable system (Barrington *et al.* 2009; Chopin 2010; 2013). In this way, the nutrients and energy recovered

or captured present a means to decrease nutrient loading to the ecosystem, increase the profitability of aquaculture operations, and act as a tool to actively manage anthropogenic loading to the environment (DFO 2013).

The trophic levels of an IMTA system and species selection criteria.

Integrated multitrophic aquaculture species are selected based on their ecological function, habitat characteristics, established husbandry practices, economic value, high potential for development, bio-mitigation ability, and consumer acceptance (Soto 2009; Zamora *et al.* 2014). An integrated culture system is principally made up of the fed aquaculture and nutrient extractive components (Chopin *et al.* 2001).

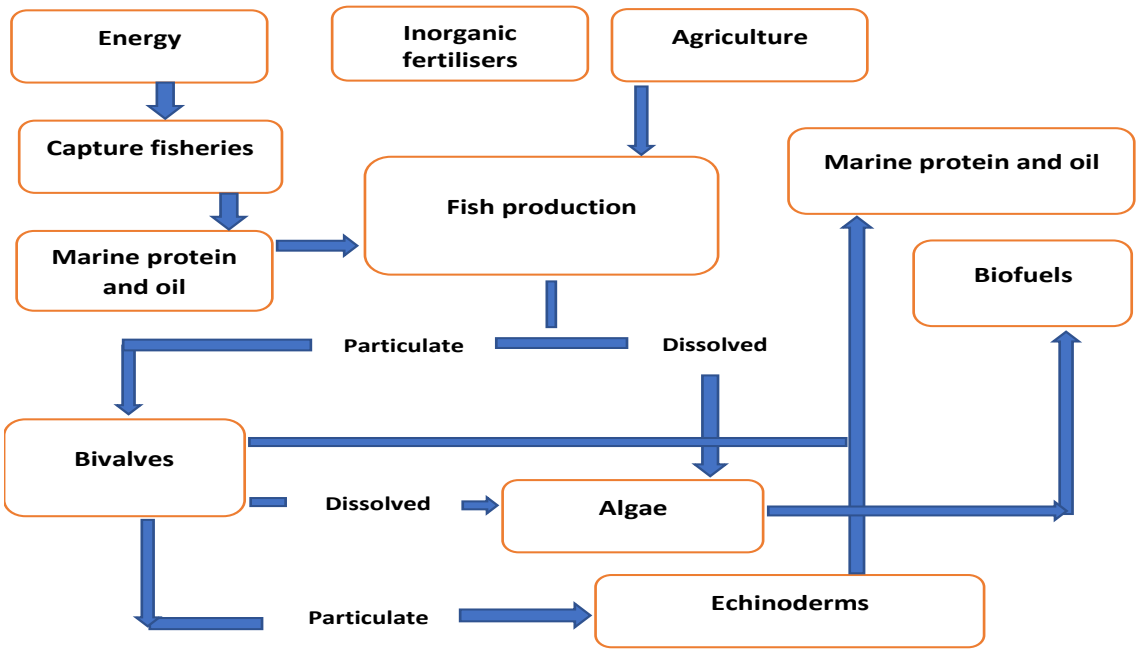


Figure 1.1. The concept of integrated multitrophic aquaculture (Hughes *et al.* 2016).

The fed component of the IMTA are the species which derive their nutrients and energy from sustainable external sources (Chopin 2010). Nutrients are introduced into the system in form

of protein and energy-enriched formulated diets comprising of marine proteins, marine oil and other ingredients of agricultural origin (Figure 1.1) (Chopin 2010). Although majority of IMTA systems have high trophic species (finfish and shrimp) as the fed aquaculture component, low trophic species such as abalone are currently cultured in different regions (China, France, Spain, Chile, Israel, South Africa) as the fed component of their IMTA systems (Neori *et al.* 2000; Schuenhoff *et al.* 2003; Fang *et al.* 2009; Xiaolong *et al.* 2018; Gao *et al.* 2019a, b).

Extractive species are organisms which make use of organic, inorganic materials and by-products from fed species for their growth and development (Chopin *et al.* 2001). Three extractive species components are needed in an IMTA system if all forms of nutrient wastes (dissolved, settleable, and suspended) are to be captured and converted into other products of economic value (Robinson and Reid 2014). They are classified as the filter feeders (e.g., bivalves) (Figure 1.1), the dissolved nutrients scrubber (e.g., algae) and the settleable solids feeders or detritivores (e.g., echinoderms and sea urchins) (Kang *et al.* 2003; Chopin *et al.* 2001; Hannah *et al.* 2013; Zamora *et al.* 2018; Buck and Langan 2017). These extractive species derive their nutrients and energy only from the waste nutrients of the fed organisms without any feed supplementation (Chopin *et al*. 2001).

Forms of integrated multitrophic aquaculture system.

Integrated farming can be practiced in closed containments along seashore (shore-based IMTA) and in the open-sea (offshore IMTA) (Chopin *et al.* 2012; Chopin 2013). Additionally, the principles of IMTA can be applied in freshwater aquaculture systems (aquaponics) both in temperate and tropical regions (Neori *et al.* 2004; Chopin *et al.* 2012; Chopin 2013).

The offshore IMTA technique involves the production of aquaculture species which have complementary trophic niches in the open sea or exposed water sites (Buck *et al.* 2018). This production system provides room for enhanced production, a higher area carrying capacity, reduced conflict with other water body users, less pollution, and a reduced adverse environmental impact of coastal fish farming (Buck 2002). Open sea locations also have the advantage of less biofouling of cages and pens, which is an expensive maintenance operation for onshore aquaculture (Atalah *et al.* 2016; Buck *et al.* 2018). Most offshore IMTA systems are found in Europe, Asia, and the USA where shellfish species are cocultured with seaweeds and finfish (Buck *et al.* 2017). For example*,* in Germany, offshore production of seaweeds *species* (*Laminaria*, *Palmaria* and *Ulva*) with bivalves have been in operation since the early 1990s (Buck *et al.* 2017). Likewise in Belgium, mussel hang culture system with seaweeds (*Undaria pinnatifida*, *Saccharina species* and *Porphyra species*) is practiced at different offshore IMTA sites (Buck *et al.* 2017). In France, offshore longline production system for mussels and seaweed is operational (Buck *et al.* 2017). In Canada an offshore IMTA site in the Bay of Fundy produces salmons, mussels, and kelp (Chopin 2017). In China, IMTA system has gained significant attention as a sustainable approach to aquaculture. The IMTA system implemented along the coastline of China involves the integrated farming of Japanese disc abalone (*Haliotis discus hannai*), Kelp (*Saccharina japonica*) and sea cucumber, *Apostichopus japonicus* (Fang *et al.* 2016; Zhang *et al.* 2016). However, for different reasons ranging from non-granting of offshore farming permits, risk aversion, inadequate capital and finance, clear identification of ownership and regional acceptance of the technology in various parts of the world, as well as opposition from environmental and non-governmental organisations, the development of open-ocean aquaculture has been limited (Buck *et al.* 2003). The development of offshore IMTA faces constraints, such as the inability of the production

system and farmed organisms to withstand prevailing hydrodynamic forces in open seas as both fed and extractive components of the system can be strained by water flows caused by breaking waves and swell, thus reducing their fitness for aquaculture (Buck *et al.* 2018). Prevailing vertical and horizontal currents in open-water IMTA can also move dissolved nutrients and organic particles out of the reach of extractive species (Buck *et al.* 2018). A more controlled multi-species production technique is the onshore or land-based IMTA production system.

Shore based IMTA.

Shore based IMTA is characterised by the combination of complementary species in sequential arrangement with seawater flowing from the fed aquaculture component to the extractive species component of the production system (Figure 1.2). The fed species receives formulated feed, natural feed, or combination of both, while the solid and dissolved waste nutrients are captured by lower trophic level organisms in the system. The effluent from fed aquaculture ponds, which carries dissolved nutrients, is typically channelled into microalgae, macroalgae ponds, or constructed wetlands (Shpigel *et al.* 2013). Both the micro- and macroalgae components of the IMTA are effective in the uptake of nutrient in land based IMTA systems, but neither is 100% efficient in dissolved nutrient removal and the algae cannot remove particulate waste (Shpigel 2013). The particulate waste, which is a combination of uneaten feed, faeces, algae, and bacteria, sinks to the bottom of the rearing tanks to become nutrient-rich sludge. The sludge can be effectively utilised by deposit feeders (e.g., sea cucumber, polychaetes, and sea urchins) of the IMTA (Shpigel 2013). These detritivores assimilate the nutrient waste into their body while serving to reduce treatment cost and at the same time serving as additional crops.

Figure 1.2. A shore-based IMTA abalone farm with effluent from the abalone culture tanks (the fed species component) flowing into the macroalgae (extractive species component) paddlewheel raceways.

Land-based IMTA systems were pioneered in the 1970s when household effluents were treated with extractive species such as suspension feeder molluscs and seaweeds (Goldman *et al.* 1974; Ryther *et al.* 1975). Later in Israel in 1981, techniques for land-based integrated culture of marine finfish, shellfish, and macroalgae was developed (Porter *et al.* 1987; Neori *et al.* 2004; Kim *et al.* 2015; Wei *et al.* 2017). However, while land-based integrated farming is practically possible in many coastal regions with the required environment variables for optimum production performance, the costs incurred from pumping seawater ashore generally limits the economic viability of shore based IMTA to high value species such as abalone (Fiander *et al.* 2014).

The ecological effectiveness of shore-based or offshore IMTA site is determined by several factors including the species combinations, the configuration and design of the IMTA site, the effectiveness of the selected extractive organisms to capture and ingest wastes, food

availability for organisms at different trophic levels, the site condition, background nutrient concentrations and the relationship between the combined species (DFO 2013; Robinson & Reid 2014). The selected species at each trophic level must ingest and efficiently utilise absorbed nutrients by converting them to tissue or metabolic waste. It is also important to select species that assimilate targeted nutrients (DFO 2013; Robinson & Reid 2014).

Bivalves as particulates extractive species of an IMTA system.

Bivalves such as oysters, clams, scallops and mussels are fine particulate filtering species that can filter water containing suspended particles at an average of 15 to 55 litres per day (Powell *et al.* 1992; Rice 2008). They can efficiently remove natural suspended particles of 1-7 mm (Gallardi 2014). Bivalves are able to sequestrate nitrogen in their shell and meat and also regulate the abundance of phytoplankton in water through their moderation of ammonia cycling in water (Gallardi 2014). The grazing habit of bivalves in an IMTA site reduces water turbidity and increase light penetration for seaweed growth (Lander *et al.* 2013). While studying the growth of *Mytilus galloprovincialis* cultured at a fish/bivalve IMTA site within the Tyrrhenian Sea, the culture of mussels in close proximity to fish cages resulted in increased total length and weight of the shellfish above those at a control site (far from the fish farms) (Sara *et al.* 2009). This growth of mussels was attributed to their ability to exploit the organic particles emanating from the fish cages, showing that organic emission loads from fed aquaculture component of the IMTA can be converted effectively into secondary products of high economic importance at little or no cost (Sara *et al.* 2009). This is because mussels are effective general consumers of particulate organic matter and are able to utilise organic matter from different sources (Dame 1996; Lindahl *et al.* 2005; Sara *et al.* 2009). The direct use of suspended particulates by bivalves contributes to the reduction of the potential

environmental impact of organic waste materials from an aquaculture site (Lindahl *et al*. 2005; Sara *et al.* 2009). At an integrated aquaculture site for the sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), Mediterranean mussel (*Mytilus galloprovincialis*), rayed pearl oyster (*Pinctada imbricata radiata*), and sea cucumber (*Holothuria polii*), positive weight and length increase of mussel (33-42%) and pearl oysters (55-56%) were recorded in the IMTA system above those cultured in a typical mussel farm (Chatzivasileiou 2022). The significant growth was attributed to the filter feeder's ability to utilise particulate from fed aquaculture (Chatzivasileiou 2022). The integration of mussels with finfish can potentially reduce nitrification processes at the culture site due to their ability to capture, ingest and absorb excess particulates from synthetic fish food (MacDonald *et al.* 2011).

In addition to the filtration efficiency, bivalve culture with macroalgae can increase the growth and biomass yield of the seaweed (Hargrave *et al.* 2022). For instance, in an IMTA culture of the sugar kelp *(Saccharina latissimi*) and the blue mussel, (*Mytilus edulis*) on the Swedish West coast, the cultivation of kelp 10 m downstream of blue mussel farms enhanced a notable yield of kelp blade length and biomass by 22% and 28% above those cultured alone (Hargrave *et al.* 2022). Additionally, culturing kelp in IMTA site reduced epiphyte growth and also increased chlorophyll, fucoxanthin and phaeophytin production of the kelp (Hargrave *et al.* 2022). Similarly, in a closed recirculating integrated culture system of blue mussel, (*Mytilus edulis*) and the western king prawn (*Penaeus latisulcatus Kishinouye*), significant amounts of the total nitrogen, total bacteria and suspended solids in the culture system was filtered by the mussels (Fotedar 2012). Although mussel culture with prawns did not improve the growth and survival of the crustacean, the growth of mussels significantly improved in the coculture system (Fotedar 2012). Some other studies have also shown that the filter feeding habit of bivalves efficiently remove nitrogen, phosphorus, suspended solids and bacteria

concentration in prawn effluent (Jones and Preston 1999; Jones *et al.* 2001). Mussel farming with fed species can improve water quality by reducing the net transport of both dissolved and particulate nitrogen and in the process generate seafood, fodder and agricultural fertilisers (Lindahl *et al.* 2005). However, excretion by mussels can increase the level of total phosphorus concentration in coculture effluent (Asmus *et al.* 1995).

The growth and performance efficiency of bivalves in a coculture system can be site, culture conditions and season specific (Cheshuk *et al.* 2003). Additionally, the distance at which the mussels are held from fish cage can prevent them from intercepting and grazing on settling particulate materials from fish cages (Cheshuk *et al.* 2003).

The studies reviewed above demonstrated that the integration of filter feeding species with fed species can be beneficial to the IMTA system and can potentially reduce the environmental impact of the farming system. However, while the smaller particulates can be removed by filter feeding species of the IMTA, there remains heavier particulates or pond bottom settlers which cannot be removed by filter feeders. Therefore, another species component of the IMTA is required to process these settled solids and of such low trophic species organisms are echinoderms.

Particulate waste extractive species component of the IMTA.

Echinoderms (e.g., sea stars, sea urchins, sea cucumber) and polychaete blood worms can feed on heavier particulates in aquaculture ponds. This has stimulated research and commercial interest in their application as recyclers and processors of sedimented waste nutrients of an IMTA system (Zamora *et al.* 2018). These low trophic species are considered ideal candidates of the IMTA due to their synergistic effect and resource utilisation efficiency as they are able to feed on uneaten and undigested food waste of fed aquaculture species

(Zamora *et al.* 2018). Detritivores can cause changes to sediment characteristics in a culture pond or farm site, resulting in substantial changes in the composition of benthic communities (Mente *et al.* 2006; Zamora *et al.* 2018). Their grazing ability on bottom sediments can reduce benthic organic loads and aid the reworking of bottom soil (Slater & Carton 2009; Zamora *et al.* 2018). They play important nutrient cycling roles in both temperate and tropical environments with the recycled nutrients significantly improving primary productivity and bringing about environmental and economic benefits (Uthicke & Klump 1998; MacTavish *et al.* 2012; Zamora *et al.* 2018). The coculture of detritivores with fed species have been documented in several studies. For example, the 91 days integrated culture of the silver kob (*Argyrosomus inodorus*) and bloodworm (*Arenicola loveni loveni*) in abalone farm effluent resulted in the bloodworm benefiting from the coculture system as they utilised enriched organic solids in the effluent for faster growth (0.39 ± 0.07% BW d^{-1}) compared to bloodworms cultured in clean seawater which lost weight (-0.19 ± 0.04% BW d^{-1}) over the culture period (Yearsley *et al.* 2011). The superior growth of *Arenicola loveni* cultured in abalone tank effluent was attributed to the high protein (33.5%) composition of suspended solids in the effluent which the bloodworm utilised as food (Yearsley *et al.* 2011). Polychaetes are effective in mitigating the adverse impact of aquaculture as they can feed on ca. 15-65% of fish organic waste and recycle nutrients from aquaculture waste material (Kinoshita *et al.* 2008; Fang *et al.* 2017).

The ability of holothurians to utilise the detritus waste from fish farming operations is well documented (Maxwell, Gardner & Heath 2009; Slater & Carton 2009; MacDonald *et al.* 2013; Qi *et al.* 2013; Zamora *et al.* 2018; Neofitou *et al.* 2019; Fang *et al.* 2009). In a 12 months open-sea IMTA culture of *Holothuria poli* at 0, 10 and 25 m away from sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), and greater amberjack (*Seriola dumerili*)

cages, positive growth of *H. poli* was recorded when cultured at 10 m (0.18 ± 0.02% d^{-1}) and 25 meters (0.20 ± 0.01% d^{-1}) away from the fish cages (Cutajar *et al*. 2022). The superior growth and higher survival of *H. poli* at this distance from the fish farm site was due to the fish particulate waste not settling directly on the sea cucumbers at these distances and the organic farm waste being enriched with nutrients for the holothurians. Likewise, in a recirculating IMTA system of the sea urchins (*Paracentrotus lividus*) and subadult sea cucumber (*Holothuria tubulosa*), the detritivore efficiently utilised 54% of organic matter from *P. lividus* as food (Grosso *et al.* 2021). A large sea cucumber can absorb up to 17.5 kg of sediment annually illustrating their ability to maximize resources utilisation efficiency (Coulon and Jangoux 1993; Tolon *et al.* 2017). Similarly, high daily growth of juvenile sea cucumber (*Apostichopus japonicus*) fed dried bivalve faeces has been reported (Yuan *et al.* 2006).

The benefits of coculturing macroalgae-foraging species such as abalone with the deposit-feeding sea cucumber have been demonstrated in Asia. For instance, successful integrated culture of the disk abalone, *H. discuss hannai* with the sea cucumber, *Apostichopus japonicus* in artificial reefs, cages and intertidal ponds is practiced along the coast of the Shandong Peninsula in eastern China (Kim *et al.* 2015). Likewise, in the Republic of Korea, the successful IMTA culture of *H. discuss* and *A. japonicus* in circulating culture tanks was recorded during the overwintering period where sea cucumbers integration with abalone culture reduced the level of ammonia and nitrite in the production system with a corresponding increase in the growth of abalone and the sea cucumbers (Kang *et al.* 2003). The integrated culture of abalone and the sea cucumber is now operated at a commercial scale in intertidal ponds and offshore in the eastern provinces of China and the practice has yielded a growing number of both species under aquaculture conditions (Yang *et al.* 2015).

Apart from their ecosystem service, the economic benefits of integrating echinoderms into IMTA systems are attractive. They are known for their high export trade value (Purcell *et al.* 2012). Sea cucumbers are traded in over seventy countries globally, with several hundred species currently exploited (Sicuro and Levine 2011; Purcell *et al.* 2012). Sea cucumbers and urchins are high valuable seafoods consumed in Asia (Zamora *et al.* 2018; Grosso *et al.* 2021). While the IMTA production of abalone and seaweed (kelp) yielded USD 107 541 ha^{-1} annually in a commercial IMTA farm in China, the integration of sea cucumber into the production system increased the yield by 31.6% (Fang *et al*. 2009). Similarly, a 600% profit increase was recorded when the sand fish (*Holothuria scabra*) was integrated into a functioning lagoon-based macroalgae farm (Beltran-Gutierrez *et al.* 2016).

While the particulate waste component of an IMTA system is remediated by detritus extractive organisms, the dissolved nutrient waste component can be removed by photosynthetic plants such as micro and macroalgae.

Macroalgae as an inorganic extractive species component of the IMTA

Dissolved nutrients (nitrogen, phosphorus) enter aquaculture tanks primarily from the leaching of nutrients from fed or unconsumed formulated diets. They are also re-mineralized into water columns from the biodeposits on the production tanks floor. Although nitrogen and phosphorus constitute high composition of aquaculture diet, less than 50% of these nutrients is utilised by fed species while the larger percentage is lost as waste to the culture medium where it constitutes nuisance with a potential impact when released into the environment (Piedrahita 2003; Dauda *et al*. 2019). Dissolved nitrogen is released into production tanks as ammonia-nitrogen which is a form that is readily utilised by seaweed for growth. Macroalgae are therefore cultured as dissolved nutrients extractive organisms of

IMTA systems to utilise the dissolved nitrogen and phosphorus in fed species effluent as food, thus reducing the pollution effect of aquaculture on receiving coastal environment with cultured macroalgae meeting the growing food demand for cultivated algal feeders (Neori *et al.* 2004; Robertson-Anderson *et al.* 2007; Neori 2008; Abreu *et al.* 2011; Lavania-Baloo *et al.* 2014; Wei *et al.* 2017; Kinney 2017).

The bioremediation potential of seaweed was first investigated by Ryther *et al.* (1975) in an oyster/*Ulva lactuca* biofilter system. In their system, about 90% of the total nitrogen was stripped from the wastewaters. It was concluded that macroalgae are potentially viable as nutrient removers in aquaculture systems to produce stable water quality conditions for fed aquaculture and extract nutrients from the final effluent before discharge (Ryther *et al.* 1975). Over the years, several studies have integrated macroalgae into aquaculture systems to remove excess nutrients from farm effluents while producing additional harvest at little cost (Gordin *et al.* 1981; Krom & Neori 1989; Neori *et al. 1991*; Krom *et al.* 1995; Buschmann *et al*. 1996; Brzeski and Newkirk 1997; Buschmann *et al.* 2001; Schuenhoff *et al.* 2003; Neori *et al*. 2004; Seema and Jayasankar 2005; Yongjian *et al.* 2008; Rabiei *et al.* 2014; Rabiei *et al.* 2016; Biwas *et al.* 2020). For example, about 99.2% of ammonia nitrogen and 93.3% of soluble reactive phosphate were removed by the red seaweed, *Spirulina platensis* when cultured in digested sago starch factory effluent (Phang *et al.* 2000). Likewise, in an integrated semi-recirculating system with abalone (*Haliotis discus hannai*), sea urchin *(Paracentrotus lividus*), fish (*Sparus aurata*), and seaweed biofilter (*Ulva lactuca*), the effect of the algae on bioremediation was remarkable, as the seaweed removed ca. 70% of ammonia from the wastewater and also increased the oxygen balance of the fishpond daily (Schuenhoff *et al.* 2003). An investigation on the nutrient bioremediation efficiency of *Ulva reticulata* and *Gracilaria crassa* on tidal fishponds effluent at Makoba bay, Tanzania, revealed that the

macroalgae were able to restore the pH and dissolved oxygen level of aquaculture wastewater (Msuya and Neori 2002). This water cleaning service by seaweeds in land-based aquaculture farms creates scope for more nutrient input from compound feeds, thus increasing farm productivity (Nobre *et al.* 2010; Lawton *et al.* 2013).

The environmental impact of aquaculture effluent on coastal ecosystem is minimised when suitable seaweed species are integrated into an aquaculture system. The use of macroalgae to serve bioremediatory functions in land-based aquaculture is globally accepted and considered highly efficient and cost-effective. However, it is worthwhile noting that an optimised bioremediatory service is only realised by selecting appropriate seaweed species for a particular location (Neori *et al.* 2004). This is important for the bioremediation service provided by these plants and their economic values (Lawton *et al.* 2013).

In addition to their scalable carbon drawdown solution to climate change, culturing seaweed as secondary product and on-site feed for marine grazers helps to reduce feeding cost and improve the health of cultured animals (Butterworth 2010; Bansemer *et al.* 2016a). It is therefore of paramount importance while integrating seaweed with fed aquaculture to select seaweed with a high growth rate and short production cycle (Barrington *et al.* 2009), able to withstand fluctuating environmental conditions (de Paula Silva *et al.* 2012); and endemic to the aquaculture site or with wide geographical distribution (Neori *et al.* 2004; Barrington *et al.* 2009). The ecological features of the selected seaweed species must also match the environmental conditions at the culture site (Kang *et al.* 2008; Kang *et al.* 2011). It is also essential to consider the nitrogen produced at an aquaculture site and the tissue carbon/phosphorus ratio when picking seaweed species for an integrated culture system as aquaculture effluent mainly contains ammonium, nitrate, and nitrite nitrogen (Kang *et al.*

2011). This is true for South African abalone industry where green (*Ulva species*), and the red seaweed (*Gracilaria spp*) are cultured in large biofilter tanks fed with abalone effluent from upstream.

The ecosystem service function of seaweed is not limited to nutrient scrubbing from culture environment. Their ability to photosynthesize makes them net producers of oxygen hence, they help to prevent coastal hypoxia (Chopin 2013). In photosynthesis, seaweed utilise carbon dioxide for food production, thus participating in carbon sequestration in a transitory manner (Chung *et al.* 2011; Chopin 2013). This process of atmospheric CO_2 removal reduces global warming (Chopin 2013). Although minor in comparison to phytoplankton, sequestration of dissolved CO_2 by seaweed in oceanic waters is a means of minimising coastal acidification (Clements and Chopin 2017).

The sea lettuce (*Ulva* species) has proven its usefulness in treating aquaculture effluents and has been acclaimed as the most widely distributed and ideal macroalgae for waste nutrient bioremediation (Neori *et al.* 2003; Copertino *et al.* 2009; Robertson-Anderson *et al.* 2007; Kang *et al.* 2011). Positive attributes, which include their abilities to effectively absorb and metabolize inorganic nitrogen and phosphorus, fast growth rates, broad tolerance for adverse environmental conditions coupled with low vulnerability to epiphytes, makes *Ulva species* the ideal candidate for the bioremediation of aquaculture wastewater (Taylor and Rees 1999; Neori *et al.* 2000, Chopin *et al.* 2001; Msuya and Neori 2002; Mata *et al.* 2010; Lawton *et al.* 2013). Sea lettuce can efficiently take up different forms of nitrogen (ammonia and nitrate) in wastewater, with higher ammonia uptake than nitrate (Guttman *et al.* 2019). The reason being that *Ulva* species have strong affinity for ammonium nitrogen, a characteristic that makes them the right bioremediation candidate for ammonium-rich aquaculture effluents

(Copertino *et al.,* 2009; Shpigel *et al.* 2018). The performance of *Ulva* species in terms of growth and nutrient loading is principally a function of nitrogen concentration (Msuya and Neori 2008). However, when cultured under nitrogen limitation, other factors such as light exposure, temperature, salinity, agitation, and aeration become determinants for optimum growth (Msuya and Neori 2008). They are also characterised by high surface area to volume ratio which gives them an equally high exposure to light flux, nutrient uptake, biomass yield, and growth rate (Littler & Littler 1980; Martínez-Aragón *et al.* 2002, Neori *et al.* 1991, 2003; Copertino *et al.* 2009). They have defense mechanisms against epiphytes contending for nutrients and light, and can survive periods of freezing, anoxia, exposure to sulphide compounds, and prolonged dark periods (Vermaat and Sand-Jensen 1987; Collén and Pedersén 1996; Wang *et al.* 2007). Most species of *Ulva* are characterised by short life cycles and massive sporulation events, which can lower their biomass yield in production ponds and, at the same time, affect their nutrient removal efficiency (Hiraoka and Oka 2008). The bioremediatory performance of *Ulva* varies among the different species and is dependent on the effluent nutrient composition, the local environmental conditions, the culture techniques employed, and the water flowrate (Copertino *et al.* 2009). High aeration, water exchange rate, and continuous flow of effluents can also boost the biomass yield of *Ulva* species (Lüning and Pang 2003; Schuenhoff *et al.* 2003). They can adapt to variations in photoperiod and chlorophyll availability (Copertino *et al.* 2009). However, a notable reduction in nutrient uptake efficiency can be experienced in *Ulva* when irradiance falls below 2.5 µmol m^2 s^{-1} (Sand-Jensen 1988). Compared to many algal species regarding reproductive performance and resistance to salinity fluctuation, *Ulva* cells uniformity gives it a higher reproduction rate and swift colonization of newly cleared substrate (Little 1980). They are also noted for their

ability to withstand broad salinity fluctuation (3 -15 ppt) (Lobban and Harrison 1997). These attributes of *Ulva* have contributed to their increasing rate of production globally.

1.1.3. Land based IMTA in South Africa

Integrated mariculture in South Africa is restricted to the shore-based culture of abalone, *Haliotis midae* (Fig. 1.3), and macroalgae and the practice has been in existence for over a decade (Shuuluka 2011; Bolton *et al.* 2013; Bachoo 2021). In this system, effluent from single-pass abalone raceways located upstream is channelled into large seaweed tanks to remove dissolved nutrients from the wastewater before a final discharge to the environment (Fig. 1.2). *Haliotis midae* forms the fed animal component of the IMTA in South Africa where they are fed formulated feed, mixed algal species or combination diets of macroalgae and formulated feed pellets with protein, carbohydrate, lipid and minerals forming the bulk of essential growth nutrients in their diet (Naidoo *et al.,* 2006; Dlaza *et al.* 2008; Robertson-Andersson *et al.* 2011). Being a demersal, slow, and messy grazer, only a portion of fed diet is ingested by *H. midae*, while the remainder ends as waste solids in the form of faeces and uneaten feed on the production tanks floor. This has prompted abalone farmers in some parts of South Africa to test the integration of other lower trophic extractive species (e.g., polychaetes bloodworm, sea cucumbers) in their culture system to help capture these heavier solids (Yearsley *et al.* 2011).

Figure 1.3. The South African abalone, *Haliotis midae*

Unlike the fed aquaculture activities in many regions, the South African abalone industry has not recorded any negative impact of effluents discharged into the environment due to the high energy nature of the coastline which rapidly disperses effluent streams, hence there is no measurable environmental burden (FAO 2009). Therefore, seaweed cultivation on South African abalone farms is not primarily practiced for bioremediation but for the production of live macroalgae as food supplement for farmed abalone. Apart from *Ulva*, South African abalone farmers also culture the red seaweed (*Gracilaria gracilis*) as food for their animals. *Gracilaria* production tends to make significant contributions not only to the ecosystem, but also to economic development through their valuable by-products such as phyco-colloid agar (Neori *et al.* 2003). However, seaweeds are predominantly cultured in South Africa as food for farmed abalone.

On many South African abalone farms, macroalgae are cultured downstream of abalone production tanks, in paddlewheel raceways at a stocking density of 1-2 kg m^{-2}. *Ulva* species are selected for culture due to: (1) their local occurrence all year round and adaptation to high temperature; (2) their ability for fast growth both in warm and cold seasons even after they are detached from their thallus; (3) the short lifecycle and the ease of cultivation; (4) efficient nitrogen uptake even at low substrate concentrations and the ability to retain a high level of nitrogen or protein in the thalli and (5) *Ulva* biomass utilisation as food for macroalgivores like abalone and sea urchins (Zhou *et al.* 2006; Robertson-Andersson *et al.* 2007; Copertino *et al.* 2009; Yokoyama & Ishihi 2010; Ale *et al.* 2011).

1.1.4 Impact assessment of IMTA in aquaculture using life cycle analysis (LCA)

As IMTA adoption continues to expand due to its potential to improve farming efficiency and sustainability, the associated environmental footprints of this culture technique must be continuously assessed and reduced to the minimum. Environmental impact assessment is now deployed as a means of quantifying the effect of various farming activities and techniques on the ecosystem. This method, which is backed by precise data, helps policy makers to make informed decisions on the impact of an activity and to also offer viable mitigation measures and strategies to deal with the impact of a production system. It also ensures that an aquaculture technique meets criteria for sustainability and best practices (Henriksson *et al*. 2012; Cao *et al.* 2013).

One important environmental impact assessment tool which has found its application in aquaculture, capture fisheries and aquafeed industries to evaluate the footprint of different production technologies is the life cycle analysis (Seppala *et al*. 2001; Aubin *et al.* 2009; Pelletier *et al.* 2009; Ayer and Tyedmer 2009; Roque D'Orbcastel *et al*. 2009; Iribarren *et al.* 2010; Jerbi *et al.* 2012; Abdou *et al.* 2017). The life cycle analysis (LCA) is a robust and standardized analytical method specifically designed for the assessment of a product system through its life cycle stages (Guinee *et al.* 2002; ISO 2006; Abdou *et al*. 2017). It is a well-established international standard organization (ISO) certified tool that is used when quantifying resource utilisation, emissions, and the environmental impact associated with a product or service (Taelman and Sfez 2015; Patrick and Malcolm 2016). This flexible assessment process accounts for the entire life cycle of a product from the extraction of raw materials, production processes, use, recycling of the product, and waste disposal stage (Guinee *et al.* 2001; Rebitzer *et al.* 2004). Due to its flexibility and the ability to use diverse

impact assessment methods, LCA can evaluate different environmental impacts for most value chains, and the goal is not only for data collection but also decision facilitation (Chester *et al.* 2010).

Every production system is characterised by its footprint, which must be reduced to the barest minimum using a technical approach. Life cycle analysis, otherwise referred to as the "cradle-to-grave" analysis, is that framework that can be used to examine the footprint (environmental impact) of any production process or service (D'orCastel *et al.* 2009). This assessment method has emerged as a valuable support tool for policymakers and industries in quantifying the cradle-to-impacts of their products, services, or production process (D'orbcastel *et al.* 2009). The cradle-to-grave impacts include the extraction of raw materials, processing, manufacturing, and fabrication of a product, its distribution, usage, disposal, and recovery (recycling) after its useful life (Guinee *et al.* 2001). Therefore, an effective LCA study of a product system allows analysts to calculate the environmental impact of a production process and identify hotspot related to this production process while finding opportunities for improvements (PRé Sustainability 2020). This information is used to improve processes, support policies, and provide a sound basis for inferred decisions (PRé Sustainability 2020).

Although there are several assessment tools used for quantifying the environmental impact of aquaculture production systems, life cycle assessment has distinguished itself as an all-encompassing assessment tool (UNEP 1996). This is due to its two distinct features: the cradle-to-grave analysis and the functional unit (UNEP 1996). The cradle-to-grave implies that all crucial steps required to assess a product system's performance are included in the analysis (UNEP 1996). The functional unit describes the role of the product systems under comparison quantitively (Klopffer and Grahl 2009; 2014). The functional unit is the premise of comparing

goods and services that provide similar functions (Guinee *et al.* 2002; ISO 2006). Applying these two features of the LCA allows the comparison of two or more products or systems while fulfilling a similar purpose (Guinee *et al.* 2002).

Apart from product and service improvement, LCA can serve different functions outside the LCA framework (Klopffer 2014). These non-exhaustive lists of direct applications include product development and implementation, strategic planning, public policymaking, marketing of consumer goods, teaching, and learning (Klopffer 2014). However, some conditions must be fulfilled and strictly adhered to while performing impact assessment using LCA as a study tool. A life cycle assessment study must be made of all the stages of impact assessment from goal and scope definition to result interpretation and must contain a set of global and localised impact categories (Klopffer 2014). In addition, the framework of the international standard organization, ISO 14040, cannot be used alone but in conjunction with ISO 14044 framework. Finally, an LCA study must be critically reviewed before its recommendation for decision or policymakers in private and government organizations (Finkbeiner *et al.* 2010; Klopffer 2012).

Life cycle assessment was first intended to measure the environmental performance of manufactured goods. However, in recent times, it has found application in food production sectors, including agriculture (Vanderwerf, Petifard and Sanders 2005; Cederbeg 2004; Mattson and Sonesson 2003), food processing effluent treatment (Taylor *et al.* 2021); capture fisheries (Thrane 2004; Hospido *et al.* 2006; Ziegler *et al.* 2003; Ziegler 2006) and aquaculture (Seppala *et al.* 2001; Papatryphon *et al.* 2004; Munkung 2005; Aubin *et al.* 2006). The extensive suitability of LCA to several product systems including food production and the

possibility of comparing data among similar systems makes LCA an important environmental assessment tool (Klopffer 2006, 2012).

Although aquaculture has been dubbed the most sustainable and efficient technique for meeting the increasing demand for fish (Pauly *et al.* 2002), the social, economic and the range of environmental concerns (viz., habitat destruction, nutrient enrichment, greenhouse gas emission, introduction of invasive species, genetic alteration, pest, and disease introduction) associated with captive production of fish have called for the need to weigh the benefits against the damages using an appropriate assessment technique (Diana 2009; Henriksson *et al.* 2013). Different tools, and frameworks such as Risk Assessment, Emergy Analysis, Fish-print and Ecological Footprint have been proposed as tools to quantify the impacts of aquaculture (Henriksson *et al.* 2013). Sets of Indicators measuring the economic, social and environmental sustainability of aquaculture have also been used individually or as aggregated indices (Valenti *et al.* 2018). However, most of these tools are limited in their operations as they only evaluate a restricted number of environmental burdens associated with aquaculture (Bartley *et al.* 2007; Henriksson *et al.* 2013).

Life cycle assessment is an all-encompassing framework that is currently employed not only as a tool to quantify the impact of aquaculture but also for mapping good farming practices (Henriksson *et al.* 2013). With aquaculture production requiring inputs in form of nutrients and energy and emitting solids, dissolved nutrients and waste gases into the environment, the LCA is an assessment tool designed to quantify a broad range of resource use and emissions-related issues in aquaculture (Henriksson *et al.* 2013). As an assessment framework, the LCA provides a scientific basis for developing aquaculture certifications and eco-labelling criteria for continuous sustainable seafood production (Cao *et al.* 2013). It differs

from other impact assessment tools used for quantifying aquaculture production because it makes a detailed assessment of relevant environmental impact along the whole life cycle of an aquaculture product based on a functional unit (Cao *et al.* 2013).

Life cycle assessment has found its application in different aquaculture techniques, especially in integrated aquaculture systems. It has been used in freshwater and marine aquaponic systems to assess the environmental impact of fish (rainbow trout/tilapia) and water lettuce integrated culture, where the technique reduced the of farming on the environment (Forchino *et al.* 2017; Cohen *et al.* 2018). Life cycle analysis has also been employed as an assessment tool in open-water IMTA systems of *Laminaria digitata* and salmon in the north Atlantic Island of Ireland where seaweed biomethane system yielded 70% reduction of greenhouse gas emission compared to gasoline (Czyrnek-Delêtre *et al*. 2017). Likewise, the integrated culture of the sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and Pacific oyster (*Crassostrea gigas*) lessened the impact of aquaculture on the environment compared to monoculture systems of the finfish (Ferreira *et al.* 2012).

Overall, applying LCA as an assessment tool for the impact of an integrated culture system helps to identify the different factors, inputs, and production processes contributing to the system's environmental burden and major production hotspots. In addition, conducting life cycle assessment for IMTA-produced seafood improves the products outlook in terms of certifications, eco-labelling and market acceptance.

Although different studies have shown that IMTA production of abalone and extractive species eliminate waste, increase food production and economic benefits, an understanding of the environmental benefits and burdens of this culture technique using LCA is still lacking in abalone/seaweed IMTA systems in South Africa. A portion of the present study therefore

sought an understanding of the impact of this production system from an environmental point of view.

1.1.5 Formulated abalone feed development in South Africa

While the production of seaweed in abalone effluent has been documented to improve nutrient utilisation and production efficiency, the environmental performance, growth and economic efficiency of abalone farming can further be enhanced by improving compound diet formulations for farmed abalone. This improvement can be achieved by reducing the use of fish and terrestrial grains in formulated diets and replacing them with farmed algal meal. This approach to formulated feed production will reduce abalone farms' dependence on fish and terrestrial grains resources, lower feed cost and minimise the impact of abalone farms on the environment. A study was therefore designed to develop a novel artificial feed by partly replacing conventional ingredients with farmed algal meals.

The development of formulated diets for South African abalone *Haliotis midae*, started in the early 1990's at the Department of Ichthyology and Fisheries Science, Rhodes University to enable captive production of this endemic animal (Britz *et al.* 1994; Knauer *et al.* 1994). This involved the identification of locally accessible protein-rich materials such as fishmeal, torula yeast, casein, *Spirulina spp*, and soya bean meal as feed ingredient for abalone. Exploratory feeding trials were later carried out to test the feeding response and growth of *H. midae* to the artificial diets using the specifications of semi-purified diet for Japanese abalone (*H. discus hannai*) as baseline, followed by a long-term research, development and optimization of diet for South African abalone (Britz 1996; Shipton & Britz 2001; Sales & Janssens 2004; Nel *et al.* 2017; Wu *et al.* 2019; Wu 2020). Better growth performance on artificial diets compared to

live macroalgae were recorded for juvenile *H. midae* (Dixon 1992; Britz 1995). Like other abalone species, *H. midae* are unselective feeders and they accept different feed ingredients of plant and animal origin. This facilitated the development of formulated feed for *H. midae* which is now marketed as Abfeed® (Marifeed Pty Ltd, South Africa).

1.1.6 *Improving feed efficiency with algal meals*

As South African abalone farms continue to expand their production capacities, the demands for conventional feed ingredients (fish and soya meal) in commercial formulated feed for abalone have increased and the trend is expected to continue with a corresponding rise in production cost. This is because fish meal, which is the most expensive ingredient in abalone feed, has no guaranteed future availability due to the continuous decline in capture fisheries. Also, soya bean, which has sealed its position as a major plant protein ingredient in abalone diet, is currently affected by increased demand from other sectors and unprecedented rainfall pattern caused by global climate change (Lupatsch and Kissil, 2004; Ansary *et al.* 2019). If abalone aquaculture is to be economically and environmentally sustained, the industry must limit its reliance on these ingredients with high production cost and unassured future availability (Britz 1996). It is therefore imperative that the industry continues to search for economically and environmentally sustainable alternatives that can partially substitute conventional feed resources or used as supplement to improve the feed utilisation efficiency of farmed *H. midae*, increase their market value and conserve natural ecosystems. One such alternative protein and natural supplement is algal meal (Bansemer *et al.* 2016a).

Live algae form the bulk of natural diet for abalone in the wild and they are also fed alone or in combination with pellets under culture conditions (Troell *et al.* 2006; Naidoo *et al.* 2006). Although the growth of abalone is enhanced when fed mixed algae diet or algae combined

with pellets (Naidoo *et al.* 2006; Viera *et al*. 2011; Dlaza *et al.* 2008; Kemp *et al.* 2015; Bansemer *et al.* 2015b; Robertson-Andersson *et al.* 2011), the biosecurity threats associated with the use of live food can sometimes be problematic (Britz 1996; Bansemer *et al.* 2016a). Feeding live algae can introduce pests, diseases and predators to production systems and also impact on abalone's growth rate due to their low nutrient density and high moisture content (Bautista-Teruel *et al.* 2011; Bansemer *et al.* 2016a). Therefore, to avoid these complications associated with using live feed and, to gain more benefits (improved growth, feed utilisation, health and marketability) of feeding algae to cultured abalone, nutrient-enriched algae are currently dried and incorporated in artificial diets (Bansemer *et al.* 2016a; Bansemer *et al.* 2016b). Previous trials on macroalgae meal inclusion in formulated diets for different species of abalone yielded positive results such as high feed acceptance, low feed conversion ratio, improved utilisation and growth rate, and better nutrient profile of abalone (Britz 1996; Allen *et al.* 2006; Lange *et al.* 2014; O'Mahoney *et al*. 2014; Bansemer *et al*. 2016b; Bates *et al.* 2017; Hoang *et al.* 2017; Nel *et al.* 2017; Ansary *et al.* 2019; Santizo-Taan *et al.* 2020; Duong *et al*. 2021; Sun *et al*. 2021). This is because concentrated algal meals are good sources of biologically active compounds such as polysaccharides, proteins, and polyphenols which are antimicrobial, antiviral, antioxidant and prebiotic in nature (Chojnacka *et al.* 2012; Bansemer *et al.* 2016a). They can act to stimulate the production of growth and health-enhancing bacteria in the digestive tract of fed species (Chojnacka *et al.* 2012; Nel *et al*. 2017).

Macroalgal meal inclusion in the diet of abalone can influence the digestive enzymes activities in the gut of abalone (Bansemer *et al.* 2016b; Nel *et al.* 2017). The Haliotids digestive system is conditioned to digest and utilise macroalgae (Nel *et al.* 2017). High trypsin and β-galactosidase activity in *H. laevigata* fed 5% *Ulva* and *Gracilaria* inclusion diet has been reported (Bansemer *et al.* 2016b). The up-regulation of digestive enzymes, especially the α-

amylase and β-galactosidase activities in abalone could elevate the rate at which carbohydrate is used for energy production which spares protein for growth (Bansemer *et al.* 2016b). A superior growth rate and dietary protein utilisation due to trypsin upregulation was recorded for greenlip abalone fed 5% *Ulva* meal inclusion diet (Bansemer *et al.* 2016b). Dietary *Ulva* inclusion in abalone diet can impact gut microbiota composition and influence the secretion of more digestive enzymes for better feed utilisation (Nel *et al.* 2017).

Macroalgae are also rich in omega-3 polyunsaturated fatty acids (PUFAs) such as phospholipids and glycolipids which are essential for the growth of *Haliotids* (Holdt and Kraan 2011). Higher growth rate was recorded in *H. laevigata* fed *Gracilaria spp.* or *Ulva spp.* meal inclusion diet than the control group fed a commercial diet with no macroalgae meal (Bansemer *et al.* 2016b). This superior growth was attributed to the high levels of arachidonic acid (ARA; 20:4*n*–6) and eicosapentaenoic acid (EPA; 20:5*n*–3) in the macroalgae. Arachidonic acid also functions to combat infections, preventing blood coagulation and inflammation (Nelson *et al.* 2002).

Algal meals are known for their feeding stimulation benefits in abalone (Bansemer *et al.* 2016a). For instance, the New Zealand Blackfoot abalone, *Haliotis iris*, fed algal (*Gracilaria spp*) stimulant diet spent 80% of their time feeding with 15% growth improvement above those without algal meal inclusion (Allen *et al.* 2006). Likewise, the Australian abalone, *H. laevigata* fed diets with 5-20% *Ulva spp*. or *Gracilaria spp*. meal displayed active feeding attributes in the light phase, a behavioral response not observed in *H. laevigata* fed 0% macroalgae meal diet (Bansemer *et al.* 2016b). This feeding stimulation by algae meal resulted in higher growth rate in weight and shell length of treated greenlip abalone. These

associated benefits of algal meal inclusion in the diet of other species of abalone show that it is beneficial to supplement the artificial diet for abalone with algal meal.

The use of algal meal as plant-protein ingredient in the diet of South African abalone (*H. midae*) started later in 1996 after an investigative trial by Britz (1996). This researcher tested the suitability of different protein sources (casein, fishmeal, soya oil cake, torula yeast and *Spirulina spp.*) in semi-purified diet for *H. midae* and documented that *spirulina sp.* inclusion in the diet significantly improved the feed utilisation and growth of *H. midae*. Over the years, some studies have built on the findings of Britz (1996) by testing different algal species inclusion in the diet of *H. midae* and the results were promising (Britz 1996; Shipton and Britz 2001; Ismail *et al.* 2009; Nel *et al.* 2017; *Madlala et al. in press*). For example, the inclusion effect of *Spirulina spp.* in weaning diet for *H. midae* revealed that the animals performed best when fed a combination of fish and *Spirulina spp.* Meal (Ismail *et al.* 2009). However, the use of *Spirulina* on a commercial scale was discontinued due to the high production cost of the algae (*Pete Britz, personal communication*). Another study tested the combination of different macroalgae meal (*Gracilaria gracilis; Laminaria spp; kelp, Ecklonia maxima; Gelidium spp and Phorphyra capensis*) as an all macroalgal formulated diet for juvenile and sub-adult *H. midae* and the macroalgal diet improved feed consumption by *H. midae* (Tsanigab 2009). Likewise in 2017, the inclusion effect of kelp (*E. maxima*) meal in the diet of sub-adult (43mm shell length) *H. midae* demonstrated that dietary kelp inclusion in pellets improves feed conversion, protein utilisation, growth, and also increases gut-bacteria homeostasis of *H. midae* (Nel *et al.* 2017). These researchers recommended a 3.5 % dry mass inclusion of kelp in the diet as its presence in formulated diets stimulates constant gut environment that is related to the gut microbiome. Kelp inclusion in artificial diet improved gut bacterial community which resulted in higher feed utilisation efficiency by *H. midae* (Nel *et al.* 2017).

