Using *Ulva* (Chlorophyta) for the production of biomethane and mitigation against coastal acidification

Albert Oluwatobi Amosu

A thesis submitted in fulfilment of the requirement for the degree PhD in the Department of Biodiversity and Conservation Biology, University of the Western Cape.

Supervisor: Professor Gavin W. Maneveldt (University of the Western Cape)
Co-supervisors: Dr. Deborah V. Roberson-Andersson (University of KwaZulu-Natal)
Professor John J. Bolton (University of Cape Town)

June 2016

I declare that

“*Using Ulva (Chlorophyta) for the production of biomethane and mitigation against coastal acidification*”

is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

24th June 2016

Albert Oluwatobi Amosu Date

I dedicate this thesis to

the blessed memory of my mother

Princess Nimotalai Abiodun Akanke Adejumobi Amosu *GRD II, NCE, B.Ed (Hons), RSA (London)*

Abstract

In South Africa the green macroalga *Ulva armoricana* is the main species of macroalgae cultured. The species is currently the largest aquaculture (2884.61 tonnes) product by weight with a corresponding capacity for biogas (CH_4) production. We have shown that biotransformation of *U. armoricana* to Liquefied Petroleum Gas (LPG) is viable and economically feasible as a clean fuel. pH toxicity tests showed that *U. armoricana* can be used as a health index, under potentially increased CO_2 concentrations that can occur in IMTA carbon sequestration. We have shown sporulation to be the morphological response to environmental stress, which is indicative of chlorophyll degradation and a reduction in the photosynthetic activity of the alga. With the exception of Cadmium (Cd), the physico-chemical values obtained and the dissolved nutrient/heavy metals uptake by the alga all fell within the FAO/WHO permissible standards. Our Cd values therefore negate the use of these macroalgae for human consumption. We have also shown that *U. armoricana* can be used in eco-monitoring by playing a significant role in wastewater filtration and bioaccumulation. Nutrient utilization and proximate composition results show that African mud catfish (*Clarias gariepinus*) grow well on a protein-enriched *Ulva* diet, suggesting that enriched *Ulva* has the potential to be a successful fish feed. This thesis suggests among others, that South Africa could take advantage by being the first African country to propose specific standards for edible macroalgae as its successful research innovations and development provides a template for other African countries to further their aquaculture sectors. Additional benefits (bioremediation, ocean de-acidification through the capture of atmospheric and dissolved CO_2 during growth to assist in climate change mitigation) from *Ulva* farming activities bode well for the aquaculture industry.

List of Papers and Contributions by Authors

In addition to the General Introduction and General Discussion, this thesis is largely prepared as a series of articles (see below) by publication.

I. **A.O. Amosu**, D.V. Robertson-Andersson, G.W. Maneveldt, R.J. Anderson and J.J. Bolton (2013). South African Seaweed Aquaculture: A sustainable development example for other African coastal countries. *African Journal of Agricultural Sciences* 8(43): 5260-5271.

II. **A.O. Amosu,** D.V. Robertson-Andersson, E. Kean and G.W. Maneveldt (2014). Aquaculture benefits of macroalgae for green energy production and climate change mitigation. *International Journal of Scientific & Engineering Research* 5(7): 146-152.

III. **A.O. Amosu,** D.V. Robertson-Andersson, E. Kean, G. W. Maneveldt and L. Cyster (2016). Biofiltering and uptake of dissolved nutrients by *Ulva armoricana* (Chlorophyta) in a land-based aquaculture system. *International Journal of Agriculture and Biology* 18: 298-304.

IV. **A.O. Amosu,** D.V. Robertson-Andersson, J.L, Knapp, G.W. Maneveldt, L. Auersward and J.J. Bolton (under review). Environmental response and pH tolerance of induced CO_2 in *Ulva rigida* (Chlorophyta) under controlled conditions. *International Journal of Environmental Pollution*.

V. **A.O. Amosu,** A.M. Hammed, G.W. Maneveldt and D.V. Robertson-Andersson (under review). Comparative analysis of nutrient utilization and proximate composition of *Ulva armoricana* (Chlorophyta) and formulated feeds on the growth performance of African Mudcatfish *Clarias gariepinus* (Burchell) fingerlings. *Animal Nutrition and Feed Technology*.

It is important to note that the planning and preparation of the articles for publication is a process separate from the actual project (laboratory and fieldwork). Besides managaing the practical and theoretical components of the thesis, as the first author I was responsible for the data analyses presented in the papers, preparing the first working draft, accommodating all editorial comments by the supervisors (co-authors) of the project, submitting the manuscripts for peer-review, and revising the manuscript based on the peer-reviewed process. My supervisors and other co-authors on the project provided only editorial comments to an original version of the manuscripts. I therefore take sole and full responsibility for the quality and condition of the final accepted versions of these peer-reviewed papers that had been derived from my thesis.

TABLE OF CONTENTS

Chapter 1. General Introduction and Literature Review

Comparative analysis of nutrient utilization and proximate composition of *Ulva armoricana* (Chlorophyta) and formulated feeds on the growth performance of African Mudcatfish *Clarias gariepinus* (Burchell) fingerlings

Chapter 1

General Introduction and Literature Review

1.1 Background

Biofuel is a fuel produced through contemporary biological processes (such as agriculture and anaerobic digestion) rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels (coal and petroleum) from prehistoric biological matter (Hammerschlag, 2006). The first biofuel was derived from firewood, mainly for cooking and heating, during the evolution of early man (Sims *et al*., 2007). The history of modern biofuels, however, can be traced to the first generation biofuels produced in the late 1800's. These biofuels were derived directly from agricultural food products and included such crops as soybeans, corn, sugar cane/beet, wheat, cassava and sorghum (Prieler and Fischer, 2009; Kapazoglou *et al*., 2013).

One of the first inventors in 1876 to make use of ethanol as a biofuel was the German engineer Nikolaus August Otto who built the first internal-combustion engine (Winter, 2009). Still later in 1896 the American industrialist Henry Ford developed and manufactured the first automobile that middle class Americans could afford, namely the Model T automobile that ran on ethanol derived from a corn product (Ford and Crowther, 1922; Lewis, 1976; Bak, 2003; Ford, 2003; Howard, 2008). Later in 1900 the German mechanical engineer Rudolf Christian Karl Diesel invented and built the diesel engine, which was powered by peanut oil (Damirbas, 2009). With cheaper and more efficient fuel produced between 1903 and1926, Ford's Model T automobile was designed to use a hemp-derived biofuel as a second generation biofuel.

Second generation biofuels, also known as advanced biofuels, were used as early as 1900 (Specht, 2011). These are fuels that have already been used and are no longer considered to be dependable feedstock or fit for human consumption (food). Such fuels include: grasses like switch grass, myscanthus, and Indian grass; waste vegetable oil; municipal solid waste; human waste; and farm yard manure (Specht, 2011). Although it was not common practice, vegetable oils, for example, were used in place of diesel fuel during the 1930s and 1940s (Sarker, 2012). During World War II in particular, the high demand for biofuels was due to its increased use as an alternative fuel (Nag, 2007). During this period, countries like Germany underwent a major fuel shortage, prompting the introduction of various other inventions such as the use of gasoline along with alcohol derived from potatoes (Nag, 2007). Eventually, petroleum entered the picture and proved to be the most logical fuel source, based on supply, price and efficiency, among other considerations (Albrecht and Hallen, 2011). While the two world wars were the periods when the various major technological changes took place and when biofuels were widely in use, the periods of peace allowed for cheap oil from the gulf countries and the Middle East to be imported to ease off the pressure (Nag, 2007).

It was not until the 1970s and 1980s that the idea of using biofuels was revisited in the USA. During this period the USA promulgated the Clean Air Act (of 1970) by the Environmental Protection Agency (EPA) (Hammes and Wills, 2005). This Act allowed for more precise regulations of emissions standards for pollutants like sulphur dioxides, carbon monoxide, ozone, nitrogen oxides (NOx) and greenhouse gases. This period set the stage for developing cleaner-burning fuels and minimum standards for fuel additives. In addition, international pressures such as the 1973 - 1974 Arab oil embargo, the 1978 - 1979 Iranian Revolution, and a serious fuel crisis during 1973 and 1979 (because of geopolitical conflict with the Organization of the Petroleum Exporting Countries (OPEC) making a heavy fuel cut in exports to the non-OPEC nations), served to drive petroleum prices up (Hammes and Wills, 2005). With petroleum prices increasing, nations began to look elsewhere for alternate fuel sources.

With a steady increase in the human population and the associated increases in resource usage and requirements, renewable forms of energy to sustain increased production has become an inevitable challenge, and this has set the stage for the development of new biofuels. By July 2015 it was estimated that the world's population reached 7.3 billion people (United Nations Department of Economic and Social Affairs, 2015) all of whom require renewable resources for their existence. This demand is set to increase as only about 2.27 billion people worldwide currently have access to electricity (Bhattacharyya, 2006; Sims *et al.*, 2007; International Energy Agency, 2011; United Nations Environment Programme, 2012; World Energy Outlook, 2015). The ongoing market demand and constant fuel shortages, the fluctuations and increases in the price of Liquefied Petroleum Gas (LPG) (Figure1.1) (United Nations Environment Programme, 2010), the diminishing life span of oil reserves, and the emissions of greenhouse gases, have all been some of the reasons presented for the new-found interest in the use of biofuels as alternate energy sources.

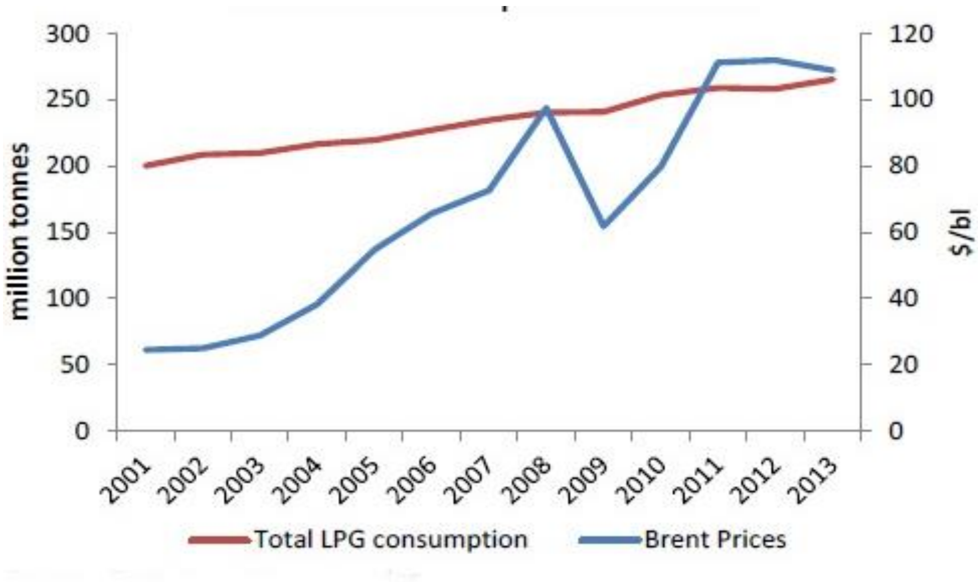

Figure 1.1: The consumption of Liquefied Petroleum Gas and the price of oil between 2001 and 2013 (Source: www.lngworldnews.com).

1.1.1 Macroalgae as an alternative fuel source

Diverse literatures have addressed a wide range of issues regarding conventional biofuel production ranging from crops for production to ethical use. The twentieth century saw investigations into a third generation biofuel derived from algae. Algae production is showing promise as the next generation feedstock, capable of much higher yields with lower resource inputs than other feedstocks currently used for biofuel production (Duffy *et al*., 2009). The lists of fuels that can be derived from algae include biodiesel, butanol, gasoline, methane, ethanol, vegetable oil and jet fuel (Duffy *et al*., 2009).

There are about 36,000 species of algae, with several species being exploited from the wild as the technology for their propagation is on the increase (Food and Agriculture Organization, 2006; Millar, 2009; Ralph, 2012), although significant strides have been made more recently (Butterworth 2010; Carl *et al.*, 2014). In the last 50 years, about 100 macroalgal species from the genera *Gracilaria*, *Euchema*, *Sachhraina*, *Laminaria*, *Undaria*, *Ulva*, *Chondrus*, *Porphyra*, *Palmaria* and *Monostroma* have been commercially cultivated (Zemke-White *et al*., 1999; Fleurence, 2004; Sahoo and Yarish, 2005; Bruton *et al*., 2009; Klaus *et al*., 2009; Mohammad *et al*., 2009; FAO, 2010; Paul and Tseng, 2012). Currently over 92 % of the world's macroalgae production comes from aquaculture species (Ozer *et al*., 2005; Chopin and Sawhney, 2009; Paul and Tseng, 2012).

The idea of growing matter to produce fuel has been controversial (Biello 2013). The production of algae for biofuel, however, does not require arable land needed for food production. Such production entails little land resources and does not compete with food production. In addition, algae can be grown on non-arable, nutrient-poor land that will not support conventional agriculture. Most commercial algal production is done in open waters. Furthermore, algae generally grow quickly at a large scale depending on species and can potentially generate up to 50 times more oil per acre than row crops like corn and soybeans, which produce vegetable oil (Duffy *et al*., 2009). Macroalgae generally have less growing costs than microalgae due to the surface area per unit of algae and may yield up to 20 % extracted oil per kg of dry matter (Aresta *et al*., 2004).

1.1.2 Global problem of increased CO_2

The earth's radiative energy balance is undergoing changes due to the increase in greenhouse gases, primarily CO_2 from fossil fuel combustion and from anthropogenic aerosols (Wuebbles *et al*., 2001). The long term trend of increasing atmospheric CO_2 has become a focal point in current research across atmospheric, terrestrial, and marine science disciplines (Le Treut *et al*., 2007). It is an established phenomenon that CO_2 emission is increasing geometrically in the atmosphere mainly through anthropogenic sources such as industrialization (Rapley, 2012). CO_2 emission is expected to rise by 20 billion tons/year by

2100 and will probably double by the next century (Philip *et al.*, 2010; Rapley, 2012) if CO_2 emissions continue unabated. The ongoing effects on global climate and the associated deleterious environmental effects that no doubt would result from such high carbon levels have led to much research interest. The impact of increased CO_2 may lead to deleterious environmental effects on the ecophysiology of aquatic 'plants' and the ecosystems they inhabit (Marchal *et al.*, 2011). Research have revealed that this expected increase in CO_2 will increase global warming through green house gases (GHG) with more environmental consequences before the 2050 projected date (IPCC AR4 SYR, 2007; Marchal *et al.*, 2011; Biron, 2012).

1.1.3 CO_2 Uptake in macroalgae

As bioremediators, marine algae are significant components of the carbon cycle of coastal ecosystems. Their responses to increased atmospheric CO_2 are therefore of considerable importance (Zou and Gao, 2002). Crops grown to produce biofuels take up a great deal of CO_2, and this is seen as a positive justification. Macroalgae grown for biofuel production, however, have a greater potential for reducing greenhouse gasses. Macroalgae absorb 50 % of tissue CO_2 (conversion of atmospheric CO_2 during growth) (Berger *et al.*, 1989; Mann and Lazier, 1991; Kativu, 2011) and produce CH_4 as a clean fuel under anaerobic conditions (Andersson *et al.*, 2009; FAO, 2009; Gouveia, 2011). Macroalgae fix CO_2 to create their biomass, thereby sequestering CO_2 (Gao and McKinley, 1994). Macroalgae are able to take up CO_2 for growth in varying conditions, both in fresh or salt-water bodies, and are tolerant of a diverse range of pH conditions (International Energy Agency, 1994). Macroalgae are capable of producing more biomass per square meter than any fast growing terrestrial plant and are the second-most cultured (*Saccharina japonica*) species of aquatic organisms after finfish (Briggs and Fung-Smith, 1993; Adams, 2011).

1.2 Macroalgae and their culture

Macroalgae are currently the most significant aquatic 'plants' that have contributed to the development of aquaculture (FAO, 2010). Presently 93.8 % of the total world macroalgae production is from aquaculture (FAO, 2011; 2012). Globally macroalgal biomass accounted for 23 % of the world aquaculture output in 2007 (FAO, 2012; Paul and Tseng, 2012) and represents close to half of the biomass of the world mariculture production (Chopin, 2007). Future supplies are even expected from countries without a tradition of macroalgae culture, these resulting due to improvements in culture techniques and genetically improved culture stock, and the development of improved culture techniques for new culture species (Tseng and Borowitzka, 2003).

Macroalgae aquaculture occurs in about 31 countries and comprises about 221 commercially exploited species, including 145 species for food and 101 species for phycocolloid production (Dhargalkar and Verlecar, 2009; FAO, 2012). The main genera of commercially exploited macroalgae include *Gracilaria* (and *Gracilariopsis*), *Eucheuma* (and *Kappaphycus*), *Saccharina* (previously *Laminaria*), *Undaria*, *Ulva*, *Chondrus*, *Pyropia* (previously *Porphyra*), *Palmaria*, *Caulerpa* and *Monostroma* (Zemke-White, *et al*., 1999; Fleurence, 2004; Bruton *et al.*, 2009; Klaus *et al.*, 2009; Mohammad *et al.*, 2009; Pia *et al.*, 2009; FAO, 2010b; Paul and Tseng, 2012). Currently the most cultivated macroalga is the Eucheumoids, which accounts for over 60 % of the total cultured macroalgae, with species from the genera *Pyropia*, *Kappaphycus*, *Undaria*, *Saccharina* and *Gracilaria* making up 99 % of the balance of all macroalgae cultured (Barsanti and Gualtieri, 2006).

1.2.1 Macroalgae in fish nutrition

As the global population continues to rise, the need for sustainable alternative sources of protein also increases (FAO, 2004a). It has been estimated that the worldwide requirement for food will increase to 50 % by 2030 (Tidwell and Allen, 2001). Juxtaposing the production input efficiencies of aquaculture against several fisheries and terrestrial agriculture systems, shows that non-fed aquaculture like macroalgal culture is amongst the world's most efficient mass producer of protein (Costa-Pierce *et al*., 2011).

Protein is the most expensive constituent of fish feed and global expenditure (7.05 million tonnes) exceeds US$1.12 billion (€1 billion) per annum (Hardy and Tacon, 2002; Hardy 2006; World Ocean Review, 2013). Fishmeal is a high-protein animal feed used extensively in aquaculture. It is made of mainly wild fish stocks and is used to feed farmed fish. The practice is considered unsustainable, as long as more fish is used in fishmeal and fish oil than is produced by farming (a calculation called FIFO). The ability of fishmeal supply to meet future demands is a global concern, especially given that aquaculture production is growing at a steady rate of nearly 9 % per annum, a growth that is unmatched by the supply of fishmeal and fishoil from capture fisheries (FAO, 2009; 2012). Macroalgae have the potential to replace a fraction of the demand for these ingredients, as they are relatively underexploited, contain comparatively high amounts of protein and can be cultured in a sustainable, environmentally-friendly manner that has the potential to increase their protein (Omega 3 lipids) contents.

The nutritional properties of macroalgae are both unique and interesting (MacArtain *et al.*, 2007; Madhusudan *et al.*, 2011; Sebaaly *et al.*, 2012). The biochemical quality and diversity of some macroalgae may have advantages with respect to fish nutrition, compared with some microalgae and terrestrial crops as they contain all the essential amino acids (Brown *et al.*, 1997; Wong and Peter, 2000; Ortiz *et al.*, 2006; Dawczynski *et al.*, 2007). Protein contents resported for macroalgae typically range from roughly 18 to 47 % (Černá, 2011; Samarakoon and Jeon 2012; Cyrus *et al.*, 2014a).

1.2.2 Macroalgae in sustainable aquaculture production

Macroalgae have contributed to the development of fisheries and the aquaculture industry (FAO, 2010). The world production has been dominated by macroalgae farmed in brackish and marine waters (FAO, 2003; 2012). Macroalgae are among the fastest growing photosynthetic organisms and are available all year round in multitrophic aquaculture. Macroalgae produce more photosynthetic efficacy per square metre (6 – 8 %, average) than any fast growing terrestrial plant producing as much as 1.8 – 2.2 % more biomass than terrestrial crops (Halford and Karp, 2011). Since 1970, the production of aquatic 'plants' (macroalgae and angiosperms) worldwide has increased consistently at a rate of 7.7 % per annum, with about 93.8 % of the total world macroalgal production being from cultivation (McHugh, 2001; FAO, 2003; 2009; 2010; 2011). This figure is higher than any other group of marine organisms (FAO, 2010; 2012). The production of macroalgae reached 19.9 million tonnes in 2010, of which aquaculture produced 19 million tonnes with a total market value estimated at US$5.7 billion (FAO, 2012).

1.2.3 Macroalgae *in Integrated MultiTrophic Aquaculture (IMTA)*

IMTA systems combine the cultivation of fed aquaculture species (e.g. finfish / shrimp) with that of organic extractive aquaculture species (e.g., shellfish) and inorganic extractive aquaculture species (e.g. macroalgae) for more balanced ecosystem management (Chopin and Robinson, 2004). This method of polyculture comprises organisms from several trophic levels in the same system under a manageable ecosystem in which each species utilizes the waste products or biomass generated by members from the other trophic level(s) (Nobre *et al*., 2010). An important factor to consider in IMTA design is that all of the individual components within this system must be marketable for the system to be commercially viable (Chopin *et al*., 2008).

The general benefit from IMTA (reduction of nutrient release to the environment) is also true for integrated macroalgal culture. Macroalgae act as bio-accumulators, bio-indicators and bio-detectors of pollutants (pesticides and heavy metals) such as Fe, Cu, Mn, Zn, Pb and more importantly Cd that reduces the rate of photosynthesis inducing a loss in pigment production as a result of the pigment reduction (Ho, 1990). Macroalgae grown in fish and shellfish wastewater have been shown to have increased nitrogen content, resulting in value-added seaweeds with often over 40 % protein dry weight content (Cohen and Neori, 1991; Lawrence and Shpigel, 2005; Hammer *et al*., 2006; Abreu *et al*., 2011). IMTA with *Glacilaria vermiculophylla* productivity and nutrient removal performance of the macroalgae in a land based pilot scale system showed excellent quality feed for shellfish (Jones and Iwama, 1991; Feldman *et al.*, 2000; Han *et al.*, 2001; Newell *et al.*, 2002). Several studies have demonstrated the technical and economic feasibility of using macroalgae as biofilters for excess ammonium removal (90 % removal efficiency) and other inorganic nutrient in IMTA units (Cohen and Neori, 1991; Shpigel and Neori, 1996; Shpigel *et al.*, 1997; Msuya, 1998; Neori *et al.*, 1998; Alieth, 2008). These studies have shown that macroalgae have a positive impact on moderately eutrophic water by absorbing nutrients from the surrounding waters.

1.3 The genus Ulva

Ulva is a cosmpolitan genus of green algae that has been used in aquaculture production (BenAri *et al.,* 2014). With the current technology and extensive available sea areas, requiring little to no terrestrial land, freshwater or fertilizers, *Ulva* production is set to expand rapidly and sustainably to the scale of agriculture (Radulovich *et al*., 2015).

1.3.1 Taxonomy

The green algal genus *Ulva* belongs to the order *Ulvales*, phylum Chlorophyta, family Ulvaceae, class Ulvophyceae (Hoek *et al*., 1995; Guiry and Guiry, 2013). The genus was described by Linnaeus in 1753 (Guiry, 2013; Guiry and Guiry, 2013). Members of the genus are distributed worldwide in all oceans and estuaries (Guiry and Guiry, 2015. *Ulva* are thin flat green algae growing from a small discoid holdfast that may reach 18 cm or more in length, though generally much less, and up to 30 cm across. Thalli are one cell thick, soft and translucent. Several taxonomic works have been done in the 60 – 70s on morphological and anatomical features, which created confusion for proper identification of *Ulva* species. However, the need to be exact and identify species based on individual molecular properties is inevitable (Prasad *et al.,* 2009; Bast *et al.,* 2014). Nowadays phycologists utilize the genetic information in individual identification as used in RAPD – PCR technique to establish a unique paternity testing from DNA band profiles (Dutcher and Kapraun, 1994; Yong *et al*., 2000; Prasad *et al.*, 2009). According to Prasad *et al.* (2009) RAPD – PCR is useful in assessing the classification of wild populations, determining relationships between species with the genus *Ulva* and for developing individual fingerprints.

There are 8 families in the order Ulvales containing 24 genera and 175 species. The family Ulvaceae has 11 genera including *Ulva* (Hoek *et al*., 1995). There are currently 557 species of *Ulva* listed in the AlgaeBase database including synonyms, of which only 100 are taxonomically accepted (Guiry and Guiry, 2015). Recent research, however, has concluded that many species from the genus may show a high degree of phenotypic plasticity as well as ecotypic variation (Silva *et al.*, 1996; Lobban and Harrison, 1997; Lee, 1999), which could explain some of the misconceptions in the taxonomic classification of specimens from the genus *Ulva*. The taxonomy of the genus *Ulva* is therefore not stable and it is currently not possible to assign specimens of *Ulva* grown in aquaculture systems without molecular sequencing (Cyrus *et al*., 2014b).

Morphological (e.g. size, branching, colour, texture) and anatomical (e.g. cell size, gradation of cell, cell size and shape) characteristics have mostly been used to identify species of *Ulva* (Critchley, 1993; Silva *et al.*, 1996). Consequently (due to their phenotypic plasticity) incorrect identification of several specimens has been inevitable (Lobban and Harrison, 1997). What complicates matters even further is that many cytological and morphological characters used in the identification of species are not always consistently applied (Steffensen, 1976; Bird *et al.*, 1982; Tanner, 1986; Critchley, 1993; Woolcott and King, 1999).

Currently developmental patterns in culture, reproductive characteristics (e.g. development of both female and male gametangia and post fertilization events) and the inability of species to interbreed, have been used to separate species from another (Critchley, 1993; Guiry and Guiry, 2013). However, some studies have shown that even these characteristics can vary across several different abiotic and biotic factors. As with most taxonomic studies, molecular data is proving far more useful in distinguishing between specimens of seemingly similar species, and the nuclear ribosomal DNA internal transcribed spacer (ITS) sequence is proving most useful for the accurate identification of species within the Chlorophyta (Hoek *et al*., 1995; Blomster, 1998; Coat *et al*., 1998; Malta *et al*., 1999).

1.3.2 Ecology, distribution and habitat

Species from the genus *Ulva* are found in marine and estuarine habitats, and occur mostly from the upper to mid-intertidal zones, but also in the sub-tidal zone (South and Whittick, 1987; Sze, 1993; Adams, 1994; Blomster *et al.*, 1998; Lee, 1999). A single species of freshwater *Ulva*, *U. limnetica*, has been recorded from the Ryukyu Islands, Japan (Ichihara *et al*., 2009). Species of *Ulva* are generally epilithic, but may also be entirely epiphytic (e.g. *U. rhacodes*, Stegenga *et al*., 1997). Most epilithic species, however, are often also found to be epiphytic, epizoic and free-living (Littler and Littler, 1999). Species of *Ulva* are prolific where waters are rich in nutrients (around outfalls that introduce organic matter), and where wave action and herbivore densities are low (South and Whittick, 1987; Vermaat and Sand-Jensen, 1987; Sze, 1993; Lee, 1999). Temperature generally determines growth and reproduction and this in turn influences their biogeographical distribution (Littler and Littler, 1999). While many factors influence the local distribution of species, their tolerance of desiccation and temperature stress more notably determine their capacity to occur higher up the shore than most other species of macroalgae (Bolton, 1986; Vermaat and Sand-Jensen, 1987; Breeman, 1988; Fong and Zedler, 1993; Poole and Raven, 1997; Fong *et al.*, 1998).

1.3.3 Life history

The ability of species from the genus *Ulva* to dominate the intertidal zone is coupled with an affinity to grow opportunistically on a range of substrates; they are also green tide forming species (Littler and littler, 1981; Niesenbaum, 1988). Species from the Ulvales colonise substrates through the production of large numbers (10^5 - 10^6 per thallus per day) of microscopic free-swimming zoospores (Maggs and Callow, 2003). The zoospores are naturally pyriform in shape and ~5 µm long with four anterior flagella (Figure 1.2) that disappear upon adhesion to a substrate (Hoek *et al*., 1995). In the wild, individual specimens take about 4 months to reach maturity (Oza and Rao, 1977). In warmer months or seasons, however, especially when farmed in high temperature media, production of zoospores and gametes increase (Niesenbaum, 1988).

The life cycle of *Ulva* species is characterized by a free living diploid sporophyte and an identical-looking free living haploid gametophyte. For this reason the life cycle it is often referred to as isomorphic and diplohaplontic because the two free living stages are morphologically identical (Searles, 1980; Tseng, 1987; Phillips, 1990). The two free living generations are multicellular and reproduction is more often vegetative (Hoek *et al.*, 1995) (Fig. 1.2). Parthenogenesis (whereby gametes develop into parthenosporophytes) is also common amongst members from the family Ulvaceae (Hoek *et al*., 1995). Under this scenario about 1 – 2 % of all gametes mature directly into gametophytes of the parent stock (Tanner, 1981, Park *et al*., 1998).

Growth, reproduction and spore germination/sporulation anatomy is determined by ecological factors such as temperature, light intensity, tidal levels, nutrients and other endogenous factors necessary to complete its life cycle (Dring, 1988; Adey and Hayek, 2011; Agrawal, 2012). Spore release is often augmented by tidal regime (e.g. as observed in *U. lobata*) (Smith, 1947; Christie and Evans, 1962; DeBusk *et al.*, 1986; Lüning, 1990; Lüning *et al.*, 2008). Still many species of *Ulva* can prolong the release, dispersal and viability of their spores and gametes (Reed *et al.*, 1988; Duke *et al*., 1989; Pedersen and Borum, 1997).

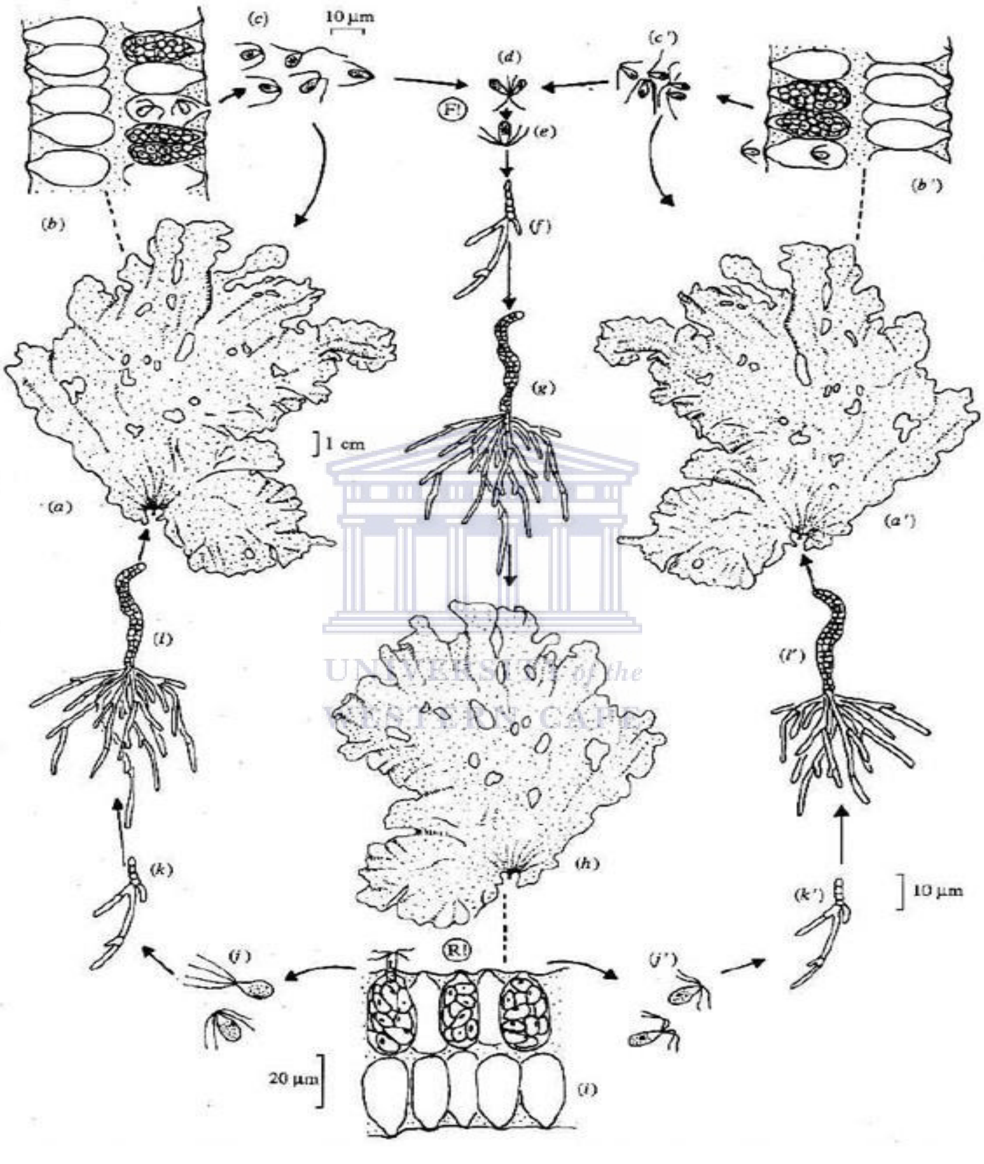

Figure 1.2: The life cycle of the genus *Ulva*. (*a*, *a'*) Flat blade-like gametophytes. (*b*, *b'*) Division of the cell contents into biflagellated gametes; these are unequal, copulation being anisogamous. (*c*) Female gametes. (*c'*) Male gametes. (*d*) Anisogamous copulation. (*e*) Quadriflagellated planozygote. (*f*) Uniseriate filamentous germling of the sporophyte generation attached via branched rhizoids. (*g*) Tubular germling of the sporophyte generation. (*h*) Developed blade-like sporophyte (diploid). (*i*) Meiotic division of sporophyte cells to form haploid quadriflagellate zoids (meiospores). (*j*, j') Quadriflagellated meiospores. (*k*, k') Uniseriate filamentous germlings of the female and male gametophytes. (*l*, *l'*) Tubular germlings of the female and male gametophytes. F! = fertilization; R! = reduction division (meiosis). Source: Hoek *et al.* (1995).

1.4 Commercial use and value of *Ulva*

Species of *Ulva* were generally consumed as salads or in soups (FAO, 2002). According to research, people living in areas where large quantities of macroalgae (including species of *Ulva*) were consumed were reported to have lived longer and had lower incidences of hypertension and arteriosclerosis (Tietze, 2004). Globally Japan, Korea and China rank as the major producers as well as the principal consumers of *Ulva* (Abowei and Tawari, 2011). According to the FAO (2012), species of *Ulva*, like most vegetables, are among the healthiest foods on the planet, containing essential vitamins, minerals and antioxidants. For example, natural *U. lactuca* comprises 3 – 27 % protein, 50 % sugar and starch, less than 1 % fat, is 11 % water when dried, and is useful as roughage in the human digestive system (Alzaablawy, 2005; Tacon *et al*., 2009). Furthermore, many species of *Ulva* are high in iron, protein, iodine, aluminum, manganese and nickel, and contain vitamins A, B1, C, sodium, potassium, magnesium, calcium, soluble nitrogen, phosphorous, chloride, silicon, rubidium, strontium, barium, radium, cobalt, boron and various trace elements (Alzaablawy, 2005; White and Keleshian, 1994; FAO, 2003).

1.4.1 Utilization as a feed

An over dependence on fish meal and fish oil may have threatened the aquaculture industry (Naylor *et al.*, 2001; Wijkström, 2009; FAO, 2011). This realization has prompted investigation into other cheaper and non-human competing food ingredients as alternative feedstuffs for protein and energy sources in fish diets (Behnassi *et al.*, 2011). Macroalgae with a high protein level can readily be used in the production of fish feed. Several research investigations have shown that 5 - 15 % *Ulva* meal can be included in the diets of fin fish (e.g. carp, mullet, channa, sea bream, sea bass, tilapia), shellfish (e.g. abalone, sea urchin, shrimp) (Nakagawa *et al*., 1987; Hashim and Mat Saat, 1992; Hashim and Hassan, 1995; Mustafa *et al.*, 1995; Wassef *et al.*, 2001; Azad *et al.*, 2002; Valente *et al.*, 2006; Francis *et al.*, 2008; Cruz-Suarez *et al.*, 2009; Azad and Xiang, 2012) and farm animals (Ventura and Castarion, 1998; Heuze *et al.,* 2016).

1.4.1.1 Ulva monoculture

Monoculture is the culture of a single species of fed or extractive aquatic organisms (fauna or flora) in a culture system of any intensity be it in any type of water body (fresh, brackish or marine). The environmental and economic consequences of monoculture are better understood when the organisms cultured are categorized into fed and extractive species (Chopin *et al.,* 2001; Neori *et al*., 2004). Fed organisms such as fin and shellfish are nourished by man-made aquafeed. Extractive organisms like macroalgae, as the name implies, extract their nourishment from the water body or from the cultured environment. It has been estimated that the monoculture of *U. lactuca,* grown in an area of about 180,000 km^2 could produce enough protein for the entire world population (Plant life, 2010). Paddle wheel ponds, which move suspended algae along raceways, can produce *Ulva* in large quantities (Chopin *et al*., 2008; Butterworth, 2010; Amosu *et al.,* 2015). The monoculture of *Ulva* species is mostly done for feed and prevalent in Asia, South America, South Africa and East Africa (Chopin *et al.*, 1999; Buschmann *et al*., 2001; Tseng, 2001; Amosu *et al.,* 2015).

1.4.1.2 Polyculture

Polyculture, as the name implies, is the culture of several species of aquatic fed or extractive organisms in the same water body. Polyculture began in China more than 1000 years ago (NACA, 1989) and the practice has spread throughout Southeast Asia and into other parts of the world. The polyculture of *Ulva* species began as early as 1991 in Israel in which the macroalgae grew in fishpond effluents, sometimes culture alongside bivalves, abalone and macroalgivores (Neori, 1991; Shpigel and Neori, 1996). Since then, several studies (e.g. Smith and Renard, 2002; Tseng and Borowitzka, 2003; Teas 2005; Abowei and Tawari 2011; Ihsan 2012; Boxman 2013) explored increasingly complex polyculture systems.

Common to these studies is the improvement by the polyculture with *Ulva* in the quality of the water by adding O_2, and removing excess CO_2 and nutrients, improving the water quality so that recirculation into the fed component of the polyculture system is possible (Troell *et al*., 2003; Shpigel and Neori, 2007; Butterworth 2010; Kirkendale *et al*., 2010; Ihsan 2012; Nirmala *et al*., 2012).

1.4.1.3 Macroalgae in Integrated MultiTrophic Aquaculture

Macroalgae-based integrated aquaculture systems improve water quality and environmental conditions by extracting nutrients, and have the potential to yield additional profits from macroalgae production (National Academy of Agricultural Sciences 2003; Robertson-Andersson, 2007; Abowei and Tawari 2011; Fakoya *et al*., 2011). Integrating optimal macroalgae species into an aquaculture system improves its sustainability and environmental friendliness. Biofiltration by *Ulva* species adds to the assimilative capacity of the aquaculture environment (Troell *et al*., 1999; Chopin 2001; Neori *et al*., 2004; Troell, 2009; Ihsan 2012). For example, *Ulva* species for use in an integrated aquaculture system must involve consideration of both their economic value (e.g. marketable species) and their biofiltration capacity (e.g. nutrient uptake rate, growth rate, and tissue nitrogen concentration) (Nobre *et al*., 2010; Neori *et al.*, 2004). The choice of *Ulva* species depends on a comparison between the ecophysiological characteristics of the species and the environmental conditions present in the cultivation area (Kang *et al*., 2008).

1.4.2 Methanogenic potential

Biomass, such as algae can generate biogas by anaerobic digestion, the degradation of organic material by bacteria in the absence of oxygen. When the process is controlled in digesters the biogas (60 % methane) can be trapped and used to produce electricity, or compressed and used as a transport fuel just like compressed natural gas (Kelly, 2012). Methane production in species from the genus *Ulva* varies between 163 - 227 ml CH_4 g^{-1} VS (Sludge volume) with similar properties and chemical composition as LPG (Habiq *et al.*, 1984; Briand and Morand, 1997; Bruhn *et al.*, 2011). Still other studies showed CH_4 yields in the range of 180 - 330 ml CH_4 g-^{1}, which is comparable to the yields obtained for livestock manure or medium yield energy crops (Briand and Morand, 1997; Bruhn *et al.*, 2011).

The methanogenic potential of fresh and macerated *U. lactuca* yielded up to 271 ml of CH_4 g^{-1} VS, which was similar to the methane production from livestock manure and terrestrial agricultural crop (Bruhn *et al.*, 2011). Sarker *et al.* (2012), in an experiment using *U. lactuca* and the brown seaweed *Laminaria digitata* as a co-digester with cattle manure, found that *U. lactuca* yielded about 122 ml of CH_4 g^{-1} VS. This latter study demonstrated that *Ulva* species are interesting substrates for use as gaseous biofuels through anaerobic co-digestion.

1.5 *Ulva* cultivation techniques

Species of *Ulva* are opportunistic in nature due to their simple life cycle (Budd and Pizzola 2008; Prue, 2009; Romero 2009; Carl *et al*., 2014), their ability to take up nutrients quickly (Santelices and Ugarte, 1987; Costa-Pierce *et al.* 2011, Klinger and Naylor 2012, Boxman, 2013, Redmond *et al.* 2014), their competitiveness relative to other algal species (Burges Watson, 1999; Amosu *el al.*, 2013), and their comparatively fast growth rate (Pádua *et al.*, 2004; Critchley and Ohno, 1997; Ben Chekroun *et al*., 2013). These characteristics make *Ulva* species suitable aquaculture candidates for large scale cultivation. In nature, seasonality influences their growth, with summer production being higher due to higher water temperatures and higher light intensities (Menendez *et al.*, 2001). Increases in the market demand for species of *Ulva* as food and feedstock, and bioremediation of coastal effluent, have largely been the reason for studies into their cultivation (Darcy-Vrillon, 1993; Davies *et al*., 1997). To produce a large quantity of biomass that is economically feasible and reliable in terms of quality and quantity, a clear understanding of the biology (growth, reproduction and recruitment), productivity and the environmental conditions necessary for optimum growth is of paramount importance (Lobban and Harrison, 1994). Depending on the growth responses (w.r.t. light, temperature, salinity, flow rate, pH, nutrient levels, etc.) different cultivation techniques have been employed for different species of *Ulva* (Lobban and Harrison, 1997; Raffaelli *et al*., 1998 Taylor *et al*., 2001; Raven and Taylor, 2003).

1.5.1 Open water cultivation

This is a seawater nutrient enriched method. Here a porous cylinder containing fertilizer is placed in a basket or the basket is placed in a waste stream together with *Ulva*. Several baskets are often strung together to form a raft. The fertilizer containers are replaced periodically (Bardach *et al.*, 1972).

1.5.2 Land-based or semi-closed cultivation

The principal advantage of land-based or semi-closed cultivation is that overall control is possible through integration of all system components (living and non living components). Here the growth rate can be regulated by environmental parameters that include aeration, pH, epiphyte/grazer/stocking density, water flow rate, irradiance, salinity, temperature, nutrient supply and carbon supply (Duke *et al.*, 1989; Lüning, 1990; Critchley, 1993; Lobban and Harrison, 1994). The fast growth rates and broad geographical distribution of *Ulva* species make them ideal candidates for bioremediation on land-based aquaculture facilities (Soto 2009; Lawton *et al*., 2013; Cyrus *et al*., 2015). Land-based or semi-closed cultivation systems can be well managed to prevent the problems (e.g. ability to withstand the strong drag forces of open oceans, exposed nature of the Open Ocean and economic challenges) associated with offshore *Ulva* mariculture (Shpigel and Neori, 1996, Troell *et al*., 2009). Some of the associated disadvantages to semi-closed cultivation include: available and suitable terrestrial land space; a capital intensive system characterized by available technology, water, pest and disease control; etc. (Lutz *et al.*, 2009). Epiphytic growth is probably the single greatest problem facing this type of *Ulva* cultivation technique (Ryther, 1977; Fletcher, 1995).

1.5.2.1 Pond cultivation

Pond cultivation can be classified into intensive and non-intensive cultivation systems. Non-intensive ponds are basically out door, uncovered, and are often without an artificial water agitation system (such as aeration pipes), while intensive cultivation ponds are made of a hard surface (concrete, plastic, etc.) and have a water agitation system (Friedlander and Levy, 1995; Capo *et al.*, 1999). The advantages of intensive culture methods include: high yield; the ability to have control over the operations; and the *Ulva* can act as biofilters for various effluents. Some disadvantages, however, include their high operational costs (including the pumping of seawater) and the costs of fertilizers (Boyd, 1990; 1998; Friedlander and Levy, 1995). Apart from epiphyte growth, the major problems include the burial of algal thalli in low oxygen sediments, large temperature and salinity fluctuations in shallow ponds, and low water movement (Boyd, 1998; Oliveire *et al.*, 2000). In such systems, *Ulva* ponds have been used as bioreactors in which both nitrogen assimilation and denitrification processes occur simultaneously under low aeration regimes, with substantially lower operational costs that considerably offset the reduced *Ulva* yield (BenAri *et al*., 2014). Filamentous species of *Ulva* are particularly ideal for pond cultivation because they are robust with high growth rates (Hayden *et al*., 2003; Msuya 2007; Carl *et al*., 2014).

1.5.2.2 Tank culture

Tank cultivation is characterized by high yield, with its efficiency dependent on aeration, water quality, flow rate, etc. (Kepenyes and Váradi, 1984; Masser *et al.*, 1999; Ozigbo *et al.*, 2014; Makkar *et al.*, 2015). Tanks are usually made from treated wood, concrete or PVC plastic, and fiberglass, with capacities ranging from a few hundred litres to several thousand cubic metres (Oliveire *et al.*, 2000). Even though this cultivation technique is a capitally intensive venture, the input is usually relatively quickly recovered if the system is well managed (Critchlery, 1993; Oliveire *et al.*, 2000).

In tank culture, the shape of the tank is very important as it is designed to influence water movement during aeration. To this end, several research works have used species of *Ulva* to demonstrate various physicochemical characteristic in response to tank cultivation (Morgan and Simpson, 1981; Rosenberg and Ramus, 1982; Hanisak, 1983; Wallentinus, 1984; Thomas and Harrison, 1985; Duke *et al.*, 1986; 1989; Lüning, 1990; Vandermeulen and Gordin, 1990; Critchley, 1993; Lobban and Harrison, 1994; Jiménez *et al.*, 1995; Braud and Amat, 1996; Flores-Moya *et al.*, 1997; Dawes 1998; Stitt and Krapp, 1999; Talarico and Maranzana, 2000; Tisserat, 2001; Villares *et al*., 2002; Mata *et al.*, 2003; Hong Yan *et al*., 2008; Nagle *et al.*, 2009; Mata *et al.*, 2010; Roleda *et al.*, 2010; Markelz *et al*., 2013). *Ulva* culture tank shapes are generally either U-shaped (with flat or round floor) (Neori *et al*., 2003; Robertson-Andersson 2007) or V-shaped (with a tapered floor) (Critchley, 1993; Hanniffy and Kraan, 2006). Aeration is provided by means of perforated PVC pipes secured to or embedded in the bottom of the tank (Figure: 1.3).

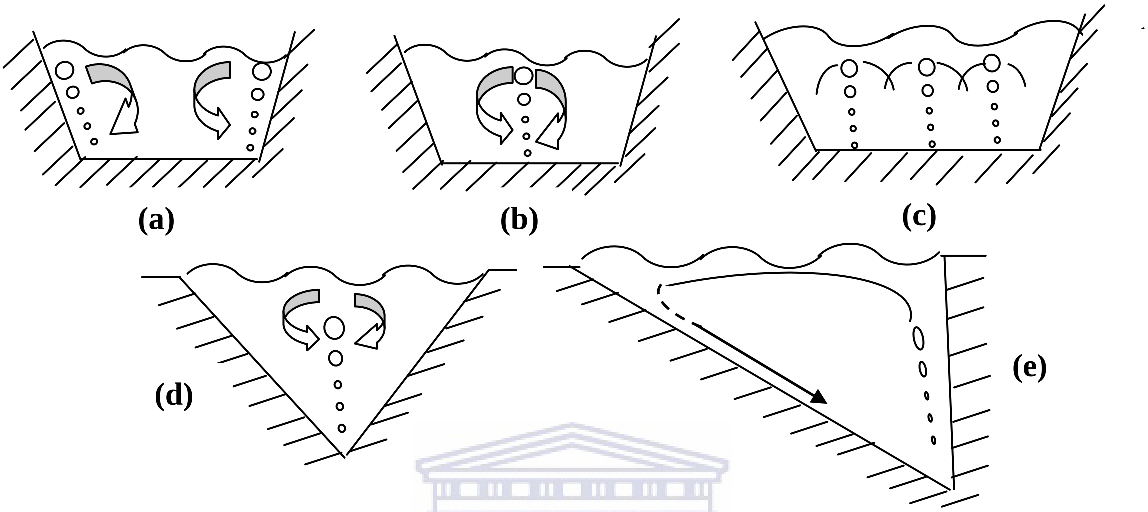

Figure 1.3: U- (a-c) and V- (d; e) shaped tank configurations with differential aeration to aid with circulation (Source: Critchley, 1993).

1.5.2.3 Raceways, spray cultivation and very high-high–intensive systems

Raceway cultivation is based on the continuous flow of water. Here the stocking density can be increased with greater flow rates. Raceways can be hard surfaced or simply dug into the earth and lined with a plastic liner to prevent seepage. The raceway usually consists of a double ended ‘D’ structure with a paddlewheel for circulation. Raceways can be enriched with fertilizers (such as nitrogen phosphorus and inorganic salt). Depending on the nutrients required by *Ulva* species, raceways have been used effectively in polyculture with shellfish (Lapointe *et al*., 1976; Ryther *et al*., 1978; Shpigel *et al*., 1997; Algae Energy, 2013).

In spray cultivation, *Ulva* thalliums are placed onto nets, which are then suspended over a box that collates the spray water before it is returned to a reservoir. The disadvantages of spray cultivation are two-fold. Firstly the soft, thin *Ulva* thallium form clumps on the nets resulting in self-shading (Littler and Littler 1980; Binzer and Sand-Jensen 2002). Secondly the lack of a suitable medium prevents boundary layers (result in nitrogen deficiency) from having effective gaseous exchange (Lignell *et al*., 1987; Robledo and Garcia Reina, 1991; Haglund *et al.*, 1991; Pickering *et al.*, 1995; Salinas, 1999; Msuya and Neori, 2010).

High light intensities generally increase *Ulva* production (Riccardi and Solidoro 1996; .Plettner *et al*., 2005; Rautenberger *et al*., 2015). Consequently submerged light sources are used in addition to very powerful overhead light sources. Epiphytes are reduced by using filters, and blue-green algae and diatom numbers are generally kept low by vigorous aeration, forcing the macroalgae to rub against each other (Bolton *et al*., 2008; Butterworth, 2010; Kirkendale *et al*., 2010; Sadek 2011). An obvious drawback of such a system is the high cost making the cultivation system uneconomically viable for commercial-scale set up (Lignell *et al*., 1987).

1.5.2.4 Polyculture

Aquaculture, like several other industries, is an important industry worldwide that has been supporting human demands for feed/protein products for decades (Odum, 1974; Chopin and Yarish 1998; Naylor *et al*., 2001). However, aquaculture production on an intensive scale has caused many aquatic problems associated with waste products from the aquaculture systems (Wu 1995; Chopin and Yarish 1998). Modern intensive aquaculture requires high inputs of water, feeds, fertilizers and chemicals and inevitably produces considerable pollutants (United Nations Environment Programme, 2012). It cannot be over emphasized that many fish farming operations put enormous pressure on coastal habitats (Chopin and Yarish, 1999; Black, 2001; Naylor *et al.*, 2001; Tacon *et al.*, 2006).

The current system that has taken favour among many aquaculture operations in the last 2 - 3 decades is integrated aquaculture that specializes in the incorporation of *Ulva* and additional genera into polyculture of macroalgae operations where the macroalgae are farmed in wastewater (Noeri *et al.*, 1991; Msuya, 2004; Rubino, 2008; Troell, 2009; Dean, 2010; Bruhn *et al.*, 2011). Macroalgal biofilter/production systems are being developed to reduce the environmental impact of marine fish-farm effluents in coastal ecosystems as part of an integrated aquaculture system. Many algal cultivars have been considered as suitable biofilter organisms based on their species-specific physiological properties (e.g. resilience, nutrient uptake kinetics, economic value). Species of *Ulva* have proven to be excellent candidates for biofiltration as they show efficient nutrient extraction properties, where the water from the culture system can be returned to the natural aquatic environment with approximately the same nutrient qualities (and even better under certain conditions) and temperature as the initial resource waters (Neori *et al.*, 1998; Troell *et al.*, 1997; 1999; Abreu *et al.*, 2011). The significant potential of *Ulva* species as an addition to abalone farming operations have already proven successful in aquaculture operations using aeration or paddlewheel ponds in Israel and South Africa in which the macroalgae have proved effective due to the high costs associated with the production of macroalgae in aerated tank cultures (Neori et al., 2009; Bolton *et al.*, 2006; Troell *et al.*, 2006; Bolton *et al.*, 2008; Robertson-Andersson *et al.*, 2008; Butterworth, 2010; Kirkendale *et al.*, 2010).

1.6 Factors affecting *Ulva* cultivation

1.6.1 Temperature

As with other macroalgae, species of *Ulva* also respond to varying temperature regimes. More importantly, however, are the responses of the different life cycle stages to variable temperature and other physical factors. *Ulva intestinalis*, for example, showed a good germling growth at comparatively high temperature (25 °C), but sporogenesis only occurred at 30 °C. This implies that temperature increases may not generally promote zoosporangia formation in *Ulva* (Ruangchuay *et al*., 2012). Altamirano *et al.* (2003) demonstrated that high temperatures in combination with a high dose of ultraviolet B radiation caused the death of the germlings. This study showed that a growth-related temperature dependence of sensitivity to ultraviolet B radiation. Similarly, in *U. fasciata* water temperatures < 20 °C favoured gamete formation and temperatures between 20 – 25 °C promoted zoospores formation (Kalita and Titlyanov, 2011). In *U. lactuca* 16 °C and 19 °C are optimal for the growth of isolated fragments of attached and unattached thallium respectively (Kalita and Titlyanov, 2011). Also in *U. rotundata* growth was photoinhibited at irradiances above 40 % sunlight at temperatures below 15 ^{o}C but not above 20 ^{o}C (Henley and Ramus, 1989).

1.6.2 Light

Light availability is a function of momentary, diurnal, seasonal and global changes both in irradiance and in spectral distribution (Talarico and Maranzana, 2000). Light penetration shows great variation as a result of scattering and absorption by the atmosphere and depth of the aquatic system. Light is an essential and uncontrollable growth determinant of *Ulva* in the aquatic environment, but can be manipulated in intensive culture system (Hanisak, 1983; Bidwell *et al*., 1985; Duke *et al*., 1986). It is also a limiting factor that influences algal development and various physiological responses (Duke *et al*., 1986; Lüning, 1990). Species of *Ulva* respond and show different adaptations to varying light intensities that vary from geographical location and seasons (Roleda *et al*. 2006; Wiencke *et al*., 2006). The features responsible for their survival tendencies include algal pigment ratio, activities of Calvin (Carbon reducing cycle) enzymes, Chlorophyll-a ratios, all of which increase with a decrease in light intensity (Reiskind *et al*., 1989). Photosynthetic output is a direct function of light intensity (Stefels 2000; Binzer and Sand-Jensen 2002; Shuuluka 2011; Yang 2013). Most species of *Ulva* are able to photoacclimatise within days to lower light levels and can maintain relatively good growth rates even if total irradiance is reduced slightly e.g. by self-shading (Vandermeulen and Gordin, 1990; Altamirano *et al*., 2000b). *Ulva lactuca*, for example, growing under lower illumination than in natural conditions contains high chlorophyll-a levels and showed higher photosynthetic rates at a lower rate of photon flux density, but have a lower maximal rate of photosynthesis (Israel *et al*., 1995).

Light quality, duration and availability are known to affect *Ulva* biomass, photosynthesis and morphogenesis (Rosenberg and Ramus, 1984a; Lüning and Dring, 1985; Israel *et al*., 1993; Fillit, 1995). *Ulva lactuca* growing under lower photon flux density than in natural conditions contains higher chlorophyll concentrations and show higher photosynthetic uptake of CO_2 for starch production (Israel *et al*., 1995; Vergara *et al*., 1997).

Photosynthetic light-response curves are widely reported for various *Ulva* species (Ramus, 1978; Platt *et al*., 1980; Jimenez *et al*., 1998; Henley, 1993; Perez-Llorens *et al*., 1996; Rodrigues *et al*., 2000). *Ulva lactuca* growing in continuous low light possess a photosynthetic capacity in excess of photosynthetic performance and would benefit from short-term exposure to high light (Sand-Jensen, 1988). Species of *Ulva* can use light energy efficiently to drive photosynthesis at high irradiances, but they dissipate less energy as non-radioactive processes (Mouget and Tremblin, 2002). Also, the dark respiration is partially inhibited in the light in immersed *U. lactuca* (Zou *et al*., 2007) due to low photosynthetic activities in the abscent of light. Ecologically, photosynthetic pigments stabilize chlorophyll - protein complexes to protect the organism against excessive light (Siefermann-Harms, 1987; Humbeck *et al*., 1989; Marquardt, 1998; Demmig-Adams, 1990). Light is also well recognized as an important factor in *Ulva* growth. If *Ulva* is stocked at very high densities its growth is impeded due to self-shading (Bartoli *et al*., 2005). Jiménez del Río *et al*. (1996) recommended that *Ulva* should not be stocked at densities greater than 1.5 grams of wet weight per litre.

1.6.3 Aeration

Aeration has a dual role of enhancing mass transfer and mixing. It maintains the alga in circulation and brings them to the surface where they are exposed to light for photosynthesis. Aeration has a secondary effect on growth by breaking down diffusive boundary layers at the surface of the thallium that would otherwise slow the uptake of nutrients and inorganic carbon (Hanisak and Ryther, 1984). In addition, aeration introduces some CO_2 from the atmosphere into the culture system to improve growth (Hanisak and Ryther, 1984; DeBusk *et al*., 1986; Vandermeulen and Gordin, 1990). Aeration results in rotation, which causes abrasion of the thallium against the culture vessel; this may serve as a mechanism to control epiphytes (Hanisak and Ryther, 1984). According to Bruhn *et al*. (2010) cultivation of *U. lactuca* in 1 m^2 tanks and water exchange of 6 times per day can achieve a production potential of 4 kg m^{-2}.

1.6.4 Salinity

Salinity changes are considered to be an important factor limiting the growth of *Ulva*. It is necessary to understand how *Ulva* responds and adapt to salinity stress as a result of osmotic pressure, fluctuating ionic balances and shortages of essential metabolites (Kirst, 1990; Parida and Das, 2005; Fan-Lu *et al*., 2006). *Ulva* in intertidal zones are able to withstand relatively high changes in salinity (and temperature) over short periods of time (Stegenga et *al*., 1997; Shuuluka - 2011; Park *et al*., 2014). These sudden changes have a dramatic effect on the algae's growth. Species of *Ulva* that naturally occur under such condition are adapted to salinity tolerances ranging from 3 ‰ to as much as 115 ‰ (Lobban and Harrison, 1997). The reason they are able to survive is because *Ulva* are able to regulate the amounts of dissolved internal salts, keeping their internal osmotic pressures somewhat higher than the surrounding medium, allowing them to maintain a constant turgor at higher salinities (Lobban and Harrison, 1997). However, despite this adaptation, *Ulva* can be negatively affected by low salinity resulting in decreased growth rates and a defence mechanism to cope with the oxidative stress induced by salinities below 20 - 30 ‰ (Murthy *et al*., 1988; Friedlander, 1992; Fan-Lu *et al*., 2006).

1.6.5 Carbon

Carbon nutrition is an essential requirement for successful algal cultivation. Carbon is converted to growth in macroalgae, which leads to increased CO_2 concentration as a result of higher biological productivity with an envisaged increase in the photosynthetic storage and nutrient uptake from the process (Surif and Raven, 1989; Maberly, 1990; Gao *et al*., 1991; Levavasseur *et al*., 1991; Kubler *et al*., 1999; Stitt and Krapp, 1999; Ding-Hui and Kun-Shan, 2002; Langdon *et al*., 2003; Zou, 2005; HongYan *et al*., 2008; Roleda *et al*., 2010; Markelz *et al*., 2013). *Ulva* utilize dissolved gaseous CO_2 from the atmosphere (De Boer, 1981).

Upon entering the aqueous environment, atmospheric CO_2 reacts with water leading to the formation of ionic forms of carbon (carbonic acid, bicarbonate, and carbonate) in equilibrium with CO_2, as described in the equation by Falkowski and Raven (2007).

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3- \leftrightarrow 2H^+ + CO_3^{2-}$$

However, depending on pH changes in the seawater (Bjork *et al*., 1993), the equilibrium shifts, with high concentrations of CO_2 at lower pH and relatively more bicarbonate and carbonate at higher pH. At pH 7.8 - 8.2, salinity 35 ‰, and water temperature of 25 ^oC for example, more than 95 % of inorganic carbon present is in the form of bicarbonate (Kremer, 1981; Falkowski and Raven, 2007). Elevated CO_2 concentrations in air enhance photosynthesis of intertidal species of *Ulva* (Zou *et al*., 2007). *Ulva rigida*, for example, having an efficient ability of HCO_{3-} utilization, was found to have its photosynthesis saturated at the Ci (Intercellular CO_2 concentration) concentration of seawater. In addition to growth and photosynthesis, increasing CO_2 levels enhances the activity of nitrogen reductase (NR) in *U. rigida* (Björk *et al*., 1993).

In dense biomass cultivation systems the *Ulva* depletes the dissolved inorganic carbon in the water, suffering from carbon malnutrition, consequently resulting in low rates of production (Bidwell *et al*., 1985; McLachlan *et al*., 1986; Jiménez *et al*., 1995). Accelerated thallus disintegration at high CO_2 concentrations under conditions of limited water exchange indicates additional CO_2 effects on the life cycle of *U. lactuca* living in rock pools, while slight enhancement of photosynthetic performance and significantly elevated growth of the alga at increase CO_2-concentrations (Olischläger *et al*., 2013). The induced CO_2 concentrations in culture media have one of the most important economic impacts on *Ulva* production (Kübler *et al*., 1999; Zou, 2005; Xu *et al*., 2010). Higher growth in many aquatic macrophytes, stimulated by induced CO_2 concentration, usually lead to greater biological productivity with an expected increase in the photosynthetic storage of carbon (Morison and Lawlor 1999). CO_2 can be utilized for stimulating the growth of wild *Ulva* (Gordillo *et al*., 2001) and possibilities also exist for promoting growth of cultured marine macroalgae. Increased CO_2 levels in seawater have been shown to promote the photosynthetic activity of *U. intestinalis* Linnaeus (Pajusalu *et al*., 2013). Elevated CO_2 levels are suggested to promote the production of fast-growing filamentous species, which thus may indirectly enhance the effect of eutrophication in the shallow coastal brackish waters (Pajusalu *et al*., 2013). Most algae improve their growth and calcification from increased $CO_{2;}$ this observed effect can vary between strains of the same species and similar species of *Ulva* (Gao *et al.*, 1999; Kübler *et al*., 1999; Iglesias – Rodriguez *et al*., 2008; Langer *et al*., 2009; Riebesell *et al*., 2007).

1.6.6 pH

In most *Ulva* cultivation tanks, carbon nutrition can be controlled by pH-regulated additions of carbon. The variables to control are the chemical form (bicarbonate or carbon dioxide) in which the carbon is added to the cultures and the pH set point at which it is added (Bidwell *et al*., 1985; Craigie and Shacklock, 1989; Amat and Braud, 1993; Demetropoulos and Langdon, 2004). The use of extra carbon sources therefore represents a major operational cost of traditional *Ulva* cultivation systems (Braud and Amat, 1996).

According to Drechsler and Beer (1992) the photosynthetic performance observed in *U. lactuca* at lower pH largely followed that prediction, with a slight discrepancy probably reflecting a minor diffusion barrier to CO_2 uptake. Changes in carbon concentration can result in a shift in pH outside the normal neutral range. In *Ulva* incubations, Menendez *et al*.

(2001) observed optimum photosynthesis between pH 6 and 7.5. At pH above 7.5, the photosynthetic rate declined rapidly while below 6 (12.30 %) this decline was less pronounced than above pH 8 (81.03 %); this difference may be related to the origin of the *Ulva*. Therefore, it is important that in *Ulva* cultivation, hydrogen ion concentration must be managed at a range close to pH 7.5 – 8.5 (Nagle *et al*., 2009).

1.6.7 Nitrogen

After carbon, nitrogen is the most important growth factor for macroalgae and the uptake rate of the different N forms is affected by environmental conditions including light and temperature (Lapointe and Ryther, 1978; Valiela, 1984; Duke *et al*., 1989). Nitrogen is available to macroalgae in three different forms; nitrite, nitrate and ammonium. Ammonium is often taken up at higher rates than nitrate (D'Elia and DeBoer, 1978; Morgan and Simpson, 1981; Wallentinus, 1984; Thomas and Harrison, 1985; Rees, 2003), despite the fact that nitrate is almost always the more abundant source of inorganic nitrogen in the aquatic environment (De Boer, 1981). However, ammonium, which can be toxic for some macroalgae at concentrations above 30 – 50 μm, is the preferred form of nitrogen for *Ulva* (Shuuluka - 2011). Light intensity, temperature fluctuation or changing stocking density generally does not affect nitrogen uptake (DeBusk *et al*., 1986; Duke *et al*., 1989; Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Lobban and Harrison, 1994).

For high yield and high nitrogen content, *U. lactuca* for example, should be kept at stocking densities of 1 - 2 kg m^{-2} and at ammonia fluxes of about 0.5 moles m^{-2} d^{-1} (Neori *et al*., 1991). Nitrogen content of cultivated *U. lactuca* can be increased further, but at reduced yield, by increasing the stocking density to 4 or 6 kg m^{-2} (Neori *et al*., 1991). Fast-growing cholorophyts like *Ulva* are usually characterized by high SA: V (surface area: volume), using a high Nitrogen uptake/requirement to promote growth, possibly facilitating Nitrogen uptake even at low substrate concentrations (Hein *et al*., 1995; Pedersen and Borum, 1997). Ammonium uptake in green algae such as *Ulva* can be regulated by Nitrogen accumulation due to their high tissue-N properties over periods of excess availability (Rosenberg and Ramus, 1982; Fujita, 1985; Duke *et al*., 1989).

1.6.8 Phosphorus

Phosphorus in the form of orthophosphate ions is an important primary component of phosphate-related reactions, as well as Calvin cycle enzymes available to aquatic macroalgae (De Boer, 1981; Müller *et al*., 2005; Ghannoum and Conroy, 2007). Naturally, the growth of macroalgae in most culture media is often determined by phosphorus in the water (Howarth, 1988; Chopin *et al*. 1990a; Flores-Moya *et al*., 1997; Lobban and Harrison, 1997). According to Lee (2000) thallus discs of *U. lactuca* grown under different NaH_2PO_4 levels suggest that the phosphorous deficiency induction of between acid phosphatise (ACP) activity may be correlated to phosphorous availability. The nitrogen-to-phosphorus (N:P) ratio is an important nutritional component for algal growth and the optimum ratio varies from species to species, and may provide a basis for competitive elimination and co-existence of algal species (Wu and Suen, 1985).

According to Björnsäter and Wheeler (1990) the growth rates of *Ulva* decrease faster under phosphorus-limitation than during nitrogen-limitation. The growth rates of *U. fenestrate*, for example, decrease faster under phosphorus-limitation than during nitrogen-limitation (Björnsäter and Wheeler, 1990). Under these conditions, a NH_4^+ is often taken up by the alga at higher rates than nitrate (D'Elia and DeBoer, 1978; Haines and Wheeler, 1978; Hanisak and Harlin, 1978; Topinka, 1978; Morgan and Simpson, 1981; Wallentinus, 1984; Thomas and Harrison, 1985; Rees, 2003). However, sustainable growth is the preferred nitrogen form for *Ulva* and other species of macroalgae (Lobban and Harrison, 1994).

Lee (2000) demonstrated the linear correlation between acid phosphatise (ACP) activity and intracellular Pi (oxyanion phosphate - Pi). According to Lee (2000) total Phosphorus concentrations show that in *U. lactuca*, ACP activity can be an indicator of Phosphorus deficiency. The increment on ACP activity in inorganic Pi-limited conditions suggests that ACP could be involved in polyphosphate degradation in *U. lactuca*, as has been suggested for some higher plants (Dewald *et al*., 1992; Duff *et al*., 1994). The concentrations of intracellular polyphosphates in *U. lactuca* decrease after transfer to phosphorus-deficient conditions, indicating that the intracellular polyphosphates could be hydrolyzed to meet phosphorus requirements (Weich and Graneli, 1989). In the case of *U. lactuca*, the activity of extracellular alkaline phosphatise (AP) increased during cultivation in a P-deficient medium (Weich and Graneli, 1989).

1.6.9 Heavy metals

Nutrient concentration at both macro and micro levels are important for growth improvements in macroalgae cultivation. Although, macroalgae contains several trace elements (e.g. alganic acid, vitamins, auxins, gibberllins, cytokines and antibiotics) they naturally take up elements like Na, K, Ca, Mg, Cl, I and Br from the surrounding water bodies (Rafia *et al*., 2006). The ability of macroalgae to accumulate metals depends on several factors such as the bioavailability of the metals in the surrounding water and their seasonal variations (Sanchez- Rodriguez *et al*., 2001; Villares *et al*., 2001; Lozano *et al*., 2003). The major metallic pollutants implicated in culture systems and coastal waters are Pb, Cr, Hg, U, Se, Zn, As, Cd, Au, Ag, Cu and Ni (Alya *et al*., 2004) of which Cd is highly absorbed by *Ulva* (Farr, 2009). Consequently species of *Ulva* have been used as biological indicators of heavy metal contamination (Ho, 1990; Villares *et al*., 2001; 2002; Mamboya 2007; Rybak *et al*., 2012; Saeed and Moustafa 2013; Chakraborty *et al*., 2014).

1.6.10 Water flow and movement

It has long been known that high water motion increases algal photosynthetic rates through the filtration of inhibiters like OH ions (Gonen *et al*., 1995). Water motion affects exchanges of nutrients and CO_2 between the water column and the algal thallus. This is so because water motion influences the algae's boundary layer (Gonen *et al*., 1995). The influence of water movement is crucial to culture technology systems for species like *Ulva* and other marine algae, since nutrient uptake is essential in the initial, early development of spore formation. *Ulva lactuca* may well be one of the most advantaged algal species if wave velocities are high, dislodgment is low, and sun and wind exposure is relatively low or of short duration (Seaborn, 2014). *Ulva* cultured in a flow-through system performs better than in a closed unit precisely because the former system ensures that both water and algae are in constant motion to maintain continuous exposure to light, water for nutrient uptake and gas exchange (Msuya, 2001; Mwandya, 2001; Butterworth, 2010).

1.6.11 Stocking density

Several studies have shown that stocking densities for species of *Ulva* have an important influence on the algae's growth rates. Very high stocking densities (> 6 $kg.m^{-2}$) generally reduce aeration and result in improper circulation leading to reduced biomass production (DeBusk *et al*., 1986; Bartoli *et al*., 2005). The optimal stocking density is therefore a function of light availability due to aeration (Vandermeulen, and Gordin 1990). If the density is too high, the algae will be shaded. Conversely, if the stocking density is too low, the algal thalli will receive too much light and energy will be wasted (McLachlan, 1991; Msuya, 2004; Bruhn *et al*., 2011). Mmochi *et al*. (2002) estimated that the optimal stocking density for *U. fasciata* and *U. reticulate* in their studies was 1 kg m^{-2}. Similarly many other studies have shown that the optimal stocking density for *U. lactuca* is also near 1 kg m^{-2} (Ryther *et al*., 1984; Neori *et al*., 1991). In still others studies, the optimal stocking density for *U. lactuca* was reported to be 4 kg m^{-2} (Bruhn *et al*., 2010). However, Jiménez del Río *et al*. (1996) recommended that *Ulva* should not be stocked at densities greater than 1.5 g of wet weight per litre. It thus appears more likely that the stocking density is often also a function of the specific culture system the species is grown in.

1.6.12 Seasonality

Environmental factors (light intensity, temperature, nutrient supply, etc.), in conjunction with seasonality, influences the survival of macroalgae (Luning and Dieck, 1989; Duke *et al*., 1989). Macroalgae differ in their nutrient content in relation to the light environment and their photosynthetic efficiency (Gerard, 1988). The relationship between nutrients such as carotenoids and Vitamin A has established that some carotenoids have proVitamin A activity, which can be transformed to Vitamin A in aquatic fauna (Carvalho, 1993). These pigments in macroalgae provide an indication of the level of Nitrogen available in surrounding water. Research experiment by Vergara and Niell (1993), for example, showed that the proportion of pigmented and non-pigmented proteins vary with Nitrogen concentrations and light availability. This phenomenon shows that macroalgae have the physiological mechanism to store Nitrogen for subsequent seasons when nutrients are possibly in short supply.

In general, biomass of *Ulva* shows minimum values in winter and maximum values (200 and 800 g dry weight (dw) per m^2) in late spring to early summer (Altamirano *et al*., 2000;

Viaroli *et al.*, 2005; Martins *et al.*, 2007; Perrot *et al.*, 2014). Altamirano *et al.* (2000) suggested that the metabolic stress due to UV-B radiation has little impact on the seasonal growth cycle of *U. olivascens*, for example, but has significant impact on the pigment concentrations and the internal carbon and nitrogen content. In their studies the relative growth rate of *U. olivascens* was 68 % higher in spring and early summer than in mid-summer. In the same spring-to-summer period, photosynthetic pigment concentrations (chlorophyll *a*, chlorophyll *b*, and carotenoids) decreased by 70 - 80 %. In *U. lactuca*, growth per incident photon (α_g) can vary from 1 to 22 mmol C (mmol incident photon)$^{-1}$, depending on the time of the year and level of shading (Geertz-Hansen and Sand-Jensen, 1992). In this regard, the α_g is high under nutrient saturation and higher temperatures in August-September (Southern Hemisphere spring) and low during nutrient limitation and sporulation.

1.7 *South African Ulva culture*

In South Africa various macroalgae, notably brown macroalgae, have long been used commercially as feed for the abalone industry and as feedstock for the production of phycocolloids (Troell *et al.*, 2006). In the abalone industry, high-quality macroalgal protein extraction has been developed for incorporation to varying degrees into the formulated feeds Abfeed™ and Midae Meal™ (Anderson *et al.*, 2003; Robertson-Andersson *et al.*, 2006; Troell, *et al.*, 2006). Among African nations, South Africa is currently spearheading research innovations into macroalgae, and their use for the commercial production of the plant growth stimulants Kelpak®, Afrikelp®, and more recently Phloroglucinol® and Eckol® (Rengasamy *et al.*, 2015). Currently, Carraguard®, a non-spermicidal microbicide containing carrageenan (a red macroalgae derivative) has been clinically tested to be a promising product capable of blocking the transmission of HIV/AIDS and lowering the risks of women in contracting the disease (Robertson- Andersson, 2007). However, of all macroalgae in South Africa, species from the genus *Ulva* are the most promising from an energy point of view (Shipton and Britz, 2007; Bolton *et al*., 2009; Bruton, *et al*., 2009; Department of Agriculture, Forestry and Fisheries 2011).

1.7.1 Ulva culture and their commercial uses

The cultivation of *Ulva* in South Africa started in the early 1990's and has shown considerable success since then (Robertson-Andersson 2007). Wild Coast Abalone® farm (East coast province), Irvin & Johnson^{Tm} Cape Cultured Abalone mariculture farm (South coast province), and Abagold® (West coast province) have cultivated most of the South Africa's *Ulva*, making the country a world leader in *Ulva* cultivation (FAO (2011). The *Ulva* species cultivated in South Africa comprise largely three species (*Ulva* armoricana, *Ulva* rigida and *Ulva* c*apensis*), with the free-floating *U. armoricana* grown more widely and forming an important feed source, particularly for integrated abalone (*Haliotis midae* L) farming (Robertson-Andersson *et al*, 2007; Department of Agriculture, Forestry and Fisheries 2012; Cyrus *et al*., 2014a).

In 2009, 2010 and 2011, *Ulva* production was 1900 tonnes, 2015 tonnes and 2884.61 tonnes respectively from only four species grown in flow-through integrated *Ulva*/abalone systems (Bolton *et al*., 2006; Robertson-Andersson *et al*., 2008; Department of Agriculture, Forestry and Fisheries, 2011b; 2012; FAO, 2012). This industry has grown rapidly since then (Shuuluka, 2011). The on-land integrated culture unit, with paddle wheel raceways, is widely viewed in South Africa as the preferred method of *Ulva* production for the industry (Robertson-Andersson, 2007).

The growth of *Ulva* and its culture in South Africa has been well researched (Robertson-Andersson, 2003; Robertson-Andersson *et al.*, 2007). Cultivated *Ulva* widely are used as a food source for cultured abalone has generated a wealth of information about the alga's growing conditions and its suitability as a food source. Feeding trials have showed that abalone growth is greatly improved by *Ulva* specimens with a high protein content, which is attained by culturing the algae under high ammonia levels (Shuuluka *et al*., 2013). Nutrient enriched *Ulva* have been shown to have comparatively high protein content of 36 – 44 % as opposed to their wild counterparts that have protein contents of only 3 – 24 % (Robertson-Andersson, 2003; Robertson-Andersson *et al*., 2007). Work on the south African abalone *H. midae* (e.g. Naidoo *et al*., 2006; Francis *et al*., 2008; Robertson-Andersson *et al*., 2011) and the sea urchin *Tripneustes gratilla* (e.g. Cyrus *et al*., 2012; 2014a; 2015) fed these higher protein enriched *Ulva* spp, whether fed a single diet of *Ulva* or in combination with other seaweeds or with formulated feed, showed that these protein enriched *Ulva* species out-performed their wild or their non-nutrient enriched counterparts.

1.7.2 Taxonomic concerns of Ulva culture

The South African species of *Ulva* have been well studied along the west coast region. The recent synonymy of the genus *Enteromorph*a with *Ulva* (which has nomenclatural priority) meant that the latter genus now comprises fifteen species in South Africa, namely: *U. armoricana* P.Dion, B.de Reviers & G.Coat, *U. atroviridis* Levring, *U. compressa* Linneaeus, *U. capensis* Areschoug, *U. fasciata* Delile, *U. flexuosa* Wulfen, *U. intestinalis* Linneaeus, *U. linza* Linneaeus, *U. marginata* (J.Agardh) Le Jolis, *U. minima* Vaucher, *U. prolifera* O.F.Müller, *U. rigida* C.Agardh, *U. rhacodes* (Holmes) Papenfuss and *U. uncialis* (Kützing) Montagne (Joska, 1992; Stegenga *et al*., 1997; Coat *et al*., 1998; Cyrus *et al*., 2014b; Dion *et al*.,1998; Guiry and Guiry, 2015). Previously thought to be *U. lactuca*, molecular evidence

(L. Kandjengo & J.J. Bolton, unpublished data) has shown that the main species of *Ulva* grown in South Africa is the free-floating *U. armoricana*. Other South African specimens reported to be "*U. lactuca*" are different from those reported from other parts of the world and will require an in depth (taxonomic and molecular) assessment to determine their taxonomic status (Cyrus *et al*., 2014b).

The first molecular study of South Africa species of *Ulva* (see Kandjengo, 2003) revealed that there are more species of *Ulva* than previously reported. This study suggested that some of these cryptic species may have been introduced into the country in the last decade. The mis-identification of several species has resulted in a number of misconceptions. *Ulva lactuca* is one such example (Stegenga *et al*., 1997) as we now know it to be genetically different to the European type specimen (Kandjengo, 2003; Cyrus *et al*., 2014b).

The taxonomy of species from the genus *Ulva* is currently in flux. Thus it is not possible currently to identify precisely species of *Ulva* in the various aquaculture systems across South Africa without any molecular sequencing (Cyrus *et al*., 2014b). "*Ulva lactuca*" is a name widely used in aquaculture studies around the world, and more than likely there are a number of species residing under this name. The *Ulva* species grown in the current study has been identified by DNA sequencing to match the holotype of *U. armoricana* P.Dion, B.de Reviers & G.Coat, a member of the *U. rigida* complex (L. Kandjengo and JJ Bolton, unpublished data; Cyrus *et al*., 2014b). Although *U. armoricana* is currently regarded as a heterotypic synonym of *U. rigida* (Brodie *et al*., 2007; Guiry and Guiry, 2015) South African aquaculture material identified as *U. armoricana* are not molecularly identical to material of *U. rigida* that occurs on South African seashores (J.J. Bolton, pers. comm.). It will take a while for the taxonomy of the genus *Ulva* to be properly sorted. Until such time, we are obliged to use the name *U. armoricana* for the material grown in aquaculture systems.

1.8 Aims of this study

Biomass is the term for renewable energy from a plant source. Energy in this form is very commonly used throughout the world. Unfortunately the most popular form of this energy is the burning of trees for cooking and warmth. This process releases copious amounts of CO_2 into the atmosphere and is a major contributor to unhealthy air in the environment. Macroalgae are an important form of biomass with the production of more modern kinds of energy as methane (CH_4) generation that could be used for fueling electric power plants. Macroalgae are currently the most significant aquatic 'plant' that has contributed to the development of the fisheries and the aquaculture industry both globally and locally in South Africa (FAO, 2010b). Harvests from wild populations are generally affected by overexploitation and climatic change. South Africa, for example, is listed as one of the top producers of wild stocks globally (FAO, 2014). If the South African macroalgal industry continues to grow at the current rate, there will be a need to improve the cultivation of *Ulva* spp to increase productivity to meet the various industry demands.

1.8.1 Rationale

South Africa has the highest energy consumption per capita in Africa, with the total energy consumption in 2003 of 4230 Peta joule (PJ) (Department of Minerals and Energy (2004). As the natural reserves of oil-producing countries decline, it is of immense importance to look towards other sources of energy generation, particularly from renewable sources. Presently, the renewable contribution to South Africa's energy supply is relatively limited

(Banks and Schäffler, 2006). The country uses coal and crude oil, which together contributed in 2000 about 88 % (3720 PJ) of the energy (Mathu 2014). However, South Africa has extensive coal reserves that are estimated to last for only one more century if used at current rates (Jeffrey, 2005). Oil reserves are small and the country imports almost all its crude oil requirements (Ward and Walsh 2010). South Africa thus has an energy-intensive economy and as a result, per capita CO_2 (and other 'greenhouse' gas) emissions are amongst the highest in the world (Mwakasonda, 2007; Parker and Blodgett 2008; Department of National Treasury 2010). With natural crude oil and other fossil fuel reserves declining, and the serious environmental pressures associated with these industries rising, industries have been forced to explore other renewable options for obtaining LPG.

1.8.2 Objectives

Among the goals of this study was to investigate the effect of physicochemical parameters (pH, oxygen, temperature, nutrients, and light quality) on the biomass production of *Ulva* used in aquaculture. Due to their high starch contents, *Ulva* species have been shown to be prime candidates for biofuels production (Bruton *et al*., 2009). This information should provide base-line data in the cultivation of *Ulva* to produce macroalgae for large-scale renewable energy production.

This study also examined the survival ability of *Ulva w*ith respect to pH in determining the extent of physiological adaptation to increased CO_2 levels. The ability to withstand reduced or higher pH levels should reflect the extent to which *Ulva* can survive pH levels in both closed and flow-through aquaculture systems. These physiological studies are of importance since increased inorganic CO_2 can result to reduce the photosynthetic ability of *Ulva* species in closed, semi-closed and flow-through aquaculture systems.

South Africa currently ranks among the 20 highest contributors to CO_2 emissions overall and produces approximately 2 % of the global GHG emissions, and yet has only 0.7 % of the world's population (Banks and Schäffler, 2006). Another part of this study was designed to measure the efficacy of different production outputs of *Ulva* cultivated under various treatment conditions. Ultimately the aim was to demonstrate whether *Ulva*'s uptake of anthropogenic CO_2 can be used as an effect carbon deposit.

The following, more precise, objectives were the basis for the research conducted during the current study.

- To evaluate the large scale production of *Ulva* cultivated in a flow-through system in order to investigate the relationships between environmental factors and growth, and to aid in the prediction of growth.
- To identify the ideal culture conditions that promotes the optimum growth of *Ulva*. In this study we investigated the optimal testing of CO_2 uptake via algae for biogas and biomass maximization for biomethane and its mitigation against coastal acidification.
- To identify whether the widely cultured green macroalga *Ulva armoricana* might have a significant contribution to South Africa's renewable energy supply.

Through these objectives, this research will attempt to explain how *Ulva* grown under more environmentally friendly and high sustainable technology systems might be better used for transforming macroalgal biomass into potentially consumable products like renewable energy sources such as LPG. By converting macroalgal biomass to useful fuel, it decreases our dependence on fossil fuels.

Chapter 2: Paper 1

South African Seaweed Aquaculture: A sustainable development example for other African coastal countries

This chapter is based on:

Amosu A.O., Robertson-Andersson D.V., Maneveldt G.W., Anderson R.J. and Bolton J.J. (2013). South African Seaweed Aquaculture: A sustainable development example for other African coastal countries. *African Journal of Agricultural Sciences* 8(43): 5260-5271.

Abstract

The green seaweed *Ulva* is one of South Africa's most important aquaculture products, constituting an important feed source particularly for abalone (*Haliotis midae* L). Besides *Ulva* spp, *Gracilaria* spp are also cultivated. Wild seaweed harvest in South Africa totals 7,602 mt, compared to 2,015 mt of cultivated *Ulva*. To mitigate for the reliance on wild harvesting, the South African seaweed aquaculture industry has grown rapidly over the past few decades. On-land integrated culture units, with paddle-wheel raceways, are now widely viewed as the preferred method of production for the industry. The success of seaweed aquaculture in South Africa is due to a number of natural and human (industrial) factors. The development of the seaweed aquaculture industry has paralleled the growth of the abalone industry, and has been successful largely because of bilateral technology transfer and innovation between commercial abalone farms and research institutions. In South Africa seaweeds have been used commercially as feedstock for phycocolloid production, for the production of abalone feed, and the production of Kelpak® and Afrikelp®, which are plant-growth stimulants used in the agricultural sector. Additionally, *Ulva* is being investigated for large-scale biogas production and utilized as a bioremediation tool and other benefits such as integrated aquaculture. The South African seaweed industry provides a template that could be used by other coastal African nations to further their undeveloped aquaculture potential.

Key words: Aquaculture, resources, seaweed, *Ulva*, South Africa

Introduction

Fisheries and mariculture provide significant food and income for the world's coastal countries, constituting the livelihoods of over 3 billion people (FAO, 2009; Smith *et al*., 2010). Fisheries rely upon renewable harvest from the aquatic environment, while aquaculture is the cultivation of desirable aquatic organisms in open, closed, or semi enclosed bodies of water (Lorenzen *et al.*, 2001). Aquaculture is currently the fastest growing primary industry (FAO, 2009) and with macroalgae aquaculture is larger than capture fisheries (FAO, 2010a; 2012). Active coastal aquaculture development with high production has been practiced in Asia, Europe and South America for several decades, whereas only minimal production has thus far been achieved in Africa (FAO, 2012). Currently the African continent accounts for less than 1% of the annual total global aquaculture production (FAO, 2010a; 2012) and the vast majority of Africa's aquaculture is in fresh water.

Macroalgae is currently the most significant aquatic plant that has contributed to the development of fisheries and the aquaculture industry (FAO, 2010b). Since 1970, the production of aquatic plants (seaweed and angiosperm) worldwide has consistently increased at an annual rate of 7.7%; 93.8% of the total world macroalgae production is now from aquaculture (McHugh, 2001; FAO, 2003; 2009; 2010b; 2011), a higher figure than for any other group of marine organism. Globally, the production of macroalgae increased from 11.66, 16.83 and 19.9 million mt in 2002, 2008 and 2010 respectively, while seaweed biomass accounted for 23% of the world aquaculture output in 2007 (FAO, 2012; Paul and Tseng, 2012). In recent years the total global annual macroalgae harvest, produced by over 30 countries, ranges from 3.1–3.8 million mt (FAO, 2010b). Aquaculture production of aquatic plants in 2008 was estimated at US$7.4 billion (99.6% quantity and 99.3% value) (FAO, 2009).

Even though Africa is the second largest continent and has a shoreline of about 30,000km, it has yet to contribute significantly to the development of the macroalgae industry despite its rich seaweed diversity (FAO, 2002; 2010b). Abalone farming in South Africa has developed rapidly and the country is now the largest producer outside Asia, partly achieved due to seaweed production. From a macroalgae perspective, *Eucheuma* farming is well established in Zanzibar where commercial interests have assisted the establishment and development of the industry (FAO, 2014), making Tanzania the largest macroalgae producing country in Africa, and among the top ten producers and one of only a few countries around the world producing more than 8,000 mt of (Eucheuma) macroalgae per annum (FAO, 2014).

Africa offers numerous aquaculture opportunities, including integrated macroalgae aquaculture production, which has been on the increase since 2001 (Table 1). The African continent produced 133,000 mt of farmed seaweeds in 2014, with Tanzania (mainly Zanzibar), Madagascar, South Africa, Mozambique, and Namibia as the leading producers (FAO, 2014). This paper examines the current status of seaweed aquaculture in South Africa, the philosophy behind the country's achievements, prospects for the future, and the lessons for other African coastal countries.

Table 1: World aquaculture production of aquatic plants by producers in 2014 (FAO 2014).

Country Pays Pais		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
World total Total mondial Total mundial	**Q**	**13 503 584**	**14 312 112**	**14 973 207**	**15 858 483**	**17 339 057**	**18 992 860**	**20 786 734**	**23 561 107**	**26 867 633**	**27 306 965**
	V	**3 887 269**	**3 994 177**	**4 194 152**	**4 314 985**	**4 907 251**	**5 641 903**	**5 374 034**	**6 333 665**	**6 491 049**	**5 637 414**
China	Q	9 494 229	9 744 210	9 745 025	9 933 886	10 496 905	11 092 270	11 549 555	12 832 060	13 561 445	13 326 315
	V	2 038 723	2 082 169	2 067 950	2 311 139	2 357 839	2 533 196	2 502 025	2 852 190	3 040 904	2 307 646
Indonesia	Q	910 636	1 170 000	1 728 475	2 145 061	2 963 556	3 915 017	5 170 201	6 514 854	9 298 474	10 076 992
	V	127 489	210 600	392 980	300 309	811 822	1 268 367	1 143 653	1 347 538	1 742 233	1 653 108
Philippines	Q	1 338 597	1 468 905	1 505 070	1 666 556	1 739 995	1 801 272	1 840 833	1 751 071	1 558 378	1 549 576
	V	109 801	173 963	136 850	291 039	201 154	256 715	263 110	231 735	233 618	256 293
Korea Rep	Q	621 154	765 595	792 953	921 024	858 659	901 672	992 283	1 022 326	1 131 305	1 087 048
	V	262 523	269 657	332 524	311 306	252 112	327 823	344 276	391 705	411 137	496 496
Korea D P Rp	Q	444 295	444 300	444 300	444 300	444 300	444 300	444 300	444 300	444 300	444 300
	V	244 362	244 365	244 365	66 645	66 645	66 645	66 645	66 645	66 645	66 645
Japan	Q	507 742	490 062	513 964	456 337	456 426	432 796	349 737	440 754	418 365	363 400
	V	1 080 450	939 490	957 384	972 037	1 081 155	1 143 130	994 352	1 397 119	933 056	746 508
Malaysia	Q	40 000	60 000	90 269	111 298	138 857	207 892	239 450	331 490	269 431	245 332
	V	1 584	2 454	3 940	6 686	7 884	17 444	21 919	23 616	25 671	63 752
Zanzibar	Q	73 620	76 760	84 850	107 925	102 682	125 157	130 400	150 876	110 438	133 020
	V	638	740	579	1 265	1 327	1 781	1 668	1 915	1 399	1 658
Solomon Is	Q	3 260	1 690	1 080	1 440	5 100	8 000	8 000	12 850	16 700	18 000
	V	87	33	21	58	190	298	315	804	1 052	1 123
Viet Nam	Q	15 000	15 000	15 000	15 000	15 000	18 221	14 019	18 544	13 561	14 327
	V	2 365	2 438	2 422	2 393	2 285	2 545	1 880	2 448	1 781	1 863
Chile	Q	15 493	38 219	26 387	27 703	88 193	12 179	14 694	4 126	12 512	12 836
	V	11 621	61 660	43 307	46 731	114 678	15 841	25 118	9 512	27 702	33 104
Madagascar	Q	900	5 300	3 650	3 650	3 600	4 000	1 699	1 400	3 575	8 363
	V	122	716	493	493	486	540	143	109	275	1 735
Tanzania	Q	3 000	3 200	4 000	5 000	5 520	6 885	6 601	6 510	6 689	6 705
	V	24	31	27	65	168	196	168	164	180	203
Kiribati	Q	5 000	8 837	1 112	1 083	1 788	4 745	4 290	8 280	2 250	3 580
	V	200	353	44	91	139	428	434	600	131	226
India	Q	5 748	1 954	2 522	4 706	6 922	4 242	4 502	4 502	4 502	3 002
	V	491	50	84	177	251	163	170	169	155	99
Papua N Guin	Q	-	-	-	-	-	100	250	1 400	2 500	3 000
	V	-	-	-	-	-	4	13	81	134	183
Russian Fed	Q	245	818	300	260	739	614	821	1 584	642	2 386
	V	294	982	360	312	887	737	985	1 901	770	2 863
Myanmar	Q	...	...	...	288	1 200	2 094	2 336	3 200	1 600	2 100
	V	...	...	...	11	45	79	88	120	60	79
South Africa	Q	3 000	3 000	3 000	1 834	1 900	2 015	2 885	2 000	2 000	2 000
	V	1 340	1 265	1 208	756	807	744	1 078	659	561	498
Timor-Leste	Q	...	...	370	1 000	1 500	1 500	1 500	1 500	1 500	1 500
	V	...	...	28	75	113	113	113	113	113	113
China,Taiwan	Q	2 438	5 949	9 390	6 879	4 383	4 888	4 883	3 496	3 234	958
	V	502	447	8 311	1 206	5 161	3 158	1 765	860	657	398
Brazil	Q	...	...	...	320	520	730	730	730	730	730
	V	...	...	...	26	39	62	65	56	51	47
Fiji	Q	450	1 190	650	660	440	560	450	560	470	550
	V	25	65	36	46	31	56	45	56	47	55
France	Q	45	32	35	53	125	120	380	350	304	300
	V	16	17	16	30	66	61	336	286	179	179
Namibia	Q	67	70	27	132	130	130	130	130	130	130
	V	62	65	25	97	93	107	108	95	81	72
Greece	Q	...	...	...	...	...	...	198	174	93	126
	V	...	...	...	...	...	...	1 517	971	548	678
Ireland	Q	...	...	3	...	...	3	3	9	42	100
	V	...	...	2	...	...	2	2	11	55	133
Burkina Faso	Q	10	50	50	70	70	70	100	100	120	100
	V	5	220	220	308	280	280	400	400	480	400
Denmark	Q	...	...	...	1 000	1 001	1 000	1 000	1 000	1 800	100
	V	...	...	...	591	567	534	559	518	192	64
Other countries Autres pays Otros paises	Q	18 655	6 971	725	1 021	547	388	504	932	543	90
	V	4 544	2 397	975	1 094	1 028	856	1 087	1 271	1 181	1 192

V = Value in USD 1 000   Q = Quantity

The South African Seaweed Industry

South Africa has had a macroalgae industry for over 60 years. The commercial exploitation of macroalgae in South Africa is based largely on beach-cast collecting and cutting of kelp. Harvesting of *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria pallida* Greville ex J. Agardh started in the 1940's as a result of the scarcity of kelp during the Second World War (McHugh, 1987). When supplies of agar from Japan became unavailable, various potential resources were identified. However, commercial exploitation only began in the early 1950s (McHugh, 1987), followed by hand-picking of *Gelidium* sp. in the Eastern Cape since 1957. Most of this harvest was shipped to Europe, North America, and Asia for alginate extraction (Anderson *et al.,* 1989). South African kelps yield alginate concentrations of between 22 – 40% (Anderson *et al.*, 1989). Some trade figures showed that powdered kelp was also exported to Japan for use in formulated fish-feed (Zhang *et al*., 2004). Since 1975, fresh – wet kelp has been harvested from Concession Area 9 (Figure 1) along the west coast solely for the production of Kelpak®, which is a plant-growth stimulant[1] and soil conditioner (Khan *et al.,* 2009).

Similar harvesting of fresh – wet kelp in small quantities started in 1979 on the west coast and later on the south coast for the production of Afrikelp®, which is also a plant-growth stimulant. This harvesting continues today (Anderson *et al.,* 1989; 2003; Robertson-Anderson *et al.* 2006; Troell, *et al.,* 2006).

---

[1] Kelp contains active ingredients (cytokinins and auxins) that have been shown to improve the growth performance and efficacy of many food and agricultural crops (Troell *et al.*, 2006).

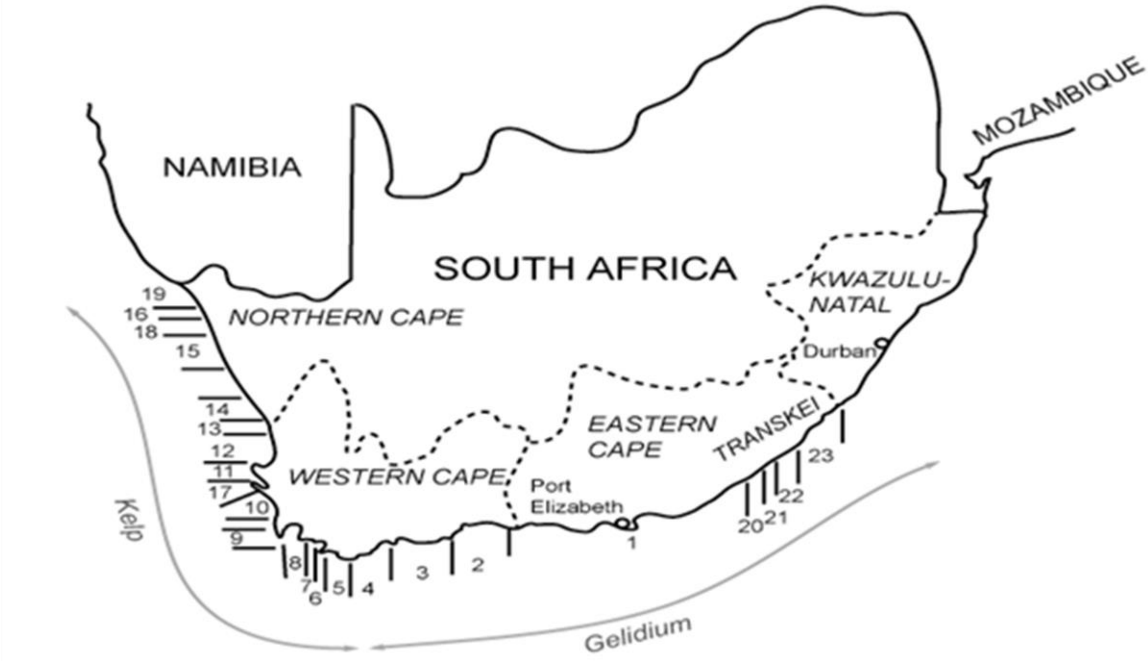

Figure 1: Map of South Africa showing area of potential commercial macroalgae concession right areas (Anderson *et al.* 2003).