More recently, a study had tested the inclusion effect of IMTA-grown *Gracilaria spp*. meal in the diet of grow-out (30-40g) *H. midae* where low inclusion (0.75 – 3%) of *Gracilaria* meal in the diet improved feed acceptance and growth of *H. midae* (Madlala *et al*. *in press*).

1.2 Research approach

The land-based integrated multitrophic system of aquaculture has continued to gain popularity in the South African abalone industry with many abalone farms fully integrated by co-culturing seaweed with *Haliotis midae*. This intensive production technique has necessitated the continuous inputs of nutrients in form formulated feed and inorganic fertilisers for cultured abalone and seaweed respectively. The production process also generates both solid and dissolved waste nutrients that are not absorbed by cultured organisms but discharged to the environment. This study was set out to quantify the production performance of an IMTA farm, the Wild Coast Abalone Pty Ltd and investigate different optimisation techniques to improve farming efficiency of the IMTA farm. These were through the removal of abalone biodeposits by detritus extracting species, using farmed macroalgae meal as supplement in compound feed, and finally, replacing chemical fertilisers with non-polluting and eco-friendly bio-fertilisers.

1.2.1 Description of Wild Coast Abalone Pty Ltd

The Wild Coast Abalone (32° 45' 02.7" S 28° 16' 29.0" E) is a 200 MT per annum land-based integrated abalone farm in the East Coast region of South Africa. The farm operates a high-density pump-ashore flow through system where eight pumps supply 6250 m^3 $h^{-1}$of seawater from the Indian Ocean into the land-based culture tanks. A proportion of the wastewater from abalone production tanks is channelled into fifty-seven (57) D-ended concrete paddlewheel

raceways for the culture of the seaweed *Ulva lacinulata* (Figure 1.3). Nutrients enter abalone and seaweed production tanks primarily from formulated feed pellets fed to farmed abalone. The seaweed culture system partially removes the dissolved nutrients in the abalone tank effluent before it is discharged into coastal environment. Apart from the cultured seaweed receiving dissolved nutrients (nitrogen and phosphorus) from the abalone effluent, nutrients also enter the system from mineral fertilisers used to supplement the dissolved waste nutrients in abalone tank effluent. Addition of inorganic fertilisers was necessary because abalone effluent is very dilute and additional nutrients are required for optimal seaweeds culture (Troell *et al*. 2006). Apart from the IMTA system at WCA, a small 'monoculture' section also exists where smaller size (< 30g) abalone and *Ulva* were cultured separately in fresh seawater for biosecurity reasons (Figure 1.3). Therefore, the nutrients for the monoculture system *Ulva* culture tanks were only supplied by inorganic fertilisers (Figure 1.3). The existence of the two systems provided a research opportunity to compare their nutrient utilisation efficiencies.

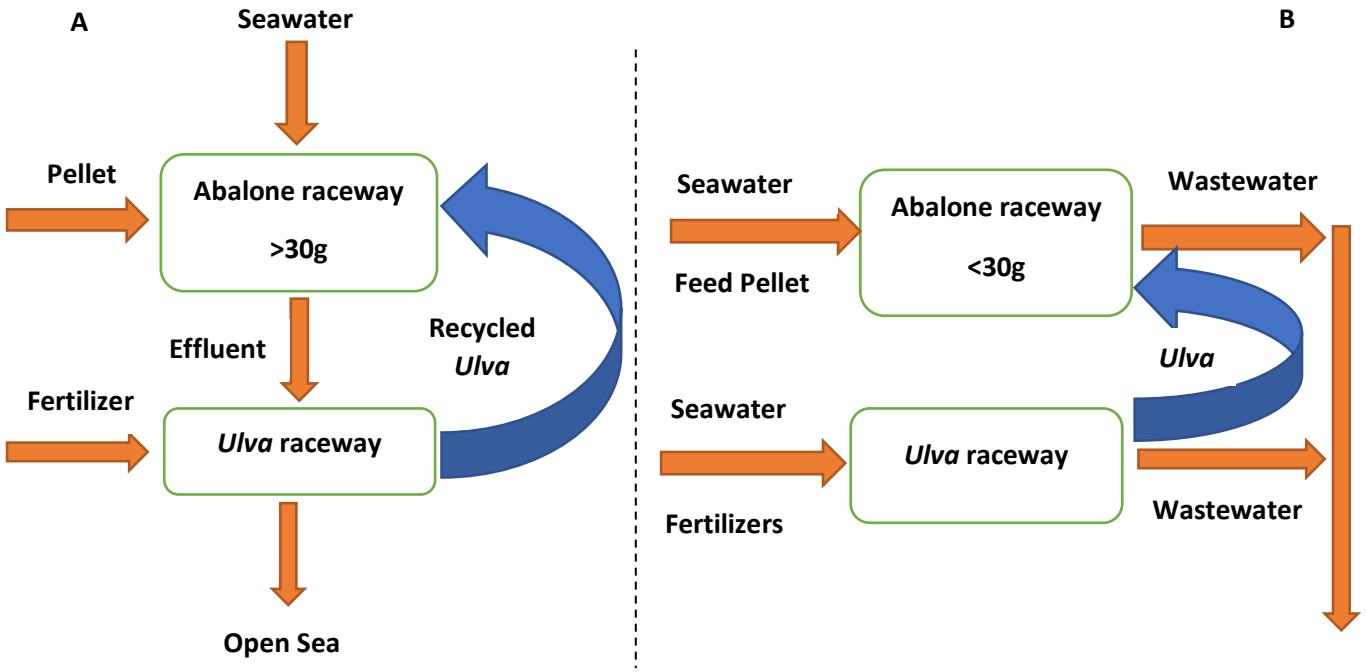


Figure 1.4. Abalone and macroalgae production at Wild Coast Abalone in (a) Integrated multitrophic aquaculture (IMTA) and (b) monoculture systems.

When the present project was initiated, the utilisation efficiency of the mineral fertilisers by seaweed in both systems was unknown and there were concerns that they may be contributing significantly to the overall discharge of nutrients by the farm. It was therefore decided to quantify the uptake efficiency of nutrients by the seaweed using a nutrient mass balance approach.

Abalone as the fed component of the IMTA system produce solids and dissolved waste nutrients. While the seaweed component of the current IMTA system absorbed a proportion of the dissolved nutrients in the abalone tank effluent, the waste solids originating from uneaten feed and faecal waste was flushed twice weekly during tank cleaning directly to the receiving coastal environment. It was known that the settled waste solids on production tanks floor could be removed biologically by organisms which feed on detritus as research in other regions such as China has shown that abalone waste solid is an excellent food source for

economically relevant species like the sea cucumbers (Kang *et al.* 2003; Fang *et al.* 2009; Grosso *et al*. 2023). It was therefore hypothesised that an endemic sea cucumber species could be used to remove detritus from *H. midae* tanks. This could reduce the particulate nutrient loading from abalone farms to the environment and produce another crop of economic importance.

Another identified means to potentially enhance the production efficiency of South African abalone farms was the inclusion of farmed macroalgae (*Ulva species*) as ingredient in commercial feed pellet (Abfeed®) for *H. midae*. Abfeed® contains fish and soya bean meals which the industry would like to partially substitute with locally available protein alternatives. With farmed *Ulva* having a relatively high protein level and well utilized by abalone, it was hypothesized that cultured *Ulva* meal could be included as a dry feed ingredient in formulated feeds for *Haliotis midae*. This approach will help reduce the biosecurity concerns of feeding fresh macroalgae as food supplement for farmed abalone.

Previous studies on partial replacement of conventional feed ingredients with *Ulva* meals in other cultured abalone species yielded promising results. For example, when *Ulva* meal was included in formulated diets of the Australian greenlip abalone (*H. laevigata*), the Donkey's ear abalone (*H. asinina*) and disk abalone (*H. discuss hannai*) the growth and feed utilisation efficiency of the abalone was enhanced (O'Mahoney *et al.* 2014; Bansemer *et al.* 2016b; Santizo-Taan *et al.* 2020). The response of abalone to algal meals in these previous trials was species specific (i.e., algae and abalone sp.) and dose dependent. However, no studies previously investigated the potential of farmed macroalgae (IMTA and monoculture) as dry feed ingredient for *H. midae* formulated feeds. Likewise, their optimal inclusion level has not been documented. Therefore, two long-term growth studies were conducted in this research

to test the inclusion effect of effluent and seawater-cultured *Ulva spp.* in formulated diet for *H. midae.*

The use of live microbial fertilisers to substitute for inorganic fertilisers could be another means of improving farming efficiency on South African abalone farms. Macroalgae culture in abalone effluent requires the supplementation of nutrients from inorganic fertilisers due to the low dissolved nutrient composition of the wastewater of *H. midae* and the large-scale production of seaweed (Troell *et al.* 2006). Mineral fertiliser supplements such as urea, monoammonium and monopotassium phosphates are utilised because of their growth and health benefits (Jacob and Kumar 2020). However, the continuous use of chemical fertilisers results in net outflow of dissolved nutrients from farms due to their inefficient uptake by farmed macroalgae (Robertson-Andersson 2003). The quest to improve nutrient uptake by seaweed and reduce the pollution effect of farms on the environment has necessitated a search for alternatives to nitrogen and phosphorus-based chemical fertilisers. As a replacement for mineral fertilizers in crop production, the use of biofertilisers has gained popularity recently, and they are established as a crucial part of the integrated nutrient delivery system (Chen 2006). This is because, by giving nutrients via ecologically benign methods, biofertilisers have a huge potential to increase crop yield and reduce nutrient release to the environment (Chen 2006; Das *et al.* 2007). They make inorganic dissolved nutrients more readily available to plants by using natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth promoting substances (Jacob and Kumar 2020). Unlike chemical fertilisers, live microbial fertilisers are biodegradable, non-toxic and eco-friendly (Renuka *et al.* 2018). Although they do not provide nutrients to plants, biofertilisers increase plants' absorption of inorganic nutrients and make essential nutrients more readily available (Wainwright *et al*. 2014).

Though used for arable crops production, information on its use for seaweed production is relatively scanty. Therefore, an experiment was set out in this research to test the efficacy of biofertilisers in macroalgae production. It was hypothesised that its application to seaweed increases the uptake of nutrients from mineral fertilisers and reduces the discharge of waste nutrients to coastal environments.

1.3 Aims and objectives.

This thesis sets out to investigate and improve the production performance of an existing abalone and seaweed IMTA system on a commercial abalone farm. This was achieved by quantifying the nutrient utilisation efficiency and the environmental impacts of the production systems using a mass balance approach and life cycle assessment. The different techniques to improve the production performance of the IMTA system were examined. To address the overall aim, the following objectives were adopted:

i. Nutrient production and flow patterns within the farming systems at Wild Coast Abalone were evaluated (Chapter 2). This baseline study examined the potential factors contributing to nutrient production in a continuous flow system of abalone farming. The disintegration potential of commercial formulated diet fed on SA abalone farms, and its contribution to dissolved and organic nutrients production in abalone production tank was examined. The diurnal variations in dissolved nutrient production were also examined.

ii. The nutrient utilisation efficiencies in integrated and monoculture systems of abalone/macroalgae were compared. Abalone and seaweed farming requires inputs of nutrients from feed pellet, mineral fertilisers, abalone tank effluent and seawater. These inputs go through nutrient cycling processes to produce harvestable products

(abalone, seaweed) and waste outputs (dissolved and organic waste). Resources utilisation within the production systems was evaluated using the nutrient mass-balance approach (Chapter 3).

iii. The relative impacts of the two production systems (IMTA and monoculture) on the environment were compared using life cycle analysis (Chapter 4). This was set out to document the environmental hotspot of each production system and to quantify the impact of each compartment of the two production systems on the environment.

iv. A comparison of the nutritional effect of macroalgae produced from the two production systems (IMTA and monoculture) was conducted (Chapter 5). This chapter investigated the inclusion effect of effluent and clean seawater-cultured *Ulva* meal (both included at 12%) on the growth, feed acceptance and nutrient utilisation of *H. midae*. These novel diets were compared with standard Abfeed® S34 (as control diet) and reference diets of pellet and fresh seaweeds at Wild Coast Abalone. The trial was conducted as a means of improving farming efficiency.

v. A comparison of the growth of abalone fed graded inclusion level of IMTA *Ulva spp.* meal in commercial abalone pellet was made (Chapter 6). This was a follow-up experiment to the experiment in objective five. Different dietary levels of IMTA-grown *Ulv*a meal were included in the diet for *H. midae* to compare their growth and nutrient utilisation efficiency.

vi. A pilot study designed to evaluate the effect of sea cucumber (*Neostichopus grammatus*) integration into an existing abalone/seaweed IMTA system on nutrient and energy flow (Chapter 7). Two production systems (i) abalone/*Ulva* and (ii) abalone/sea cucumber/*Ulva* integrated system were assessed and compared for

efficiency of nutrient utilisation using the nutrient mass balance approach. The effect of sea cucumbers inclusion into the production system on organic waste production was evaluated.

vii. The substitution effect of mineral fertilisers with live microbial fertilisers on the uptake and discharge of nutrients from seaweed culture system was evaluated. Additionally, the growth, yield, and nutrient compositions of *Ulva* were quantified as a means of improving the production performance of the IMTA system at Wild Coast Abalone (Chapter 8).

Chapter 2: Nutrient conversion pathways in abalone production tanks at Wild Coast Abalone

2.1 Introduction

The waste nutrients in the effluent of South African abalone farms originate primarily from the feed and metabolic processes of abalone. These waste load dynamics have been investigated in previous studies with respect to the level of discharged nutrients. In general, low concentrations of nutrients were recorded in effluents exiting abalone farms (Samsukal 2004; Sankar 2005; Yearsley *et al.* 2009; Probyn *et al.* 2017). However, the nutrients produced and discharged from abalone farms can have localised effects on certain flora and fauna species in the vicinity of the farm discharge point. For example, tracking the environmental pathway of abalone farm effluent using carbon and nitrogen stable isotope analysis revealed that the wastewaters from farms along the coast of Jacobsbaai and Mauritzbaai in the west coast region of South Africa have localised effect on brown seaweed, *Ecklonia maxima* and the Mediterranean mussels, *Mytilus galloprovincialis* (Thomas 2007). This confirms that nutrient production pattern and waste loading from abalone farms is site specific, determined by local coastal oceanography and recurring input of nutrients on farms. The present preliminary study was therefore set out to quantify the production of waste nutrients (organic and dissolved) produced on a standard land-based *H. midae* farm. This was necessary to improve the performance efficiency of the existing IMTA system by determining where and how to improve the utilisation of nutrients and reduce the discharge of unabsorbed nutrients to the environment.

Two forms of waste nutrients emanate from 'fed' abalone rearing tanks. These include the dissolved nutrients leaching out of formulated feed or abalone faeces and secondly, dissolved waste nutrients from abalone metabolic processes (Yearsley *et al.* 2009).

The water quality dynamics and nutrient waste production on *Haliotis midae* farms follow diurnal pattern due to the nocturnal feeding habit of abalone and the variations in metabolic processes caused by temperature changes (Yearsley 2007). Abalone have been recorded to excrete particulate waste for 60 h post-feeding (Shipton and Britz 2001). During the digestion of food, abalone consume dissolved oxygen to produce ammonia and carbon dioxide as main metabolites (Barkai & Griffiths 1987; Yearsley 2007; Naylor *et al.* 2011). The production of carbon dioxide also affects water quality by lowering the hydrogen ion concentration (Sanni & Forsberg 1996). Hydrogen ion concentration on abalone farms follows a diurnal pattern with lowest and highest levels recorded at pre-dusk and midnight (Yearsley 2007). Likewise, the consumption of oxygen and the production of ammonia by abalone vary over 24 h due to their aerobic metabolism with highest dissolved oxygen consumption occurring at night (Barkai & Griffiths 1987). An investigation of the diurnal water quality dynamics at Wild Coast Abalone was carried out to establish the period of peak concentration for dissolved nutrients exiting the abalone production tanks. The result from the monitoring was required to develop an appropriate nutrient monitoring programme in the next chapter.

Particulate waste nutrients may alter the quality of water in abalone tanks and can also affect the peak period of nutrient concentration (Yearsley 2007). A clear understanding of the factors responsible for waste nutrient production at the study site was required to characterise the potential nutrients held within abalone tanks bottom sediments and give insight to the utilisation efficiency of combination diets fed to farmed abalone.

Nutrients produced on abalone farms are usually a product of wastes associated with feed following grazing, ingestion, excretion, and biodegradation by microorganisms. At Wild Coast Abalone (WCA), particulate waste generated from feeding a combination of pellet and seaweeds settles in the *H. midae* production tanks between cleaning events. Sediments are removed from each culture tank twice weekly and discharged to the receiving coastal waters without any treatment. Before a tank cleaning event, part of the solid waste is re-mineralized into water column. Since abalone are slow feeders and the applied feed may remain uneaten for several hours after application, it was hypothesised that the breakdown and leaching of nutrients from formulated diet was probably a major source of dissolved and particulate waste nutrients on the farm. Additionally, the breakdown and the rapid loss of nutrients from formulated diet could be exacerbated by the vigorous tank aeration technique employed on abalone farms coupled with the physical grasping of pellet by *H. midae*. Therefore, quantifying the water stability of pellets and measuring the dissolved nutrients and solids leaching losses was necessary.

The solid waste materials in abalone tanks are degraded by microorganisms and higher chemoheterotrophs to produce nutrients which may be remineralised into water column or settled on tank floor. The accumulation of these organic materials in abalone tanks result in the increase of free ammonia-nitrogen (FAN) concentration in water and increase total ammonia nitrogen production by approximately 44% if not removed (Yearsley *et al.* 2009). Free ammonia nitrogen concentration of 9.8 – 16.4 µg L^{-1} can result in 50% mortality of farmed *H. midae* (Reddy-Lopata *et al.* 2006). Detritus waste also makes suitable growth environment for abalone's ectoparasites (Yearsley *et al.* 2009). Accumulated sludge forms the main food of the problematic polychaete worm, *Terebrasabella heterouncinata* with high preference for solids from abalone fed formulated feed (Chalmers 2002). This preference is

attributed to the particle size, high organic content, protein and energy of formulated feed waste solids (Chalmers 2002). Therefore, a strict adherence to regular removal of sludge and constant cleaning of abalone production tanks is required to prevent these detritus filtering shell parasites from deforming abalone shells and becoming a major threat to abalone aquaculture (Simon *et al.* 2004).

The organic waste materials in abalone production tanks contain a significant concentration of nutrient (Chalmer 2002; Ho 2006; Yearsley *et al.* 2011). This concentration varies depending on the nutrient composition of the feed consumed by the abalone and is usually high for abalone fed formulated diets and low for those fed seaweed (Chalmers 2002). For example, not less than 55% of the nitrogen, 69% phosphorus, and 45% of carbon in a commercial diet (Adam and Amos™) for the greenlip abalone (*H. laevigata)* were lost to the particulate waste settling on the production tank floor (Ho 2006). Likewise, approximately 66-88% nitrogen in formulated diet (Abfeed®) for *H. midae* was lost to organic waste materials (Chalmers 2002; Yearsley *et al*. 2009). However, a lower nitrogen level (1.69%), representing 33% of feed nitrogen was documented for kelp produced accumulated sludge (Chalmers 2002). No study has documented the production pattern and nutrient (nitrogen, phosphorus and carbon) composition of the accumulated sludge of *H. midae* fed the combination of pellets and seaweeds. It was hypothesized that this feeding technique produces a high solid waste load and that the replacement of a portion of pellet with live macroalgae could reduce the nutrient levels of the waste solids.

The nutrients in the effluent of *H. midae* vary diurnally with the feeding and excretion cycle of abalone. The seasonality in seawater temperature which affects the metabolic rate of abalone also affects the nutrient composition of abalone waste (Yearsley 2007). Therefore, to

determine the aggregate and average load of nutrients in abalone waste, it was important to understand the diurnal and seasonal cycle variations in nutrient load from abalone tanks.

Although a diurnal rhythm in water quality of Cape abalone farms with much colder water temperature was reported (Yearsley 2007), the east coast abalone farms experience much warmer water temperature all year round which affects the metabolism and microbiota of abalone in this region (*Pete Britz, pers com*). A knowledge of the Wild Coast Abalone farm nutrient export over a 24-hour cycle was therefore necessary to determine the peak periods of nutrient concentration. Hence, a part of this study investigated the diurnal variation of dissolved nutrients exiting abalone production tanks at WCA.

Overall, the current study investigated the potential pathways of waste nutrients production and patterns on a shore-based abalone farm in the east coast region of South Africa. This was achieved by examining:

i. the disintegration rate of commercial feed pellet (Abfeed®) used on Wild Coast Abalone by quantifying the proportion of particulate and dissolved nutrients that pellets contribute to abalone farms waste stream.
ii. the quantity of solid waste (from uneaten feed and faeces) produced by abalone in a standard production raceway tank, and the nutrient (nitrogen, phosphorus, carbon) composition of these waste solids.
iii. the diurnal variation in the concentration of dissolved nutrients in the effluent exiting abalone tanks in order to determine the peak discharge periods.

2.2 Materials and methods

To achieve the experimental objectives listed in Section 2.1, four experiments were conducted. These include:

Experiment 1: Effect of abalone tank aeration on pellet dry matter and nutrient loss.

The effect of culture tank aeration on the stability of abalone feed pellets was evaluated to determine the loss of dry matter and nutrients from the pellet fed on South African abalone farms.

Experimental System

The experiment was conducted using four standard abalone flow-through tanks (5.0 m x 2.2 m x 1.05 m; length x width x depth), each with 16 oyster mesh baskets (0.87 m x 0.48 m x 0.5 m; length x width x depth) suspended in the tank (Fig. 2.1). The tanks were washed, filled with seawater and maintained at a flowrate of 3.0 $m^3 h^{-1}$ and at ambient temperature. The baskets were not stocked with abalone for this trial. Air was supplied in two tanks, using a farm blower via polyethylene pipes, that were perforated at 55 mm intervals, positioned lengthwise under abalone baskets, a system which is commonly used to aerate tanks on abalone farms in South Africa (Robertson-Anderson *et al.* 2011). The other two tanks were not aerated as they served as the control tanks.

Figure 2.1. Standard abalone production tanks with oyster mesh baskets.

Experimental trial

Commercially available abalone feed (Abfeed® S34 leaf; 30 mm x 30 mm, x 1.2 mm; length x width x thickness; 34% protein; 4.5% lipid; Marifeed Pty Ltd, South Africa) was used for the trial. Abfeed® S34 was selected as it forms the most widely used product on abalone farms at the time of the experiment (Dirk Weich, General Manager, Marifeed Pty Ltd, *pers. com*.). About 30 g of the feed pellet was weighed into each of the sixty-four baskets in the prepared tanks with 32 baskets (i.e., two tanks) allotted to the aerated treatment and the other 32 baskets to the non-aerated treatment. A temperature logger (Ebro, EBI 300) was deployed into one aerated and non-aerated tank. Water temperature ranged between 17.4°C – 19.3°C and 17.0°C- 19.1°C for the non-aerated and aerated tanks respectively. At pre-established sampling times of 0, 15, 30 min and after 1, 4, 8, 12, 24 and 36 h, three randomly selected baskets containing soaked pellets were removed from the aerated and non-aerated experimental tanks and allowed to drain for 10 min. The pellets were rinsed with distilled water (to remove salt) and then transferred into pre-weighed Petri-dishes. They were then oven-dried at 65 °C (PROLAB-PL010) for 36 h to achieve a constant weight and thereafter,

they were transferred into air-tight containers and stored at -4°C. The percentage of dry matter leaching (DM*i*), at the respective immersion times, was calculated using Equation 2.1 (Carvalho and Nunes 2006):

$$DM_i = [1 - (Wd_i / Wf)] \times 100 \qquad \text{Equation 2.1}$$

where: DM*i* = percentage of dry matter leaching at time *i* (%); Wf = weight of dry feed before immersion in seawater (g), and Wd*i* = weight of feed (g) after immersion in seawater at time *i*. Dry feed weight refers to feed weight after drying at 65 °C for 36 h

Finally, triplicate samples of Abfeed® pellets at the start and after 4, 8, 12, 24, and 36 h of immersion and aeration were analysed for their nitrogen, phosphorus, and carbon compositions using standard procedures described in Jimenez & Ladha (1993) and Zasoski & Burau (1977). The analysis was carried out at the ARC-institute for soil, climate and water, Pretoria, South Africa.

Carbon and nitrogen determination in samples of feed pellet

For carbon and nitrogen analysis, each pellet sample was milled to a fine powder using a laboratory mortar and pestle. Approximately 10 mg of powdered sample was weighed into a thin-walled tin foil container and used directly for C and N determinations on a Carlo Erba NA 1500 C/N/S analyser following the Dumas dry oxidation method. The analyser was calibrated against a pure organic compound (ethyl ester 4-aminobenzoic acid). The sample in the tin container was ignited at 1020 °C in oxygen (on a chrome oxide catalyst) to produce carbon dioxide, nitrogen gas, and oxides of nitrogen. To remove oxides of sulphur and halogens, the nitrogen gas that was produced was passed through silver cobalt (II) oxide and a column of copper at 550 °C. This helped to disintegrate nitrogen oxides into N_2 gas while free oxygen

gas was removed in the process. Later, a trap of anhydrous magnesium perchlorate (Cl_2MgO_8) was employed to remove water vapour. The N_2 gas and CO_2 were finally separated by gas chromatography using a helium carrier gas. A thermal conductivity detector detected carbon and nitrogen in the samples.

ICP Determination of Phosphorus

Phosphorus determination in the samples followed the inductive coupled plasma optical emission spectrometry (ICP-OES) method, using Agilent 700 series (Jimenez & Ladha 1993). About 1.00 g of powdered sample was weighed in a digestion tube and digested with 14 ml of concentrated nitric acid (HNO_3) and 6ml of perchloric acid ($HClO_4$) at 200 °C and was later brought to 100 ml in a volumetric flask (Zasoski and Burau 1997). An aliquot of the digested solution was used for P determination at a wavelength of 213.618 nm after calibrating the instrument against standard solutions containing all elements of interest.

Experiment 2: Effect of grazing by H. midae on the leaching rate of nutrients from feed pellet

The effect of grazing by abalone on the loss of solids and nutrients from formulated diet was quantified.

Two abalone production tanks, each stocked with 16 baskets of 65-75 g abalone (200.86 ± 0.20 kg basket^{-1}) were used for this trial. Before the trial, the experimental *H. midae* in their baskets were sprayed with a jet of water to flush away leftover feed and debris and the animals were allowed to purge for 48 h to increase their food demand when the trial commences. The flow of water to each tank was kept at 1.5 L s^{-1} while temperature in the tank was monitored using the EBI-300 temperature logger. At 1600 h, 40 g of feed pellet was

fed to experimental abalone in each basket. This quantity was equivalent to two-thirds of the daily ration, to ensure that every pellet given was grazed by abalone within 24 h of the study. Samples of grazed pellets were collected from all 32 baskets after 4, 8, 12 and 24 h post-feeding. The pellets were oven-dried and stored using the same methods described in Experiment 1. The grazed and dried pellets were analysed for their nitrogen, phosphorus, and carbon composition following the same procedure described in the previous trial.

Experiment 3: Abalone solid waste production and nutrient composition

This experiment was designed to determine the quantity of solid waste produced in abalone production tank over 72 h, and to determine the nutrient composition of the waste.

Three tanks with dimensions described in Experiment 1 were stocked with 200 ± 0.6 kg of 65-75 g abalone with water exchange in the tanks maintained at 1.50 ± 0.22 L s^{1}. Before the trial, all the tanks and abalone holding baskets were cleaned and flushed of debris following the farm's standard husbandry practice and were then re-filled with seawater. After preparation and stocking, the animals were left unfed for 48 h to allow them to purge their gut content. Thereafter, each abalone basket was fed combination diet of Abfeed® S34 formulated diet (50 g) and fresh *Ulva spp* (325 g w/w) and *Gracilaria gracilis* (120 g) for 72 h until the end of the trial. This feeding regime simulated the standard feeding protocol on the farm. The animals were not fed on the fourth day to allow them to purge their guts for 24 hours before the tanks were cleaned. The trial was terminated at the end of the fourth day, and the baskets in each tank were first sprayed down with water to remove all solid forms. Later, the water level in the experimental tanks was reduced to two-thirds of their volume and the baskets transferred to another tanks. Thereafter, the experimental tank base outlet where the

detachable standpipe connects to the raceway was clogged with a sponge, and the pipe was completely lowered. Clogging the outlet with the sponge allowed only water to drain through the sponge while the accumulated sludge was retained in the tank. When the water had completely drained from the tanks, the leftover sludge was collected in three 10 L buckets and allowed to stand for three hours. After this period, the clear layer of water above the sediments was filtered through a muslin cloth while the leftover sludge was collected in pre-weighed heat-resistant aluminum containers. The containers were later oven dried at 65 °C for 36 hours. The weights of dried sediments were recorded and then stored for nitrogen, phosphorus and carbon analyses as described in Experiment 1a.

Experiment 4: Diurnal water quality dynamics in abalone production tanks

The monitoring of diurnal water quality in abalone production tanks was conducted for 24 hours in June 2020. The trial was carried out to document the peak period of dissolved waste nutrients in abalone post-tank effluent.

The experiment started at 1600 h, one-hour post-feeding at WCA. Three tanks (dimensions described in Experiment 2.2.1), that were stocked with 65-75 g abalone (200 kg ± 0.11 kg/tank) and gravity-fed with drum-filtered seawater at an average flow of 1.50 ± 0.12 L s^{-1} were monitored for this trial. Seawater entered the tanks at one end and the resulting effluent flowed out through a standpipe at the opposite end of the tank. After one-hour of feeding, the temperature, pH, and dissolved oxygen concentration of the incoming and outflowing water from each tank were recorded in-situ using pre-calibrated Ebro temperature logger (model EBI 300, serial number 73268609 by Xylem Analytics, Germany), Thermo Scientific Eutech Expert pH meter (serial no. 2913115, Singapore) and Oxyguard handy Polaris DO

meter (Model v. 2.51, serial no. 015BODE0140000 by Oxyguard International A/S, Denmark) respectively. Water samples were collected for dissolved nutrient analysis from the inlet and outlet of each raceway (n=3) using acid-rinsed 500 ml bottles at one hour after feeding and the process was repeated every four hours for 24 hours. After collection, the water samples were filtered (0.45 µm; Whatman filters, CAT no: 1001185) and analysed for total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3-N), and reactive phosphate (PO_4-P) using a spectrophotometer (Palintest automatic wavelength photometer, model 7100, Palintest Ltd, England) and commercial Palintest test reagents (AP163 Nitratest and AP152 Ammonia; batch: CEO59/CE060). Analysis followed standard methods and procedures for the examination of natural water and wastewater (APHA 2005).

2.2.4 Statistical analyses

The repeated measure multi-factorial analysis of variance (F-RM ANOVA) was performed to analyse and compare the stability and leaching loss of dry matter from the test pellets over 36 h of immersion in water. The diurnal variations in the concentration of water nutrients and physical parameters were compared with the one-way analysis of variance. All analyses were carried out using STATISTICA® software for windows and Microsoft excel (version 13.4; Statistica, Tulsa, USA). The assumptions of homogeneity of variances and normal distribution were tested using the Levene's and Shapiro-Wilk tests, respectively. Where the assumptions of these parametric tests were violated and the data transformation failed, the non-parametric Kruskal-Wallis test was performed as an alternative test to the one-way analysis of variance tests. A significant level of $p < 0.05$ was used for all statistical tests and Tukey's multiple range post-hoc analysis was employed if a significant difference was detected across treatments. The results from all the trials are presented as mean ± standard error.

2.3 Results

Experiment 1: Dry matter loss from feed pellet for South African abalone

The result showing the effect of basket aeration on the leaching loss of dry matter from Abfeed® pellets is presented in Figure 2.2. Overall, pellets from both treatments (aerated and unaerated) were stable in water over 36 h of water immersion, losing weight gradually and retaining 77.4% (aerated) and 80.1% (non-aerated) of their original weights. There was no significant difference in the disintegration between the two treatments over time (RM-ANOVA, $F_{(8, 36)} = 7.639$, $p = 0.636$; Figure 2.2).

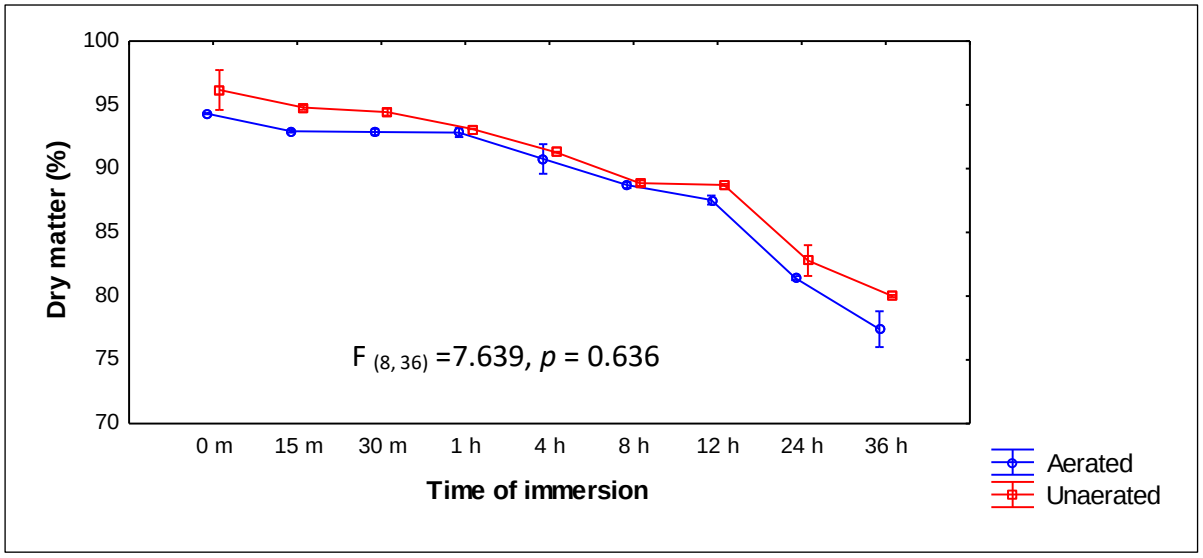
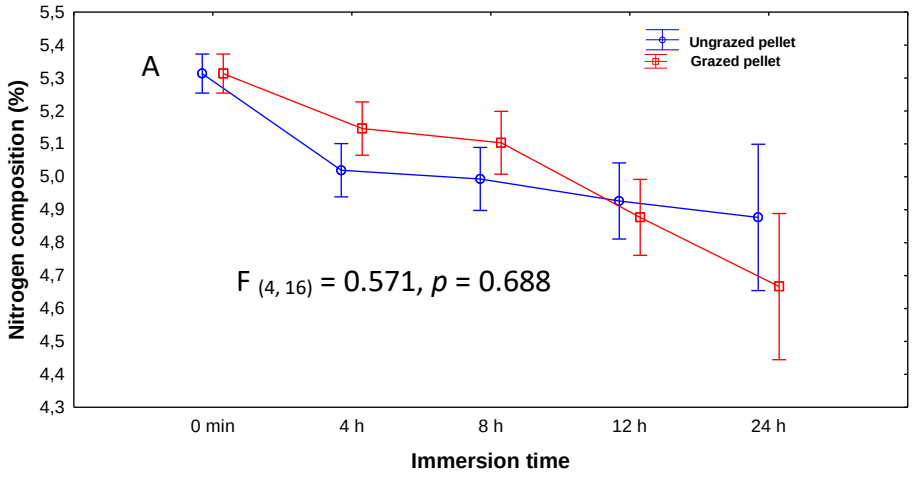


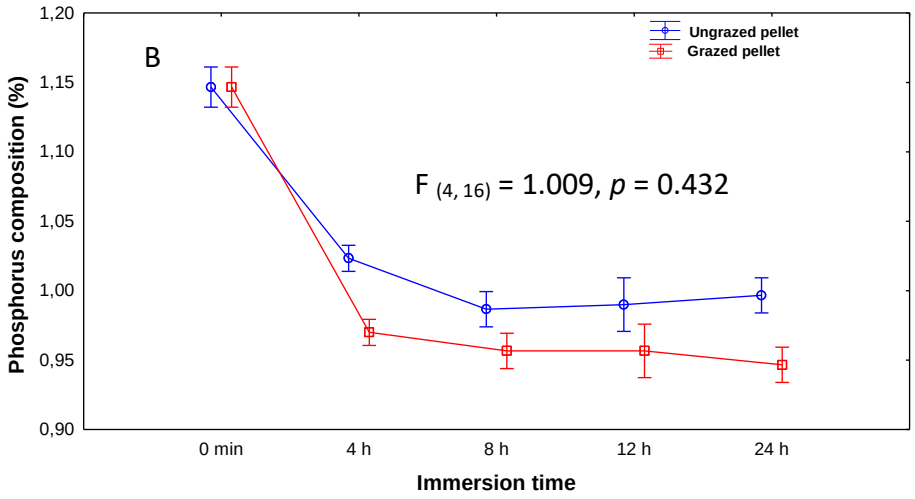
Figure 2.2. Solid loss (means ± standard error) from Abfeed® pellet over 36 hours of immersion in seawater with and without tank aeration.

Experiment 2: Nutrient compositions of grazed and un-grazed pellets in seawater

A similar nutrient loss was recorded for both grazed and un-grazed pellets (Figure 2.3). Nitrogen losses after 24 h immersion was 8% for the un-grazed pellet and 12.5% for grazed pellets and no significant difference was observed between treatments over time (RM-

ANOVA, $F_{(4, 16)} = 0.571$, $p = 0.688$; Figure. 2.3a). A rapid loss of phosphorus was recorded for both grazed (15.65%) and un-grazed pellet (11.3%) between 0 - 4 h immersion (Figure 2.3b). Thereafter, phosphorus composition remained relatively stable for both treatments and after 24 h, and approximately 17.39% and 13.91% phosphorus were lost from the grazed and un-grazed pellets respectively. The loss of phosphorus from both treatments was similar after 24 h (RM-ANOVA, $F_{(4, 16)} = 1.009$, $p = 0.432$; Figure 2.3b). The carbon composition of un-grazed and grazed pellet decreased from 41% at 0 min of immersion to 38.9% and 38.56% after 24 h respectively (RM-ANOVA, $F_{(4, 16)} = 2.101$, $p = 0.522$; Figure 2.3C).





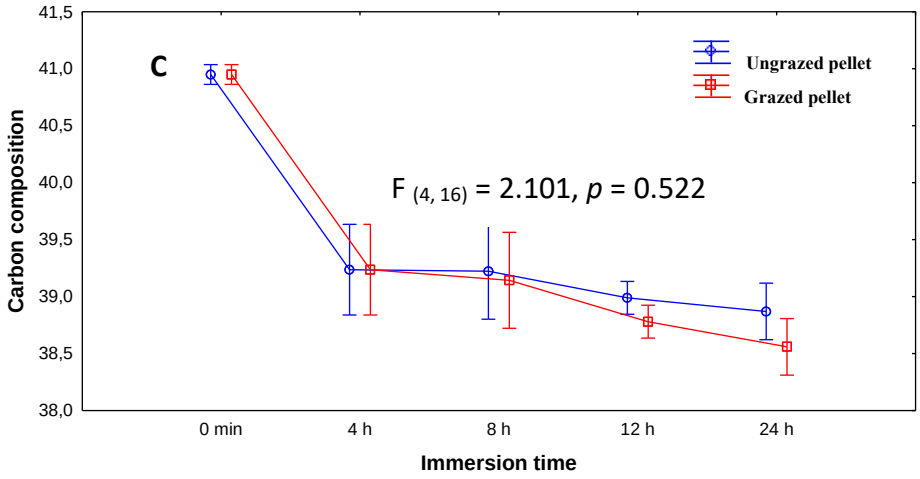


Figure 2.3. Percentage (mean ± standard error) (A) nitrogen (B) phosphorus and (C) carbon composition of grazed and un-grazed pellets at different immersion times in seawater.

Experiment 3: Abalone solid waste production and nutrient (nitrogen, phosphorus, carbon) composition

The nitrogen, phosphorus, and carbon composition of solid waste from abalone tanks were compared with the nutrient composition of the fed diet (Abfeed, *Ulva* and *Gracilaria*) and presented in Table 2.1. The accumulated sludge had a low nitrogen (1.45 %) and carbon (9.30 %) content in comparison with the nitrogen and carbon of Abfeed (N-5.31%, C-40.95%), *Ulva* (N-4.65%, C-34.63%), *Gracilaria* (N-3.16%, C-22.59%). However, the phosphorus composition of the accumulated sludge (0.86%) was higher than the P of *Ulva* (0.36%) and *Gracilaria* (0.33%) but lower than that of the pellet (1.15%).

Table 2.1. Mean (± standard error) nutrient composition of feed pellet, seaweed species, and *H. midae* solid waste.

Nutrient	Feed type			Solid waste
	Abfeed pellet	*Ulva*	*Gracilaria*	
Nitrogen (%)	5.31 ± 0.06	4.65 ± 0.05	3.16 ± 0.03	1.45 ± 0.02
Phosphorus (%)	1.15 ± 0.15	0.36 ± 0.09	0.33 ± 0.07	0.86 ± 0.05
Carbon (%)	40.95 ± 0.09	34.63 ± 0.12	22.59 ± 0.39	9.30 ± 0.07

A tank stocked with 200 kg abalone and fed 1.7 kg mix of pellet and seaweed (d/w) yielded approximately 0.67 ± 0.31 kg (dry weight) of solid waste per day (Table 2.2). This shows that 1000 kg of abalone fed 8.3 kg combination of pellet and seaweed (dry weight) per day produces approximately 3.3 kg dry weight of solid waste. From this trial, it was estimated that about 39.75% of the feed fed to *H. midae* goes to waste either through uneaten feed dropping through holding basket holes or from faecal materials while the remaining 60% is either assimilated or lost as dissolved organic waste compounds from metabolism and leaching from feed.

Table 2.2. Mean (± standard error) quantity of feed fed and solid waste retrieved from 200kg *H. midae* tank after three days of feeding.

	Feed type			Solid waste
	Abfeed pellet	*Ulva*	*Gracilaria*	
Quantity (kg)	3.12 ± 0.11	1.54.63 ± 1.4	0.44 ± 0.27	2.02 ± 1.17

Experiment 4: Diurnal water quality dynamics in abalone production tanks

Physical parameters

There were significant diurnal patterns in the dissolved oxygen, temperature and pH levels of effluent exiting abalone production tanks with lowest and peak levels occurring at different times over 24 hours. The dissolved oxygen (DO) level reduced at midnight and pre-dawn hours while significantly higher DO concentration was recorded at midday (1200 h) (One-way ANOVA: F = 448.32, $P < 0.05$, $df = 12$; Figure 2.4). The highest temperature was observed in the late afternoon (1600 h), which was closely followed by midday's temperature. Both hours (1200 and 1600 h) temperatures were significantly higher than other sampling hours (ANOVA, F = 95.015, $P < 0.05$, $df = 12$). The highest mean pH levels were recorded late afternoon (16h00) and at midday (12h00) and in the afternoon while the lowest was recorded at midnight.

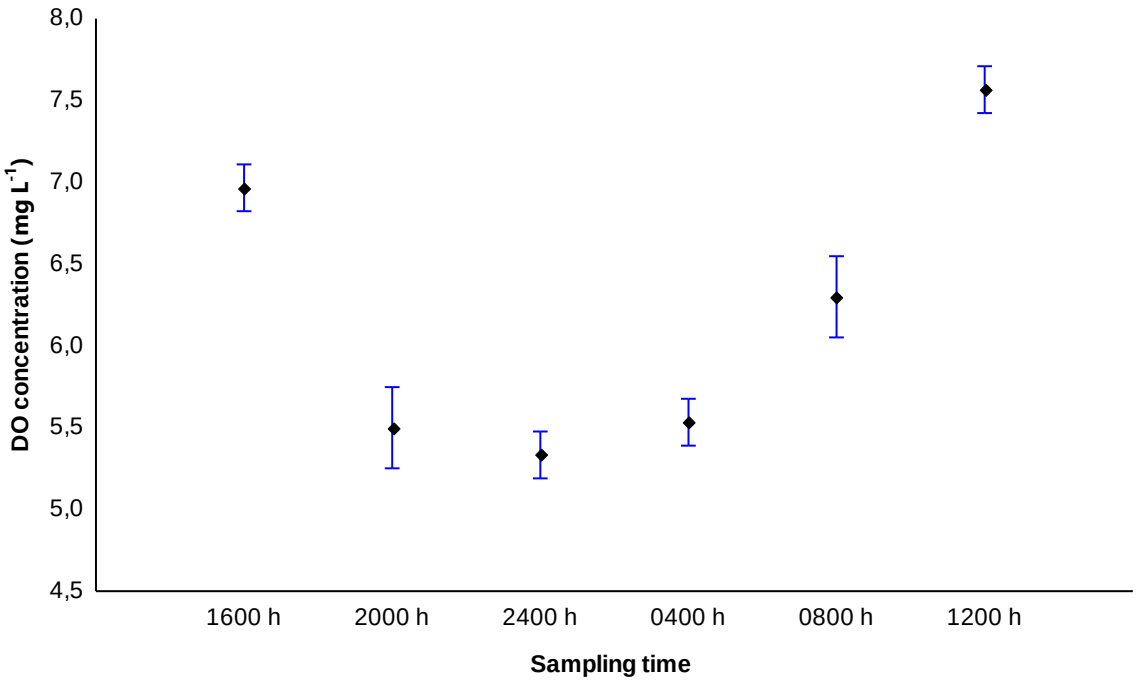


Fig 2.4. Mean (± standard error) dissolved oxygen (DO) level in the effluent exiting abalone tanks over 24h monitoring period.

Dissolved nutrients

The variation in the level of dissolved nutrients in abalone effluent also followed a diurnal pattern over 24 h of sampling (Table 2.3; Figure 2.4). The highest mean concentration of dissolved nitrogen (total ammonia nitrogen + nitrite-nitrogen + nitrate-nitrogen) was recorded just before dawn (0400 h), and then declined through the day to a low at 0200 h. Similar nitrogen concentrations were recorded at 0400 h and 0800 h and these concentrations were significantly higher than other sampling hours (One-way ANOVA: $F_{(5, 12)} = 28.65$, $P < 0.01$; Figure 2.5a). Phosphate concentrations of effluent at 0400 h, 0800 h and 1600 h were significantly higher than other sampling hours (One-way ANOVA $_{(5, 12)} = 32.17$, $P < 0.01$; Figure 2.5b).