The bulk of the fresh – wet harvested kelp forms the major fresh feed ingredient for cultured abalone in South Africa. The use of kelp for abalone feed has fluctuated since 2005 (Table 2), but in 2010 a total of 5,542 mt of fresh kelp fronds were supplied to farmers (DAFF, 2011a). Despite the large quantities of kelp supplied for use as abalone feed (Ad libitum feeding method), some farmers also use formulated feeds, such as Abfeed™ and Midae Meal™, and some do not use kelp at all. In 2010 commercial quantities of *Gelidium* were collected only from Concession Area 1; *G. pristoides* (Turner) Kuetzing comprises more than 90% of the harvest. Abundant endemic species such as *G. pristiodes*, *G. pteridifolium* Norris, Hommersand & Fredericq and *G. abbottiorum* R. E. Norris have been harvested from Eastern Cape intertidal areas since the mid 1950s and have been identified for possible exploitation from other Concession Areas[2] (viz. 1, 20, 21, 22 and 23).

---

[2] The coastline between the Orange and Mtamvuna Rivers is divided into 23 macroalgae rights areas. In each area, the rights to each group of seaweeds (e.g. kelp, *Gelidium*, or Gracilarioids) can be held by only one company, to prevent competitive overexploitation of these resources. Different companies may hold the rights to different resources in the same area(Figure 2).

Table 2: Annual yields of commercial macroalgae in South Africa, 2000-2010

Year	Gelidium (kg dry wt)	Gracilaria (kg dry wt)	Kelp Beach cast (kg dry wt)	Kelp fronds harvest (kg fresh wt)	Kelp fresh beach cast (kg fresh wt)	Kelpak (kg fresh wt)
2001	144 997	247 900	845 233	5 924 489	0	641 375
2002	137 766	65 461	745 773	5 334 474	0	701 270
2003	113 869	92 215	1 102 384	4 050 654	1 866 344	957 063
2004	119 143	157 161	1 874 654	3 119 579	1 235 153	1 168 703
2005	84 885	19 382	590 691	3 508 269	126 894	1 089 565
2006	104 456	50 370	440 632	3 602 410	242 798	918 365
2007	95 606	600	580 806	4 795 381	510 326	1 224 310
2008	120 247	0	550 496	5 060 148	369 131	809 862
2009	115 502	0	606 709	4 762 626	346 685	1 232 760
2010	103 903	0	696 811	5 336 503	205 707	1 264 739
Totals	1 140 374	633 089	8 034 189	45 494 533	4 903 038	10 008 012

Kelp beach cast' (column 4) refers to material that is collected in a semi-dry state, whereas 'kelp fresh beach cast' (column 6) refers to clean wet kelp fronds that, together with 'kelp fronds harvest' are supplied as abalone feed. 'Kelp fresh beach cast' was only recorded separately since 2003. Source: DAFF 2011a.

Yields vary with demand from a few to about 120 mt dry weight annually. The sheltered waters of Saldanha Bay (macroalgae Rights Area 17) and St Helena Bay (Areas 11 and 12 in parts) contain commercially viable amounts of Gracilarioids. *Gracilaria* gracilis (Stackhouse) Greville wash-ups from Saldanha Bay on the west coast were exported for extraction of agar. Although some *Gracilaria* cultivation was attempted in the 1990's in Saldanha Bay and St Helena Bay, these commercial ventures failed (Anderson *et al.*, 1989; 2003). Only beach-cast *Gracilaria* material may be collected commercially, because harvesting of the living beds is not sustainable. In Saldanha Bay, large yields (up to 2,000 mt dry weights in 1967) were obtained until the construction of the iron ore jetty and breakwater in 1974, after which yields fell dramatically (Anderson *et al.*, 1989; 2003). Occasional small wash-ups are obtained in St Helena Bay. Because total annual yields of Gracilarioids range from zero to a few hundred tonnes dry weight, this resource is regarded as unreliable (Anderson *et al.*, 1989; 2003). Accordingly, no gracilarioids have been collected commercially since 2007. From the start of commercial seaweed exploitation in South Africa in the 1950's, only six macroalgae genera (*Ecklonia*, *Laminaria*, *Gracilaria*, *Gelidium*, *Gigartina* and *Porphyra*) have been harvested, with most of this material being exported for use in the phycocolloid industry. *Ulva* has also been harvested in small amounts, but mostly for seaweed salt.

Today seaweed aquaculture in South Africa started as an offshoot of the abalone (*Haliotis midae* L) farming industry in the 1990's and has increased accordingly. Within South Africa twelve macroalgae species are currently being exploited: *Ulva* sp., *Porphyra* sp., *E. maxima*, *Laminaria pallida*, *Gracilaria gracilis*, *Gracilariopsis longissima* (S. G. Gmelin) M. Steentoft, L. M. Irvine & W. F. Farnham, *Gelidium abbottiorum*, *G. pteridifolium*, *G. pristoides, G. capense* (S.G. Gmelin) P. C. Silva, and *Plocamium corallorhiza* (Turner) Harvey (ESS, 2005; Troell *et al*., 2006; Robertson-Andersson, 2007).

South Africa's macroalgae resources are well protected under the Marine Living Resources Act of 1998 and are conserved from a concessional perspective[3] (Anderson *et al*., 1989; 2003; GPR, 2005; Anderson *et al*., 2006). In certain Concession Areas, limitations are placed on the quantity that can be harvested. These sustainable limits are termed Maximum Sustainable Yields (MSY) and equate to 10% of the estimated kelp accessible (non-reserve) biomass, a value that was estimated to equal the annual mortality rate for the kelp *E. maxima* (Simons and Jarman, 1981). A large amount of this harvested macroalgae is exported for the

---

[3] The macroalgaeresources are managed in terms of both a Total Applied Effort (TAE) and a Total Allowable Catch (TAC)

extraction of gums and 42.7% of the total harvest of fresh kelp fronds was supplied to abalone farmers as feed, this harvested kelp fetching a market value of R6 million (~ US$750 000) in 2010 (DAFF, 2012). Within the 23 Concession Areas, currently 14 areas are for kelp rights (Figure 2); no commercial activity was reported in five of these areas (DAFF, 2012).

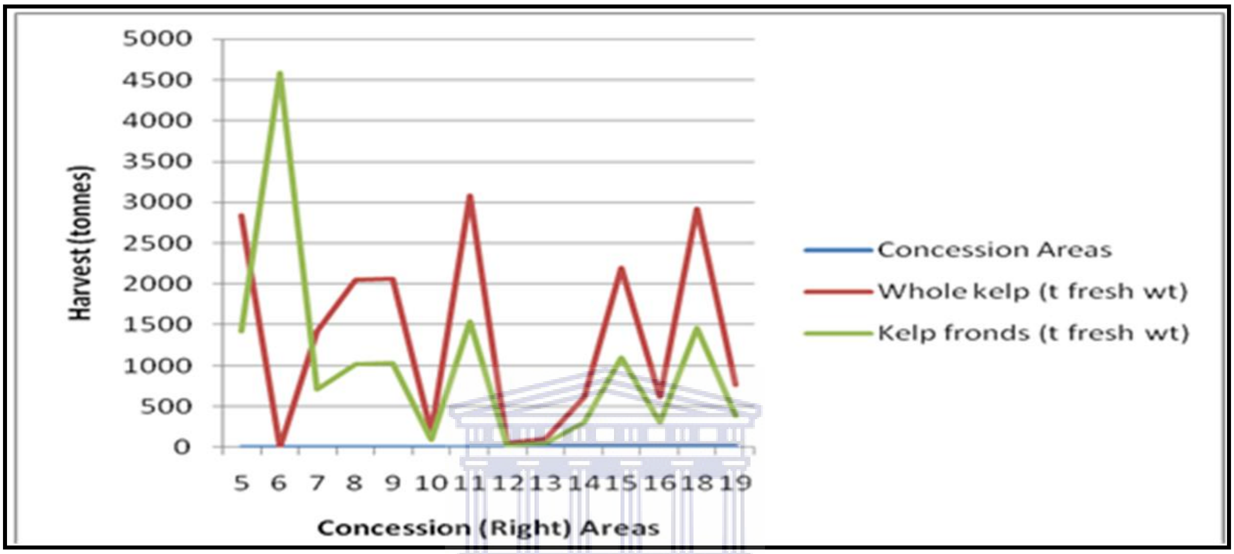

Figure 2: Maximum sustainable yield (MSY) of harvested kelp for all areas for 2010.

Importance, uses and benefits

Seaweed is produced (cultivated) in 25 countries globally, comprising 145 species used in food production and 101 species used in phycocolloid production (Dhargalkar and Verlecar, 2009). There are some commercial macroalgae species being cultivated in each of the main genera, which includes *Caulerpa*, *Chondrus*, *Eucheuma* (and *Kappaphycus*), *Gracilaria* (and *Gracilariopsis*), *Palmaria*, *Pyropia* (formerly *Porphyra*), *Monostroma*, *Saccharina* (formerly *Laminaria*), *Ulva* and *Undaria* (Zemke-White *et al.*, 1999; Fleurence 2004; Bruton *et al.*, 2009; Klaus *et al.*, 2009; Mohammad and Chakrabarti, 2009; FAO, 2010b; Paul and Tseng, 2012).

Agar and carrageenan are commercially valuable substances. The best quality agar, and its associated derivative agarose, comes from red algae belonging to the family Gelidiaceae, while lower-quality agars are mainly found in other families, mainly the Gracilariaceae. Globally agarose is used extensively in gels for electrophoresis in molecular biology. Carrageenans are generally employed for their viscous properties in gelation, as stabilization of emulsions, in suspensions and foams, and in the control of crystal formation in dairy products and frozen foods.

Macroalgae have been called the medical food of the 21st century (Khan and Satam, 2003). According to the World Health Organization (WHO) macroalgae are among the healthiest foods on the planet as they contain vitamins, over 90 minerals and many antioxidants (FAO, 2003). Historic evidence shows that seaweeds have been eaten by coastal communities of many countries since ancient times (FAO, 2002) and according to research, such communities who have historically consumed large quantities of seaweed, on average lived longer and had a lower incidence of hypertension and arteriosclerosis **(**Tietze, 2004).

Macroalgae are also used in the manufacture of pharmaceuticals and cosmetic creams (Bhakuni and Rawat, 2005; Leonel, 2011; Lewis *et al.*, 2011). For example, *Digenea* spp (Rhodophyta) produce an effective vermifuge (kainic acid) (Smit, 2004). *Laminaria* and *Sargassum* species have been used for the treatment of cancer (Khan and Satam, 2003). Anti-viral compounds discovered in *Undaria* spp have been used to inhibit the Herpes simplex virus (Barsanti and Gualtieri, 2006). Research is now being carried out into using seaweed extracts to treat breast cancer and HIV (Schaeffer and Krylov, 200; Synytsya *et al.*, 2010). Several calcareous species of *Corallina* have been used in bone-replacement therapy (Stein and Borden, 1984). *Asparagopsis taxiformis* and *Sarconema* spp. are used to control and cure goiter while heparin, a seaweed extract, is used in cardiovascular surgery (Khan and Satam, 2003).

Global seaweed trade

Globally the macroalgae industry is estimated to have an annual value of some US$6 billion (McHugh, 2003) and the largest share of this is for food products. Currently there are 42 countries across the world with reports of commercial macroalgae activity (cultivation and harvesting) (Khan and Satam, 2003; Bixler and Porse, 2011). The primary wild-harvested genera include *Chondrus*, *Furcellaria*, *Gigartina*, *Sarcothalia*, *Mazzaella*, *Iridaea*, *Mastocarpus*, and *Tichocarpus* (Bixler and Porse, 2011). As already mentioned, macroalgae are an important food source, especially in Japan (FAO, 2003). Popular macroalgae food stuffs include Wakame, Quandai-cai (*Undaria pinnatifida*), Nori (*Porphyra* spp), Kombu or Haidai (*Laminaria japonica*), Hiziki (*Hizikia fusiforme*), Mozuku (*Cladosiphon okamuranus*), Sea grapes or Green caviar (*Caulerpa lentillifera*), Dulse (*Palmaria palmata*), Irish moss or Carrageenan moss (*Chondrus crispus*), Winged kelp (*Alaria esculenta*), Ogo, Ogonori or Sea moss (*Gracilaria* spp), Carola (*Callophyllis variegata*), Leafy sea lettuce (*Ulva* spp), Arame (*Eisenia bicyclis*), and Kanten (agar-agar). Macroalgae products for human consumption contribute about US$ 5 billion of which nori is worth US$ 2 billion per annum (FAO, 2003).

The production of macroalgae and other aquatic algae reached 19.9 million mts in 2010, of which aquaculture produced 19 million mt. Japanese kelp was the most cultivated macroalgae species (5.1 million mt) in 2010 and most of it was grown in China (FAO, 2012). Major macroalgae aquaculture production come from China, Indonesia, Philippines, North Korea, South Korea, Japan, Malaysia, Chile, India, and Tanzania (Barsanti and Gualtieri, 2006, Bixler and Porse, 2011; FAO, 2010b; 2012). The most cultivated macroalgae is the kelp *Saccharina japonica*, which accounts for over 60% of the total cultured macroalgae ; species from the genera *Porphyra*, *Kappaphycus*, *Undaria*, *Eucheuma*, and *Gracilaria* make up the majority of the remaining total (Barsanti and Gualtieri, 2006).

High demand for carrageenan has similarly triggered the development of *Kappaphycus alvarezii* and *Eucheuma denticulatum* farming in several countries, the largest producers being the Philippines, Indonesia, Malaysia, Tanzania, Kiribati, Fiji, Kenya, and Madagascar (Bixler and Porse, 2011). World carrageenan production exceeded 50,000 mt in 2009, with a value of over US$527 million (Bixler and Porse, 2011). About 32,000 – 39,000 mt of alginic acid per annum is extracted worldwide from approximately 50,000 mt (wet weight) annual production of kelp (Barsanti and Gualtieri, 2006). Agar is relatively cheap, usually around US$18 per kg. In 2009, about 86,100 mt of hydrocolloids were traded comprising 58% of carrageen, approximately 31% alginates, and approximately 11% agar (10,000 mt with a value of $175 million); the major genera included *Ahnfeltiopsis*, *Gelidium*, *Gelidiella*, *Gracilaria*, *Pterocladiella* and *Pterocladia* (Bixler and Porse, 2011).

Africa regional seaweed aquaculture development

The African continent comprises 29 coastal countries and five island nations, few of which are practicing some form of macroalgae aquaculture (Machena and Moehl, 2001). However, the biogeographical features and shore characteristics in several of these countries suggest a high potential for macroalgae resources exploitation, culture and utilization.

West Africa

Excluding Ghana (200 species), Senegal (241 species), and Sierra Leone (112 species), which have high macroalgae diversities associated with upwelling events and rocky shores (Bolton *et al.*, 2003), West Africa generally has a low macroalgae diversity (John and Lawson, 1991). Nigeria (49 species), Benin (16 species), Togo (37 species) and Guinea Bissau (12 species) have coastlines characterized by sandy beaches and extensive mangroves, deltas, estuaries, and lagoons with correspondingly low algal diversity (John and Lawson, 1997). Recent research (Fakoya *et al.*, 2011; Abowei and Tawari, 2011) has shown the potential of macroalgae resources for exploitation, culture and utilization for Nigeria but as yet, no targeted commercial harvesting and cultivation has commenced.

North Africa

North Africa (Morocco – 197 species, Libya – 178 species, Tunisia – 87 species, Western Sahara – 81 species, Sudan – 18 species) has variable macroalgae species richness. The Moroccan coast, however, has been most studied, due to its proximity to European countries (Gallardo *et al.*, 1993) and this may explain the high species numbers. None the less, Morocco has a well-established macroalgae industry based on the extraction of agar from wild *Gelidium* species. Steps are also being taken to identify suitable protected natural sites for macroalgae cultivation, presumably with a view to cultivating *Gracilaria* to supplement the natural resources of *Gelidium* for agar production (FAO, 2003).

East Africa

The East African coastline is about 9500 km long and comprises the tropical coasts of Somalia (211 species), Kenya (403 species), Tanzania (428 species), Mozambique (243 species) and Madagascar (207 species). Macroalgae aquaculture is a recent development in

East Africa, occurring in all East African countries except Somalia. Tanzania's aquaculture production has increased steadily to become the largest producer of aquaculture products in Africa (FAO, 2012). *Eucheuma denticulatum* (previously *E. spinosum*) and *Kappa-phycus alvarezii* (previously *E. cottonii*) have been farmed in the region since 1989. These two species are found naturally in East Africa, and were previously collected from the wild for export to USA and Europe. Although the species are found locally, the farmed strains are mainly imported from the Philippines (FAO, 2012). Madagascar currently accounts for a very small proportion (about 4,000 mt of macroalgae per year) of global seaweed production, despite the fact that much of its 5,000 km coastline provides perfect conditions for macroalgae cultivation (FAO, 2012). With assistance from commercial sources, macroalgae cultivation is proving to be promising in Mozambique (FAO, 2012). This will make Mozambique only the fourth macroalgae producing nation in Africa (FAO, 2012). To support this industry and to promote aquaculture, the Mozambique government recently (2011) approved a decree establishing the marine aquaculture reserve. Approximately 10,600 hectares have been set aside for macroalgae aquaculture, potentially yielding 641,000 mt of seaweed (Nkutumula, 2011). The macroalgae of Kenya are well-studied relative to other East African/Indian Ocean countries (Bolton *et al.*, 2003). However, Kenya does not present good prospects for a macroalgaeindustry. None of the pilot studies carried out have produced any promising results that would encourage investors to venture into macroalgae farming for Kenya**.**

Southern Africa

Namibia’s proximity to South Africa greatly influenced the documentation of the former country’s macroalgae resources. Both countries have developed through technology sharing, but the macroalgae aquaculture industry in Namibia is still not as developed as in South

Africa. The 196 macroalgae species of Namibia have been studied and documented (Engeldow, 1998; Rull Lluch, 1999; 2002; Engeldow and Bolton, 2003). As in South Africa, Namibian macroalgae harvesting companies operate under a system of Concessions Areas. The industry provides employment opportunities for over 250 people in an area where job opportunities are severely lacking. Investment in polyculture of macroalgae and crustaceans has also been promoted in Namibia (Hasan and Chakrabarti, 2009). Of the 196 Namibian species of macroalgae, nine have shown potential use as animal feed supplements. Beach-cast *Gracilaria* is also collected and cultivation is being developed by a local company; the current market, however, is depressed.

The macroalgae of South Africa have been extensively detailed (Stegenga *et al*., 1997; De Clerck *et al.*, 2005; Maneveldt *et al*., 2008). The known macroalgae diversity of South Africa has increased from 547 species in 1984 to around 900 species in 2012, making the region one of the richest marine floras in the world, with a high level of endemism (Payne *et al*., 1989; Bolton, 1999; Bolton *et al*., 2003; Maneveldt *et al*., 2008; pers. obs.). South African macroalgae aquaculture is focused on the abalone industry, particularly the abalone, *Haliotis midae* (Bolton *et al*., 2006; Troell *et al*., 2006). By far the most cultivated macroalgae species is *Ulva* spp. The aquaculture of *Ulva* spp occurs on many abalone farms (DAFF 2010) and here paddle-wheel raceways have proven to be the most suitable device for growing *Ulva* spp in large quantities (Chopin *et al*., 2008).

The South African abalone aquaculture industry has grown rapidly over the past few decades along the west coast of South Africa where suitable rocky habitat exists (Troell *et al*., 2006). On-land integrated culture units, which use shallow raceways, are the preferred method of production for the abalone industry (Bolton *et al*., 2006). There is growing evidence that suggests a mixed diet of kelp and other macroalgae can induce growth rates that meet or exceed those attained with artificial feed (Naidoo *et al*., 2006; Dlaza *et al*., 2008; Francis *et al*., 2008; Robertson-Andersson *et al*., 2011). Moreover, a natural diet can improve abalone quality and reduce parasite loads (Robertson-Andersson, 2003; Naidoo *et al*., 2006; Al-Hafedh *et al*., 2012).

Problems and prospects of macroalgae aquaculture in Africa

Failures of some ill-conceived pilot projects (e.g. South-west Madagascar - De San, 2012) continue to remain a major constraint in convincing farmers and investors of the economic viability of macroalgae aquaculture in most African coastal countries. Several other constraints have prolonged the development of the industry in many African countries, and these can be summarized as: weak economies; poor aquaculture development policies; inappropriate technologies; weak extension services; weak impact of research institutions; inadequate information management systems; limited coordination between research and production sectors; scanty reliable production statistics and the high value/cost of coastal land; and the associated competition for this land from other coastal industries (Troell *et al*., 2011). In the countries (South Africa, Tanzania, Madagascar, Mozambique, Namibia, Burkina Faso, Central Africa Republic and Senegal) where thriving macroalgae cultivation practices have been achieved, these industries provide a meaningful form of income for communities that might otherwise not be employable in the traditional sense (Troell *et al*., 2011).

Lessons for other coastal nations

The general benefit of integrated multitrophic aquaculture[4] (IMTA) is the reduction of nutrient release to the environment (Neori *et al*., 2004; Bolton *et al*., 2009). This phenomenon is also true for integrated macroalgae -abalone culture in South Africa. The technical and economic feasibility of IMTA using macroalgae as biofilters is already well established in South Africa (Nobre *et al*., 2010). Macroalgae grown in abalone effluent have an increased nitrogen content (sometime as much as 40% protein dry weight content), resulting in value-added macroalgae of excellent quality to feed abalone (Naidoo *et al*., 2006; Robertson-Andersson, 2007; Robertson-Andersson *et al*., 2011). Not only in South Africa but elsewhere, the increasing demand for abalone feed has seen the need for sustainable production of macroalgae in IMTA aquaculture with aquatic animals (Brzeski and Newkirk, 1997; Troell *et al*., 1999; Buschmann *et al*., 2001), especially with abalone (Neori *et al*., 1991; 1996; 1998; 2004; Bolton *et al*., 2009). To improve macroalgae biomass estimations and to document the relative macroalgae distributions, GIS mapping and diver-based sampling of the resource is regularly undertaken in South Africa as a government requirement. Monthly harvests of fresh kelp are routinely checked against the prescribed MSY as set in the annual permit conditions of all rights holders. Visual inspections by South African government officials, and reports received from right-holders, show that the kelp resource is stable and healthy (DAFF, 2011a).

Although the South African macroalgae sector is small in comparison to similar fisheries, it is currently worth US$3.7 million, generates approximately US$2 million[5] per year, but nevertheless employs up to 400 people, the majority of whom are women who earn an

---

[4] Integrated Multitrophic Aquaculture (IMTA) is defined as an ecosystem based management approach that effectively mitigates the overabundance of nutrients introduced by fish farming.

[5] 1US$(US dollar) equals R9.55 (SA Rand) as at 22 May 2013.

average annual salary of US$ 5 000 (Payne *et al*., 1989; DAFF, 2011a; 2011b; DAFF, 2012). More importantly, high proportions (92%) of the employees in the sector are classified as historically disadvantaged persons[6] (DAFF, 2011a). The South African aquaculture sector thus has an important local impact within previously disadvantaged coastal communities, where any increase in employment is valuable largely because such communities are generally characterized by high rates of unemployment (85.7%) and low skill levels (50%) (Nobre *et al*., 2010).

South Africa is currently spearheading a number of other research innovations. Research has shown that abalone farms incorporating an IMTA macroalgae-abalone system can significantly reduce their green-house gas (GHG) emissions (Nobre *et al*., 2010; Troell *et al*., 2011). Due to their high carbohydrate contents, macroalgae can be fermented to CH_4 (biogas) and have subsequently been considered a potential CO_2-neutral and renewable energy supply (Bartsch *et al*., 2008; Roesijadi *et al*., 2008; Bruhn *et al*., 2011; Chung *et al*., 2011). Furthermore, recent research findings have shown that *Laminaria* and *Ulva* species are important prospects from an energy point of view (Bruton *et al*., 2009; Klaus *et al*., 2009; Abowei and Tawari, 2011; Bruhn *et al*., 2011; Chanakya *et al*., 2012). This important benchmarking knowledge could propel the commencement of research on macroalgae as a substitute for liquefied petroleum gas (LPG). Aside from being a renewable resource and reducing CO_2 emissions (especially if macroalgae cultivation is incorporated with a source of CO_2 production), macroalgae cultivation could potentially have a major positive impact on global warming and ocean acidification. As a consequence of these findings, South Africa is

---

[6] Historically disadvantaged persons are persons so classified as underdeveloped populations targeted by the SA Government for accelerated development (www.polity.org.za/html/govdocs/rd/rdp2.html).

.

currently investigating large-scale anaerobic digestion of macroalgae to methane as well as the local species of macroalgae potential for mitigation of ocean acidification.

Conclusion

Despite the fact that South Africa is currently not Africa's highest macroalgae aquaculture producer, the country has the highest regional macroalgae diversity and one of the richest in the world. As a third-world country with many first-world technologies, South Africa provides many important lessons for less developed coastal African nations. The South African macroalgae aquaculture industry is well researched and has developed steadily due to the increasing demand for abalone feed that has seen the need for sustainable production of seaweed in IMTA. The South African macroalgae aquaculture industry provides raw materials for other sectors of the economy, as well as the potential for bioremediation of both the atmospheric and aquatic environment.

Acknowledgements

We thank the Department of Biodiversity and Conservation Biology at the University of the Western Cape, and the Department of Biological Sciences at the University of Cape Town for providing funding and research equipment. The South African National Research Foundation (NRF) and Department of Agriculture, Forestry and Fisheries (DAFF) are thanked for research grants and the required permits to GWM, JJB and RJA.

References

Abowei JFN, Tawari CC (2011). A Review of the Biology, Culture, Exploitation and Utilization Potentials Seaweed Resources: Case Study in Nigeria. Research Journal of Applied Sciences, Engineering and Technology 3(4): 290-303.

Al-Hafedh YS, Alam A, Buschmann AH, Fitzsimmons KM (2012). Experiments on an integrated aquaculture system (seaweeds and marine fish) on the Red Sea coast of Saudi Arabia: efficiency comparison of two local seaweed species for nutrient biofiltration and production. Reviews in Aquaculture 4: 21-31.

Amosu AO, Bashorun OW, Babalola OO, Olowu RA, Togunde KA (2012). Impact of climate change and anthropogenic activities on renewable coastal resources and biodiversity in Nigeria. Journal of Ecology and the Natural Environment 4(8): 201-211.

Anderson RJ, Rothman MD, Share A, Drummond H (2006). Harvesting of the kelp *Ecklonia maxima* in South Africa affects its three obligate, red algal epiphytes. Journal of Applied Phycology 18: 343-349.

Anderson RJ, Bolton JJ, Molloy FJ, Rotmann KW (2003). Commercial seaweeds in southern Africa. In: Chapman ARO, Anderson RJ, Vreeland VJ, Davidson I (Eds) Proceedings of the 17th International Seaweed Symposium. Oxford University Press, Oxford, pp. 1-12.

Anderson RJ, Simons RH, Jarman NG (1989). Commercial seaweeds in southern Africa: A review of utilization and research. South African Journal of Marine Science 8: 277-299.

Barsanti L, Gualtieri P (2006). Algae Anatomy, Biochemistry, and Biotechnology Taylor and Francis Group LLC. Boca Raton London New York CRC Press.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schumann R, Schubert H, Valentin K, Weinberger F, Wiese J (2008). The genus *Laminaria* sensu lato: recent insights and developments. European Journal of Phycology 43(1): 1-86.

Bhakuni DS, Rawat DS (2005). Bioactive marine natural products. Anamaya Publishers, New Delhi, India.

Bixler HJ, Porse H (2011). A decade of change in seaweed hydrocolloids industry. Journal of Applied Phycology 23: 321-335.

Bolton JJ (1999). Seaweed systematic and diversity in South Africa: An historical account. Transactions of the Royal Society of South Africa 54 (1): 167-177.

Bolton JJ, De Clerck O, John DM (2003). Seaweed diversity patterns in Sub-Saharan Africa. Proceedings of the Marine Biodiversity in Sub-Saharan Africa: The Known and the Unknown, 23-26 September, Cape Town, South Africa, pp. 229-241.

Bolton JJ, Robertson-Andersson DV, Shuuluka D, Kandjengo L (2009). Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. Journal of Applied Phycology 21: 575-583.

Bolton JJ, Robertson-Andersson DV, Troell M, Halling C (2006). Integrated systems incorporate seaweeds in South African abalone culture. Global Aquaculture Advocate 9: 54-55.

Bruhn A, Dah J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011). Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion. Bioresource Technology 102: 2595-2604.

Bruton, T, Lyons H, Yannick Lerat, Y, Rasmussen MB (2009). A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Sustainable Energy Ireland, Glasnevin, Dublin 9. Ireland. Retrieved from www.sei.ie

Brzeski V, Newkirk G (1997). Integrated coastal food production systems - a review of current literature. Ocean Coast Manage 34: 66-71.

Buschmann AH, Troell M, Kautsky N (2001). Integrated algal farming: a review. Cahiers de Biologie Marine 43:615-655.

Chanakya HN, Mahapatra D.M, Ravi S, Chauhan SV, Abitha R (2012). Sustainability of large-Scale algal biofuel production in India. Journal of the Indian Institute of Science 92(1): 63-98.

Chopin T, Robinson SMC, Troell M, Neori A, Buschmann AH, Fang J (2008). Multitrophic integration for sustainable marine aquaculture. In: Jørgensen SE, Fath BD (eds) Encyclopedia of ecology, vol 3, Ecological engineering. Elsevier, Oxford, pp. 2463-2475.

Chung IK, Beardall J, Mehta S, Sahoo D, Stojkovic S (2011). Using marine macroalgae for carbon sequestration: a critical appraisal. Journal of Applied Phycology 23: 877-886.

Department of Agriculture, Forestry and Fisheries DAFF (2010). Marine Aquaculture Annual Farm Operation Report 2010.

DAFF (2011a). Status of the South African Marine Fishery Resources, 2010. Unpublished report, South African Department of Agriculture, Forestry and Fisheries.

DAFF (2011b). Aquaculture annual report 20011 South Africa. South African Department of Agriculture, Forestry and Fisheries.

DAFF (2012). Agriculture, Forestry and Fisheries- Integrated Growth and Development Plan 2012. South African Department of Agriculture, Forestry and Fisheries.

De Clerck O, Bolton JJ, Anderson R.A, Copperjans E (2005). Guide to the seaweeds of KwaZulu-Natal. Scripta Botanica Belgica 33: 1-294.

De San M (2012). The farming of seaweeds. Implementation of a regional fisheries strategy for the eastern - southern Africa and India Ocean Region. Report: SF/2012/28.

Dlaza TS, Maneveldt GW, Viljoen C (2008). The growth of post-weaning abalone (*Haliotis midae* Linnaeus) fed commercially available formulated feeds supplemented with fresh wild seaweed. African Journal of Marine Science 30(1): 199-203.

Engeldow HE (1998). The biogeography and biodiversity of the seaweed flora of the Namibian intertidal seaweed flora. PhD Dissertation, University of Cape Town, South

Africa.

Engledow HE, Bolton JJ (2003). Factors affecting seaweed biogeographical and ecological trends along the Namibian coast. Proceedings of the 17th International Seaweed Symposium. Oxford University Press. 285-291.

ESS, 2003.Mather D, Britz P J, Hecht T, Sauer, WHH (2003). An economic & sectoral study of the South African Fishing Industry. V .1. Economic & regulation principles, survey results, transformation and socio-economic impact. Report prepared for Marine and Coastal Management, Department of Environmental Affairs and Tourism by Rhodes University, Grahamstown, South Africa. pp. 300.

Fakoya KA, Owodeinde FG, Akintola SL, Adewolu MA, Abass MA, Ndimele PE (2011). An Exposition on Potential Seaweed Resources for Exploitation, Culture and Utilization in West Africa: A Case Study of Nigeria. Journal of Fisheries and Aquatic Science 6: 37-47.

FAO (2002). Prospects for seaweed production in developing countries, FAO Fisheries Circular No. 968 FIIU/C968 (En). FAO Rome.

FAO (2003). A guide to the seaweed industry, FAO fisheries technical paper 44, Rome.

FAO (2009). The state of world fisheries and aquaculture 2008. FAO fisheries and aquaculture department. Food and Agriculture Organization of United Nations. Rome. 2009.

FAO (2010a). The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department. Food and Agricultural Organization of the United Nations. Rome.

FAO (2010b). FishStat fishery statistical collections: aquaculture production (1950-2008; released March 2010). Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fishery/statistics/software/fishstat/en

FAO (2011). FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FishStatPlus. Universal Software for fishery statistical time series. Version 2.3 in 2000. Last database update in April 2011. Retrieved from http://www.fao.org/docrep/013/i1820e/i1820e00.htm.

FAO (2012). The State of World Fisheries and Aquaculture. ISBN: 978-92-5-107225-7. ftp://ftp.fao.org/FI/brochure/SOFIA/2012/english_flyer.pdf

Fleurence J (2004). Seaweed proteins, in: R.Y. Yada (Ed.), Proteins in Food Processing, Woodhead Publishing, pp. 197-210.

Francis TL, Maneveldt GW, Venter J (2008). Growth of market-size abalone (*Haliotis midae*) fed kelp (*Ecklonia maxima*) versus a low-protein commercial feed. African Journal of Aquatic Science 33(3): 279-282.

Gallardo T, Garreta GA, Ribera MA, Cormaci M, Furnari G, Giacconi G, Boudouresque CF (1993). Checklist of Mediterranean seaweeds. II Chlorophyceae. Botanica Marina 36: 399-421.

GPR (2005). General reasons for the decisions on the allocation of rights in the seaweed sector. DEAT publication. Retrieved from https: www.mcm-deat.gov.za.

Hasan MR, Chakrabarti R (2009). Use of algae and aquatic macrophytes as feed in small-scale aquaculture: a review. FAO Fisheries and Aquaculture Technical Paper. No. 531. Rome, FAO. 2009. pp.123.

John DM, Lawson GW (1991). Littoral ecosystems of tropical western Africa. In: Mathieson, A.C. & Nienhuis, P.H. (eds) Intertidal and Littoral Ecosystems. Ecosystems of the World 24: 297-322.

John DM, Lawson GW (1997). Seaweed biodiversity in West Africa: a criterion for designating marine protected areas S.M. Evans, C.J. Vanderpuye, A.K. Armah (Eds.), The Coastal Zone of West Africa: Problems and Management, Penshaw Press, Sunderland U.K (1997), pp. 111-123.

Khan SI, Satam SB (2003). Seaweed Mari culture. Scope and Potential in India. *Aquaculture* Asia 8 (4): 26-29.

Khan W, Rayirath UP, Subramanian S, Jithesh NM, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009). Seaweed Extracts as Biostimulants of Plant Growth and Development. Journal of Plant Growth Regulation 28: 386-399.

Klaus JH, Uwe F, Rocio H, Anja E, Morchio R, Suzanne H, Boosya B (2009). Aquatic Biomass: Sustainable Bio-energy from Algae? - Issue Paper -,The workshop is co-funded by two German research projects “bio-global” (sponsored by the Federal Ministry for Environment, BMUthrough the Federal Environment Agency, UBA) and “conCISEnet”(sponsored by the Federal Ministry for Education and Research, BMBF) Bio-global / conCISEnet. Retrieved from www.oeko.de

Leonel P (2011).A Review of the Nutrient Composition of Selected Edible Seaweeds. In: Seaweed (Ed) by Vitor H. Pomin. Nova Science Publishers, Inc.

Lewis J, Salam F, Slack N, Winton M, Hobson L (2011). Product options for the processing of marine macroalgae. Summary Report’.The Crown Estate, pp.44.

Lorentzen K, Amarasinghe US, Bartley DM, Bell JD, Bilio M, de Silva SS, Garaway CJ, Hartman WD, Kapetsky JM, Laleye P, Moreau J, Sugunan VV, Swar DB (2001). Strategic review of enhancement and culturebased fisheries. pp. 211-237 In Subasinghe, RP., Bueno, PB., Phillips, MJ., Hough, C,, McGladdery, SE. and Arthur, JR. (eds.). Aquaculture in the third millennium. Technical proceedings of the conference on aquaculture in the thirdmillennium, Bangkok, Thailand. 2025 February 2000. NACA, Bangkok and FAO, Rome.

Machena C, Moehl J (2001). Sub-Saharan African aquaculture: regional summary. In R.P. Subasinghe, P., Bueno, M.J., Phillips, C., Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 341-355. NACA, Bangkok and FAO, Rome.

Maneveldt GW, Chamberlain YM, Keats DW (2008). A catalogue with keys to the non-geniculate coralline algae (Corallinales, Rhodophyta) of South Africa. South African Journal of Botany 74: 555-566.

McHugh DJ (1987) (ed.). Production and utilization of products from commercial seaweeds. FAO FisheriesTechnical.Paper No.288. Rome, Italy.

McHugh DJ (2001). Prospects for Seaweed Production in Developing Countries. Food and Agriculture Organization of United Nations, Rome, Italy.

McHugh DJ (2003). A guide to the seaweed industry. FAO Fisheries Technical Ppaper No. 441. Rome: Food and Agricultural Organisation of the United Nations.

Mohammad RH, Chakrabarti R (2009). Use of algae and aquatic macrophytes as feed in small-scale aquaculture: A review. FAO, Fisheries and Aquaculture Technical Paper No. 531. Rome.

Naidoo K, Maneveldt GW, Ruck K, Bolton JJ (2006). A comparison of various seaweed-based diets and artificial feed on growth rate of abalone in land-based aquaculture systems. Journal of Applied Phycology 18: 437-443.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern aquaculture. Aquaculture 231: 361-391.

Neori A, Krom M, Ellner S, Boyd C, Popper D, Rabinovitch R, Davison P, Dvir O, Zuber D, Ucko M, Angel D, Gordin H (1996). Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture* 141: 183-199.

Neori A, Ragg NLC, Shpigel M (1998). The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. Aquaculture Engineering 17: 215-239.

Neori A, Cohen I, Gordin H (1991). *Ulva lactuca* biofilters for marine fish pond effluents. II. Growth rate, yield and C: N ratio. Botanica Marina 34: 483-489.

Nkutumula A (2011). Mozambique: Government Promotes Aquaculture, Fisheries Ministry, National Aquaculture Development Institute (INAQUA), Retrieved from http://allafrica.com/stories/201109141399.html

Nobre AM, Robertson-Andersson DV, Neori A, Sankar K (2010). Ecological-economic assessment of aquaculture options: comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture 306: 116-126.

Paul NA, Tseng CK (2012). Seaweed and Microalgae .Aquaculture: Farming Aquatic Animals and Plant, Second edition, edited by John S Lucas, C Southgate. Blackwell Publishing Ltd.

Payne AIL, Crawford RJM, Van Dalsen A (1989). Oceans of Life off Southern Africa. Vlaeberg Publishers, Cape Town.

Robertson-Andersson DV, Maneveldt GW, Naidoo K (2011). Effects of wild and farm-grown macroalgae on the growth of juvenile South African abalone *Haliotis midae* (Linnaeus). African Journal of Aquatic Science 36(3): 331-337.

Robertson-Andersson DV (2007). Biological and economical feasibility studies of using seaweeds *Ulva lactuca* (chlorophyta) in recirculation systems in abalone farming. PhD Dissertation, University of Cape Town, South Africa.

Robertson-Andersson DV, Leitao D, Bolton JJ, Anderson RJ, Njobeni A, Ruck K (2006). Can kelp extract (KELPAK™) be useful in seaweed mariculture? Journal of Applied Phycology 18: 315-321.

Robertson-Andersson DV (2003). The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. MSc Dissertation, University of Cape Town, South Africa.

Roesijadi G, Copping AE, Huesemann MH, Forster J, Benemann JR (2008). Techno-economic feasibility analysis of offshore seaweed farming for bioenergy and biobased products. Independent research and development report IR # PNWD-3931, Battelle Pacific Northwest Division, pp. 115.

Rull Lluch JR (1999). Algues benthõniques marines de Namibia. PhD Dissertation. University of Barcelona, Spain.

Rull Lluch JR (2002). Marine benthic algae of Namibia. Scientia Marina 66 (suppl. 3): 5-256.

Schaeffer DJ, Krylov VS (2000). Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicology and Environmental Safety 45: 208-227.

Simons RH, Jarman NG (1981). Subcommercial harvesting of kelp on a South African shore. Proceedings of the Tenth International Seaweed Symposium. De Gruyter, Berlin, pp. 731-736.

Smit AJ (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review, Journal of Applied Phycology 16: 245-262.

Smith MD, Roheim CA, Crowder LB, Halpern BS, Turnipseed M, Anderson JL, Asche F, Bourillón L, Guttormsen AG, Kahn A, Liguori L.A, McNevin A, O'Connor M, Squires

D, Tyedemers P, Brownstein C, Carden K, Klinger DH, Sagarin R, Selkoe KA (2010) Sustainability and Global Seafood, Science 327: 784-786.

Stegenga H, Bolton JJ, Anderson RJ (1997). Seaweeds of the South African west coast. Contributions from the Bolus Herbarium No. 8, University of Cape Town, pp.655.

Stein JR, Borden CA (1984). Causative and beneficial algae in human disease conditions: a review. Phycologia 23: 485-501.

Synytsya A, Kim W, Kim S, Pohl R, Synytsya A (2010). Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. Carbohydrate Polymers 81(1): 41-48.

Tietze HW (2004). Spirulina - Micro Food Macro Blessing, Harald W. Tietze Publishing, Bermagui NSW 2546. Australia.

Troell MD, Robertson-Andersson DV, Anderson RJ, Bolton JJ, Maneveldt G, Halling C, Probyn T (2006). Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Journal of Applied Phycology 257(4): 266-281.

Troell MD, Ronnback P, Halling C, Kautsky N, Buschmann A (1999). Ecological engineering in aquaculture: use of seaweeds for removing nutrients form intense mariculture. Journal of Applied Phycology 11: 89-97.

Troell MD, Hecht T, Beveridge M, Stead S, Bryceson I, Kautsky N, Mmochi A, Ollevier F (eds.) (2011) Mariculture in the WIO region - Challenges and Prospects. WIOMSA. Book Series No. 11. Viii, pp .59.

Zemke-White WL, Clements KD, Harris PJ (1999). Acid lysis of macroalgae by marine herbivorous fishes: myth or digestive mechanism? Journal of Experimental Marine Biology and Ecolog 233: 95-113.

Zhang G, Que H, Liu X, Xu H (2004). Abalone mariculture in China, Journal of Shellfish Research 23: 947-950.

Chapter 3: Paper 2

Aquaculture benefits of macroalgae for green energy production and climate change mitigation

This chapter is based on:

Amosu A.O., Robertson-Andersson D.V., Kean E. and Maneveldt G.W. (2014). Aquaculture benefits of macroalgae for green energy production and climate change mitigation. *International Journal of Scientific & Engineering Research* 5(7): 146-152.

Abstract

It is an established fact that climate change caused by human-induced concentrations of greenhouse gases (GHG), especially CO_2 emissions is increasing in the earth's atmosphere and is the greatest challenge the world is currently facing. Algae play significant roles in normal functioning of the atmospheric environment and are important candidates for climate change mitigation. Macroalgae (over 20 commercial seaweed species) are the second most cultured species of aquatic organisms after finfish. More than 92 % of the world's macroalgae production comes from mariculture. Macroalgae have a higher photosynthetic efficacy (6 – 8 %) than that of terrestrial plants (1.8 – 2.2 %). An investigation into seaweed as a food source for the South African abalone (*Haliotis midae* L.) has led to an increased knowledge of its fisheries and aquaculture conditions. *Ulva* spp are grown on a large scale in paddle wheel ponds and is currently South Africa's largest aquaculture product. Its growth rate, ease of harvesting, resistance to contamination by other algal species and minimal production loss make it preferable to microalgae and to other macroalgae for large scale renewable energy production and CO_2 capturing systems. Of all macroalgae, *Ulva* spp are exciting prospects in terms of energy efficiency. Findings have further revealed that biotransformation of *Ulva armoricana* to Liquefied Petroleum Gas (LPG) is viable. Large scale aquaculture production of *Ulva* spp is occurring in South Africa and biotransformation to LPG is possible and economically feasible with additional benefits from farming activities including bioremediation, ocean de-acidification, mineral-rich plant stimulants, and the capturing of atmospheric and dissolved CO_2 during growth to assist in climate change mitigation.

Index Terms — Aquaculture, climate change, CO_2, green energy, mitigation, seaweed, South Africa, *Ulva armoricana*

Introduction

The Earth's radiative energy balance is undergoing change due to the increase in greenhouse gases, primarily CO_2 from fossil fuel combustion, and from anthropogenic aerosols (Wuebbles and Jain, 2001). The long term trend of increasing atmospheric CO_2 has become a focal point in current research across atmospheric, terrestrial, and marine science disciplines. An evolved understanding of how our current global climate is being and will be influenced by continuing increases in CO_2 emissions and subsequent global warming, is required to predict how climate change will impact our livelihood and the future health of ecosystem integrity. In response, several developed and developing nations like the EU, USA, Canada, Brazil, Argentina, Colombia, China, New Zealand and Japan have incorporated biofuel targets into their renewable energy policies in recent years (Steenblik, 2007). Meanwhile in Africa, South Africa was one of the very first countries to provide the necessary political will and desire to explore opportunities for a green economy, through the National Green Economy summit in 2010 (Tshangela and Roman, 2012). South Africa emits approximately 400 million tons of CO_2 annually, ranks among the 20 highest contributors to CO_2 emissions overall, and produces approximately 2 % of global greenhouse gas (GHG) emissions, yet it has only 0.7 % of the world's population, and produces 0.9 % of the world GDP (Douglas and Schaffler, 2006; Sinha *et al.,* 2010; Khoza, 2012).

Energy supply in South Africa is primarily coal-based. South Africa is therefore a CO_2 intense economy, with the country's major energy requirement sourced from fossil fuels (Douglas and Schaffler, 2006). It is necessary, at an industrial scale, to shift the dependence on fossil fuel-based energy to that of renewable and sustainable practices (Douglas and Schaffler, 2006). The seaweed aquaculture industry as a biomass source for the production of biomethane gas is feasible in South Africa and could help promote this needed shift. The fact that fossil fuel prices are increasing and that macroalgae production costs will inevitably fall as algal production expands (Douglas and Schaffler, 2006; Khoza, 2012), make large scale macroalgae cultivation financially feasible. Unlike the first generation biofuels, macroalgae have additional advantages that make them environmentally sustainable. The high oxygen (by-product of photosynthesis) amounts dissolved in the paddle ponds enable the water to be re-used for integrated polyculture with aquatic animals.

Biomass energy is the conversion of biomass into useful forms of energy such as electricity, heat, and liquid fuels (Mckendry, 2002). Macroalgae or seaweeds, undergo CO_2 fixation to attain a high biomass production, and may assist in sequestering atmospheric sources of CO_2. Of all macroalgae in South Africa, the algae *Ulva* spp are one of the most promising prospects from an energy point of view. *Ulva* spp are grown on a large scale, and are currently South Africa's largest aquaculture product (Shipton and Britz, 2007; Bolton *et al.*, 2009; Bruton *et al.*, 2009; DAFF, 2011).

Macroalgae are able to grow in varying conditions, both in fresh or salt-water bodies, and are tolerant of a diverse range of pH conditions (IEA, 1994). There are about 36000 species of algae, and most species are exploited from the wild as the technology for their propagation is yet to be fully developed (FAO, 2006; Miller, 2009; Ralph, 2012), although significant strides have been made more recently. Macroalgae are capable of producing more biomass per square meter than any fast growing terrestrial plant and are the second most cultured aquatic organisms after finfish (Briggs and Fung-Smith, 1993; Adams, 2011). In the last 50 years, about 100 macroalgal species have been commercially cultivated from the genera *Gracilaria*, *Euchema*, *Laminaria*, *Undaria*, *Ulva*, *Chondrus*, *Porphyra*, *Palmaria* and *Monostroma* (Zemke-White and Ohno, 1999; Fleurence, 2004; Sahoo and Yarish, 2005; FAO, 2009; Klaus *et al.*, 2009; Mohammad and Chakrabarti, 2009; Paul and Tseng 2012). Currently over 92 % of the world's macroalgae production by weigtht comes from aquaculture species (Ozer, 2005; Chopin and Sawhney, 2009; Paul and Tseng 2012). Macroalgae aquaculture in South Africa started as an off shoot of the abalone (*Haliotis midae* L) farming industry (Troell *et al.*, 2006). Since its inception in the 1990s, abalone aquaculture in South Africa has developed rapidly and the country is currently the second largest producer outside Asia (FAO, 2000; Troell *et al.*, 2006). This rapid development was partly achieved due to demand being driven by the decline of South African wild abalone collection due to poaching. By 2006 several South African seaweed concession areas had harvested up to 99 % of their MSY (Troell *et al.*, 2006). This lead the industry to explore alternative abalone feed. One of the alternatives proposed were seaweeds cultivated in aquaculture effluent (Robertson-Andersson 2007). Since then over 2000 tons of *Ulva* spp. were cultivated as feed. Researchers performed a strength, weaknesses, opportunities and threats (SWOT) analysis of the seaweed cultivation industry and stated that *Ulva* product diversification is needed to increase its potential in South Africa (Bolton *et al.*, 2009). The objective of this work was to investigate the potential for large scale anaerobic digestion of *Ulva* spp to produce methane gas from a readily available aquaculture product. If the large scale production of biomethane proved environmentally and economically feasible and sustainable, it could serve as an alternative to the dwindling oil supply and help mitigate global CO_2 emissions.

Materials and Methods

Biomass Production

Ulva armoricana production experiments were carried out during winter at the Benguela Abalone Group aquaculture farm on the West Coast of South Africa in four 32 m X 8 m (180

m^3) concrete paddle ponds, filled to approximately 0.55 m depth with unfiltered seawater on a flow through system. Ponds received 2 volume exchanges per day. The ponds set up were characterized as follows:

A – *U. armoricana* + standard seawater (control)
B – *U. armoricana* + nutrients added to improve growth (single fertilizer ratio)
C – *U. armoricana* + nutrients added to improve growth (double fertilizer ratio)
D – *U. armoricana* + nutrients added to improve growth (triple fertilizer ratio)
E – *U. armoricana* + nutrients added to improve growth (quadruple fertilizer ratio)
F – *U. armoricana* + nutrients added to improve growth (sextuple fertilizer ratio)
G – *U. armoricana* + nutrients added to improve growth (octuple fertilizer ratio)

Figure 1: Flow-through, paddle-wheel raceways are the preferred method for growing *Ulva*.

Initial biomass of 500 kg/*Ulva* spp were stocked in each pond and growth rates were measured every 21 days (~3 weeks) for a period of 3 months. The stocked *Ulva* spp in ponds B to G were fertilized (every 7 days in order to allow assimilation) with a mixture of (10:16:0) Maxipos® and Ammonium sulphite at 100g/kg providing both nitrogen and phosphorous respectively. Fertilization was carried out in the evenings with the incoming water turned off and the paddle wheel remaining in motion. Four physico-chemical parameters were measured per hour for 24 hours and included temperature (Temp °C), pH, dissolved oxygen (DO, mgl^{-1}) and light ($\mu E\ m^{-2}\ s^{-1}$). The Waterproof CyberScan Series 300 Dissolved Oxygen meter specially designed to measure oxygen and temperature simultaneously was used to detect DO and temperature values. pH was determined with the aid of a portable pH meter model 8414 (Wincom company Ltd) that also measures temperature at 0.1 °C. Irradiance levels were measured using a Biospherical Instruments probe (QSP200).

Wet to Dry Weight Ratios

Samples were taken after 21 days, washed in distilled water to remove any impurities, weighed, and then oven dried for 3 days at 50 ˚C or until weight stopped decreasing. Wet to dry weight ratios were calculated by the following equation:

(Dwt/Wwt X 100)

Wwt = wet weight,

Dwt = dry weight

Biogas Production

1 kg of harvested samples of *U. armoricana* were prepared for biomethane analysis by rinsing in clean water and stored frozen at - 25 °C until analysis. Samples were anaerobically digested in batch cultures for 25 days using the Matsui *et al.* (2006) methods of methane fermentation of macroalgal biomass. The analysis were done at an independent laboratory (Biogas Nord, Bielefeld, Germany. www.biogas – nord.com) using an X day bacterial digestion.

Statistical Analyses

All data were analyzed statistically on graphpad prison V statistical software using one way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) (Duncan, 1955). All tests were considered statistically significant at $p < 0.05$.

Results and Discussion

Yield of *Ulva* differed substantially among treatments from one pond to another (Table 1). The lowest value was recorded in the control, which contained no fertilizer and also produced the least biomass with a 113 % increase at harvest due to the nutrient content of the seawater. A progressive increase in weight gain was seen with reference to fertilizer increase from one pond to another, with the highest weight being recorded in the quadruple fertilizer experiment of 691 % increase. Yield differed substantially among treatments from one pond as a result of the previous week's fertilization. This result is consistent with other published works (Robertson-Andersson *et al.,* 2007). Marine algae accumulate nutrients by means of a two stage process consisting firstly of a rapid and reversible physico-chemical process of adsorption on the surface of the algae, and then secondly of a slower metabolically arranged intracellular uptake (Garnham *et al.,* 1992; Barreiro *et al.,* 1993). Thus the effects of a fertilization regime are often felt in the second growth period. As this trial was performed in winter, periods of sunlight influenced growth, with higher growth rates being experienced towards spring (i.e. the end of the trial). Findings showed that *Ulva* growth rates are seasonal and so we can assume that production would increase in summer (Robertson-Andersson, 2007). These increases are slightly lower than those obtained using smaller tanks (Robertson-Andersson, 2007), however, the CAPEX and OPEX costs of the paddle ponds provide the greatest production per unit areas and is more efficient than any other type of farming (Hanisak, 1987; Sahoo and Yarish, 2005).

TABLE 1: Composition and biogas yield from *Ulva armoricana*

Element	Unit of Measure	Biogas
Methane (CH_4)	%	53
CO_2	%	47
Hydrogen	%	1
H_2S	ppm (Vol)	325
NH_3	ppm (Vol)	75
Water (H_2O)	Dew point, °C	3
Gas yield	Nm^3 Biogas/t FM	77.4
Gas yield	Nm^3 Biogas/t DM	691

FM=fresh matter, DM= dry matter

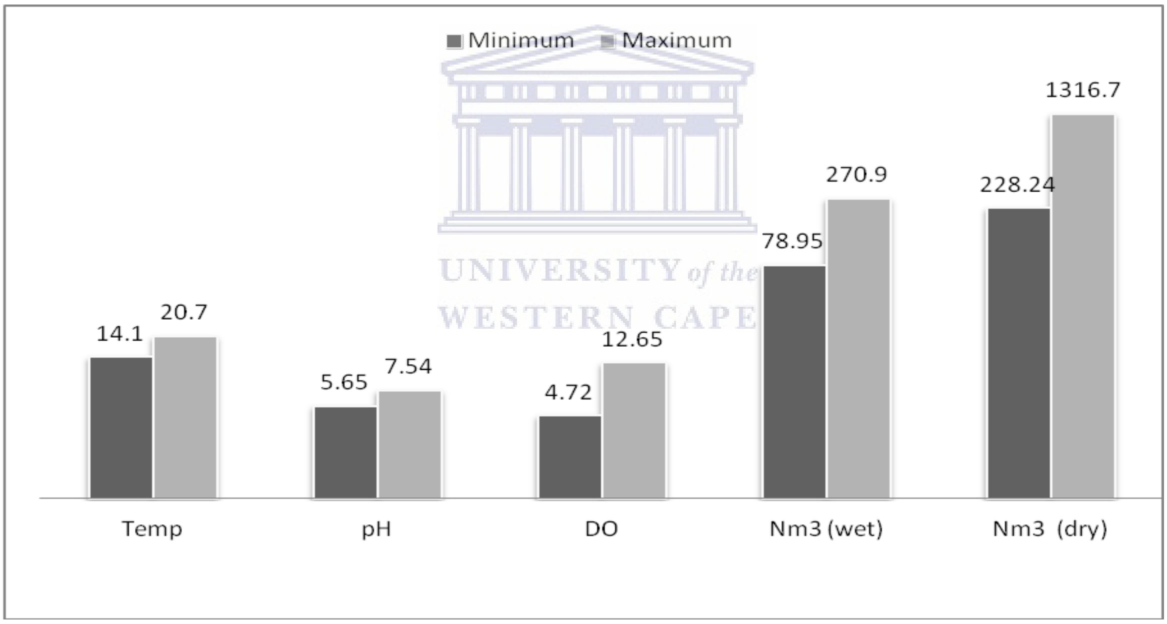

Figure 2: Comparative maximum and minimum values of physico-chemical parameters and gas yield. Minimum values were recorded, during the dark phase (night), while maximum values were recorded during the light phase (day) excluding gas production values (just minimum and maximum values are illustrated).

In aquaculture, biomass accumulation is generally dependent on both on external factors (pH, salinity, inorganic and organic complex molecules) and on physico-chemical parameters (temperature, light, dissolved oxygen and nutrients) that control the metabolic rate. Figure 1 illustrates the maximum and minimum values of physico-chemical variables experienced in the ponds, the mean and standard deviation were as follows; temperature (17 ± 2.03), pH (6.53 ± 0.39), DO (8.07 ± 2.32), light (910 ± 2.32) there was no significant different ($p < 0.05$) in these variables across the different treatments. Temp, pH, DO and light show a diurnal variation (Figure 2). Similar ranges were previously reported on by (Matsui *et al.,* 2006) for *Ulva spp* production in similar systems. Other research showed that *Ulva lactuca* could be cultured at 15– 20 °C and 400 – 1000 $\mu Es^{-1}m^{-2}$[(Bidwell *et al.*, 1985; Sand Jensen and Gordon, 1984; Cohen and Neori, 1991). The lower values of pH 5.65, DO 4.72 mgl^{-1} and $0.0\mu Es^{-1}m^{-2}$ were recorded during the dark phase at night when the biochemical activities was minimal due to the absence of sunlight and photosynthesis. These values similarly agree with the results and findings of (Garnham *et al.*, 1992). Light range values in the pond were recorded as 0 – 1800 $\mu Es^{-1}m^{-2}$. This result falls within the range of results reported in similar research (Xu and Lin, 2008). The wet to dry weight for the samples was 36.48 ± 21.35; this figure is within the range noted in earlier research (Matsui *et al.*, 2006).

Table 2: Composition and biogas yield from *Ulva armoricana*.

Element	**Unit of Measure**	**Biogas**
Methane (CH_4)	%	53
Carbon Dioxide (CO_2)	%	47
Hydrogen (H_2)	%	1
H_2S	ppm (Vol)	325
NH_3	ppm (Vol)	75
Water	Dew point, °C	3
Gas yield	Nm^3 Biogas/t FM	77.4
Gas yield	Nm^3 Biogas/t DM	691

FM=fresh matter, DM= dry matter

Biogas is primarily a mixture of methane (53 %) and CO_2 (47 %); the CO_2 was the initial atmospheric CO_2 absorbed by *Ulva* during culture. This result is comparable to 60 – 70 % for LPG (Table 2), but better than LPG on major harmful emission like CO_2, hydrocarbon and nitrogen oxide (NO_x) produced (Net technologies 2012). The potential difference in the nutrient content of each pond (A to G) effects the biogas production with an optimum biogas output at sextuple fertilizer ratio (*Ulva* + nutrients added to improve growth) (Table 1).

Conclusion

Utilizing cultivated seaweed as a sustainable and renewable feedstock for biogas production would be a great advantage for South Africa and could potentially lead the way in renewable energy development. Additional benefits from such projects might include: capturing industrially emitted CO_2 to use for enhanced seaweed growth to mitigate climate change, decreasing ocean acidification through carbon sequestration, as well as uptake of excess nutrients from industrial and agricultural effluent discharges; and reducing coastal eutrophication. All these practices ultimate support change towards more environmentally sound practices.

Acknowledgements

We thank the management and staff of I & J cape Cultured Abalone and Benguela Abalone Group, West coast, South Africa for the paddle wheel ponds used in this study. The Department of Biodiversity and Conservation Biology at the University of the Western Cape, and the Department of Biological Sciences at the University of Cape Town are also appreciated for providing funding and research equipment.

References

Adams, J. (2011). Seaweed may prove a viable future biofuel, EurekAlert. AAAS, the Science Society, retrieved from http:webmaster@eurekalert.org**.**

Barreiro, E., C. Real and A. Carballeira (1993). Heavy-metal accumulation by *Fucus ceranoides* in a small estuary in north-west Spain. Mar. Environ. Res., 27, 789-814.

Bidwell, R.G.S., J. McLachlan and N.D.H. Lloyd (1985). Tank cultivation of Irish Moss, *Chondrus crispus* Stackh. Bot. Mar., 28, 87-97.

Bolton, J.J., D.V. Robertson-Andersson, D. Shuuluka and L. Kandjengo (2009). Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. J. App. Phycol., 21(5), 575-583.

Briggs, M.R.P and S.J. Fung-Smith (1993). Macroalgae in aquaculture: An overview and their possible roles in shrimps culture, Paper presented at the proceedings of Marine biotechnology in the Asia pacific region, Bangkok, Thailand. 16 - 20 November 1993.

Bruton, T., L.H. YannickLerat and M.B. Rasmussen (2009). A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Sustainable Energy Ireland, Glasnevin, Dublin 9. Ireland, retrieved from www.sei.ie

Chopin, T and M. Sawhney (2009). Seaweeds and their maricultureIn: *The Encyclopedia of Ocean Sciences*. J.H. Steele, S.A. Thorpe & K.K. Turekian (eds.). Elsevier, Oxford. pp. 4477-4487.

Cohen, I and A. Neori (1991). *Ulva lactuca* biofilters for marine fishpond effluents .1.Ammonia uptake kinetics and nitrogen-content. Bot. Mar., 34, 475-482.

DAFF (Department of Agriculture, Forestry and Fisheries) (2011). South Africa aquaculture annual report 2011. South African Department of Agriculture, Forestry and Fisheries.

Douglas, B and J. Schäffler (2006). The potential contribution of renewable energy in South Africa. Sustainable Energy & Climate Change Project (SECCP). A project of Earthlife Africa Jhb, 2006.

FAO (Food and Agricultural Organization) (2010). The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department. Food and Agricultural Organization of the United Nations. Rome.

FAO (Food and Agriculture Organization) (2000). Agricultural Trade and Food Security: Issues and Options in the WTO Negotiations from the Perspective of Developing Countries, Vol.II Country Case Studies. FAO, Rome.

FAO (Food and Agriculture Organization) (2006). State of World Aquaculture, FAO Fisheries Technical Paper No. 500, FAO Fisheries and Aquaculture Department, Rome, 134.

Fleurence, J. (2004). Seaweed proteins, in: R.Y. Yada (Ed.), Proteins in Food Processing, Woodhead Publishing, pp. 197-210.

Garnham, G.W., G.A. Codd and G.M. Gadd (1992). Accumulation of Co, Zn and Mn by the estuarine green micro alga *chlorella salina* immobilized with alginate micro beads. Environ. Sci. Technol., 26, 1764-1770.

Hanisak, M.D. (1987). Cultivation of *Gracilaria* and other macroalgae in Florida for energy production. In K. T. Bird & P. H Benson (eds), Seaweed Cultivation for renewable resources. Elsevier, New York. pp.191-218.

IEA (International Energy Agency) (1994). A paper series on carbon dioxide capture, disposal and utilization, IEA Greenhouse gas R & D programme. Gloucestershire. UK. Retrieved from http//www.ieagree.org.uk

Khoza, D. (2012). Quantifying South Africa’s carbon storage potential using geophysics. S. Afri. J. Sc., 108, 9-10.

Klaus, J. H.., F. Uwe, H. Rocio, E Anja, R. Morchio, H. Suzanne and B. Boosya (2009). Aquatic Biomass: Sustainable Bio-energy from Algae? - Issue Paper -,The workshop is co-funded by two German research projects “bio-global” (sponsored by the Federal Ministry for Environment, BMU through the Federal Environment Agency, UBA) and “conCISEnet”(sponsored by the Federal Ministry for Education and Research,BMBF) Bio-global / conCISEnet, 2009. Retrieved from www.oeko.de

Matsui, J.T., T. Amano, Y. Koike, A. Saiganji and H. Saito (2006). Methane fermentation of seaweed biomass**.** In *American institute of chemical engineers*. San Francisco, 2006.

McKendry, P. (2002). Energy production from biomass (part 2): conversion technologies. Biores. Tech., 83(2), 47-54.

Millar, D. (2009). Macroalgae Profitable and Sustainable Primary Industry**,** PRIMEFACT-MACROALGAE, NSW ISSN 1832-6668e, 2009. Retrieved from www.dpi.nsw.gov.au/primefacts

Mohammad, R.H and R. Chakrabarti (2010). Use of algae and aquatic macrophytes as feed in small-scale aquaculture: A review. FAO, Fisheries and Aquaculture Technical Paper 531. Rome.

Nett technologies Inc (2012). Fact sheet. Catalytic mufflers for LPG Engines, Ontario, Canada, 2012, retrieved from http://www.nettinc.com/docs/nett_factsheet_lpg_catalytic_mufflers.pdf

Ozer, A., G. Akkayaa and M. Turabik (2005). Biosorption of Acid Red 274 (AR 274) on *Enteromorpha prolifera* in a batch system, J. Hazard. Mater., 126, 119-127.

Paul, N.A and C.K. Tseng (2012). Seaweed and Microalgae Aquaculture: Farming Aquatic Animals and Plant, Second edition, edited by John S Lucas. C South gate. Blackwell Publishing Ltd.

Ralph, E.T. (2012). Marine Macrophytic Algae, Department of Plant Biology and

Department of Geological Sciences, Michigan State University, 2012, retrieved from http://taggart.glg.msu.edu/bot335/seaweed.htm

Robertson-Andersson, D.V. (2007). Biological and economical feasibility studies of using seaweeds *Ulva lactuca* (chlorophyta) in recirculation systems in abalone farming. PhD Dissertation, University of Cape Town, South Africa, 2007.

Robertson-Andersson, D.V., M. Potgieter, J. Hansen, J.J Bolton, M. Troell, R. Anderson, C. Halling and T. Probyn (2007). Integrated seaweed cultivation on an abalone farm in South Africa. J. App. Phycol., 20(5), 579-595.

Sahoo, D and C. Yarish (2005). Mariculture of seaweeds. In R. Andersen (Ed.) Phycological Methods: Algal Culturing Techniques. Ch. 15. Academic Press, Elsevier Publ. pp. 219-237.

Sand-Jensen, K and D.M. Gordon (1984). Differential ability of marine and freshwater macrophytes to utilize HCO_3^- and CO_2. Mar. Biol., 80, 247-253.

Shipton, T and P. J. Britz (2007). A Study on the Status of Aquaculture Production and Trade in South Africa. Volume 1: Industry Status and Diagnostic Report. A report for the Department of Trade and Industry produced by Enviro-Fish Africa (Pty.) Ltd. pp.90.

Sinha, V.R.P and B.S. Fraley (2010). Chowdhry, “Carbon dioxide utilization and seaweed production.World Bank Project, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh, retrieved from http://www.netl.doe.gov/publications/proceedings/01/carbon_seq/p14.pdf

Steenblik, R. (2007). Biofuels - at what cost? Government support for ethanol and biodiesel in selected OECD countries: A synthesis of reports addressing subsidies for biofuels in Australia, Canada, the European Union, Switzerland and the United States. IISD, Geneva, Switzerland.

Troell, M., D.V Robertson-Andersson, R.J Anderson, J.J Bolton, G. W Maneveldt, C. Halling and T. Probyn (2006). Abalone farming in South Africa: An overview with perspectives on kelpresources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aqua., 257, 266-281.

Tshangela, M and H. Roman (2012). Ecoomy and environment. Env. 13, 26-27.

Wuebbles, D.J and A.K. Jain (2001). Concerns about climate change and the role of fossil fuel use. Fuel Proc. Tech., 71, 99-119.

Xu, Y and L. Lin (2008). Effect of temperature, salinity and light intensity on the growth of the green macroalga, *Chaetomorpha linum.* J. World Aqua. Soc., 39(6), 847-851.

Zemke-White, W.L and M. Ohno (1999). World seaweed utilization: An end-of-century summary. J. App. Phycol., 11, 369-379.

Chapter 4: Paper 3

Biofiltering and uptake of dissolved nutrients by *Ulva armoricana* (Chlorophyta) in a land-based aquaculture system

This chapter is based on:

Amosu A.O., Robertson-Andersson, D.V., Kean E., Maneveldt G.W. and Cyster L. (2016). Biofiltering and uptake of dissolved nutrients by *Ulva armoricana* (Chlorophyta) in a land-based aquaculture system. *International Journal of Agriculture & Biology* 18: 298-304.

Abstract

An on-land flow-through cultivation system was designed for the macroalgal species *Ulva armoricana* (Chlorophyta) to reduce the environmental impact of aquaculture effluent in coastal ecosystems as part of an integrated aquaculture system. The macroalgae was cultured in various enriched media at a stocking density of 500kg wet weight/pond. Overall, *U. armoricana* was able to remove a greater percentage of inorganic nitrogen in the double fertilizer ratio. The total dissolved phosphate was higher in standard seawater. *Ulva armoricana* showed preference for bioaccumulation, with ranges as follows: zinc (9.908 – 32.942 $mg.kg^{-1}$); copper (1.893 – 5.927 $mg.kg^{-1}$); cadmium (0.254 – 1.500 $mg.kg^{-1}$); and lead (none detected). Apart from the presence of cadmium (Cd), the algal biomass produced at the end of the experiment was of a relatively good quality with limited heavy metal contamination so that *U. armoricana* could be successfully used as a plant stimulant but not as part of a feed formulation for livestock and for the food industry. This study showed that *U. armoricana* can effectively be used as a biological filter for dissolved nutrient uptake from aquaculture effluents. The prospect of better management practices, based on the utilization of *Ulva* mariculture designs, bodes well for the aquaculture industry.

Key words: Dissolved nutrients, fertilizer, heavy metals, Integrated Multitrophic Aquaculture (IMTA), macroalgae, *Ulva armoricana*

Introduction

Global aquaculture production continues to improve at about 10% annually, outpacing terrestrial livestock production and capture fisheries (FAO, 2010). However, the rapid development of intensive aquaculture along coastal areas throughout the world has raised increasing concerns on environmental degradation and specifically the impact of nutrient loading if these industrial production practices are not sustainably managed using the best available technology (BAT) (Haylor and Bland 2001; Pauly *et al*., 2003; Troell 2009; Zhou *et al*., 2006; Ihsan 2012). Waste products from aquaculture activities consist mainly of $CO_{2,}$ nitrogen, phosphorus, and heavy metals.

Aquaculture waste can result in pollution that contributes to the degradation of the environment through (organic and inorganic inputs) agro-allied and industrial activities that can lead to a substantial increase of organic matter and nutrient loading into adjacent water bodies. Modern integrated aquaculture systems like (non-fed aquaculture) macroalgae-based aquaculture contribute to eco-monitoring by playing a significant role in coastal wastewater filtration and bioaccumulation (Costa-Pierce *et al*., 2011; Klinger and Naylor 2012; Boxman, 2013; Redmond *et al*., 2014). This is due largely to the ability of macroalgae to achieve high biomass and have a significant potential as nutrient bioremediators (Msuya and Neori 2002; Tyler and McGlathery, 2006; Marinho-Soriano *et al*., 2009; Winberg *et al*., 2011).

In aquatic environments, nitrogen and phosphorus (major aquaculture contaminants), are the two most important nutrients that usually limit biomass production of macroalgae (Smith and Smith 1998; GESAMP 2001; UNEP and Gems Water 2006). Nitrogenous compounds (NH_4^+, NO_3^-, and NO_2^-) have been indicted as a source of pollution in aquaculture effluent due to discharge of untreated non-point aquaculture run-off, animal waste and failed technology practices. According to estimates, 78 kg N and 9.5 kg P per ton of fish are released into water bodies per year. This is because about 72% N and 70% P constituent of feed are not utilized in the fish physiology (Ackefors and Enell 1994; Chopin *et al*., 1999).

In the past few decades, increasing emphasis have been placed on developing sustainable approaches to coastal aquaculture development of large-scale Integrated Multitrophic Aquaculture (IMTA) seaweed farming (Robertson-Andersson 2007; Smith *et al*., 2010; Redmond *et al*., 2014). The integrated culture system provides mutual benefits for the cultured organisms and improves water quality of the aquaculture system. Macroalgae take up inorganic nutrients for growth and can thus alleviate the seasonal nutrient depletion from aquaculture (Chopin *et al*., 2001; Neori *et al*., 2004). Several aquaculture research and development efforts have shown the efficiency and benefits of integrating macroalgae in on-land treatment systems for treating aquaculture waste effluents before being discharged into open water bodies (Buschmann *et al*., 1996; Neori 1996; Winberg *et al*., 2011; Dittert *et al*., 2012; Renzi *et al*., 2014).

Macroalgae have found applications in the removal of nutrients from effluent waters of sewage, industry and aquaculture farming (Neori *et al*., 2004; Robertson-Andersson 2007; Dittert *et al*., 2012; Redmond *et al*., 2014). More recently, it has been demonstrated that using different dissolved CO_2 concentrations in seawater has the potential to improve nutrient uptake, a possible solution to the problems associated with coastal eutrophication around the world (Zou and Gao 2009). This is so because in the polyculture of integrated fauna and macroalgal mariculture, the wastes from one consumer become a resource for the other in the mutually beneficial system. This integrated approach gives nutrient bioremediation efficacy, mutual benefits to co-cultured organisms, and results in a more stable aquaculture environment (Neori *et al*., 2000; Chopin *et al*., 2001).

Tissue metal contents are also potential hazard prediction indices for organisms and the environment when natural concentrations are higher than the maximum standard recommended by monitoring agencies (Ayers and Westcot 1994; Almela *et al*., 2002; 2006; Smith 2009; Sánchez-Bayo *et al*., 2011). Macroalgae naturally take up elements like Na, K, Ca, Mg, Cl, I and Br from the surrounding water bodies. The major metallic pollutants implicated in culture systems and coastal waters are Pb, Cr, Hg, U, Se, Zn, As, Cd, Au, Ag, Cu and Ni among which Cd is readily absorbed in a combined state with S, Cl and O and stored in the algal thalli (Komjarova 2009; Dittert *et al*., 2012; Renzi *et al*., 2014). Green macroalgae (Chlorophyta) are known to be a significant biological indicator of heavy metal contamination in marine ecosystems (Nelson *et al*., 2010). Various studies have demonstrated the use of green macroalgae from the genus *Ulva* as a bio-filter/monitor of coastal contamination because of their relatively simple morphology, high tissue bioaccumulation, and widespread distribution (Alkhalifa *et al*., 2012; Zoll and Schijf 2012; Renzi *et al*., 2014).

In IMTA, bio-filtration processes easily remove considerable amounts of pollutants contained in the out flowing water, resulting in a reduced permissible discharge into open water bodies. The development of such systems requires the removal of solid compounds and dissolved metabolites contained in the outlet water of the systems. The specific justification of this research has evolved from aquaculture's environmental consequences, and the nutrient enrichment of the outlet water systems associated with more general aquaculture practices. Aquaculture practices generally lead to high nutrient loading that can facilitate changes in the natural dynamics of water bodies and can lead to oxygen depletion, green tide (harmful algal blooms) events, eutrophication, fish kills, low productivity, increased risks of infectious diseases, and deterioration of the groundwater with serious consequences for human health, the environment and economic development (Van Alstyne *et al*., 2007; Nelson *et al*., 2010; DEC 2014; Redmond *et al*., 2014). In this study, we investigated the nutrient uptake potential, efficiency and bioaccumulation potential of the green macroalga *Ulva armoricana* in an outdoor, on-land flow-through paddle wheel system. The philosophy behind this part of the research was to: 1) deduce if the SA seaweed industry can produce macroalgae for direct human consumption; and 2) determine the implications for the abalone industry due to metal compounds in macroalgae.

Materials and Methods

Ulva Materials

Ulva armoricana used in this experiment was sampled from the I & J Cultured Abalone farm (34^{o}34′60 S; 19^{o}21′0 E) and were transported to the research farm at Benguela Abalone Group (32°54'24" S; 17°59'17" E) on the West Coast of South Africa. Samples were rinsed with filtered seawater and gently scrubbed to remove sediments and any epiphytes. The specimens were then stabilized in a culture for 3-4 days (acclimatization) under a continuous flow of seawater pumped from the ocean (mean nutrient concentrations were 0.6 µM NH_4^+, 0.5 µM NO_3^-, NO_2^-, and 0.7 µM PO_4^{3-}) and kept at 20 °C in concrete paddle ponds.

Experimental Systems

Macroalgae production experiments were carried out during winter in four 32 m X 8 m (180 m^3) concrete paddle ponds and filled to approximately 0.55 m depth with unfiltered seawater in a flow-through system (Figure 1). Ponds received two volume exchanges per day. The experimental treatments were as follows:

A - *U. armoricana* + standard seawater (control)

B - *U. armoricana* + nutrients added to improve growth (double fertilizer ratio)

C - *U. armoricana* + nutrients added to improve growth (quadruple fertilizer ratio)

Figure 1: Flow-through, paddle-wheel raceways are the preferred method for growing *Ulva*.

Initial *Ulva* biomass of 500 kg wet weight was stocked in each pond and growth rates were measured after 21 days. The algae were fertilized (7 days before the experiment in order to allow assimilation) with a mixture of (10:16:0) Maxipos® and ammonium sulphite at 100g/kg providing both nitrogen and phosphorous respectively (algae need N & P in a ratio: 16 atoms of N for every 1 atom of P - Greenfield *et al*., 2012). Fertilization was carried out in the evenings with the incoming water turned off and the paddle wheel remaining in motion. The mean physico-chemical parameters measured during the experiment include temperature (17 °C), pH (6.53), and dissolved oxygen (8.07mg L^{-1}).

Water Sampling and Analysis

Water samples were collected from 10:00am and every (intervals) hour thereafter for 24 h to determine the inorganic nutrients concentrations. Four inorganic nutrients were measured 12 times at different intervals and included Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-), and phosphorus (PO_4^{3-}). Analysis of the various inorganic nutrients Ammonium, Nitrate, Nitrite and Phosphorus were determined using a Spectroquant® Pharo 300M. The detailed chemical analysis methods were done photometrically based on the manufacturer's manual – Merck KGaA, (Germany) www.merck-chemicals.com/test-kits, www.merck-chemicals.com/photometry. The amount of light (µE m^{-2} s^{-1}) was also recorded as irradiance levels and was measured using a Biospherical Instruments probe (QSP200). Algal tissue metal content was determined every 21 days for 3 months. The heavy metals tested for included cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb), using an Atomic Absorption Spectrophotometer (AAS), Unicam Atomic Absorption – M Series), Unicam Limited, U.K.

Statistical Analysis

The design of the experiment was completely randomized with three replications. Apart from light and temperature data collected every hour, other inorganic nutrients were sampled every three hours for 24 hours. For heavy metals, % N and % P significance differences were used to juxtapose with standards. Data are presented as means ± standard deviation (SD). All data were analyzed using GraphPad PrismV.

Results

Our findings show that nutrient availability followed the fertilizer ratio (Figure 2 to 7 and Table 1). Availability of Ammonium (NH_4^+) showed a diurnal variation with the different treatments, the highest being observed during the day (0.18mg L^{-1}) in the quadruple fertilizer ratio (12:00pm) and reducing with time, its lowest (0.04 mg L^{-1}) value recorded at 6:00 pm (seawater), 6:00 pm and 4:00 am (double fertilizer ratio), and 10:00 pm (quadruple fertilizer ratio) respectively. Nitrate (NO_3^-) was highest in the quadruple fertilizer ratio (8 mg L^{-1}), with the lowest value (0.11 mg L^{-1}) occurring at 10:00 pm and 10:00 pm in the double fertilizer ratios. Nitrite (NO_2^-) was stable in the treatments and ranged from 0.01 – 0.02 mg L^{-1}, but was highest in the seawater control at 0.03 mg L^{-1} at 12.00pm. Phosphorus (PO_4^{3-}) availability in the different treatments increased with day time and attained a peak (0.44 mg L^{-1}) at 2:00 pm (quadruple fertilizer ratio), while the lowest value (0.06 mg L^{-1}) was observed in the seawater control at 10:00 am. Temperature in this study ranged between 14.1 – 20.7 °C and was a function of the availability of day light, which showed a gradual decrease in photoperiod (0 – 1900 µE m^{-2} s^{-1}) with time (16:8 hr light : dark). With regards to heavy metals, *U. armoricana* showed a preference for bioaccumulation, which ranged as follows: zinc (9.908 – 32.942 $mg.kg^{-1}$); copper (1.893 – 5.927 $mg.kg^{-1}$); cadmium (0.254 – 1.500 $mg.kg^{-1}$); and lead (none detectable). The results also showed that *U. armoricana*'s assimilation affinity decreased as follows: double > quadruple > 0 (Table 1). Apart from cadmium, heavy metal contamination levels in cultured *U. armoricana* showed safe uptake mechanisms in all fertilizer ratios compared to various local and international standards.

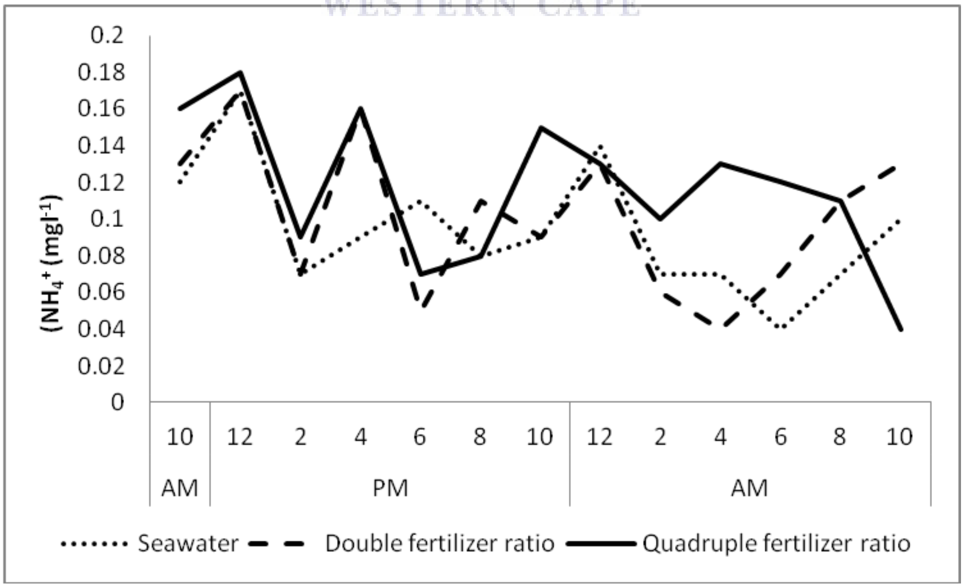

Figure 2: Ammonium (NH_4^+) time graph for the different fertilizer ratios.

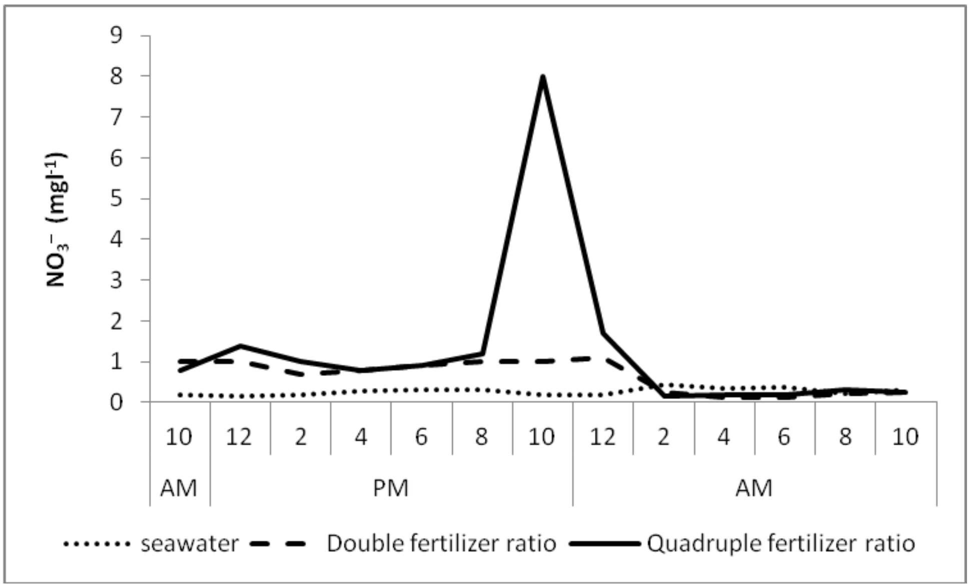

Figure 3: Nitrate (NO_3^-) time graph for the different fertilizer ratios.

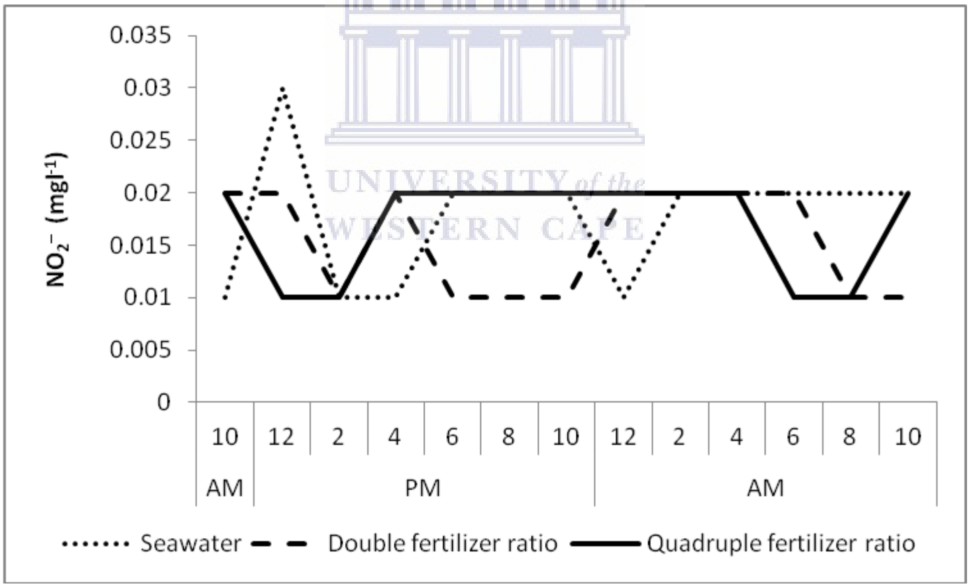

Figure 4: Nitrite (NO_2^-) time graph for the different fertilizer ratios.

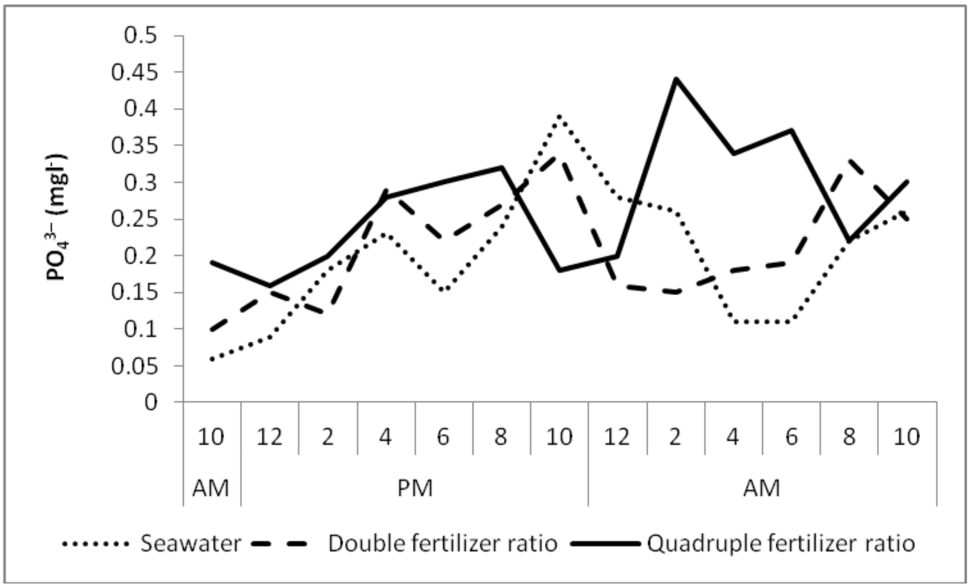

Figure 5: Phosphorus (PO_4^{3-}) time graph for the different fertilizer ratios.

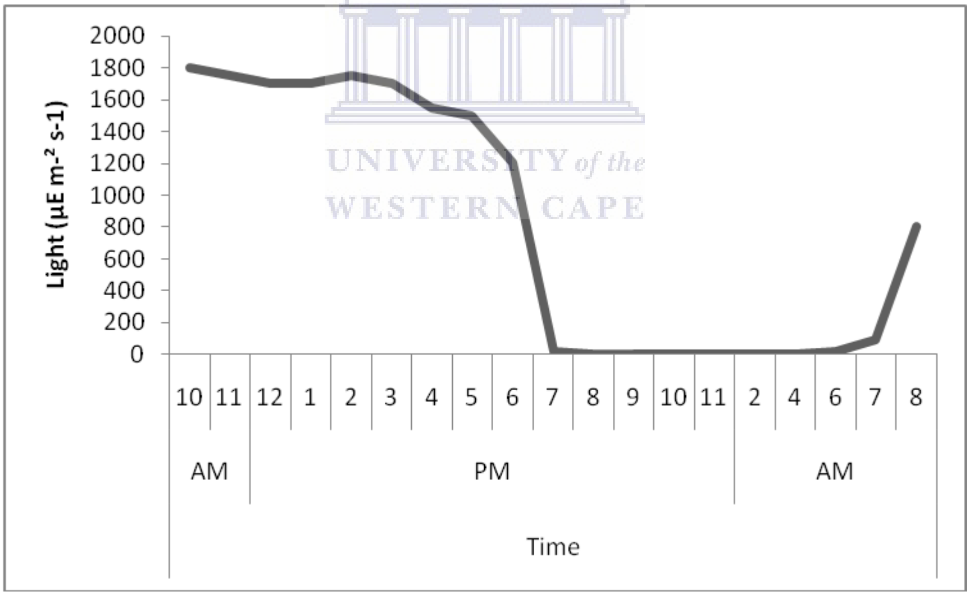

Figure 6: The mean amount of light available over a 24 hour period for *U. armoricana* biomass production in the culture ponds from this study.

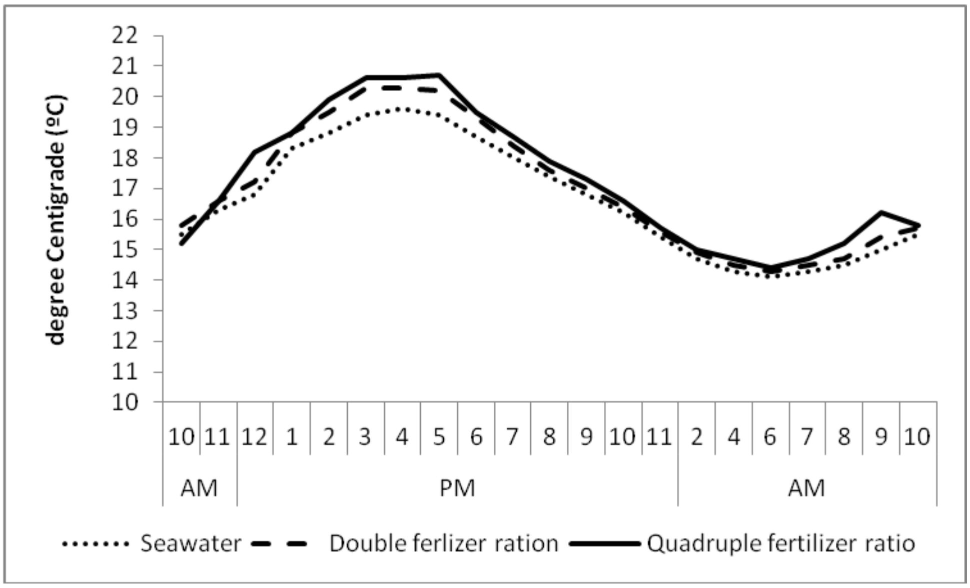

Figure 7: The mean temperature over a 24 hour period in the culture ponds from this study.

Table 1: Heavy metals and nutrient composition in *U. armoricana* grown in the various experimental treatments.

Heavy metals/Nutrient	Experimental Treatments (Mean ± SD)			Standards	
	Seawater (0 fertilizer)	Double fertilizer ratio	Quadruple fertilizer ratio	*SA Permissible Limit (mg.kg^{-1}) (Lettuce)	**FAO/WHO Permissible Limit (mg.kg^{-1})
Cd	0.639±0.023^{a}	1.166±0.360^{b}	0.8451±0.566^{b}	0.1	0.2
Cu	4.619±1.193^{a}	5.676±1.367^{a}	4.687±1.148^{a}	30.0	0.1
Pb	ND	ND	ND	0.5	0.3
Zn	18.640±4.814^{a}	20.244±2.011^{a}	22.158±8.991^{a}	40.0	.015-.030 ***
% N	2.122±0.862a	3.220±0.494^{b}	2.350±1.039^{b}	-	GMP
% P	1.789±0.082^{a}	1.711±0.318^{a}	1.700±0.269^{a}	-	2200

Means in the same row with the same superscript are not significantly different ($p > 0.05$),* South Africa Government Gazette, 9 September, 1994, metals in foodstuffs, cosmetics and disinfectants act, (Act no. 54 of 1972), **FAO/WHO (2001) standard for seaweed/vegetable, ***Australia recommended leaf nutrient concentrations, GMP = Good manufacturing practices (GMP) must be followed (hygiene, low temperature, and disinfection) as in packaging gas. ND = None detected.

Discussion

Aquaculture effluents are rich in NH_4^+ that could come from feed and nutrients in the inlet water. Although NH_4^+ concentrations > 2.0 mg L^{-1} can be detrimental (Lazur 2007), aquaculture effluents are highly suitable as a nutrient source for *Ulva* species. Values of NH_4^+ in this study were comparatively low. NH_4^+ in winter is typically low due to the lower mean temperatures (fig. 6 & 7) and pH. These results are consistent with those reported by Robertson-Andersson (2007). Nitrate-nitrogen concentrations above 3 mg L^{-1} and any detectable amounts of total P (above 0.025mg L^{-1}) may be indicative of pollution from fertilizers, manures or other nutrient-rich wastes (Cole *et al.*, 2014). Nitrogen and phosphorus are nutrients that may cause increased growth of aquatic plants and algae. Dissolved inorganic phosphorus (DIP) obtained in this study corroborated the outcome of a related experimental investigation by Robertson-Andersson (2007). Nitrites from feed are not toxic to seaweed. Several authors have reported assimilation rates of NH_4^+ in the range of 50−90 μmol N g^{-1} DW h^{-1} among different *Ulva* species, and these species have been verified as successful biofilters of aquaculture wastewaters (Hernández *et al*., 2002; Neori *et al*., 2003; Copertino *et al*., 2009; Cahill *et al*., 2010). Nitrite results from enriched nutrients and there is evidence of nitrate uptake during the day (Potgieter 2005; Robertson-Andersson 2007). The inorganic nutrients observed in this study were below the South African water quality guidelines for Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-), and phosphorus (PO_4^{3-}) (DWAF 1996).

Light intensity is well correlated with temperature, which is largely subject to diurnal and seasonal changes both in irradiance and photoperiodic systems. This finding differs from those of Lüning (1993) and Kirk (1994) who showed that light intensity correlated with day length and not temperature. Most outdoor culture systems research on *Ulva* showed that the alga could readily be cultured at 15 – 20 °C and at 400 – 1000 µE$s^{-1}m^{-2}$ (Winberg *et al*., 2011; Corey *et al*., 2012; 2014). These values are similar to the irradiance and photoperiod ranges found in the present study using *U. armoricana*. This study showed that double fertilized *U. armoricana* had high Cd, Cu and Zn values, but that values for Cu, Pb and Zn were lower than the permissible South African limits for lettuce (Table 1). Concentrations of Cd in all treatments were, however, higher than SA limits for lettuce. This may be due to the rate of fertilizer application. Only low/trace levels of Pb were found in seawater and in fertilized *U. armoricana*. Apart from Pb that was not detected in all treatments, the other heavy metals had values higher than the FAO/WHO (2001) standard for seaweed/vegetable. The main observation here seems to be that cultured *U. armoricana* at Benguela Abalone Group tended to have higher levels of heavy metals than *Ulva* from the unfertilized/seawater tanks. This result is contrary to the findings of Shuuluka (2011).

The Cd values in this research were higher than the maximum recommended level for Cd in the FAO/WHO (2001) standard for seaweed/vegetable, the South African limits for lettuce, the French limits for edible seaweeds (< 0.5 µgg^{-1} dw, Besada *et al*., 2009) and the Australian and New Zealand limits for edible seaweeds (0.2 µgg^{-1} dw, Almela *et al*., 2002; 2006; Besada *et al*., 2009). The high Cd concentrations in the current study could well have originated from the unfiltered seawater and/or the fertilizer (Shuuluka 2011). Irrespective of the source, our Cd values negate the use of these seaweeds for human consumption.

Conclusion

As human health is directly affected by ingestion of vegetables, the biomonitoring of trace elements in macroalgae needs to be continually monitored because these algae are the main sources of food for humans in many parts of the world. It is therefore of great importance that South Africa implements a continuous update of its seaweed safety monitoring by formulating a standard guideline and permissible limits of nutrients in macroalgae that must be strictly adhered to by all industries.

Acknowledgement

We thank the management and staff of I & J Cultured Abalone and Benguela Abalone Group, West coast, South Africa for the paddle wheel ponds used in this study. Also we thank Robert J Anderson of the Department of Agriculture, Forestry and Fisheries (DAFF), Seaweed Unit, Marine and Coastal Management, University of Cape Town, Cape Town, South Africa. The Department of Biodiversity and Conservation Biology at the University of the Western Cape, and the Department of Biological Sciences at the University of Cape Town are also thanked for providing funding and research equipment.

References

Ackefors, H. and M. Enell, 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. J. Appl. Ichthyol., 10: 225-241.

Alkhalifa, A.H., A.A. Al–Homaidan, A.I Shehata, H.H. Al–Khamis, A.A. Al-Ghanayem and A.S.S. Ibrahim, 2012. Brown macroalgae as bioindicators for heavy metals pollution of Al-Jubail coastal area of Saudi Arabia. Afr. J. Biotechnol., 11: 15888-15895.

Almela, C., S. Algora, V. Benito, M.J. Clemente, V. Devesa, and M.A. Súñe, 2002. Heavy metals total arsenic and inorganic arsenic contents of algae food products. J. Agric. Food Chem., 50: 918-23.

Almela, C., M.J. Clemente, D. Vélez and R. Montoro, 2006. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. Food Chem. Toxicol., 44: 1901-1908.

Ayers, R.S. and D.W. Westcot, 1994. Water quality for agriculture. FAO IRRIGATION AND DRAINAGE PAPER. 29 Rev. 1. Food and Agriculture Organization of the United Nations. Rome, Italy.

Besada, V., J.M. Andrade, F. Schultze and J.J. González, 2009. Heavy metals in edible seaweeds commercialised for human consumption. J. Mar. Syst., 75: 305-313.

Boxman, S., 2013. Evaluation of a pilot land-based marine integrated aquaculture system. Graduate School Theses and Dissertations. University of South Florida. U.S.

Buschman, A., D.A. Lopez and A.A. Medina, (1996). A review of the environmental effects and alternate production strategies of Chile. Agric. Eng., 15: 397-402.

Cahill, J.F., G.G. McNickle, J.J. Haag, E. G., Lamb, S.M. Nyanumba and C.C. St. Clair, 2010. Plants Integrate Information About Nutrients and Neighbors. Sci., 238: 1657.

Chopin, T., C. Yarish, R. Wilkes, E. Belyea, S. Lu and A. Mathieson, 1999. Developing Porphyra/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. J. Appl. Phycol., 11: 463-472.

Chopin, T., A.H. Buschmann, C. Halling, M. Troell, N. Kautsky, A. Neori, G. Kraemer, J. Zertuche-Gonzalez, C. Yarish and C. Neefus, 2001. Integrating seaweeds into aquaculture systems: a key towards sustainability. J. Phycol., 37: 975-986.