Table 2.3. Mean (± standard error) diurnal fluctuations of dissolved nutrient, temperature and pH in abalone production tanks over 24 h of sampling. Values with different superscripts in the same row are significantly different (P ≤ 0.05).

Parameters	Time (h)					
	1600	2000	2400	0400	0800	1200
TAN [1] (mg/l)	0.16 ± 0.03^{b}	0.11 ± 0.02^{b}	0.05 ± 0.01^{b}	0.48± 0.07^{a}	0.38 ± 0.02^{a}	0.17 ± 0.03^{b}
Temperature (°C)	21.33 ± 0.15^{a}	20.10 ± 0.15^{b}	19.47 ± 0.07^{b}	20.60± 0.06^{a}	18.67± 0.03^{c}	19.37 ±0.07^{b}
pH	7.93 ± 0.03	7.83 ± 0.03	7.73 ± 0.03	7.83 ± 0.03	7.87 ± 0.03	7.97 ± 0.03

[1] TAN = Total ammonia nitrogen

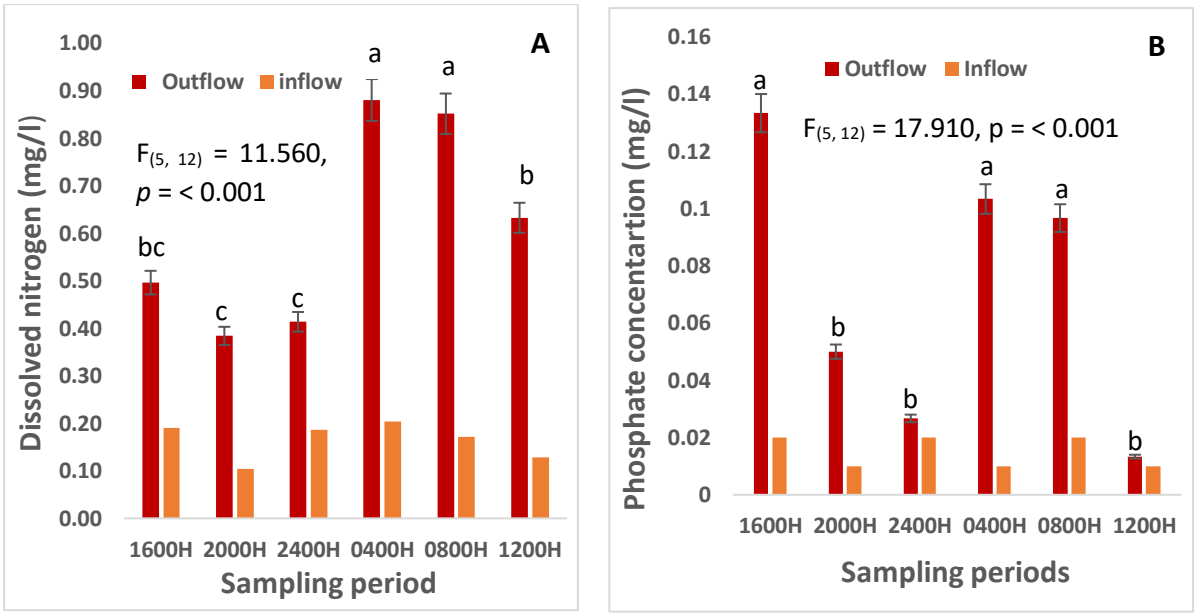


Fig. 2.5. Mean (± standard error) diurnal variations of (A) dissolved nitrogen (TAN + NO_3-N + NO_2-N) and (B) phosphate concentrations of abalone tank effluent at Wild Coast Abalone. Bars with different superscripts are significantly different ($p < 0.05$).

2.4 Discussion

The experiments to quantify the solids and nutrient losses from the 'fed' abalone production tank component of the IMTA system established a baseline for determining how much nutrient was potentially available for extractive IMTA components.

Overall, the commercial feed pellet maintained good stability in seawater with only 10% of the feed lost between the time the pellet was submerged in water and midnight when the bulk of the feed was consumed by farmed abalone. Additionally, tank aeration added an approximate 3% loss to the overall weight loss of tested pellet. However, similar weight loss was recorded for aerated and non-aerated pellets. The feed leaching loss of dry matter from the current study compares well with other feed stability studies. For instance, the dry matter loss of the aerated and non-aerated pellets after six hours of immersion were similar to the

10.15% dry matter loss from commercial feed pellet, (Adam and Amos™) for Australian greenlip abalone (*H. laevigata*) after seven hours of immersion in seawater (Ho 2006). It also compares with the 9.25% dry matter loss (by weight) from an experimental diet for *H. midae* (Sales *et al.* 2003).

The grazing habit of *H. Midae* made a low (4%) contribution to the loss of nitrogen from the test pellet during the period of active feeding (6-8 hours post-pellet application). Like other nutrients, nitrogen loss was predominantly caused by the 2-6 hours of pellets immersion before feeding commenced.

From the current results, the total nitrogen loss from feed pellets for the entire Wild Coast Abalone Farm was calculated. For the 180 tonnes per annum grow out section of the farm, approximately 585.5 kg of pellet was fed daily (*Pers. comm., Daphne Taylor, production manager, WCA*) and assuming that feeding began four hours after feed application (Sales and Janssen 2004; Ho 2006), an average of 17.6 kg N d^{-1} (6424 N kg y^{-1}) was leached from feed pellet to the tank environment before the pellets were consumed. This significant loss of nitrogen was thus available to the cultivated seaweed in the IMTA *Ulva* raceways.

The contribution by *H. midae* to the leaching of phosphorus (P) from Abfeed pellet was very low and not significant. After 8 h of feed immersion and grazing, approximately 14% and 17.4% phosphorus loss were recorded for un-grazed and grazed pellets respectively. From the current study, it could be inferred that the loss of phosphorus from pellet is primarily caused by its immersion in water with a minor contribution (about 3.5%) caused by the grazing of abalone. Overall, phosphorus loss from Abfeed® commercial diet was relatively low when compared to other formulated diets. For instance, the 14.0 -17.4% P loss recorded in this study is lower than the 48.7% phosphorus loss in a practical diet made from conventional feed

ingredients (soy, fish, and cottonseed meal) without phosphorus supplementation (Sales *et al.* 2003). Similarly, the P loss in the current study is lower than the 31% phosphorus loss from Australian Adam and Amos commercial abalone pellet after 15 minutes of submersion in seawater (Ho 2006). The low leaching loss of dietary P in this study indicates an effective binding of Abfeed pellet.

The carbon loss from both the grazed and un-grazed pellets when feeding started (4 hours post immersion), was low (ca. 4.29%) probably because most pellet carbon was in organically bound form such as protein, carbohydrates and lipids, whereas there was likely a higher proportion of phosphorus and nitrogen in the form of small molecule soluble compounds (*Pete Britz, pers com*.).

Investigation into Solid waste production and nutrient composition

Approximately 11% of nitrogen from all feed types (*pellet, Ulva,* and *Gracilaria*) fed to the experimental abalone was retained in the accumulated sludge. The 1.45% nitrogen composition of accumulated sludge was lower than the 3.6% N observed for the biodeposits of *H. midae* (47mm) fed exclusively with formulated feed (Yearsley *et al.* 2009). A similarly low particulate waste nitrogen (1.93 – 3.38%) was reported for the greenlip abalone (*Haliotis laevigata*) fed artificial feed (Ho 2006). The nitrogen content of organic solids in the current study falls below the 7-32% total nitrogen in particulate waste fraction of finfish farms (Foy and Rosell 1991; Cripps & Bergheim 2000).

Approximately 47% of the total phosphorus supplied from the diets fed to farmed abalone was found in the biodeposits on production tank floor. This is an indication of the strong affinity of sediments for phosphorus (Adhikari *et al.* 2014; Guo *et al.* 2017; Gao *et al.* 2019a). Previous studies documented biodeposits in ponds as the main source of phosphorus output

in pond culture systems and may account for 39 to 67% of the total phosphorus inputs (Thakur and Lin 2003; Sahu *et al.* 2015; David *et al.* 2017; Flickinger *et al.* 2020a). This indicated the scope to further optimise the IMTA system at WCA by integrating organic nutrients extractive organisms into the farming system.

From the current results, it was inferred that about 61% of the feed (formulated and natural) fed to *Haliotis midae* is either utilised for growth and maintenance or lost as dissolved nutrients while 39% of the feed is deposited on tanks floor as waste in the form of uneaten and undigested feed. These relatively dilute waste solids are currently flushed from shore-based farms during tank cleaning events to coastal environment (Yearsley *et al*. 2011). This meant approximately 270 g (dry weight) of food per square meter of a tank per day was available for the proposed sea cucumber coculture (Chapter 7).

Diurnal Water Quality Dynamics at Wild Coast Abalone

A diurnal pattern of dissolved oxygen concentration was recorded in the current study, with the lowest and highest concentrations recorded at midnight and midday respectively. The low midnight concentration falls above the 5 mg L^{-1} minimum concentration recommended for abalone and other saltwater shellfishes (Meade 1989; Anzecc 2000; ASC 2012). Abalone uses significant amount of DO at night for metabolic activities such as feeding (Fleming 1996; Yearsley *et al.* 2009). This, together with the absence of oxygen production from algal photosynthesis in the culture tanks reduces the DO concentration at night (Fleming 1996).

The peak concentrations of dissolved nitrogen at tank outflow were recorded at 13 to 17 h post-feeding in the period 04h00 – 08h00, most likely as a result of the excretion of nitrogenous waste compounds by *H. midae* (Knauer *et al.* 1993). The diurnal variation of dissolved nitrogen and phosphate in the current study is similar to that recorded at the

abalone farms Australia, where the peak concentrations of dissolved nitrogen and phosphate were recorded at sunrise (06h00) and 12-15 hours post-feeding (Ho 2006).

The dissolved nitrogen species monitored in this study were dominated by nitrate (70-80%), followed by total ammonia nitrogen (TAN) and a barely measurable nitrite concentration. The dominance by nitrate would be due to the aerobic autotrophic bacteria (e.g., Nitrosomonas and Nitrobacter) within the tank system, which converted ammonia to nitrite and later to non-toxic nitrate. Both nitrite and nitrate levels recorded in this study were markedly lower than the documented safe limits (nitrite: < 5mg/l; nitrate: 100-250 mg/l) for European abalone, *Haliotis tuberculata* (Basuyaux and Mathieu 1999). The highest concentration of total ammonia nitrogen (0.48 mg l^{-1}) in this study falls below the 1mg/l TAN safe level for grow-out *H. tuberculata* (Basuyaux and Mathieu 1999). It also falls below the sub-lethal TAN level (0.56 mg/l) for *H. midae* (Reddy-Lopata *et al.* 2006).

The peak concentration of phosphate was recorded in the late afternoon after the animals were fed. This resulted from the rapid leaching of phosphorus from fed pellets. Previous studies also documented a rapid loss of phosphorus within an hour or two of feeding (Sales *et al.,* 2003; Ho 2006). Additionally, high P level was also recorded at pre-dawn hours (13-17 hours post-feeding) which could have resulted from faecal release after night grazing.

The results of this trial indicate that the maximum concentrations of dissolved nitrogen in the effluent exiting the abalone farm would be best recorded at sunrise and before midday. Likewise, phosphate samples can be collected at sunrise as concentration at this hour was similar to the peak phosphorus in the late afternoon after feeding.

2.5 Conclusion

This baseline study examined the factors contributing to nutrient production on land-based abalone farms. Nutrient loss from formulated diet and organic waste material were the main source of dissolved and particulate nitrogen and phosphorus in effluent exiting the IMTA system at Wild Coast Abalone. Not less than 39% of the feed (pellet and macroalgae) ended up as detritus on tank floor while the remaining was either assimilated by fed abalone or lost as dissolved nutrients in the effluent. The production of these dissolved waste nutrients followed a diurnal pattern with the peak concentrations recorded at 13-17 h post-feeding. The high solid waste production by *H. midae* shows that sufficient food would be available to the detritus waste extractive organisms when the IMTA system of abalone and seaweed at WCA abalone is optimised with sea cucumbers integration into the production system. This production approach was hypothesized to not only improve nutrient utilisation on the farm, but also reduce the ecological footprint of the farming system.

CHAPTER 3: Quantification of a seasonal nutrient budget in abalone-seaweed IMTA and monoculture systems at Wild Coast Abalone

3.1 Introduction

The present study which builds on the baseline understanding of the diurnal waste nutrient dynamics in abalone production tanks (Chapter 2), examined the overall flow, conversion and discharge of nutrients in abalone-seaweed production systems at WCA. This was an important baseline study conducted to improve the nutrient utilisation and production efficiency of the IMTA farm.

On many shore-based abalone/seaweed farms in South Africa, nutrients enter the production system majorly from the artificial feed fed to farmed abalone and through applied mineral fertilisers for macroalgae culture. Mineral fertilisers are applied due to the low nutrient composition of abalone wastewater (Troell *et al.* 2006). A few previous studies have quantified the portion of fed nutrients that is ingested and utilised by farmed South African abalone (Robertson-Anderson *et al*. 2007; Yearsley *et al.* 2009; Probyn *et al.* 2017). For example, an average of 33.0% of the nitrogen and 12.1% phosphorus from the formulated diet fed to *H. midae* were recovered in the tissue of harvested abalone in Cape abalone farms while the largest portions were lost as particulate and dissolved waste to the environment (Probyn *et al.* 2017). Likewise in other regions, about 68.5% nitrogen and 12.1% phosphorus in the diet were absorbed by *H. discuss hannai* cultured in an IMTA system with *Apostichopus japonicus* and *Sebastes schlegeli* while approximately 31.5% N and 87.9% P were lost as dissolved and solid waste to the environment (Gao *et al.* 2019a). However, the nutrient budget for the integrated culture system of South African abalone and seaweed has not been documented.

Therefore, to optimise the production performance at the Wild Coast Abalone, it is important to quantify the efficiency of nutrient utilisation by cultured species (abalone and macroalgae) and have a baseline data on the particulate and dissolved waste nutrients outputs from the farm. The present study was therefore set out to quantify the performance efficiency of the IMTA system at WCA using the nutrient budget approach.

The information on the fate of nutrients supplied into an aquacultures system is achieved through the nutrient budget approach. This technique helps to quantitatively model the efficiency of nutrient utilisation, distribution, the dynamics of water quality and other processes that occur in aquaculture systems, especially in pond bottom sludge (Anvimelech and Lacher 1979). It also helps to evaluate the effect of aquaculture activities on surrounding aquatic ecosystem and for management recommendations (Islam *et al.* 2004; Sahu *et al.* 2013). Additionally, preparing a nutrient balance budget will help quantify the efficiency of nutrient utilisation by the different components of the production systems and also provide a tool to evaluate the progress towards achieving low levels of waste discharge to the receiving coastal waters.

Nutrient production and water quality in abalone production systems also vary seasonally due to the variations in water temperature (Yearsley 2007; Robertson-Andersson *et al*. 2007). For example, the rate of conversion of ammonia nitrogen to nitrate in *H. midae* tanks was higher in summer than in winter (Yearsley 2007). Similarly, Robertson-Andersson *et al.* (2007) recorded higher ammonia concentration in abalone flowthrough units during winter than summer. Therefore, to compile an aggregate nutrient mass balance for an abalone farm, it is important to quantify the seasonal variation of dissolved nutrient levels.

This study monitored and quantified the seasonal nutrient utilisation in 1) the integrated abalone-seaweed (IMTA) system and 2) monoculture system of abalone and seaweed at Wild Coast Abalone Pty Ltd. Due to the differences between the systems, the study is analogous to an ecological study of the nutrient flow in two existing and functional systems. It was hypothesised that (1) the nutrient composition of effluent exiting the IMTA, and monoculture systems is affected by the seasonality in environmental parameters, (2) the growth and productivity of *Ulva* cultivated in the two systems is affected by seasonal environmental changes and (3) the nutrient utilisation efficiency of the IMTA system is more efficient than the monoculture (non-IMTA) system.

The specific objectives were to:

1. Characterize the seasonal dynamics of nutrients in effluent exiting the farm's IMTA and monoculture systems of abalone and *Ulva*.
2. Evaluate the growth, yield and nutrient composition of *Ulva lacinulata* cultured in monoculture and IMTA system.
3. Apply a nutrient budget approach to quantify the flow and conversion of nutrients through the two production systems at WCA.

3.2 Materials and method

A more detailed description of the operating systems at Wild Coast Abalone and the nutrient monitoring protocol is provided in this section.

3.2.1 *Description of the IMTA and monoculture systems at WCA and their operating protocols.*

IMTA and monoculture abalone tank systems

The IMTA section at WCA consisted of 72 abalone production tanks (length X width X depth: 32 m x 2.2 m x 1.05 m, 110 baskets capacity) covering a total pond area of 0.507 ha. All tanks were lined with black tarpaulin which prevented the growth of algae on tank walls. Drum-filtered seawater entered at one end of the tanks through 100 mm polyvinyl chloride (PVC) pipe while the post abalone effluent drained out at the other end of the tank through an overflow standpipe that maintained water depth at 1.05 m. The tanks were constantly aerated by two PVC air diffusers (punctured at 55 mm equidistance) which were permanently fixed to the base of the tanks and connected to an air blower. Each tank was stocked with approximately 1210 ± 0.40 kg of 65-75 g abalone and fed daily with 8-9 kg of pellet (Abfeed® E26, i.e., 26% crude protein and < 5% lipid; Marifeed Pty Ltd), *Gracilaria* (cultured elsewhere on the farm*)* and *Ulva*. The wastewater from these tanks were channeled into double D-ended paddlewheel raceways for *Ulva lacinulata* culture and the seaweed recycled as feed supplement for farmed abalone. Water exchange in IMTA abalone tanks was maintained at 5 L s^{-1} (Table 3.1).

The monoculture abalone section on the other hand comprised of 98 production tanks (length X width X depth: 6.4 m x 2.2 m x 0.9 m) made from fiberglass housing 22 abalone (< 30 g) baskets at a stocking density of 165 ± 0. 20 kg. The flow of water and exchange in the monoculture tanks was kept at 1.5 L s^{-1} to achieve seven exchanges per day. The wastewater from these tanks was directly released to the sea as there was no connection between abalone and seaweed tanks in the monoculture section. Abalone in the monoculture system

were also fed combination diet of pellet (Abfeed® S34, i.e., 34% CP and < 5% lipid), *Ulva* and *Gracilaria* produced by using monoculture methods.

Table 3.1. Characteristic features of abalone culture in IMTA and monoculture production systems.

	IMTA	Monoculture
Tank dimension	(L x W x D: 32 m x 2.2 m x 1.05 m)	L x W x D: 6.4 m x 2.2 m x 0.9 m
Stocking density	1210 ± 0.40 kg $tank^{-1}$	165 ± 0.20 kg $tank^{-1}$
Water flowrate	5.0 L s^{-1}.	1.50 L s^{-1}
Abalone size	> 30 g	< 30 g
Formulated feed type	Abfeed® E26 (26% CP)	Abfeed® S34 (34% CP)
Fed seaweed	Effluent cultured *Ulva, Gracilaria*	Seawater-grown *Ulva* and *Gracilaria*
Feeding frequency	Daily	Daily
Tank cleaning frequency	Twice weekly	Twice weekly
Abalone effluent channeled to seaweed tanks	Yes	No

*CP = crude protein.

IMTA and monoculture seaweed tank systems

There were 65 *Ulva* paddlewheel raceways (length X width X depth: 36.74 m x 8.4 m x 0.85 m) at WCA at the time of this study, with 57 of the raceways fed with effluent from abalone tanks upstream (IMTA *Ulva*) and the remaining eight supplied with clean seawater (*Ulva* monoculture). The D-ended raceways were made from concretes and lined with high-density white polyethylene liner to reflect high light and facilitate cleaning. Each *Ulva* raceway was aerated by a paddlewheel, controlled by an 8 kVA electric motor. The paddlewheel circulated

impounded water at ten rotations per minute which continuously exposed the plants to light in moving water. All IMTA seaweed raceways received independent water supply and a common channel (0.8m wide) discharged the post-seaweed effluent to the sea. After thirty days of culture, the seaweed was harvested by draining the pond into a concrete harvesting chamber beside the effluent channel. The flow of water in each IMTA *Ulva* raceway was manually controlled with graduated rectangular board to allow regulated flow to the required flow rate, while the flow of fresh seawater into the monoculture *Ulva* raceways was controlled by means of a valve.

3.2.2 Production tanks preparation and nutrient Monitoring Protocol

Abalone culture tanks

At the start of the nutrient monitoring experiment in June 2020 (winter), three abalone tanks were selected from the IMTA and monoculture systems, prepared (washed, filled with seawater, stocked with abalone) and monitored for 30 days, coinciding with *the Ulva* production cycle on the farm. The process was repeated in September 2020 (spring) and January 2021 (Summer). Autumn sampling was not carried out during the monitoring as this season is transitional and very short. Flowrates of incoming seawater to abalone tanks and the post abalone wastewater from the raceway of the two systems were recorded at each sampling event using a graduated 50L bucket and stopwatch (TFA Dostmann, D-97877).

Seaweed paddlewheel raceways

Three *Ulva* raceways each from the IMTA and monoculture sections were selected, washed and filled overnight with abalone effluent or clean seawater. After preparation, each raceway was seeded with 250kg (wet weight) of *Ulva* weighed out from previous harvest with the best performance in terms of growth, green coloration, and thallus' thickness. After 16 -17 h of

stocking, the seaweeds were fertilised with the combination of granular urea (N-460 g/kg; Gaviloc, South Africa), monobasic potassium phosphate, MKP (KH_2PO_4; P-221 g/kg, K- 282 g/kg, Petrow Agri, South Africa) & monoammonium phosphate, MAP (N-121 g/kg; P- 269g/kg, Petrow Agri, South Africa) fertilisers. The cultured *Ulva* were fertilised three (IMTA) and five (monoculture) times every week following the farm's standard fertilisation rate and practice (Table 3.2). Prior fertilisation, water inlets to the seaweed raceways were closed and thereafter, they were fertilised between 0800 h and 0900 h. The raceways were then left for 8 h without inflow and outflow of water while the paddle mixed the fertilized water with the *Ulva*. In the late afternoon (1600 h – 1630 h), inlets to the ponds were opened until the next fertilization day.

Table 3.2. Characteristic features of *Ulva* culture in IMTA and monoculture *Ulva* production systems.

	IMTA	Monoculture
Raceway dimension	(L x W x D: 36.74 m x 8.4 m x 0.85 m)	(L x W x D: 36.74 m x 8.4 m x 0.85 m)
Stocking density	250 kg $tank^{-1}$	250 kg $tank^{-1}$
Fertilisation Frequency	3 times per week	5 times per week
Fertilisation method	Closed fertilisation	Open fertilisation
Culture cycle	30 days	30 days
Water flowrate	200 – 250 L min^{-1}	350 L min^{-1}
	(Measured at every sampling period)	(Measured at every sampling period)
Type of harvest	Total (at the end of culture cycle)	Total (at the end of culture cycle)

Water sample collection

Water sampling from abalone tanks and *Ulva* raceways in the two production systems was carried out simultaneously. Physiochemical parameters including dissolved oxygen (DO; mg/l), pH, and temperature (°C), were recorded weekly at the point of sampling using appropriate devices described in Chapter two (Section 2.3). The water samples for analyses of nutrients were collected every week between 0700h and 0800 h in 500ml bottles that have been washed and rinsed four times in the water samples before actual collection. Weekly sampling was carried out according to the technique described in Al-Hafedh *et al.* (2012) and Shpigel *et al*. (2018). The water samples were collected at the inlet and outlet of abalone and seaweed tanks for the four-weeks *Ulva* production cycle on the farm. The samples for dissolved nutrients were immediately taken to an onsite laboratory, filtered (45 μm; Whatman GF filters) and analysed for ammonia-nitrogen (NH_3-N, mg/l), nitrate-nitrogen (NO_2^-N, mg/l), nitrate-nitrogen (NO_3^-N, mg/l), and reactive phosphate (PO_4^-P, mg/l). Additionally, unfiltered samples were prepared for total nitrogen (TN, mg/l), total phosphorus (TP, mg/l), and total organic carbon (TOC, mg/l) analyses. All nutrients except carbon were analysed using the Palintest photometer and commercial test kits (Chapter 2, Section 2.2.3), while TOC was analysed by direct reading with a colorimeter (Hach DR900 colorimeter, Colorado, USA) and commercially available test kits (mid-range TOC test N tube reagent set 26159-45) after acid digestion.

3.2.3 Suspended solids and sediment sampling

Suspended solids concentrations (> 10 μm) were quantified gravimetrically using 47 mm by 0.45 μm filter papers (Macherey-Nagel, NGF-3, LoT. 180203; Reference no: 4130047, GmbH & Co, Germany) that were first rinsed with deionized water (to remove friable materials) and

dried at 65°C in a hot-air oven for 1 h. The filters were allowed to cool off and later weighed to the nearest 0.001g using KERN PCB 350-3 analytical balance (KERN & SOHN GmbH Ziegelei, Balingen, Germany). About 500 ml of the incoming seawater, abalone effluent, and post-seaweed effluents were collected, vigorously shaken to mix and 100ml of each water sample filtered on the pre-weighed filter papers. The filter papers and their residues were rinsed with deionized water (Improchem, South Africa) to eliminate sea salt on the filter matrix and dried at 65 °C until a constant weight was achieved and re-weighed. Suspended solids concentration (mg L^{-1}) was calculated as the dry weight increase of the filter paper (mg) divided by the quantity (volume) of filtered water sample (L) (EPA 2007). Sediment samples from abalone tanks were collected weekly and were made as composite samples of each raceway after four samplings in a month. The sampling method followed the procedure described in Chapter two (Section 2.2.2).

3.2.4 Ulva harvest, biomass production and growth rate

At the end of each production cycle, *Ulva* biomass from the paddle ponds were harvested, graded into their respective harvest crates, and the weights recorded after draining for two hours. Biomass daily yield (Y) expressed in g wet weight m^{-2} day^{-1} was calculated as the difference between the initial stocking weight and harvested *Ulva* weight:

$$Y \text{ (g wet weight } m^{-2} \text{ day}^{-1}) = [(W_t - Wo) / t] / SA \qquad \text{Equation 3.1}$$

The specific growth rate (SGR) of *Ulva* was computed following the equation of Evans (1972):

$$SGR (\%) = 100 \times [(\text{Ln } W_t - \text{Ln } W_0)] / t. \qquad \text{Equation 3.2}$$

The net yield (NY) or productivity (g wet weight day^{-1}) of *Ulva* (w/w) was calculated with the equation of Al-Hafedh *et al.* 2012:

NY (g wet weight day^{-1}) = [final – initial] biomass (m^3)] / time (days) Equation 3.3

where W_0 = initial biomass weight (g), W_t = final biomass weight (g) with t expressing the days of culture of the seaweed biofilter and SA being the surface area of paddle pond.

3.2.5 Nutrient composition of inputs and outputs

Ulva samples from each paddle pond were collected at seeding and harvest of each monitoring period for nutrients analysis after they have been rinsed in distilled water to remove salt and visible epiphytes. The spun *Ulva* samples were dried for 48 hours at 40 °C in a laboratory oven (PROLAB, E60 series, PROLAB, Kuwait) and stored in a cool dry place. They were later analysed for total tissue nitrogen, phosphorus, and carbon content using the methods described in Chapter 2 (Section 2.2.3). Additionally, samples of all feed types used (Abfeed S34 and E26 pellets, IMTA and monoculture *Ulva*, and *Gracilaria*), the three fertilisers (MEP, urea, and MPK), accumulated sludge and abalone tissue at the start and end of the monitoring period were collected and analysed for their nitrogen, phosphorus, and carbon compositions.

3.2.6 Nutrient budget calculation

The flow of nutrients in abalone-*Ulva* IMTA and monoculture systems was quantified during the seaweed production cycle based on the input and output of nutrients per tank using the data pooled from the three seasons monitored. To describe the nutrient budget, both the IMTA and monoculture systems were divided into inputs and outputs of each production system. Farm records were used to quantify the total quantity of abalone pelleted feed, wet feed (*Ulva* and *Gracilaria*) fed at each section, and the quantity of fertilisers applied per *Ulva* production cycle. Budgets for nitrogen, phosphorus, and carbon were calculated for each unit

(abalone and *Ulva*) of the two systems based on all inputs (incoming water, feed pellets, fed *Ulva,* fed *Gracilaria*, all fertiliser types used and stocked abalone biomass); and outputs (abalone effluent, post-seaweed effluent, harvested *Ulva*, suspended solids, pond accumulated sludge and harvested abalone at the end of each production cycle). The differences between the total nutrient input and output from the systems were used to estimate the unaccounted nutrients in the production systems. The equation of Neori *et al.* (2000) was employed to estimate the budget of nutrients and the unaccounted portion:

$$\text{Nutrient budget} = \Sigma \text{ Nutrient}_{In} - \Sigma \text{ Nutrient}_{out} \pm \textit{unaccounted} \qquad \text{Equation 3.4}$$

Positive and negative unaccounted values represented unaccounted output and input of each culture system. In the current study, direct absorption of gases and their subsequent removal from the production systems was not quantified. These include nitrogen gas' absorption through fixation and loss through ammonia volatilization and denitrification processes. Likewise, absorption and emission of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) were not quantified directly but assumed as part of the unaccounted portion of input and output of the nutrient budget.

3.3 Statistical analyses

The repeated measure multi-factorial analysis of variance (F-RM ANOVA) was performed to analyse the effect of production systems (IMTA and monoculture) and seasons (winter, spring, summer) on the quality of effluent exiting abalone and seaweed production tanks. The effects of seasons and production systems on the growth, production and nutrient composition of *Ulva* cultured in the two systems were also examined using factorial ANOVA

at P < 0.05 after they have been checked for normal distribution of residuals and homogeneity using the Shapiro-Wilk's (Shapiro & Wilk 1965) and Levene's test (Levene's, 1960). The nutrient loads of inputs and outputs of the IMTA and monoculture abalone were not compared statistically due to the differences in pond sizes and biomass between the two systems. All analyses were performed with STATISTICA® software, version 13.0 (Statasoft, Tulsa, OK, USA).

3.4 Results

The result of the nutrient composition of effluent exiting each production system, macroalgae production and the overall nutrient budget are presented below.

3.4.1 Nutrient composition and physico-chemical parameters of effluent exiting abalone tanks in IMTA and monoculture systems.

The levels of ammonia nitrogen, nitrate, phosphate, total nitrogen and total phosphorus in the effluent from abalone tanks varied seasonally.

The highest concentrations of total nitrogen (TN) in IMTA and monoculture abalone effluent was recorded during summer, followed by winter and the lowest in springtime. Additionally, the means TN in the effluent was significantly influenced by the production system as higher concentration was recorded for IMTA abalone than the monoculture (F-RM ANOVA: $F_{(2, 12)}$ = 15.361, p = 0.002; Figure 3.1b). However, there was no significant interaction between production systems and the seasons of monitoring in the nitrogen levels in effluent exiting abalone tanks (F-RM ANOVA: $F_{(2, 12)}$ = 2.01, p = 0.176; Figure 3.1a).

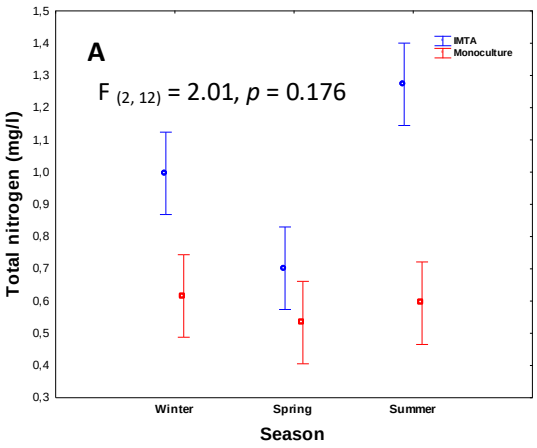
The concentration of ammonia in abalone tanks wastewater was also influenced by the seasonal variations as the highest TAN levels for both systems were during winter, and the lowest in summer. Additionally, NH_4-N concentration was significantly influenced by an interaction between the season and the production systems (F-RM ANOVA: $F_{(2, 12)} = 4.595$, $p = 0.03$; Figure 3.1c). Overall, IMTA abalone effluent was higher in TAN concentration all seasons than the monoculture.

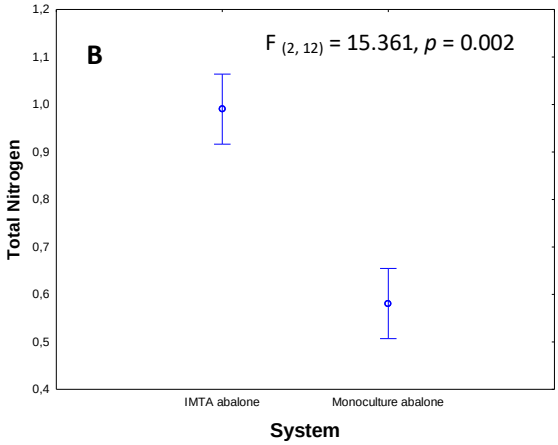
The nitrite concentration in the effluent did not follow a regular seasonal pattern for both systems and there was no significant interaction between production systems and seasons in the level of nitrite of effluent exiting the two systems F-RM ANOVA: $F_{(2, 12)} = 2.85$, $p = 0.097$; Figure 3.1d).

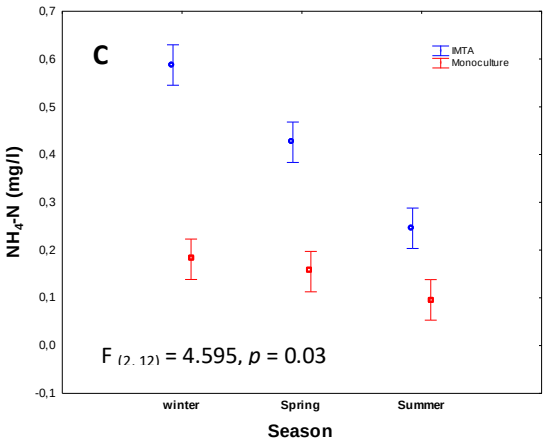
The highest and lowest nitrate levels for both systems were recorded during winter and spring respectively. Additionally, there was a significant effect of production systems on the concentration of nitrate with higher nitrate level recorded in IMTA effluent (F-RM ANOVA: $F_{(2, 12)} = 12.34$, $p = 0.004$; Figure 3.1f). However, no significant interaction was recorded between seasons and systems with regards to nitrate levels (F-RM ANOVA: $F_{(2, 12)} = 1.38$, $p = 0.35$; Figure 3.1e).

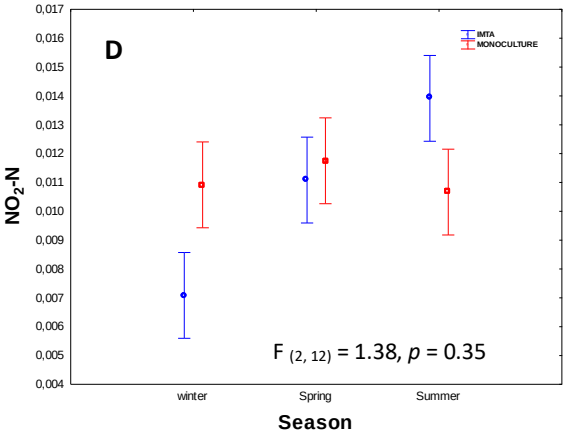
The concentration of soluble reactive phosphate in abalone effluent varied significantly between production systems with higher phosphate concentrate in IMTA than monoculture (F-RM ANOVA: $F_{(2, 12)} = 8.422$, $p = 0.013$; Figure 3.1h).

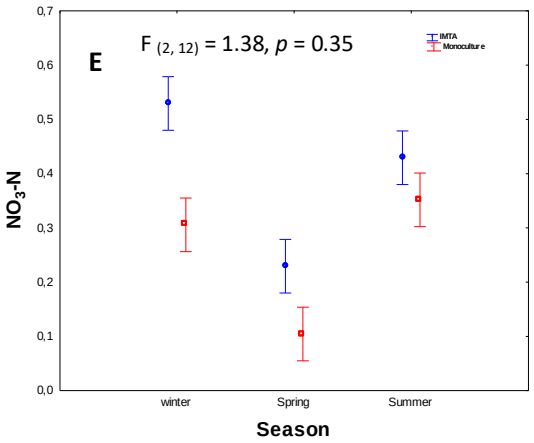
The highest and lowest total phosphorus levels were recorded for both systems during spring and winter respectively. Also, the IMTA abalone system produced significantly higher total phosphorus than the monoculture system (F-RM ANOVA: $F_{(2, 12)} = 12.378$, $p= 0.004$; Figure 3.2j).

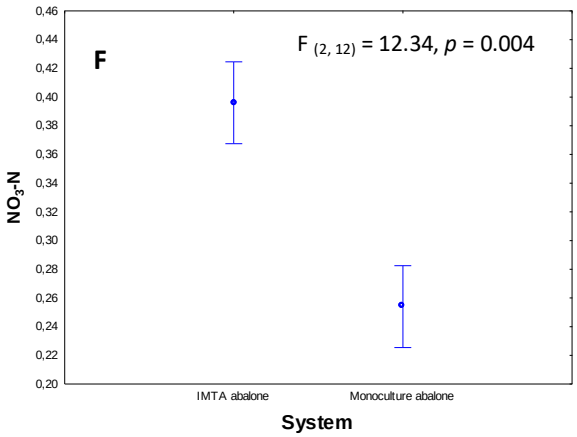

A
F (2, 12) = 2.01, p = 0.176
IMTA
Monoculture
Total nitrogen (mg/l)
Winter
Spring
Summer
Season


B
F (2, 12) = 15.361, p = 0.002
Total Nitrogen
IMTA abalone
Monoculture abalone
System


C
IMTA
Monoculture
NH4-N (mg/l)
F (2, 12) = 4.595, p = 0.03
winter
Spring
Summer
Season


D
IMTA
MONOCULTURE
NO2-N
F (2, 12) = 1.38, p = 0.35
winter
Spring
Summer
Season


E
F (2, 12) = 1.38, p = 0.35
IMTA
Monoculture
NO3-N
winter
Spring
Summer
Season


F
F (2, 12) = 12.34, p = 0.004
NO3-N
IMTA abalone
Monoculture abalone
System

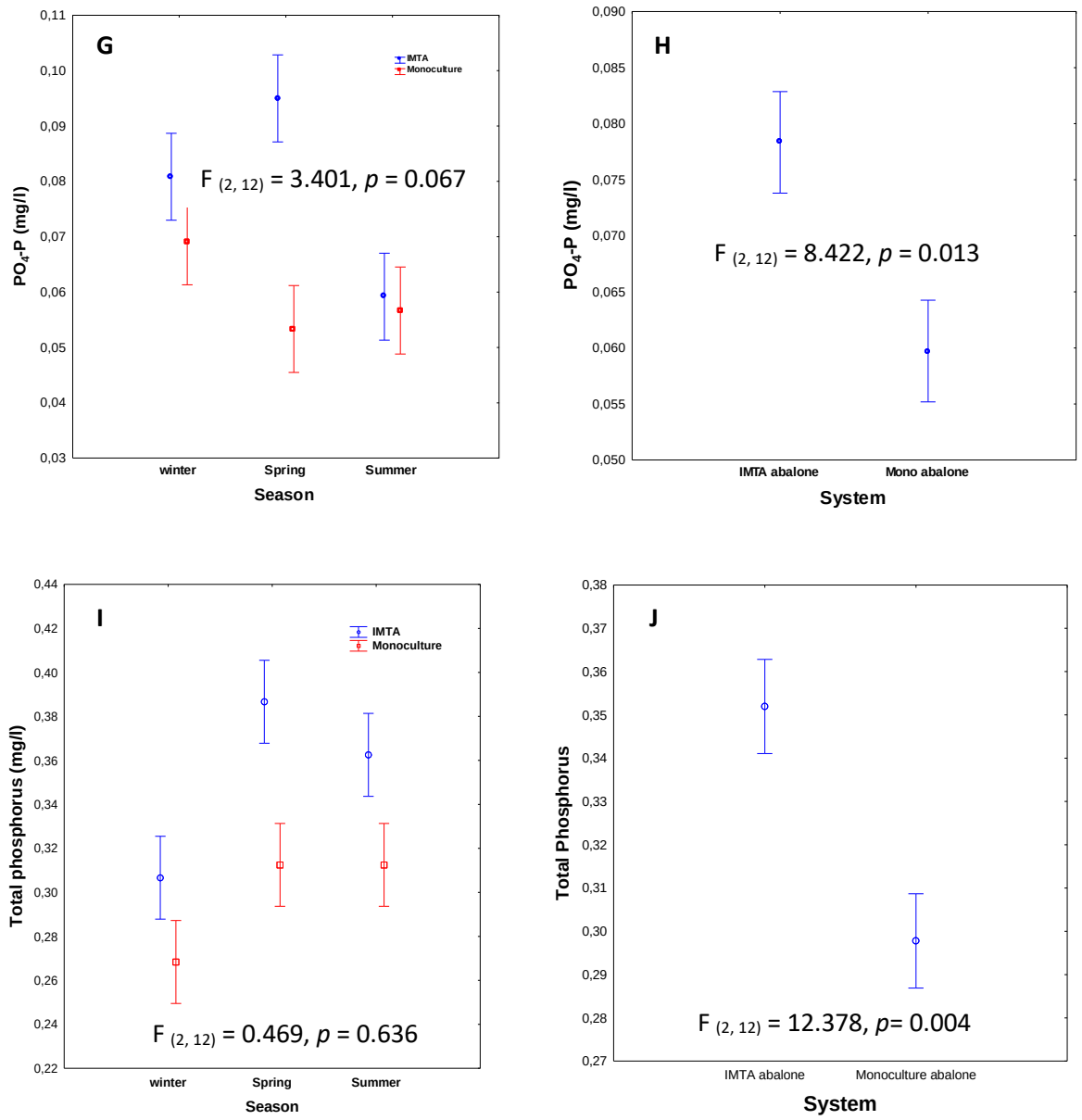


Figure 3.1. Mean (± SE) nutrient composition of effluent exiting abalone tanks of the IMTA and monoculture systems of Wild Coast Abalone over.

Higher concentration of total organic carbon TOC was recorded for the IMTA abalone effluent than the monoculture (F-RM ANOVA: $F_{(2, 12)} = 5.901$, $p = 0.032$; Figure 3.2b). Total organic carbon concentration of in the effluent was similar for all seasons monitored (F-RM ANOVA: $F_{(2, 12)} = 1.806$, $p = 0.206$).

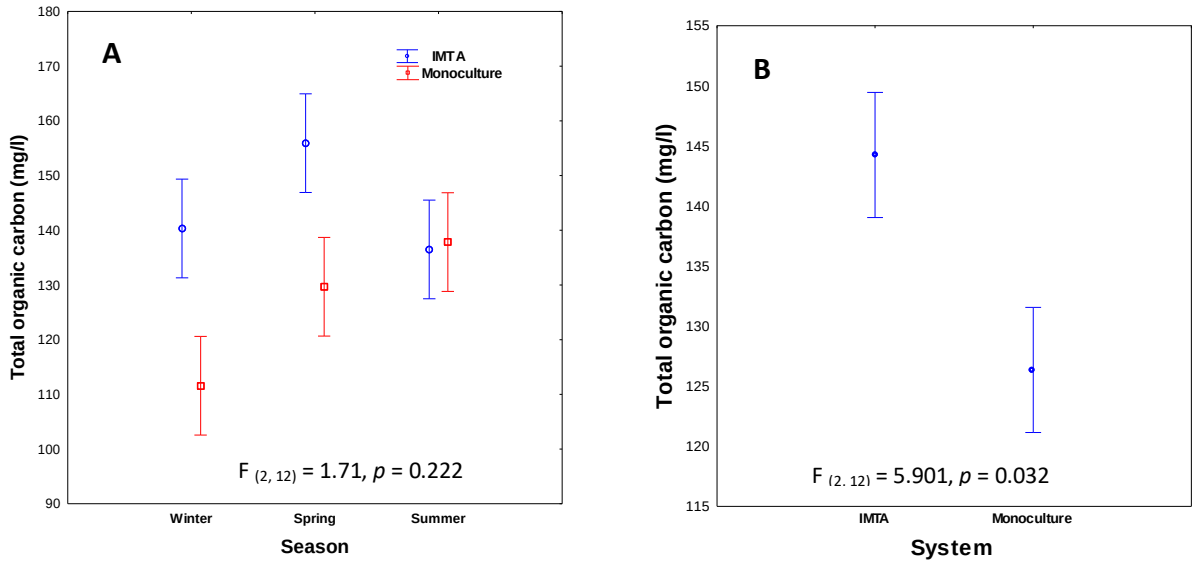


Figure 3.2. Mean (± standard error) total organic carbon concentrations of effluent exiting abalone tanks of the IMTA and monoculture systems of Wild Coast Abalone over.

Physico-chemical water quality parameters in abalone tanks

The highest pH levels for both systems (7.90 – 8.14) were recorded during summer and the lowest (7.69 – 7.70) in spring (Table 3.3). The highest water temperatures were recorded in summer, followed by winter temperatures. Dissolved oxygen in abalone tank was highest during spring (7.07 – 7.09 mg/l), and lowest during summer (6.55 – 6.66 mg/l) (Table 3.3).

Table 3.3. Mean (± standard error) maximum and minimum values of monitored water quality parameters in abalone tanks of the IMTA and monoculture systems during seasonal monitoring at Wild Coast Abalone Pty Ltd.

	Winter		Spring		Summer	
Parameters	Range	Mean	Range	Mean	Range	Mean
Temperature						
IMTA	19.75 - 20.10	19.95 (0.10)	17.85 - 17.98	17.93 (0.04)	19.58 - 19.73	19.68 (0.05)
Monoculture	19.55 - 20.00	19.78 (0.13)	17.98 - 18.23	18.13 (0.08)	20.38 - 20.73	20.52 (0.10)
pH						
IMTA	7.85 - 7.89	7.88 (0.08)	7.65 - 7.73	7.69 (0.02)	8.13 - 8.15	8.14 (0.08)
Monoculture	7.75 - 7.85	7.80 (0.03)	7.68 - 7.73	7.70 (0.01)	7.89 - 7.95	7.90 (0.03)
Dissolved oxygen						
IMTA abalone	6.83 - 6.90	6.86 (0.02)	7.01 - 7.25	7.09 (0.08)	6.58 - 6.70	6.66 (0.04)
Monoculture	6.70 - 6.78	6.75 (0.03)	6.98 - 7.23	7.07 (0.07)	6.48 - 6.63	6.55 (0.04)
Suspended solids						
IMTA	3.68 - 4.18	4.01 (0.17)	1.01 - 1.14	1.05 (0.04)	0.81 - 1.07	0.97 (0.08)
Monoculture	5.72 - 7.22	6.49 (0.43)	1.50 - 2.22	1.93 (0.22)	1.32 - 2.49	1.84 (0.35)

3.4.2 Nutrient composition and physical parameters of effluent exiting the Ulva raceways of IMTA and monoculture systems.

In the seaweed culture tanks, the highest TAN level exiting the system in the post-*Ulva* effluent was recorded during spring and the lowest in summer. Additionally, similar TAN concentrations were recorded in the post-seaweed effluent from both systems and there was no significant interaction between seasons and production systems (F-RM ANOVA: $F_{(2, 12)}$ = 1.194, p = 0.337; Figure 3.3a). However, a significant effect of seasons (F-RM ANOVA: $F_{(2, 12)}$ = 7.939, p = 0.006; Figure 3.3b) and a non-significant effect of production system (RM ANOVA: $F_{(2, 12)}$ = 2.930, p = 0.113) on the discharge of ammonia in the effluent were recorded.

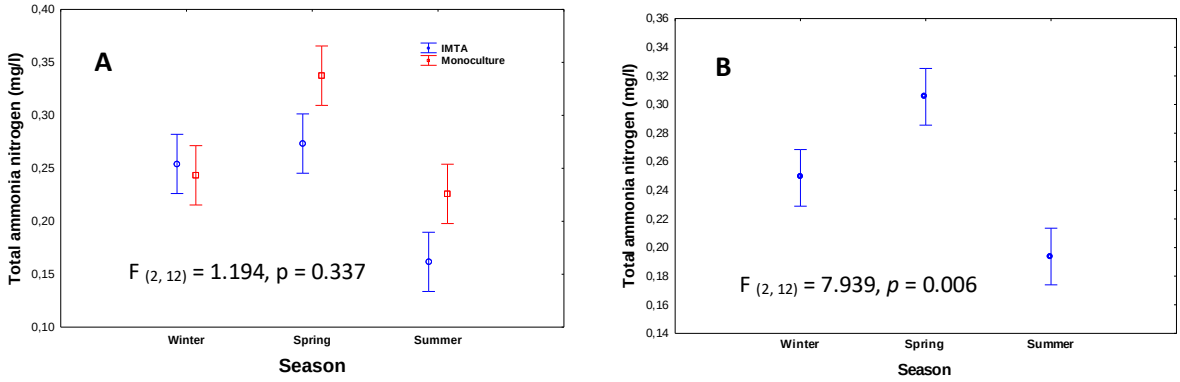


Figure 3.3. Mean (± standard error) ammonia nitrogen concentrations of effluent exiting seaweed raceways of the IMTA and monoculture systems.

For both production systems, the highest discharge of total nitrogen in the post-seaweed effluent was recorded during summer and the lowest in winter. The concentration of total nitrogen (TN) discharged in the post-seaweed effluent of IMTA *Ulva* raceway was significantly lower than the monoculture system (F-RM ANOVA: $F_{(2, 12)}$ = 8.653, p = 0.01; Figure 3.4a). Additionally, TN discharge was significantly higher during summer than other seasons (F-RM ANOVA: $F_{(2, 12)}$ = 11.069, p = 0.002; Figure 3.4b).

There were no significant variations between the two systems in terms of the concentration of nitrite (F-RM ANOVA: $F_{(2, 12)} = 1.178$, $p = 0.839$), and nitrate (F-RM ANOVA: $F_{(2, 12)} = 2.919$, $p = 0.093$) concentration discharged in the post-seaweed effluent.

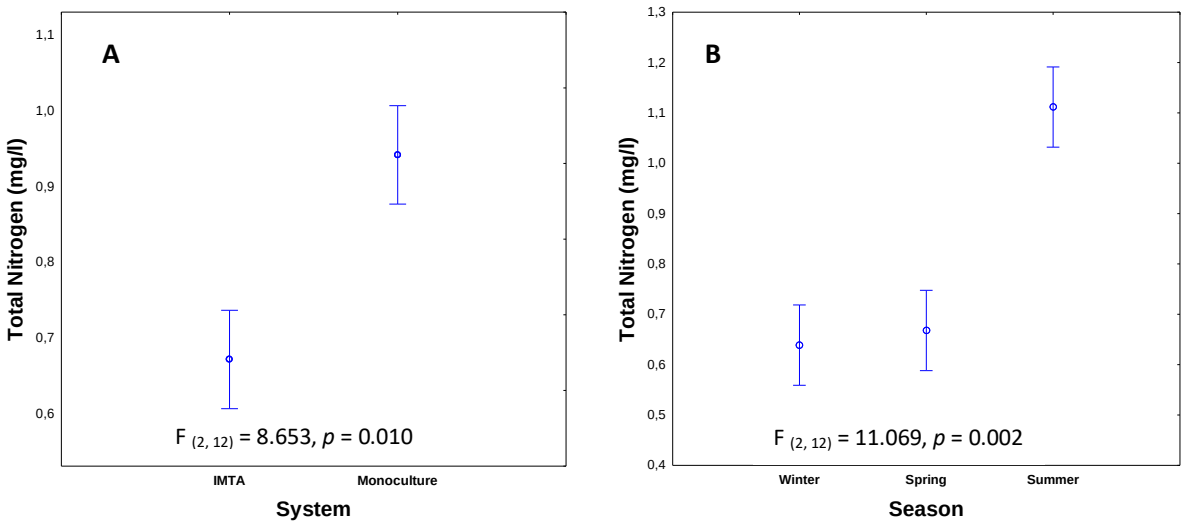


Figure 3.4. Mean (± standard error) total nitrogen concentrations of effluent exiting seaweed raceways of the IMTA and monoculture systems.

The concentration of total phosphorus in the post-seaweed effluent was also affected by seasonal variations with highest discharge recorded during spring and the lowest in winter (Figure 3.5). A higher phosphorus discharge was recorded for both systems in spring compared to summer and winter time (F-RM ANOVA: $F_{(2, 12)} = 60.037$, $p < 0.001$ Figure 3.5c). Additionally, the production systems influenced the discharge of dissolved phosphorus from seaweed raceways with significantly lower TP discharge recorded in the IMTA than the monoculture system (F-RM ANOVA: $F_{(2, 12)} = 49.283$, $p = 0.01$; Figure 3.5b). Overall, there was no significant interaction between the production systems and the seasons of the year on the concentration of total phosphorus in the post-seaweed effluent (F-RM ANOVA: $F_{(2, 12)} = 3.747$, $p = 0.0544$; Figure 3.5a).

The organic carbon composition of the post-seaweed effluent showed no significant variation between the systems (F-RM ANOVA: $F_{(2, 12)} = 2.761$, $p = 0.103$; Figure 3.5e) and the seasons (F-RM ANOVA: $F_{(2, 12)} = 1.913$, $p = 0.988$; Table 3.2; Figure 3.5f).

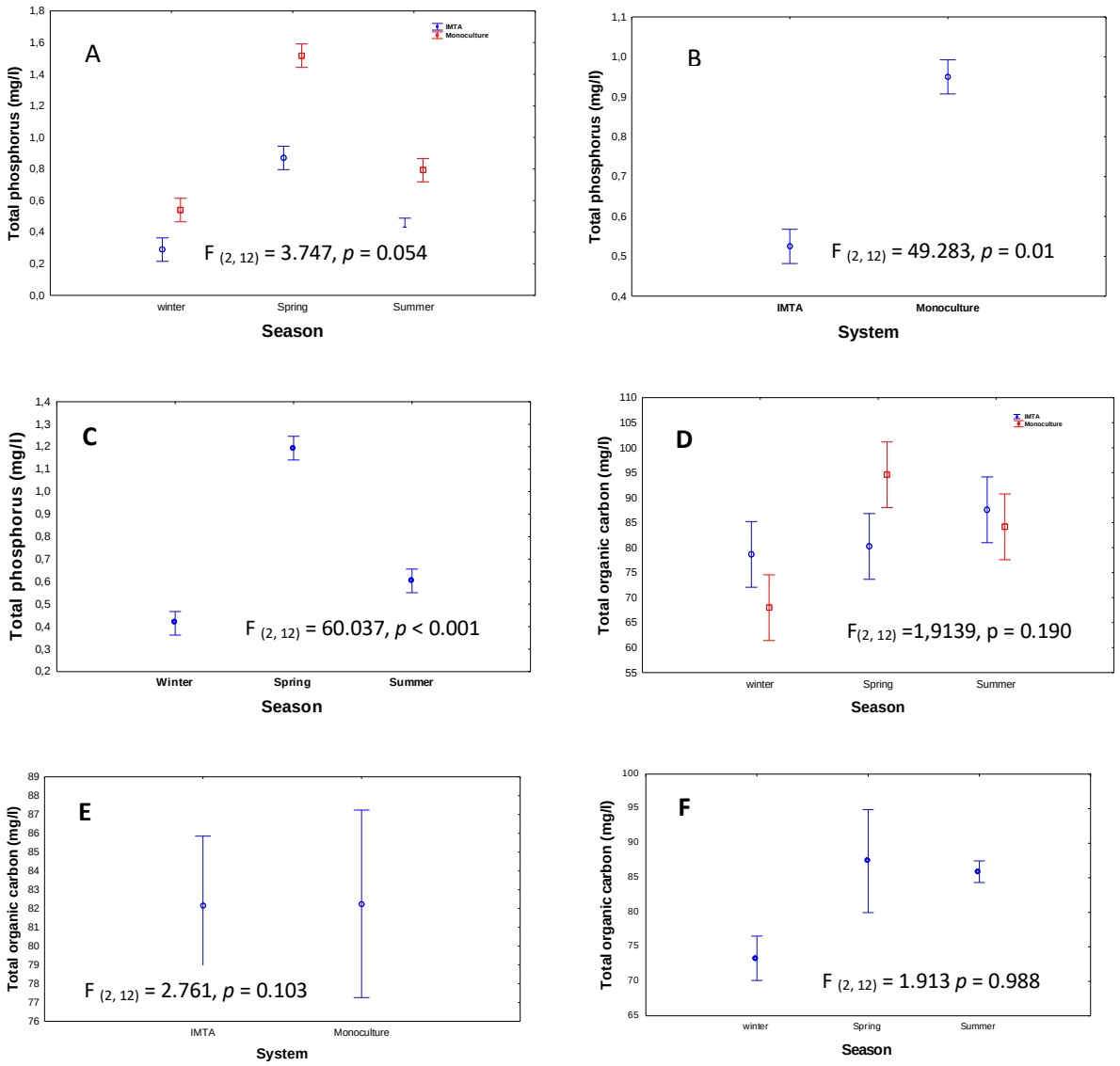


Figure 3.5. Mean (± standard error) total phosphorus and organic carbon concentrations of effluent exiting seaweed raceways of the IMTA and monoculture systems.

Physical water quality parameters in Ulva tanks

The highest and lowest pH levels for both seaweed culture systems were recorded during summer and spring (Table 3.4). The water temperature also followed a similar trend where the highest temperatures were recorded in summer, followed by winter temperatures and the least in spring. The dissolved oxygen concentrations in the seaweed tanks were highest during summer (8.18 – 8.58 mg/l), and lowest during winter (7.35 – 7.43 mg/l) (Table 3.4).

Table 3.4. Mean (± SE), maximum and minimum values of monitored water quality parameters in *Ulva* compartment of the IMTA and monoculture systems during seasonal monitoring at Wild Coast Abalone Pty Ltd.

	Winter		**Spring**		**Summer**	
Parameters/system	Range	Mean	Range	Mean	Range	Mean
Temperature						
IMTA	19.40 - 19.55	19.48 (0.04)	17.93 - 18.30	18.09 (0.11)	21.28 - 21.93	21.64 (0.19)
Monoculture	18.90 - 19.15	19.00 (0.08)	18.08 - 18.43	18.25 (0.10)	21.53 - 21.98	21.75 (0.13)
pH						
IMTA	8.15 - 8.25	8.20 (0.03)	8.08 - 8.10	8.09 (0.08)	8.30 - 8.63	8.48 (0.09)
Monoculture	8.30 - 8.35	8.33 (0.01)	8.08 - 8.23	8.15 (0.04)	8.48 - 8.65	8.56 (0.05)
Dissolved oxygen						
IMTA abalone	7.40 - 7.48	7.43 (0.03)	7.93 - 8.00	7.97 (0.02)	8.28 - 9.05	8.58 (0.24)
Monoculture	7.28 - 7.40	7.35 (0.04)	7.50 - 8.45	7.98 (0.27)	7.73 - 8.63	8.18 (0.26)
Suspended solids						
IMTA	3.68 - 4.18	4.01 (0.17)	1.01 - 1.14	1.05 (0.04)	0.81 - 1.07	0.97 (0.08)
Monoculture	5.72 - 7.22	6.49 (0.43)	1.50 - 2.22	1.93 (0.22)	1.32 - 2.49	1.84 (0.35)

3.4.3 Seaweed production, growth rate, and tissue composition

Seaweed production and growth rate

The mean growth rate of *Ulva* was significantly influenced by an interaction between culture systems and seasons of the year as the highest seaweed growth in both production systems was recorded for IMTA *Ulva* during summer (F-RM ANOVA: $F_{(2, 12)} = 4.310$, $p = 0.039$; Figure 3.6a).

There was no significant interaction between production systems and seasons on the yield of *Ulva* (F-RM ANOVA: $F_{(2, 12)} = 3.716$, $p = 0.055$; Figure 3.6b). However, the yield of *Ulva* was highest and lowest during summer and winter time respectively (F-RM ANOVA: $F_{(2, 12)} = 12.210$, $p = 0.001$; Figure 3.6d).

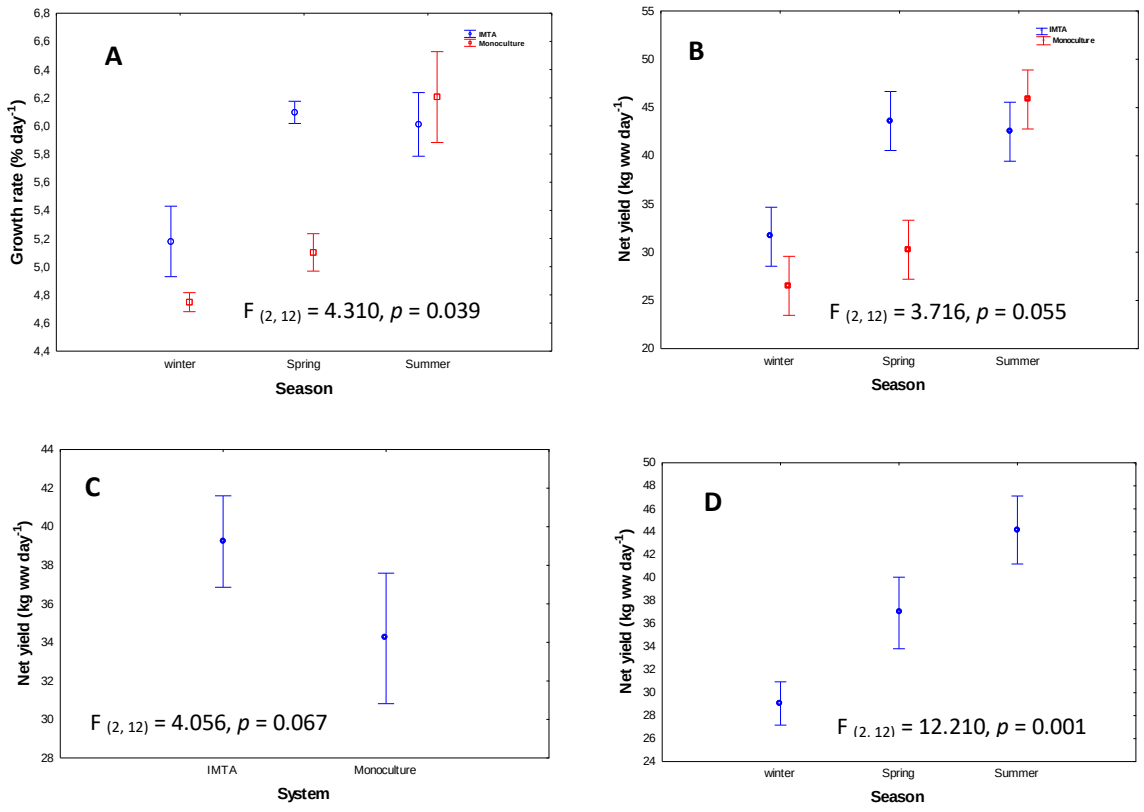
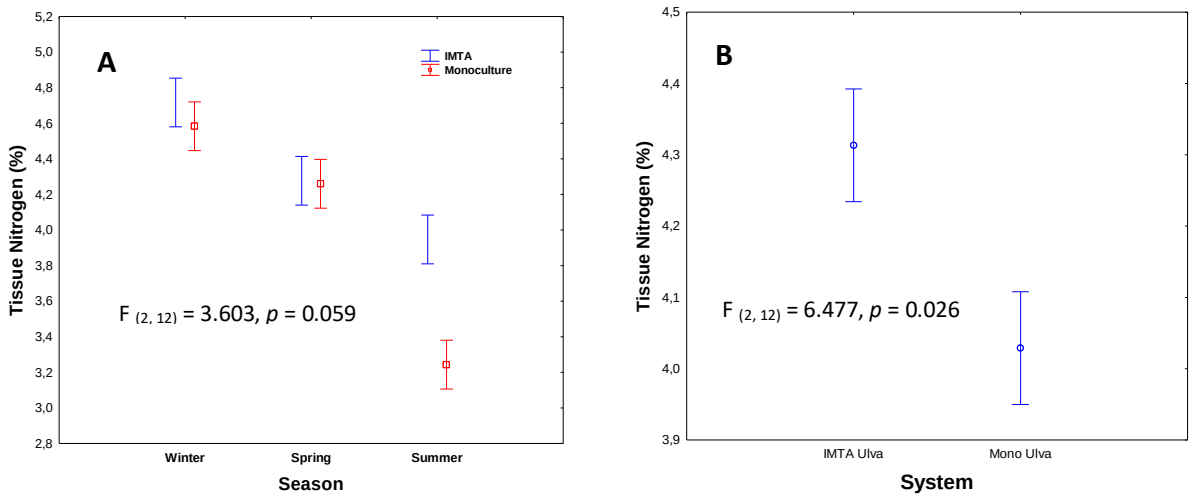


Figure 3.6 (a) The mean (± standard error) growth and yield of *Ulva lacinulata* cultured in abalone effluent and seawater.

Ulva tissue nutrient (nitrogen, phosphorus and carbon) composition

The nutrient composition of *Ulva* cultured in the two systems was affected by the difference in seasons. The highest tissue nitrogen for both systems was recorded during winter and the lowest in summer (Figure 3.7a). In addition, the production system affected the nutrient compositions of *Ulva* as significantly higher ($p < 0.05$) nitrogen composition was recorded in the IMTA than the monoculture system (F-RM ANOVA: $F_{(2, 12)} = 6.477$, $p = 0.026$; Figure 3.7b). However, the nitrogen composition was not significantly influenced by the interaction between production system and seasons (F-RM ANOVA: $F_{(2, 12)} = 3.603$, $p = 0.059$; Figure 3.7a).

There was a significant interaction between season and production system on tissue phosphorus (F-RM ANOVA: $F_{(2, 12)} = 25.528$, $p < 0.001$; Figure 3.9c) and carbon (F-RM ANOVA: $F_{(2, 12)} = 13.060$, $p < 0.001$; Figure 3.7d).



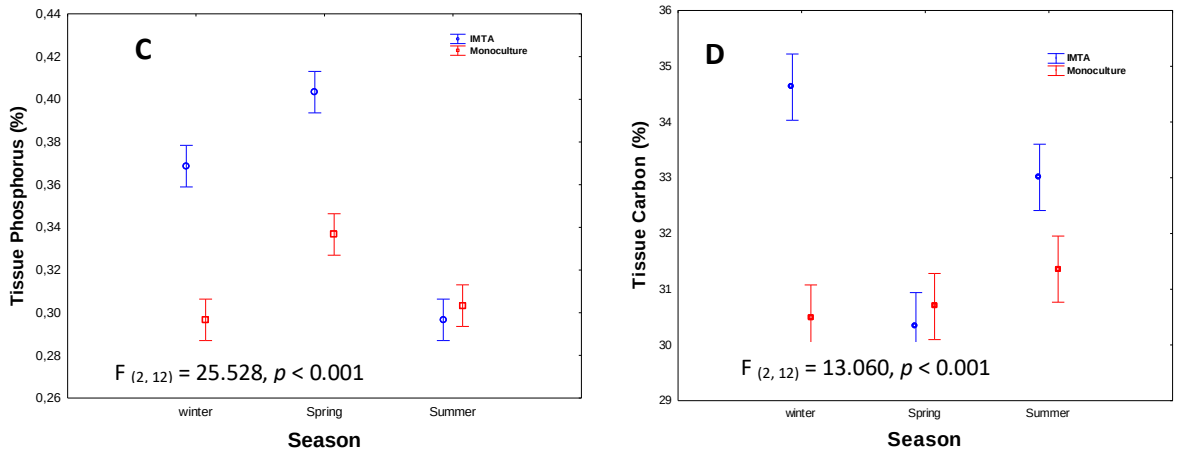


Figure 3.7. The mean (± standard error) tissue nitrogen, phosphorus and carbon composition of Ulva *lacinulata* cultured in IMTA and monoculture systems.

3.4.4 Budget of nutrients (N, P, C) in abalone-Ulva IMTA and monoculture systems

The nutrient load (i.e., % nutrient composition of inputs and outputs x quantity/volume) for the production systems was mapped as percentage flow of inputs and outputs / losses over the production cycles of *Ulva* monitored.

Nitrogen budget in abalone and Ulva units of the IMTA and monoculture production systems

In abalone tanks of both production systems, the formulated diet fed to farmed abalone accounted for the highest input of nitrogen being approximately 74% (9.29 ± 0.05 kg N/tank/month) and 81% (29.37 ± 0.08 kg N/tank/month) for the monoculture and the IMTA system, respectively (Table 3.5). The seawater flowing into the production tanks also accounted for ca. 15-19% nitrogen input into the two systems. Approximately 48-50% of the nitrogen from the diet and seawater was recovered in harvested abalone from both systems while about 31.4 and 20.2% of the nitrogen were lost to abalone biodeposits in the IMTA and

monoculture systems respectively. Additionally, 31.6% N (4.48 ± 0.34 Kg N/tank/month) was lost as dissolved waste in the monoculture system (Table 3.5).

In the seaweed culture units, the highest nitrogen (N) input for both systems were the mineral fertilisers which supplied ca. 89% (66.66 ± 2.01 Kg N/tank/month) and 63.5% (50.80 ± 0.69 Kg N/tank/month) nitrogen in the monoculture and IMTA *Ulva* raceways respectively. Approximately 36.6% (29.26 ± 2.60 Kg N/tank/month) and 10.5% (7.78 ± 0.32 Kg N/tank/month) of the nitrogen entering the IMTA and monoculture *Ulva* culture tanks were supplied by abalone effluent and intake seawater respectively.

A higher nitrogen recovery (68.6%, 54.93 ± 0.30 Kg N/tank/month) of input N in *Ulva* unit, was recorded for IMTA *Ulva* compared to the 52.2% (38.84 ± 0.27 Kg N/tank/month) N recovery by monoculture *Ulva*. While ca. 25.5 and 46.2% N in IMTA and monoculture seaweed raceways were lost in the post-*Ulva* effluent, approximately 1.7-5.9% nitrogen loss to other N-forms in the two *Ulva* culture systems was unaccounted.

Table 3.5. Mean (± standard error) nitrogen load and percentage contributions in each production tank compartment for the nitrogen budget of South African abalone (*H. midae*) and *Ulva species* reared in integrated multitrophic aquaculture (IMTA) and monoculture systems.

		IMTA SYSTEM		Monoculture System	
UNIT	**N-form**	**kg N/month**	**%**	**kg N/month**	**%**
Abalone					
Input:	Feed input	29.37 ± 0.08	80.97	9.29 ± 0.05	73.84
	Intake water	6.90 ± 0.31	19.03	2.13 ± 0.06	15.02
	Unaccounted input	-	-	1.58 ± 0.02	11.14
Output:	Abalone harvest	18.63 ± 0.55	51.36	6.82 ± 0.06	48.10
	Abalone biodeposits	11.39 ± 0.09	31.40	2.86 ± 0.06	20.17
	Abalone wastewater	-	-	4.48 ± 0.34	31.59
	Suspended solids	0.02 ± 0.01	0.09	0.02 ± 0.01	0.14
	Unaccounted output	6.23 ± 0.29	17.16	-	-
Ulva:					
Input:	Fertilisers	50.80 ± 0.69	63.45	66.66 ± 2.01	89.54
	Dissolved nitrogen from abalone effluent/seawater	29.26 ± 2.60	36.55	7.78 ± 0.32	10.45
Output:	*Ulva* harvest	54.93 ± 0.30	68.61	38.84 ± 0.27	52.18
	Post-seaweed effluent	20.37 ± 0.99	25.45	34.37 ± 2.39	46.17
	Unaccounted output	4.73 ± 2.0	5.94	1.23 ± 0.33	1.65

Phosphorus budget in abalone and Ulva units of the IMTA and monoculture systems

The budget of phosphorus (P) in the monoculture and IMTA systems followed the same trend as the nitrogen budget (Table 3.6).

In abalone tanks, the fed diet contributed the highest input of phosphorus in the IMTA (78.1%, 8.94 ± 0.10 kg P/tank/month) and monoculture (74.3%, 2.20 ± 0.03 kg P/tank/month) systems. Other sources of phosphorus in abalone culture tanks of both systems were the intake water (ca. 11.8-21.9%), and phosphorus from unquantified sources (ca.13.9%). Approximately 12.5 and 19.4% of the phosphorus inputs from the feed and supplied seawater was absorbed by farmed monoculture and IMTA abalone while the largest portions were lost to accumulated waste solids (33.8-44.6%) and the effluent flowing out of abalone tanks (53.7%).

In the seaweed culture raceways, the highest inputs of phosphorus were the phosphate fertilisers which supplied 93.6% (24.01 ± 0.61 kg P/tank/month) and 66.4% (21.38 ± 1.48 kg P/tank/month) phosphorus in the monoculture and IMTA *Ulva* tanks respectively (Table 3.6). Additionally, the abalone effluent channeled into IMTA *Ulva* raceways contributed ca. 33.4% dissolved phosphorus in the system. At the end of the culture cycle, the cultured *Ulva* in the two systems absorbed ca. 11.4-15.2% phosphorus while the largest portion (76.9-88.6%) was lost to the environment (Table 3.6).

Table 3.6. Mean (± standard error) phosphorus loads and percentage phosphorus by each components for the phosphorus budget of South African abalone (*H. midae*) and *Ulva species* reared in Integrated multitrophic aquaculture and monoculture systems.

		IMTA SYSTEM		Monoculture System	
UNIT	**P-form**	**Kg P/month**	**%**	**Kg P/month**	**%**
Abalone					
Input:	Feed input	8.94 ± 0.10	78.14	2.20 ± 0.03	74.32
	Intake water	2.50 ± 0.11	21.85	0.35 ± 0.01	11.83
	Unaccounted input	-	-	-0.41 ± 0.02	13.85
Output:	Abalone harvest	2.22 ± 0.09	19.41	0.37 ± 0.01	12.50
	Abalone biodeposits	5.10 ± 0.16	44.58	1.00 ± 0.01	33.78
	Abalone wastewater	-	-	1.59 ± 0.11	53.72
	Suspended solids	0.00 ± 0.00	0.00	0.00 ± 0.01	0.00
	Unaccounted output	4.12 ± 0.04	36.01	-	-
Ulva:					
Input:	Fertilisers	21.38 ± 1.48	66.44	24.01 ± 0.61	93.64
	Dissolved phosphorus from abalone tanks	10.74 ± 0.40	33.40	1.63 ± 0.02	6.36
	Unaccounted input	-0.06 ± 0.24	0.19	-	-
Output:	*Ulva* harvest	3.68 ± 0.10	11.44	3.89 ± 0.13	15.20
	Post-seaweed effluent	28.50 ± 2.02	88.56	19.73 ± 0.73	76.91
	Unaccounted output	-	-	2.02 ± 0.23	7.89

Carbon budget in abalone and Ulva culture units of the IMTA and monoculture systems

The carbon budget for each compartment of the two production systems was calculated in kilogram and also mapped as percentage flows of inputs and outputs of carbon over the monitoring period (Table 3.7). The fresh seawater flowing into abalone tanks contributed the highest load of carbon in the monoculture (53.0%, 78.40 ± 0.56 kg C/tank/month) and IMTA

(59.4%, 405.65 ± 17.94 kg C/tank/month) abalone tanks, followed by the feed carbon in the monoculture (45%, 67.31 ± 0.70 kg C/tank/month) and IMTA (40.6%, 259.53 ± 7.16 kg C/tank/month) systems. Approximately 19.8-24.4% carbon load from the feed and impounded water was absorbed by *H. midae* in the two systems while ca. 12% organic carbon was recorded in the biodeposits on production tanks floor. Additionally, 68.5% carbon load in the monoculture system was lost to the wastewater flowing from monoculture abalone tanks while 71.7% carbon was recorded in the effluent flowing into IMTA seaweed raceways (Table 3.7).

In the seaweed raceways, the highest input of carbon was the intake seawater in monoculture raceways (ca. 67.4%) and abalone effluent in IMTA (ca. 70.7%). Other sources of carbon in *Ulva* raceways were the chemical fertilisers (ca. 4%), and inputs (ca. 24.0-28.5%) from unquantified sources. At the end of culture cycle, approximately 50.8% (monoculture) and 58.0% (IMTA) carbon input from water, fertilisers and other sources was sequestered by the seaweed while 42-49% C was lost to the environment.

Table 3.7. Mean (±SE) carbon loads in each production tank and percentage carbon contribution in tank compartment for the carbon budget of South African abalone (*H. midae*) and *Ulva species* reared in Integrated multitrophic aquaculture and monoculture systems.

		IMTA SYSTEM		Monoculture System	
UNIT	**C-form**	**Kg C/month**	**%**	**Kg C/month**	**%**
Abalone					
Input:	Feed input	259.53 ± 7.16	40.63	67.31 ± 0.38	45.53
	Intake water	379.31 ± 0.65	59.37	78.40 ± 0.56	53.03
	Unaccounted input	-	-	19.60 ± 0.16	1.44
Output:	Abalone harvest	155.66 ± 2.77	24.36	29.25 ± 0.28	19.78
	Abalone biodeposits	77.81 ± 1.53	12.18	17.36 ± 0.07	11.75
	Abalone wastewater	-	-	101.21 ± 1.97	68.45
	Suspended solids	0.02 ± 0.01	0.00	0.03 ± 0.00	0.02
	Unaccounted output	233.49 ± 3.56	63.45	-	-
Ulva:					
Input:	Fertilisers	24.94 ± 0.33	4.26	25.16 ± 0.93	4.12
	Influent carbon	419.60 ± 16.63	71.70	411.79 ± 1.01	67.35
	Unaccounted input	140.54 ± 5.42	24.02	174.44 ± 0.33	28.53
Output:	*Ulva* harvest	339.25 ± 5.65	57.98	310.62 ± 6.53	50.81
	Post-seaweed effluent	245.83 ± 5.89	42.02	300.77 ± 14.25	49.19

3.5 Discussion

3.5.1 Waste discharge from monoculture and IMTA systems.

The culture of seaweed in abalone effluent significantly reduced the demand for mineral fertilisers at Wild Coast Abalone as the dissolved nutrients in abalone wastewater provided significant amount of dissolved nutrients (34-38%) required by IMTA seaweed. This production technique reduced the discharge of dissolved waste nutrients to the environment and also limited the use of inorganic fertilisers in the system. While the monoculture *Ulva* raceways were fertilised fifteen times in a production cycle, the IMTA *Ulva* received fertilizer only nine times within the same culture period. From farm data, each monoculture raceway annually receives about 306 kg of Urea, 270 kg of monoammonium phosphate (MAP) and 270 kg of monopotassium phosphate (MKP) fertilisers while the IMTA *Ulva* raceway is fertilised with 183.6 kg, 162 kg and 162 kg of Urea, MAP and MKP respectively. This translates to a 35 - 40% reduction in the demand for mineral fertilisers in the IMTA compared to the monoculture system.

The IMTA *Ulva* system appeared to perform better than the monoculture system in terms of nutrient uptake, as the level of dissolved nitrogen and phosphors in IMTA post-seaweed effluent was 26% and 12% lower respectively than the monoculture system. This could have resulted from the lower water exchange (200 L min^{-1}) maintained in the IMTA *Ulva* raceways compared to the 350 L min^{-1} waterflow of the monoculture system. This lower water flow allowed the supplemented nutrients to reside for an extended period in the raceways while bioremediation took place before a final discharge.

Nonetheless, the percentage of input nitrogen (ca. 25.5 and 46.2%) and phosphorus (76.9 and 88.6%) discharged from both seaweed production systems was relatively high, indicating an excess nutrient supplementation (especially of phosphorus) that was beyond the uptake ability of the *Ulva* stock. The continuous discharge of these waste nutrients represents an economic loss and also contributes negatively to the environmental footprint of the farm. Optimising the water flow and exchange rate in the seaweed raceways could improve dissolved nutrient removal efficiencies of *Ulva species* (Macchiavello & Bulboa 2014). In addition to water exchange optimisation, reducing fertilisation rate by subjecting the seaweed to periodic or recurrent fasting could also improve nutrient removal efficiency of seaweeds (Lobban *et al.* 1985; Lobban and Harrison 1994; Neori *et al.* 2003; Robertson-Andersson *et al.* 2007). However, macroalgae starved of nutrients, especially nitrogen do not make for good quality feed (Troell *et al.* 2003). For a commercial seaweed farm like the Wild Coast Abalone where monthly turnover of macroalgae is prioritized over nutrient removal efficiency, regular fertilisation and high-water exchange is required. Although this production process results in high biomass yield, a lower efficiency of nutrients removal by seaweed is achieved. An alternative means to enhance the uptake of inorganic fertilisers by the seaweed could be by the application of biofertilisers. Biofertilisers are living microorganisms capable of increasing the uptake and availability of chemical nutrients when applied to plants (Vessey 2003; Suhag 2016). They do not provide nutrients to cultured plants but through nitrogen fixation, and phosphorus solubilization, biofertilisers provide high uptake of nutrients from inorganic fertilisers (Suhag 2016; Jacob and Kumar 2020). Information on the potential of biofertilizers in seaweed culture is still scanty

therefore, a pilot trial to test the efficacy of biofertilisers in *Ulva culture* was carried out (Chapter 8).

Phosphorus removal by both IMTA and monoculture *Ulva* was very low (less than 15%) when compared with the uptake of nitrogen and carbon. Apart from the high waterflow which exported nutrients out of the seaweed tanks, excessive supplementation of phosphorus-based inorganic fertilisers (monoammonium phosphate and monopotassium phosphate) beyond the level required by the seaweed could have contributed to their low uptake by the macroalgae. The poor uptake of phosphorus by *Ulva lactuca* have been reported on a South African abalone farm (Robertson-Andersson *et al.* 2007). This was attributed to the high preference of *Ulva* for nitrogen species and most importantly ammonium before other nutrients (Robertson-Andersson *et al.* 2007). Therefore, it is important that the frequent application of monoammonium and monopotassium phosphate fertilisers be minimised at WCA.

3.5.2 Macroalgae production and growth in IMTA and monoculture systems

The growth of seaweed in the current study was affected by the production system type and the season, with higher average monthly *Ulva* production recorded for IMTA seaweed system compared to the monoculture *Ulva*. This higher production in the IMTA *Ulva* system could have resulted from the strong affinity of *Ulva* for bound nutrients from organic residues of fed aquaculture species metabolism (Nardelli *et al*. 2019). Seaweed growth rates were highest for both production systems during summer and lowest during the winter. The higher growth in summer could have resulted from the longer photo period and higher water temperatures which increased the metabolism of the plants (Hasanuzzaman *et al*. 2013). Overall, the 4.75- 6.21% daily

growth of *Ulva* in the current study are similar to the 5.78% growth of *Ulva lactuca* cultured in an IMTA system with *Oreochromis spirulus* (Al-Hafedh *et al.* 2015). Additionally, the 4.75 - 6.32% day^{-1} growth rates of *U. lactuca* cultured in an IMTA system with Cobia fish (*Rachycentron canadum*) and mussels (*Perna perna*) (Nardelli *et al*. 2019) compares with the growth rate of *U. lacinulata* in the present study. Likewise in a pilot recirculating IMTA system of the South African abalone and *U. lactuca*, approximately 6.25% daily growth of *Ulva* cultured in *H. midae* effluent was recorded (Robertson-Andersson *et al.* 2007). Nevertheless, the growth rates of IMTA and monoculture *Ulva* in the present study are lower than the 18.6% daily growth of *U. lactuca* cultured at 2 kgm^{-2} stocking density in fish effluent (Neori *et al.* 1991). They are also lower than the 20% day^{-1} growth of *U. clathrata* cultured in shrimp effluent (Copertino *et al.* 2009). The high levels of inorganic nitrogen and ammonium in fish and shrimp effluents could have contributed to the higher growth of *Ulva* in these previous studies. Additionally, the smaller seaweed culture tanks used in Copertino *et al.* (2009) and Neori *et al.* (1991) could have accounted for the higher growth rates recorded in these studies. This is because seaweed growth reduces when culture tanks dimensions are increased (Robertson-Andersson 2003; Robertson-Andersson *et al.* 2007). This suggests that macroalgae production at Wild Coast Abalone can be improved if realistic optimisation measures are adopted. One of such measures would be by carrying out a periodical or batch harvesting of seaweed before their production cycle is completed. This has been reported to enhance the uptake of dissolved nutrients by seaweed (Robertson-Andersson *et al.* 2007).

The daily biomass yield of *Ulva* was affected by the production system type as biomass yield of IMTA *Ulva* was 12.8% higher than the monoculture *Ulva*, thus indicating that the IMTA system is

more efficient in nutrient utilisation and production than the monoculture. Different factors including lower water exchange of IMTA seaweed tanks and the continuous supply of dissolved nutrients from abalone wastewater could have contributed to the higher yield of IMTA *Ulva*. Overall, the 85.95 (monoculture) and 148.53 m^{-2} g (IMTA) daily *Ulva* yield are similar to the 150 g m^2 day^{-1} biomass yield for *U. lactuca* cultured at a water flow of 5.4 m^3 day^{-1} in land-based IMTA system with *Oreochromis spirulus* (Al-Hafedh *et al.* 2015). However, when the water exchange of this system was increased from 5.4 to 10.8 4 m^3 day^{-1} at a stocking rate of 3 kg m^{-3}, the 12% daily growth and 250 – 300 g m^2 daily biomass yield of *U. lactuca* were twice the growth and yield of *Ulva* in the current study. This suggests that the production performance of the seaweed culture systems at WCA can be improved if the current stocking density of 1.05 kg m^{-3} is optimised. The alteration of the stocking density of seaweed could significantly impact their growth and production quality at harvest (Robertson-Andersson *et al.* 2007). The optimum efficiency of nutrient utilisation was recorded when the stocking density of *Ulva lactuca* was reduced from 4.5 kg m^{-2} to 3.5 3.5 kg m^{-2} (Robertson-Andersson 2003). Moreover, the water flow in IMTA and monoculture *Ulva* tanks is 50-64% higher than the optimum exchange rate of 125 liters per minute for *Ulva* production (Al-Hafedh *et al.* 2015). Therefore, to improve the efficiency of seaweed production at WCA, water flow and exchange rate in seaweed raceways should be investigated in future studies.

3.5.3 Ulva tissue nutrient composition

Higher tissue nitrogen, phosphorus and carbon compositions were recorded for IMTA-cultured *Ulva* than the monoculture *Ulva*. This could have resulted from the higher nutrients supplied into this system from abalone effluent. While the monoculture *Ulva* received nutrients primarily from

inorganic fertilisers supplied every other day, the IMTA *Ulva* continuously received nutrients from abalone effluent flowing into seaweed raceways. This is one of the advantages of culturing seaweed in IMTA system with abalone as they help strip nutrients from abalone effluent before a final discharge to the environment and in the process build-up their tissue nutrients level. Likewise, the organically bound nitrogen and phosphorus in abalone effluent could have contributed to the higher nutrient uptake and composition of IMTA *Ulva*. Seaweed is characterised by their strong affinity for organically bound nutrients in aquatic ecosystems (Park *et al*. 2021). Additionally, the closed fertilisation technique adopted for IMTA *Ulva* raceways could have also contributed to the higher nutrient composition of *Ulva* cultured in this system. At Wild Coast Abalone, while *Ulva* raceways in the monoculture system were opened to water flow during fertilisation (Open fertilisation technique), IMTA *Ulva* raceways were closed to incoming and outflowing effluents for eight hours. Preventing water exchange during this period results in longer retention of nutrients from abalone effluent and mineral fertilisers in the raceways while bio-extraction of nutrients takes place.

The higher tissue nitrogen of IMTA *Ulva* translated into significantly better tissue protein than the monoculture *Ulva*. Overall, the average tissue proteins of IMTA and monoculture *Ulva* in the current study are significantly higher than the 3.7-24% protein values for wild harvested *Ulva* (Simpson and Cook 1998; Wilkinson 2001; Wong and Cheung 2001; Robertson-Andersson *et al*. 2011). The average protein levels for both the IMTA and monoculture *Ulva* also compare with the 36% protein level considered most beneficial for South African abalone (Sales and Britz 2001).

The nutrient composition of the seaweed was also affected by seasonal changes in environmental conditions as *Ulva* nitrogen, phosphorus and carbon were highest for both systems during winter

and spring. Although growth was highest during summer, nutrient compositions of *Ulva* was lowest at this season of the year. This is an indication of an inverse relationship between seaweeds growth rate and tissue nutrient composition (Duke *et al.* 1986; 1989, Cohen and Neori, 1991; Robertson-Andersson 2003; Robertson-Andersson *et al.* 2007). The improved light and temperature conditions during summer significantly decreased the nitrogen level of *Ulva curvata* and *Codium decorticatum* (Duke *et al.* 1989). Coastal oceanographic events also contribute to the seasonal changes in nutrient composition of seaweed tissue (Robertson-Andersson *et al.* 2007). By drawing a comparison between the protein composition of *Ulva* in this study and the reported 40-44% CP in other studies (Goldburg and Triplett 1997; Neori *et al.* 1991), it can be inferred that it is possible to increase the protein level of the *Ulva* at Wild Coast Abalone beyond the current 30-34% level. This can be achieved by making substantial improvements to the system's design. Some of these improvement techniques include the optimisation of waterflow and exchange rate in seaweed tanks to allow more uptake of nutrients by the seaweed, partial or batch harvesting of *Ulva*, phasing out the current stock of *Ulva* and replacing with improved breeds and changing fertilisation protocols.

3.5.4 The budget of nutrients in abalone and seaweed units of the IMTA and monoculture production systems

In the current study, the percentage input of nutrients, uptake and discharge from abalone tank of both production systems were similar.

Nitrogen budget in the compartments of abalone-Ulva IMTA and monoculture system

The daily application of feed pellet for *H. midae* accounted for the highest input of nitrogen (ca. 74-81%) in abalone tanks of both production systems under study. This corresponds with other IMTA and monoculture systems where the feed accounted for the highest input of nitrogen in abalone culture tanks (Neori *et al.* 2000, Gao *et al.* 2019a, b). For instance, the seaweed (*Ulva lactuca*) protein accounted for the only significant (100%) input of nitrogen in abalone tanks of an IMTA system with fish and seaweed (Neori *et al.* 2000). Likewise, the formulated diet fed to Japanese abalone *H. discuss hannai* accounted for the highest input of nitrogen (83.8%) in a recirculating aquaculture system (Xiaolong *et al*. 2018). The seawater flowing into abalone tanks also contributed significant portion of nitrogen (11-19%) to abalone tanks in the current research.

The uptake of nitrogen from fed diet was similar for both monoculture and IMTA abalone. The 48-51% N uptake by South African abalone (*H. midae*) in the present research is similar to the nitrogen uptake by other species of abalone. For example, 68.5 and 64.7% nitrogen uptake were recorded for *H. discuss hannai* that was cultured in a monoculture and IMTA system with the sea cucumber *Apostichopus japonicas* respectively (Gao *et al.* 2019b). Additionally, 40% nitrogen assimilation was recorded for *H. discuss hannai* cultured in an integrated mariculture system with sea bream (*Sparus aurata*), seaweed (*Ulva species*) and *Gracilaria* (Neori *et al.* 2000). Likewise, 31% N recovery was documented for *H. discus hannai* fed 51% CP diet in an IMTA system with *Apostichopus japonicas* and *Sebastes schlegeli* (Goa *et al.* 2019a). However, the nitrogen uptake by *H. midae* in the current study is higher than the 14% N uptake documented for the European abalone (*H. tuberculata*) cultured in an IMTA system with *Ulva* and fed the recycled *Ulva* (Neori *et al.* 1998).

The accumulated waste solids from faecal material and uneaten food deposited on production tanks floor accounted for approximately 20 and 31% nitrogen loss from feed in the monoculture and IMTA abalone tanks respectively. This was a significant loss of nitrogen from the production systems as organic waste nutrients from abalone are currently not utilised but discharged into neighboring coastal environment. To improve the farming efficiency at WCA, it is important to reduce the production of organic waste from the production systems by using the waste nutrient for the production of other species of economic importance. Integrating a detritus waste extractive species (e.g., sea cucumbers) into the farming system could further reduce the discharge of solids waste nutrients into the environment and reduce the footprint of the farm on the environment (Fang *et al*. 2009; Gao *et al.* 2019a, b).

A significant portion of the nitrogen supplied into abalone tanks of the monoculture (32%) and IMTA system (29%) was lost to the wastewater flowing out of the production tanks which is typical of a flowthrough pellet-fed system. This loss resulted from the continuous exchange of seawater which rapidly transports potentially toxic nutrients out of abalone tanks (Nhan et *al.* 2008). While the nitrogen in the post-abalone tank effluent of the monoculture system was discharged to the environment, the effluent of IMTA abalone was captured downstream and used for seaweed production. Integrating seaweed with abalone production significantly reduced the dissolved nutrient export to the environment and lessened the impact of the IMTA farm on the environment (Chapter 4).

The production efficiency of IMTA seaweed culture system was substantially better than the monoculture system as nitrogen supplementation from inorganic fertilisers was lower for this system. While ca. 90% of the nitrogen load in the monoculture seaweed raceways was supplied

by added chemical fertiliser (urea), only 63% of the nitrogen in IMTA *Ulva* pond was supplied by the fertilisers and 37% N by impounded abalone effluent. This demonstrates the positive effects of IMTA adoption on commercial abalone farms both economically and environmentally, as effluent from abalone tanks provides more than one-third of the necessary dissolved nitrogen.

The absorption of nitrogen by IMTA seaweed was 16.4% higher than the monoculture *Ulva*. This could have resulted from the continuous inflow of nutrients from abalone effluent into IMTA seaweed raceways and the strong affinity of *Ulva* for organically bound nitrogen in fed aquaculture effluent than the nitrogen in supplemented chemical fertilisers (Park *et al*. 2021). This further confirms the benefits of integrating seaweed and abalone culture into a single system. The nitrogen uptakes by both IMTA (ca. 68.6%) and monoculture *Ulva* (52.2%) are similar to the 58% N recovery by *U. lactuca* cultured in a land based IMTA system with *Haliotis tuberculata* (Neori *et al.* 1998). Likewise, approximately 49-56% ammonia-nitrogen was removed by *U. lactuca* cultured in marine fishpond effluents (Cohen and Neori 1991).

The post-seaweed effluent of the IMTA was 21% lower in waste nitrogen than the monoculture system. Some possible reasons for this could have been the lower utilisation of mineral fertilisers in the IMTA system than monoculture system. Additionally, the lower water exchange and flowrate in IMTA seaweed raceways could have contributed to the lower discharge of nitrogen from the integrated unit than the monoculture system. Similarly, the affinity of *Ulva* for organically bound nitrogen in aquaculture effluent could have contributed to the lower discharge of this nutrient in the IMTA system (Park *et al*. 2021). However, the percentage nitrogen discharge (ca. 25-45%) in the post-seaweed effluents from both systems shows a high export of dissolved nitrogen from Wild Coast Abalone to surrounding coastal environment. It is therefore

important that the farm source for ways to further reduce the discharge of dissolved waste nutrients to coastal environment.