Cole, A.J., R. de Nys and N.A. Paul, 2014. Removing Constraints on the Biomass Production of Freshwater Macroalgae by Manipulating Water Exchange to Manage Nutrient Flux. PLoS ONE., 9(7): e101284. http://dx.doi.org/10.1371/journal.pone.010284.

Copertino, M.S., A. Cheshire and T. Kildea, 2009. Photophysiology of a turf algal community: integrated responses to ambient light and standing biomass. J. Phycol., 45: 324-336.

Corey, P., J.K. Kim, D.J. Garbary, B. Prithiviraj and J. Duston, 2012. Cultivation of red macroalgae for potential integration with Atlantic halibut: effects of temperature and nitrate concentration on growth and nitrogen removal. J. Appl. Phycol., 24: 441-448.

Corey, P., J.K. Kim and D.J. Garbary, 2014. Growth and nutrient uptake by *Palmaria palmate* integrated with Atlantic halibut in a land-based aquaculture system. Alg., 29: 35-45.

Costa-Pierce, B.A., D.M. Bartley, M. Hasan, F. Yusoff, S.J. Kaushik, K. Rana, D. Lemos, P. Bueno and A. Yakupitiyage, 2011. Responsible use of resources for sustainable aquaculture. Global Conference on Aquaculture 2010, Sept. 22-25, 2010, Phuket, Thailand. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Department of Water Affairs and Forestory (DWAF), 1996. South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems, Edited by S Holmes, CSIR Environmental Services. Second Edition.

Division of Energy and Climate (DEC) 2014. Climate Change Impact Assessment, Delaware Department of Natural Resources and Environmental Control. Retrieved from www.dnrec.delaware.gov/energy/documents/climate%20change%202013-2014/DCCIA%20interior.full dated.pdf

Dittert, I.M., V.J.P. Vilar, Eduardo, A.B. da Silva, M.A. Selene, G. de Souza, A.A.U de Souza, C.M.S. Botelho and R.A.R. Boaventura, 2012. Adding value to marine macro-algae *Laminaria digitata* through its use in the separation and recovery of trivalent chromium ions from aqueous solution. Chem. Eng. J., 193: 348-357.

Food and Agriculture Organisation (FAO), 2010. World Review of Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO/WHO, 2001. Standard programme, codex alimenttarius commission, twenty-fourth SessionGeneva, Switzerland, 2-7 July 2001 and report of the 33rd session of the codex committee on food additives and contaminants. The Hague, the Netherlands 12-16.

GESAMP, 2001. Protecting the Oceans from Land-based Activities - Land-based Sources and Activities Affecting the Quality and Uses of the Marine, Coastal and Associated Freshwater Environment, GESAMP Reports and Studies 71.

Greenfield, D.I., C. Keppler, L.M. Brock, S. Kacenas, S. Hogan and R. Van Dolah, 2012. Assessing biological responses to nitrogen and phosphorus levels across the South Carolina coastal zone. Proceedings of the 2012 South Carolina Water Resources Conference, Columbia.

Haylor, G. and S. Bland, 2001. Integrating aquaculture into rural development in coastal and inland areas. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery and J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the

Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp.73-81. NACA, Bangkok and FAO, Rome, Italy.

Hernández, L.P., M.J.F. Barresi and S.H. Devoto, 2002. Functional morphology and developmental biology of zebrafish: reciprocal illumination from an unlikely couple. J. Int. Comp. Biol., 42: 222-231.

Ihsan, Y.N., 2012. Nutrient fluxes in multitrophic aquaculture systems. PhD Dissertation, Christian-Albrechts-Universität. Sweden.

Kirk, J.T.O., 1994. Light and photosynthesis in aquatic ecosystems, Cambridge University Press, UK.

Klinger, D. and R. Naylor, 2012. Searching for Solutions in Aquaculture: Charting a Sustainable Course. Annu. Rev. Environ. Res., 37: 247-276.

Komjarova, I., 2009. Uptake of Trace Metals in Aquatic Organisms: A Stable Isotopes Experiment. Dissertation for Doctor of Sciences, University of Antwerp. Germany.

Lazur, A., 2007. Growout Pond and Water Quality Management, JIFSAN Good Aquacultural Practices Program (Joint institute for food safety & applied nutrition), University of Maryland, Symons Hall, College Park, MD 20742.

Lüning, K., 1993. Environmental and internal control of seasonal growth in seaweeds, Hydro., 260/261: 1-14.

Marinho-Soriano, E., S.O. Nunes, M.A.A. Carneiro and D.C. Pereira, 2009. Nutrients removal from aquaculture wastewater using the macroalgae *Gracilaria birdiae*. Bio. Bioe., 33: 327-331.

Msuya, F.E. and A. Neori, 2002. *Ulva reticulata* and *Gracilaria crassa*: Macroalgae that can biofilter effluent from tidal fishponds in Tanzania. W. Ind. Oc. J. Mar. Sci., 1: 117-126.

Nelson, T.A., J. Olson, L. Imhoff and A.V. Nelson, 2010. Aerial exposure and desiccation tolerances are correlated to species composition in "green tides" of the Salish Sea (northeastern Pacific). Bot. Mar., 53: 103-111.

Neori, A., (1996). The form of N-supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Isr. J. Aqua., Bamidgeh, 48: 19-27.

Neori, A., T. Chopin, M. Troell, A.H. Buschmann, G.P. Kraemer, C. Halling, M. Shpigel and C. Yarish, 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aqua., 231: 361-391.

Neori, A., F.E. Msuya, L. Shauli, A. Schuenhoff, F. Kopel and M. Shpigel, 2003. A novel three stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculature. J. Appl. Phycol., 15: 543-553.

Neori, A., M. Shpigel and D.M. Ben-Ezra, 2000. A sustainable integrated system for culture of fish, seaweed and abalone. Aqua., 186: 279-291.

Pauly, D., J. E. Alders, V. Bennett, P. Chrisensen, P. Tyedemers and R. Watson, 2003. The Future of Fisheries. Sci., 302: 1359-1361.

Potgieter, M., 2005. A comparison of suspended particle size and sediment loading produced by artificial and seaweed diets in integrated flow through and recirculating aquaculture systems on a commercial South Africa abalone farm. M.Sc Dissertation, University of Cape Town, South Africa.

Redmond, S.L., C. Green, J. Yarish, J. Kim, and C. Neefus, 2014. New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01. pp. 92. Retrieved from http://seagrant.uconn.edu/publications/aquaculture/handbook.pdf

Renzi, M., A. Giovani and S. Focardi, 2014. Responses of aquatic vegetation to pollution: preliminary results on ecotoxicological effects and bioenrichment factors. J. Environ. Prot., 5: 274-288.

Robertson-Andersson, D.V., 2007. Biological and econoical feasibility studies of using seaweeds I (Chlorophyta) in recirculation systems in abalone farming. PhD Dissertation. Department of Botany, University of Cape Town, South Africa.

Sánchez-Bayo, F., P.J. van den Brink and R.M. Mann, (Eds.) 2011. Ecological Impacts of Toxic Chemicals. Bentham Science Publishers Ltd. The Netherlands. Benthan eBooks. pp. 281.

Shuuluka, D., 2011. Ecophysiological studies of three South African *Ulva* species from integrated seaweed/abalone aquaculture and natural. PhD Dissertation. University of Cape Town. South Africa.

Smith, R.L. and T.M. Smith, 1998. Elements of Ecology, San Francisco USA. Addison-Wesley. Longman.

Smith, M.D., C.A. Roheim, L.B. Crowder, B.S. Halpern, M. Turnipseed, J.L. Anderson, F. Asche, L. Bourillón, A.G. Guttormsen, A. Kahn, L.A. Liguori, A. McNevin, M. O'Connor, D. Squires, P. Tyedemers, C. Brownstein, K. Carden, D.H. Klinger, R. Sagarin and K.A. Selkoe, 2010. Sustainability and global seafood. Sci., 327: 784-786.

Smith, S.R., 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ. Int., 35: 142-156.

Troell, M., 2009. Integrated marine and brackish water aquaculture in tropical regions: research, implementation and prospects. In D. Soto (ed.). Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper. No. 529. FAO, Rome, Italy. pp. 47-131.

Tyler, A.C. and K.J. McGlathery, 2006. Uptake and release of nitrogen by the macroalgae *Gracilaria vermiculophylla* (Rhodophyta). J. Phycol., 42: 515-525.

UNEP and Gems Water Programme, 2006. Water Quality for Ecosystem and Human Health, National Water Research Institute, Burlington, Ontario Canada. Retrieved from http://www.unep.org/gemswater/Portals/24154/digital_atlas/digital_atlas.pdf

Van Alstyne, K.L., L. Koellermeier and T.A. Nelson, 2007. Spatial variation in dimethylsulfoniopropionate (DMSP) production in *Ulva lactuca* (Chlorophyta) from the northeast Pacific. Mar. Biol., 150: 1127-1135.

Winberg, P.C., D. Skropeta and A. Ullrich, 2011. Seaweed cultivation pilot trials – towards culture systems and marketable products. Australian Government Rural Industries Research and Development Corporation, RIRDC Publication No. 10/184. PRJ - 000162. Retrieved from http:// rirdc.infoservices.com.auitems/10-184.

Zhou, Y., H. Yang, H. Hu, Y. Liu, Y. Mao, H. Zhou, X. Xu and F. Zhang, 2006. Bioremediation potential of the macroalga *Gracilaria Lemaneiformis (*Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aqua., 252: 264-276.

Zoll, A.M. and J. Schijf, 2012. A surface complexation model of YREE sorption on *Ulva lactuca* in 0.05–5.0 M NaCl solutions. Geochim. Cosmochim. Acta., 97: 183-199.

Zou, D.H. and K.S Gao, 2009. Effects of elevated CO_2 on the red seaweed *Gracilari lemaneiformis* (Gigartinales, Rhodophyta) grown at different irradiance levels. Phycol., 48: 510-517.

Chapter 5: Paper 4

Environmental response and pH tolerance of induced CO_2 in *Ulva rigida* (Chlorophyta) under controlled conditions

This chapter is based on:

Amosu A.O., Robertson-Andersson D.V., Knapp J.L., Maneveldt G.W., Auersward L. and Bolton J.J. (under review). Environmental response and pH tolerance of induced CO_2 in *Ulva rigida* (Chlorophyta) under controlled conditions. *International Journal of Environmental Pollution*.

Abstract

Macroalgal biomass can be manipulated by several different techniques to influence their functions. Effect of increased CO_2 levels on water pH and the corresponding physiological responses in the macroalgae *Ulva rigida* C.Agardh were tested under controlled conditions. pH toxicity experiment was conducted on *U. rigida* to determine morphological and physiological changes resulting from varied CO_2 induced stress. Our results show that increasing CO_2 concentrations decreases water pH (4.71 - 6.67) as expected, causing a significant inhibition in growth, and leading to different sporulation responses. Furthermore, we show that *U. rigida* in flow through system showed a gradual degeneration in specific growth rate from day 7 at different rates until the end of experiment in the following sequence pH 7.20 > 8.20 > 7.50 > 7.80. The treatment set at a pH of 7.20, yielded the greatest specific growth rate as well as having the greatest yield. A stocking density of 5 $g.l^{-1}$ of seawater proved to be suitable for cultivation. Our results show that pH toxicity response testing is a useful tool for assessing the health of macroalgae grown under aquaculture conditions under potentially increased CO_2 concentrations that typically can occur in IMTA carbon sequestration of large scale *Ulva* cultivation.

Keywords: *CO_2, macroalgae, pH, physiochemical characteristics s, sporulation, Ulva rigida*

Introduction

The world's oceans have retained about 125 billion tons of carbon (one quarter of the 500 billion tons) emitted into the atmosphere mainly through anthropogenic sources such as industrialization (Rapley, 2012). This figure is expected to rise by 20 billion tons/year by 2100 and will probably double by the next century (Philip *et al*., 2010; Rapley, 2012). The ongoing effects on global climate change and the associated deleterious environmental effects that no doubt would result from such high carbons levels has lead to much research interest into the impact of increased CO_2 on the ecophysiology of aquatic plants and the ecosystems they inhabit. As bioremediators, marine algae are significant components of the carbon cycle of coastal ecosystems and their responses to increased atmospheric CO_2 are of considerable importance. Growth in most aquatic plants is stimulated by increased CO_2 concentrations, which leads to higher biological productivity with an envisaged increase in the photosynthetic storage and nutrient uptake from the process (Surif and Raven, 1989; Maberly, 1990; Gao *et al*., 1991; Levavasseur *et al*., 1991; Kubler *et al*., 1999; Stitt and Krapp, 1999; Ding-Hui and Kun-Shan, 2002; Langdon *et al*., 2003; Zou, 2005; HongYan *et al*., 2008; Roleda *et al*., 2010; Markelz *et al*., 2013). Macroalgae grown in increased CO_2 environments typically exhibit increased rates of photosynthesis and biomass production (Logothetis *et al*., 2004; Andersen *et al*., 2006; Cornelisen *et al*., 2007; Suarez-Alvarez *et al*., 2012).

In the wild all aquatic plants use CO_2 for photosynthesis, while some also use HCO_3^- (Raven, 1991a; b), macroalgae are important carbon sinks as they absorb carbon throughout their lives; they enhance their photosynthetic capacity through the diffusion of greater amounts of CO_2 from the environment to the active site of rubisco, and depending on the pH, they utilize CO_3^{2-} and HCO_3^- as dissolved CO_2 (Becker, 1994; Giordano *et al*., 2005). Increased amounts of CO_2 give rise to more H^+ ions, thus lowering the pH.

The prolonged effects of increased CO_2 concentration and the resulting lower pH are varied (HongYan *et al*., 2008). This observed variation could be due to the availability of nutrients and or the methods of utilization by the marine algae since increase growth with high CO_2 will give rise to an increased nutrients demand under a normal seawater pH (Andria *et al*., 1999). Contrary to this Samesi *et al*. (2009) found that pH had significant effects on both the calcification and photosynthetic processes, with an increase in dissolved CO_2 concentration closer to 26 Mu mol kg^{-1} (bubbling with air at 0.9 mbar CO_2) resulting in a lower water pH, which resulted in 20 % less calcification in marine algae after 5 days of exposure. Recent research has also showed CO_2 to be a major limitation to large-scale algal biomass production and utilization, especially in closed systems (Ugoala *et al*., 2012). In aquatic environments with very high CO_2 concentrations, the pH can be as low as 4 - 6. The required pH for optimum algae cultivation is, however, within the range 7.5 – 8.5 (Nagle *et al*., 2009). Toxic effects from low pH values, which result in acidification, have been reported to both stimulate and reduce marine algal biomass (Berge *et al*., 2010).

South African species of *Ulva* have been well studied and about fourteen species are currently known to occur along country's west, south and east coast region (figure 1) due to merging of all *Enteromorpha* spp with *Ulva* (Joska, 1992; Stegenga *et al*., 1997; Cyrus *et al.*, 2014; J.J. Bolton, University of Cape Town, pers. comm). Commercial cultivation of *Ulva* spp has long been carried out in South Africa where the algae have been used as biofilters in the local aquaculture industry, as feed for commercially farmed abalone, and more recently in efforts geared towards using CO_2 to increase algal growth (Troell *et al*., 2006, Robertson-Andersson, 2007, Amosu *et al*., 2013). Several studies using algae have shown that super-elevated atmospheric CO_2 concentrations are not detrimental to fresh and marine micro- and macroalgae when using either a flow through or a recirculation system (Friedlander and Levy, 1995; Tisserat 2001; Andersen and Andersen, 2006; Gao *et al*., 2012). However, information about the physiological responses and the ability of macroalgae to withstand prolonged lowered pH as a result of increased CO_2 concentrations is scarce. Knowledge of the effects of elevated CO_2 concentrations on macroalgal physiology are therefore necessary to be able to determine the actual pH at which growth in these algae is inhibited. The aim of this study was to determine the survival of South African *Ulva rigida* C.Agardh under different pH scenarios resulting from varied CO_2 concentrations in controlled laboratory conditions.

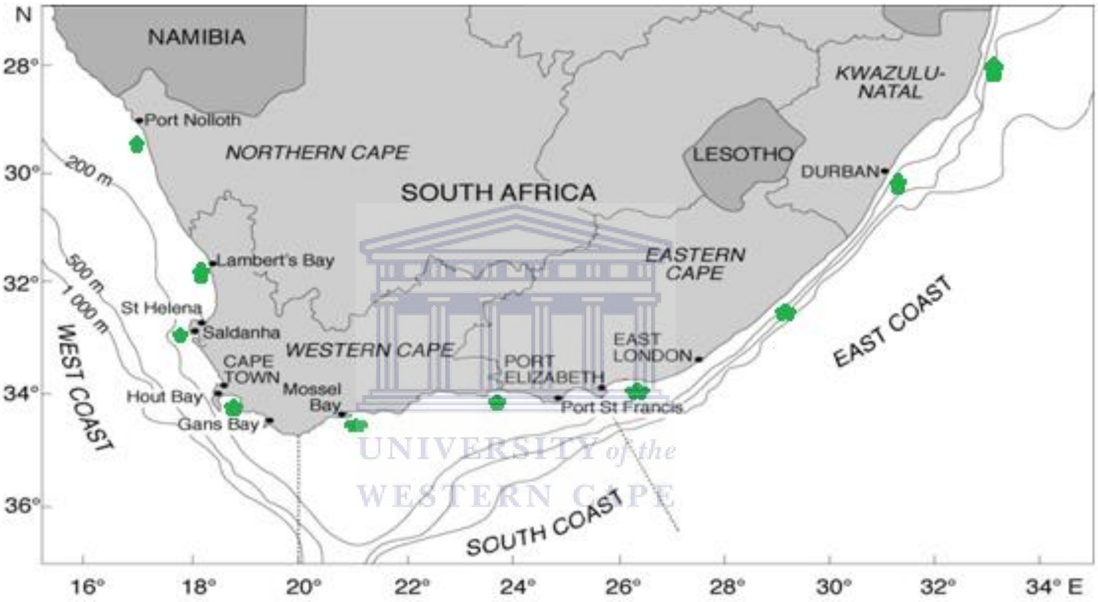

Figure 1. Location where *Ulva* species occured along South Africa coast.

Materials & Methods

Experimental material

A. *Closed experiment* - *Ulva* spp was collected at Simon’s town between latitude 34° 14’ S and longitude 18° 26’ E during low tide. *Ulva rigida* was used as a test alga due to its availability, ability to grow in reduced light conditions, and the fact that it is widely used in the South African aquaculture industry (Boarder and Shpigel, 2001; Robertson-Andersson, 2003; Troell *et al*., 2006). In the laboratory, thalli were cut into 1cm discs using a stainless steel cork borer (Figure 2a). Filtered seawater used in the experiment was collected from the Research Aquarium (Sea point, Cape Town) and enriched seawater (EW) medium was prepared using a standard Provosoli protocol (Provosoli, 1968 – Appendix 1). Glassware was sterilized in an autoclave before the commencement of the experiment and irradiation was provided by means of white fluorescent tubes at 16:8h (i.e light: dark) photoperiod.

Culture in air bubbles containers

The *in situ* survival response of *U. rigida* was carried out in a specialized temperature (15 ± 0.1 °C) and light (60 µmol.m^{-2}.s^{-1}) controlled cold room. Six discs (Figure 2a) were placed into each of 39 conical flasks (500 ml) filled with either enriched seawater (n = 18), or non-enriched seawater (n=18) (Figure 2b) and control (n=3) mediums for a period of 7 days (Figure 3c). The experiment was conducted in two phases. In the first phase, the mediums were continuously pumped with CO_2 in serial numbers of bubbles /second (s^{-1}) as follows: 0 s^{-1}, 1 s^{-1}, 2 s^{-1}, 3 s^{-1}, 4s^{-1}, 5 s^{-1} and 6 s^{-1} (Figure 2c) to determine the sporulation response to varied CO_2 concentrations. The second phase was characterized by the setting up of high and low CO_2 bubbling regimes run continuously for 48 hours with new thallium discs. In the high bubbling regimes, CO_2 was bubbled continuously to allow for constant movement and rotation of the discs. In the low bubbling regimes very little CO_2 was bubbled without inducing any movement of the discs. Both experimental treatments were run in triplicate (3) for both non-enriched seawater (SW) and enriched seawater (EW) at (1 bars) 10 l.min^{-1} CO_2 pressure gauge. The growth media were replaced every day. Water chemistry and ambient aerial light was measured daily and included salinity (‰), pH, temperature (°C), dissolved oxygen (DO, mg.l^{-1}) and light (µmol.m^{-2}.s^{-1}). The Waterproof CyberScan Series 300 (Eutech Instruments Pte Ltd, Singapore) - Dissolved Oxygen meter, specially designed to measure oxygen and temperature simultaneously, was used to measure DO and temperature to determine consistency in the measurements. pH was determined with the aid of a portable digital pH meter (model 8414, Hangzhou rock biological technology Co. LTD,) that also measureed temperature at 0.1 °C. Salinity was determined with the aid of refractometer (S/Mill-E 0 ~ 100 ‰, ATAGO, Japan). Irradiance levels were measured using a skye quantum sensor (Skye Instruments Ltd, UK).

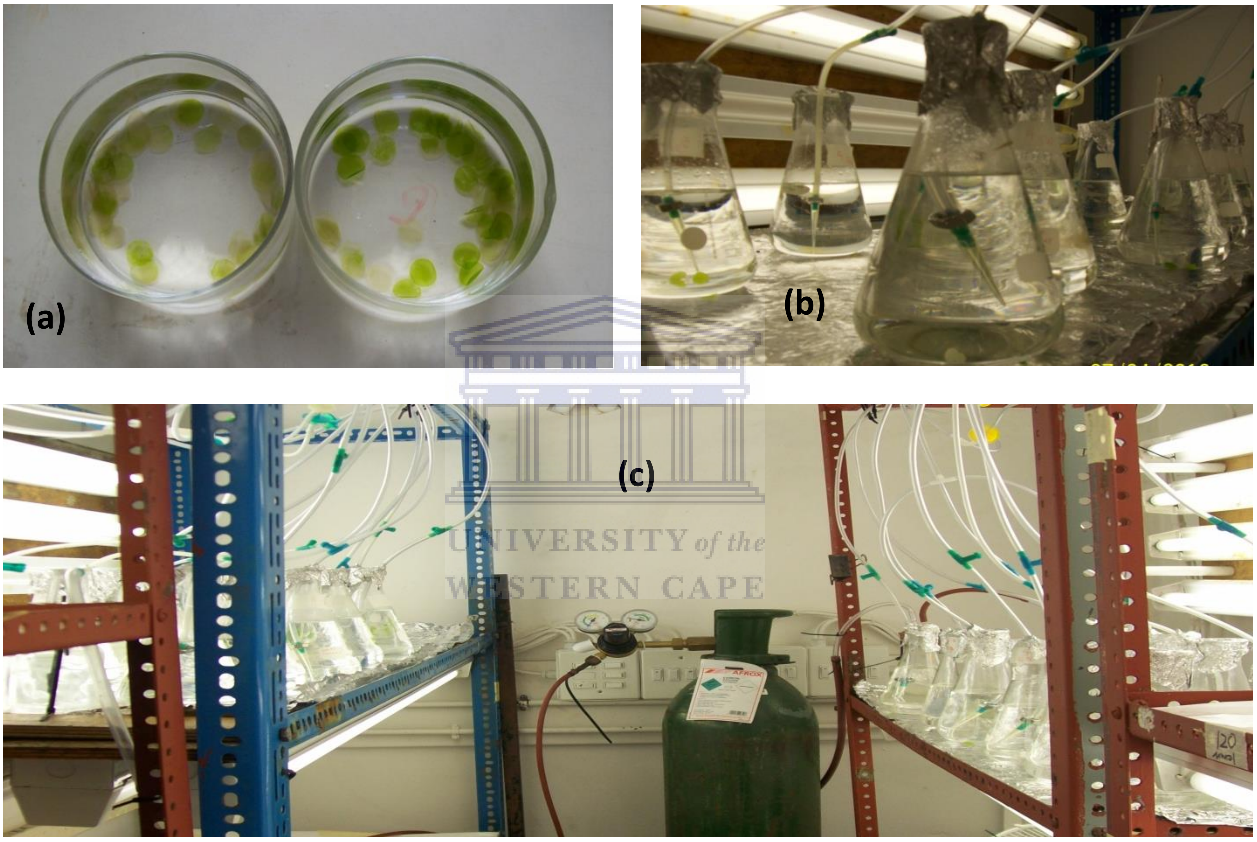

Figure 2: (a) Algal discs, (b) *Ulva* discs placed in different flasks with different bubbling regimes and (c) Co_2 induced replicates of enriched and non enriched media.

B. *Flow through experiment* - The experiments were conducted in flow-through seawater systems (~ 3 $L.min^{-1}$ at Sea point research Aquarium) were water was delivered at a consisted flow rate into each individual rearing tank. The experimental pH was regulated by injection of pure CO_2 gas via an air stone which was controlled via a Tunze system (Tunze pH controller 7070/2- The Age of Aquariums, Germany). Tanks were continuously bubbled with O_2 to aid mixing and to maintain dissolved oxygen. Vibrator propeller pumps was used to induce mixing and movement by SUNSUN New Design 24W Dual Propeller Circulation Pump (JVP-202A/B), Guangzhou Weierma Electrical Co., Ltd, Zhejiang, China (Mainland). Seawater within the tanks was at pH 8.20 with two replicates. Water chemistry was measured with a YSI Professional Plus Multiparameter Water Quality Meter (YSI Incorporated, US).

Culture in flow through media

The second experiment was performed in three 111.1 L (85.3 cm X 28 cm X 46.5 cm) tanks, stocked at 5 g. l^{-1} specially designed for flow through culture of *Ulva* (figure 3). The source of growth medium and nutrients were from provided from the unfiltered seawater at sea-point aquarium, which was the same as that used during the stock maintenance period with pH 8.20. Seawater within the tanks was maintained at several pH levels (namely; 7.20, 7.50, 7.80 and a control of 8.20), with three replicates each in the different treatments. Light was provided at 92 µmol.m^{-2}.s^{-1} and controlled by means of white fluorescent tubes attached to a controller, set to a 16:8 h (i.e light: dark) photoperiod. A pH electrode measured the seawater pH, if the seawater pH moved above that of the set value (determined by set value on controller, which is done according to the required value) a magnetic valve was opened and CO_2 from the cylinder is bubbled into the seawater via the air stone. This then mixes with the surrounding water with the help of the circulating pumps; the magnetic valve continually opens and closes until the set pH value is acquired. The one way valve is placed in the system to prevent water moving from the tank into the magnetic valve. O_2 is continually pumped into the system via another air stone.

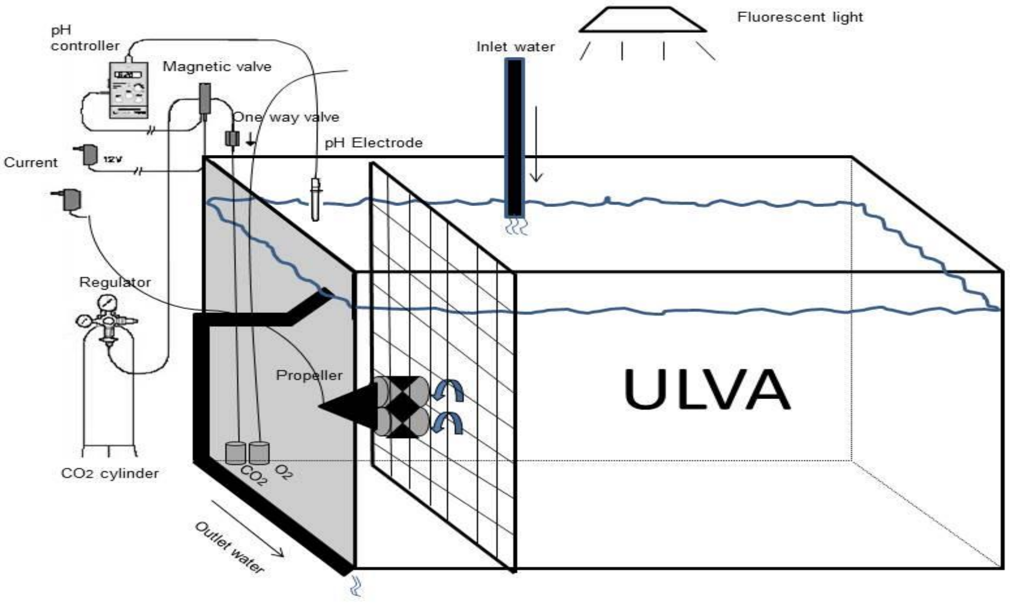

Figure 3: Flow through experimental design.

Specific growth rate data acquisition

The seaweed was harvested every 3 days for 16 days, blotted dry and weighed for restocking. Specific growth rate (SGR, %) was calculated as:

$$\mathbf{SGR = (wt - wo)\ 100/t}$$

Where **wo** is the initial biomass and **wt** is the biomass after **t** culture days. Biomass yield (fresh weight) was calculated as the difference between initial and final weights and expressed in units of $\mathrm{g.\ l^{-1}\ day^{-1}}$, based on the areas of the culture from Duke *et al*, (1986).

Statistical analysis

Two way ANOVA was used after data was examined for normality and meeting the assumptions of ANOVA to test the differences among treatments. A significance level of $p < 0.05$ was applied for statistically different data. All data were analyzed statistically in GraphPad Prism5.

Results

Closed experiment - Our results show that, irrespective of the treatment (enriched vs. non-enriched seawater) and the number of days from commencement of the experiment, the higher the CO_2 concentration, the greater the degree of sporulation. The high CO_2 concentration gave rise to low (acidity) pH, resulting in thalli disintegration, the process known as sporulation. Survival was therefore a function of the pH of the culture medium. Control thalli (zero CO_2 bubbling) showed minor sporulation even after 7 days (Table 1).

Table 1: Degree of sporulation in *U. rigida* under different CO_2 consecutive numbers of bubbles per second. Empty cells represent no noticeable sporulation.

Day	Media	CO_2 consecutive numbers of bubbles (s^{-1})						
		0	1	2	3	4	5	6
1	SW		×		×	×	××	××
	EW				×	×	×	××
2	SW		×	×	×	×	××	××
	EW		×	×	×	×	×	××
3	SW		×	××	××	××	××	××
	EW		×	×	×	×	××	××
4	SW		×	×	××	××	××	×××
	EW		×	×	×	×	××	×××
5	SW		×	×	××	××	×××	n
	EW		×	××	××	××	×××	n
6	SW	×	×	××	××	×××	n	n
	EW	×	××	××	××	×××	n	n
7	SW	×	××	××	N	n	n	n
	EW	×	××	××	N	n	n	n

× = minor sporulation (one disc or spherical sporulate), ×× = moderate sporulation (more than one disc or sporulate), ××× = significant sporulation (5/6 discs turning pale), n = no algae in media

Thalli under high CO_2 concentrations sporulate sooner and expend their resources in a shorter period of time. Initially (days 1 - 4), thalli grown in non-enriched seawater sporulated sooner (than those grown in enriched seawater) with an increase in CO_2 concentration. Towards the end of the experiment (days 5 - 7), there was a severe degree of sporulation irrespective of the treatments. After 48 hours exposure, our results revealed that apart from salinity, which was within seawater range (for Simonstown) irrespective of the bubbling regime, pH and DO show a diurnal variation at the different bubbling regime with time (see Figure 4, 5 and 6).

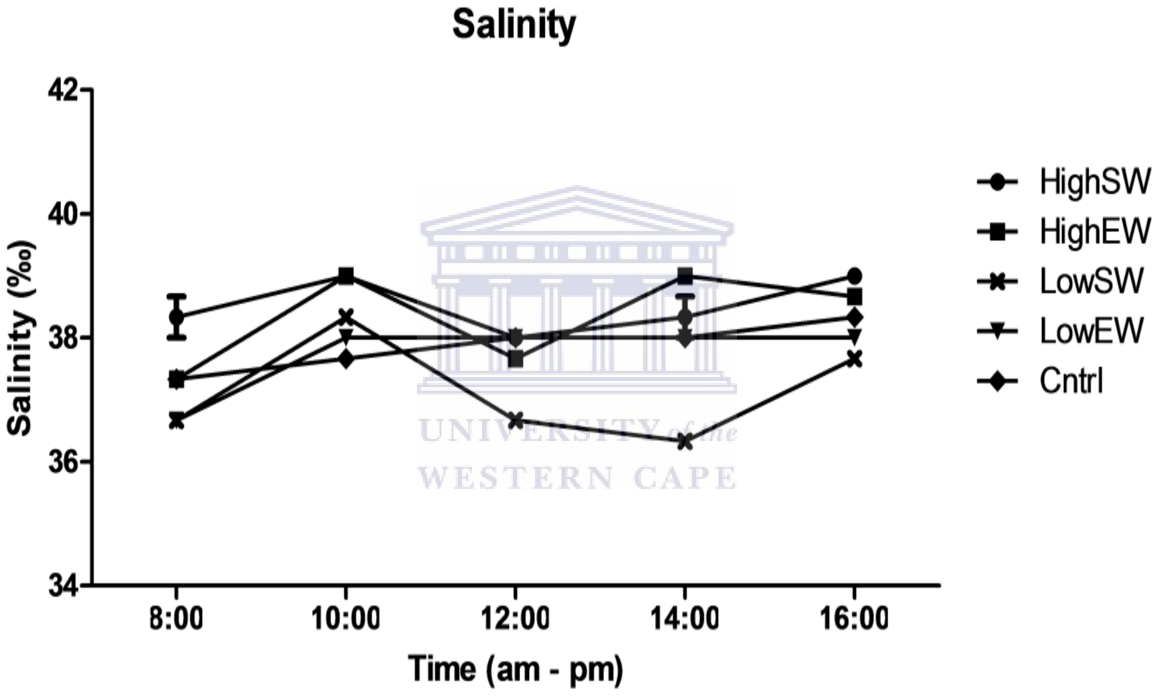

Figure 4: Salinity time graph of Co_2 concentration in *U. rigida*.

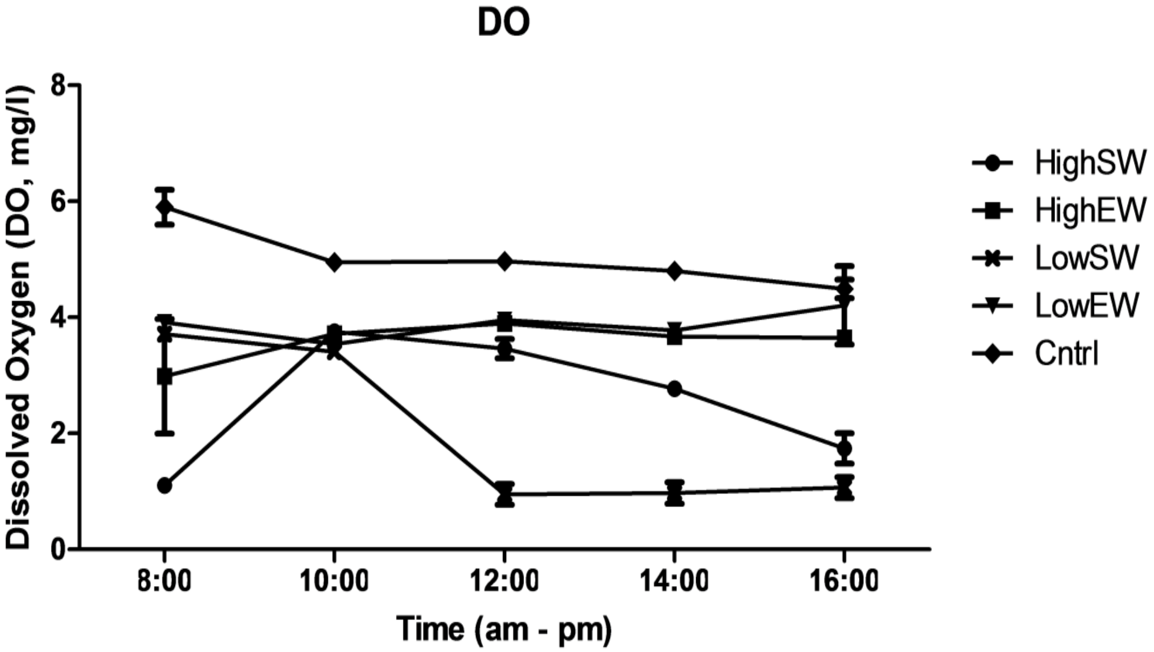

Figure 5: Dissolved oxygen time graph of Co$_2$ concentration in *U. rigida*.

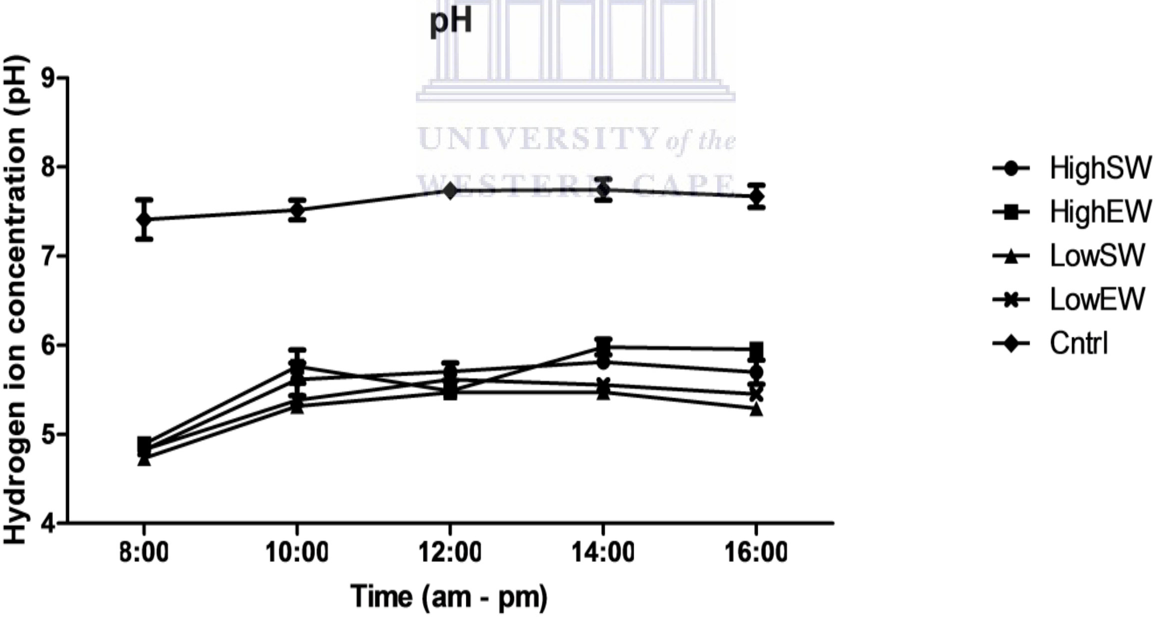

Figure 6: pH time graph of Co$_2$ concentration in *U. rigida*.

Graph key: High bobbling regimes for non-enriched seawater = High sw, High bobbling regimes for enriched seawater = High ew, Low bobbling regimes for non-enriched seawater = Low sw, Low bobbling regimes for enriched seawater = Low ew, Control = cnt.

The salinity values observed in the study fluctuate in the range between 36.6 – 39 ‰ despite the bubbling regime. The lowest salinity (36.6 ‰) was observed in the low bubbling regime irrespective of the treatment and time of the day, while the highest salinity 39 ‰ were common in the high bubbling regime at the hours of 10h00, 14h00 and 16h00 of the days irrespective of the treatments. This was likely due to the bubbling increasing evaporation resulting in increased salinity. During this experiment DO was low at 8h00 and 16h00 in non-enriched seawater and decreased with time irrespective of the treatments forming a normal skewed curved (Figure 4), while in the control it decreases with time. The control pH recorded was slightly higher (7.07) than neutral and normal (8.14) seawater throughout the study. Values for both treatments remained slightly acidic irrespective of the treatment. At low pH (4.73 - 6.67) sporulation was not a function of nutrient availability (Figure 6).

Flow through experiment - On the aspects of the flow through experiment, water chemistry revealed conducive physico-chemical parameters of *Ulva rigida* (Table 2). Dissolved Oxygen values in the tank were similar despite the addition of CO_2. The temperature of the growth media increased from 12°C at the beginning of experiment with a gradual increase of 0.10 °C to attain the maximum 16.9 °C at the end of cultivation. The pressure result recorded in the study ranged from 101.85 to 103.54 KPa. The salinity tolerance was narrow between 34.52 to 35.27 ppt. The salinity increased slightly in different treatment for 3rd day. Total Dissolved Solids ranged from 34125 to 34710 TDS mg.L^{-1}.

Table 2: Some physicochemical parameters of *Ulva rigida* cultured in flow through system.

	Treatment 1 (pH 8.20)	Treatment 2 (pH 7.20)	Treatment 3 (pH 7.50)	Treatment 4 (pH 7.80)
pH	8.22 ± 0.10^a	7.39 ± 0.13^b	7.61 ± 0.15^c	7.74 ± 0.14^c
A_T (µmol kg^{-1})	2159 ± 32^a	2127 ± 85^a	2160 ± 22^a	2169 ± 21^a
Temperature (°C)	13.0 ± 0.7^a	13.0 ± 0.7^a	15.0 ± 1.1^b	15.1 ± 1.2^b
Salinity (‰)	34.8 ± 0.2^a	34.8 ± 0.2^a	35.1 ± 0.1^a	35.1 ± 0.1^a
pCO_2 (µatm)	232 ± 71^a	1952 ± 673^b	1192 ± 384^b	860 ± 278^c
Ca^{2+} (mmol l^{-1})	10.4 ± 0.3^a	10.9 ± 0.3^a	11.3 ± 0.3^a	11.1 ± 0.1^a
Mg^{2+} (mmol l^{-1})	46.9 ± 1.1^a	45.7 ± 1.9^a	52.5 ± 3.4^b	50.8 ± 1.1^b
Pressure (KPa)	102.91 ± 0.420^a	102.95 ± 0.418^a	102.42 ± 0.318^a	102.43 ± 0.310^a
DO (mg l^{-1})	8.32 ± 0.344^a	8.44 ± 0.340^a	7.37 ± 0.707^b	7.65 ± 0.378^b
SPC	52.89 ± 0.253^a	52.87 ± 0.251^a	53.24 ± 0.147^a	53.23 ± 0.142^a
TDS (mg l^{-1})	34382 ± 160^a	34386 ± 164.6^a	33600 ± 5597^b	34607 ± 96^a

Means in the same row having the same superscript are not significantly different ($p > 0.05$)

Discussion

Closed experiment - Little is known about the effects of elevated CO_2 concentrations on *Ulva* cultivation. Our results therefore suggests that CO_2 concentration more so than the growth medium (enriched vs. non-enriched) determines the degree of sporulation in *U. rigida*. With the current knowledge, we can predict positive effects on growth, reproduction and variable effects (such as positive effect of increasing CO_2 , while it adverse effect of decreasing pH (slightly acidic) as observed in our study) on biomass development (Harley *et al*., 2012; Roleda *et al*., 2012). Although, Nordby (1977) reported that the optimum pH for sporulation lies between 8.0 and 8.5. Outside this range the rate of sporulation was found to decrease.

However earlier work conducted by Lersten and Voth (1960) found that a pH value of between 6.5 and 7.5 gave the highest degree of sporulation. These corroborate our findings of an acidic pH (4.73 - 6.67) range observed for sporulation. The varied responses observed could be due to the fact that sporulation starts from a slightly acidic to a slightly alkaline state of water.

As experienced in this study *Ulva* spp often show warning signs of impending sporulation for various environmental stresses (Beach *et al*., 1995; Kalita and Titlyanov, 2003; Kalita and Titlyanov, 2011). Increased CO_2 concentrations are proving to be one such a stress and here we have shown the increased degree of sporulation as a consequence of decreased pH resulting from increased CO_2 concentrations. The toxicity responses of *Ulva* spp. reported by various authors (e.g. Han and Choi, 2005; Han *et al*., 2007) support our findings. Our findings show that, at low pH (4.73- 6.67) sporulation is not a function of nutrient availability. This may be reason why marine algae could survive at pH 7.9 – 8.4 as a result of high CO_2 give rise to an increased nutrients demand as reported by Andria *et al.* (1999). This result is in agreement with that of Han and Choi, (2005) that reported on sporulation inhibition in *U. pertusa*. Naturally in the aquatic ecosystem, survival in macroalgae is function of water chemistry as been influenced by physico-chemical parameters that control the metabolic activities and chemical speciation of the contaminant. These have been observed in this experiment. Observed sporulation (Table 1) was not linked to water chemistry (Figure 4, 5 and 6) in these variables at the different bubbling regime. Similar ranges were previously reported on by Robertson-Andersson, (2007) for *Ulva* production in flow through systems. Continuous bubbling of CO_2 to the experimental medium affected the pH values since CO_2 supplied is dissolved, hydrated forming carbonic acid, and disintegrated to bicarbonate, releasing H+ ions and causing a decrease of pH, which has been reported in previous works (Samesi *et al*., 2009; Ugoala *et al*., 2012). This was also revealed in our present study where the low value of pH 4.73 was recorded in the non enriched medium at high (8h00 – early morning) bubbling regime, when the biochemical activities was minimal and about rising for photosynthesis activities. The low pH experienced in both (enriched/non enriched) media supplied with CO_2-enriched air may be caused not only by respiration but also by the continuous influx and dissolution of CO_2 into the medium. This shows a physiological and biochemical responses as reported in recent work with Ulvales in *Ulva prolifera* O.F.Müller and *Ulva linza* Linnaeus to Cadmium Stress (Jiang *et al*., 2013). This value similarly agrees with the results and findings of Garnham *et al*. (1992). pH values also vary significantly with time at different bubbling regime (Two-way ANOVA: $F = 55.53$, $p < 0.0001$). Other recent works showed that *Ulva* spp has survived at 15 – 20 °C and 60 $\mu mol.m^{-2}. s^{-1}$ as observed in the present study (Ruangchuay *et al*., 2012; Jiang *et al*., 2013). Dissolved oxygen shows a diurnal trend, which could be as a result of photosynthesis response with time of the day. The summary of result analysis conducted on DO varies significantly with time Table 3 (Two-way ANOVA: $F = 7.041$, $p = 0.0001$). Macroalgae usually uptake CO_2 via the C_3 biochemical pathway with rubisco as the carboxylating enzyme a process that is competitively inhibited by dissolved Oxygen via photorespiration (Raven, 1989; 1997). According to Choi *et al*. (2010) Growth decreased by low salinity and also at salinities greater than 25 ‰, which may lead to sporulation as a toxic effect of increased CO_2 (Boyd 1998). Salinity values vary significantly with time at different bubbling regimes (Two-way ANOVA: $F = 17.93$, $p < 0.0001$). However, the salinity range of 36.6 - 39 ‰ observed in this study are the osmotic consequence of the movement of water molecules along water-potential gradients, and the flow of ions along electrochemical gradients (Lobban and Harrison 1994). The fluctuating salinity also corroborate Dickson *et al*. (1982) as they studied the responses of *U. lactuca* to salinity fluctuations and concluded that changes in internal solute concentrations closely follow salinity fluctuations, reducing the changes in turgor pressure.

Table 3: Physico-chemical parameters analyzed on GraphPad PrismV

Analyzed Salinity (‰)						
Source of Variation	Df	Sum-of-squares	Mean square	% of total variation	P value	F
Interaction	16	10.75	0.6717	20.86	0.0003	3.598
Treatment	4	18.05	4.513	35.04	P<0.0001	24.18
Concentration with Time	4	13.39	3.347	25.98	P<0.0001	17.93
Residual	50	9.333	0.1867			
Analyzed Dissolved Oxygen (DO)						
Source of Variation	Df	Sum-of-squares	Mean square	% of total variation	P value	F
Interaction	16	38.28	2.392	27.76	P<0.0001	10.77
Treatment	4	82.24	20.56	59.64	P<0.0001	92.54
Concentration with Time	4	6.258	1.564	4.54	0.0001	7.041
Residual	50	11.11	0.2222			
Analyzed Hydrogen ion concentration (pH)						
Source of Variation	Df	Sum-of-squares	Mean square	% of total variation	P value	F
Interaction	16	1.171	0.07321	1.77	0.0025	2.838
Treatment	4	57.83	14.46	87.60	P<0.0001	560.6
Concentration with Time	4	5.728	1.432	8.68	P<0.0001	55.53
Residual	50	1.290	0.02579			

Flow through system - In the aquatic environment, survival in macroalgae is function of water chemistry as been influenced by physico-chemical parameters that control the biophysiological activities (Table 2). Similar ranges were previously reported for *Ulva* production in flow through systems (Robertson-Andersson, 2007). *Ulva rigida* cultured in this flow through system show decreased yields after 1 week. The gradual degeneration was noticed after day 4. Nikolaisen *et al*. (2011), in mass cultivation of *Ulva lactuca* found this decrease leading to sporulation and reduction in biomass within few days of culture. Despite this (pH 8.2) being within the optimum pH for algae cultivation (Björk *et al*., 1992; Robertson Andersson *et al*., 2008). According to research, increasing stocking density above 1 kg.m^{-2} decreases growth rate and yield (Neori *et al*., 1991; Bruhn *et al*., 2011; Nikolaisen *et al*., 2011). Ryther *et al*. (1984) suggested the optimal stocking density of *Ulva lactuca* to be near 1 kg m^{-2}. However Prue, (2009) found that stocking densities of between 5 - 20 g of *Ulva* per litre along with the addition of the soluble fertilizer (Aquasol) at a rate of 87 g.l^{-1} of seawater was ideal for achieving a desired doubling of growth per week (Our stocking density of 5g.l^{-1}, *Ulva* was well within this range and thus sporulation and subsequent degeneration were not due to stocking density.

The biomass and yield of *Ulva rigida* ranged between 467 – 1433 g in sixteen days without enriched nutrient (Figure 7). The growth rates of *Ulva rigida* observed in this study differs to some previously reported growth rates of *Ulva lactuca* and *U. fasciata* in outdoor flow-through tanks (Lapointe and Tenore 1981; Neori *et al*., 1991) but similar to the results obtained by Corey *et al*. (2014). However, some findings with *Ulva* cultivation have shown an array of disparities in the cultivation conditions. For example, both Neori *et al*. (1998) and Robertson-Andersson *et al*. (2008) noted that as cultivation volume increased, total yield was reduced along with growth rates. This supports our present findings in this research.