Phosphorus budget of abalone-Ulva IMTA and monoculture systems

As with nitrogen, the fed pellet (Abfeed) also accounted for the highest (74-78%) input of phosphorus in abalone tanks of both production systems. This agrees with previous studies on abalone IMTA and monoculture systems where the feed accounted for the highest input of phosphorus in abalone culture systems (Xiaolong *et al.* 2018; Gao *et al.* 2019a, b). However, due to different management practices, feeding regimes, and site-specific conditions, variations exist in the percentage contribution and loss of nutrients between systems (David *et al.* 2017b). Therefore, comparing data of different production systems is difficult due to the differences in management (David *et al.* 2017b).

The uptake of phosphorus by IMTA (19.4%) and monoculture abalone (12.5%) was lower than nitrogen uptake, showing a low utilisation of diet phosphorus by *H. midae*. This compares well with other abalone species in different production systems. Approximately 14% P uptake was recorded for *H. discus hannai* cultured in an IMTA system with the sea cucumber (*Apostichopus japonicus*) and Rockfish (*Sebastes schlegeli*) (Gao *et al.* 2019a). Likewise, ca. 7–14% phosphorus uptake was documented for *H. discus hannai* that were was cultured at different water exchange rates in a recirculating system (Xiaolong *et al.* 2018). Similarly, 9-11% phosphorus uptake was recorded for *H. discus hannai* cultured with the *A. japonicus* (Gao *et al.* 2019b).

Not less than 33.8 and 44.5% of the phosphorus input from the diet and intake water was lost to the accumulated solids in the monoculture and IMTA systems respectively. This high level of

organic phosphorus in the systems shows the strong affinity of aquaculture waste solids for phosphorus (Boyd 1995; Sahu *et al*. 2015; Flickinger *et al.* 2020a). Accumulated solid waste represents the main output of phosphorus in pond culture system and could account for about 39-93% P expenditure in aquaculture systems (Jackson *et al.* 2003; Sahu *et al*. 2015). The high P level in organic waste is related to phosphorus involvement in sedimentary cycle which allows most P to precipitate with mineral ions deposited in the sediment (Alongi *et al*. 2009; Xiaolong *et al.* 2018). Presently at WCA, these biodeposits are flushed twice weekly from abalone tanks and released into coastal environment thus contributing negatively to the environmental performance of the farm. It is therefore important to source for sustainable ways of remediating these solid waste. One of such would be through the integration of solid waste extractive organisms such as sea cucumbers into abalone production system.

The wastewater from abalone tanks accounted for 54% phosphorus outflow from abalone tanks in the monoculture systems and 33.4% in the IMTA. While the dissolved waste in the monoculture was emptied directly to the sea, the dissolved phosphorus in the IMTA was used for seaweed culture.

The highest input of phosphorus in the seaweed tanks were the chemical fertilizers which supplied approximately 94% and 64% phosphorus in the monoculture and IMTA *Ulva* tanks respectively. This suggests that the demand for phosphorus supplement from inorganic fertilisers is reduced by 35% in the IMTA system as this portion of required phosphorus is supplied by abalone effluent channeled into seaweed raceways. This production technique has both economic and environmental benefits for commercial abalone farms where IMTA is adopted.

This implies that for Wild Coast Abalone, the demand for phosphorus-based mineral fertilisers is 35% lesser in the IMTA system than the monoculture system.

The uptake of dissolved phosphorus by farmed *Ulva* from both production systems was relatively low (12-15%), indicating that the phosphorus not absorbed by the macroalgae was exported out of the system into coastal environment thus contributing to the overall pollution effect of the farm. Low phosphate/phosphorus uptake by *Ulva lactuca* have been reported in previous studies (Neori *et al.* 1998; Schuenhoff *et al.* 2003; Robertson-Andersson *et al*. 2007; Fotedar 2011; Fan *et al.* 2014). For instance, in a closed recirculating IMTA system of the western King prawn (*P. latisculcatus Kishinouye*) and *U. lactuca,* only 2-14% phosphorus in prawns effluent was absorbed by farmed *Ulva* (Fotedar 2011). It was assumed in the current study that since nitrogen is the most limiting nutrient for *Ulva* growth (Robertson-Anderson *et al.* 2007; Roleda & Hurd 2019), phosphorus uptake by IMTA *Ulva* at WCA would be higher if it only receives dissolved phosphorus from abalone effluent and not supplemented with excess phosphorus from two mineral fertiliser (monoammonium phosphate and monopotassium phosphate). To obtain efficient phosphorus utilisation, it is crucial to apply the basic system optimization approaches proposed for efficient nitrogen uptake. Additionally, the use of synthetic phosphorus from two sources could be discontinued while the quantity of phosphorus fertilisers applied per fertilisation period is reduced for both systems. Additionally, the use of non-polluting and biodegradable microbial fertilisers could be adopted. These biofertilisers improve the uptake of inorganic phosphate fertilisers (Jacob and Kumar 2020).

Carbon budget in abalone-Ulva IMTA and monoculture systems

The pumped seawater contributed the highest input of carbon (53-39%) in abalone tanks followed by the ca. 41-46% carbon load from the different feed types. Only 20-24% of carbon supplied from the different feed types and the incoming sweater was retained in abalone tissue. Previous studies documented that less than 30% of the nutrients in the feed fed to aquaculture species are absorbed by the animal with a large percentage lost to the sediment and effluent (Dauda *et al.* 2019). The carbon uptake by *H. midae* in the present study is similar to the 15-19% uptake by other culturable fish species (Sahu *et al*. 2013; Adhikari *et al.* 2014; David 2015).

A large percentage (68-71%) of carbon supplied into abalone tanks of both systems was lost to the wastewater flowing out of the production tanks while a smaller portion (ca. 12%) was lost to the solid waste component of the two production systems. Approximately 63% of the carbon supplied from feed and impounded water was unaccounted for in IMTA abalone tanks. This portion of carbon could have been lost as greenhouse gases (CO_2 and CH_4) which diffused out of the system (Dallas Flickinger, *personal communications*). Previous studies documented loss of carbon from aquaculture systems as diffused greenhouse gases in aquaculture ponds (Flickinger *et al.* 2020b; David *et al.* 2021).

The two major sources of carbon in the seaweed tanks were the influent water from IMTA abalone (70%), and inputs from (ca. 24-28%) from unaccounted sources such as absorbed or trapped greenhouse gases in the production system (CO_2, CH_4).

The sequestration of dissolved carbon by seaweed was marginally higher for IMTA *Ulva* (58%) than the monoculture *Ulva* (ca. 51%). However, a large percentage (42-49%) of the carbon

entering the systems was lost in the post-seaweed effluent. This shows that seaweed only removed about half of the organic and atmospheric carbon entering the seaweed production systems.

3.6 Conclusion

Overall, it can be inferred that the IMTA system at WCA was more efficient than the monoculture system in terms of nutrient utilisation because:

- About 33-37% dissolved waste nutrients (nitrogen and phosphorus) from abalone tanks was used for seaweed culture thereby reducing nutrients discharge from this system to coastal environment.
- The inorganic fertilisers requirement was lower for the IMTA system with over 37% of N and 33% P required for seaweed culture supplied by nutrient-laden abalone wastewater.
- Nutrient (nitrogen, phosphorus and carbon) absorption and retention by IMTA *Ulva* was higher than the monoculture *Ulva*.

While the IMTA system was more efficient in terms of the efficiency of nutrient utilisation, there are nonetheless areas where improvement can be made. Some of these include:

- A more efficient application of fertilisers in seaweed tanks as the current study revealed excessive use of chemical fertilisers in the two systems which results in high nutrient levels (especially phosphorus) in the post-seaweed effluent discharged to the environment. It was therefore suggested that the farm limits its application of phosphorus-based fertilisers to monoammonium phosphate (MAP) and not in

combination with mono-potassium phosphate since the seaweed can readily absorb potassium from seawater which is known to contain about 380 mg K per liter of water.

- The use of live microbial fertilisers as a substitute for mineral fertilisers should be tested. Biofertilisers are relatively cheap when compared with granular fertilisers in terms of the quantity applied per production cycle of *Ulva*. They are eco-friendly (non-polluting), and they increase the solubilization and efficient uptake of inorganic nitrogen and phosphorus in mineral fertilisers. An experiment was conducted to test the efficiency of liquid fertilisers for seaweed production on the farm and it is reported in Chapter 8.
- The phasing out of the current stock of *Ulva* at WCA was suggested due to the low nutrient uptake and growth rate of this current stock which has been recycled for more than two decades.
- The integration of solid waste extractive organism into existing abalone-*Ulva* IMTA system was suggested to further improve nutrient utilisation efficiency of the production system. While *Ulva* is currently cultured downstream to ameliorate dissolved nitrogen and phosphorus waste from abalone production, capturing the waste solids with detritus extractive organisms will reduce particulate discharge, improve feed utilisation and also produce a secondary crop of commercial relevance at little or no cost. A portion of this research tested the integration of sea cucumber with *H. midae* (Chapter 7).

Chapter 4: Life cycle assessment of abalone-seaweed monoculture and integrated aquaculture systems

4.1 Introduction

The present study assessed the performance efficiency of the existing farming systems at Wild Coast Abalone in terms of the relative impact of these systems on the environment. This was conducted to assess how the farming systems affect environmental impact indicators such as global warming, ozone layer depletion, ocean acidification and eutrophication potential. This approach will help determine the areas of the farming system where improvements could be made in order to reduce the effect of South African abalone farms on the environment.

In recent years, majority of South African abalone farms have adopted the integrated abalone-seaweed culture technique to improve their production efficiencies (Bolton *et al.* 2009). Seaweeds are now cultured in abalone effluents to produce fresh feed for farmed *H. midae* and reduce the level of dissolved nutrient discharged to the environment. This culture technique has greatly reduced the dependence of South African Abalone farms on fishmeal resources. However, input such as inorganic fertilisers are still required for seaweeds production due to the low dissolved nutrients composition of abalone wastewater (Troell *et al.* 2006). This results in the discharge of unabsorbed nutrients from farms to the coastal environment. Additionally, the production system also generates high volume of solid wastes from uneaten feed and faecal materials which has not received much attention on any *H. midae* farm as they are directly discharged to coastal environment without any pre-treatment.

Apart from the input of nutrients and the discharge of associated waste, the land-based production of abalone in South Africa requires a very high input of direct energy to supply seawater into abalone and seaweed production tanks and to power water filtration systems.

Although no adverse effect of discharged waste and energy consumption by abalone farms has been reported by environmental agencies, there is a need to quantify the environmental impacts of production practices in order to achieve regulatory compliance and as evidence to obtain various sustainability certifications. Therefore, to achieve this economic and environmental sustainability goal, it is important to conduct an assessment of production processes and techniques employed on farms using a holistic approach. One such approved method for assessing the environmental impact of a product or production process including aquaculture operations is the life cycle assessment.

Life cycle assessment (LCA) is an internationally recognized framework that is used for quantifying the potential impacts associated with a production system, product or service based on the resources utilisation and subsequent waste emission (Guinee *et al.* 2002; Aubin 2013; Abdou *et al.* 2017). It is an assessment process or tool which accounts for the full life cycle of a product or production process from extraction of raw materials to its end-of-life (waste disposal stage) (Guinee *et al.* 2001; Guinee *et al.* 2002). Since its standardization as a competent framework, LCA is increasingly finding its use as an assessment tool in other sectors different from the environment. These include Agriculture, food production, animal feed industry, land and water use, renewable energy, bioenergy, renewable energy, packaging and waste management, product development and improvement, marketing, constructions, fisheries, and

aquaculture (Henriksson *et al.* 2013). Private organizations and government agencies have also found LCA framework as an essential tool in policy decision making (Heijungs and Guinée 2012).

Life cycle assessment can be categorized into four interdependent analytical phases in accordance with ISO 14040 standards (Figure 4.1). These include: (1) goal and scope definition, (2) life cycle inventory, (3) Impact assessment and (4) interpretation of results (Guinee *et al.* 2002; Chester *et al.* 2010; Taylor 2019).

Goal and scope definition

The goal and scope definition in life cycle assessment provides background information or introduction to the study (Guinee *et al.* 2002). This is the phase where the basis and the scope of the evaluation is defined as it answers the question "Why the assessment" through a careful selection of a system boundary and functional unit of measurement for the product or processes under consideration (Rebitzer *et al.* 2004; Ayer and Tyedmers 2009). This phase of the LCA ensures consistency in the assessment process and decides the intended audience of the analysis (Rebitzer *et al.* 2004). It also determines whether the outcomes should be used in comparative assertions unveiled to the public (Heijungs and Guinée 2012). It is very important when defining the goal and scope of an LCA study to create SMART (specific, measurable, achievable, realistic, and timely) goals and carefully choose a system boundary (delineations of processes to be included in the analysis), and a functional unit that allows similar goods and services to be compared and analysed (ISO 2006; Samuel-Fitwi *et al.* 2013).

Inventory Analysis

The life cycle inventory analysis (LCI) forms the second phase and the bedrock of an LCA study (Guinee *et al.* 2001; Heijungs and Guinée 2012; Klopffer & Grahl 2014). It is the data collection phase where environmental inputs, outputs and emission data that are associated with a product or service are compiled and quantified (Heijungs and Guinée 2012). This unit process forms the building block of an LCA as it converts a sum of inputs into a bundle of outputs (Heijungs and Guinée 2012). The LCI involves the creation of inventory flows for a product system, related to the functional unit defined in the goal and scope definition (Guinee *et al.* 2001). The LCI result provides information on all inputs and outputs as an elementary flow to and from the environment (Heijungs and Guinée 2012).

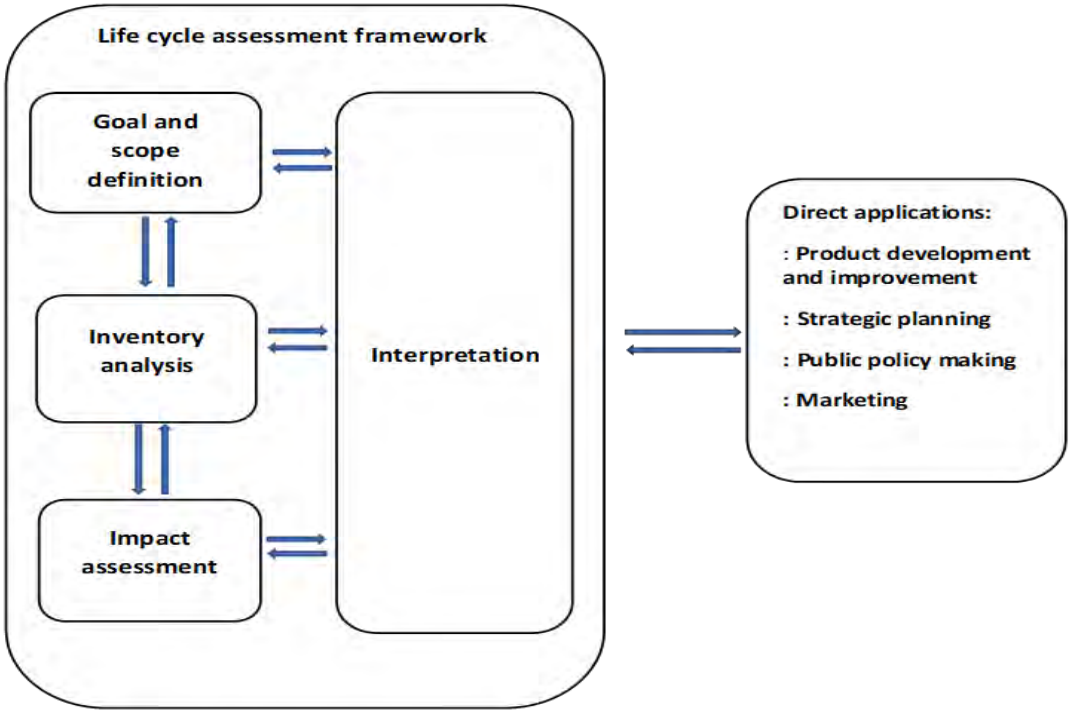


Figure 4.1: Life Cycle Assessment Framework (from ISO 14040 Standards)

Life cycle impact assessment

This phase of the LCA aims at evaluating the potential environmental impact of a product system based on the inventory flow results of the product system under consideration (Guinee *et al.* 2001; Heijungs and Guinée 2012). The LCI is made of some mandatory elements which include: the selection of impact categories; the classification stage (where inventory parameters are stored, grouped, and assigned to specific impact categories) and the impact measurement where the categorized LCI flows are characterized (Samuel-Fitwi *et al.* 2013). An impact category is the central element in impact assessment which clusters various product emissions into global or regional scale (Heijungs and Guinée 2012; Samuel-Fitwi *et al.* 2013). Some of these universally applicable impact categories include acidification potential (AP), eutrophication potential (EP), global warming potential (GWP), ozone depletion potential, primary energy use, net primary product use (NPPU), water dependence (WD), and surface use (Guinee *et al.* 2002; Roque d'orbcastel *et al.* 2009; Aubin *et al.* 2009; Papatryphon *et al.* 2004). The Life cycle impact assessment (LCIA) allows an analyst to draw conclusions for better business decision making based on the outcome of the inventory analysis and an expert knowledge of the inventory table (Heijungs and Guinée 2012; Pré Sustainability 2020).

Result Interpretation

The result interpretation phase is the final stage of the LCA, and it is the outcome of the defined goal and scope of the LCA (ISO 2006). This is the phase where the outcome of either life cycle inventory / life cycle impact assessment or both phases are combined together with the goal and scope of the assessment to identify the "hotspot", reach a concise conclusion, and make

necessary recommendations (Samuel-Fitwi *et al*. 2012; Heijungs and Guinée 2012). This phase ensures that other phases of the assessment are well tuned in and consistent with each other (Guinee *et al*. 2002).

For an aquaculture system, LCA offers a complex but efficient model which transparently quantifies the inefficiencies and the damaging effect of a production process (Sun 2009). It evaluates the overall impact of an aquaculture production technique by evaluating the energy and material input into production, the process as well as the waste and other emissions generated from the culture system (Sun 2009). However, one crucial challenge with using LCA in aquaculture is the challenge of selecting the right allocation method for environmental burdens between products and co-products (Samuel-Fitwi *et al.* 2013). It is considered challenging as different techniques will yield different results, hence different interpretations (Samuel-Fitwi *et al.* 2013).

4.1.1 LCA application in aquaculture

Life cycle analysis has been applied as an environmental impact assessment tool in aquaculture and aquafeed industries where nutrients input and direct energy form the major contributing factors to the overall impacts of aquaculture systems (Seppala *et al.* 2001; Aubin *et al.* 2006, 2009; Pelletier *et al.* 2009; Roque d'Orbcastel *et al.* 2009; Ayer and Tyedmer 2009; Roque D'orbcastel *et al.* 2009; Iribarren *et al.* 2010; Jerbi *et al.* 2012; Abdou et *al.* 2017; Forchino *et al.* 2017). Feed impact is directly related to fish meal and oil production and the release of dissolved and particulate waste into aquatic environment (Aubin *et al.* 2006; Abdou *et al.* 2017). However, the choice of a production system plays a major role in the impact of an aquaculture system on

the environment (Sun 2009). These impacts are often related to nutrients and solids emissions both on local and regional scale (Aubin *et al.* 2006). Other environmental impact indicators such as green-house gas production, energy use and biotic resources reduction are often neglected due to their impact being evident only on global scale (Aubin *et al.* 2006). For instance, while assessing nutrient emissions of recirculating and flowthrough systems for turbot production using the nutrient measurement accounting approach, both culture methods produced similar phosphorus and solids emissions while nitrogen emission was higher in the recirculating system (Aubin *et al.* 2006). Additionally, the non-renewable energy use, acidification and global warming potentials of the recirculating system were significantly higher than the flow-through system (Aubin *et al.* 2006). Similarly, a cradle to grave assessment of the efficiencies and environmental impact of traditional Indonesian tilapia/lettuce production system and aquaponics revealed a significantly lower impact of the aquaponics on freshwater eutrophication when compared with conventional agriculture technique (Cohen *et al.* 2018). In addition to this, the endpoint damage categories showed the aquaponic system to have reduced damage to human health, ecosystem, and resources by 88.2%, 75.2% and 50.16% respectively (Cohen *et al.* 2018). However, the aquaponic production technique resulted in higher mineral resources scarceness and ionizing radiation due to energy and material inputs in the system.

To date, the environmental impact of abalone and seaweed integrated culture system has not been assessed on any South African abalone farm using the life cycle analysis. The present study therefore aimed to evaluate and compare the environmental impacts associated with the culture of abalone and *Ulva* in integrated and monoculture systems using LCA. This will help to determine which of these two functionally equivalent production systems is environmentally friendly and

also document those components of the production systems contributing significantly to environmental impacts.

4.2 Materials and methods

4.2.1 Data collection and environmental indicators

The LCA was conducted using the primary data collected at the experimental site in Chapter 3 (data for feed, fertilizer application, abalone biomass, dissolved nutrients and solid waste outputs) and secondary data from databases. Environmental contributions of all compartments of each production system were evaluated from input to output. After collecting relevant data, they were aggregated into impact categories on global and regional scale as described by Guinee *et al.* (2002). Global impact indicators (global warming potential, ozone layer depletion, energy use) and regional impact indicators (eutrophication potential, ocean acidification, abiotic depletion, marine aquatic ecotoxicity) were quantified using the OpenLCA .10.3 as described by Carlos-Hernández & Díaz-Jiménez (2022).

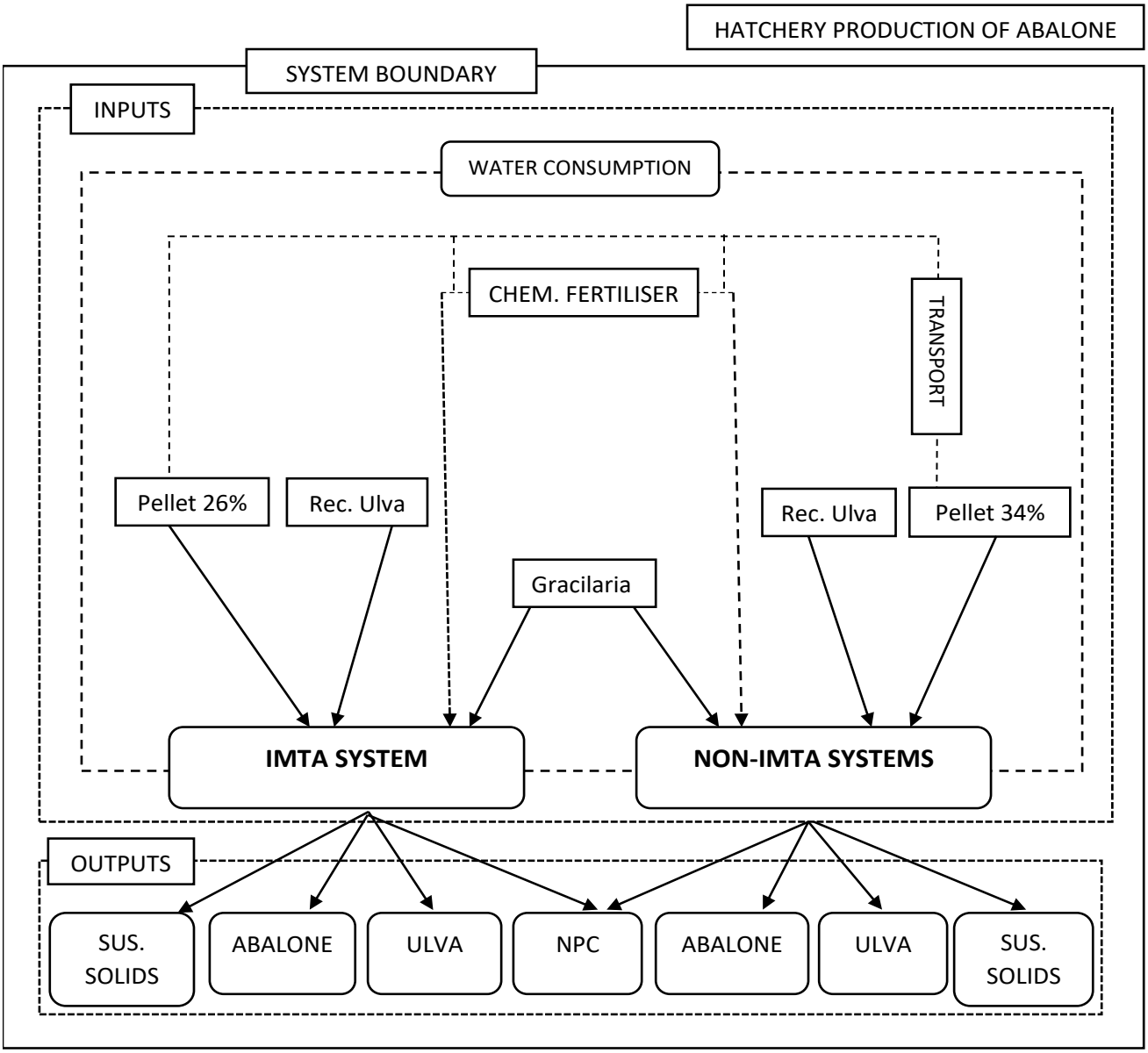
Goal and Scope of the study

The goal of this LCA study was to evaluate the environmental impact associated with the production of South African abalone, *H. midae* and the green seaweed *Ulva lacinulata* in integrated aquaculture and monoculture systems described in Chapter 3 by identifying the components of the production systems with the most significant impact on the environment. Abalone production starts with raw material extraction for feed ingredients, feed production, transportation, hatchery and grow-out production of abalone and ends up with harvest and

processing or live sale of adult abalone at the farm gate. Raw material extraction varies from resources extracted for the supply of energy to mineral resources utilised as inorganic fertilisers for farmed seaweed. At Wild Coast Abalone, abalone spat, juvenile, grow-out, adult production, and seaweed culture occur in different facilities but in the same location. Therefore, to compare the monoculture and integrated culture systems, one functional unit of 1 kg of *H. midae* and *Ulva* at the farm gate was used. The methodological attributional approach employed was the "Cradle-to-abalone farm gate" assessment and the system boundaries included the processes taking place in grow-out phase to the farm gate (Figure 4.2).

The LCA was conducted using the OpenLCA 1.10.3 software, leveraging inventory data from Ecoinvent v3.9 and Agribalyse (version 301_27052021) databases as background data. Comparisons between impact categories were carried out using the CML-IA baseline assessment method with normalization. This assessment method was selected due to its ability over other LCA methods to standardize both the midpoint and endpoint impact indicators. The system boundary was set to include all production processes and impacts related to abalone rearing, feedstuff production and the input needed to set (water, nutrients, air) and maintain (electricity, filters) the abalone-*Ulva* production systems (Figure 4.2). Hatchery production (characterized by an entirely different process) and abalone's post-production phases (e.g., processing, packaging, exportation, sales, and consumption) were not included in the system boundary (Figure 4.2). Also, the various infrastructure and building materials which were similar for the two production systems remained outside the scope of this LCA study. Nutrient emissions associated with abalone/*Ulva* production were quantified using nutrient balance modelling in chapter 3. Nitrogen, phosphorus, and carbon (N, P, C) calculation as well as solid emission were based on

the differences between input of nutrients in the diet which was converted to abalone flesh in terms of specific growth rate and weight gain. Solids and dissolved fractions of nutrients were used to quantify the emission of N, P and C.



*Rec *Ulva*: recycled *Ulva*

Figure 4.2. System boundary for the IMTA and monoculture production of abalone and seaweed at Wild Coast Abalone Pty Ltd.

Life Cycle Inventory (LCI)

The collection of primary data (consumption and emission data) for this study was carried out during the monitoring period in chapter 3. Materials and background processes (emissions from materials, chemicals, electricity and transport) which were not collected directly from the study site were extracted from the Agribalyse and Ecoinvent database (Table 4.1 - 4.2). The South African electricity mix was used for all electricity requirement and the inventory analysis was carried out according to the framework of ISO 14040 and ISO 14044 (ISO 2006a, b).

Life Cycle Impact Assessment

After collecting primary and farm data, the data were aggregated into impact categories on both global and regional scale applying the characterization factor of Guinee *et al.* (2002). Impact categories quantified were eutrophication potential (EUP), abiotic depletion (ABD), fossil fuels depletion (FFD), global warming potential (GWP), marine aquatic ecotoxicity (MAE), ozone layer depletion (ODP), ocean acidification potential (OAP), terrestrial ecotoxicity (TE), and freshwater ecotoxicity. Energy consumption for the two production systems corresponds to the operational energy consumption incurred from seawater pumps, filtration, and aeration system (Table 4.1- 4.2).

Table 4.1. Inventory of inputs and outputs in abalone culture tanks of the IMTA and monoculture production systems. Values are representative of the functional unit (1.0 kg of abalone culture).

Input	**Unit**	**IMTA**	**Monoculture**
Feed pellet (Abfeed E26)			
Fish oil (Anchovy)	g/kg	$1.06E^{3}$	$3.00E^{2}$
Fish meal (63-65%)	g/kg	$1.70E^{4}$	$2.01E^{4}$
Kelp (E. maxima)	g/kg	$3.54E^{3}$	$9.00E^{2}$
Lysine HCL	g/kg	$5.0E^{-3}$	$5.0E^{-3}$
Maize starch	g/kg	$5.61E^{4}$	$4.85E^{2}$
Soybean	g/kg	$2.20E^{4}$	$3.01E^{2}$
Vitamin premix	g/kg	$1.30E^{2}$	$1.30E^{2}$
Recycled *Ulva* production	kg/kg	$1.65E^{-2k}$	$1.58E^{2}$
Gracilaria gracilis	kg/kg	$5.80E^{-3}$	$4.90E^{-3}$
Feed transportation	km kg	$1.035E^{3}$	$1.03E^{3}$
Electricity for air	kWh/kg	$1.04E^{1}$	$5.03E^{-1}$
Electricity for water supply	KWh/kg	$1.05E^{1}$	$5.03E^{-1}$
Pumped seawater	m^3/kg	$6.12E^{1}$	$1.8E^{-2}$
Output			
Emission to water			
Nitrogen, total	g/kg	$2.12E^{1}$	$2.22E^{1}$
Phosphorus, total	g/kg	$7.80E^{2}$	$7.13E^{-3}$
Organic carbon	g/kg	$3.04E^{3}$	$4.54E^{-1}$
Waste solids	kg/kg	$4.06E^{-3}$	$1.57E^{-1}$
Emission to air	g/kg	$5.40E^{-4}$	$4.13E^{-1}$

Table 4.2. Inventory of inputs and outputs in *Ulva* culture tanks of the IMTA and monoculture production systems. Values are representative of the functional unit (1.0 kg of abalone culture).

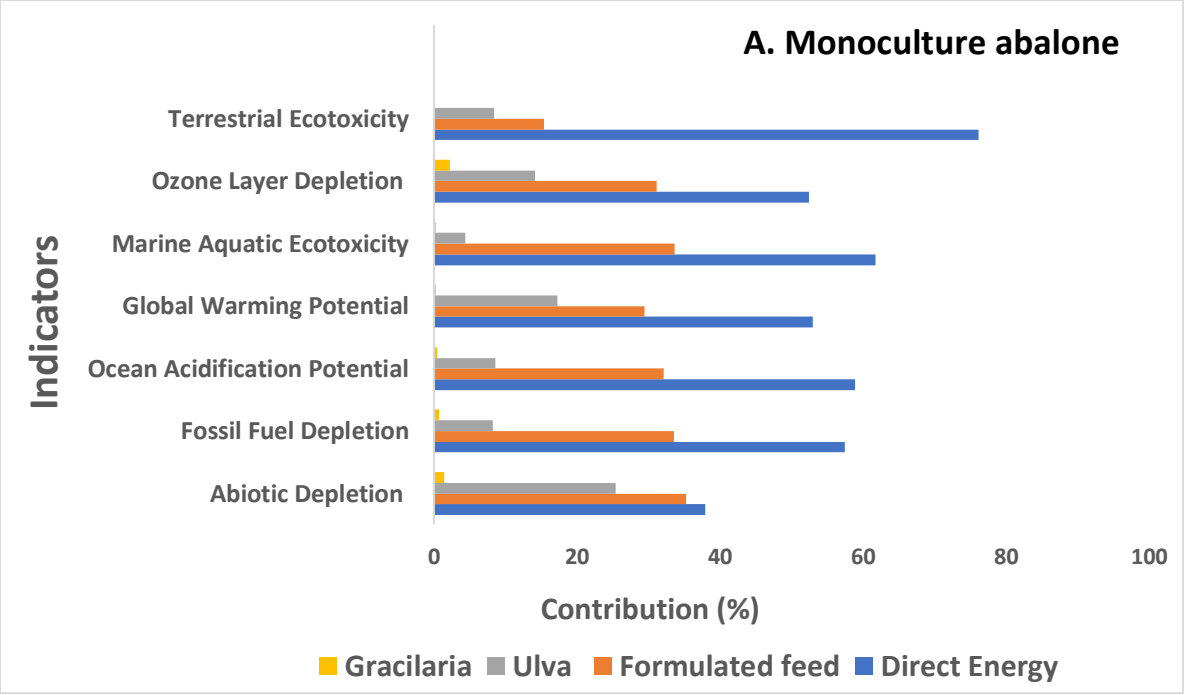
Input	**Unit**	**IMTA**	**Monoculture**
Monoammonium phosphate	kg/kg	$1.10E^{-2}$	$1.67E^{-2}$
Monopotassium phosphate	kg/kg	$5.00E^{-3}$	$1.32E^{-2}$
Urea (46% N)	kg/kg	$2.66E^{-2}$	$4.10E^{-2}$
Transport, freight, sea, transoceanic	km kg	$6.48E^{3}$	$6.42E^{3}$
Transport, freight, lorry (16-32 metric ton)	km kg	$2.72E^{2}$	$1.53E^{0}$
Electricity (air supply)	KWh/kg	$1.34E^{0}$	$1.56E^{0}$
Electricity (water supply)	KWh/kg	-	$1.17E^{0}$
Water	m^3/kg	$7.76E^{0}$	$1..12E^{1}$
Output			
Emission to water			
Nitrogen, total	g/kg	$3.2E^{1}$	$9.02E^{1}$
Phosphorus, total	g/kg	$1.99E^{-2}$	$1.61E^{1}$
Organic carbon	g/kg	$6.50E^{-2}$	$2.45E^{2}$
Emission to air	g/kg	$1.25E^{0}$	$2.89E^{1}$

4.3 Results

The contribution of all inputs and production processes in the IMTA and monoculture systems of abalone and seaweed to overall environmental impact categories such as abiotic depletion; fossil fuel depletion; ocean acidification potential; global warming potential; marine aquatic ecotoxicity; ozone layer depletion; terrestrial ecotoxicity were analysed and compared between the two systems.

4.3.2 Comparison of IMTA and monoculture abalone culture systems

For both abalone culture systems (IMTA and monoculture), the highest contributor to all assessed impact categories was the direct energy input for air and water supply to the production tanks. This was followed by the contribution from formulated feed application and the seaweed (Figure 4.3a, b). The contribution by direct energy supply to all impact categories varied from 55-86% in the IMTA and ca. 38-76% in monoculture tanks. The formulated feed diet fed to the abalone contributed 11-40% and 15.4-35% to the impact categories in the IMTA and monoculture systems respectively. The fresh *Ulva* fed to the abalone contributed between 5.2-10.8% to analysed impact categories in the IMTA and 4.3-25% in the monoculture system. The contribution by the production process of fed *Gracilaria* was very low ranging from 0.08-1.9 for the IMTA and 0.05-2.2% for the monoculture abalone (Figure 4.3a, b).



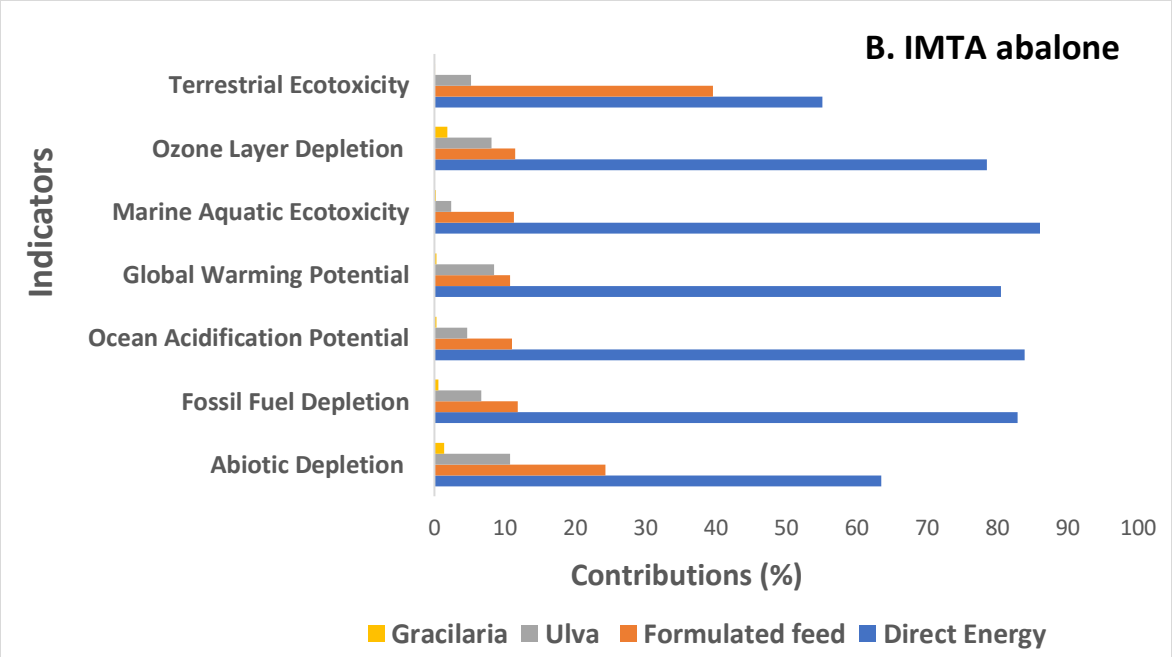


Figure 4.3. Contribution analysis for the different inputs to (A) abalone monoculture, (B) abalone Integrated culture raceways. Impact categories and relative contributions (%) for the two production systems were aggregated in macro-categories such as Abiotic Depletion; Fossil Fuels Depletion; Ocean Acidification Potential; Global Warming Potential; Marine aquatic ecotoxicity; Ozone layer depletion; Terrestrial ecotoxicity.

Impact comparison

There were variations between the monoculture and IMTA abalone production units with the IMTA abalone (> 30 g) culture system with its higher abalone biomass having higher impacts on the environment than the more lightly stocked monoculture abalone units (< 30 g) (Table 4.3 and Figure 4.4). The results also revealed that the highest and the most substantial impact produced by both production systems was marine aquatic ecotoxicity being 33090 and 2070 kg 1,4-DB eq for IMTA and monoculture system respectively (Figure 4.4). Other characterisation categories were very low.

Table 4.3. Comparison of abalone culture in monoculture and IMTA production systems using the CML-IA impact method.

.	Monoculture abalone	IMTA abalone	Unit
Abiotic depletion	0.00	0.00	kg Sb eq
Abiotic depletion (fossil fuels)	10.40	15.00	MJ
Ocean acidification	0.01	0.02	kg SO_2 eq
Eutrophication	0.05	0.05	kg PO4--- eq
Global warming (GWP100a)	1.57	2.16	kg CO2 eq
Human toxicity	0.60	0.86	kg 1,4-DB eq
Marine aquatic ecotoxicity	2070.00	3090.00	kg 1,4-DB eq
Ozone layer depletion (ODP)	0.00	0.00	kg CFC-11 eq
Photochemical oxidation	0.00	0.00	kg C2H4 eq
Terrestrial ecotoxicity	0.03	0.02	kg 1,4-DB eq

The relative contributions of the IMTA and monoculture production systems to the different impact indicators were compared (Figure 4.4). Depending on the impact category tested, the impact of the abalone in the IMTA system with its higher stocking biomass was 7-33% higher than the monoculture system. Impact categories such as marine water eutrophication, abiotic depletion, global warming potential and marine aquatic ecotoxicity were 7%, 19%, 27% and 33% higher for IMTA abalone system than the monoculture abalone system (Figure 4.4).

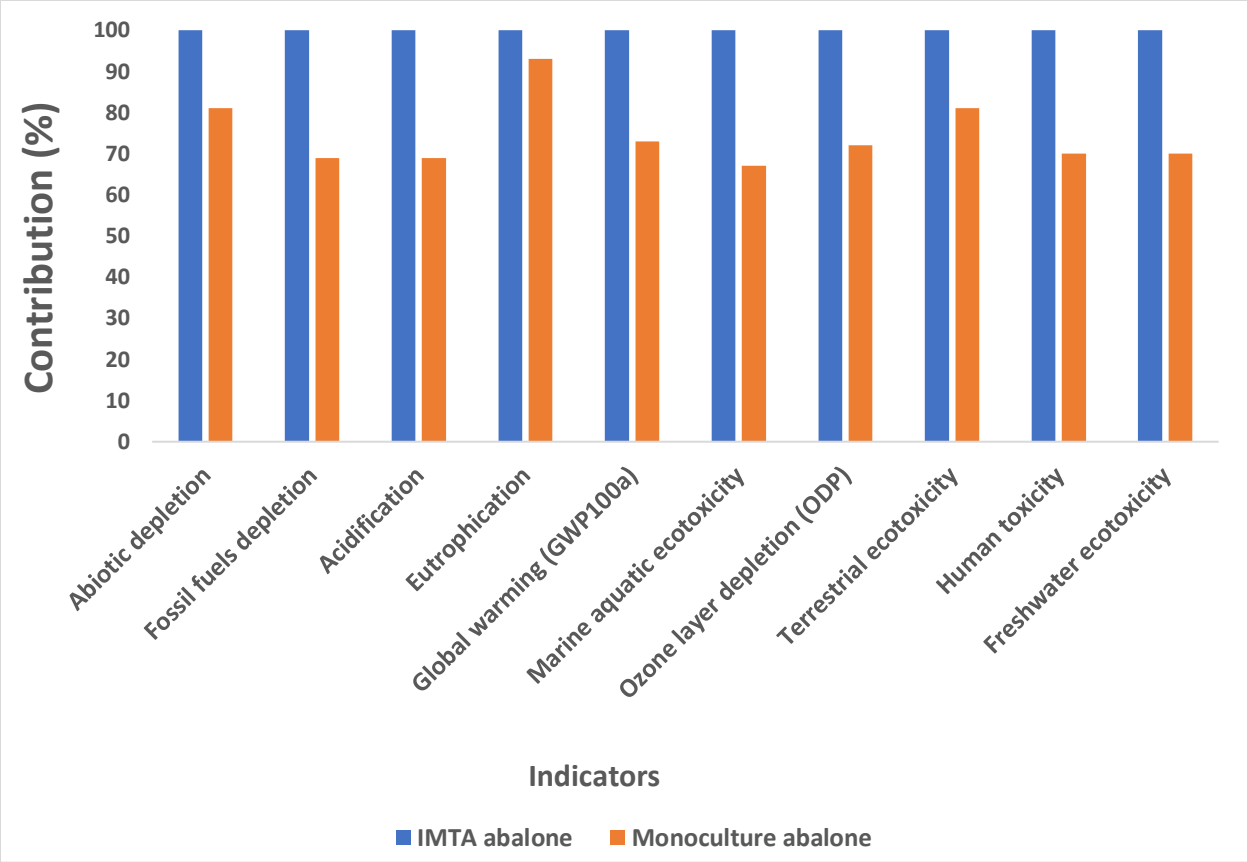


Figure 4.4. Comparison of relative impacts of the IMTA and Monoculture abalone systems for selected impact indicators using CML-IA baseline calculation method.

4.3.1 Comparison of IMTA and monoculture Ulva culture systems

In the seaweed culture systems, the direct energy for water and air supply in the paddlewheel raceways made the most significant contribution to all analysed impact categories in the two production systems (Figure 4.5a, b). This was followed by the impact from the different mineral fertilisers (urea, monoammonium phosphate and monopotassium phosphate) input. Depending on the impact category, energy consumption by seaweed production system contributed approximately 80-98% and 75-98% to environmental impact in the monoculture and IMTA *Ulva* systems respectively. The contributions by fertiliser application to impact categories ranged

between 1.4-20% and 1.8-24.5% in the IMTA and monoculture systems respectively (Figure 4.5a, b).

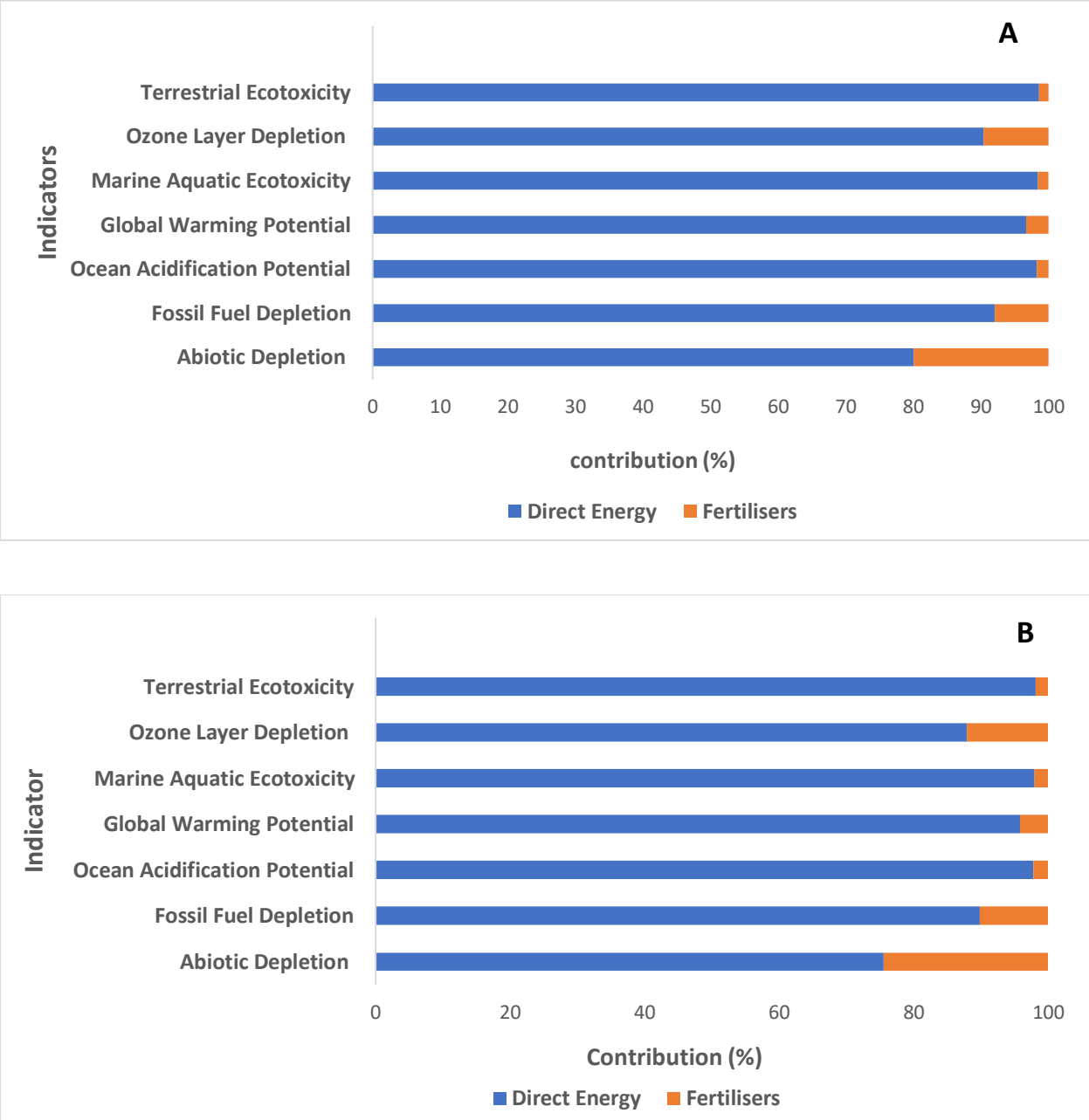


Figure 4.5. Contribution analysis for (A) *Ulva* monoculture, (B) *Ulva* IMTA. Impact categories and relative contributions (%) for the two production systems were aggregated in macro-categories. Abiotic Depletion; Fossil Fuels Depletion; Ocean Acidification Potential; Global Warming Potential; Marine aquatic ecotoxicity; Ozone layer depletion; Terrestrial ecotoxicity.

Impact comparison

The impact of IMTA *Ulva* production system at WCA on the environment was considerably lower than the monoculture system (Table 4.4). The most significant effect of both production systems on the environment was marine aquatic ecotoxicity, being 4430 and 2110 kg 1,4-DB eq. for monoculture and IMTA system respectively (Table 4.4).

Table 4.4. Comparison of abalone culture in monoculture and IMTA production systems using the CML-IA impact method.

Indicator	Monoculture *Ulva*	IMTA *Ulva*	Unit
Abiotic depletion	0.00	0.00	kg Sb eq
Abiotic depletion (fossil fuels)	22.20	10.80	MJ
Ocean Acidification	0.02	0.01	kg SO_2 eq
Eutrophication	0.08	0.08	kg PO_4 eq
Global warming (GWP100a)	2.94	1.41	kg CO_2 eq
Human toxicity	1.20	0.58	kg 1,4-DB eq
Marine aquatic ecotoxicity	4430.00	2110.00	kg 1,4-DB eq
Ozone layer depletion (ODP)	0.00	0.00	kg CFC-11 eq
Photochemical oxidation	0.00	0.00	kg C_2H_4 eq
Terrestrial ecotoxicity	0.02	0.01	kg 1,4-DB eq

*IMTA: Integrated multitrophic aquaculture system

The relative contributions of the production systems to the different impact indicators were compared (Fig. 4.6). The contribution of IMTA *Ulva* production system to abiotic depletion, fossil fuel depletion, ocean acidification, global warming potential, marine aquatic ecotoxicity, ozone

layer depletion, terrestrial ecotoxicity was 48-52% lower than the monoculture system while eutrophication potential was 4% lower (Figure 4.6).

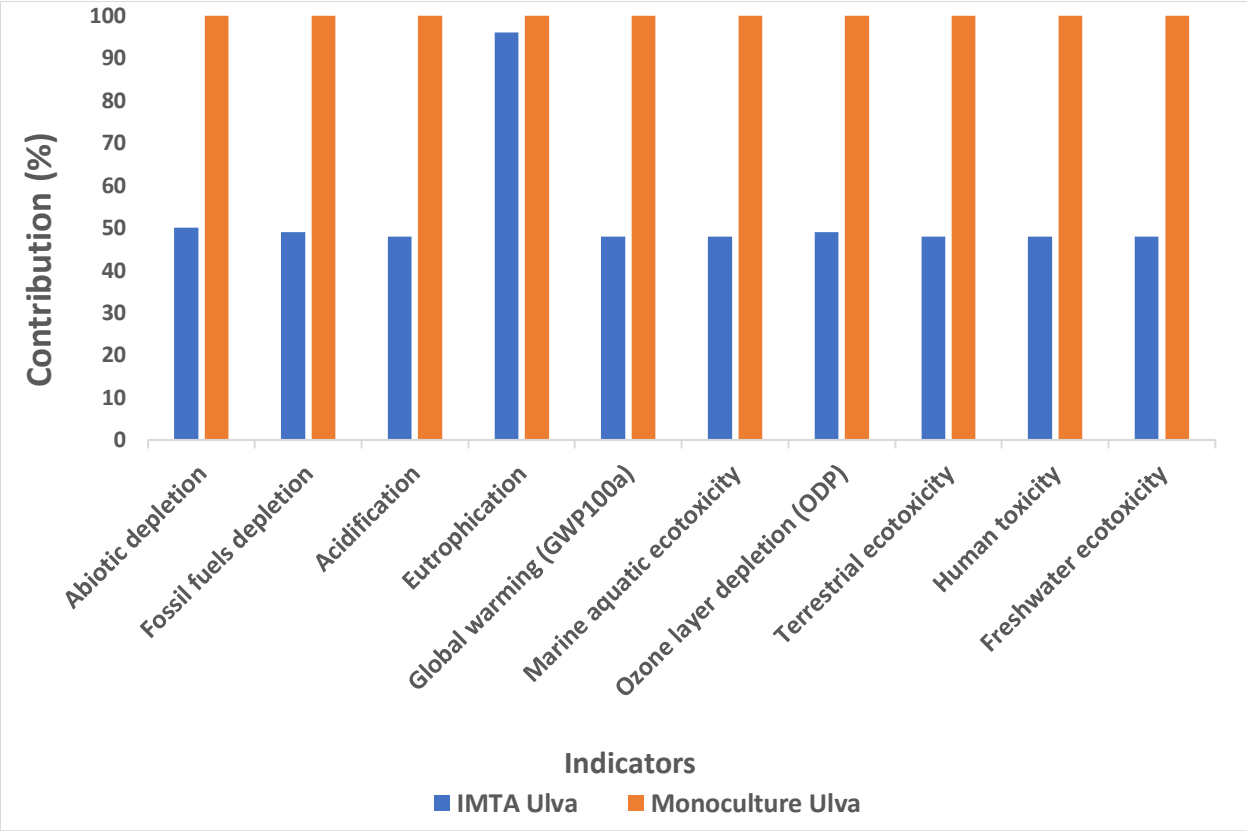


Figure 4.6. Comparison of relative impacts of the IMTA and Monoculture *Ulva* systems for selected impact indicators using CML-IA baseline calculation method.

4.4 Discussion

A life cycle assessment was conducted in this study to compare the two techniques of growing abalone and seaweed on a commercial South African abalone farm. This was carried out to establish the main environmental burdens associated with the two production systems

(integrated culture and monoculture) at the Wild Coast abalone by assessing their impacts on coastal environment.

From the contribution analysis, the direct energy supplied to both abalone and seaweed production tanks (for air and water supply) constituted the highest contribution (hotspot) to all the impact categories analysed for the two production systems. This was due to Wild Coast Abalone operating a continuous pump-ashore flow-through production technique where seawater is pumped onshore into land-based production tanks situated at 8 m static height above sea level. This hourly pumping of 6250 m^3 of seawater resulted in 647-655 kw/h energy consumption by the farm. Apart from this, a substantial amount of electricity was required to power the water filtration systems, water chillers, high-pressure pump for tank cleaning, and seaweed tanks paddlewheel motors. This resulted in a high monthly farm expenditure of about 1.5 million South African Rand on electrical energy, constituting 65% of the farm production costs (*Richard Clark, WCA Director, pers com.*). The impact by electricity in this study was highly influenced by the South African energy mix as 84.4% of electricity generated in South Africa is from coal-fired production plants which are characterised by a high environmental impact (PRé sustainability 2020). This measured impact is similar to intensive aquaculture systems such as aquaponics where energy consumption constitutes a great environmental impact (Cohen *et al.* 2018). Previous studies of integrated aquaculture production systems show that the major environmental impacts are electrical energy consumption, nutrients and infrastructure (Forchino *et al.* 2017). Direct energy consumption contributes more than 50% to abiotic depletion potential, global warming potential and the cumulative energy demand in aquaponic systems (Aubin *et al.* 2006; Forchino *et al.* 2017; Maucieri *et al.* 2018; Ghamkhar *et al.* 2021). However,

the energy consumption in commercial South African abalone farms is about ten times higher than that required in aquaponics. Considering the effect of direct energy utilisation by abalone farms on the characterised impact categories, it is thus imperative that *H. midae* farms source for alternative forms of energy such as renewable energies to decrease their environmental footprint and improve economic performance. Some land-based abalone farms in the Western cape region of South Africa now generate 70% of their energy from solar-powered systems (*Matt Naylor, HIK Pty Ltd., pers. Comm.*). The Wild Coast Abalone has advanced plans to install a 3MW wind farm to generate a major portion of its direct energy requirements which will reduce its environmental impact.

In the present study, formulated diets (Abfeed® S24 and E26) fed to abalone constituted the second highest environmental burden (hotspot) in abalone tanks of both production systems, followed by the impact from the seaweeds production processes. Additionally, the contribution to environmental impact from artificial feed and seaweed was higher for the monoculture system where abalone were fed 34% protein diet (Abfeed® S34) supplemented with seawater-grown *Ulva* than the IMTA abalone fed 26% CP diet (Abfeed® E26) and effluent-cultured *Ulva* seaweed. This resulted from the higher fish meal and fish oil inclusion in 34% CP diet than the 26% CP diet which had part of its animal protein substituted with terrestrial grain proteins, hence the high (ca. 40%) contribution of IMTA artificial feed (Abfeed® E26) to terrestrial ecotoxicity compared to the 15.7% contribution by Abfeed® S34 (Figure 4.2a-b). Apart from the low contribution of the 34% CP diet to terrestrial ecotoxicity, its contributions to other environmental indicators (abiotic depletion, fossil fuel depletion, ocean acidification potential, global warming potential, marine aquatic ecotoxicity, ozone layer depletion) were higher than the 26% protein diet. The high

impacts of both formulated feeds can be attributed to their production life cycle which is made up of series of fishing, farming and industrial processes to produce fish meal, fish oil, soya meal and cereal crop derived feed ingredients. For example, fish meal and oil production are associated with the combustion of fossil fuels noted for damaging biodiversity and ecosystem, thus contributing to global environmental impacts (Smetana *et al.* 2019). The high contribution of artificial feed to the environmental impacts in the current study agrees with previous research where fishmeal-based diets accounted as a major environmental burden in aquaculture production (Aubin *et al.* 2006; Cohen *et al.* 2018). Comparing the contributions of fed diets in IMTA and monoculture systems on analysed impact categories, it can be concluded that culturing abalone in IMTA system where the animals are fed low-fishmeal diets supplemented with effluent-cultivated seaweed is an efficient technique to reduce the environmental impact of abalone aquaculture. However, much more needs to be done to further reduce the degree of environmental burden by formulated feed utilisation in abalone aquaculture. This can be achieved by harnessing the potential of locally produced and sustainable animal and plant protein (e.g., insect and microalgae meal) as an alternative to fish. For instance, insect protein utilisation in aquafeed is characterised as a solution to the unsustainable inclusion of fishmeal in aquaculture diets due to their lower environmental consequences than fish proteins (Smetana *et al.* 2019; Quang *et al.* 2022).

The effluent cultured *Ulva* used as feed for abalone also contributed to the overall environmental impact emanating from both abalone production systems due to the high input of nutrients from mineral fertilisers and the electrical energy consumption by the production system during seaweed culture. About 3349 and 1913.76 kwh of electricity is consumed by each monoculture

and IMTA *Ulva* paddlewheel raceway respectively during the 30-day culture cycle at Wild Coast Abalone. In addition, approximately 71 and 42.3 kg of inorganic fertilisers are applied to monoculture and IMTA seaweed during each culture period thus contributing to the impact of recycled seaweed.

The higher stocking biomass, feed and energy inputs in IMTA abalone tanks accounted for their higher contributions to eutrophication potential, abiotic depletion, global warming potential and marine aquatic ecotoxicity than the monoculture abalone. However, utilising IMTA abalone's waste nitrogen and phosphorus for seaweed production reduced the footprint of this production system on the environment.

Contrary to the expectation, the life cycle assessment revealed the overall impact of fertilisers application to be relatively small with only 1.41–20% and 1.83–24% contributions to the impact categories in the IMTA and monoculture systems respectively.

While seaweed was cultured in continuously renewed clean seawater in the monoculture system, water dependence was substantially reduced in the IMTA system due to *Ulva* production in abalone effluent, thus reducing the footprint of the system on the environment. Additionally, this technique of culturing seaweed downstream in abalone effluent lowered direct energy requirement of the IMTA system as abalone wastewater flows by gravity into the seaweed raceways without requiring electrical energy input to supply effluents in the raceways. This is contrary to the monoculture system where approximately 1500 KWh electrical energy is required to move clean water from the sea into *Ulva* raceway per culture cycle.

The reduced energy and nutrients demand by IMTA seaweed production system lowered the impact of this system by 52% compared to the monoculture system. This suffice to say that culturing seaweeds in aquaculture effluent is an important avenue to offset the negative impact of dissolved aquaculture waste on the environment as the seaweeds utilise the waste nutrients for their growth. The eutrophication potential of IMTA *Ulva* culture systems (0.08 kg PO4 eq) could have been lower if they only received their phosphorus intake from impounded abalone effluent and not supplemented with excess phosphorus from monoammonium and monopotassium phosphate fertilisers.

4.5 Conclusion

Cultivating seaweed in abalone effluent using Integrated Multi-Trophic Aquaculture (IMTA) significantly reduced the environmental impact of abalone and seaweed farming. This is attributed to the efficient use of resources, reduced water consumption and fewer nutrient emissions in the IMTA system. This points to better long-term environmental sustainability of integrated culture when compared with conventional monoculture system. However, in land-based commercial abalone farms like the wild coast abalone, energy consumption contributes significantly to the environmental burdens of abalone and seaweed farming. Therefore, much needs to be done to reduce the environmental impact emanating from electrical energy consumption. One important way of reducing the impacts related to direct energy utilisation is to harness the potential of renewable energies such as wind and solar as substitute for the current hard coal-generated energy mix. In this way, the electrical energy demand for air and water supply on the farm could be drastically reduced.

Additionally, reducing the input of nutrients from mineral fertilisers will further reduce the impacts of seaweed production on the environment. The life cycle assessment indicated a substantial impact of nitrogen and phosphorus-based chemical fertilisers utilisation on eutrophication and marine aquatic ecotoxicity. Therefore, substituting inorganic fertilisers with biodegradable, non-toxic and ecofriendly biofertilisers could reduce the footprint of the farm on the environment.

Chapter 5: Evaluation of IMTA and monoculture produced *Ulva* meal in a commercial feed pellet for *Haliotis midae*

5.1 Introduction

On many South African abalone farms, seaweeds are cultured as secondary crops in nutrient-rich effluents flowing from abalone tanks located upstream. This integrated culture technique (IMTA) enhances the nutritional profile of farmed seaweeds and reduces dissolved waste nutrients discharged from shore-based abalone production systems (Troell *et al.* 2006; Bolton *et al.* 2009; Robertson-Anderson *et al.* 2011). Farmed seaweeds have been considered as an excellent non-conventional ingredient in artificial feed for abalone due to their strong potential for growth under high nitrogen conditions, resulting in a high tissue protein and amino acid composition (Bansemer et al. 2016a; Santizo-Taan *et al.* 2020). However, the potential to use IMTA cultured *Ulva* as a meal in commercially formulated feeds for abalone has not been evaluated. If cultured *Ulva* meal could be included in formulated feeds, it could increase the contribution of abalone farm IMTA systems to more efficient abalone production and reduce the environment impact of abalone farming by reducing the levels of fishmeal and soya in the current commercial formulations. IMTA cultured *Ulva* meal was thus tested in formulated feeds in a series of feeding trails at the Wild Coast Abalone Farm (Chapter 5 and 6 of the present thesis).

The supplementation of compound feed pellets with seaweeds and seaweed meal has been documented to improve the growth, nutrient utilisation, and health of farmed H*aliotids* (Naidoo *et al.* 2006; Stone *et al.* 2014; Bansemer *et al.* 2015a; Nel *et al.* 2017; Kemp 2018; Ansary *et al.* 2019; Santizo-Taan *et al.* 2020). While one commercially produced compound feed supplemented with 5% seaweed (*Ecklonia maxima*) meal is currently used on South African

abalone farms, the inclusion of other macroalgae meals, such as *Ulva species* in the diet of *Haliotis midae* remains untested.

Macroalgae are an excellent source of biologically active compounds such as polysaccharides, proteins, pigments, and polyphenols, which have antimicrobial, antiviral and antioxidant properties (Chojnacka *et al.* 2012; Bansemer *et al.* 2016a). The polysaccharides contained in macroalgae are prebiotic as they stimulate the production of growth-promoting and health-enhancing bacteria in the digestive tract (Chojnacka *et al.* 2012). The inclusion of the kelp (*Ecklonia maxima*) in the diet of sub-adult South African abalone (*H. midae*) promoted their gut-bacteria homeostasis resulting in faster growth and improved feed and protein utilisation efficiencies (Nel *et al.* 2017). Macroalgae are also known for their feeding stimulation and disease prevention benefits in abalone (Lange *et al.* 2014; Stone *et al.* 2014). The Blackfoot abalone, *Haliotis iris* fed *Gracilaria species* stimulant diet spent 80% of their time feeding with 15% growth improvement above those fed diet without algal meal inclusion (Allen *et al.* 2006).

Ulva seaweed is an excellent source of growth-enhancing nutrients (Garcia-Casal *et al.* 2007; Abdel-Warith *et al.* 2016), and it contains all dietary essential amino acids (Wong & Cheung 2000). Its inclusion effect in the artificial diet of abalone has been reported for the Donkey's hair abalone, *Haliotis asinina* (Santizo-Taan *et al.* 2020); Japanese abalone, *Haliotis discus hannai* (Ansary *et al.* 2019), and the greenlip abalone, *H. laevigata* (Bates *et al.* 2017; Bansemer *et al.* 2016b). Its partial substitution for fish and soybean meal in the diet of *H. asinina* increased feed acceptance with growth rate, shell length, feed conversion and protein efficiency ratio of the animals not compromised even as inclusion increased to 30% (Santizo-Taan *et al.* 2020). Similarly, the 5-20% replacement of soybean meal, dehulled lupins, and wheat flour with *Ulva* protein

extract in the diet of juvenile greenlip abalone, *H. laevigata* did not compromise their growth and nutrient utilisation (Bates *et al.* 2017). Additionally, the 5-20% inclusion of *Ulva* in the diet of the greenlip abalone upregulated trypsin activities which improved feeding stimulation and caused higher growth in weight and shell length of *H. laevigata* (Bansemer *et al.* 2016b). This feeding stimulation and growth improvement in other abalone species suggest that it might be beneficial to supplement formulated feed for South African abalone with *Ulva species* meal.

The South African abalone culture industry is based on a single species of abalone, *Haliotis midae*, cultured in shore-based, flowthrough tanks and fed compound formulated feed, live macroalgae (*Ulva*, Kelp & *Gracilaria*) or a combination of pellet and macroalgae. The fresh *Ulva* is grown in abalone effluent channeled into paddlewheel raceways located downstream. However, for biosecurity reasons, some farms also culture a portion of their seaweed (*Ulva* and *Gracilaria*) in fresh seawater enriched with nutrients from mineral fertilisers. Previous research that investigated the use of seaweeds in the diet of *H. midae* have predominantly been conducted with live macroalgae (Naidoo *et al.* 2006; Dlaza *et al.* 2008; Robertson-Andersson *et al*. 2011; Kemp 2018). A few studies have also included dried *Spirulina species* and *Ecklonia maxima* as part of the ingredient for compound feed formulation for *H. midae* (Britz 1996; Shipton and Britz; 2001; Ismail *et al*. 2009; Nel *et al.* 2017). No previous investigation has reported on the inclusion effect of farmed *Ulva* meal in the diet of *H. midae*.

It was hypothesised that the partly replacement of fish and soybean meal with farmed (effluent or seawater-grown) *Ulva* meal in artificial diet of *H. midae* would not compromise their growth and nutrient utilisation, thus serving to optimise farm production efficiency. This initial study, therefore, examined the effect of 12% *Ulva* IMTA and monoculture *Ulva lacinulata* meal inclusion

in commercially formulated diet on the performance of farmed *H. midae*. The performance of the abalone fed the experimental formulated diets was compared with the standard farm diet of the commercial pellet supplemented with fresh cultured *Ulva.* In a follow-up study reported in Chapter 6, graded levels of *Ulva* meal were fed in formulated diets to determine an optimal level of *Ulva* meal inclusion.

5.2 Materials and methods

5.2.1 Macroalgae meal and experimental diets production

Ulva meal production

Cultured *Ulva* from the IMTA and monoculture paddlewheel raceways was harvested, rinsed in freshwater to remove unwanted materials and visible epiphytes and transported to an on-site drying factory. *The Ulva* was dried onsite at 40 °C in an electric dryer until a constant weight was obtained. Afterward, they were carefully packed in their respective boxes, sealed, and couriered to Marifeed Pty Ltd, Hermanus, South Africa, for experimental diet manufacture.

Experimental diets formulation and pelleting.

All diets were formulated and manufactured to experimental specifications by Marifeed (Pty) Ltd. Three isoenergetic (15.9 kJ/kg) and isonitrogenous (34% protein) practical diets supplemented with 0% (Abfeed-S34 plain/basal), 12% IMTA *Ulva* meal (Abfeed®-S34 IMTA) and 12% monoculture *Ulva* meal (Abfeed®-S34 non-IMTA) were formulated (Table 5.1). The ingredients used to make the Abfeed®-S34 basal diet for the "S34-Ulva 12" and "Control" treatments were

adjusted to ensure that total protein, total lipid, and total lysine remained constant in the formulation of all diets. Abfeed®-S34 is a commercial abalone diet produced by Marifeed Pty Ltd (Hermanus, South Africa), the formulation of which remains proprietary. The main protein sources in the basal diet were fishmeal and soya, and the total volume of both ingredients was adjusted between diets to ensure that total protein remained constant; however, the ratio of fishmeal to soya was maintained between all diets. The total lipid content was also maintained, and this was done with the addition of fish oil to the basal diet (Table 5.1). The starch in Abfeed®-S34 basal diet includes terrestrial grains, and this detail remains proprietary. The diets also consisted of a standard inclusion of vitamin mix (also standardized between treatments), the content of which remains proprietary. The diets were analysed for their composition before the trial commenced with the analysis carried out at the ARC-Irene Agricultural research laboratory (SANAS accredited), Irene, South Africa. All analyses followed the standard methods of food analysis by the Association of Official Analytical Chemists (AOAC 1996).

Table 5.1. The target formulation (i.e., isonitrogenous and isoenergetic experimental diets) and the proximate composition of the with 0% and 12% *Ulva* meal inclusion used for the growth trial with farmed adult South African Abalone (*Haliotis midae*).

	Abfeed®-S34 Control	S34-Ulva 12 Non-IMTA	S34-Ulva 12 IMTA
Target formulation (dry weight basis)			
Ulva meal non-IMTA (%)	-	12	-
Ulva meal IMTA (%)	-	-	12
Abfeed®-S34 basal diet	100.0	88.0	88.0
Total	100.0	100.0	100.0
Total protein (%)	34.0	34.0	34.0
Lysine (%, g lysine/100 g diet)	2.3	2.3	2.3
Total lipid (%)	4.6	4.6	4.6
Energy (kJ/kg)	16.2	15.9	15.9
Proximate composition (dry weight basis)			
Total protein (%)	33.89	33.72	33.83
Total lipid (%)	4.60	4.47	4.49
Energy (kJ/kg)	19.48	18.99	19.03

IMTA *Ulva*; protein: 33.29; lipid: 3.63; energy: 15.13 k/kg. Monoculture *Ulva*; protein: 31.82; lipid: 2.50; dry matter: 90.82; energy: 16.19 k/kg

[a] Proximate compositions were analysed using standard methods – Crude protein: Duma's combustion method, lipid: AOAC 996.06 (gas chromatography).

5.2.2 Experimental culture system

The feeding trial was conducted at WCA for eight months, from August 2020 to April 2021, using standard farm production materials and procedures. Moderately aerated and drum-filtered seawater was gravity-fed into abalone grow-out concrete tanks (length x breadth x height; 5 500 mm × 1300 mm × 550 mm, 1200 L) in a single pass and continuous flow-through system at a flow

rate of 75.80 ± 0.53 L min^{-1} to allow for seven exchanges per day. Each abalone tank contained fifteen polyethylene abalone surface suspended culture baskets (57 cm × 96 cm × 50 cm; length x breadth x height). The basket was subdivided by five vertically oriented high-density acrylonitrile butadiene styrene (ABS) plates (L × B × H: 760 mm × 4mm × 310 mm) to increase the surface area available for abalone attachment. The culture basket with a horizontal corrugated feeder plate (60 cm x 45 cm; length x breadth) was placed over vertical plates about 2 cm below the water surface. Each tank was completely drained, washed, scrubbed, and refilled with fresh seawater twice every week, following the farm's standard husbandry practice. During the washing of the tanks, water flow to each raceway was turned off, and the holding baskets shifted to newly prepared raceways. The photoperiod of the abalone farm followed the natural period.

Seawater temperature (17.55 ± 0.32 °C) was monitored daily for the period of the growth trial using a temperature logger (Ebro, model EBI 300, serial number 73268609 by Xylem Analytics, Germany) while the pH (7.7 – 8.3) and dissolved oxygen (7.7 – 8.8 mg L^{-1}) of abalone raceway tanks were monitored every week at 08h00 with pH meter (Thermo Scientific Eutech Expert; serial no. 2913115, Singapore) and Oxyguard DO meter (handy Polaris; model v. 2.51, serial no. 015BODE0140000 by Oxyguard International A/S, Denmark) respectively. Also, ammonia nitrogen (0.06 – 0.25 mg L^{-1}), nitrite nitrogen (0.00 – 0.04 mg L^{-1}) and nitrate nitrogen (0.30 – 0.65 mg L^{-1}) concentrations were periodically monitored using Palintest automatic wavelength photometer (model 7100, Palintest Ltd, England) and test kits (AP152, AP163, AP184).

5.2.4 Experimental animals and stocking

Abalone used for this trial were hatchery-bred *H. midae* (of the same cohort) that were fed a combination diet of Standard Abfeed® S34pellets (34 % protein, 4.2 % lipid, Marifeed Pty Ltd., Hermanus, South Africa), and live macroalgae (*Ulva lactuca* and *Gracilaria gracilis*) for six months prior to the experiment. The experimental abalone (65-75g) were stocked in 75 baskets, and each basket housed about 160 – 170 abalone (66.28 ± 0.48 g ablone^{-1}) to attain the target production basket biomass of 12500 ± 0.72 g/basket. After stocking, the animals were acclimated to the culture raceway tanks for fourteen days without feeding. After acclimation and purging, 30 representative abalones from each basket were carefully removed using a flat, blunt metallic spoon, blotted dry, weighed to 0.01 g accuracy and their individual shell length measured to the nearest 0.01 mm with a digital caliper. The animals were returned to their respective baskets after the baseline measurements.

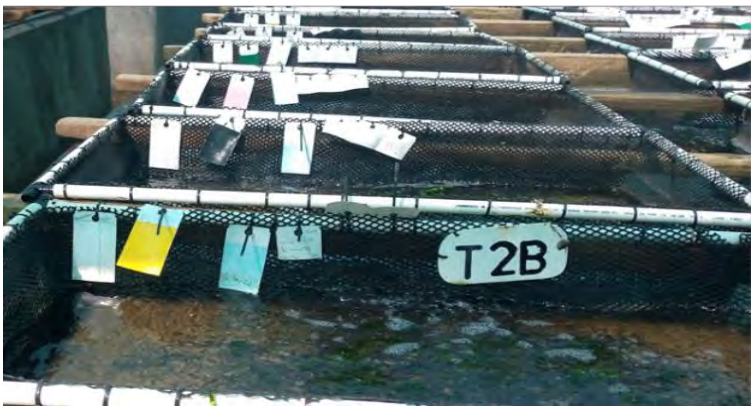


Figure 5.1. Abalone holding baskets with colour tags differentiating each experimental treatment.

5.2.5 Experimental design and treatments

The 75 abalone baskets for the trial were distributed in randomized block design in the five tanks prepared for the experiment, such that each tank contained at least three replicate baskets per treatment, so that each dietary treatment was represented in the five experimental tanks. Five dietary treatments were tested in this study (Table 5.2) with each treatment replicated five times (n=5).

Table 5.2. Experimental treatments under study (formulation in Table 5.1).

Treatment	Diet type	Description
1	Abfeed®-S34 Control	Basal 34% CP diet with 0% *Ulva* meal inclusion
2	S34-*Ulva* 12 IMTA	Abfeed® S34 pellet supplemented with 12% effluent-grown *Ulva* meal
3	S34-*Ulva* 12 Non-IMTA	Abfeed® S34 pellet supplemented with 12% Ulva meal cultured in clean seawater (monoculture *Ulva*)
4	Combination diet 1	Standard farm diet of commercial Abfeed® S34 plain pellet with fresh IMTA *Ulva* and fresh *Gracilaria*
5	Combination diet 2	Standard farm diet of commercial Abfeed® S34 pellet with fresh monoculture *Ulva* and fresh *Gracilaria*

Diet 4 and 5 were the feeding protocols employed at WCA at the time of this research. Hence, they were not part of the controlled experimental formulations but were included as reference

treatments which are referred to 'combination diets' in this study. Throughout the feeding trial, abalone were daily fed on demand with their pellets between 1600 and 1800 h. In addition to feeding pellets, the farm's feeding protocol treatments received supplements of fresh seaweed three times every week. These two treatments were fed pellet (64%) and *Ulva* (36%) on days 1 and 5 of the week, while *Gracilaria* (17%) and pellet (83%) were given every third day of each week as this simulated the standard feeding practice on the farm (Table 5.3). On days, when the reference treatments were fed combination diet of algae and pellets, each basket of abalone first received pellets, and after one hour, they were fed seaweeds after they (algae) have been drained of water for 30 mins and weighed.

Table 5.3. Feeding regimes for the five dietary treatments investigated in this study.

	Control	S34-Ulva IMTA	S34-Ulva Non-IMTA	Combination diet 1	Combination diet 2
Formulated feed					
Pellet's introduction to basket	days 1-7	days 1-7	days 1-7	days 1-7	days 1-7
Fresh macroalgae					
Ulva spp	n/a	n/a	n/a	days 1 & 5	days 1 & 5
Gracilaria gracilis	n/a	n/a	n/a	day 3	day 3

*n/a: not applicable

5.2.6 Abalone growth measurements and data collection

The feeding trial was carried out for 244 days, and the animals were weighed three times during this period. Data collection was carried out at the start of the experiment in August 2020 (after acclimation), 122 days into the trial when the stocking density of each basket was reduced to the initial biomass (termed splitting), and at the end of the trial. During splitting, abalone was randomly removed from each basket to achieve the target stocking density of 12.5 kg abalone per basket. The biomass weight gain of *H. midae* for each basket was recorded on an electronic scale after allowing the baskets to drain excess water for 15 minutes. To establish individual abalone weight and size variation, representative abalone samples (n=30) were randomly selected from each basket with their weight established on electronic balance (0.001g). In addition, the shell length of abalone for each basket (n=30) was measured along the longest axis of the shell and recorded to the nearest 0.01 mm using a digital Vernier caliper (SHA 1920, OMNI-TECH).

The specific growth rate (SGR; % body weight d^{-1}) was computed with the equation:

$$SGR = ([\ln (W_f) - \ln (W_i)] /t) \times 100 \text{ (Britz 1996)} \qquad \text{Equation 5.1}$$

where W_f, W_i represents final weight and initial weight of abalone, and t represents the number of days the animals were fed.

The condition factors (CFs), an index to account for the relationship existing between individual abalone's weight and the shell length were determined using the equation of Britz (1996):

$$CF = [\text{body weight} / \text{shell lenght}^{2.99}] \times 5\ 575 \qquad \text{Equation 5.2}$$

Apparent feed conversion ratio (FCR) was computed using the equation of Naylor *et al.* (2011):

FCR = [dry weight of feed fed ⁄ biomass weight gain per basket of abalone] Equation 5.3

The FCR was not corrected for leaching and uneaten feed loss as the design of abalone basket and the standard farm husbandry practice does not allow the collection of uneaten feed. However, animals were only fed on demand throughout the experiment, significantly minimising leftover feed from the previous feeding.

The Apparent Protein Efficiency Ratio (PER) was calculated according to Nel *et al.* (2017):

PER = [abalone biomass gain/ grams feed protein applied] Equation 5.4

Apparent energy efficiency ratio (EER) was calculated with Bansemer *et al.* (2016a):

EER = abalone weight gain / energy consumed Equation 5.5

5.3 Statistical analyses

All **s**tatistical analyses were performed using STATISTICA software version 13.5 (Statsoft, Tulsa, Oklahoma). Treatments were compared using a one-way analysis of variance (ANOVA). The assumptions of homogeneity of variances and normal distribution of the residuals were tested using the Levene's and Shapiro-Wilk tests, respectively. Where the assumptions of these parametric tests were violated and the data transformation failed to address this, then the non-parametric Kruskal-Wallis test was performed as an alternative test to the ANOVA. A significant level of $p < 0.05$ was used for all statistical tests. Tukey's multiple range post hoc analysis was

employed if a significant difference was detected in the data tested. The results are presented as mean ± standard error.

5.4 Results

5.4.1 *Feed consumption and nutritional indices*

Abalone fed the two pellet treatments with *Ulva* meal inclusion consumed significantly ($P < 0.05$) more feed than the control and the reference abalone fed combination diets of pellets and live macroalgae (Kruskal–Wallis ANOVA: $H_{(4, 25)} = 18.033$; $p = 0.001$; Figure 5.2). However, the apparent feed conversion efficiency of abalone fed the two pelleted diets with *Ulva* mean inclusion (IMTA and monoculture) was significantly poorer than that of the control (Abfeed S34 basal diet) and the reference treatments fed the combination diets (One-way ANOVA $F_{(4, 20)} = 5.716$; $p = 0.003$; Table 5.4).

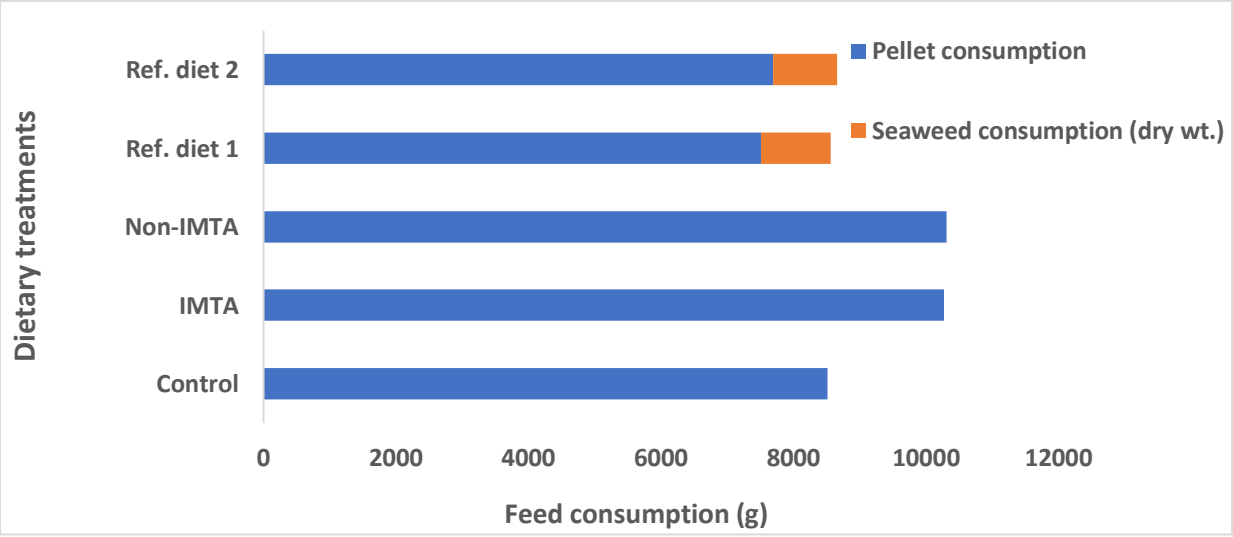


Figure 5.2: Total feed consumption by abalone fed the different feed types over the period of the growth study.

The Protein efficiency ratio (PER) followed a similar trend to the FCR as the dietary protein utilisation by the control and reference groups (combination diets) of abalone were significantly better than the abalone fed 12% IMTA and monoculture *Ulva* meal inclusion diets (one- way ANOVA: $F_{(4, 20)} = 13.147$; $p < 0.001$; Table 5.4). The energy efficiency ratio (EER) of *H. midae* also varied across the treatments with the highest recorded for abalone fed the combination diets (i.e., reference group), followed by the control (basal treatment) and the Ulva meal treatments. The *H. midae* fed 0% and 12% *Ulva* meal diets recorded similar EER (one- way ANOVA: $F_{(4, 20)} = 23.349$; $p < 0.001$; Table 5.4).

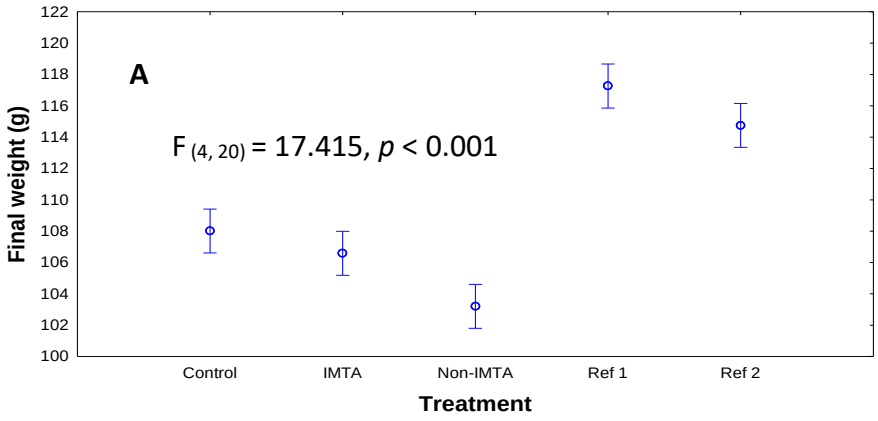
Table 5.4. Nutrient utilisation indices for *H. midae* reared for 244 days on Standard Abfeed® S34 plain (Control), S34 + 12% IMTA *Ulva* meal, S34 + monoculture *Ulva* meal, Abfeed® S34 plain + fresh IMTA *Ulva* + fresh *Gracilaria* (combination diet 1) and Abfeed® S34 plain + fresh monoculture *Ulva* + fresh *Gracilaria* (combination diet 2). Values reported are the treatment means of five replicates and standard error. Values with similar superscripts on the same column are not significantly different (p > 0.05).

	Diet					
	AbfeedS34 (Control)	S34- *Ulva*12 IMTA	S34- *Ulva*12 Non-IMTA	Combination diet 1	combination diet 2	P value
FCR	1.08 ± 0.04 ^{bc}	1.54 ± 0.09 ^{ab}	1.81 ± 0.20 ^{a}	0.99 ± 0.05 ^{c}	1.02 ± 0.14 ^{c}	0.003
PER	2.74 ± 0.11 ^{ab}	1.94 ± 0.11 ^{bc}	1.68 ± 0.14 ^{c}	3.66 ± 0.48 ^{a}	3.55 ± 0.18 ^{a}	0.000

FCR = feed conversion ratio and PER = protein efficiency ratio

5.4.2 Growth responses of H. midae to the different diets

The abalone fed treatments with 12% *Ulva* meal in the dry pellets (IMTA and monoculture) grew at a similar rate with those fed Abfeed S34 basal diet (control treatment), while those fed the combination diets of pellets and live macroalgae (reference treatments 1 &2) grew significantly faster than the pellet only treatments (one-way ANOVA: $F_{(4, 20)} = 22.547$, $p < 0.001$; Figure 5.2). Likewise, *H. midae* fed combination diets recorded significantly higher final weight and shell length (one-way ANOVA: $F_{(4, 20)} = 17.415$, $p < 0.001$; Fig. 5.4, Figure 5.5). The condition factors of abalone fed the control and *Ulva* meal diets were also similar but lower than the reference treatments (Kruskal-Wallis ANOVA: $H_{(4, 25)} = 11.823$ $p = 0.019$; Table 5.5). Similar shell length increment was recorded for all dietary treatments over the period of the study (one-way ANOVA: $F_{(4, 20)} = 1.084$, $p = 0.391$; Table 5.5).



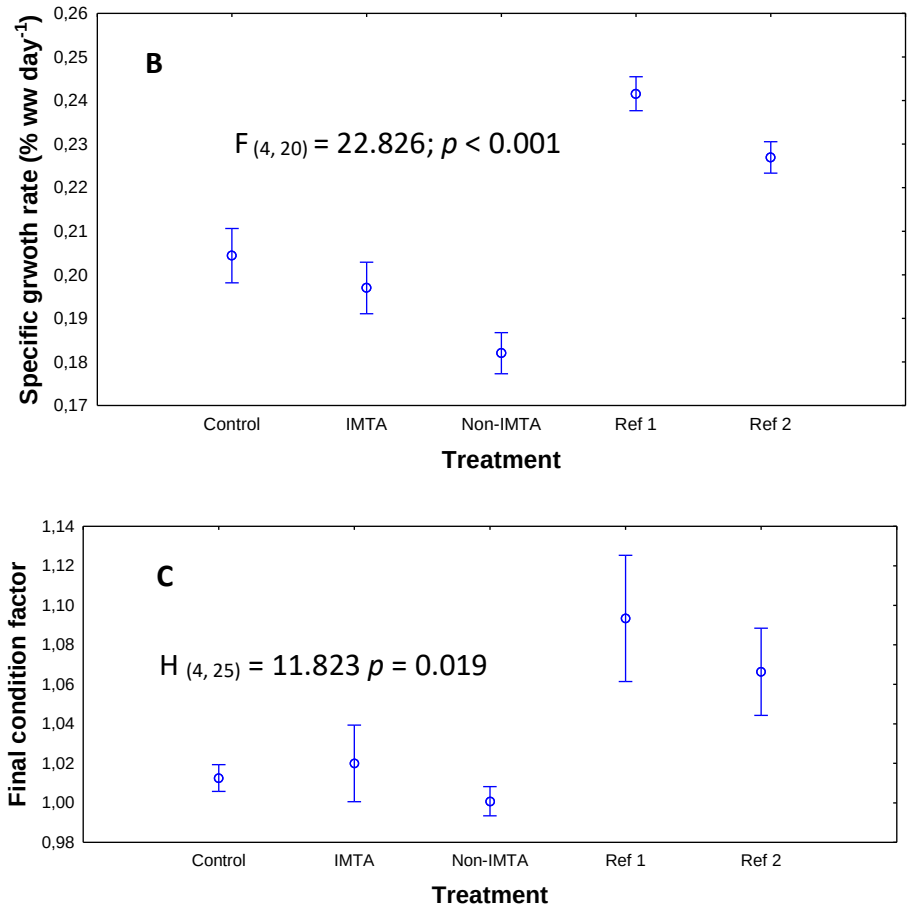


Figure 5.3. The mean (± 95% confidence interval) (A) final weight (B) daily increase in body weight (C) body weight gain (D) specific growth rate (E) final condition factor of *H. midae* fed different diets for 244 days.

*Control: Basal 34% CP diet with 0% *Ulva* meal inclusion.
IMTA: Abfeed® S34 pellet supplemented with 12% effluent-grown *Ulva* meal.
Non-IMTA: Abfeed® S34 pellet supplemented with 12% *Ulva* meal cultured in clean seawater.
Ref.1: Standard farm diet of commercial Abfeed® S34 plain pellet with fresh IMTA *Ulva* and fresh *Gracilaria*
Ref 2: Standard farm diet of commercial Abfeed® S34 pellet with fresh monoculture *Ulva* and fresh *Gracilaria*

Table 5.5. Somatic growth parameters of *H. midae* raised for 244 days on Standard Abfeed S34 plain (Control), S34 + 12% IMTA *Ulva* meal, S34 + non-IMTA *Ulva* meal, Abfeed S34 plain + fresh IMTA *Ulva* + *Gracilaria* (combination diet 1) and Abfeed S34 plain + fresh non-IMTA *Ulva* + *Gracilaria* (combination diet 2). Values reported are the treatment means of five replicates and standard error. Values with different superscripts on the same row are significantly different ($p < 0.05$).

	AbfeedS34 (Plain)	S34- *Ulva*12 IMTA	S34- *Ulva*12 Non-IMTA	Combination diet 1	Combination diet 2	P value
Initial shell length (mm)	69.73 ± 0.46	69.69 ± 0.45	70.35 ± 0.42	69.59 ± 0.84	70.12 ± 0.28	0.818
Final shell length (mm)	85.34 ± 0.56	84.79 ± 0.60	84.39 ± 0.39	85.56 ± 0.78	85.62 ± 0.29	0.465
MISL (mm/month)	2.02 ± 0.10	1.95 ± 0.85	1.81 ± 0.05	2.06 ± 0.12	2.00 ± 0.07	0.391

*MISL = Monthly increase in shell length

5.5 Discussion

While the experimental diets with 12% inclusion *Ulva* meal (IMTA and monoculture) had no statistically significant effect on the growth rate of the abalone and resulted in significantly higher feed consumption and poorer feed and protein conversion efficiencies compared to the control diet with no *Ulva* meal. This suggests that the abalone consumed more food to compensate for a lower nutrient availability and possible imbalances in the *Ulva* supplemented experimental diets. This result contrasts with other authors who reported good performance in abalone fed pelleted diets with seaweed meal inclusion. For example, the replacement of conventional feed ingredients with 20% *Ulva* meal in the diet of juvenile (1.82g) *H. laevigata* resulted in similar feed utilisation and growth rate with those fed diets without *Ulva* inclusion (Bates *et al.* 2017); and the inclusion of 7 % *Ulva lactuca* meal in a fish-based diet and 11% *Ulva sp.* meal in mixed

seaweed (*Ulva lactuca, Laminaria digitata*, and *Palmaria palmata*) diet for *Haliotis discus hannai* resulted in significantly better feed conversion than the control (0% seaweed), with no growth difference (Mahoney *et al.* 2014). Likewise, when fish and soybean meal were replaced with 10-30% nutrient-enriched *Ulva pertusa* meal in the diet of juvenile *H. asinine*, there were no significant differences in the body weight gain, shell growth rate, feed conversion, and protein efficiency ratio of the animals even at 30% inclusion of *Ulva* in the diet (Santizo-Taan *et al.* 2020). These contrasting results suggest that the ability of abalone to utilise macroalgae meal might be species (abalone and algae) specific or that the quality of the *Ulva* meal used in the present study was somehow deficient.