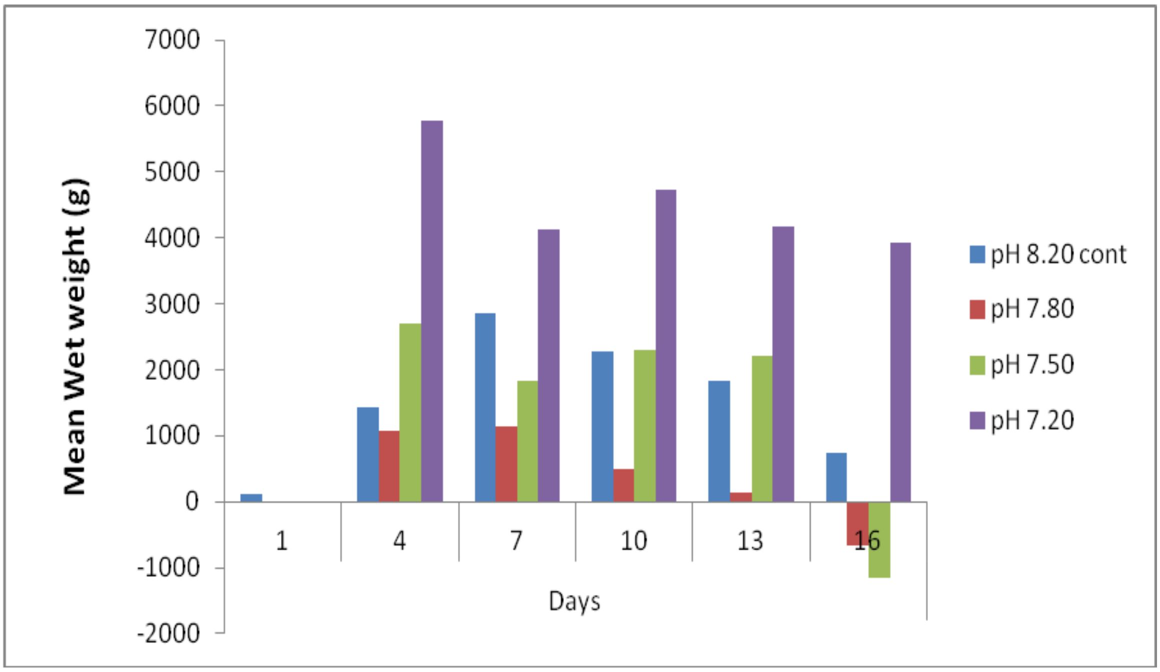

Figure 7: Biomass of *Ulva rigida* at different pH.

Conclusion

This work adds to baseline evidence for sporulation and toxicity tolerance of *Ulva* spp in a high-CO_2 situation. It has demonstrated that the response of *Ulva rigida* to increase CO_2 enrichment is complex and potentially multifactorial. An *in situ*, ecosystem based approach, incorporating flexible and controlled conditions, provides more accurate insights into the responses of macroalgae, highlighting the importance of increase CO_2 gradients as a valuable tool in the study of pH tolerance. Elevated CO_2 accumulates in the growth media increasingly affects marine photosynthetic processes in direct and/or indirect ways. Different physiological processes are involved, whether or not macroalgae will benefit from increased CO_2 remains controversial. Low pH and/or increased CO_2 can inhibit growth relative to the medium or the oxidation-reduction potential. Sporulation is seen here as a morphological response to environmental stress in macroalgae. Increased CO_2 levels in production unit may decrease pH and photosynthetic activity of macroalgae with influential effects on other physicochemical parameters under closed conditions. In flow through system, despite sporulation in the majority of the treatments, the results show that *Ulva rigida* is able to tolerate a sustained low pH of 7.2. This implies that in large scale cultivation systems CO_2 could be added and pH maintained at 7.2. In addition, the application of soluble fertilizer could achieve a better growth and help prevent sporulation. Therefore, it will be important to conduct future experiments on rates of fertilizer application at low pHs, as well as to follow the responses of multiple generations to elevated CO_2 under conditions which simulate growth.

Acknowledgements

We thank the management and staff of the Sea point aquarium, Department of Agriculture, Forestry and Fisheries (DAFF) and the Seaweed Unit, Marine and Coastal Management, University of Cape Town, Cape Town, South Africa. The Department of Biodiversity and Conservation Biology at the University of the Western Cape, and the Department of Biological Sciences at the University of Cape Town are also thanked for providing funding and research equipment.

References

Amosu, A.O., Robertson-Andersson, D.V., Maneveldt, G.W., Anderson, R.J. and Bolton J.J. (2013). South African Seaweed Aquaculture: A sustainable development example for other African coastal countries. African Journal of Agricultural Research, 8(43): 5260-5271.

Andersen, T. and Andersen, F.O. (2006). Effects of CO_2 concentration on growth of filamentous algae and *Littorella uniflora* in a Danish softwater lake. Aquatic Botany, 84: 267-271.

Andersen, T., Andersen, F.O. and Pedersen, O. (2006). Increased CO_2 in the water around *Littorella uniflora* raises the sediment O_2 concentration. Aquatic Botany, 84: 294-300.

Andría, J.R., Pérez-Llorens, J.L. and Vergara, J. (1999). Mechanisms of inorganic carbon acquisition in *Gracilaria gaditana* nom. prov. (Rhodophyta). Planta, 208: 564-573.

Beach, K.S., Smith, C.M., Michael, T. and Shin, H. (1995). Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa*: implications for ecological success. Marine Ecology Progress Series, 125: 229-237.

Becker, E.W. (1994). Microalgae: Biotechnology and microbiology, Cambridge Univeristy Press, USA.

Berge, T., Daugbjerg, N., Andersen, B.B. and Hansen, P.J. (2010). Effects of lowered pH on marine phytoplankton growth rates. Marine Ecology Progress Series, 416: 79-91.

Boarder, S.J. and Shpigel, M. (2001). Comparative growth performance of juvenile *Haliotis roei* fed on enriched *Ulva rigida* and various artificial diets. Journal of Shellfish Research, 20: 653-657.

Boyd, C.E. (1998). Pond Aquaculture Water Quality Management. *Aquaculture Series* (*Chapman & Hall Aquaculture Series*). ed. by C S Tucker. Springer. pp.700.

Choi, T.S., Kang, E.J., Kim, J. and Kim, K.Y. (2010). Effect of salinity on growth and nutrient uptake of *Ulva pertusa* (Chlorophyta) from an eelgrass bed. Algae, 25(1): 17-26.

Cornelisen, C.D., Wing, S.R., Clark, K.L. and Bowman, M.H. (2007). Patterns in the d13C and d15N signature of Ulva pertusa: Interaction between physical gradients and nutrient source pools. Limnology Oceanography, 52(2): 820-832.

D'Elia, C.F. and DeBoer, J. (1978). Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. Journal of Phycology, 14: 266-272.

Dickson, D.M.J., Wyn-Jones, R.G. and Davenport, J. (1982). Osmotic adaptation in *Ulva latuca* under fluctuating salinity regimes. Planta, 155: 409-415.

Ding-Hui, Z. and Kun-Shan, G. (2002). Photosynthetic responses to inorganic carbon in *Ulva lactuca* under aquatic and aerial state. Acta Botanics Sinica, 44(11): 1291-1296.

Friedlander, M. and Levy, I. (1995). Cultivation of *Gracilaria* in outdoor tanks and ponds. Journal of Applied Phycology, 7: 315-324.

Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T. and Kiyohara, M. (1991). Enhanced growth of the red alga Porphyra yezoensis Ueda in high CO_2 concentrations. Journal of Applied Phycology, 3: 355-362.

Gao, K., Helbling, E.W., Häder, D.P. and Hutchins, D.A. (2012). Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Marine Ecology Proress Series, 470: 167-189.

Giordano, M., Beardall, J. and Raven, J.A. (2005). CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation and evolution. Annual Review of Plant Biology, 56: 99-131.

Harley, C.D., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A. and Graham, M.H. (2012). Effects of climate change on global seaweed communities. Journal of Phycology, 48: 1064-1078.

Han, T. and Choi, G. (2005). A novel marine algal toxicity bioassay based on sporulation inhibition in the green macroalga *Ulva pertusa* (Chlorophyta). Aquatic Toxicology, 75: 202-212.

Han, Y., Brown, M.T., Gyoung, S. and Han, T. (2007). Evaluating Aquatic Toxicity by Visual Inspection of Thallus Color in the Green Macroalga *Ulva*: Tesing a Novel Bioassay. Environmental Science Technology, 41: 3667-3671.

HongYan, W., DingHu, Z. and KunShan, G. (2008). Impacts of increased atmospheric CO_2 concentration on photosynthesis and growth of micro- and macro-algae. Science in China Series C: Life Science, 51(12): 1144-1150.

Jiang, H., Gao, B., Li, W., Zhu, M., Zheng, C., Zheng, Q. and Wang, C. (2013). Physiological and Biochemical Responses of *Ulva prolifera* and *Ulva linza* to Cadmium Stress. The Scientific World Journal, 2013: 11-150.

Kalita, T.L. and Itlyanov, E.A. (2003). Effect of temperature and illumination on growth and reproduction of the green alga *Ulva fenestrate*. Russian Journal of Marine Biology, 29(5): 316-322.

Kim, J.K., Kang, E.J., Park, M.G., Lee, B.G. and Kim, K.Y. (2011). Effects of temperature and iradiance on photosynthesis and growth of green-tide-forming species (*Ulva linza*) in the Yellow Sea. Journal of Applied Phycology, 23: 421-432.

Kubler, J.E., Johnston, A.M. and Raven, J.A. (1999). The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*. Plant, Cell and Environment, 22: 1303-1310.

Langdon, C., Broecker, W.S., Hammond, D.E., Glenn, E., Fitzsimmons, K., Nelson, S.G., Peng, T.S., Hajdas, I. and Bonani, G. (2003). Effect of elevated CO_2 on the community metabolism of an experimental coral reef. Global Biogeochemical Cycles, 17: 10-21.

Lersten, N.R. and Voth, P.D. (1960). Experimental control of zooid discharge and rhizooid formation in the green alga E*nteromorpha*. Botanical Gazzet, 122: 33-45.

Levavasseur, G., Edwards, G.E., Osmond, C.B. and Ramus, J. (1991). Inorganic carbon limitation of photosynthesis in *Ulva rotundata* (Chlorophyta). Journal of Phycology, 27: 667-672.

Lobban, C.S. and Harrison, P.J. (1994). Seaweed ecology and physiology. Cambridge University Press, Cambridge, pp.366.

Logothetis, K., Dakanali, S., Ioannidis, N. and Kotzabasis, K. (2004). The impact of high CO_2 concentrations on the structure and function of the photosynthetic apparatus and the role of polyamines. Journal of Plant Physiology, 161: 715-724.

Maberly, S.C. (1990). Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. Journal of Phycology, 26: 439-449.

Malta, E.J., Draisma, S.G.A. and Kamermans, P. (1999). Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. European Journal of Phycology, 34: 443-454.

Markelz, R. (2013). Interactive effects of elevated carbon dioxide concentration with nutrient availability and leaf development on plant carbon metabolism. PhD Dissertation. University of Illinois. U.S.

Nordby, Ø. (1977). Optimal conditions for meiotic spore formation in *Ulva Mutabilis* Føyn. Botanica Marina, 20: 19-28.

Philip, O., Laban, A., Ogallo, R., Anyah, J., Muthama, M. and Ininda, J. (2013). Linkages between global sea surface temperatures and decadal rainfall variability over Eastern Africa region. International Journal of Climatology, 33(8): 2082-2104.

Provasoli, L. (1968). Media and prospects for cultivation of marine algae. In *Cultures and Collections of Algae* (Watanabe, A. & Hattori, A., editors), 47-74. *Japanese Society of*

Plant Physiology, Tokyo.

Rapley, C.G. (2012). The Science of Climate Change. British Medical Journal, 344(7849): 23-25.

Raven, J.A. (1989). Overview of transport systems in algae and bryophytes. In: Fleischer S, Fleischer B, eds. Methods in enzymology: biomembranes/biological transport, Vol. 4, Part V (Vol. 174). San Diego: Academic Press, pp.366-390.

Raven, J.A. (1991a). Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. Plant, Cell & Environment, 14: 774-794.

Raven, J.A. (1991b). Implications of inorganic carbon utilization: ecology, evolution and geochemistry. Canandian Journal of Botany. 69: 908-924.

Raven, J.A. (1997). Inorganic carbon acquisition by marine autotrophs. Advances in Botanical Research, 27: 85-20.

Robertson-Andersson, D.V. (2003). The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. M.Sc Dissertation, University of Cape Town, South Africa.

Robertson-Andersson, D.V. (2007). Biological and economical feasibility studies of using seaweeds *Ulva lactuca* (chlorophyta) in recirculation systems in abalone farming. PhD Dissertation, University of Cape Town, South Africa.

Roleda, M., Stecher, A., Gutow, L., Bartsch, I. and Wiencke, C. (2010). Differential effects of increased CO_2 concentration and acidification on photosynthesis and growth of green and brown macroalgae, *58th Meeting of the British Phycological Society*. Scottish Association for Marine Science (SAMS). Oban, Scotland. 6-8th January 2010.

Roleda, M.Y., Morris, J.N., McGraw, C.M. and Hurd, C.L. (2012). Ocean acidification and seaweed reproduction: increased CO_2 ameliorates the negative effect of lowered pH on meiospore germination in the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). Global Change Biology, 18: 854-64.

Ruangchuay, R., Dahamat, S., Chirapat, A. and Notoya, M. (2012). Effects of culture conditions on the growth and reproduction of Gut Weed, *Ulva intestinalis* Linnaeus (Ulvales, Chlorophyta) Songklanakarin. Journal of Science and Technology, 34(5): 501-507.

Semesi, I.S., Kangwe, J. and Bjork, M. (2009). Alterations in seawater pH and CO_2 affect calcification and photosynthesis in the tropical coralline alga, *Hydrolithon* sp (Rhodophyta). Estuarine Coastal and Shelf Science, 84: 337-341.

Stengenga, H., Bolton., J.J. and Anderson, R.J. (1997). The Seaweed flora of the South West Coast. Contributions from the Bolus Herbarium no. 18. Bolus Herbarium, University of Cape Town, Cape Town, South Africa.

Steyn, P. (2000). A comparative study of the production and suitability of the two *Ulva* species as Abalone fodder in a commercial mariculture system. M.Sc thesis. University of Porth Elizabeth. South Africa.

Stitt, M. and Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environment, 22: 583-621.

Strickland, J.D.H. and Parsons, T.R. (1968). A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin, 167: 311-325.

Suarez-Alvarez, S., Gomez-Pinchetti, J.L. and Garcia-Reina, G. (2012). Effects of increased CO_2 levels on growth, photosynthesis, ammonium uptake and cell composition in the macroalga *Hypnea spinella* (Gigartinales, Rhodophyta). Journal of Applied Phycology, 24: 815-823.

Surif, M.B. and Raven, J.A. (1989). Exogenous inorganic carbon sources for photosynthesis in seawater by members of the *Fucales* and the *Laminariales* (Phaeophyta): ecological and taxonomic implications. Oecologia, 78: 97-103.

Tisserat, B. (2001). Influence of ultra-high carbon dioxide concentrations on growth and morphogenesis of Lamiaceae species in soil. Journal of Herbs, Spices & Medicinal Plants, 9: 81-89.

Tseng, C.K. (1983). Common Seaweeds of China. Science Press, Beijing, pp. 258.

Ugoala, E., Ndukwe, G.I., Mustapha, K.B. and Ayo, R.I. (2012). Constraints to large scale algae biomass production and utilization. Journal of Algal Biomass Utilization, 3(2): 14-32.

Zou, D. (2005). Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). Aquaculture, 250: 726-735.

Appendix A: PROVASOLI'S ENRICHED SEAWATER (ES)

ADDITIVE	CONCENTRATION
$NaN0_3$ (Stock A)	35g/100ml
Na_2 glycerophosphate (Stock B)	5g/100ml
Vitamin B_{12} (Stock C)	1mg/100ml
Thiamine (Stock D)	50mg/100ml
Biotin (Stock E)	0.5ml/100ml
Fe (as EDTA 1: molar) (Stock F)	
$Fe (NH_4)_2 (SO_4) + 6H_2O$	351mg/100ml
Na_2 EDTA	300mg/500ml
P11 Trace metals (Stock G)	
H_2BO_3	1.14g/l
$FeO_3 + 6H_2O$	49mg/l
$MnSO_4 + 4H_2O$	164mg/l
$Zn\ SO_4 + 7H_2O$	22 mg/l
$Ca\ SO_4 + 7H_2O$	4.8 mg/l
Na EDTA	1g/l

Seawater is sterilized by filtration or autoclaving, enrichments assembled into a single solution, and added aseptically to the medium. Mix 10 ml of each stock solution A - E and 250 ml of each stock solution F and G and bring total volume to 1250 ml with distilled or deionized water. Add 20 ml of the above stock solution mixture to 1000 ml of filtered seawater to prepare full - strength medium.

Source: Provasoli, L. (1968). Media and prospects for cultivation of marine algae. In Cultures and Collections of Algae (Watanabe, A. & Hattori, A., editors), 47 - 74. Japanese Society of Plant Physiology, Tokyo.

Chapter 6: Paper 5

Comparative analysis of nutrient utilization and proximate composition of *Ulva armoricana* (Chlorophyta) and formulated feeds on the growth performance of African Mudcatfish *Clarias gariepinus* (Burchell) fingerlings

This chapter is based on:

Amosu A.O., Hammed A.M., Maneveldt G.W. and Robertson-Andersson D.V. (under revision). Comparative analysis of nutrient utilization and proximate composition of *Ulva armoricana* (Chlorophyta) and formulated feeds on the growth performance of African Mudcatfish *Clarias gariepinus* (Burchell) fingerlings. *Animal Nutrition and Feed Technology*.

Abstract

We evaluated the effects of feed fortification with different nitrogen content of *Ulva armoricana* on growth, nutrient utilization and proximate composition in *Clarias gariepinus* fingerlings using cultivated macroalgae as a protein substitute and feeding attractant. The fish were monitored in indoor hatchery for 21 days. The treatments were: {Commercial FeedX}, {35% crude protein diet (CD) + non enriched *Ulva* (NEU) (CD + NEU)}, {CD+ enriched *Ulva* (EU) (CD +EU)} and {Control CD)}. The fish grew well and utilized *Ulva* enriched diet (CD + NEU) with no losses iin the weight gain, % weight gain, specific growth rates and nitrogen metabolism, compared with the other diets. There was significant difference ($p < 0.05$) in the food conversion ratio (FCR) and gross food conversion ratio (gFCR) across the experimental diets. The best FCR was noticed on fish fed diet CD (0.79 ± 2.39) followed by fish fed diet CD +EU (1.75 ± 1.34) indicating that the experimental fish utilized the enriched (CD +EU) diet better than non-enriched (CD + NEU) and the FeedX diets. This suggests that *Ulva armoricana* has the potential to be a successful feed stimulant if the diets include macroalgae grown with enriched nutrient.

***Key words*:** Aquaculture, *Clarias gariepinus*, diet, growth, macroalgae, *Ulva* s *armoricana*

Introduction

Aquaculture production has been sustained and rapid, between 8.8 - 10 %.a^{-1} per annum for over thirty years, while the take from wild fisheries has been essentially flat for the last decade (FAO, 2012a; 2014). Over 300 aquatic spp are farmed worldwide for production in a variety of facilities of varying input intensities and technological sophistication, using fresh, brackish and marine water (FAO 2014). Aquaculture remains one of the fastest-growing agro-industrial activities in the last four decades and is projected to outpace population growth (OECD/FAO 2013). In fact, it is estimated that the worldwide requirement for food will increase up to 50 % by 2030 (Tidwell and Allen, 2001). The most prominent species in aquaculture include: finfish, crustaceans and molluscs (FAO, 2014). Finfish in particular has come under attack by environmentalists for its reliance on fishmeal and the wild fisheries and is seen as being unsustainable (Naylor *et al.*, 2000; 2009; FAO 2012b; FUS 2012; Waite, *et al.*, 2014). As the global population continues to rise and the demand for aquaculture products rises, the need for sustainable, alternative sources of fish protein also increases.

Protein is the most expensive constituent of fish feed and global expenditure exceeds (7.05 million MT) €1bn per annum (Hardy and Tacon, 2002; Hardy 2006; WOR 2013). In 2006 a global survey put aquaculture consumption of fishmeal at 3724 thousand tonnes (Tacon and Metian 2008). Fishmeal is a high-protein animal feed used extensively in aquaculture but uses wild fish stocks to feed farmed fish and is an unsustainable feed resource. The ability of fishmeal supply to meet future demand is a massive global concern – especially given that aquaculture production is growing at a rate of nearly 10 % per annum (FAO, 2009; 2012a; 2013). As natural wild fish stocks decline, the aquaculture industry faces a massive challenge to identify cost-effective and environmentally-friendly alternatives to fishmeal on which it is so heavily reliant. Macroalgae protein has the potential to provide a solution to this problem as it is relatively underexploited, contains high amounts of protein and can be cultured in a sustainable, environmentally-friendly manner. Comparisons of production input efficiencies of aquaculture versus several fisheries and terrestrial agriculture systems shows that non-fed aquaculture (macroalgae; molluscs) are among the world's most efficient mass producer of proteins (Costa –Pierce and Page, 2010; Costa-Pierce *et al*., 2011). Certainly, the nutritional properties of macroalgae would make good candidates to serve as alternatives to fishmeal in fish/livestock feeds, with some macroalgae having protein levels as high as 47 % and considerable differences exist in the protein content of brown, green and red macroalgae (Samarakoon and Jeon 2012). The functional biological properties of macroalgae protein make it an excellent candidate for a natural, sustainable alternative to fishmeal in aquaculture (Henry 2012). Macroalgae provide the deficient amino acids in fishmeal and most terrestrial crop such as lysine, methionine, threonine, and tryptophan (Li *et al*., 2008; Henry 2012), whereas analyses of the amino acid content of numerous algae have found that although there is significant variation, they generally contain all the essential amino acids (Rosell and Srivastava 1985; Wong and Peter 2000; Lourenço *et al*., 2002; Ortiz *et al*., 2006; Dawczynski *et al*., 2007).

In South Africa, macroalgae have been used commercially as feedstock for phycocolloid production and the production of abalone feed (Troell *et al*., 2006). Its aquaculture started as an off-shoot of the abalone (*Haliotis midae* L) farming industry, (Troell *et al*., 2006). The capacity for large-scale production of macroalgae is feasible in South Africa (Amosu *et al*., 2014; Robertson-Andersson *et al*., 2014) , together with the high-quality macroalgae protein feeds developed as Abfeed™ and Midae Meal™ further enhances the future potential of macroalgae (Anderson *et al.*, 1989; 2003; Robertson-Andersson *et al*., 2006; Troell, *et al*., 2006).

The green macroalgae, *Ulva armoricana* L., (kandjengo and Bolton, unpubl.; Bolton *et al*., 2013; Cyrus *et al.*, 2014a) has been fed to abalone and feeding trials showed that abalone growth is greatly improved by high protein content, and this is attained by culturing the macroalgae with high levels of ammonia present (Naidoo *et al*., 2006; Robertson-Andersson *et al*., 2011; Henry 2012). The growth of *U. armoricana* and its culture within farming systems has been well studied and documented (Neori *et al*., 1998; 2004; Shpigel *et al*., 1999; Robertson-Andersson, 2003; Robertson-Andersson *et al.*, 2007). In particular, this macroalgae has a nutrient uptake capacity that is one of the highest known among macroalgae (Shpigel *et al*., 1999; Neori *et al*., 2004). Nutrient enriched *U. lactuca* have been shown to have a higher protein content of 36 – 44 % as opposed to their wild counterparts that have a protein content of only 3 – 24 % (Neori and Shpigel, 1999; Shpigel *et al*., 1999; Robertson-Andersson, 2003; Robertson-Andersson *et al*., 2007).

There are reservoirs of knowledge on the macroalgae utilization in integrated multi-trophic aquaculture (IMTA), where fish/shellfish and macroalgae are all grown together in an ecologically-based aquaculture farm design (see for examples - Bolton *et al*., 2006; Buschmann *et al*., 2007; Chopin *et al*., 2001; Neori *et al*., 2004; Troell *et al*., 2009; Kangmin 2012). Macroalgae can be used as a feed ingredient: to reduce cost and to increase the growth and survival in animal production. However, information is scarce on the effects of nutrient-enriched macroalgae diets on the growth of carnivorous fishes. Additionally, little is known on comparing seawater cultured or non-nutrient enriched macroalgae with nutrient enriched marcroalgae when fed used in feed. Work on the south African abalone and the sea urchin *Tripneustes gratilla* L. with (*Ulva* spp) and Abfeed® indicated that protein enriched macro algae out performed wild or non-nutrient enriched algae both in a whole or compound feed diet (Naidoo *et al*., 2006; Francis *et al*., 2008; Robertson-Andersson *et al*., 2011; Cyrus *et al*., 2014a;b). Several other research works on *Ulva* include *Ulva* fed at 5 to 15 % meal inclusion in diets to Carp, Channa, Tilapia, Shrimp, Sea Bream, European Sea Bass, Striped Mullet, Gilthead Sea Bream, Sea urchin (Nakagawa *et al*., 1987; Hashim and Mat Saat 1992; Hashim and Hassan, 1995; Mustafa *et al*., 1995; Mustafa and Nakagawa 1995; Wassef *et al*., 2001; Wassef *et al*., 2005; Valente *et al*., 2006; Diler *et al*., 2007; Soyutu *et al*., 2009; Cruz-Suarez *et al*., 2010; Azad and Xiang 2012; Cyrus *et al*., 2014) revealed significant benefit in herbivores like molluscs and echinoderms. The aims of this present study was to use an African ecotype cat fish to determine the efficacy of nutrient utilization and proximate composition of macroalgae based and formulated feeds on African mud catfish *Clarias gariepinus* (Burchell) fingerlings.

Materials and Methods

Macroalgae production

Initial production stock of *Ulva armoricana* was collected from Irvine & Johnson (I&J) (34^{o}34′60 S; 19^{o}21′0 E) Cape Abalone farm. Macroalgae production experiments were carried out during winter (Southern Hemisphere) at Benguela Abalone Group (32°54'24" S; 17°59'17" E) on the West Coast of South Africa in four 32 m X 8 m (180 m^3) concrete paddle ponds, filled to approximately 0.55 m depth with unfiltered seawater on a flow through system. Ponds received 2 volume exchanges per day. Initial biomass of 500 kg/*Ulva* spp were stocked in each pond and growth rates were measured every 21 days (~3 weeks) for a period of 3 months. The stocked *Ulva* spp in ponds A and B were fertilized (every 7 days in order to allow assimilation) with a mixture of (10:16:0) Maxipos® and Ammonium sulphite at 100g/kg providing both nitrogen and phosphorous respectively. Fertilization was carried out in the evenings with the incoming water turned off and the paddle wheel remaining in motion. The set up used were characterized as follows:

- Pond A - Non enriched (*Ulva* spp) in standard seawater and
- Pond B - Enriched (*Ulva* spp) added to improve growth (triple fertilizer ratio)

About 5kg of *Ulva* from each pond were oven dry at 50°C for 4 days to prepare them for inclusion in the experimental diets.

Experimental fish

Clarias gariepinus (Burchell) is a freshwater species of air breathing catfish from the family Clariide. The fish is found throughout Africa and the Middle East and is widely cultivated in Africa. They are carnivorous, feeding on a wide variety of prey items, from zooplankton, to small crustaceans to other fish. On commercial systems they have been habituated to an ominivorous feeding behavior. The species is very hardy and can tolerate both well and poorly oxygenated water (Hammed *et al*., 2013a).

Preparation of the experimental fish samples

240 healthy fingerlings of *Clarias gariepinus* were purchased from Daplay fish farm in Iba area of Lagos, transported in aerated plastic bags within 10 minutes to the fish hatchery unit of the Department of Fisheries, Lagos State University, Nigeria. The experiment was conducted in line with Boyd (1998) pond aquaculture water quality management guidelines and practice.

Feed composition, formulation and feeding trials

The control feed (CD) (Table 3) with 35 % crude protein level (fish meal, soybean meal, white maize, palm oil, Ca_2So_4 and premix) was formulated using the Pearson's square method as described Wagner and Stanton, (2006) and reported by Hammed *et al*. (2013a, b). Four different experimental diets were used in (Table 1) in this research work. The differences in feeds composition were chosen to test a commercial feed against the control and then an enrich *Ulva* feed against a non enriched feed against the control and commercial feed. FeedX is a commercial feed (a fish meal based feed, soya bean, starch, vitamin & minerals with some kelp and about 1% *Ulva*) available in South Africa used in feeding cultivated abalone. CD + NEU comprise the control diet (10% inclusion) and non-enriched *Ulva*. CD + EU contain the control diet (10% inclusion) and enriched *Ulva*. Preparations of the diets were based on the description by Royes and Chapman (2012).

A total of 20 fingerlings of *Clarias gariepinus* with average weight 1.60 ± 0.05g and standard length of 4.50 ± 0.07cm were used in each of four treatments and three replicates were weighed and stocked in $5000m^3$ (50 L) culturing bowl between the period of June and July 2013. Prior to feeding trials, all fish were starved for 24 hours to ensure that their guts were emptied. The feed was administered twice (per day at 08:30 and 17: 30 hr) daily at 4 % body weight. Weight changes were measured every 3 days and the feeding rate adjusted accordingly to accommodate the change (Hogendoorn and Koops, 1983). *In situ* parameters, such as water temperature (^oC), dissolved oxygen (mg/ L), and pH were measured with multi-probe analyser Hanna HI 9828.

Table 1: Gross composition of the experimental diets.

Ingredient (%)	Treatments			
	FeedX	CD + NEU	CD + EU	CD
Fish meal	NR	28.56	28.56	31.415
Soybean meal	NR	28.56	28.56	31.415
White maize	-	27.88	27.88	30.670
Palm oil	-	4.5	4.5	5
Ca_2So_4	-	0.45	0.45	0.5
Premix	-	1	1	1
FeedX	100	0	0	0
NEU spp	0	9.09	0	0
EU spp	0	0	9.09	0
Total	**100**	**100**	**100**	**100**

FeedX (Commercial feed), NEU (Non-enriched *Ulva*), EU (Enriched *Ulva*), CD (Control diet: 35% CP), NR (Not revealed)

Chemical evaluation of the experimental feed and fish

Dry, milled *Ulva* samples of experimental diets and fingerlings were analyzed for proximate composition according to the methods of Animal Science Laboratory, Institute for Animal Production, Department of Agriculture, Forestry and Fisheries; Elsenberg, Western Cape Province, South Africa for Ash, Carbohydrate, Crude Fat, Crude Fibre, Crude Protein, Gross Energy (Mj/ kg), Nitrogen (N), Nitrogen Free Extract (NFE), Total Digestible Nutrients (TDN) and Moisture.

Determination of growth and nutrient utilization

The evaluation of experimental diets for growth and nutrient utilization was carried out using growth indices such as: Weight gain (WG), percentage weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), gross feed conversion ratio (GFCR) and Nitrogen metabolism (Nm) as described by Fashina-Bombata *et al.* (2010), Hammed (2012) and Hammed *et al*. (2013a; b).

Determination of weight gain (WG)

The weight gained by the fish was calculated weekly from the differences between the final mean weight and the initial mean weight.

WG = $(W_2) – (W_1)$

Where:

W_2 = Final mean body weight (g)

W_1 = Initial mean body weight (g)

Percentage weight gain (%WG)

The Percentage weight gain was calculated using the formula:

% weight gain = $(X_2) – (X_1) \times 100 / (X_1)$

Where:

X_2 = Final mean body weight (g)

X_1 = Initial mean body weight (g)

Specific growth rate (SGR)

SGR: an instantaneous growth (% /day) was calculated as:

$$SGR = Log_e W_2 – Log_e W_1 / T_2 – T_1$$

Where:

W_2 = Weight of fish at time T_2 in days

W_1 = Weight of fish at time T_1 in days

Log_e = Natural log of base e

Feed Conversion Ratio (FCR)

The food conversion ratio (FCR) is expressed as the proportion of dry food fed per unit live weight gain of fish:

$$\text{FCR} = \text{Weight of dry fed (g)} / \text{Live weight gain (g)}$$

Gross Feed Conversion Ratio (GFCR)

The gross feed conversion ratio was calculated as percentage of the reciprocal of feed conversion ratio:

$$\text{GFCR} = 1 \times 100 / \text{FCR}$$

Nitrogen metabolism (Nm)

This was calculated as:

$$\text{Nm} = (0.54)\,(b - a)\,h / 2$$

Where:

a = Initial weight of fish (g)

b = Final weight of fish (g)

h = Experimental period in days

0.54 = Experimental constant

Statistical Analysis

All data collected were analysed for significant differences ($P>0.05$) (ANOVA) on Graph Pad Prism V. The results were expressed as mean ± SD. Determined differences among treatments were partitioned by the Least Significant Difference (LSD) and the Duncan's New Multiple Range Test (DNMRT) (Duncan, 1955).

Results

The water quality parameters in all the culture units were within the range of 23 – 24 ^{o}C, 6.5 - 8.5 mg.l^{-1}, 0.60 – 0.27 mg.l^{-1} and 7.8 - 8.4 for temperature, dissolved oxygen, ammonia and pH respectively. *Ulva* biomass differed substantially among the two treatments from pond A to B. The lowest value was recorded in the standard seawater, which contained no fertilizer and also produced the least biomass (1045 ± 32.5 kg) with a 113 % increase at harvest. An increase in weight gain was seen with reference to fertilizer increase from one pond to another, with a higher weight (1235 ± 162.6 kg) being observed in the triple fertilizer experience 134 % increase. Growth rates differed substantially in pond (B) as a result of the weekly fertilization. The proximate composition (per g dry matter) of the initial stock of *Ulva* (Pond A & B) collected for the production of the different biomass incorporated in the diet composition (crude protein 18.310, ash 32.660, crude fibre 6.024, crude fat 0.380, nitrogen free extract 30.259, P 0.172, K 1.897, Ca 1.034, Mg 4.310, Na 5.172, Fe 0.007, Cu 0.001, Zn 0.001, Mn 0.001, Br 0.006, Al 0.006 %) is presented in Table 2. The highest mean weight gain (Table 3) was observed in fish fed diet 3 CD +EU (0.56 ± 0.22) and the lowest in diet 1 Feed X (0.13 ± 0.05). There was a significant difference ($P>0.05$) in the weight gain of fish fed diet 1 compared to fingerlings fed diets 2, 3, 4. The result (Table 4) also shows performance of experimental diets on tested fish. There was no significant difference ($p >$

0.05) amongst the weight gain, % weight gain, specific growth rates and nitrogen metabolism of diets 2, 3 and 4 while significant difference ($p < 0.05$) was noticed in the food conversion ratio and gross food conversion ratio among the fish across the different experimental diets. The best of the FCR was noticed on fish fed diet 4 (0.79±2.39) followed by fish fed diet 3 (1.75±1.34) indicating that the experimental fish was able to utilize control + non-enriched *Ulva* diet better than enriched as well as the commercial FeedX diets.

Table 2: Proximate composition (per g dry matter) of the *Ulva* before cultivated in enriched water.

Nutrients composition	**% dry matter**
Crude protein	18.310
Ash	32.660
Crude fibre	6.024
Crude fat	0.380
Nitrogen free extract (calc)	30.259
Phosphorus (P)	0.172
Potassium (K)	1.897
Calcium (Ca)	1.034
Magnesium (Mg)	4.310
Sodium (Na)	5.172
Iron (Fe)	0.007
Copper (Cu)	0.001
Zinc (Zn)	0.001
Manganese (Mn)	0.001
Bromine (Br)	0.006
Aluminium (Al)	0.006

Table 3: Proximate composition of the formulated feed, macroalgal supplemented diets including all-macroalgae-based commercially formulated FeedX and *Clarias gariepinus* fingerlings used in this study.

Proximate composition of the experimental feeds											
Feeds	Moisture	Dry matter (%)	Ash (%)	Protein (%)	Fibre (%)	Fat (%)	N (%)	TDN (%)	NFE (%)	Gross Energy Mj/ kg	Carbohydrate (%)
FeedX	~10	NA	32.9	19.2	10.9	0.7	NA	NA	NA	NA	47.30
CD + NEU	~10	91.02	10.22	42.03	3.55	Nil	6.73	66.74	35.21	17.81	47.75
CD + EU	~10	92.72	10.89	44.03	3.25	Nil	7.05	68.52	35.55	18.38	45.08
CD	~10	93.49	9.90	45.38	2.70	Nil	7.05	69.37	35.55	18.40	44.72

Proximate composition of *Clarias gariepinus* fingerlings fed with experimental diets						
Fish Fed Diet	Dry matter (%)	Ash (%)	Protein (%)	Fat (%)	N (%)	Gross Energy Mj/ kg
FeedX	91.50	17.66	63.44	Nil	10.15	19.08
CD + NEU	86.60	12.44	65.63	Nil	10.50	17.95
CD + EU	91.45	13.11	65.00	Nil	10.40	20.31
CD	89.24	13.86	68..13	Nil	10.90	17.77

Key: NA= Not Analysed, Nil= Negligible

Table 4: Cumulative growth rate of *Clarias gariepinus* fingerlings fed with different diet composition

	Diet composition			
Growth indices	Commercial FeedX	*Ulva armoricana* –non enriched CD + NEU	*Ulva armoricana* – enriched CD + EU	Control - 35% Crude protein diet CD
Mean body weight g	2.40±0.28^{a}	3.72±1.21^{b}	3.88±1.36^{b}	3.90±1.22^{b}
Weight gain g	0.13±0.05^{a}	0.49±0.17^{b}	0.56±0.22^{b}	0.52±0.2 3^{b}
% weight gain	5.46±1.72a^{a}	17.35±8.83^{b}	18.25±5.6^{b}	18.72±7.80^{b}
Specific Growth Rate	0.32±0.07^{a}	0.12±0.06^{b}	0.09±0.06^{b}	0.11±0.08^{b}
Food Conversion Ratio	8.18±3.08^{a}	3.23±2.17^{b}	1.75±1.34^{c}	0.79±2.39^{c}
Gross Feed Conversion Ratio	6.86±1.7 8^{a}	14.96±8.30^{b}	41.60±24.39^{c}	62.72±21.68^{c}
Nitrogen metabolism	0.11±0.07^{a}	0.40±0.67^{b}	0.45±0.55^{b}	0.42±0.75^{b}

Measurements in the same row with the same superscript are not significantly different ($p > 0.05$). Data are presented as means ± Standard Error.

Discussion

The reason for insignificant difference (Table 4) between the *Ulva* supplemented diets (diet 2 & 3) could be due to quality decline in the cultured macroalgae after double fertiliser ratio as revealed in our previous work (Amosu *et al*., 2014). However, the effect of supplementing the formulated feeds with macroalgae significantly improved the growth of all *Clarias gariepinus* fingerlings. This was on account of the contribution of macroalgae active ingredient to the nutrients that may have been lacking in the commercial FeedX. Although proximate composition of macroalgae typically have low protein content but have been shown to be valuable supplementary feeds that promote good growth in aquaculture animals (Stepto and Cook 1993; Simpson 1994; Fleming *et al*., 1996; Robertson-Andersson 2003; Schoenhoff *et al*., 2003; Najmudeen and Victor 2004; Johnston *et al*., 2005; Dlaza *et al*., 2008). However, the relatively high nutritional value of the formulated feed (control) and supplemented feeds probably accounted for the enhanced growth recorded with the supplemented diets. The all-macroalgae-based commercially formulated feed (FeedX) performed extremely poorly compared to others. The major nutritional requirements for optimum growth in fish include the necessary carbohydrate and protein ratios (Fleming *et al*., 1996; Guzmán and Viana 1998; Nelson *et al*., 2002). Whereas the carbohydrate content of the best performing formulated feed (Control, 44.72 %) and the worst performing Commercial FeedX (47.30 %) are both within the optimal range for fish requirements (Tacon 1987), differences in their performance may be explained by the differences in their crude protein contents. Although the optimum dietary crude protein requirements of Clarias *gariepinus* was reported to be about 35 – 45 % (Tacon, 1987),finding shows tha not only did Commercial FeedX (19.2 %) have a substantially lower crude protein content than (control) locally formulated feed (45.38 %), but also animal-based or animal-supplemented proteins were more readily digested than plant-based proteins (Durazo-Beltrán *et al*., 2003). Commercial FeedX consists of dried macroalgae and seal meal, and it has been shown that fish fed on dry macroalgae only grow poorly like other aquatic animals (Britz 1996; Naidoo *et al*., 2006). Originally, FeedX contains kelp which is naturally low in protein (Troell *et al.*, 2006). The protein and carbohydrate contents of the locally formulated feed supplemented *Ulva* feeds were relatively similar to those of locally formulated feed, so those factors could not have accounted for the poorer growth obtained with the locally formulated feeds supplemented with *Ulva*. In addition to the low protein content, a low palatability could also have accounted for the poor growth obtained with commercial FeedX (figure 1). When the culturing bowls were cleaned, more uneaten remnant of the commercial FeedX were found relative to other feeds, indicating that the fish fingerlings consumed less of the feed. This reason may be due to low palatability as a result of other active inhibiting substance in the commercial FeedX. It has been reported that less palatable feeds become wasted, and because feed intake is low, slower growth rates are achieved (Kautsky *et al*., 2001; Lee *et al*., 2004). The probable lower palatability of commercial FeedX could have been on account of the absence, or reduced effects, of attractants that may otherwise be active in locally formulated feed (Fleming *et al*., 1996; Sales and Janssens 2004; Dlaza *et al*., 2008).

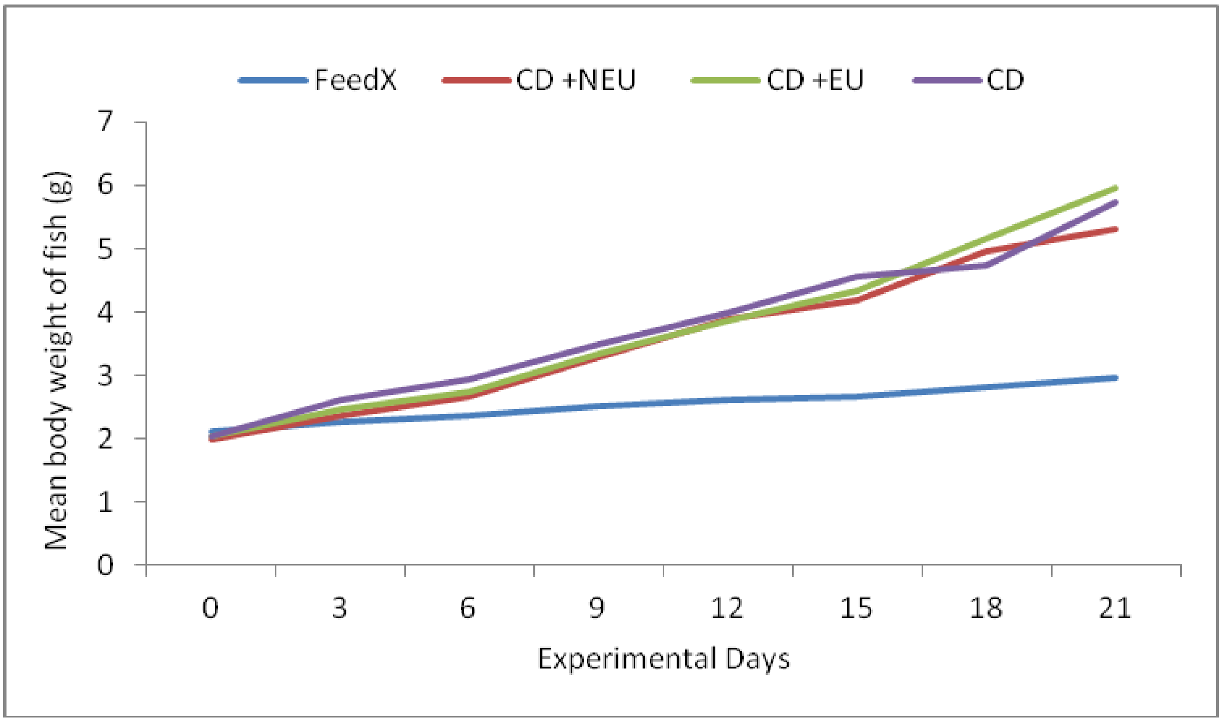

Figure 1: Growth response of *C. gariepinus* fingerlingsto the different feed formulations.

Similar to our findings, there is information that shows that a mixed diet of macroalgae can induce growth rates in aquatic animals that meet or exceed those attained with artificial feeds (Naidoo *et al*., 2006; Dlaza *et al*., 2008; Francis *et al*., 2008; Robertson-Andersson *et al*., 2011). This implies, a natural diet can improve livestock quality and reduce parasite infections as reported by Robertson-Andersson, 2003; Naidoo *et al*., 2006; Al-Hafedh *et al*., 2012. Therefore, combine formulation of different *Ulva* additive that was incorporated into the experimental diets (Table 3), suggests that protein enriched or fertilized *Ulva* spp has the potential to be a successful fin fish feed on African fish farms (Table 4).

In addition to Urchin diets, *Ulva* has proven beneficial as an additive in aquatic feeds (Cyrus *et al*., 2014), *U. rigida* as a supplementary diet for Carp *Cyprinus carpio*. L and *Oreochromis niloticus*, showed peak growth performance with a 5 – 15 % diet inclusion (Diler *et al*., 2007;

Ergun *et al*., 2009). This negates the research by Sherrington (2013) that *Ulva* is more beneficial for algivorous fish species than carnivorous fish as experienced in this present studies with *Clarias gariepinus*. However, further dietary investigation is required for fresh and marine water farmed finfish species because most cultured aquactic fish species in Africa are fresh water fishes compared to the more expensive mariculture systems. The overall economic benefits is similar to other research findings (e.g. Robertson-Andersson, 2003; Bolton *et al*., 2008; Robertson-Andersson *et al.*, 2008; Cruz-Suarez *et al.*, 2010), that have identified the many direct and indirect economic benefits of integrating *Ulva* spp as an inorganic extractive component and direct feed for shellfish like abalone (*Haliotis midae,* L) and shrimp (*Litopenaeus vannamei*). This is an ecosystem-based integration approach that effectively mitigates the excessive amounts of nutrients that may lead to fish kill, but also significantly reduce their green house gas emissions and thus their carbon footprints. The highest food conversion ratio (8.18±3.08) was observed and recorded in diet 1 containing (FeedX **-** fishmeal based with some kelp and about 1% *Ulva*) and the lowest food conversion ratio (0.79±2.39) recorded in diet 4 containing (Control diet: 35% Crude Protein) at the end of the experiment. This shows that fish fed diet 4 (control 35% Crude protein diet) converted the diet well with good performance.

Conclusion

Our results support the idea that animal based protein feeds yield better growth rates than all-macroalgae based protein feeds (FeedX). The locally formulated feed outperformed all other formulated feeds tested in this experiment, and supports that it is a good formulated feed for cat fish (*Clarias gariepinus*) fingerlings. The formulated feed contains fish meal that supplies the surphure (lysine and methionine) amino acid lacking in plant protein. Also, supplementing the formulated feeds with enriched and non-enriched *Ulva* spp resulted in better growth due to the inclusion of fish meal. Our study shows the benefits of supplementing existing formulated feeds with *Ulva* spp combinations for *Clarias gariepinus* fingerlings. In this present study our findings suggest that plant protein *Ulva armoricana* supplemented diet has the potential to be a successful feed stimulant.

Acknowledgement

The authors are grateful to the Department of Biodiversity and Conservation Biology at the University of the Western Cape, Cape Town, South Africa. Also we appreciate Benguela Abalone Group, I & J Cape Cultured Abalone and Wild Coast Abalone on the West Coast of South Africa, The hatchery unit of the Department of Fisheries at the Lagos State University, Lagos, Nigeria for facilities provided during this research. Also we thank Robert J Anderson, Department of Agriculture, Forestry and Fisheries (DAFF), Seaweed Unit, Marine and Coastal Management, University of Cape Town, Cape Town, South Africa.

References

Abd El-Baky, H.H., El-Baz, F.K. and El-Baroty, G.S. 2008. Evaluation of marine alga *Ulva lactuca L*. as a source of natural preservative ingredient. *American-Eurasian Journal of Agriculture and environmental Science*, 3(3): 434-444.

Al-Hafedh, Y.S., Alam, A., Buschmann, A.H. and Fitzsimmons, K.M. 2012. Experiments on an integrated aquaculture system (seaweeds and marine fish) on the Red Sea coast of Saudi Arabia: efficiency comparison of two local seaweed species for nutrient biofiltration and production. *Reviews in Aquaculture*, 4: 21-31.

Amosu, A.O., Robertson-Andersson, D.V., Kean, E. and Maneveldt, G.W. 2014. Aquaculture Benefits of Macroalgae for Green energy Production and Climate change Mitigation. *International Journal of Scientific & Engineering Research*, 5(7): 147-152.

Anderson, R.J., Simons, R.H. and Jarman, N.G. 1989. Commercial seaweeds in southern Africa: A review of utilization and research. *South African Journal of Marine Science*, 8: 277-299.

Anderson, R.J., Bolton, J.J., Molloy, F.J. and Rotmann, K.W. 2003. Commercial seaweeds in Southern Africa. In: Chapman A.R.O., Anderson R.J., Vreeland V.J., Davidson I. (eds.) *Proceedings of the 17th International Seaweed Symposium*. Oxford University Press, Oxford, pp. 1-12.

Azad, S.A. and Xiang, T.Z. 2012. Suitability of Seaweed Meals Incorporated With Rhodovulum Sp. Bacterium as Feed Supplement for Finfish Larvae. *Science*, 30: 57-64.