The performance of the abalone fed the two combination diets with fresh *Ulva* (reference treatment) was superior in terms of growth and efficient feed utilisation efficiency compared to the control and *Ulva* meal treatments highlighting the nutritional value of a fresh algal supplement. Previous nutrition studies on *H. midae* and *H. rufescens* also reported higher growth performance for abalone fed a combination diet of pellets and fresh macroalgae than abalone placed on a single diet of either pellets or seaweed (Naidoo *et al*. 2006; Dlaza *et al.* 2008; Hernandez *et al.* 2009; Kemp *et al.* 2015; Kemp 2018, Hernandez *et al.* 2009). The role played by live macroalgae in abalone growth enhancement can be either nutritional or functional (Kemp 2018). Nutritionally, essential nutrients can be provided by macroalgae directly or as coproducts from the activities of the gut microbiome (Kemp 2018). Some of these are amino acids, minerals, fatty acids, and vitamins deficient in formulated feed, compensating for nutritional deficiencies in the formulation and production of feed pellets (Iehata *et al.* 2009; 2014; Kemp 2018). From a functional point of view, seaweeds are known for its biologically active compounds

(polysaccharides, proteins, pigments, omega-3 polyunsaturated fatty acids and polyphenols), potent as antiviral, antibacterial, anti-inflammatory and antioxidant agents in aquaculture (Chojnacka *et al.,* 2012; Dang *et al.* 2012; Silva *et al.* 2015; Stone *et al.* 2013; Bansemer *et al.* 2016a). The present results suggest that some of these nutritional properties were lost during the seaweed drying and pelleting process for the manufacture of the experimental diets containing *Ulva* meal.

5.6 Conclusion

The protein and energy sources in commercial diet (Abfeed S34) for South African abalone can be partly replaced with nutrient-enriched *Ulva* meal without compromising the growth of the animals. However, the poor feed conversion and nutrient utilisation recorded at 12% inclusion of *Ulva* meal in the diet makes its supplementation economically unattractive. Given that lower inclusions (0.44-3.54%) of kelp (*E. maxima*) has been documented to improve growth and nutrient utilisation by *H. midae*, a follow-up study (reported in Chapter 6) was carried out to test lower inclusions of *Ulva* meal in formulated diet for South African abalone (*H. midae*).

Chapter 6: An evaluation of graded inclusion levels of IMTA-cultured *Ulva species* meal in the formulated diet for South African abalone (*Haliotis midae*)

6.1 Introduction

The inclusion of different levels of *Ulva* meal in formulated diets on the performance of different species of farmed abalone has been documented by a few authors (O'Mahoney *et al.* 2014; Bansemer *et al.* 2016b; Bates *et al.* 2017, Ansary *et al.* 2019a, b; Santizo-Taan *et al.* 2020). For example, 5% *Ulva* meal was documented as the best inclusion level in the diet of the greenlip abalone (Bansemer *et al*. 2016b) and 20% *Ulva* protein extract was recommended for inclusion in the diet of the same species (Bates *et al.* 2017). Additionally, up to 30% inclusion of *Ulva pertusa* meal in the diet of *H. asinina* did not negatively affect their growth performance and nutrient utilisation efficiency (Santizo-Taan *et al.* 2020). However, the 12% inclusion of IMTA and monoculture *Ulva lacinulata* meal in the diet of South African abalone yielded poor nutrient utilisation and growth performance (Chapter 5). This result suggested that 12% *Ulva* meal inclusion is too high for *H. midae* hence, a need was identified to test different inclusions of the seaweed. The current study thus tested graded levels of effluent-grown (IMTA) *Ulva* meal in the diet of *H. midae*. It was hypothesised that a lower inclusion level of farmed *Ulva* in the compound feed might significantly improve the nutrient utilisation efficiency and growth *H. midae*.

6.2 Materials and methods

6.2.1 Macroalgae meals and experimental diets production

Ulva species meal production

Ulva meal production followed the same process described in Chapter 5, Section 5.2.1. The same batch of *Ulva* used in Section 5.2.1 was used in the current experiment.

Experimental diet formulation and manufacture

Six isoenergetic (16.2 kJ/kg) and isonitrogenous (34% protein) practical diets were prepared to contain graded inclusion levels of IMTA *Ulva* ranging from 0.00 – 12.00%. The dietary *Ulva* inclusion levels were 0% (commercial control diet Abfeed®-S34, IMTA-U0), 0.75% (IMTA-U0.75), 1.5% (IMTA-U1.5), 3% (IMTA-U3), 6% (IMTA-U6), and 12% (IMTA-U12%) (Table 6.1). Abfeed®-S34 is a commercial abalone diet produced by Marifeed Pty Ltd (Hermanus, South Africa), the formulation of which remains proprietary. Fixed portions of the ingredients used to manufacture the control diet (IMTA-U0) were replaced to ensure that total protein, total lipid, and total lysine remained constant in the IMTA-U0.75, IMTA-U1.5, IMTA-U3, IMTA-U6, IMTA-U12 formulations. The ratio of fishmeal to soya was maintained across the diets. The total lipid content was also maintained, and this was done with the addition of fish oil to the basal diet. The diets included terrestrial grain and a standard inclusion of a vitamin mix (also standardized between treatments) the content of which remains proprietary. The protein and lipid compositions of the experimental diets were analysed at ARC-Irene Agricultural research laboratory, using the standard methods the Association of Official Analytical Chemists (AOAC 1996). The analysed protein contents for all the diets were close to the calculated 34% inclusion level. The lipid content of the control diet

was similar to the target lipid level of 4.6%, but slightly lower for the *Ulva* treatments ranging from 4.21-4.29%.

6.2.2 *Experimental animals and culture system*

The feeding trial was conducted for 242 days from December 2020 – July 2021 using standard farm production materials and procedures described in Chapter 5 (Section 5.2.2).

The seawater temperature in culture tanks (18.75 ± 0.41 °C) was daily monitored for the period of the growth trial using Ebro temperature logger (model EBI 300, serial number 73268609 by Xylem Analytics, Germany) while the pH (7.5 – 8.1) and dissolved oxygen (7.1 – 8.2 mg L^{-1}) were monitored every week at 08h00 Thermo Scientific Eutech Expert pH meter (serial no. 2913115, Singapore) and Oxyguard handy Polaris DO meter (Model v. 2.51, serial no. 015BODE0140000 by Oxyguard International A/S, Denmark) respectively. Ammonia nitrogen (0.10 – 0.25 mg L^{-1}), nitrite nitrogen (0.00 – 0.02 mg L^{-1}) and nitrate nitrogen (0.32 – 0.71 mg L^{-1}) concentrations were periodically monitored using Palintest automatic wavelength photometer (model 7100, Palintest Ltd, England) and test kits (AP163 Nitratest and AP152 Ammonia; batch: CEO59/CE060).

Table 6.1. Target formulation and proximate and lysine composition (g / 100g) of experimental diets containing graded *Ulva* meal.

	IMTA U0	IMTAU0.75	IMTAU1.5	IMTAU3	IMTAU6	IMTAU12
Target formulation						
IMTA Ulva meal (%)	0.00	0.75	1.50	3.00	6.00	12.00
Abfeed®-S34 basal diet	100.0	99.25	98.50	97.00	94.00	88.00
Total	100	100	100	100	100	100
Total protein (%)	34.0	34.0	34.0	34.0	34.0	34.0
Lysine (%, g lysine/100 g diet)	2.30	2.30	2.30	2.30	2.30	2.30
Total lipid (%)	4.60	4.60	4.60	4.60	4.60	4.60
Energy (kJ/kg)	16.20	15.90	15.90	15.90	15.90	15.90
Proximate composition (dry mass)						
Total protein (%)	33.88	34.13	34.15	34.07	33.85	33.89
Total lipid (%)	4.59	4.27	4.22	4.21	4.22	4.29
Energy (kJ/kg)	19.20	19.12	19.08	18.99	18.42	18.67

Proximate compositions were analysed using standard methods – Crude protein: Duma's combustion method, lipid: AOAC 996.06 (gas chromatography).

6.2.3 *Experimental abalone and stocking*

Prior to the study, the experimental abalone (35-45g) were stocked in 18 culture baskets in a tank with each basket housing about 250-350 animals to attain the target production basket biomass (10500 ± 1.50 g / basket) for this size class. They were acclimated to the culture tanks for fourteen days following the standard experimental procedures on the farm before any baseline data collection. At the start of this investigation, all individual *H. midae* were active, healthy, and with clean shells. After acclimation, 30 randomly selected animals from each basket were carefully removed using a flat, blunt metallic spoon, blotted dry, and weighed to 0.01 g accuracy and their individual shell length measured to the nearest 0.01 mm with a digital caliper.

6.2.4 *Experimental design and treatments*

The eighteen abalone baskets for the growth study were assigned colour tags after the baseline measurements and randomly distributed in the tank assigned for the trial, such that each dietary treatment was replicated three times. There were no differences in abalone weight and shell lengths between treatments at the start of the experiment (one-way ANOVA; $F_{(5,12)} = 0.54711$, $p = 0.738$ and $_{(5,12)} = 1.383$, $p = 0.298$, respectively; Table 6.2). Each diet type was fed to satiation to replicate baskets of abalone for the eight months of the trial between 1600 – 1800 hours daily. Four months into the experiment (April 2021), the eighteen baskets were split by randomly removing animals from each basket to reduce the weight of each basket to the biomass weight at the start of the growth trial.

6.2.4 *Abalone growth measurements*

The feeding trial lasted for 242 days, and growth and feed utilisation measurements followed the same procedure described in Chapter 5.

The specific growth rate (SGR; % body weight d^{-1}) was computed with the equation (Britz 1996):

$$SGR = ([In (W_f) - ln (W_i)] /t) \times 100 \quad \text{Equation 6.1}$$

where W_f, W_i represents final weight and initial weight of abalone, and t represents the number of days the animals were fed.

The condition factors (CFs), an index to account for the relationship existing between individual abalone's weight and the shell length were determined using the equation of Britz (1996):

$$CF = [\text{body weight} / \text{shell lenght}^{2.99}] \times 5\,575 \text{ (Britz 1996)} \quad \text{Equation 6.2}$$

The feed intake and conversion ratio were corrected for leaching and calculated using the equations of Britz (1996):

$$\text{Daily feed consumption (\% Body weight per day)} = C_g/W_t \times 100 \quad \text{(Britz 1996)} \quad \text{Equation 6.3}$$

The Apparent Protein Efficiency Ratio (PER) was calculated using the equation of Nel *et al.* (2017):

$$PER = [\text{abalone biomass gain}/ \text{grams feed protein applied}]: \quad \text{Equation 6.4}$$

6.2.5 *Proximate composition of abalone tissue*

At the end of the feeding trial, feed was withheld from the abalone for 48 hours to purge them of their gut content. Five abalone were randomly selected from each basket, with a total of fifteen animals collected from each treatment and anaesthetized with an overdose of ethylene-

glycol-monophenyl-ether (Kemp 2018) before the shell was shucked and the gut removed. The animals were then dried at 40 ℃ for 72 hours, blended into powder and stored at -20 ℃. Triplicate samples of the stored abalone were analysed for their proximate (protein, lipid, ash, crude fibre, Nitrogen free extract) and energy compositions at a private laboratory (ARC-Irene, South Africa) using standard methods (AOAC 1996).

6.3 Statistical analyses

All statistical analyses were performed using STATISTICA software version 13.5 (Statsoft, Tulsa, Oklahoma). Assumptions of homogeneity of variances and normal distribution were tested using the Levene's and Shapiro-Wilk tests, respectively. Where the assumptions of these parametric tests were violated and the data transformation failed, the non-parametric Kruskal-Wallis test was performed as an alternative test to the one-way analysis of variance tests. A significant level of $p < 0.05$ was used for all statistical tests. Tukey's multiple range post hoc analysis was employed if a significant difference was detected in the data tested. The results are presented as mean ± standard error.

6.4 Results

6.4.1 *Feed consumption and nutrient utilisation indices*

Feed consumption was similar for abalone fed 0, 0.75 and 1.5% *Ulva* inclusion diets, but increased sharply from 3-12% *Ulva* inclusion (One-way ANOVA $F_{(5,12)} = 30.552$, $p = 0.005$, Figure 6.1).

The feed conversion ratio of *H. midae* decreased significantly from 0-1.5% *Ulva* meal inclusion before a sharp increase from 3-12% (one-way: ANOVA $F_{(5, 12)} = 4.121$, $p = 0.021$; Figure 6.2).

The inclusion of *Ulva* meal in the diet also increased the protein utilisation efficiency of *H. midae* from 0 -1.5% before a decline at 3-12% (one-way: ANOVA $F_{(5, 12)} = 1.897$, p= 0.169; Figure 6.3).

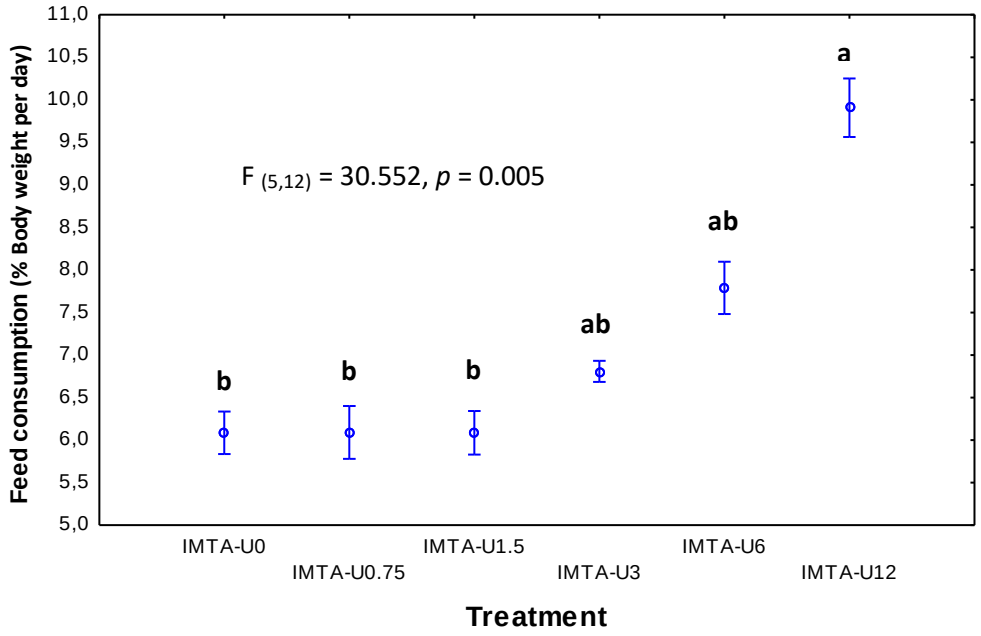


Figure 6.1. The mean (± 95% confidence interval) feed intake of *H. midae* fed different inclusions of IMTA-grown *Ulva* meal for 242 days. Treatments with similar superscripts are not significantly different ($p > 0.05$).

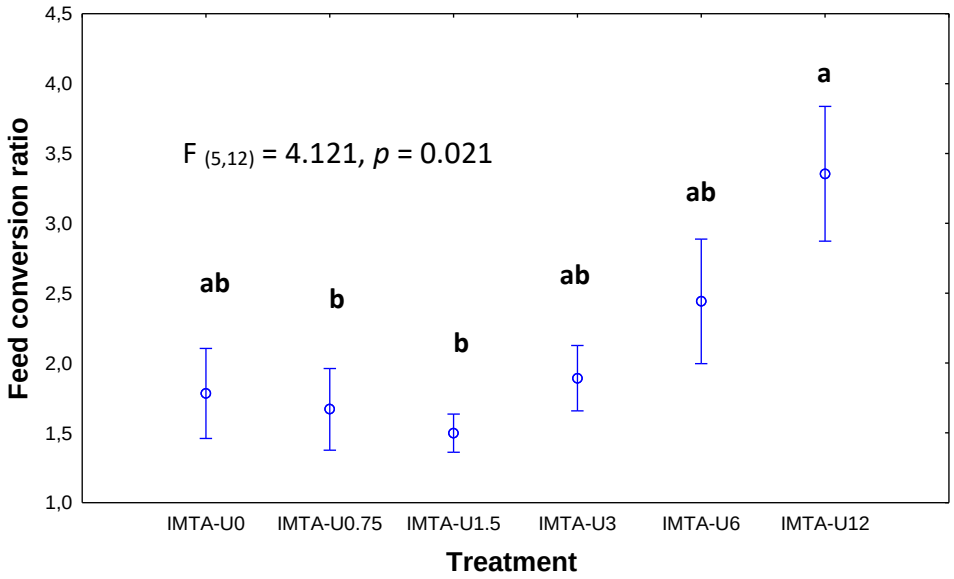


Figure 6.2. The mean (± 95% confidence interval) feed conversion ratio of *H. midae* fed different inclusions of IMTA-grown *Ulva* meal for 242 days. Treatments with similar superscripts are not significantly different ($p > 0.05$).

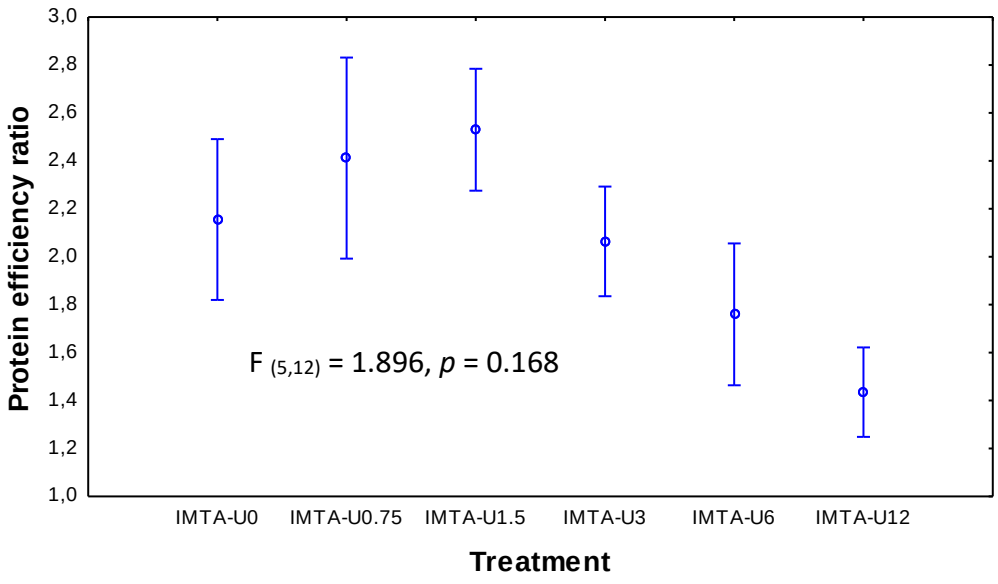
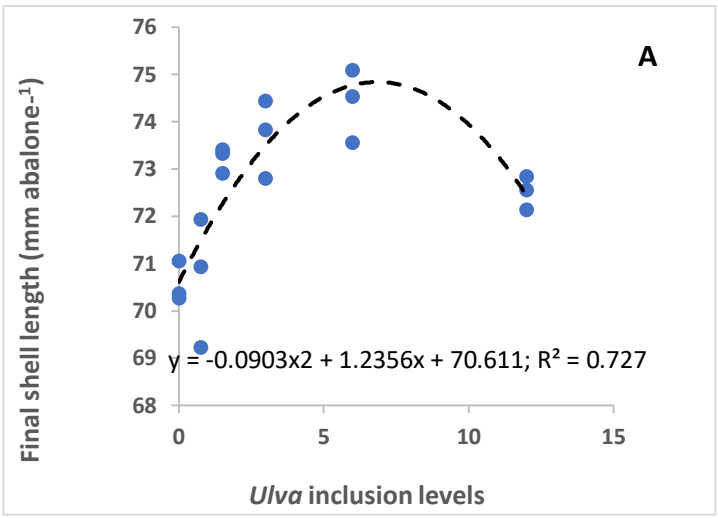
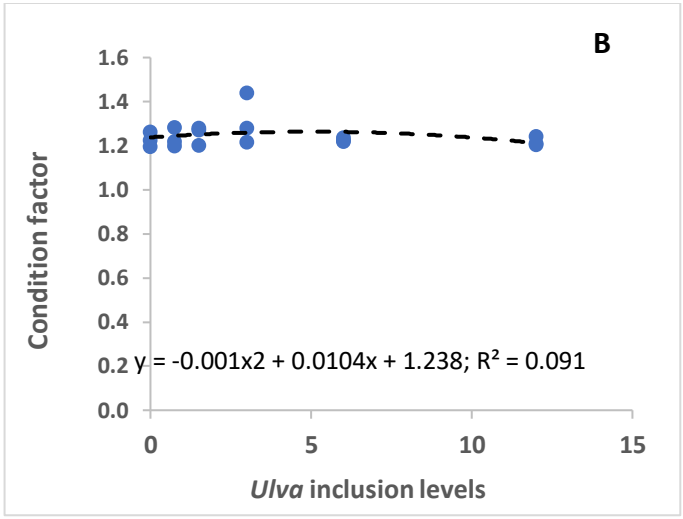


Figure 6.3. The mean (± 95% confidence interval) protein efficiency ratio of *H. midae* fed different inclusions of IMTA-grown *Ulva* meal for 242 days.

6.4.2 *Growth performance of experimental H. midae*

The growth rate of the experimental abalone, expressed as SGR, final weight and shell length, improved with the level of *Ulva* inclusion from 0-6%, and thereafter decreased to 12% inclusion (Figure 6.4). The final condition factor was similar ($P > 0.05$) for all the experimental treatments (Figure 6.4d).





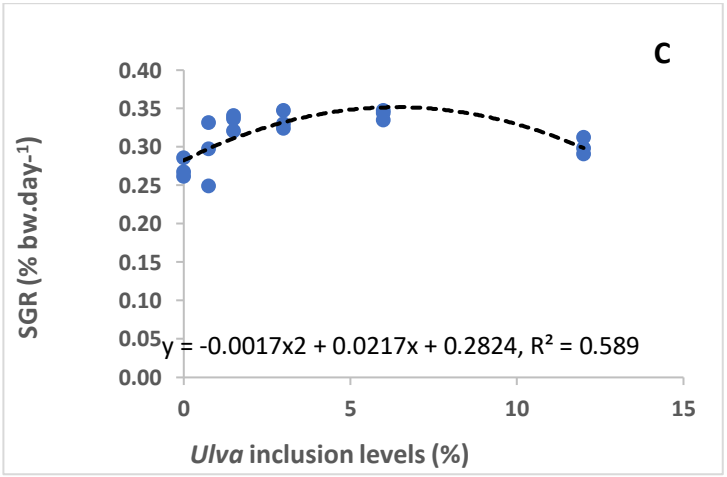


Figure 6.4. A second order Polynomial regression of abalone (A) shell length (B) specific growth rate and (C) condition factor against inclusion levels of IMTA *Ulva* in commercial pellet fed to the animals.

6.4.3 *Biochemical proximate composition of H. midae fed experimental diets*

Overall, similar dry matter (One-way: ANOVA $F_{(5, 12)} = 1.213$, $p = 0.361$; Table 6.2), ash (One-way: ANOVA $F_{(5, 12)} = 1.874$, $p = 0.962$; Table 6.2), lipid (One-way: ANOVA $F_{(5, 12)} = 2.676$, $p = 0.922$; Table 6.2) and energy (One-way: ANOVA $F_{(5, 12)} = 3.386$, $p = 0.387$; Table 6.2) were recorded for fed *H. midae* at all levels of IMTA *Ulva* inclusion in the diet. However, the soft tissue protein decreased with increasing level of *Ulva* in the diet (One-way: ANOVA $F_{(5, 12)} = 23.253$, $p < 0.001$; Table 6.2).

Table 6.2. Tissue composition of *H. midae* fed graded inclusions of IMTA *Ulva* meal for 242 days. Values reported are the treatment means of three replicates and standard error. Values with different superscripts on the same row are significantly different ($p < 0.001$).

	Diet						
	IMTA-U0	IMTA-U0.75	IMTA-U1.5	IMTA-U3	IMTA-U6	IMTA-U12	*P* value
Parameters							
Dry matter	94.21± 0.15	93.99 ± 0.33	92.99 ± 0.86	93.39 ± 0.01	93.87 ± 0.26	93.49± 0.24	0.962
Ash (%)	4.55 ± 0.11	4.36 ± 0.33	4.46 ± 0.34	4.21 ± 0.28	4.31 ± 0.29	4.40 ± 0.19	0.622
Protein (%)	64.50 ± 1.10 [a]	63.77 ± 0.10 [a]	61.27± 0.97 [a]	58.21±0.32 [b]	57.92 ± 0.20 [b]	49.66 ± 1.87 [c]	0.001
Lipid (%)	4.35 ± 0.10	4.11 ± 0.31	4.16 ± 0.31	3.95 ± 0.27	4.06 ± 0.27	4.13 ± 0.17	0.922
Energy (MJ/kg)	18.50± 0.12	18.36 ± 0.02	17.67 ± 0.17	17.89 ± 0.32	18.42 ± 0.09	18.18± 0.18	0.387

6.5 Discussion

The present study demonstrated that for sub-adult South African abalone, up to 6% of IMTA *Ulva* meal can be included in the diet formulation without impacting their growth performance negatively. After 242 days of intensive feeing, the final body weight, shell length and specific growth rate of *H. midae* markedly increased with the increasing inclusion levels *of* Ulva meal from 0 - 6% in the diet and thereafter, a sharp decline in growth was recorded. This finding is consistent with the result of the growth trial in Chapter 5 where *H. midae* fed 12% IMTA and monoculture *Ulva* performed poorly in growth and nutrient utilisation efficiency. The peak in growth at 6% suggests that the inclusion of *Ulva* above this level compromises the nutritional quality of formulated diet for *H. midae*.

Similarly, the increases in consumption with increasing level of *Ulva* in the diet did not translate into efficient nutrient utilisation. Though the feed conversion and protein efficiency ratio of *H. midae* significantly improved at 0.75-1.5% inclusion of IMTA *Ulva* meal in the diet, there was a sharp decline in conversion efficiency as the inclusion level increased from 3-12%. The drop in growth and feed conversion efficiencies above 6% *Ulva* inclusion could have resulted from a growing imbalance in the proportion of essential amino acids required for optimal abalone growth. The poor feed conversion at high (3-12%) inclusion of IMTA *Ulva* meal in the diet could have also resulted from the poorer stability of test pellets in seawater as the dietary level of macroalgae increased in the pellet. High inclusion of plant ingredients in formulated diets reduces the homogeneity of particle size with resultant loss of dry matter from pellet when immersed in water (Mahoney *et al.* 2014)

The increase in pellet consumption rate indicates that *H. midae* probably consumed more to compensate for the low dietary amino acid and possibly other nutritional imbalances. A relatively low content of amino acids such as methionine, threonine, cysteine and phenylalanine have been reported in *Ulva* meal diets (Azaza *et al.* 2008). However, while the reasons for the decline in performance remain speculative, the results indicate clearly that the availability of dietary nutrients for growth declined with an inclusion level of IMTA *Ulva* above 6%.

The improved feed utilisation efficiency at low (0.75-1.75%) *Ulva* inclusion levels is consistent with the findings of O'Mahoney *et al.* (2014); Kemp *et al*. (2015) and Nel *et al*. (2017) where the efficiency of feed utilisation by abalone was improved when they were fed low level of fresh seaweed and macroalgae meal supplements.

The tissue protein of *H. midae* was similar at 0-1.5% but significantly declined as macroalgae meal inclusion increased from 3-12%. The decreasing tissue protein confirms the decline in protein conversion efficiency as a result of amino acid imbalance or the unavailability of seaweed protein to *H. midae* at high inclusions. Previous studies also documented that the nutrient composition of abalone diets could affect the biochemical composition of abalone soft tissue (Kim *et al*. 2016; Lee *et al.* 2017; Santizo-Taan *et al.* 2020).

6.6 Conclusion

The present results suggest that the utilisation of the nutrients in commercial diet for South African abalone can be significantly improved if farmed *Ulva* meal is included in the formulation at low (0.75-1.5%) inclusion level. Additionally, to achieve the best growth performance by

farmed *H. midae,* IMTA *Ulva* meal can be added to Abfeed pellet at a level not more than 6%. This formulation technique will reduce the dependence of abalone farms on fish resources and improve their production efficiency.

Chapter 7: Effect of the integration of detritivore sea cucumber on waste nutrient removal from an abalone-seaweed integrated culture system

7.1 Introduction

The monoculture system of abalone production on South African farms produces solid waste from uneaten feed and faecal materials which fall through the culture baskets and accumulate on the tank floor (Potgieter 2005; Troell *et al*. 2006). The waste solids are flushed weekly during production tanks cleaning and released directly into the coastal environment. Approximately 100kg (dry weight) of accumulated tanks sediments is released annually per tonne of abalone farm production (Potgieter 2005). While the dissolved waste nutrients in abalone wastewaters are currently used for the production of seaweeds on many South African abalone farms, the suitability of the waste solids as a culture medium for detritus extractive species remains largely untested. Sea cucumbers have been identified as a potentially suitable co-culture species with the South African abalone as they are deposit-feeding, bottom-dwelling species capable of ingesting and absorbing nutrients from anoxic sediments (Li *et al.* 2014; Tolon *et al.* 2017). To further improve IMTA production efficiency on South African abalone farms and reduce the production and discharge of organic waste from land-based farms, the integration of solid waste extractive sea cucumbers into abalone culture system was tested in the present study.

The integrated culture of sea cucumber with fed aquaculture species has been documented for different species of sea cucumber. These include the western Pacific Sea cucumber *Apostichopus japonicus* (Zhou *et al.* 2006; Li *et al.* 2014); the California sea cucumber, *Parastichopus californicus Stimpson* (Paltzat *et al.* 2008), and New Zealand species, *Australostichopus mollis* (Slater and

Carton 2007; Slater *et al.* 2009; Zamora and Jeffs 2011). Despite their successful culture in other regions and the environmental and economic to aquaculture, information about the integrated culture of sea cucumber with abalone and macroalgae is scant. Therefore, the current study tested the feasibility of culturing endemic species of sea cucumber (*Neostichopus grammatus*) with the South African abalone (*Haliotis midae*) and macroalgae (*Ulva lacinulata*). The effect of the multi-species group on the flow of nutrients and energy was investigated. The specific objectives were to:

1. Examine the solid waste and organic nutrients removal efficiency of *N. grammatus* in the production system.

2. Compare the flow of nutrients in abalone-*Ulva* and abalone-sea cucumber-*Ulva* IMTA systems using the nutrient budget approach.

3. Test the effect of integrating sea cucumbers into an abalone-*Ulva* IMTA system on the growth, biomass yield, and the nutrient composition of *Ulva lacinulata*.

7.2 Materials and methods

7.2.1 *Experimental facility*

Two experimental systems: (i) abalone-*Ulva* (AU**)** and (ii) abalone-sea cucumber-*Ulva* (ASU) were installed at the research site of Wild Coast Abalone (Pty) Ltd between September and December 2021 using high-density polyethylene (HDPE) tanks. The systems comprised of six rectangular-shaped HDPE flow bins (1.2 m x 0.88 m x 0.76 m; length x width x height, 0.80 m^3) as abalone

culture tanks and six transparent rectangular basins (0.75 m x 0.35 m x 0.40 m; length x width x height, 0.11 m^3) as seaweed culture tanks. Drum filtered seawater was supplied from a sump (10500 L) into abalone tanks through 500 mm polyvinyl chloride (PVC) pipe at one end, and a standing pipe connected to the flow bin served as a water outlet at the other end of the flow bin. The seaweed tank had one side fitted with a 500 mm PVC standing pipe, which served as an outlet for post-seaweed effluent. Each *Ulva* tank was placed very close to the base of the abalone tanks such that the water outlet (standpipe) of abalone tanks served as an inlet to the seaweed tank. Both abalone and *Ulva* tanks were fitted with an airline made from 500 mm PVC pipes perforated at equidistance (55mm apart) to provide constant aeration in the culture tanks.

7.2.2 *Sea Cucumbers Collection and Holding Conditions*

Sub-adult *Neostichopus grammatus* (8.47 ± 0.25 g) were collected from the shallow rock pools (depth of about 30cm-50cm) in the intertidal zone along the coast opposite the Wild Coast Abalone Pty Ltd. They were then transferred to the quarantine section of the farm for acclimation and conditioning to aquaculture conditions. The sea cucumbers were held in a 12000 L flowthrough tank, that was filled to one quarter of its total capacity and fed with solid waste from abalone culture tanks for 21 days before the trial commenced. The animals appeared to adapt well to the captive conditions as no mortality or weight loss was recorded during the conditioning period. Similarly, the sea cucumbers fed readily on abalone tank waste in the holding tanks.

7.2.3 *Experimental design and growth trial*

The two experimental treatments tested in this study were replicated three times (Fig. 7.1). Each abalone tank replicate was stocked with 10.5 ± 0.12 kg of 35-45 g *H. midae* of the same cohort in culture baskets. In addition to this, the ASU treatment was stocked with 50 sea cucumbers (600 ± 0.22g) per replicate tank on the tank floor under abalone culture baskets (Fig. 7.1). This stocking biomass of *N. grammatus* was selected based on daily faecal waste production by *H. midae* (Section 2.3.3). The abalone were fed daily with a commercial abalone feed (protein 34%, lipid 4.5%; Standard Abfeed® S34 pellet, Marifeed Pty Ltd, South Africa) supplemented with fresh *Ulva* and *Gracilaria species* as described in Chapter 5 (Section 5.2.5). Supplemental feed was not provided for the sea cucumbers throughout the trial so that they depended solely on the feed and faecal waste from the abalone. The weekly cleaning of abalone tanks and trapping of sedimented sludge waste was carried out throughout the ninety days culture period using the method described in Chapter 2 (Section 2.2.2).

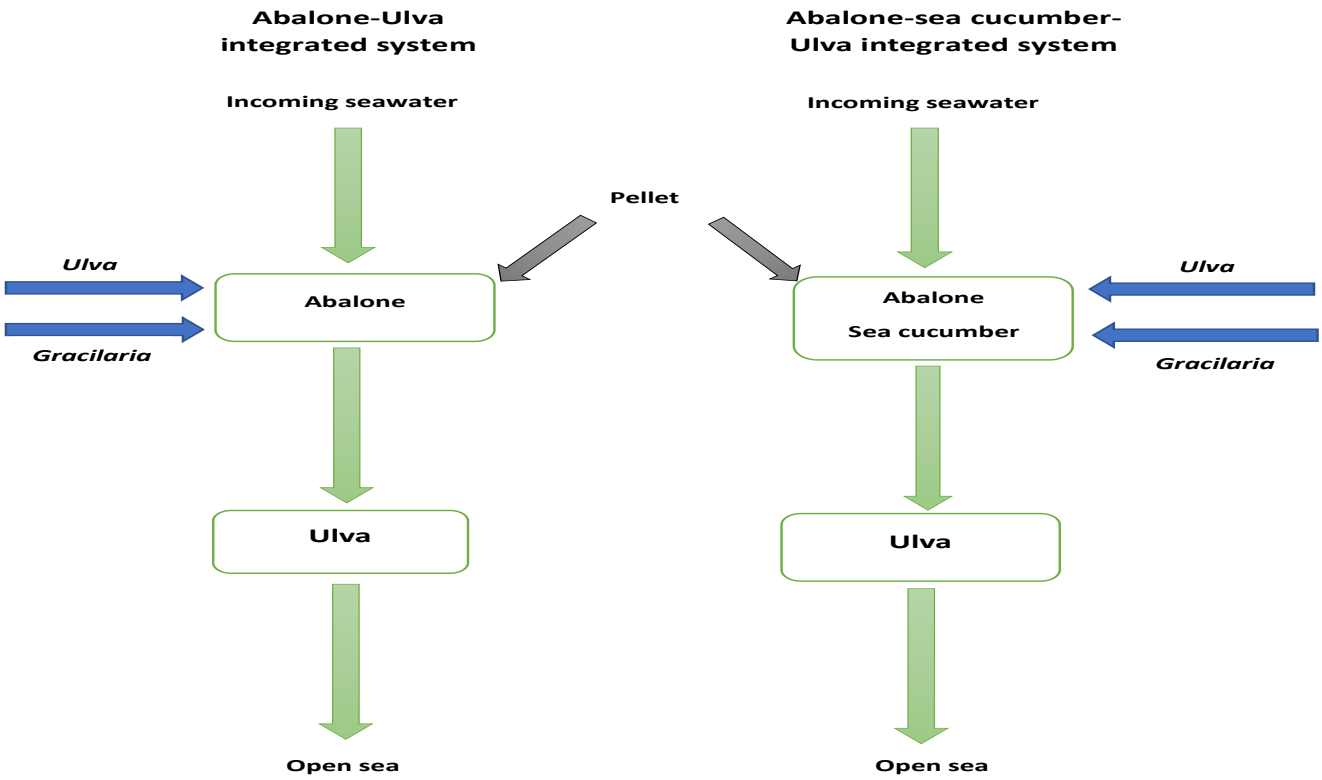


Figure 7.1. Pilot-scale design of IMTA systems of abalone-*Ulva* and abalone-sea cucumber-*Ulva* at WCA. Both systems were replicated three times.

7.2.4 *Ulva culture, water quality monitoring and sediment samplings*

Fresh *Ulva* seed stock was collected from the rocky shores of the neighboring Indian Ocean, cleaned of debris, and cultured for two weeks before the start of the investigation. At the commencement of the trial, each seaweed tank was stocked with *Ulva* at a density of 1 kg m^{-3} as recommended by Neori *et al.* (1991; 2003). The effluent exiting abalone and abalone-sea cucumber tanks flowed into the seaweed culture tanks at a flow (171.8 L h^{-1}) (Fig. 7.2). *Ulva* biomass was harvested every ten days, drained in a salad spinner, hand-pressed to remove excess water, and then weighed on a tabletop weighing scale. After each harvest event, the seaweed tanks were restocked at the initial density with *Ulva* from the harvested biomass of the same

culture tank. In this trial, inorganic fertilisers were not applied to the seaweed tanks to supplement the nutrients provided by abalone effluent. This is because the flow rate and adopted aeration technique allowed the availability of more nutrients and air for *Ulva* growth.

Figure 7.2. Integrated culture system of abalone/sea cucumber and seaweed. The effluent from abalone or abalone-sea cucumber tanks was used for seaweed culture**.**

Weekly water nutrient sampling for was carried out throughout the experimental period by collecting water samples from the inlet and outlet of abalone and seaweed tanks, with temperature, pH, and dissolved oxygen concentration measured i*n-situ* using appropriate methods (Chapter 2, Section 2.2.3). At the same time, ammonia nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and reactive phosphate (PO_4-P) were analysed using Palintest photometer and test kits (Chapter 2, Section 2.2.3). Also, total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) were analysed using a spectrophotometer (DR900, Hach Lange, Dusseldorf, Germany).

7.2.5 *Macroalgae growth*

The specific growth rate (SGR = % wet wt. day^{-1}), biomass yield (Y) (g wet wt. m^{-2} day^{-1}) and net yield (NY) (wet weight day^{-1}) of *Ulva* from the two culture systems were calculated by using the equations of Evans (1972):

$$SGR\ (\%) = 100 \times [(W_{t.} - W_0)]/\ t \qquad \text{Equation 7.1}$$

$$\text{Biomass yield (g wet wt. } m^{-2}\ day^{-1}) = [(W_{t.} - W_0)\ /\ t]\ /\ SA \qquad \text{Equation 7.2}$$

$$\text{Net Yield (ww } day^{-1}) = [(\text{final biomass } (m^3) - \text{initial biomass } (m^3)]/\text{time (days)} \qquad \text{Equation 7.3}$$

where W_0 and $W_{t.}$ are initial and final weights (g), t is the number of culture days and S A is the surface area of the seaweed tanks.

7.2.7 *Nutrient composition of abalone, Ulva, and accumulated sludge*

The nutrient (nitrogen, phosphorus, and carbon) composition of abalone, *Ulva*, *Gracilaria*, sea cucumber, feed, and accumulated sludge from the two treatment groups was determined at the end of the experiment using the procedures described in Chapter 2 (Section 2.2.1). The dry matter was calculated by weight loss after oven-drying at 60°C for 24-48 h.

7.2.8 *Nutrient mass balance*

The inputs and outputs of nutrients in the production systems were calculated for stocked and harvested abalone, sea cucumber, influent and effluent water, feed (wet and dry), stocked and harvested seaweed biomass, and the accumulated sludge using the mass balance approach described in Chapter 3 (Section 3.2.8).

7.3 Statistical analyses

The growth, yield, and nutrient compositions of *Ulva* from the two culture systems were compared for significant differences using the Student's *t*-test at $p < 0.05$. Likewise, the nutrient load of input and output of the two culture systems was analysed and compared using the Student's *t*-test. All data were tested for homogeneity of variance using Levene's test and for the normal distribution of residuals employing a Shapiro–Wilk plot. The Mann-Whitney U non-parametric test was employed when the data for growth, yield, nutrient uptake efficiency, nutrient uptake rate, and nutrient compositions of *Ulva* and the nutrient load into and out of the production systems did not meet the assumptions of parametric an analysis of variance. All analyses were performed using STATISTICA® software, version 13.2 (Statasoft, Tulsa, OK, USA).

7.4 Results

7.4.1 *Growth performance of abalone / sea cucumbers in abalone-Ulva and abalone-sea cucumber-Ulva integrated system and the associated accumulated solids.*

At the end of the 90 days trial, similar final biomasses (Student's *t*-test = -0.62, $p = 0.57$; Table 7.4) and weight gains (Student's *t*-test = -1.42, $p = 0.23$; Table 7.4) were recorded for abalone cultured with or without sea cucumber (Table 7.4). Additionally, the feed conversion ratio was similar for the two systems (Student's *t*-test = -1.32, $p = 0.26$; Table 7.4).

A loss in biomass (-303.41 ± 0.65 g) and a negative specific growth rate (- 0.59 ± 0.22 % day^{-1}) was recorded for the sea cucumbers in the ASU treatment at the end of the trial.

The biodeposits production and discharge in the culture system with sea cucumber (ASU) was significantly lower than the abalone-*Ulva* production system (Student's *t*-test = 3.00, *p* = 0.04; Table 7.2). There were no significant variations in the nitrogen (Student's *t*-test = 3.00, *p* = 0.95; Table 7.2), phosphorus (Student's *t*-test = 3.00, *p* = 0.52; Table 7.2) and carbon (Student's *t*-test = 3.00, *p* = 0.69; Table 7.2) compositions of biodeposits in the two systems.

Table 7.1. Growth performance parameters of abalone and sea cucumbers cultured in pilot abalone-*Ulva* and abalone-sea cucumber-*Ulva* IMTA systems.

	Abalone-*Ulva*	Abalone-sea cucumber-*Ulva*	*P*-value
Abalone			
Initial biomass wt. (g)	9756.67 ± 3.51	9383.33 ± 3.09	0.31
Final biomass wt. (g)	12910.00 ± 2.67	13153.33 ± 2.60	0.57
Biomass gain (g)	3153.33 ± 0.86	3770.00 ± 0.50	0.23
Feed conversion ratio	2.00 ± 0.02	1.61 ± 0.04	0.26
Sea cucumber			
Initial biomass wt. (g)	-	627.64 ± 2.21	-
Final biomass wt. (g)	-	324.23 ± 1.74	-
Biomass weight loss (g)	-	- 303.41 ± 0.65	-
Specific growth rate (% day^{-1})		- 0.59 ± 0.22	

Table 7.2. Organic solids production and their nutrient compositions in abalone-*Ulva* and abalone-*Ulva*-sea cucumber production systems.

Accumulated sludge (dry weight)	Abalone-*Ulva*	Abalone-sea cucumber-*Ulva*	*P*-value
Production rate (g day^{-1})	4.69 ± 0.16	4.17 ± 0.19	0.04
Nitrogen (%)	3.00 ± 0.55	2.90 ± 0.16	0.95
Phosphorus (%)	1.46 ± 0.14	1.37 ± 0.15	0.52
Carbon (%)	23.90 ± 3.52	22.88 ± 2.25	0.69

7.4.2 Nutrient budgets in abalone-*Ulva* and abalone-sea cucumber/*Ulva* production systems

The nitrogen, phosphorus and carbon load (i.e., percentage nitrogen x quantity or volume) of inputs and outputs from abalone-*Ulva* and abalone/sea cucumber-*Ulva* culture tanks and their percentage contributions are presented in Table 7.3 to 7.5.

Nitrogen budget in abalone and seaweed units of AU and ASU systems

The highest nitrogen inputs into abalone culture tanks of the two production systems was the feed fed the farmed abalone which was ca. 84 % (0.54 ± 0.04 kg N) for both production system. Additionally, the supplied seawater in abalone tanks accounted for approximately 15.6-15.9% N (0.10 kg N pond^{-1}) in the two production systems respectively.

The cultured abalone absorbed approximately 53-54% of the total nitrogen input in the two systems while the sea cucumber in the ASU retained its 3.2% nitrogen. (Table 7.4). The accumulated sediments in the AU and ASU production systems respectively accounted for ca. 34.4% and 28.6% of the N input from the abalone diets while approximately 9.4-14.3% nitrogen

input to the production systems was unaccounted loss. In the seaweed tanks of the two production systems, the influent nitrogen from abalone tanks accounted for the only source of nitrogen (100%; 0.58 ± 0.01 - 0.60 ± 0.04 kg N). Approximately 70% (0.42 ± 0.02 kg N) and 74% (0.43 ± 0.01 kg N) of the nitrogen supplied from abalone effluent was absorbed by cultured *Ulva* in the AU and ASU production system respectively and not less than 26-27% (0.15 ± 0.02 -0.16 ± 0.04 kg N) of the nitrogen input was lost in the post-seaweed effluent from the two systems (Table 7.3).

Table 7.3. Mean (±SE) nitrogen loads and percentage nitrogen in abalone and *Ulva* tank units of abalone-*Ulva* (AU) and abalone-sea cucumber-*Ulva* (ASU) IMTA systems.

		Abalone-Ulva IMTA		**Abalone-Sea cucumber-Ulva**	
UNIT	**N-form**	**Kg N/month**	**%**	**Kg N/month**	**%**
Abalone					
Input:	Feed input	0.54 ± 0.04	84.37	0.53 ± 0.03	84.13
	Intake water	0.10 ± 0.01	15.63	0.10 ± 0.01	15.87
Output:	Abalone harvest	0.34 ± 0.01	53.13	0.34 ± 0.01	53.97
	Abalone biodeposits	0.22 ± 0.00	34.37	0.18 ± 0.01	29.57
	Sea cucumber harvest	-		0.02 ± 0.01	2.17
	Suspended solids	0.02 ± 0.01	3.13	0.00 ± 0.01	0
	Unaccounted output	0.06 ±0.03	9.38	0.09 ± 0.00	14.29
Ulva:					
Input:	Influent nitrogen	0.60 ± 0.04	100	0.58 ± 0.01	100
Output:	*Ulva* harvest	0.42 ± 0.02	70.00	0.43 ± 0.01	74.14
	Post-seaweed effluent	0.16 ± 0.04	26.67	0.15 ± 0.02	25.86
	Unaccounted output	0.02 ± 0.02	3.33	0.00 ± 0.00	0

Phosphorus budget in abalone and seaweed units of AU and ASU systems

The input and output of phosphorus (P) in the two production systems were mapped as kilogram phosphorus per production tank per culture period and also as percentage contributions (Table 7.4). The highest input of phosphorus (86%) in abalone tanks of the two systems were the feed, followed by the P from intake water (13.3-14.0%).

At the end of the culture period, approximately 25.2 and 30.0% phosphorus supplied from the feed and the intake water was absorbed by cultured abalone in the AU and ASU treatments respectively while the sludge in abalone tanks accounted for approximately 66.2 and 55.9% phosphorus in the AU and ASU production systems respectively (Table 7.8). The sea cucumbers also accounted for 2.3% of phosphorus output from the system.