Bolton, J.J., Brand, M., Cyrus, M., Joubert, M. and Macey, B. 2013. Why grow *Ulva*?: a review. *11th Conference of the Aquaculture Association of Southern Africa Aquaculture, AQUACULTURE CONFERENCE 2013*. 9-13 September 2013, Stellenbosch, South Africa.

Bolton, J.J., Robertson-Andersson, D.V., Troell, M. and Halling C. 2006. Integrated systems incorporate seaweeds in South African abalone culture. *Global Aquaculture Advocate*, 9: 54-55.

Boyd, C.E. 1998. Pond Aquaculture Water Quality Management. *Aquaculture Series* (*Chapman & Hall Aquaculture Series*). ed. by C.S Tucker. Springer. pp.700.

Britz, P.J. 1996a. Effect of dietary protein level on growth performance of South African abalone *Haliotis midae*, fed fishmeal based semi-purified diets. *Aquaculture*, 140: 55-61.

Britz, P.J. 1996b. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. *Aquaculture*, 140: 63-73.

Buschmann, A.H., Varela, D.A., Hernández-González, M.C., Henríquez, L., Correa, J., Flores, R. and Gutierrez, A. 2007. The development of an integrated multi-trophic activity in Chile: the importance of seaweeds. *Aquaculture 2007 conference proceedings*, World Aquaculture Society. pp.136. Retrieved from https://www.was.org/Meetings/AbstractData.asp?AbstractId=14199

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-Gonzalez, J.A., Yarish, C. and Neefus, C. 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycolog*, 37: 975-986.

Costa-Pierce, B.A. and Page, G.G. 2010. Sustainability science in aquaculture. In: Costa - Pierce, B.A. (ed.), Ocean Farming and Sustainable Aquaculture Science and Technology .Encyclopedia of Sustainability Science and Technology. Springer Science, N.Y.

Costa-Pierce, B.A., Bartley, D.M., Hasan, M., Yusoff, F., Kaushik, S.J., Rana, K., Lemos, D., Bueno, P. and Yakupitiyage, A. 2011. Responsible use of resources for sustainable aquaculture. *Global Conference on Aquaculture 2010*, Sept. 22-25, 2010, Phuket, Thailand. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Cruz-Suárez, L.E., Leon, A., Pena-Rodriguez, A., Rodriguez-Pena, G., Moll, B. and Ricque-Marie, D. 2010. Shrimp/*Ulva* co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality. *Aquaculture*, 301(1-4): 64-68.

Cyrus, M.D., Bolton, J.J., Scholtz, R. and Macey, B.M. 2014a. The advantages of Ulva (Chlorophyta) as an additive in sea urchin formulated feeds: effects on palatability, consumption and digestibility. *Aquaculture Nutrition*, 21(5): 1-14.

Cyrus, M.D., Bolton, J.J., De Wet, L. and Macey, B.M. 2014b. The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla* (Linnaeus). *Aquaculture Research*, 45: 159-176.

Diler, I., Tekinay, A., Guroy, D., Guroy, B.K. and Soyuturk, M. 2007. Effect of *Ulva rigida* on the growth, feed intake, and body composition of Common Carp, *Cyprinus carpio* L. *Journal of Biological Sciences*, 7(2): 305-308.

Dlaza, T.S., Maneveldt, G.W. and Viljoen, C. 2008. The growth of post-weaning abalone (*Haliotis midae* Linnaeus) fed commercially available formulated feeds supplemented with fresh wild seaweed. *African Journal of Marine Science*, 30(1): 199-203.

Duncan, D.B. 1955. Multiple Range and Fultiple F Test. *Biometric*, 11: 1-42.

Durazo-Beltrán, E., D'Abramo, L.R., Toro-Vazquez, J.F., Vasquez-Peláez, C. and Viana, M.T. 2003. Effects of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). *Aquaculture*, 224: 257-270.

Ergun, S., Soyutu, M., Guroy, D. and Merrifield, D. 2009. Influence of *Ulva* meal on growth, feed utilization, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid. *Aquaculture International*, 17: 355-361.

F.A.O. 2009. Feeding the world in 2050 in world summit on food security. Natural Resources Management and Environment Department, Food and Agriculture Organization of the

United Nations, Viale delle Terme di Caracalla, Rome, Italy. Retrieved from http://food2025.ucanr.edu/files/160428.pdf

F.A.O. 2012a. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FishStatPlus. Universal Software for fishery statistical time series. Version 2.3 in 2000. Retrieved from http://www.fao.org/docrep/013/i1820e/i1820e00.

F.A.O. 2012b. The State of World Fisheries and Aquaculture - 2012. Food and Agriculture Organization of the United Nations (FAO), Fisheries and Aquaculture Department. Rome. Italy.

F.A.O. 2014. OECD and FAO see lower farm prices - livestock and biofuels outpacing crop production. The OECD-FAO Agricultural Outlook 2014-2023, FAO, Rome, Italy. Retrived from http://www.fao.org/news/story/en/item/238638/icode/

Fisheries of the United States - 2012. National Oceanic & Atmospheric Administration; Fisheries Statistics Division; National Marine Fisheries Service. 2013.

Fashina-Bombata, H.A. and Hammed, A.M. 2010. Determination of Nutrient Requirements of an Ecotype Cichlid of Epe Lagoon, Southwest Nigeria. *Global Journal of Agricultural Sciences*, 9(2): 57-61.

Fleming, A.E., Van Barneveld, R.J. and Hone, P.W. 1996. The development of artificial diets for abalone: a review and future directions. *Aquaculture*, 140: 5-53.

Francis, T.L., Maneveldt, G.W. and Venter, J. 2008. Determining the most appropriate feeding regime for the South African abalone *Haliotis midae* Linnaeus grown on kelp. *Journal of Applied Phycology*, 20: 597-602.

Guzmán, J.M. and Viana, M.T. 1998. Growth of abalone *Haliotis fulgens* fed diets with and without fishmeal, compared to a commercial diet. *Aquaculture*, 165: 321-331.

Hammed, A.M. 2012. Aspects of Biology, Biochemical Characterization and Nutrient Requirements of a Cichlid species ‘Wesafu’ from Epe Lagoon, Lagos, Nigeria. PhD.

Thesis Dissertation, Lagos State University, Nigeria.

Hammed, A.M., Amosu, A.O. and Fashina-Bombata, H.A. 2013a. Effect of partial and total replacement of Soybean meal with Pigeon pea (*Cajanus cajan*) as alternative plant protein source in the diet of juveniles African Mud catfish (*Clarias gariepinus* (Burchell, 1822). *International Journal of Food Technology Photon*, 105: 139-145.

Hammed, A.M., Fashina-Bombata, H.A. and Olowu, R.A. 2013b. Growth response and survival rate of *Clarias gariepinus* fingerlings exposed to varying metal concentrations. *Pacific Journal of Science and Technology*, 14(1): 430-438.

Hardy, R.W. 2006. Worldwide Fish Meal Production Outlook and the Use of Alternative Protein Meals for Aquaculture. En: E ditores: L. Elizabeth Cruz Suárez, Denis Ricque Marie, Mireya Tapia Salazar, Martha G. Nieto López, David A. Villarreal Cavazos, Ana C. Pue llo Cruzy Armando García Ortega. Avances en Nutrición Acuícola VIII. VIII Simposium Internacional de Nutrición Acuícola. 15-17 Noviembre. Universidad Autónoma de Nuevo León, Monte rrey, Nuevo León, México. ISBN 970-694-333-5.

Hardy, R.W. and Tacon, A.G.J. 2002. Fish meal historical uses, production trends and future outlook for sustainable supplies. In R.R. Stickney (Ed.), Sustainable Aquaculture New York: CABI.

Hashim, R. and Hassan, H.N. 1995. The use of varying level of *Ulva* sp. meal as binders for practical and their effect on growth of snakehead (*Channa striatus*) fry. *Journal of Bioscience,* 6: 123-131.

Hashim, R. and Mat Saat, M.A. 1992. The utilization of seaweed meals as binding agents in pelleted feeds for snakehead (*Channa striatus*) fry and their effects on growth. *Aquaculture*, 108: 299-308.

Henry, E.C. 2012. The use of algae in fish feeds as alternatives to fishmeal. International AquAFeed, retrieved from http://users.auth.gr/kganias/Aquaculture/AQUAFEED_selection.pdf

Hogendoorn, H. and koops W.J. 1983. Growth and production of African catfish *Clarias lazer*. Effects of stocking density, pond size and mix culture with tilapia under extensive field condition. *Aquaculture*, 34(3-4): 253-263.

Kangmin, L. 2012. Extending Integrated Fish Farming Technologies to Mariculture in China. *Biotechnology*, 9: 1-11.

Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. 2000. Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024.

Mustafa, G.M., Wakamatsu, S., Takeda, T., Umino, T. and Nakagawa, H. 1995. Effects of algae meal as a feed additive on growth performance, feed efficiency and body composition in red sea bream. *Fisheries Science*, 61: 25-28.

Naidoo, K., Maneveldt, G.W., Ruck K. and Bolton J.J. 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology*, 18: 437-443.

Najmudeen, T.M. and Victor, A.C.C. 2004. Seed production and juvenile rearing of the tropical abalone *Haliotis varia* Linnaeus 1758. *Aquaculture*, 234: 277-292.

Nakagawa, H., Kasahara, S. and Sugiyama, T. 1987. Effect of *Ulva* meal supplementation on lipid metabolism of black sea bream (*Acanthopagrus schlegeli* B.). *Aquaculture*, 62: 109-121.

National Research Council (NRC). 2001. Nutrient Requirements for Dairy Cattle. 7th revised edition. National Academy Press, Washington.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliot, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. and Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of National Academy of Science.* USA 106: 15103-15110.

Nelson, M.M., Leighton, D.L., Phleger, C.F. and Nichols, P.D. 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens,* provided specific macroalgal diets. *Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology*, 131: 695-712.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M. and Yarish, C. 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, 231: 361-391.

Nobre, A.M., Robertson-Andersson, D.V., Neori, A. and Sankar, K. 2010. Ecological-economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. *Aquaculture*, 306 (1-4): 116-126.

Li, P., Mai, K., Trushenski, K. and Wu, G. 2008. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids,* 47: 60-71.

O.E.C.D. /F.A.O. 2013. Agricultural outlook 2013- 2022. Corrigenda to OECD publications may be found online at: www.oecd.org/publishing/corrigenda. *Progressive Fish Culturist*, 39: 43-47.

Robertson-Andersson, D.V. 2003. The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. MSc Dissertation, University of Cape Town, South Africa.

Robertson-Andersson, D.V. 2007. Biological and economical feasibility studies of using seaweeds *Ulva lactuca* (chlorophyta) in recirculation systems in abalone farming. PhD Dissertation, University of Cape Town, South Africa.

Robertson-Andersson, D.V., Leitao, D., Bolton, J.J., Anderson, R.J., Njobeni, A. and Ruck, K. 2006. Can kelp extract (KELPAK™) be useful in seaweed mariculture? *Journal of*

Applied Phycology, 18: 315-321.

Robertson-Andersson, D.V., Maneveldt, G.W. and Naidoo, K. 2011. Effects of wild and farm-grown macroalgae on the growth of juvenile South African abalone *Haliotis midae* (Linnaeus). *African Journal of Aquatic Science*, 36(3): 331-337.

Robertson-Andersson, D.V., Amosu, A.O. and Maneveldt, G.W. 2014. Large Scale cultivation of macroalgae for green energy production in South Africa. ISAP 2014. Sydney 22-27 August 2014. (Book of Abstract/Postal presentation).

Royes, J.B. and Chapman, F.A. 2012. Preparing Your Own Fish Feeds , Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.Document No. Cir 97. Retrieved from http://www.edis.ifas.ufl.edu.

Sales, J. and Janssens, J.J. 2004. Use of feed ingredients in artificial diets for abalone: a brief update. *Nutrition Abstracts and Reviews Series*, 74: 13-21.

Sales, J., Truter, P.J. and Britz, P.J. 2003. Optimum dietary crude protein level for growth in South African abalone (*Haliotis midae* L.). *Aquaculture Nutrition*, 9: 85-89.

Samarakoon, K. and Jeon, Y.J. 2012.Bio-functionalities of proteins derived from marine algae - A review. *Food Research International*, 48: 948-960.

Schoenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E. and Neori, A. 2003. A semi-circulating, integrated system for the culture of fish and seaweed. *Aquaculture*, 221: 167-181.

Sherrington, N.A. 2013. *Ulva lactuca* L. as an inorganic extractive component for Integrated Multi-Trophic Aquaculture in British Columbia: An analysis of potentialities and pitfalls. M.Sc Dissertation, University of Victoria. Canada.

Shpigel, M., McBride, S.C., Marciano, S., Ron, S. and Ben-Amotz, A. 2005. Improving gonad colour and somatic index in the European sea urchin *Paracentrotus livid*.

Aquaculture, 245: 101-109.

Shpigel, M., Ragg, N.L., Lapatsch, I. and Neori, A. 1999. Protein content determines the nutritional value of the seaweed *Ulva lactuca* L. for the abalone *Haliotis tuberculata* L. And *H. discus hannai*. *Journal of Shellfish Research*, 18: 227-233.

Tacon, A.G.J. 1987. A report prepared for the FAO Trust Fund GCP/RLA/075/ITA Project Support to the Regional Aquaculture Activities for Latin America and the Caribbean, Brasilia, Brazil FAO-UN.

Troell M., Robertson-Andersson, D.V., Anderson, R.J., Bolton, J.J., Maneveldt, G.W., Halling, C. and Probyn, T. 2006. Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Journal of Applied Phycology*, 257(4): 266-281.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H. and Fang, J.G. 2009. Ecological engineering in aquaculture potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, 297: 1-9.

Wagner, J. and Stanton, T.L. 2006. Formulating Rations with the Pearson Square. No. 1.618. Colorado State University Extension. *Livestock Series Management*, 618: 1-3. Retrieved from http://www.ext.colostate.edu/PUBS/livestk/01618.html.

Wahbeh, M.I. 1997. Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. *Aquaculture*, 159: 101-109.

Waite, R., Beveridge, M., Brummett, R., Castine, R., Chaiyawannakarn, N., Kaushik, S., Mungkung R., Nawapakpilai, S. and Phillips, M. 2014. Improving Productivity and Environmental Performance of Aquaculture. Working Paper, Installment 5 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. Retrieved from http://www.worldresourcesreport.org

World Ocean Review (WOR). 2013. The Future of Fish – The Fisheries of the Future. World ocean review. Living with the oceans. Retrieved from http:// www.worldoceanreview.com/wp-content/downloads/wor2/WOR2_english.pdf

Chapter 7

General Discussion

This ultimate objective of this thesis was to mitigate for the reliance on wild harvesting of macroalgae, using the on-land integrated culture systems, with paddle-wheel raceways, as the preferred method of large scale macroalgal biomass production for the South African seaweed aquaculture industry. Our findings include some exciting prospects for energy efficiency as we have shown that the biotransformation of *Ulva armoricana* to Liquefied Petroleum Gas (LPG) is viable and economically feasible in South Africa among other additional benefits from farming activities, including ocean de-acidification and the capturing of atmospheric and dissolved CO_2 during growth to assist in climate change mitigation. Investigations into *Ulva armoricana* as an environmentally safe candidate for efficient heavy metals (Copper, Zinc, Lead) and inorganic nutrients (Ammonium, Nitrate, Nitrite, Phosphorus) extractions was also proven in this study. Feed for human and/or livestock consumption was deemed not possible due to the unsafe levels of Cadmium (Cd) found for our macroalgae. However, such enriched macrolage could be successfully used as a plant-growth stimulant. Furthermore, we investigated the growth response of *Ulva* in two culture systems (Closed and Flow through system) to reveal the best available culture characteristics for optimum production of the macroalga with induced CO_2. In order to achieve a more comprehensive overview of resource benefits, the nutrient utilization and proximate composition of cultivated, protein-enriched *Ulva armoricana* was tested to determine the alga's potential as a fish feed/feed ingredient for substitution in formulated and commercially available aquaculture feeds.

7.1 *Ulva* cultivation in South Africa

Three species (*U. armoricana*, *U. capensis* and *U. rigida*) of *Ulva* are currently cultivated in South Africa, with the free-floating *U. armoricana* (previously reported as *U. lactuca*) more widely cultured and used as an important feed source, particularly for the abalone industry (Robertson-Andersson, 2003; 2007; Department of Agriculture, Forestry and Fisheries, 2012). The taxonomy of the genus *Ulva* is currently in flux and it will take a while for this to be properly sorted. Until such time, we are obliged to use the name *U. armoricana* for the material grown in aquaculture systems notably because this material has proven to be genetically different to South African material of *U. rigida* (Cyrus *et al*., 2014b; J.J. Bolton, pers. comm.) for which the former has been suggested to be synonymous (Brodie *et al*., 2007; Guiry and Guiry, 2015). *Ulva armoricana*, *U. capensis* and *U. rigida* comprise South Africa's largest aquaculture product by weight reaching a production of 2884.61 tonnes in 2011 (Department of Agriculture, Forestry and Fisheries, 2011b; 2012; FAO, 2012). Although South Africa is not Africa's largest macroalgal producer (Zanzibar, Tanzania is the continent's largest producer), the country has the highest regional seaweed diversity and one of the richest in the world (Department of Agriculture, Forestry and Fisheries, 2012; FAO, 2012). None-the-less the South African marine macroalgal aquaculture industry is well researched and has developed steadily due to the increasing demand for abalone feed that has seen the need for sustainable production of macroalgae in IMTA. The development and innovation is reported to be similar to that which has been reported in the developed world (Hernández *et al*., 2002; Copertino *et al*., 2009; Cahill *et al*., 2010).

7.2 The South African Seaweed Industry

The South African seaweed aquaculture industry provides raw materials to other sectors of the economy, as well as the potential for bioremediation and other benefits such as biomass for biofuel, with species of *Ulva* being investigated for biogas production and for integrated aquaculture. To mitigate for the reliance on wild harvesting, the South African seaweed aquaculture industry has grown rapidly over the past few decades. On-land integrated culture units, with paddle-wheel raceways, are now widely viewed as the preferred method of *Ulva* production for the industry. Using this technology, cultured *Ulva* production currently stands at 2000 tonnes wet weight per annum as at 2012 (Department of Agriculture, Forestry and Fisheries, 2013).

The success of the seaweed aquaculture industry in South Africa is due to a number of natural and human (industrial) factors. Natural factors include a rich seaweed diversity of about 900 species, with a high level of endemism (Bolton *et al*., 2003; Amosu *et al*., 2013). Industrial factors are numerous and can be summarized as follows. The seaweed aquaculture industry has grown rapidly along the west coast where suitable rocky habitat exists, which serves as adhesive substrates for seed propagation (Troell *et al*., 2006). Collaboration among local, international phycologist and research institutes have improved the knowledge and understanding of *Ulva* aquaculture leading to the success of it sustainable domestication in South Africa (Shipton and Britz 2007). The development of the seaweed aquaculture industry has paralleled the growth of the abalone industry, and has been successful largely because of bilateral technology transfer and innovation between commercial abalone farms and various research institutions (Amosu *et al.*, 2015). The South African seaweed industry thus provides a template that could be used by other coastal African nations to further their undeveloped aquaculture potential.

7.3 *Ulva* energy production and climate change mitigation

The South African government was the first in Africa to propose a 20-50 % biofuel renewable energy (Deenanath *et al*., 2012). The fact that fossil fuel prices are fluctuating, and that macroalgal production costs will inevitably fall as macroalgal production expands, makes large scale macroalgae cultivation financially feasible (Robertson-Andersson *et al.*, 2013). *Ulva* are exciting prospects in terms of energy efficiency. Its growth rate, ease of harvesting, resistance to contamination by other algal species and minimal production loss, make it preferable to microalgae and to other macroalgae for large-scale renewable energy production and CO_2 capturing systems. Macroalgae have additional advantages that make them environmentally sustainable (Habiq *et al*., 1984; Briand and Morand, 1997; Duffy *et al*., 2009; Bruhn *et al*., 2011; Specht, 2011; Sarker *et al*., 2012). Utilizing cultivated marine macroalgae as a sustainable and renewable feedstock for biogas production would be a great advantage for South Africa and could potentially lead the way in renewable energy development for the continent. Additional benefits from such projects might include: 1) capturing industrially emitted CO_2 to use for enhanced seaweed growth in large scale aquaculture facilities to mitigate climate change; 2) decreasing ocean acidification through carbon sequestration, as well as the uptake of excess nutrients from industrial and agricultural effluent discharges; and 3) reducing coastal eutrophication. The high amounts of dissolved oxygen (the by-product of photosynthesis) in the paddle ponds further enable the aquaculture water to be reused for integrated polyculture with aquatic animals. All these benefits ultimately support changes towards more environmentally-sound aquaculture practices.

The current research has demonstrated that South Africa may well be the first African country with the capacity for large-scale production of *Ulva* to Liquefied Petroleum Gas (LPG) as our findings compared well to similar other findings (e.g. Nett, 2012). Our biogas production corroborate earlier reports from fresh and macerated *U. lactuca* yielded up to 271 ml CH_4 g^{-1} VS, which is in the range of the methane production from cattle manure and land-based energy crops such as grass clover (Bruhn, 2011). In a similar finding by Nikolaisen *et al*. (2011) the methane yield of the fresh and solid fraction of *U. lactuca* was 196 ml CH_4 g^{-1} VS and 192 ml CH_4 g^{-1} VS respectively. This latter result is comparable to 60 – 70 % for LPG, but better than LPG on major harmful emission like CO_2, hydrocarbons and nitrogen oxide (No_x) production (Nett, 2012). Additional benefits from seaweed-farming activities include capturing atmospheric and dissolved carbon dioxide during growth to assist in climate change mitigation. Presently South Africa is spearheading renewable energy production in Africa in response to several developed and developing nations like the EU, USA, Canada, Brazil, Argentina, Colombia, China, New Zealand and Japan, all of whom have incorporated biofuel targets into their renewable energy policies in recent years (Steenblik, 2007).

The energy supply in South Africa is primarily coal based. South Africa is therefore a CO_2 intense economy, with the country's major energy requirement sourced from fossil fuels. South Africa is amongst the countries with the highest per-capita emissions of greenhouse gases in the world. It is widely accepted that increases in CO_2, largely caused by human-induced emissions from the burning of fossil fuels and other activities, result in global warming, ocean acidification and climate change. Ocean acidification, in particular, represents an extremely serious environmental hazard for aquaculture organisms. Evidence suggests that the South African abalone industry will be severely influenced by ocean acidification (Amosu *et al*., 2015). Our research has demonstrated that it would be perfectly feasible to incorporate *Ulva* into abalone aquaculture on a national scale to potentially mitigate this effect.

7.4 Efficacy of *Ulva* as an effluent filter

Integration of *Ulva* in multitrophic aquaculture serves a dual purpose: (i) the production of biomass; and (ii) the removal of nutrients from the effluent waters of the aquaculture system, thereby reducing the load of dissolved nutrients to the aquatic environment. The current research has shown that the *Ulv*a biomass was of a suitable quality for biofilters of effluent wastewaters and that their heavy metal (except for cadmium) contents fell within the FAO and WHO permissible standards. This quality makes the *Ulva* suitable as a feed for aquaculture animals, but not food for direct human consumption. The high Cadmium (Cd) concentrations in the current study could well have originated from the unfiltered seawater and/or the fertilizer, possibilities already alluded to by Shuuluka (2011). Irrespective of the source, our Cd values negate the use of these seaweeds for human consumption but may be used for feeding abalone that will be used as food. *Ulva armoricana* used in this study efficiently removed dissolved inorganic nutrients from the effluents and their biofiltration capacities increased with an increase in fertilizer application. The results from this study can therefore be applied in the development of a large-scale wastewater treatment pond system for both agro-allied industries and for fish farms. The prospect of sustainable best available management practices, based on the utilization of *Ulva* mariculture designs of IMTA and paddle pods, bodes well for the South African aquaculture industry. As human health is directly affected by ingestion of vegetables, the biomonitoring of trace elements in macroalgae needs to be continually monitored because these algae could be a source of food for humans in many parts of the world.

The current research has also showed that *Ulva* can be used in eco-monitoring by playing a significant role in coastal aquaculture, especially for wastewater filtration and bioaccumulation as described by a number of previous studies (e.g. Troell, 1999; Robertson-Andersson, 2007; Msuya and Neori, 2010; Nobre *et al*., 2010). Although the Cadmium (Cd) values in this research were higher than the maximum recommended level for Cadmium (Cd) in the FAO/WHO (2001) standard for seaweed/vegetable and the South African limits for lettuce, this could well have originated from the unfiltered seawater and/or the fertilizer (Shuuluka 2011). It is therefore important that South Africa implements a continuous update of its seaweed safety monitoring by formulating a standard guideline and permissible limits of nutrients policy that must strictly be adhered to by all industries. Apart from the FAO/WHO standards, other nation-permissible limits can be emulated such as the French limits for edible seaweeds (Besada *et al*., 2009) and/or the Australian and New Zealand limits for edible seaweeds (Almela *et al*., 2002; 2006).

7.5 pH tolerance of *Ulva* under induced CO_2

pH toxicity tests were used as health indices of *Ulva* under increased CO_2 concentrations that potentially could occur in IMTA systems. The current research revealed sporulation as a physiological response to environmental stress, which is indicative of chlorophyll degradation and a reduction in photosynthetic activity. Our results showed that acidic conditions (pH 4.73 – 6.67) were responsible for sporulation, which caused a significant inhibition in growth and led to sporulation responses, especially when the medium was not enriched. However, the *Ulva* was able to withstand this slightly low pH. Toxicity tests for pH were conducted to assess the health of *Ulva* grown under extreme pH conditions. Although this finding is in contradiction to some earlier reports (e.g. Nordby (1977) reported optimum pH of 8.0 – 8.5 for sporulation) our results are largely consistent with more recent works by Harley *et al*. (2012) and Roleda *et al*. (2012) who found that a pH of 6.5 - 7.5 gave the optimum sporulation. This could be due to differences in the species involved in the experiments, seanonality and cultivation methods. We further demonstrated CO_2 to be a major limitation to large-scale algal biomass production and utilization, especially in closed systems, a sentiment already expressed by Ugoala *et al*. (2012). In our flow-through systems, despite sporulation resulting in the majority of the treatments, the results show that *U rigida* is able to tolerate a sustained pH of 7.2. This implies that in large-scale cultivation systems CO_2 could be added and pH maintained at 7.2. Incidentally, the growth rates of *U. rigida* under increased CO_2 concentrations observed in this study differs from those reported for *U. lactuca* and *U. fasciata* (see Neori *et al*., 1991; Robertson-Andersson *et al*., 2008).

The current research also found that the application of a soluble fertilizer was found to promote growth and prevent sporulation. This is in support of previous studies for the production of *U. lactuca* (Bruhn *et al*., 2011; Nikolaisen *et al*., 2011). In the future, it will be important to conduct experiments on the rates of fertilizer application at low pH, as well as to follow the responses of multiple generations to elevated CO_2 under conditions that simulate growth.

7.6 *Ulva* as fish feed

Our nutrient utilization and proximate composition results show that the African mud catfish *Clarias gariepinus* grew well when protein enriched *U. armoricana* was incorporated into its diet, proving that protein enriched *Ulva* has the potential of a successful fish feed. In addition, the current research has identified with a number of other direct (e.g. feed for shellfish like abalone (*H. midae*) and shrimp (*Litopenaeus vannamei*) and indirect (e.g. integration of protein enriched *Ulva* as an inorganic biofilter) economic benefits of integrating protein enriched *Ulva*. Similar to previous findings (e.g. Diler *et al*., 2007; Soyutu *et al*., 2009; Robertson-Andersson *et al*., 2011; Cyrus *et al*., 2014a; b) our results show that a mixed diet of macroalgae, that includes protein enriched *Ulva*, can result in growth rates in cat fish that match or even exceed those attained with some artificial feeds. These findings (including the current research), are in support of the research by Sherrington (2013) that demonstrated that *Ulva* is more beneficial for algavorous finfish than carnivorous finfish. Our research shows that an *U. armoricana* supplemented diet has the potential to be a successful feed stimulant.

Various species of macroalgae have been incorporated into fish feed formulations to assess their nutritional value, and many have been shown to be beneficial especially when the crop plant proteins, commonly used in fish feeds, have been shown to be deficient in certain amino acids such as lysine, methionine, threonine and tryptophan (Li *et al*., 2007). Whereas analyses of the amino acid content of numerous macroalgae have found that although there is significant variation, they generally contain all the essential amino acids (Wassef, 2005; Lemme, 2010). Similar findings by Wassef *et al*. (2001) demonstrated that an *U. lactuca* meal feeding trial with mullet (*Mugil cephalus*) resulted in a higher growth performance, feed intake and protein utilization efficiency in the mullet. Similarly, *U. lactuca* has been fed to European sea bass, striped mullet and seabream (*Sparus aurata*); all resulted in higher growth performance, feed intake and protein utilization efficiency in these fish species. (Wassef *et al*., 2001;Wassef, 2005; Li *et al*., 2008). Numerous other reports on the dietary benefits of using *Ulva* spp in fish feeds were summarized by Mustafa and Nakagawa (1995) in general, and by Mustafa *et al*. (1995) more specifically for red seabream among others.

7.7 Future research

In order to facilitate a more effective South African aquaculture industry that is increasingly becoming more dependent on species of *Ulva*, the following recommendations are proposed.

- A comprehensive taxonomic study, including DNA sequencing, of all species of *Ulva* (both naturally occurring and those grown in aquaculture facilities) should be undertaken to access the levels of cryptism and ultimately the diversity of the genus in South Africa.
- Detailed investigations into the liquid chromatography mass spectroscopy (LCMS) differences in the chemical composition of all 'species' of *Ulva* reported for South Africa. Species-specific differences no doubt are expected to affect the quality in respect of their potential biogas production. Identifying candidate species based on preferred chemical properties would go a long way in reducing the time and effort generally taken through aquaculture trial-and-error synarios.
- Serious consideration should be given to research programme using bacteria to enhance the methane produced from the anaerobic digestion process, using a bioaugmentation with bacteria to improve the degradation of *Ulva* biomass, to achieve

higher levels of methane. Macroalgae contain two main sugars, namely mannitol and laminaran.

- Additional studies need to be conducted to improve *Ulva* species-specific cultivation in terms of productivity, cost benefit analysis, atmospheric CO_2 sequestration during growth, adaptive mechanisms to altered pH concentrations, improving nutritional quality, biofiltration responses, and the possible mechanisms for heavy metal exclusions.

In conclusion, the results presented in this thesis suggests that South Africa could take advantage of being the first African country to propose specific standards for large-scale *Ulva* production and its use in agriculture as the country's successful research innovations and development provides a template for other developing countries to follow. We have shown that the derived benefits from *Ulva* farming activities includes bioremediation, ocean de-acidification, and the capturing of atmospheric and dissolved CO_2 during growth to assist in climate change mitigation.

Chapter 8

General References

Abreu, M.H., Pereira, R., Yarish, C., Buschmann, A.H. and Sousa-Pinto, I. (2011). IMTA with Gracilaria vermiculophylla: productivity and nutrient removal performance of the seaweed in a Land-based pilot scale system. *Aquaculture*, 312(1-4): 77-87.

Adams, N.M. (1994). Seaweeds of New Zealand. An Illustrated Guide. Canterbury University Press, Christchurch, New Zealand.

Adedeji, O.B. and Okocha R.C. (2011). Constraint to Aquaculture Development in Nigeria and Way Forward. *Journal of Applied Sciences Research*, 7(7): 1133-1140.

Adey, W.H. and Hayek, L.C. (2011). Elucidating Marine Biogeography with Macrophytes: Quantitative Analysis of the North Atlantic Supports the Thermogeographic Model and Demonstrates a Distinct Subarctic Region in the Northwestern Atlantic. *Northeastern Naturalist*, 18(8): 1-128.

Agrawal, S.C. (2012). Factors controlling induction of reproduction in algae review: the text. *Folia Microbiology*, 50: 155-160.

Alamsjah, M.A., Hirao, S., Ishibashi, F., Oda, T. and Fujita, Y. (2009). Algicidal activity of polyunsaturated fatty acids derived from *Ulva fasciata* and *U. pertusa* (Ulvaceae, Chlorophyta) on phytoplankton. *Journal of Applied Phycology*, 20(5): 713-720.

Albrecht, K.O. and Hallen, R.T. (2011). A Brief Literature Overview of Various Routes to Biorenewable Fuels from Lipids for the National Alliance of Advanced Biofuels and Bio-products NAAB Consortium. Prepared by the US Department of Energy. Retrieved from http://www.pnnl.gov/main/publications/external/technical-reports/PNNL-2027pdf

Algae Energy (2013). Algae biofuel, biodiesel and renewable energy resource. Retrieved from http:// www.algae-energy.co.uk/biofuel production/cultivation/

Alieth, M. (2008). Interactive governance approach in mariculture activities in Tanzania: A Case Study at Mlingotini Village, Bagamoyo. MSc Dissertation, University of Tromsø. Norway.

Altamirano, M., Flores-Moya, A. and Figueroa, F.L. (2003). Effects of UV radiation and temperature on growth of germlings of three species of Fucus (Phaeophyceae). *Aquatic Botany*, 75: 9-20.

Altamirano, M., Flores-Moya, A., Conde, F. and Figueroa, F.L. (2000). Growth seasonality, photosynthetic pigments, and carbon and nitrogen content in relation to environmental factors: a field study of *Ulva olivascens* (Ulvales, Chlorophyta). *Phycologia*, 39(1): 50-58.

Alzaablawy, K.M. (2005). Utilization some of seaweeds in poultry diets. M.Sc. Dissertation. Al-Azhar University, Egypt.

Amosu, A.O., Robertson-Andersson, D.V. and Maneveldt, G.W. (2015). Seaweed mariculture provides feed, green energy production, bioremediation. *Global Aquaculture Advocate* (March/April 2015 Magazine), 18(2): 66-68.

Amosu, A.O., Robertson-Andersson, D.V., Maneveldt, G.W., Anderson, R.J. and Bolton, J.J (2013). South African Seaweed Aquaculture: A sustainable development example for other African coastal countries. *African Journal of Agricultural Sciences*, 8(43): 5260-5271.

Anderson, R.J., Levitt. G. J. and Share, A. (1996). Investigations for the mariculture of *Gracilaria gracilis* at Saldanha Bay, South Africa. *Journal of Applied Phycology*, 8: 421-430.

Andersson, V., Broberg, S. and Hackl, R. (2009). Integrated Algea Cultivation for Biofuels Production in Industrial Clusters. This report is a result of the course Interdisciplinary Energy System Project that runs as a part of the interdisciplinary and national research programme and graduate school; The Energy Systems Programme/ Chalmers University of Technology/ Linköping University.

Areli, A., Sklan, D. and Kissil, G. (1993). A note on the nutritive value of *Ulva lactuca* for ruminants. *Animal Science*, 57(2): 329-331.

Asian Institute of Technology (1994). *The Promotion of Sustainable Aquaculture*. Asian Institute of Technology, Bangkok. pp.98.

Azad, S.A. and Xiang, T.Z. (2012). Suitability of Seaweed Meals Incorporated With Rhodovulum Sp. Bacterium as Feed Supplement for Finfish Larvae. *Science*, 30: 57-64.

Azad, S.A., Chong, C.V. and Vikineswary, S. (2002). Phototrophic Bacteria as Feed Supplement for earing *Penaeus monodon* Larvae. *Journal of the World Aquaculture Society*, 33(2): 158-168.

Bak, R (2003). *Henry and Edsel: The Creation of the Ford Empire*. Wiley ISBN 0-471-23487-7.

Barrington, K., Chopin, T. and Robinson, S. (2009). Integrated multi-trophic aquaculture IMTA) in marine temperate waters. In D. Soto (ed.). Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper No. 529. Rome, FAO. pp. 7-46.

Bartoli, M., Nizzoli, D., Naldi, M., Vezzulli, L., Porrello, S., Lenzi, M. and Viaroli, P. (2005). Inorganic nitrogen control in wastewater treatment ponds from a fish farm (Orbetello, Italy): Denitrification versus Ulva uptake. *Marine Pollution Bulletin*, 50: 1386-1397.

Basak, A. (2008). Effect of Preharvest Treatment with Seaweed Products, Kelpak and Goëmar BM 86, on Fruit Quality in Apple, *International Journal of Fruit Science*, 8(1): 1-14.

Bast, F., Bhushan, S. and John, A.A. (2014). DNA barcoding of a new record of epi-endophytic green algae Ulvella leptochaete (Ulvellaceae, Chlorophyta) in India. *Journal* *of Biosciences*, 39(4): 711-716.

Behnassi, M., Draggan, S. and Yaya, S. (Eds.) (2011). Global Food Insecurity-Rethinking Agricultural and Rural Development Paradigm and Policy. Springer Dordrecht New York Heidelberg London. pp. 433.

Ben Chekroun, K., Moumen, A., Rezzoum, N., Sánchez, E. and Baghour, M. (2013). Role of macroalgae in biomonitoring of pollution in (Marchica), the Nador lagoon. *International Journal of Experimental Biology*, 82: 31-34.

Ben-Ari, T., Neori, A., Ben-Ezra, D., Shauli, L., Odintzov, V. and Shpigel, M. (2014). Management of *Ulva lactuca* as a biofilter of mariculture effluents in IMTA system. *Aquaculture*, 434: 493-498.

Berger, W.H., Smetack, V.S. and Wefer, G. (1989). "Productivity of the Ocean: Present and Past", Wiley: New York.

Bhattacharyya, S.C. (2006). Energy access problem of the poor in India: Is rural electrification a remedy? *Energy Policy*, 34(18): 3387-97.

Biello, D. (2013). Can Ethanol from Corn Be Made Sustainable? Scientific American, a Division of Nature America, Inc. February 20, 2013. Retrieved from http:// www.scientificamer/.

Bindu, M.S., Sobha V. and Balasubramanian N.K. (2003). Digestive enzyme response of *Ctenopharyngodon idella* to the three species of seaweed. *Seaweed Resouces Utilization*, 25(2003): 195.

Binzer, T. and Sand-Jensen, K. (2002). Production in aquatic macrophyte communities: A theoretical and empirical study of the influence of spatial light distribution. *Limnology Oceanography*, 47(6): 1742-1750.

Bird, K.T., Habig, C. and Debusk, T. (1982). Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *Journal of Phycology*, 18: 344-348.

Biron, C.L. (2012). Carbon Dioxide Emissions Hit Record High in 2012, Inter Press Service, June 11, 2013. Retrived from http://www.ipsnews.net/2013/06/carbon-dioxide-emissions-hit.

Björk, M., Haglund, K. and Ramazanov, Z. (1993). Inducible mechanism for HCO_3^- utilization and repression of photorespiration in protoplasts and thallus of three species of *Ulva* (Chlorophyta). *Journal of Phycology*, (29): 166-173.

Black, K.D. (ed.) (2001). Environmental Impacts of aquaculture .Sheffield Academic Press, Sheffield. pp. 214.

Blomster, J. and Stanhope, M.J. (1998). Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. *Journal of Phycology*, 34: 319-340.

Bolton, J.J., Robertson-Andersson D.V., Shuuluka, D. and Kandjengo, L. (2008). Growing Ulva (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. *Journal of Applied Phycology*, 10/21(5): 575-583.

Bolton, J.J., Robertsson-Andersson, D.V., Troell, M. and Halling, C. (2006). Integrated Systems Incorporate Seaweeds. *Global Aquaculture Advocate*, July/August, pp. 54-56.

Bolton, J.J. (1996). Patterns of species diversity and endemism in comparable temperate brown algal floras. *Hydrobiologia*, 326/327: 173-178.

Borum, J. (1985). Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biological*, 87: 211-218.

Boxman, S. (2013). "Evaluation of a pilot land-based marine integrated aquaculture system" Graduate Theses and Dissertations. University of South Florida. U.S.

Breeman, A.M. (1988). Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phonological evidence. *Helgolander Messresuntersuchungen*, 42: 199-241.

Briand, X. and Morand, P. (1997). Anaerobic digestion of Ulva sp. 1. Relationship between *Ulva* composition and methanisation. *Journal of Applied Phycology*, 9: 511-524.

Brodie, J., Maggs, C.A. and John, D.M. (2007). *Green seaweeds of Britain and Ireland*. pp. [i-v], vi-xii, 1-242, 101 figs. London: British Phycological Society.

Browdy, C.L., Hulata, G., Liu, Z., Allan, G.L., Sommerville, C., Passos de Andrade, T., Pereira, R., Yarish, C., Shpigel, M., Chopin, T., Robinson, S., Avnimelech, Y. and Lovatelli, A. (2012). Novel and emerging technologies: can they contribute to improving aquaculture sustainability? In R.P. Subasinghe, J.R. Arthur, D.M. Bartley, S.S. De Silva, M. Halwart, N. Hishamunda, C.V. Mohan and P. Sorgeioos (eds.) farming the waters for people and food. Proceedings of the global conference on aquaculture 2010, Phuket, Thailand. 22-25 September 2010. pp. 149-191. FAO and NACA, Bangkok.

Brown, M.R., Jeffrey S.W. and Dunstan G.A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, 151: 315-331.

Bruhn, A., Dahl, J., Nielsen, H.B., Nkolaisen, L., Rasmussen, M.B. and Markagar, S. (2011). Bioenergy potential of *U. lactuca*: biomass yield, methane production and combustion. *Bioresources technology*, 102: 2595-604.

Budd, G. and Pizzola, P. (2008). *Ulva intestinalis*. Gut weed. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 02/11/2015]. Retrieved from http://www.marlin.ac.uk/reproduction.php?speciesID=4540

Burg, S., van den, M., Stuiver, F., Veenstra, P., Bikker, A., López Contreras, A., Palstra, J., Broeze, H., Jansen, R., Jak, A., Gerritsen, P., Harmsen, J., Kals, A., Blanco, W., Brandenburg, M., van Krimpen, A.P., van Duijn, W., Mulder, L. and van Raamsdonk, K. (2012). A Triple P review of the feasibility of sustainable offshore seaweed production in the North Sea.Wageningen, Wageningen UR (University & Research centre), LEI Report, 13-77.

Butterworth, A. (2010). Integrated Multi-Trophic Aquaculture systems incorporating abalone and seaweeds. Nuffield Australia Farming Scholar, Nuffield Australia Project No. 0914. pp. 39.

Capo, T.R., Jaramillo, J. C., Boyd, A.E., Lapointe, B.E., and Serafy, L.E. (1999). Sustainable high yields of Gracilaria (Rhodophyta) grown in intensive large scale culture. *Journal of Applied Phycology*, 11(2): 143-147.

Carl, C., de Nys, R. and Paul, N.A. (2014). The Seeding and Cultivation of a Tropical Species of Filamentous *Ulva* for Algal Biomass Production. *PLoS One*, 9(6): 1-9.

Černá, M. (2011). Seaweed proteins and amino acids as nutraceuticals. *Advance Food Nutrition Resources*, 64: 297-312.

Chakraborty, S., Bhattacharya, T., Singh, G. and Maity, J.P .(2014). Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: a biomonitoring approach for pollution assessment. *Ecotoxicology and Environmental Safety,* 100: 61-68.

Chbani, A., Mawlawi, H. and Zaouk, L. (2013). Evaluation of brown seaweed (*Padina pavonica*) as biostimulant of plant growth and development. *African Journal of Agricultural Research*, 8(13): 1155-1165.

Chojnacka, K., Saeid A., Witkowska, Z. and Tuhy, L. (2012). Biological active compounds in seaweeds extracts - the prospects from the application. *The Open Conference Proceedings Journal*, 3(Suppl 1-M4): 20-28.

Chopin, T. (2001). Intergrateing seaweed into marine aquaculture systems: A key toward sustainability. *Journal of Phycology*, 37: 975-986.

Chopin, T. and Yarish, C. (1998). Nutrients or Not Nutrients? That is the question in seaweed aquaculture and the answerdepends on the type and purpose of the aquaculture system. World *Aquaculture*, 29: 31-33.

Chopin, T. and Yarish, C. (1999). Aquaculture does not only mean finfish monoculture seaweeds must be a significant component for an integrated ecosystem approach. *Bulletin of Aquaculture Association Canada*, 99-1: 35-37.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori A., Kraemer, G.P., Zertuche-Gonzalez, J.A., Yarish C. and Neefus, C. (2001). Integrated seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycology*, 37: 975-986.

Chopin, T. and Robinson, S.M.C. (2004). Defining the appropriate regulatory and policy framework for the development of integrated multi-trophic aquaculture practices: introduction to the workshop and positioning of the issues. *Bulletin of Aquaculture Association Canada*, 104(8): 1-84.

Chung, I.K., Kang, Y., Yarish, C., Kraemer, G.P. and Lee, J.A. (2002). Application of Seaweed Cultivation to the Bioremediation of Nutrient-Rich Effluent. *Algae*, 17(3): 187-194.

Coat, G., Dion, P., Noailles, M.C., Reviers, B., de Fontaine, J.M., Berger-Perrot, Y. and Loiseaux-De Goër, S. (1998). *Ulva armoricana* (Ulvales, Chlorophyta) from the coasts of Brittany (France). II. Nuclear rDNA ITS sequence analysis. *European Journal of Phycology*, 33: 81-86.

Coleman, V.L. and Burkholder, J.M. (1995). Response of microalgal epiphyte communities to nitrate enrichment in an eelgrass (Zostera marina) meadow. *Journal of Phycology*, 31: 36-43.

Colorni, A. (1989). Perforation disease affecting *Ulva* sp. cultured in Israel on the Red Sea. *Disease Aquatic Organism*, 7: 71-73.

Cornwall, C.E., Phillips, N.E. and McNaught, D.C. (2009). Feeding Preferences of the Abalone Haliotis iris in Relation to Macroalgal Species, Attachment, Accessibility and Water Movement. *Journal of Shellfish Research*, 28(3): 589-597.

Costa -Pierce, B.A. and Page, G.G. (2010). Sustainability science in aquaculture. In: Costa - Pierce, B.A. (ed.), Ocean Farming and Sustainable Aquaculture Science and Technology. Encyclopedia of Sustainability Science and Technology. Springer Science, N.Y.

Costa-Pierce, B.A., Bartley, D.M., Hasan, M., Yusoff, F., Kaushik, S.J., Rana, K., Lemos, D., Bueno, P. and Yakupitiyage, A. (2011). Responsible use of resources for sustainable aquaculture. Global Conference on Aquaculture 2010, Sept. 22-25, 2010, Phuket, Thailand. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Critchley, A.T. (1993). *Gracilaria* (Rhodophyta, Gracilariales): An economically important agarophyte. In: Ohno, M. & Critchley, A.T. (eds.), Seaweed cultivation and marine ranching. JICA. pp. 89-112.

Crowe, T.P., Thompson, R.C., Bray, S. and Hawkins, S.J. (2000). Impacts of anthropogenic stress on rocky intertidal communities. *Journal of Aquatic Ecosystem Stress and Recovery*, 7: 273-297.

Cyrus, M., Bolton, J.J., De Wet, J. and Macey, B.M. (2014). The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: Effects on palatability, consumption and digestibility. *Aquaculture Nutrition*, 46: 1-14.

Cyrus, M.D., Bolton, J.J. and Macey, B.M. (2015). The role of the green seaweed *Ulva* as a dietary supplement for full life-cycle grow-out of *Tripneustes gratilla*. *Aquaculture*, 446: 187-197.

Cyrus, M.D., Bolton, J.J., Lourens De Wet, L., Brett, M. and Macey, B. (2014a). The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla* (Linnaeus). *Aquaculture Research,* 45: 159-176.

Damirbas, A. (2009). Green energy and technology. Springer-Velag, London Ltd. pp.149.

Danakusumah, E. and Hirata, H. (1991). Ecological Effects of *Ulva pertusa* in Recirculation Culture System of the Prawn *Penaeus japonicus*. *Aquaculture Science*, 39(2): 195-200.

Darcy-Vrillon, B. (1993). Nutritional aspects of the developing use of marine macroalgae for the human food industry. *International Journal of Food Science and Nutrition,* 44: 23-35.

Dawczynski C., Schubert R. and Jahreis G. (2007). Aminoacids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 103: 891-899.

Dean, D. (2010). Aquaculture in Vietnam: from small- scale integration to intensive production. B.Sc. Thesis, Brown University. U.S.

Deenanath, E.D., Iyuke, S.I. and Rumbold, K. (2012). “The Bioethanol Industry in Sub-Saharan Africa: History, Challenges, and Prospects”. *Journal of Biomedicine and Biotechnology*, 11: 89-97.

Department of Agriculture, Forestry and Fisheries (2012). South Africa’s aquaculture: Yearbook, 2012.

Department of Agriculture, Forestry and Fisheries (2013). South Africa’s aquaculture: Yearbook, 2013.

Department of Minerals and Energy (2004). Draft Energy Efficiency Strategy of the Republic of South Africa. Pretoria, December 2004.

Department of National Treasury (2010). Discussion paper for public comments. Reducing Greenhouse Gas Emissions: The Carbon Tax Option. Republic of South Africa.

Dewald, D.B., Mason, H.S. and Mullet J.E. (1992). The soybean vegetative storage proteins VSP alpha and VSP beta are acid phosphatases active in polyphosphates. *Journal of Biology and Chemistry*, 267: 15958-15964.

Dion, P., Reviers, B. and Coat, G. (1998). *Ulva armoricana* sp. nov. (Ulvales, Chlorophyta) from the coasts of Brittany (France). I. Morphological identification. *European Journal of Phycolog*, 33: 73-80.

Dobretsov, S.V. and Qian, P.Y. (2002). Effect of bacteria associated with the green alga Ulva reticulata on marine micro- and macrofouling. *Biofouling*, 18: 217-228.

Drechsler, Z. and Beer, S. (1992). Utilization of Inorganic Carbon by *Ulva lactuca. Plant Physiology*, 98(4): 1439-1444.

Duff, S.M.G., Sarath, G. and Plaxton, W.C. (1994). The role of acid phosphatases in plant phosphorus metabolism. *Physiology of Plant*, 90: 791-800.

Dutcher, J.A. and Kapraun, D.F. (1994). Random Applied Polymorphic DNA (RAPD) identification of genetic variation in three species of Porphyra. *Journal of Applied Phycology*, 6: 267-273.