In *Ulva* tank, the wastewater flowing from abalone tanks accounted for 81.8 (0.036 ± 0.00 kg P/tank) and 93.0% (0.040 ± 0.00 kg P) P input in the AU and ASU production systems respectively (Table 7.4). Additionally, ca. 7.0 and 18.2% P input were unaccounted for in the AU and ASU systems. The cultured *Ulva* removed 68.2-70% phosphorus load in abalone effluent from both systems while ca. 30-32% P was lost to the environment through the post-seaweed effluent.

Table 7.4. Mean (±SE) phosphorus loads and percentage phosphorus in abalone and *Ulva* tank units of abalone-*Ulva* (AU) and abalone-sea cucumber-*Ulva* (ASU) IMTA systems.

		Abalone-Ulva IMTA		Abalone-Sea cucumber-Ulva	
UNIT	**P-form**	**Kg P/month**	**%**	**Kg P/month**	**%**
Abalone					
Input:	Feed input	0.131 ± 0.01	86.75	0.123 ± 0.01	86.01
	Intake water	0.020 ± 0.00	13.25	0.020 ± 0.00	13.99
	Unaccounted input				
Output:	Abalone harvest	0.038 ± 0.00	25.17	0.040 ± 0.00	27.97
	Abalone biodeposits	0.100 ± 0.00	66.23	0.080 ± 0.01	55.94
	Sea cucumber harvest	-	-	0.004 ± 0.00	2.79
	Suspended solids	0.001 ± 0.00	0.66	0.002 ± 0.00	1.40
	Unaccounted output	0.012 ± 0.00	7.94	0.017 ± 0.00	11.89
Ulva:					
Input:	Dissolved phosphorus	0.036 ± 0.00	81.82	0.040 ± 0.00	93.02
	Unaccounted input	0.008 ± 0.00	18.18	0.003 ± 0.02	6.98
Output:	*Ulva* harvest	0.030 ± 0.00	68.18	0.030 ± 0.00	69.76
	Post-seaweed effluent	0.014 ± 0.01	31.82	0.013 ± 0.02	30.23

Carbon budget in abalone and Ulva tank units of abalone-Ulva (AU) and abalone-sea cucumber-Ulva (ASU) production systems.

The highest input (ca. 54.7%) of carbon (C) in abalone banks of both production systems was the water intake, followed by the carbon (45.3%) from fed feed (Table 7.5). Only 16% of the carbon

from these sources (feed and seawater) was absorbed by farmed abalone while ca. 18% and 13.6% carbon load were deposited in tank sediments of abalone-*Ulva* and abalone-sea cucumber-*Ulva* systems respectively. While harvested *N. grammatus* accounted for 0.72% of the total carbon output, ca. 66-70% carbon output in the two production systems were unaccounted.

The effluent flowing from abalone and abalone/sea cucumber culture tanks accounted for 100% carbon input in *Ulva* tanks of both systems (Table 7.5). Approximately 42% (3.13 ± 0.04 kg C per $pond^{-1}$) and 39% (3.09 ± 0.05 kg C per $pond^{-1}$) of total carbon load input were removed by cultured *Ulva* in the AU and ASU units respectively while 28.6 - 32.5% carbon (2.28 ± 0.12 - 2.43 ± 0.20 Kg C per $pond^{-1}$) was lost to coastal environment through the post-seaweed effluent. Additionally, about 28-33% carbon load was lost to other unaccounted processes.

Table 7.5. Mean (±SE) carbon loads and percentage carbon flows in abalone and *Ulva* tank units of abalone-*Ulva* (AU) and abalone-sea cucumber-*Ulva* (ASU) IMTA systems.

		Abalone-Ulva IMTA		Abalone-Sea cucumber-Ulva	
UNIT	**C-form**	**Kg C/month**	**%**	**Kg C/month**	**%**
Abalone					
Input:	Feed input	3.74 ± 0.04	45.27	3.74 ± 0.04	45.27
	Intake water	4.52 ± 0.25	54.72	4.52 ± 0.25	54.72
	Unaccounted input				
Output:	Abalone harvest	1.33 ± 0.03	16.10	1.23 ± 0.03	14.74
	Abalone biodeposits	1.48 ± 0.08	17.92	1.12 ± 0.03	14.56
	Sea cucumber harvest	-		0.06 ± 0.01	0.72
	Suspended solids	0.02 ± 0.01	0.24	0.00 ± 0.01	0.00
	Unaccounted output	5.43	65.74	5.78	69.98
Ulva:					
Input:	Influent carbon	7.47 ± 0.26	100	7.98 ± 0.36	100
Output:	*Ulva* harvest	3.13 ± 0.04	41.90	3.09 ± 0.05	38.72
	Post-seaweed effluent	2.43 ± 0.20	32.53	2.28 ± 0.12	28.57
	Unaccounted output	2.10 ± 0.02	28.11	2.61 ± 0.20	32.71

7.4.3 *Seaweed production, growth rate and tissue composition*

The growth and biomass production of *Ulva* from the two systems were compared for variations. Similar growth rates (Student's t-test = 2.56, p = 0.06; Table 7.6) and daily biomass yield (Student's t-test = 2.97, p = 0.05; Table 7.6) were recorded for *Ulva lacinulata* cultured in the two

production systems. However, the net yield of *Ulva* in abalone effluent (AU treatment) was significantly higher than that cultured in the abalone/sea cucumber effluent (Student's t-test = 2.97, $p = 0.04$; Table 7.6).

The nitrogen, phosphorus and carbon accumulation in the tissue of *Ulva* was not negatively affected by the integration of sea cucumber into the culture system (Table 7.6). The highest nitrogen composition was recorded for the *Ulva* cultured in abalone/sea cucumber effluent. However, this nitrogen (N) content was only 0.16% higher than the N composition of *Ulva* cultured in abalone effluent, hence, no significant difference (Student's *t*-test = -1.37, $p = 0.24$; Table 7.6). The carbon (C) compositions of *Ulva* from both systems were statistically different (Student's *t*-test = 2.92, $p = 0.04$; Table 7.6). However, similar ($P > 0.05$) phosphorus compositions were recorded for the *Ulva* cultured in the two systems (Student's *t*-test = -0.67, $p = 0.54$; Table 7.6).

Table 7.6. The mean (± standard deviation) growth rate, productivity, yield and nutrient compositions of *Ulva lacinulata* cultured in abalone and abalone-sea cucumber effluents (Student's *t*-test, $P < 0.05$; Mann-Whitney, $P < 0.05$).

	Aba-*Ulva*	Aba-SC-*Ulva*	t/*U* value	df/n	*p-Value*
Growth rate (% d^{-1})	20.56 ± 0.19	20.02 ± 0.3	t = 2.56	df = 4.00	0.06
Net yield (g ww d^{-1})	70.29 ± 1.23	66.04 ± 2.15	t = 2.97	df = 4.00	0.04
Biomass yield (g ww m^{-2} d^{-1})	26.72 ± 0.47	25.11 ± 0.82	t = 2.97	df = 4.00	0.05
Tissue Nitrogen (%)	5.31 ± 0.15	5.47 ± 0.13	t = -1.37	df = 4.00	0.24
Tissue Phosphorus (%)	0.34 ± 0.02	0.35 ± 0.01	t = -0.67	df = 4.00	0.54
Tissue Carbon (%)	29.22 ± 0.31	28.60 ± 0.20	t = 2.92	df = 4.00	0.04

7.5 Discussion

7.5.1 *Solid waste removal by Neostichopus grammatus in the ASU production system*

Despite the weight loss experienced by the sea cucumbers, the daily discharge of waste solids (uneaten food and faecal materials) from the production system with sea cucumber (ASU) was 11% lower than the AU system without sea cucumber. This reduction in the release of accumulated sludge from the ASU system resulted from the warty sea cucumbers (*N. grammatus*) utilising the abalone biodeposits as food, thus reducing the impact of the production system on the environment. Additionally, the nitrogen, phosphorus and carbon composition of the solid waste in the ASU was lower than the AU production system by 4.33, 6.16 and 4.27% respectively. Previous studies also documented the ability of sea cucumbers to utilise the biodeposits of fed aquaculture as food (Zhou *et al.* 2006; Slater and Carton 2009; Watanabe *et al.* 2012). For instance, in a recirculating IMTA system of the sea urchins (*Paracentrotus lividus*) and sub-adult sea cucumber (*Holothuria tubulosa*), the detritivore efficiently utilised 39-55% of organic matter from *P. lividus* as food resulting in 17-40% somatic growth increase (Grosso *et al.* 2021). Likewise, the integrated culture of scallops and *Apostichopus japonicus* in lantern nets improved the production systems as the sea cucumbers utilised the solid waste materials from the bivalve as food, thus reducing organic matter discharge and improving growth rates of the sea cucumbers (Zhou *et al*. 2006). Additionally, in an IMTA system combining *Haliotis discuss hannai, Laminaria japonica* and *A. japonicus*, the sea cucumbers utilised the uneaten food and faecal material from abalone as food which daily increased their growth by 0.33% (Fang *et al.* 2009).

In the current study, the *N. grammatus* displayed a 49% weight loss by the end of the trial demonstrating that the nutritional and/ or environmental requirements of the sea cucumbers were not optimal. While the abalone tank waste was readily consumed by the sea cucumbers, it was probably nutritionally deficient. Previous studies have suggested that for farm waste to provide nutritional value to a detritivore, the biodeposits should undergo an "ageing" process (Lupatsch *et al.* 2003) to allow for biosolids decomposition, as well as the build-up of microbial biomass on the organic matter (Kristensen 2000). Thus, the weekly complete removal of accumulated sludge from abalone-sea cucumber production tanks in the current study, may have precluded biodeposits sediment decomposition. The weight loss recorded in the current study mirrors previous findings on different species of sea cucumbers (Battaglene *et al.* 1999). For weight loss, weight loss and a negative specific growth rate was recorded for both large (– 32.79%, – 0.34% day^{-1}) and small size (– 20.42%, – 0.20% day^{-1}) European sea cucumber, *Holothuria polii* cultured for four months in cages under mussel (*Mytilus galloprovincialis*) farm (Grosso *et al.* 2023). Similarly, a negative growth (– 0.026 to – 0.022 g/day) and reduction in length (– 0.27 to – 0.17 mm/day) was recorded for *Holothuria scabra* cultured on hard substrate (without sand) for 21 days without sand (Watanabe *et al.* 2012). Likewise, the growth and length of *H. Scabra* reduced daily (– 0.13 g/day, – 0.42 mm/day) when cultured without sand (Kihara *et al.* 2009). Tissue-self-digestion and weight decrease take place when sea cucumbers are subjected to suboptimal food source for an extended period (Sun *et al*. 2020).

There is evidence that sea cucumbers require the organic waste they feed on to undergo an anaerobic decomposition process for the nutrients to become available to the sea cucumbers. In a study on the role of sand substrate and dietary component on the growth performance of *H.*

scabra, the sandfish grew well on abalone waste when cultured over an anoxic sand sediment, but lost weight over aerobic sand sediment (Robinson *et al.* 2013). In a parallel EUH2020 Aquavitae project study to the present one on the WCA farm, *N. grammatus* displayed positive growth when cultured on a sea sand substrate (*Senekal et al. in press*). It is thus likely the sand substrate facilitates anaerobic decomposition of the abalone waste, and that sand ingestion might physically aid the digestion and assimilation processes (Watanabe *et al.* 2012).

7.5.2 *Nutrient and energy flow in abalone-Ulva and abalone-sea cucumber-Ulva pilot IMTA systems.*

The highest input of nitrogen, phosphorus and carbon in abalone tanks of the two production systems was the feed fed to cultured abalone. This corresponds with previous findings on nutrient budget in IMTA system where the diet fed to abalone contributed the highest load of nitrogen and phosphorus in production tanks (Neori *et al.* 2000; Xiaolong *et al.* 2018; Gao *et al.* 2019a, b). The other input of nutrients in abalone tanks was the seawater flowing into the production tanks.

The poor nutrient absorption and utilisation by *N. grammatus* and the subsequent weight loss impacted their contribution to the nutrient budget as the percentage nitrogen removed by the sea cucumber was very low (2%). This nitrogen loss by *N. grammatus* contradicts previous studies where nitrogen recovery was recorded for sea cucumber. For instance, ca. 8-9% N recovery was reported for the sea cucumber, *A. japonicas* that was cocultured with *H. discus hannai* and rockfish (*Sebastes schlegeli*) (Gao *et al.* 2019a). Likewise, approximately 10.6% nitrogen recovery was documented for *A. japonicas* cultured with *H. discus hannai* in a recirculating system (Gao *et*

al. 2019b). The current study revealed that while the integration of *N. grammatus* into abalone-*Ulva* farming system did not produce a significant reduction in the level of organic nitrogen produced, it is reasonable to hypothesize that they will be a net remover of nitrogen if cultured over a sand substrate which traps the abalone waste and promotes anaerobic decomposition.

The discharge of organic phosphorus from abalone culture systems was reduced by ca. 10% in the production system with sea cucumber (ASU) compared to the AU without the detritivore. The ability of *N. grammatus* to utilise abalone solid waste as food contributed to the lower discharge of organic phosphorus from the ASU production system as accumulated sludge is known as a sink for organic phosphorus (Sahu *et al.* 2015). This shows that sea cucumbers integration into the production system improved phosphorus utilisation, thus reducing the impact of abalone farming on the environment and if adopted on a large scale could contribute toward reducing the pollution effect of commercial abalone farms on the environment. However, significant loads of supplied phosphorus (ca. 56 – 66%) from the feed and intake water were still lost to the environment through the accumulated sludge discharged from the systems. Therefore, it is important to continue sourcing for ways of reducing the discharge of organic phosphorus to the environment. One of such could be by testing the performance of other detritus extractive organisms and sea cucumber species in abalone culture systems.

Approximately 49.6% of the carbon input by *N. grammatus* at stocking was lost at the end of the trial which mirrored their weight loss. Additionally, similar organic carbon loads in accumulated sludge were discharged from both the AU and ASU production systems to the environment, indicating a relatively non-significant impact of the sea cucumber on organic carbon utilisation in the production system. The carbon removal efficiency of *N. grammatus* would likely be enhanced

if they were provided with sand sediments to promote anaerobic decomposition of abalone solid organic wastes.

Nutrient flow in Ulva culture tanks of AU and ASU production system

The nitrogen, phosphorus and carbon uptake by cultured *Ulva* l*acinulata* in the two systems were similar, which indicated that the sea cucumber did not affect the uptake of nutrient by the seaweed. Approximately 70-74% of the dissolved nitrogen in abalone effluent was absorbed by the seaweed cultured in the two systems, showing an efficient utilisation of nitrogen by the seaweed. The nitrogen uptake By *Ulva lacinulata* cultured in the two systems (AU and ASU) under study is higher than the 34 and 57% nitrogen load recovery by *U. lactuca* cultured at an IMTA site with clam, abalone, and fish (Neori *et al.* 1998; Neori *et al.* 2000). It is also higher than the 52-67% N recovery recorded in Chapter 3 (Section 3.3.4) of the current study. The higher nutrient uptake by *U. lacinulata* in the current study could have resulted from the low waterflow in the production systems.

Interestingly, the 68-70% phosphorus recovered by *Ulva* in this study is substantially higher than the 11-17% recovery recorded in Chapter 3 of the current research. Different factors could have contributed to the higher phosphorus uptake by the macroalgae in this pilot study. One of such was the use of wild-harvested *Ulva* and not the farm stock which has been recycled for two decades. In addition, the low water flow and exchange (0.057 L s^{-1}) adopted in this study could have contributed to the high uptake of phosphorus and other nutrients by *Ulva*. The water flow in this pilot system was ca. 32-51% lower than the flow in IMTA and monoculture seaweed production systems in Chapter 3 (Section 3.2.3). The low flow rate would thus have allowed a

longer retention time for nutrients to be absorbed by the seaweed. Finally, the non-supplementation of abalone effluent with excess nutrients from inorganic phosphate fertilisers could have allowed the seaweed to more efficiently strip phosphorus from the effluent. This result therefore points to the need to optimise water flow in seaweed tanks at WCA and reduce the use of mineral phosphorus fertilisers.

There was no difference in carbon removal (ca. 39-42%) by *Ulva lacinulata* cultured in the production two systems. The sequestered carbon contributes towards reducing the carbon footprint of abalone farming and making the production systems more environmentally benign as carbon removal by macroalgae is a significant bio-mechanism to reduce the rise in atmospheric carbon dioxide thereby alleviating the detrimental trends of global warming (Jana *et al.* 2013; Agarwal *et al.* 2016). It is also important that abalone farms in South Africa invest in the cultivation of different macroalgae species to increase their chances towards achieving a zero-carbon footprint and acquiring carbon trading credits.

7.6 Conclusion

The integration of *N. grammatus* in abalone tanks reduced the daily discharge of solid waste from uneaten food and faecal materials by 11%, thus contributing towards making the production system environmentally benign. However, the organic nutrient removal efficiency of the sea cucumbers was very poor with a resultant 49% sea cucumber weight loss and negative (-0.59% day^{-1}) growth rate. The lack of sand substrate in production tanks to facilitate anaerobic decomposition of the abalone tank organic waste was hypothesized to contribute to the poor

growth performance of the detritivore. Therefore, future studies should be directed towards studying the effect of substrates and anaerobic decomposition on the production performance of *Neostichopus grammatus* in IMTA system with South African abalone *H. midae*. The 11% reduction in discharged biodeposits and the slight reduction in the organic nutrients levels in the ASU production system shows that under proper rearing and management conditions, the coculture of *H. midae* with *N. grammatus* could potentially increase nutrient utilisation efficiency in abalone aquaculture and improve productivity. Additionally, it is highly recommended that the aquaculture performance of other endemic species of sea cucumber should be investigated in the future. If further studied and developed, this aquaculture technique may be eligible for nutrient trading credits.

Chapter 8: Optimising IMTA production of seaweed on abalone farm with live microbial fertilisers

8.1 Introduction

The Wild Coast Abalone (Pty) Ltd is a shore based IMTA farm where the seaweed, *Ulva spp* is cultured in paddlewheel raceway tanks using the abalone culture system wastewater. This integrated culture technique reduces the nitrogen and phosphorus in the effluent before final discharge to the receiving coastal environment. However, the low nutrient concentration of abalone effluent coupled with large scale production of seaweed for farmed abalone necessitates the use of water-soluble chemical fertilisers to supplement the nutrients from the wastewater (Troell *et al.* 2006).

In the current seaweed production technique on shored-based abalone farms in South Africa Inorganic fertilisers such as urea, monoammonium phosphate, monoammonium phosphate and anhydrous ammonia are standardly applied. Inorganic fertilisers boost seaweed growth rates and improve the plants' quality and health by minimising infestations from epiphytes (Hazra 2016; Jacob and Kumar 2020). An adverse impact of using mineral fertilisers is however an increase in the net outflow nutrients from the farm due to their inefficient uptake by the seaweed (Robertson-Andersson 2003; Troell *et al.* 2006). There is a growing awareness of the adverse impact of chemical fertilisers on the environment, which coupled with the economic cost of their application has led to a search for alternatives.

Recently, the use of biofertilisers has been embraced as an alternative to mineral fertilisers and they have emerged as an important component of the integrated nutrient supply system

(Chen 2006). This is because biofertilisers have great potential to improve crop yields by supplying nutrients through environmentally friendly techniques (Chen 2006; Das *et al.* 2007). Biofertilisers are organic substances which utilize microorganisms to increase the availability of inorganic dissolved nutrients to plants (Tilak and Reddy, 2006). These microbial inoculants are made up of the living cells of microorganisms (bacteria, algae, and fungi) that can help increase plant productivity and yield (Tilak and Reddy, 2006; Sharafzadeh and Ordookhani 2011). Biofertilisers make nutrients available to plants through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth promoting substances (Jacob and Kumar 2020). Unlike mineral fertilizers, biofertilisers are biodegradable, non-polluting, non-toxic, eco-friendly, and responsible for continuous availability of nutrients from natural sources (Chaudhary, 2010; Renuka *et al.* 2018; Jacob and Kumar 2020). They are cost effective with longer shelf life than mineral fertilisers (Singh, Kumar & Sharma, 2015). Liquid biofertilisers utilise natural processes to promote growth by increasing the supply of essential nutrients to the host plants (Vessey 2003; Jee 2009; Wainwright *et al*. 2014).

Considering the yield-promoting advantage of biofertilisers coupled with the non-polluting effect on the environment, a study was conducted to investigate the possibility of replacing inorganic fertilizers (urea, monoammonium phosphate, monopotassium Phosphate fertilizer) with live microbial fertilisers. The effect on growth rate, yield and seaweed nutrient composition was examined. It was hypothesised that biofertilisers can fully or partially replace mineral fertilisers without any adverse effect on the yield and nutrient composition of *Ulva* seaweed.

8.2 Materials and methods

8.2.1 *Ulva* production raceways preparation and stocking

At the start of the experiment in June 2021, six IMTA paddlewheel raceway*s* were selected and prepared (washed, disinfested, and filled with abalone culture tank effluent overnight). After preparation, each raceway was seeded with 250kg (wet weight) of *Ulva* weighed out from the previous harvest with the best production performance in terms of growth, deep green coloration, and thickness of thallus.

8.2.2 Experimental design

Three fertilization treatments were tested, and each treatment was replicated twice. These included: (1) 100% mineral fertilisers; (2) 50% mineral fertilizers plus 50% biofertilizer; and (3) 100% biofertilizer application. The detailed information on the quantities of fertilisers applied is proprietary, which remains the property of Wild Coast Abalone (Pty) Ltd. The live microbial bio-fertiliser (ExploGrow) used for this trial was a poly-microbial blend with natural organic carbon carrier (100 g/kg). The seaweed was fertilised three times every week until the end of the culture cycle following the protocol for each treatment. The effluent channeled into the seaweed tanks were kept constant at 200 liters per minute across the treatments.

8.2.3 Water quality monitoring and growth of seaweed

Weekly samples of water were collected at the inflow and outflow of each seaweed raceway following the procedure described in Chapter 3 (Section 3.2.4). The water samples were analysed for their total nitrogen (TN, mg/l) and total phosphorus (TP, mg/l) compositions. At the end of the culture period, the Ulva biomass from each paddle pond was harvested, drained in crates and

the weights recorded after two hours of draining. The productivity (net yield) and specific growth rate of Ulva were calculated according to Evans (1972) where:

NY (g wet weight day^{-1}) = [final biomass (m^3) – initial biomass (m^3)] / time (days) Equation 8.1

Specific growth rate, SGR (% wet weight day^{-1}) = 100 x [Ln W_t – Ln W_0)] / t Equation 8.2

where W_i and W_o are the final and initial weights of cultured *Ulva lacinulata*.

8.2.4 Nutrient compositions of *Ulva*

Samples of *Ulva* from each treatment were collected at the start and end of culture cycle for nitrogen (N), phosphorus (P) and carbon (C) analyses. They were rinsed in distilled water to remove salt and visible epiphytes. The spun *Ulva* samples were later dried at 40 °C for 48 h and stored in a cool dry place after drying. The dried *Ulva* samples were later analysed for total tissue nitrogen, phosphorus, and carbon content using standard procedures described in Chapter 2 (Section 3.2.3) of this thesis.

8.3 Statistical analyses

The growth, yield, nutrient uptake efficiency, nutrient uptake rate, and nutrient compositions of *Ulva* from the three fertilisation protocols were compared for significant differences using one-way analysis of variance (ANOVA). All data were tested for homogeneity of variance using Levene's test, and for normal distribution of residuals by employing a Shapiro–Wilk plot. All analyses were performed using STATISTICA® software, version 13.2 (Statasoft, Tulsa, OK, USA).

8.4 Results

8.4.1 Water nutrients exiting macroalgae tanks.

The mean weekly water samples varied among the treatments during the production cycle of Ulva (Figure 8.1). The average nitrogen in effluent exiting the Ulva tanks that were fertilised with 100% mineral fertilisers was significantly higher than other treatments (one-way ANOVA $F_{(2, 3)}$ =11.625, p = 0.039; Figure 8.1a). Likewise, the 50% replacement of mineral phosphorus with biofertilisers significantly reduced phosphorus discharged from the culture system to the environment (one-way ANOVA $F_{(2, 3)}$ = 9.931, p = 0.047; Figure 8.1b).

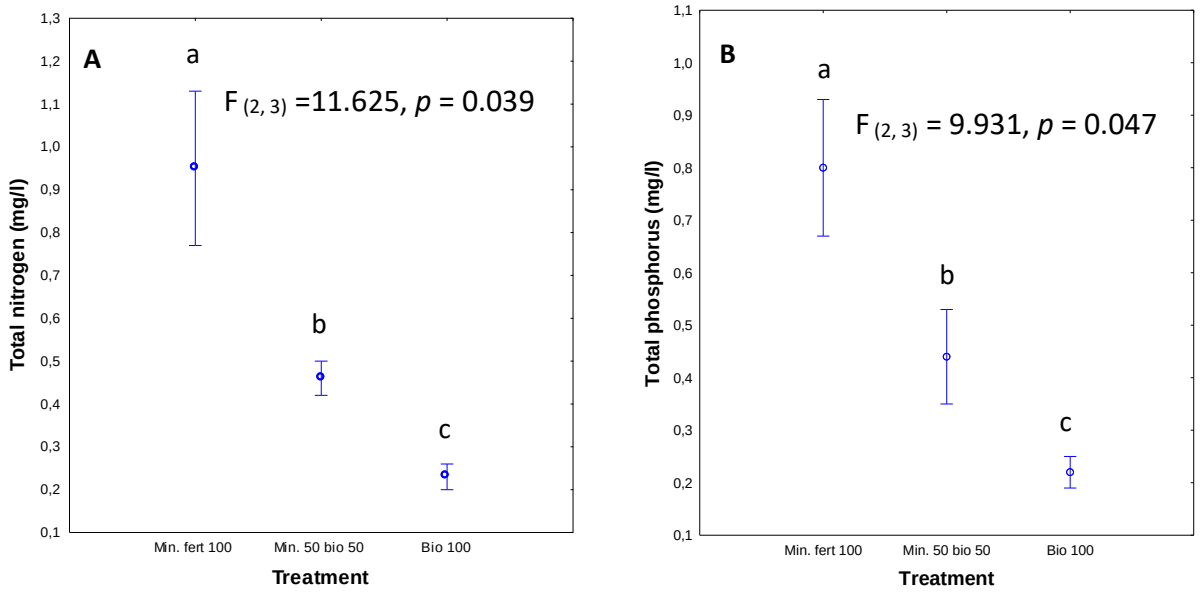
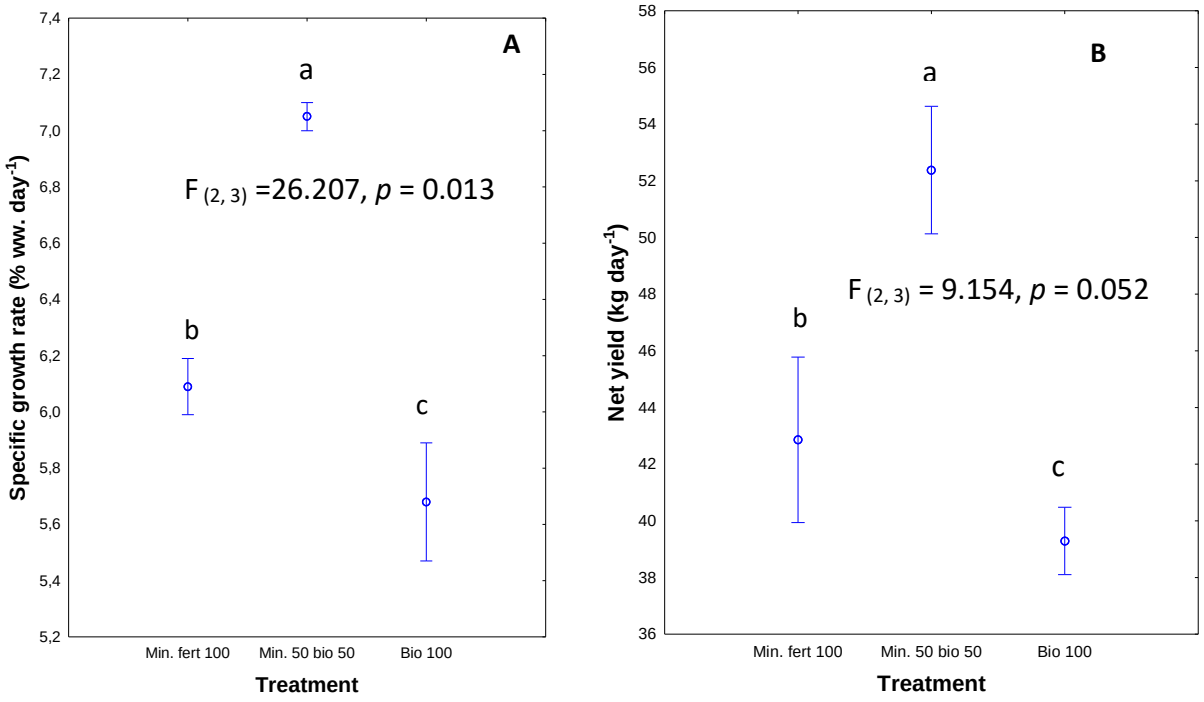


Figure 8.1. Mean (± standard error) (A) total nitrogen and (B) total phosphorus) of effluent exiting *Ulva* raceways subjected to 100% mineral fertilizers, 50% mineral fertilizers and 50% biofertilisers and 100% biofertilisers. Treatments with similar superscripts are not significantly different ($p > 0.05$).

8.4.2 Production performance of experimental *Ulva* species

The different fertilisation protocols affected the production performance of *Ulva* in this study (Figure 8.2). The highest growth of macroalgae was recorded for *Ulva* raceways fertilised with

50% mineral and 50% biofertilisers followed by those subjected to 100% mineral fertilisers and the lowest for the 100% biofertilizer treatment (one-way ANOVA $F_{(2, 3)}$ =26.207, p = 0.013; Figure 8.2a). Similarly, significantly higher yield of seaweed was recorded for the *Ulva* fertilised with 50% mineral and 50% biofertilisers (one-way ANOVA $F_{(2, 3)}$ = 9.154, p = 0.052; Figure 8.2b). Finally, the 50% replacement of mineral fertilisers with biofertilisers significantly improved the biomass gain of *Ulva* in this study (one-way ANOVA $F_{(2, 3)}$ =23.416, p = 0.015; Figure 8.2c).



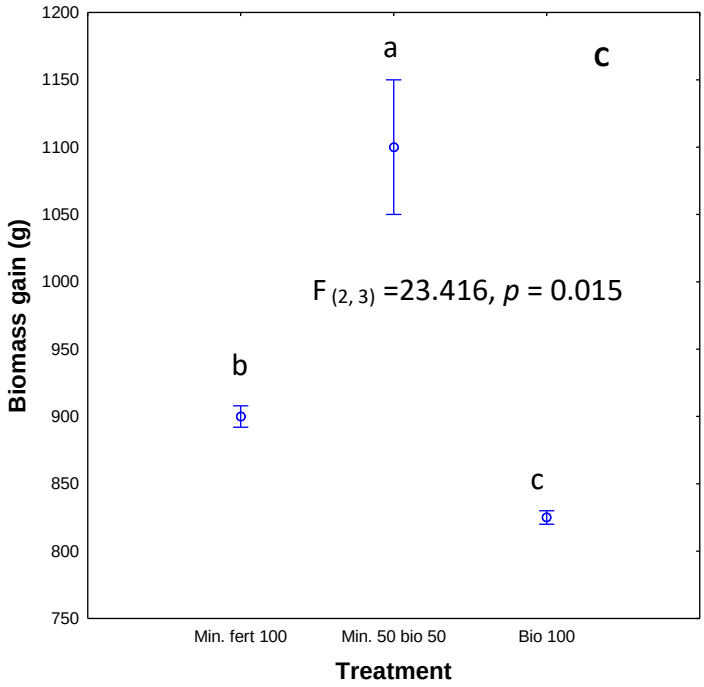


Figure 8.2. Mean (± standard error) (A) specific growth rate (b) net yield and (C) biomass gain of *Ulva lacinulata* subjected to 100% mineral fertilizers, 50% mineral fertilizers plus 50% biofertilisers and 100% biofertilisers.

8.4.3 *Nutrient composition of Ulva species*

The seaweeds that were fertilised with 100% mineral fertilisers or 50% mineral and 50% biofertilisers yielded similar nitrogen (one-way ANOVA $F_{(2, 3)} = 9.931$, $p = 0.047$; Figure 8.3a), phosphorus (one-way ANOVA $F_{(2, 3)} = 9.931$, $p = 0.047$; Figure 8.3b) and carbon (one-way ANOVA $F_{(2, 3)} = 12.135$, $p = 0.01$; Figure 8.3c) levels, which were higher than those fertilised with 100% biofertilisers.

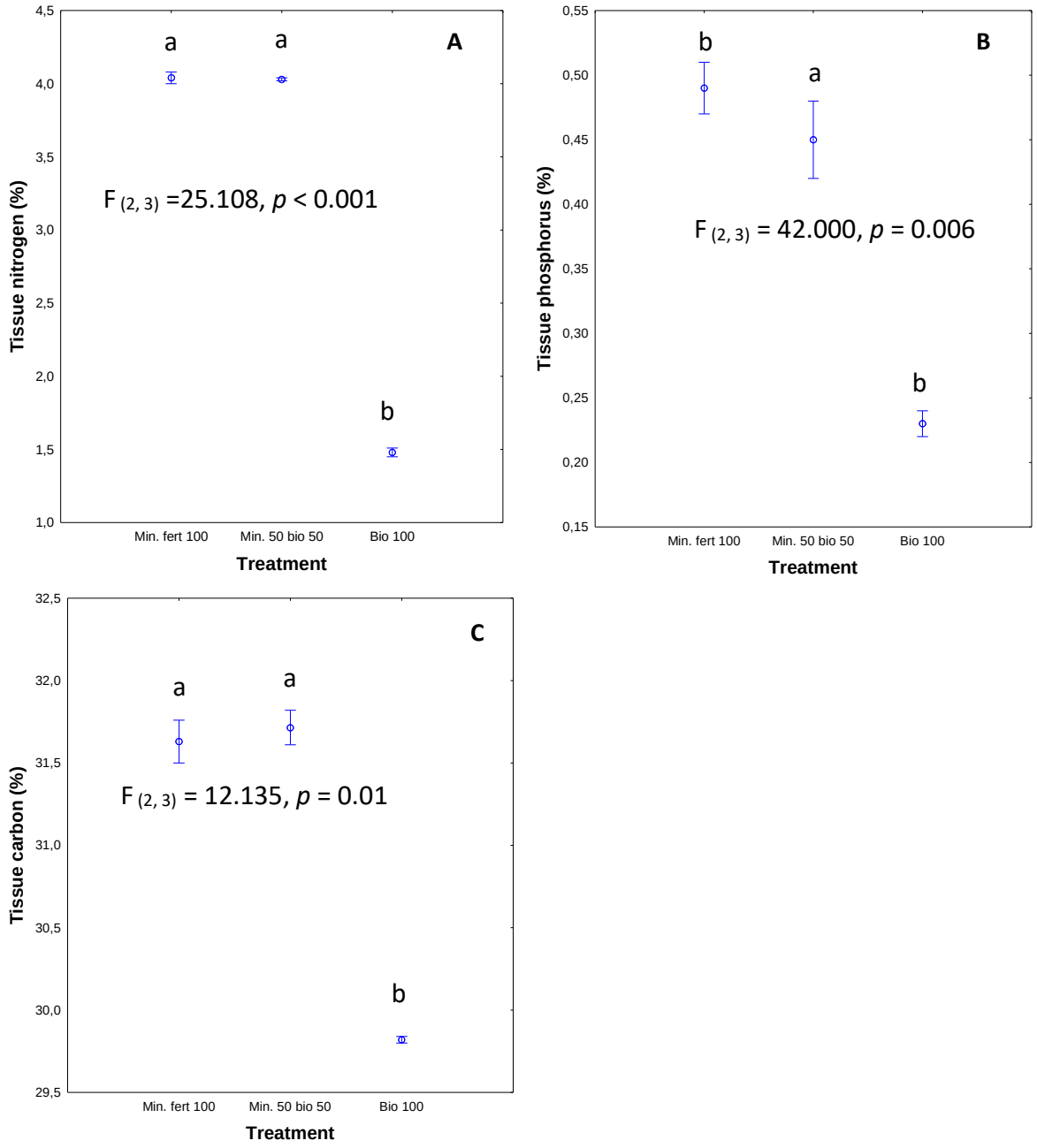


Figure 8.3. The mean (± standard error) tissue (a) nitrogen (b) phosphorus and (c) carbon of *Ulva lacinulata* subjected to 100% mineral fertilizers, 50% mineral fertilizers plus 50% biofertilisers and 100% biofertilisers. Treatments with similar superscripts are not significantly different ($p > 0.05$).

8.5 Discussion

8.5.1 *Nutrient composition of post-seaweed effluent*

The 50% substitution of mineral fertilisers with biofertilisers significantly reduced the concentrations of nitrogen and phosphorus in the post-seaweed effluent by 55% and 45% respectively while at 100% replacement, effluent nitrogen and phosphorus were reduced by 78.5 and 72.5% respectively. This further confirms that the use of biofertilisers is safe for the environment as they primarily comprise of nitrogen fixers, phosphate dissolvers and silicate bacteria (Shaheen *et al.* 2013). From the current study, it can be inferred that the pollution effect from seaweed production at Wild Coast Abalone could be further minimised by optimising fertilisation protocols. One of these ways is by substituting inorganic fertilisers with live microbial fertilisers.

This production process will also improve the economic performance of seaweed farms as less money is spent on fertilisers' application. Substituting 50% of mineral fertilisers with biofertilisers and adopting a new fertilisation protocol significantly reduced the cost of fertilisation at WCA by 41% of the initial cost (*Emmanuel Mapengo, seaweed production Manager, WCA*).

8.5.2 *Growth performance of Ulva under different fertilisation protocols*

The 50% substitution of chemical fertilisers with live microbial fertilisers produced the best growth and production performance of seaweed in the current study as approximately 15.74% growth increase was recorded over those fertilised with 100% chemical fertilisers. This agrees with previous findings which documented better performance of different plants fertilised with biofertilisers (El-Sabagh *et al*. 2011; Shaheen *et al.* 2013). For example, the application of Biogen

Rhyzobacterine and Microbene biofertilisers in combination with chemical N fertilizer increased the weight and yield of flame seedless and crimson seedless grapevines (El-Sabagh *et al*. 2011), while the partial substitution (50%) replacement of NPK mineral fertilisers with a combination of organic fertiliser, natural rock and biofertilisers improved the growth, yield, and fruit quality of superior grapevine (Shaheen *et al.* 2013). Additionally, the partial substitution of mineral nitrogen fertiliser with biofertilisers applied to Anethum graveolens plants resulted in improved vegetative growth, oil yield and nutrient (nitrogen, phosphorus, and potassium) composition of the herb (Hellal, Mahfouz and Hassan 2011). A study with *Ulva* species culture found that when 50% of the mineral fertilisers were replaced with multifunctional biofertilisers, an approximate 25% yield increase was recorded (Young *et al.* 2003). In the current study, the better growth performance of *Ulva lacinulata* fertilised with a combination of microbial and inorganic fertilisers may have resulted from the ability of biofertilisers to mobilise and increase the supply and availability of the main nutrients in mineral fertilisers to the seaweed (Hazra 2016). When the microorganisms in biofertilisers find an appropriate environmental condition for their growth, they become very efficient in dissolving nutrients in chemical fertilisers and making them available to cultured plants (Chen 2006). Also, supplementing mineral fertilisers with biofertilisers produces a more balanced nutrient supply which keeps plants healthy with an improved nutrient mobilization from both organic and inorganic sources (Roba 2018).

The lowest performance in terms of growth, productivity and yield of *Ulva* was observed for the seaweed fertilised with 100% biofertilisers. Previous study documented that nutrient input through biofertilization cannot totally substitute for mineral fertilisers as live-microbial fertilisers

are deficient in nutrients, hence the need to combine it with mineral fertilisers for optimum result (Chen 2006).

8.5.3 *Nutrient composition of Ulva lacinulata*

The partial replacement of mineral fertilisers with biofertiliser did not impact negatively on the nutrient composition of farmed seaweed as both treatments (100% mineral and 50% mineral plus biofertiliser) produced *Ulva* with similar nitrogen, phosphorus, and carbon compositions. This indicates that cutting down Wild Coast Abalone's use of urea, monoammonium and monopotassium phosphate mineral fertilisers by 50% will not affect the nutrient composition of the seaweed fed to farmed abalone if they are replaced with live-microbial fertilisers. This technique will not only reduce the discharge of dissolved waste nutrients waste to the environment but also improve the economic efficiency of the farm.

The total replacement of mineral fertilisers with biofertiliser may not produce seaweed with sufficient nutrient for farmed abalone as *Ulva* fertilised with 100% biofertiliser contained only 9.3% protein compared to the 35.3% protein of those subjected to 100% mineral fertilisers and 50:50 mineral and biofertilisers. Likewise, the phosphorus and carbon composition of *Ulva* subjected purely to biofertilisers were appreciably lower than other treatments. This shows that the best performance is achieved when 50% mineral fertilisers is combined with 50% biofertilisers.

8.6 Conclusion

From the current study, it can be inferred that the substitution of 50% inorganic fertilisers at WCA with live microbial fertilisers could potentially reduce the discharge of waste nitrogen and phosphorus to coastal environment by approximately 50% and 45% respectively. The inclusion of biofertilisers in the fertilisation protocol enhanced the uptake of inorganic nitrogen and phosphorus compounds. Additionally, this replacement level with not affect the production, growth and nutrient composition of cultured *Ulva*. Therefore, adopting this fertilization technique at Wild Coast Abalone could further minimise the footprint of the farm on the environment.

Chapter 9: Concluding discussion.

The present study aimed at improving the production performance of an integrated aquaculture system on a standard South African abalone farm, the Wild Coast Abalone Pty Ltd (WCA). The current state of the IMTA system in terms of nutrient utilisation efficiency and environmental performance was assessed in Chapter 2-4. The experiments in these chapters examined the factors responsible for nutrient production, the efficiency of nutrient utilisation by farmed organisms (abalone and macroalgae) and the overall impacts of the production techniques on the environment. In the later Chapters (5-8), the various techniques that could improve the IMTA system at WCA were investigated. These optimisation techniques included:

- the replacement of fish resources in abalone formulated diet with IMTA-grown macroalgae,
- the replacement of mineral fertilisers with live-microbial fertilisers and
- the use of abalone biodeposits for the production of another species of economic importance (sea cucumber).

Overall, the study achieved its aims and objectives and confirmed that the application of IMTA techniques improved farm production and environmental performance of Wild Coast Abalone and that there is further scope to enhance IMTA efficiencies. The novel contribution of this research and the implications of these findings towards improving the production and environmental performance of South African abalone farms as well as the considerations for future studies is discussed below.

Novel contributions of this research towards improving abalone farming in South Africa

The outcomes of the various on-farm trials conducted in the present research demonstrated the potential for improvements in the production efficiency of Wild Coast Abalone Pty Ltd and which can be replicated on other abalone farms in South Africa. For example, the low inclusion (< 6%) of farmed *Ulva species* meal in compound pellet for *H. midae* significantly improved their growth rate and nutrient utilisation efficiency. Additionally, using IMTA macroalgae meal in the diet formulation reduced fish meal inclusion level, thus potentially reducing the abalone farm's environmental impact.

Co-culturing detritus-eating sea cucumber with abalone reduced the production of detritus materials by abalone. The solid waste production experiment in Chapter 2 revealed that approximately 39.8% of the diet fed to *H. midae* at Wild Coast Abalone ends up as biodeposits on production tanks floor and flushed into the environment during tank cleaning events. However, the integration of local sea cucumber *Neostichopus grammatus* into abalone culture system reduced the daily discharge of accumulated waste solids from abalone tanks by 11.10% as the sea cucumbers utilised *H. midae* biodeposits as food. This was achieved despite the detritivores losing weight over the experimental period due to suboptimal culture conditions. This production technique if adopted on SA abalone farms could potentially reduce solid waste discharge from farms, lessen their impact on the environment and produce additional crop of economic importance for abalone farms.

Biofertiliser was tested as an alternative to chemical fertilisers in this research due to the low uptake of dissolved nutrients from inorganic fertilisers by farmed seaweed resulting in high levels of unabsorbed waste nutrients in post-seaweed effluent released to the environment. The discharge of nutrients from seaweed tanks was significantly reduced when the fertilisation protocol at Wild Coast Abalone was adjusted and 50% of the mineral fertilisers substituted with non-polluting, eco-friendly, biodegradable biofertilisers. This implies that the potential pollution effect through seaweed production at Wild Coast Abalone can be significantly reduced by 50% without affecting the production rate, yield, and quality of seaweed. In the light of the present results, Wild Coast Abalone management is considering adopting this technique as the new fertilisation protocol on the farm.

Impact of the current research on Wild Coast Abalone Pty Ltd

The present study was conceptualised with two industry partners (Wild Coast Abalone Pty Ltd and Marifeed Pty Ltd) and was conducted under real farming conditions, thus bringing about a direct transfer of technology. Through this study, the Wild Coast Abalone has been able to (1) make improvements to the seaweed production processes, (2) reduce the discharge of unabsorbed nutrients from chemical fertilisers to the environment by 50%, (3) reduce seaweed production cost as the farm saves money on fertilisation (p*er com. Richard Clerk, Managing Director of Wild Coast Abalone*), (4) make an improvement on the environmental footprint of the farm as 50% replacement of chemical fertilisers with ecofriendly biofertilisers reduces dissolved nutrients discharge to the environment. Similarly, when the result of the experiment on IMTA

seaweed meal inclusion in *H. midae* diet is adopted and fully commercialised by Marifeed Pty Ltd, the growth rate and nutrient utilisation of abalone will be improved.

Future research and considerations

In terms of future research arising from the present results, further work on using *Neostichopus grammatus* to capture detritus waste in abalone tanks is warranted despite the cultured animals losing 48% of their weight due to suboptimal culture conditions. It was subsequently found that providing a sand substate for the sea cucumbers enhanced their growth rate and survival (Senekal *et al.* in press). Thus, the co-culture of South African sea cucumber species and other detritus extractive organisms such as mullet and annelid worms and should be tested in future studies. The 38% growth increase recorded for abalone fed the 6% IMTA *Ulva* meal inclusion diet compared to those fed commercial abalone diet Abfeed® S34 indicates that effluent grown *Ulva* is a good potential dry feed ingredient and that further optimization trials are warranted. The present research clearly demonstrated the potential benefits of using biofertilisers to reduce chemical fertilizer usage and to improve performance and that follow-up research could yield further improvements. Lastly, the life cycle analysis has established a framework for monitoring the overall environmental performance of the farm and that it provides a useful tool for decision making to improve the farm's environmental footprint, particularly in respect of energy usage.

Conclusion

A takeaway lesson from the present series of experiments is that the integration of new IMTA species and circular economy techniques should not be regarded as a once-off intervention but

represents an ongoing developmental approach to improving abalone farming efficiency and environmental performance. Encouragingly, the Wild Coast Abalone Farm has integrated an ongoing IMTA based developmental approach into its business model as the economic and environmental benefits have been demonstrated, and the improvements are seen as reducing medium to long terms business risks.

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