Edwards, P. (1994). A Systems Approach for the Promotion of Integrated Aquaculture. Integrated Fish Farming International Workshop, 11-15 October 1994, Wuxi, PR China. pp. 22.

Egan, S., Holmstrom, C. and Kjelleberg, S. (2001). Pseudoalteromonas ulvae sp nov., a bacterium with antifouling activities isolated from the surface of a marine alga. *International Journal of Systematic and Evolutionary Microbiology*, 51: 1499-1504.

Fakoya, K.A., Owodeinde, F.G., Akintola, S.L., Adewolu, M.A., Abass, M.A. and Ndimele P.E. (2011). An Exposition on Potential Seaweed Resources for Exploitation, Culture and Utilization in West Africa: A Case Study of Nigeria. *Journal of Fisheries and Aquatic Science*, 6: 37-47.

FAO (2002a). Reducing poverty and hunger: the critical role of financing for food, agriculture and rural development. Paper Prepared for the International Conference on Financing for Development Monterrey, Mexico, 18-22 March 2002. Rome, Italy.

FAO (2003). Review of the state of the world aquaculture. Rome, Italy.

FAO (2004a). Protein sources for the animal feed industry. Proceedings of expert consultation and workshop, Bangkok, 29 April – 3 May 2002, FAO/UN. Rome, Italy.

FAO (2010b). FishStat fishery statistical collections: aquaculture production (1950-2008; released March 2010). Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/fishery/statistics/software/fishstat/en

FAO (2011a). Aquaculture development. 5. Use of wild fish as feed in aquaculture. FAO Technical Guidelines for Responsible Fisheries. No. 5, Suppl. 5. Rome, FAO. 2011. pp. 79.

FAO (2011b). FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FishStatPlus. Universal Software for fishery statistical time series. Version 2.3 in 2000. Last database update in April 2011.

FAO (2012). Seaweeds used as human food in A guide to the seaweed industry. FAO (Food and Agriculture Organization of the United States) Corporate Repository. Retrieved from http://www.FAO.org

FAO (2015). 2011-2015- Fisheries and aquaculture software. FishStatJ - software for fishery statistical time series. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 23 June 2015. Retrieved from http://www.FAO.org

FAO. (2002b). The State of World Fisheries and Aquaculture 2002 - SOFIA, Rome, Italy.

FAO. (2004). The State of World Fisheries and Aquaculture 2004 - SOFIA, Rome, Italy. pp.154.

FAO. (2008). Fisheries and Aquaculture Information and Statistics Service. Total fisheries production 1950 to 2006. FishStat Plus - Universal software for fishery statistical time series (online or CD-ROM). Rome, FAO. Retrieved from http://www.FAO.org

FAO. (2009). The state of world fisheries and aquaculture 2008. FAO fisheries and aquaculture department. Food and Agriculture Organization of United Nations. Rome. Italy.

FAO. (2010a). The state of world fisheries and aquaculture 2008. FAO fisheries and aquaculture department. Food and Agriculture Organization of United Nations. Rome. Italy.

FAO. (2014). *Fishery and Aquaculture Statistics. Aquaculture production 1950-2012 (FishstatJ).* In: FAO Fisheries and Aquaculture Department [online or CD-ROM]. Rome. Updated 2014. Retrieved from http://www.FAO.org

Feldman, K.L., Armstrong, D.A., Dumbauld, B.R., DeWitt, T.H. and Doty, D.C. (2000). Oysters, crabs, and burrowing shrimp: Review of an environmental conflict over aquatic resources and pesticide use in Washington State's (USA) coastal estuaries. *Estuaries*, 23(2): 141-176.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology*, 10: 25-28.

Fleurence, J., Morancais, M., Dumay, J., Decotisgnies, P., Turpin, V., Munier, M. and Jaouen, P. (2012). What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends in food Science & technology*, 27(1): 57-61.

Fong, P. and Zedler, J.B. (1993). Temperature and light effects on the seasonal succession of algal communities in shallow coastal lagoons. *Journal of Experimental Marine Biology* *and Ecology*, 171: 259-272.

Fong, P., Boyer, K.E. and Zedler, J.B. (1998). Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). *Journal of Experimental Marine Biology and Ecology*, 35: 67-73.

Food and Agriculture Organization of the United Nations (FAO) (2009). Algae-Based Biofuels: A Review of Challenges and Opportunities for Developing Countries. Environment, Climate Change and Bioenergy Division. Rome. Italy.

Ford, H. (2003). The International Jew: The World's Foremost Problem. Kessinger Publishing. ISBN 0-7661-7829-3, pp. 61.

Ford, H. and Crowther, S. (1922). My Life and Work, Garden City, New York, USA: Garden City Publishing Company, Inc. various republications, including ISBN 9781406500189. Original is public domain in U.S. Also available at Google Books.

Franks, A., Haywood, P., Holmström, C., Egan, S., Kjelleberg, S. and Kumar, N. (2005). Isolation and structure elucidation of a novel yellow pigment from the marine bacterium pseudoalteromonas tunicate. *Molecules*, 10(10): 1286-1291.

Fry, B. (1984). $^{13}C/^{12}C$ ratios and the trophic importance of algae in Florida Syringodium Filiforme seagrass meadows. *Marine Biology*, 79: 11-19.

Gao, K. and McKinley, K. (1994). Use of macroalgae for marine biomass production and CO2 remediation: a review. *Journal of Applied Phycology*, 6: 45-60.

Gao, K.S., Ji, Y. and Aruga, Y. (1999). Relationship of CO2 concentrations to photosynthesis of intertidal macroalgae during emersion. *Hydrobiologia*, 399: 355-359.

Gao, K., Helbling, E.W., Hader, D.P. and Hutchins, D.A. (2012). Reponses of marine primary producers to interactins between ocean acidification, solar radiation, and warming, *Marine Ecology Progress*, 470: 167-189.

Global Conference on Aquaculture (2010). Phuket, Thailand. 22–25 September 2010. pp. 149-191. FAO, Rome and NACA, Bangkok.

Godfray, H.C.J., Beddington, I.R.C., Haddad, L., Muir, J.F., Pretty, J., Robniso, S., Thomas, S.M. and Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, 327(5967): 812-818.

Gordillo, F.J.L., Jiménez, C., Goutx, M. and Niell, X. (2001). Effects of CO2 and nitrogen supply on the biochemical composition of *Ulva rigida* with especial emphasis on lipid class analysis. *Journal of Plant Physiology*, 158(3): 367-373.

Gouveia, L. (2011). Microalgae as a Feedstock for Biofuels . Springerbrief in Microbiology. Springer Berlin Heidelberg. SBN: 978-3-642-17996-9 (Print) 978-3-642-17997-6 (Online).

Guiry, M.D. and Guiry, G.M (2013). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Retrived from http://www.algaebase.org; searched on 23 April 2013.

Habiq, C., DeBusk, T.A. and Ryther, J.H. (1984). The effect of nitrogen content on methane production by the marine algae *Gracilaria tikvahiae* and *Ulva* sp. *Biomass*, 4: 239-251.

Haglund, K., Robledo, D., GarciaReina, G. and Pedersen, M. (1991). Increasing metal content in spray cultivated saweeds. In seaweed cellular biotechnology, physiology and intensive cultivation. *Proceedings of a COST 48 (Subgroup 1) Workshop, held at Marine Plant Biotechnology Laboratory, University of Las Palmas, Canary Islands, Spain, February 8- 17.*

Halford, N.G. and Karp, A. (Eds.) (2011). Energy Crops (RSC Energy and Environment Series No. 3), RSC Publishing, retrieved from www.rsc.org/shop/books/2010/9781849730327.asp.

Hall, J., Matos, S., Bruno, S. and Martin, M. (2011). Managing Technical and Social Uncertainties of Innovation**:** The Evolution of Brazilian Energy and Agriculture. *Technology Forcasting and Social Change*, 78: 1147-1157.

Hammes, D. and Wills, D. (2005). Black Gold- The end of bretton wood the oil price shocks of the 1970s. *The Independent Review*, 11(4): 501-511.

Hammerschlag, R. (2006). Ethanol energy return on investement: A Survey of the literature 1999 - Present. *Environmental Science Technology*, 40: 1744-1750.

Han, J., Zhang, Z., Yu, Z. and Widdows, J. (2001). Differences in the benthic-pelagic particle flux (biodeposition and sediment erosion) at intertidal sites with and without clam (Ruditapes philippinarum) cultivation in eastern China. *Journal of Experimental Marine Biology and Ecology*, 261: 245-261.

Hanniffy, D. and Kraan, S. (2006). Biopuralg: reducing the environmental impact of land based aquaculture through cultivation of seaweeds Irish Seaweed Centre, MRI National University of Ireland, Galway.

Harder, T., Dobretsov, S., and Qian, P.Y. (2004). Waterborne polar macromolecules act as algal antifoulants in the seaweed Ulva reticulata. *Marine Ecology Progress Series*, 274: 133-141.

Harder, T., Lau, S.C.K., Dahms, H. and Qian, P.Y. (2002). Isolation of bacterial metabolites as natural inducers for larval settlement in the marine polychaete Hydroides elegans (Haswell). *Journal of Chemical Ecology*, 28: 2029-2043.

Harley, C.D., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A. and Graham, M.H. (2012). Effects of climate change on global seaweed communities. *Journal of Phycology*, 48: 1064-1078.

Hashim, R. and Hassan, H.N. (1995). The use of varying level of Ulva sp. meal as binders for practical and their effect on growth of snakehead (*Channa striatus*) fry. *Journal of*

Bioscience, 6: 123-131.

Hashim, R. and Mat Saat, M.A. (1992). The utilization of seaweed meals as binding agents in pelleted feeds for snakehead (*Channa striatus*) fry and their effects on growth. *Aquaculture*, 108: 299-308.

Hauxwell, J., Cebrian, J. and Valiela, I. (2003). Eelgrass Zostera marina loss in temperate estuaries: relationship to land derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series*, 247: 59-73.

Hay, M.E. (2009). Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. *Annal Review in Marine Science*, 1: 193-212.

Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C. and Stanhope, M.J. (2003). Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycol*ogy, 38: 277-294.

Heo, S.J., Park, E.J., Lee, K.W. and Jeon, Y.J. (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology*, 96: 1613-1623.

Heuze, V., Tran, G., Giger-Reverdin S., Lessire, M. and Lebas, F. (2016). Seaweeds (marine macroalgae) Feedipedia, a programme by IRA, CIRAD, AFZ and FAO Last updaed on April 15. 2016. Retrived from http//www.feedipedia.org/node/78

Ho, Y.B. (1990). *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. *Hydrobiologia*, 203: 73-81.

Holland, P. and Brown, D. (1999). *Aquaculture Policy: Selected Experiences From Overseas*, ABARE Research Report 99.7, Canberra.

Hong, D.D., Hien, H.M. and Son P.N. (2007). Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology*, 19: 817-826.

Howard, P.S. (2008). Recasting the Machine Age: Henry Ford's Village Industries. pp. 46.

Iglesias-Rodriguez, M.D., Halloran, P.R., Rickaby, R.E.M., Hall, I.R., Colmenero-Hidalgo, E., Gittins, J.R., Green, D.R.H., Tyrrel, T., Gibbs, S.J.,Von Dassow, P., Rehm E., Armbrust, E.V. and Boessenkool, K.P. (2008). Phytoplankton calcification in a high-CO_2 world. *Science*, 320: 336-340.

IPCC, IEA, AR4, SYR. (2007). Core Writing Team; Pachauri, R.K; and Reisinger, A., ed., *Climate Change 2007: Synthesis Report*, Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, ISBN 92-9169-122-4.

International Energy Agency (2011). Energy for all: Financing access for the poor, Special early excerpt of the World Energy Outlook 2011, International Energy Agency, Paris. Retrieved from http//: www.iea.org/.

Jeffrey, L.S. (2005). Characterization of the coal resources of South Africa. *Journal of the South African Institute of Mining and Metallurgy*, 105(2): 95-102.

Jiménez del Río, M., Ramazanov, Z. and García-Reina, G. (1996). *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents. *Hydrobiologia*, 326: 61-66.

John, D.M. and Maggs, C.A. (1997). Species problems in eukaryotic algae: a modern perspective. *In* M.F. Claridge, H.A. Dawah & M.R. Wilson (eds.), *Species: Units of biodiversity*. *Systematics Association* (Special Volume), 54: 83-107.

Joska, M.A.P. (1992). Taxonomy of *Ulva* species (Chlorophyta) in the South Western Cape, South Africa. Unpublished MSc Dissertation. University of Cape Town.

Kalita, T.L. and Titlyanov, E.A. (2011). The effect of temperature on infradian rhythms of reproduction in *Ulva fenestrata* postels et ruprecht, 1840 (Chlorophyta: *Ulvales*). *Russian Journal of Marine Biology*, 37(1): 52-61.

Kandjengo, L. (2003). The Molecular systematics of *Ulva* Linnaeus and *Enteromorpha* Link (Ulvales, Chlorophyta) form the South Western Cape, South Africa. M.Sc Dissertation. University of Cape Town. South Africa.

Kang, Y.H., Shin, J.A., Kim, M.S. and Chung, I.K. (2008). A preliminary study of the bioremediation potential of Codium fragile applied to seaweed integrated multi-trophic aquaculture (IMTA) during the summer. *Journal of Applied Phycology*, 20: 183-190.

Kapazoglou, A.V., Drosou, C.K., Nitsos, I., Bossis, A., Tsaftaris, K.S., Triantafyllidis, A. and Hilioti, Z. (2013). Biofuels Get in the Fast Lane: Developments in Plant Feedstock Production and Processing. *Advance Crop Science Technology*, 1: 117. Retrieved from http//dx.doi.10.4172/2329-8863.1000117.

Karthikryan, R., Somasundram, S.T., Manivasagam, T., Balasubra-manian, T. and Anantharaman, P. (2010). Hepatoprotective activity of brown alga Paduina boergesenii against CCl4 induced oxidative damage in Wistar rats. *Asian Pacific Journal of Tropical Biomedicine*, 3: 696-701.

Kativu, E. (2011). Carbon dioxide absorption using fresh water algae and identifying potential uses of algal biomass. M.Sc Dissertation. University of the Witwatersrand. South Africa.

Kepenyes, R. and Varadi, L. (1984). Aeration and oxygenation in aquaculture. In FAO (ed.). Inland aquaculture engineering, FAO, Rome. Italy.

Khan W., Rayirath, U., Subramanian S., Jithesh, M., Rayorath P., Hodges, M., Critchley, A., Craigie, J., Norrie, J., Prithiviraj, B. (2009). Seaweed Extracts as Biostimulants of Plant Growth and Development. *Journal of Plant Growth Regulation*, 28(4): 386-399.

Khan, M.A., Khan, S. and Miyan, K. (2011). Aquaculture as a food production system: A review. *Biology and Medicine*, 3(2): 291-302.

Khotimchenko, S.V., Vaskovsky, V.E. and Titlyanova T.V. (2002). Fatty acids of marine algae from the Pacific Coast of North California. *Botanical Marina*, 45: 17-22.

Korzen, L., Abelson, A. and Israel, A. (2015). Growth, Protein and carbohydrate contents in *Ulva rigida* and Gracilaria bursapastoris integrated with an offshore fish farm. *Journal of Applied Phycology*, 1-11. Retrieved from http//dx.doi.org/10.1007/s10811-015-0691-5.

Kovac, D.J., Simeunovic, J.B., Babic, O.B., Misan, A.C. and Milovanovic, I.L. (2013). Algae in Food and Feed. *Journal of Food Feed Resources*, 40: 21-32.

Krom, M.D., Ellner, S., van Rijn, J. and Neori, A. (1995). Nitrogen and phosphorus cycling and transformations in a prototype 'non-polluting'integrated mariculture system, Eilat, Israel. *Marine Ecology Progress Series*, 118: 25-36.

Kübler, J.E., Johnston, A.M. and Raven, J.A. (1999). The effects of reduced andelevated CO_2 and O_2 on the seaweed Lomentaria articulata. *Plant, Cell and Environment*, 22: 1303-1310.

Kumari, P., Kumar, M., Gupta, V., Reddy, C.R.K. and Jha, B. (2010). Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chemistry*, 120: 749-757.

Langer, G., Nehrke, G., Probert, I., Ly, J. and Ziveri, P. (2009). Strain specific responsesof Emeliana huxleyi to changing seawater chemistry. *Biogeosciences*, 6: 2637-2646.

Lawton, R.J., Mata, L., de Nys, R. and Paul, N.A. (2013). Algal Bioremediation of Waste Waters from Land-Based Aquaculture Using *Ulva*: Selecting Target Species and Strains. *PLoS ONE*, 8(10): 10-21.

Le Treut, H., Somerville, R., Cubasch, U., Ding, Y., Mauritzen, C., Mokssit, A., Peterson, T. and Prather, M. (2007). Historical Overview of Climate Change. In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D.

Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Lemme, A. (2010). Availability and effectiveness of free amino acids in aquaculture. En: Cruz-Suarez, L.E., RicqueMarie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D.A. and Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N.L., México. ISBN 978-607-433-546-0. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 264-275.

Lewis, D.I. (1976). The Public Image of Henry Ford: An American Folk Hero and His Company. Wayne State University Press. pp. 146-154.

Li, P., Mai, K., Trushenski, J. and Wu, G. (2008). New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids, *Amino Acids*, 37(1): 43-53.

Li, P., Yin, Y.L., Li, D.F., Kim, S.W. and Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, 98: 237-252.

Littler, M.M. and Littler, D.S. (1980). The Evolution of Thallus Form and Survival Strategies in Benthic Marine Macroalgae: Field and Laboratory Tests of a Functional Form Mode *The American Naturalist*, 116(1): 25-44.

Littler, M.M., and Littler, D.S. (1981). Intertidal macrophyte communities from Pacific Baja California and the upper Gulf of California, relatively constant vs. environmentally fluctuating systems, *Marine Ecology Progress Series*, 4: 145-158.

Lobban, C.S. and Harrison, P.J. (eds.) (1997). Seaweed ecology and physiology. Cambridge University Press. Cambridge. pp.366.

Lutz, G., LeBlanc, B.D., Sheffield, R., Karen, E. and Nix, M.S. (2009). Aquaculture Environmental - BEST MANAGEMENT PRACTICES, BMPs Department of agriculture and forestry, Louisiana State University Agricultural Center, LSU AqCenter Research and extension Pub. 2894. pp.28. Retrived from www.LSUAgCenter.com

MacArtain, P., Gill, C.I.R., Brooks, M., Campbell, R. and Rowland, I.R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews,* 65: 535-543.

Madhusudan, C., Manoj, S., Rahul, K. and Rishi, C.M. (2011). Seaweeds: a diet with nutritional, medicinal and industrial value. *Research Journal of Medical Plant*, 5: 153-157.

Maggs, C.A. and Callow, M.E. (2003). Algal spores, version 1.0. In Encyclopedia of Life Sciences, Nature Publishing Group, London.

Mai, H., Foteda, R. and Fewtrel, J. (2008). Removal of inorganic nitrogen by integrating seaweed *Sargassum sp*. into western king prawn (*Penaeus latisulcatus*, Kishinouye 1896) culture Conference on International Research on Food Security, Natural Resource Management and Rural Development. Tropentag 2008 University of Hohenheim, October 7-9, 2008.

Makkar, H.P., Tran, G., Heuze, V., GigerReverdin, S., Lessire, M., Lebas, F. and Ankers, P. (2015). Seaweeds for livestock diets. A review. *Animal Feed Science and Technology*, 23(5): 447- 452.

Malta, E.J., Draisma, S.G.A. and Kamermans, P. (1999). Freefloating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. *European Journal of Phycology*, 34: 443- 454.

Mamboya, F.A. (2007). Heavy metal contamination and toxicity: Studies of Macroalgae from the Tanzanian Coast. MSc Dissertation, Stockholm University. Sweden.

Mann, K.H. and Lazier, J.R.N. (1991). Dynamics of Marine Ecosystems: Biological-Physical Interactions in the Oceans, Blackwell Scientific Publications: Boston.

Marchal, V., Dellink, R., van Vuuren, D., Clapp, C., Château, J., Lanzi, E., d Magné, B. and van Vliet, J. (2011). OECD Environmental Outlook to 2050, Climate Change Chapter (PRE-RELEASE VERSION) prepared by a joint team from the OECD Environment Directorate (ENV) and the PBL Netherlands Environmental Assessment Agency (PBL) November 2012. Retrieved from http://www.oecd.org/env/cc/49082173.pdf

Martins, I., Lopes, R.J., Lillebø, A.I., Neto, J.M., Pardal, M.A., Ferreira, J.G. and Marques, J.C. (2007). Significant variations in the productivity of green macroalgae in a mesotidal estuary: Implications to the nutrient loading of the system and the adjacent coastal area. *Marine Pollution Bulletin*, 54: 678-690.

Masser, M.P., Rakocy, T. and Losordo, M. (1999). Recirculatory aquaculture tank production system: South Regional aquaculture centre. SRAC Publicaton No. 452.

Mata, L., Santos, R., Chapman, A.R.O., Anderson, R J., Vreeland, V.J. and Davison, I.R. (2003). Cultivation of *Ulva rotundata* (Ulvales, Chlorophyta) in raceways, yield and biofiltratin. In proceedings of the 17th International Seaweed Symposium, Cape Town, South Africa, 28 January – 2 February 2001. (pp. 237-242). Oxford University Press.

Mata, L., Schuenhoff, A. and Santos, R. (2010). A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. *Journal of Applied phycology*, 22(5): 639-644.

Mathu, K. (2014). Towards Energy Sustainability in South Africa. *Mediterranean Journal of Social Sciences*, 5(27): 1686-1697.

McLaughlin, E., Kelly, J., Birkett, D., Maggs, C. and Dring, M. (2006). *Assessment of the Effects of Commercial Seaweed Harvesting on Intertidal and Subtidal Ecology in Northern Ireland.* Environment and Heritage Service Research and Development Series. No. 06/26.

Menendez, M., Martınez, M. and Comın, F.A. (2001). A comparative study of the effect of pH and inorganic carbon resources on the photosynthesis of three floating macroalgae species of a Mediterranean coastal lagoon. *Journal of Experimental Marine Biology and Ecology*, 256: 123-136.

Miller, M.R., Nichols, P.D. and Carter, C.G. (2008). n-3 Oil sources for use in aquaculture alternatives to the unsustainable harvest of wild fish. *Nutrition Research Reviews*, 21: 85-96.

Mmochi, A.J., Dubi, A.M., Mamboya, F.A. and Mwandya, A.W (2002). Effects of Fish Culture on Water Quality of an Integrated Mariculture Pond System. *Western Indian Ocean Journal of Marine Science*, 1(1): 53-63.

Morison, J.I.L. and Lawlor, D.W. (1999). Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant, Cell & Environment*, 22: 659-682.

Moss, S.M., Arce, S.M., Argue, B.J., Otoshi, C.A., Calderon, F.R.O. and Tacon, A.G.J. (2001). Greening of the blue revolution: efforts towards environmentally responsible shrimp culture. Craig L. Browdy and Darryl E. Jory editors. The new wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001. The World Aquaculture Society, Baton Rouge, LA USA.

Mouget, J.L. and Tremblin, G. (2002). Suitability of the Fluorescence Monitoring System (FMS, Hansatech) for measurement of photosynthetic characteristics in algae. *Aquatic Botany*, 74: 219-231.

Msuya, F.E. (2001). Experiments on macro algae cultivation and bio filtration capacity at Makoba and Muungoni sites, Zanzibar, Tanzania. Report submitted to the Institute of Marine Sciences under the aquaculture project. Zanzibar, Tanzania.

Msuya, F.E. (2004). The influence of culture regimes on the performance of seaweed biofilters in integrated mariculture. PhD Dissertation, Tel-Aviv University. Isreal.

Msuya, F.E. (2007). The effect of stocking density on the performance of the seaweed *recticulata* as a biofilter in earthen pond channels, Zanzibar, Tanzania. *Western Indian Ocean Journal of Marine Science*, 6(1): 65-72.

Msuya, F.E. (1998). Seaweed Farming: Livelihooddiversification in natural resource in paths for change. Experieces in particicpaionand democratization in Lindi and Mtwara regions, Tanzania. Rural integration project support (RIPS) programme. Phase II. Oy Finnagro Ab. pp. 85-86.

Msuya, F.E. and Neori, A. (2010). The performance of spray irrigated *Ulva lactuca* (Ulvales, Chlorophyta) as a crop and as a biofilter of fishpond effluent. *Journal of Applied Phycology*, 46(4): 813-817.

Murata, M. and Nakazoe, J. (2001). Production and use of marine algae in Japan. *Japan Agricultural Research Quarterly*, 35: 281-290.

Mustafa, G.M., Wakamatsu, S., Takeda, S., Umino, T. and Nakagawa, H. (1995). Effects of algae meal as a feed additive on growth performance, feed efficiency and body composition in red sea bream. *Fisheries Science*, 61: 25-28.

Mustafa, M.G. and Nakagawa, H. (1995). A review: Dietary benefits of algae as an additive in fish feed. *The Israeli Jurnal of Aquaculture, Bamidegeh*, 47(3-4): 155-162.

Mwakasonda, S.A. (2007). South Africa Low Carbon Scenario Report (2007). Energy research centre. University of Cape Town. South Africa.

Mwandya, A.W., Mtolera, M.S.P., Pratap, H.B. and Jiddaw, N.S. (2001). Macroalgae as biofilters of dissolved inorganic nutrients in an integrated mariculture tank system in Zanzibar. pp. 159-170.

Mwandya, A.W. (2001). Macroalgae as biofilters of effluents from integrated mariculture fish pond systems in Zanzibar, Tanzania. MSc Dissertation. University of Dar es Salaam. Tanzania.

NACA. (1989). Integrated Fish Farming in China. NACA Technical Manual 7. pp. 227.

Nag, A. (2007). Biofuels Refining and Performance.New York, NY: McGraw - Hill.

Nakagawa, H., Kasahara, S. and Sugiyama, T. (1987). Effect of Ulva meal supplementation on lipid metabolism of black sea bream (*Acanthopagrus schlegeli* B.). *Aquaculture*, 62: 109-121.

National Academy of Agricultural Sciences (2003). Seaweed cultivation and utilization. Policy Paper 22. National Academy of Agricultural Sciences, India. pp: 1-6. Retrieved from http://www.naasindia.org/Policy%20Papers/pp22.pdf

Robertson-Andersson, D.V. (2007). An experience of the XIX ISS. Forum Phycologium. Newsletter. *Phycological Socety of Southern Africa*, 65: 3-5.

Naylor, R.L., Goldburg, R.J., Primavera, J., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. (2001). Effects of Aquaculture on World fish Supplies: Issue in Ecology. *Ecological Society of America*, 8: 1-14.

Neilsen, M.M., Bruhn, A., Rasmussen, M.B., Olesen, B., Larsen, M.M., and Moller, H.B. (2012). Cultivation of *Ulva lactuca* with manure fro simultaneous bioremediation and biomass production. *Journal of Applied Phycology*, 24(3): 449-458.

Neori**,** A., Chopin, T., Troell, M. and Yarish, C. (2004). Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modem mariculture. *Aquaculture*, 231(1-4): 361-391.

Neori, A., Cohen, I. and Gordin, H. (1991). *Ulva lactuca* Biofilters for Marine Fishpond Effluents II. Growth Rate, Yield and C: N Ratio. *Botanica Marina*, 34: 483-489.

Newell, R., Cornwell, J. and Owens, M. (2002). Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study. *Limnological Oceanography*, 47: 1367-1379.

Nielsen, M.M., Bruhn, A, Rasmussen, M.B., Olesen, B., Larsen, M.M. and Mølle, H.B. (2011). Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *Journal of Applied Phycology*, 24: 499-458.

Nikolaisen, L., Jensen, P.D., Bech, K.S., Dahl, J., Busk, J., Brødsgaard, T., Rasmussen, M.O., Bruhn, A., Bjerre, A., Nielsen, H.B., Albert, K.R., Ambus, P., Kadar, Z., Heiske, S., Sander, B. and Schmidt E.R. (2011). Energy Production from Marine Biomass (*Ulva lactuca*) PSO Project No. 2008-1-0050.

Nirmala, A., Manjari, P. and Seepana, S. (2012). Biofiltration Efficiency of Three Sea Weed Species. *Journal of Research in Biology*, 2(4): 338-347.

Olischläger, M., Bartsch, I., Gutow, L. and Wiencke, C. (2013). Effects of ocean acidification on growth and physiology of *Ulva lactuca* (Chlorophyta) in a rockpool-scenario. *Phycological Research*, 61(3): 180-190.

Ort, D.R., Oxborough, K. and Wise, R.R. (1994). Depression of photosynthesis in crops with deficits in photoinhibition of photosynthesis from molecular mechanism to the field (Eds. NR Baker and JR Bowter), (BioScientific Publishers: Oxford), pp. 315-329.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A. and Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry*, 99: 98-104.

Ozigbo, E., Anyadike, C. and Adegbite, O.K.P. (2014). Review of aquaculture production and management in Nigeria. *American Journal of Experimental Agriculture*, 4(10): 1137-1151.

Pádua, M., Fontoura, P.S.G. and Mathias, A.L. (2004). Chemical composition of *Ulvaria oxysperma* (Kützing) bliding, *Ulva lactuca* (Linnaeus) and *Ulva fascita* (Delile). *Brazilian Archives of Biology and Technology*, 47(1): 49-55.

Pajusalu, L., Martin, G., Põllumäe, A. and Paalme, T. (2013). Results of laboratory and field experiments of the direct effect of increasing CO_2 on net primary production of macroalgal species in brackish-water ecosystems. *Proceedings of the Estonian Academy of Sciences*, 62(2): 148-154. Retrieved from www.eap.ee/proceedings.

Pangestuti, R. and Kim, S.K. (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods*, (3):255-266.

Park, S.R., Yun, H., Kang, Y.H., Lee, H.J., Ko, Y.W. and Kim J.H. (2014). The importance of substratum and elevation in recruitment and persistence of ulvoid algal blooms on rocky intertidal shores of the southern Korean coast. *Botanica Marina*, 57(1): 55-66.

Park, S.K., Howden, R. and Twell, D. (1998). The Arabidopsis thaliana gametophytic mutation gemini pollen1 disrupts microspore polarity, division asymmetry and pollen cell fate. *Development*, 125: 3789-3799.

Parker, L. and Blodgett, J. (2008). Greenhouse Gas Emissions: Perspectives on the Top 20 Emitters and Developed Versus Developing Nations. Congregational Research Service. CRS Report for congress. Order Code RL32721.

Pascoe, S. and Gréboval, D. (2003). Measuring Capacity in Fisheries. FAO Fisheries Technical Paper No. 445. Rome. Italy.

Paul, V.J., Arthur, K.E., Ritson-Williams, R., Ross, C. and Sharp, K. (2007). Chemical defenses: from compounds to communities. *Biological Bulletin*, 213:226-251.

Penaflorida, V.D. and Golez, N.V. (1996). Uses of seaweed meals from *Kappaphycus alvarezii* and *Gracilaria heteroclada* used as binders in diets for juveniles shrimp *Penaenus monodon*. *Aquaculture*, 143: 393-401.

Pereira, R., Valente, L., Pinto, I.S. and Rema, P. (2012). Apparent Digestibility of Nutrients from Seaweed in Rainbow Trout (*Oncorhynchus mykiss*) and Nile Tilapia (*Oreochromis niloticus*). *Algal Research*, 1(1): 77-82.

Perrot, T., Rossi, N., Ménesguen, A.and Dumas, F. (2014). Modelling green macroalgal blooms on the coasts of Brittany, France to enhance water quality management. *Journal of Marine Systems*, 132: 38-53.

Phang, S.M., Shaharuddin, S., Noraishah, H. and Sasekumar, A. (1996). Studies on Gracilaria changii (Gracilariales Rhodophyta) from Malaysian mangroves. *Hydrobiologia*, 326/327: 347-352.

Pickering, T.D., Gordon, M.E. and Tong, L.J. (1995). A preliminary trial of a spray culture technique for growing the agarophyte *Gracilaria chlensis* (Gracilariales, Rhodophyta). *Aquacuture*, 130(1): 43-49.

Plettner, I., Steinke, M. and Malin, G. (2005). Ethene (ethylene) production in the marine macroalga *Ulva* (Enteromorpha) *intestinalis* L. Chlorophyta, Ulvophyceae): Effect of light-stress and co-production with dimethyl sulphide. *Plant Cell and Environment*, 28(9): 1136-1145.

Poole, L.J. and Raven, J.A. (1997). The biology of *Enteromorpha*. *Progress in Phycological Research*, 12: 1-123.

Prasad, U., Deshmukhe, G., Dwivedi, A. and Singh, S.D. (2009). Detection of genetic variation in four *Ulva* species based on RAPD technique. *Indian Journal of Marine Science*, 38(1): 52-56.

Preston, N.P., Macleod, I., Rothlisberg, P.C. and Long, B. (1997). Environmentally sustainable aquaculture production: an Australian perspective, in D.A Hancock, D.C Smith, A. Grant & J.P Beumer (eds), *Developing and Sustaining World Fisheries Resources: The State of Science and Management*, Second World Fisheries Congress, Brisbane, 1996, volume 2: Proceedings, CSIRO, Melbourne. pp. 471-7.

Prieler, S. and Fischer, G. (2009). Agricultural by-products associated with biofuel production chains. Report of ELOBIO subtask 5.1. International Institute of Applied

Systems Analysis. Retrieved from http://www.iiasa.ac.at/Research/LUC/

Qing, X., Hongyuan, Z. and Yongcun, C. (2016). Multi-sensor monitoring of *Ulva prolifera* blooms in the Yellow Sea using different methods, *Frontiers of Earth Science*, 10(2): 378-388.

Radulovich, R., Neori, A., Valderrama, D., Reddy, C.R.K., Cronin, H. and Forster, J. (2015). Farming of seaweeds. In: B. Tiwari and D. Troy (Eds.). Seaweed Sustainability - Food and Non Food Applications. Elsevier. Amsterdam.

Raffaelli, D.G., Raven, J.A. and Poole, L. (1998). Ecological impact of green macroalgal blooms. *Oceanography Marine Biology*, 36: 97-125.

Rahman, A.K.A. (1989). Freshwater Fishes of Bangladesh, Zoological Society of Bangladesh, Dhaka. pp. 364.

Rahman, M.M., Varga, I .and Chowdhury, S.N. (1992). Manual on polyculture and integrated fish farming in Bangladesh. Food and Agriculture Organisation of the United Nations.

Rautenberger, R., Fernández, P.A., Strittmatter, M., Heesch, S., Cornwall, C.E., Hurd, C.L. and Roleda, M.Y. (2015). Saturating light and not increased carbon dioxide under ocean acidification drives photosynthesis and growth in *Ulva rigida* (Chlorophyta). *Ecological Evolution*, 5(4): 874-888.

Raven, J.A. and Taylor, R. (2003). Macroalgal growth in nutrient-enriched estuaries: a biogeochemical and evolutionary perspective. *Water Air Soil Pollution*, 3: 7-26.

Reed, D.C., Laur, D.R. and Ebeling, A.W. (1988). Variation in algal dispersal and recruitment: the importanceof episodicevents. *Ecology Monograph*, 58: 321-335.

Riccardi, N. and Solidoro, C. (1996). The Influence of Environmental Variables on *Ulva rigida* C. Ag. Growth and Production, *Botanica Marina*, 39(1-6): 27-32.

Riebesell, U., Schulz, K.G., Bellerby, R.G.J., Botros, B., Fritsche, P., Meyerhofer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J. and Zollnere, H. (2007). Enhanced biological carbon consumption in a high CO_2 ocean. *Nature*, 450: 545-549.

Robertson-Andersson, D.V. (2007). Biological and economical feasibility studies of using seaweeds *Ulva lactuca* (chlorophyta) in recirculation systems in abalone farming. PhD Dissertation, University of Cape Town, South Africa.

Robertson-Andersson, D.V. (2003). The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. MSc Dissertation, University of Cape Town, South Africa.

Robertson-Andersson, D.V., Amosu, A.O., Kean, E., Bauer, R. and Maneveldt, G.W. (2013). Industrial scale cultivation of *Ulva lactuca* for biofuel production. 11th Conference of the Aquaculture Association of Southern Africa Aquaculture, tagged “Fish Farm to Plate”. 9th - 13th September 2013 - Stellenbosch, South Africa. (Oral).

Robledo, D. and GarciaReina, G. (1991). Saweed spray cultivation technique. In seaweed cellular biotechnology, physiology and intensive cultivation. *Proceedings of a COST 48 (Subgroup 1) Workshop, held at Marine Plant Biotechnology Laboratory, University of Las Palmas,* Canary Islands, Spain, February 8-17, 1991. pp. 233.

Rodrigues da Mata, L.F. (2008). Integrated aquaculture of Bonnemaisoniaceae: physiological and nutritional controls of biomass production and of halogenated metabolite content. PhD Dissertation, Universidade do Algarve. Portugal.

Rodriguez, K., Jasso, R.M., Mussatto, S.I., Pastrana, L., Aguilar, C.N. and Teixeira, J.A. (2011). Microwave assisted extraction of sulfated polysaccharides (fucoidan) from brown eaweed. *Carbohydrate Polymers*, 86: 1137-1144.

Roleda, M.Y., Morris, J. N., McGraw, C.M. and Hurd, C.L. (2012). Ocean acidification and seaweed reproduction: increased CO_2 ameliorates the negative effect of lowered pH on meiospore germination in the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). *Global Change Biology*, 18: 854-64.

Romero, R. (2009). Recruitment strategies of *Ulva* and Porphyra in central California. MSc Dissertation. San Jose State University. U.S.

Ruangchuay, R., Dahamat, S., Chirapat, A. and Notoya, M. (2012). Effects of culture conditions on the growth and reproduction of Gut Weed, *Ulva intestinalis* Linnaeus (Ulvales, Chlorophyta). *Songklanakarin Journal of Science and Technology*, 34(5): 501-507.

Rubino, M. (ed.). (2008). Offshore Aquaculture in the United States: Economic Considerations, Implications & Opportunities. U.S. Department of Commerce; Silver Spring, MD; USA. NOAA Technical Memorandum NMFS F/SPO-103. pp. 263.

Rybak, A., Messyasz, B. and Łęska B. (2012). Freshwater Ulva (Chlorophyta) as a bioaccumulator of selected heavy metals (Cd, Ni and Pb) and alkaline earth metals (Ca and Mg). *Chemosphere*, 89: 1066-1076.

Sadek, S. (2011). An overview on desert aquaculture in Egypt. In V.Crespi & A. Lovatelli, eds. Aquaculture in desert and arid lands: development constraints and opportunities. FAO Technical Workshop. 6–9 July 2010, Hermosillo, Mexico . FAO Fisheries and Aquaculture Proceedings No.20. Rome, FAO. 2011. pp. 141-158.

Saeed, S.M. and Moustafa, Y.T.A. (2013). The seaweed (green macroalgae), Ulva sp. as bioindicator of metal pollution in the Mediterranean Coast, Alexandria region, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, 17(3): 57-68.

Salinas, J.M. (1999). Spray system for re attachment of *Gelidium sequipedale* (Clem.) Born. Et Thur. (Gelidiales, Rhodophyta). In International Workshop on Gelidium. Springer Netherlands. pp. 107-117.

Sanchez-Machado, D.I., Lopez-Cervantes, J., Lopez-Hernandez, J. and Paseiro-Losada, P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, 85: 439-444.

Sand-Jensen, K. (1988). Photosynthetic responses of *Ulva lactuca* at very low light. *Marine Ecology Progress Series*, (50): 195-201.

Sarker, S., Bruhn, A., Ward, A.J. and Møller, H.B. (2012). Bio-Fuel from Anaerobic Co-Digestion of the Macro-Algae *Ulva lactuca* and *Laminaria digitata*. Renewable Energy and Energy Efficiency, Biogas and biofuel production technologies. Retrieved from http://www.yumpu.com/en/biofuel.

Schonfeld Leber, B. (1979). Marine algae as human food in Hawaii, with notes on other Polynesian islands. *Ecology of Food and Nutrition*, 8(1): 47-59.

Seaborn, T. (2014). Limpets and Their Algal Epibionts: Costs and Benefits of Acrosiphonia spp and *Ulva lactuca* Growth. *Journal of Marine Biology*, 2014: 1-7.

Sebaaly, C., Karaki, N., Chahine, N., Evidente, A., Habib, Y. and Kannaan, J. (2012). Polysaccharides of the red algae Pteroclaia growing on the Lebanese coast: Isolation, structural features with antioxidant and anticoagulant activities. *Journal of Applied Pmarmacuetical Science*, 2(10): 1-10.

Searles, R.B. (1980). The Strategy of the Red Algal Life History. *The American Naturalist,* 115(1): 113-120.

Sfriso, A. (2010). Acque di transizione – Macrofite, flora acquatica. *In*: AA.VV. *Validazione sperimentale delle metodiche di campionamento e analisi degli elementi di qualità biologica per fiumi e acque di transizione ai sensi della Direttiva 2000/60/CE – Relazione finale*. Regione Emilia Romagna, Assessorato Ambiente e Arpa Emilia-Romagna Struttura Oceanografica Daphne, pp.147-154.

Shimda, S., Yokoyama, N., Arai, S. and Hiraoka, M. (2008). Phylogeography of the genus *Ulva* (Ulvophyceae, Chlorophyta, with special reference to the Japanese freshwater and brackish taxa. In *Nineteenth international seaweed symposium*. pp. 529-539.

Shipton, T. and Britz, P.J. (2007). A Study on the Status of Aquaculture Production and Trade in South Africa. Volume 1: Industry Status and Diagnostic Report. A report for the Department of Trade and Industry produced by Enviro-Fish Africa (Pty.) Ltd. pp. 90.

Shpigel, M. and Neori, A. (2007). Microalgae, Macroalgae, and Bivalves as Biofilters in Land-Based Mariculture in Israel.In book: Ecological and Genetic Implications of Aquaculture Activities, Chapter: 24, Publisher: Klewer Publications, Dordrecht, Editors: Bert, T.M., pp.433-446.

Shpigel, M., McBride, S.C., Marciano, S., Ron, S. and Ben-Amotz, A. (2005). Improving gonad colour and somatic index in the European sea urchin *Paracentrotus livid*. *Aquaculture*, 245: 101-109.

Shpigel, M., Ragg, N.L., Lapatsch, I. and Neori, A. (1999). Protein content determines the nutritional value of the seaweed *Ulva lactuca* L. for the abalone *Haliotis tuberculata* L. And *H. discus hannai*. *Journal of Shellfish Research*, 18: 227-233.

Shuuluka, D. (2011). Ecophysiological studies of three South African *Ulva* species from integrated seaweed/abalone aquaculture and natural. PhD Dissertation. University of Cape Town, South Africa.

Silva, P.C., Basson, P.W. and Moe, R.L. (1996). Catalogue of the benthic Marine algae of the Indian Ocean. University of California Press. Berkeley.

Sims, R.E.H., Schock, R.N., Adegbululgbe, A., Fenhann, J., Konstantinaviciute, I., Moomaw, W., Nimir, H.B., Schlamadinger, B., Torres-Martínez, J., Turner, C., Uchiyama, Y., Vuori, S.J.V., Wamukonya, N.N. and Zhang, X. (2007). Energy Supply In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth

Assessment Report of the Intergovernmental Panel on Climate Change [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Smith, A.H. and Renard, Y. (2002). Seaweed Cultivation as a Livelihood in Caribbean Coastal Communities. A Paper Presented at the ICRI Regional Workshop for the Tropical Americas: Improving Reef Conditions through Strategic Partnerships. Cacun, Mexico, CANARI Communication No. 309, pp.8.

Smith, M.D., Roheim, C.A., Crowder, L.B., Halpern, B.S., Turnipseed, M., Anderson, J.L., Asche, F., Bourillón, L., Guttormsen, A.G., Kahn, A., Liguori, L.A., McNevin, A., O'Connor, M., Squires, D., Tyedemers, P., Brownstein, C., Carden, K., Klinger, D.H., Sagarin, R. and Selkoe, K.A. (2010). Sustainability and Global Seafood. *Science*, 327: 784-786.

SoE (Australian State of the Environment Committee) (2001). Coasts and Oceans, Australia State of the Environment Report 2001 (theme Report), CSIRO Publishing on behalf of the Department of the Environment and Heritage, Canberra.

Soto, D. (2009). Integrated mariculture: A global review. FAO Fisheries and Aquaculture Technical Paper 529, FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy.

Specht, Z. (2011). Biofuels History and Review. Energy Discussion Group. Retrieved from internet, retrieved from http://solar.sdsu.edu/EDG_pdf/A%20Biofuels%20History%20and%20Review.pdf

Stadnik, M.J. and de Freitas, M.B. (2014). Algal polysaccharides as source of plant resistance inducers. *Tropical Plant Pathology*, 39(2): 111-118.

Stefels, J. (2000). Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *Journal of Sea Research*, 43: 183-197.

Steffensen, D.A. (1976). The effect of nutrient enrichment and temperature on the growth in culture of *Ulva lactuca* L. *Aquatic Botany*, 2: 337-351.

Stegenga, H., Bolton, J.J. and Anderson, R.J. (1997). Seaweeds of the South African West Coast. *Contributions from the Bolus herbarium*, 18: 655-662.

Tacon, G.J.A., Metian, M. and Hasan, M.R. (2009). Feed ingredients and fertilizers for farmed aquatic animals - sources and composition. Food and Agriculture Organization of the United Nations. FAO Fisheries and Aquaculture Technical Paper No. 540. Rome.

Tacon, G.J.A., Metian, M. and Primavera, J.H. (2006). Overcoming the impact of aquaculture on the coastal zone. *Ocean and Coastal Management*, 49(9): 531-545.

Tanner, C.S. (1986). Investigations of the taxonomy and morphological variation of *Ulva* (Chlorophyta): *Ulva californica* Wille. *Phycologia*, 25(4): 510-520.

Taylor, R., Fletcher, R.L. and Raven, J.A. (2001). Preliminary studies on the growth of selected 'green tide' algae in laboratory culture: effects of irradiance, temperature, salinity and nutrients on growth rate. *Botanica Marina*, 44: 327-336.

Teas, J. (2005). Dietary Brown Seaweeds and Human Health Effects. In: Critchley, A.T., O. Masao and M. Danilo (eds.), Seaweed Resources. Publisher Expert Centre for Taxonomic Identification, Amsterdam.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N. and Yarish, C. (2003). Integrated mariculture: asking the right questions. *Aquaculture*, 226: 69-90.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H. and Fang, J.G. (2009). Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, 297(1-4): 1-9.

Tseng, C.K. (1987). Laminaria mariculture in China, Case study of seven commercial seaweeds resources. FAO Fisheries Technical Paper, pp. 311.

Tseng, C.K. and Borowitzka M. (2003). Algae Culture. In: J.S. and P.C. Southgate (Eds.), Aquaculture: Farming Aquatic Animals and Plants, Lucas. Wiley-Blackwell, New York, ISBN-10: 0852382227.

Tuhy, L., Chowanska, J. and Chojnacka, J. (2013). Seaweed extracts as biostimulants of plant growth: review. *CHEMIK*, 67(7): 636-641.

Tujula, N.A. (2006). Analysis of the epiphytic bacterial community associated with the green alga *Ulva australis*. PhD Dissertation. University of New South Wales. Australia.

Turan, G. and Tekogul, H. (2014). The Turkish Mezzes Formulated with Protein Rich Green Sea Vegetable (Chlorophyta) *Ulva rigida* Cultured in Onshore Tank System. *Journal of Aquatic Food Product Technology*, 23(5): 447-452.

Ugoala, E., Ndukwe, G.I., Mustapha, K.B. and Ayo, R.I. (2012). Constraints to large scale algae biomass production and utilization. *Journal of Algal Biomass Utilization*, 3(2): 14-32.

United Nations Environment Programme (UNEP). (2010). Liquefied Petroleum Gas (LPG) Demand, Supply and Future Perspectives for Sudan, Ministry of Environment, Forestry and Physical Development – Sudan, Ministry of Petroleum – Sudan, United Kingdom Department for International Development, United Nations Development Programme and United Nations Environment Programme. Synthesis report of a workshop held in Khartoum, 12-13 December 2010. Retrieved from http//: www.unep.org/sudan.

United Nations Environment Programme (UNEP). (2012). Green Economy in a Blue World. Retrieved from www.unep.org/greeneconomy and www.unep.org/regionalseas.

Vairappan, C.S. and Suzuki, M. (2000). Dynamics of total surface bacteria and bacterial species counts during desiccation in the Malaysian sea lettuce, *Ulva reticulata* (Ulvales, Chlorophyta). *Phycological Research*, 48: 55-61.

Van den Hoek, C., Mann, D.G. and Jahns, H.M. (1995). Algae, An introduction to phycology. Cambridge University Press. Cambridge. pp. 390-408.

Van Ginneken, V.J.T., Helsper, J.P.F.G., de Visser, W., van Keulen, H. and Brandenburg, W.A. (2011). Polyunsaturated fatty acids in various macroalgal species from North Atlantic and tropical seas. *Lipids in Health and Disease*, 2011: 10:104. Retrieved from http://www.lipiworld.com/content/10/1/104.

Vandermeulen, H. and Gordin, H. (1990). Ammonium uptake using Ulva (Chlorophyta) in intensive fishpond systems: Mass culture and treatment of effluent. *Journal of Applied Phycology*, 2: 363-370.

Ventura, H.P. and Castanon, J.I.R. (1998). The nutritive value of seaweed (*Ulva lactuca) for goats. Small Ruminant Research*, 29(3): 325-327.

Vermaat, J.E. and Sand-Jensen, K. (1987). Survival, metabolism and growth of *Ulva lactuca* L. under winter conditions: a laboratory study of bottlenecks in the life cycle. *Marine Biology*, 95: 55-61.

Viaroli, P., Bartoli, M., Azzoni, R., Giordani, G., Mucchino, C., Naldi, M., Nizzoli, D. and Taje, L. (2005). Nutrient and iron limitation to Ulva blooms in a eutrophic coastal lagoon (Sacca di Goro, Italy). P. Viaroli, M. Mistri, M. Troussellier, S. Guerzoni & A.C. Cardoso (eds.), Structure, Functions and Ecosystem Alterations in Southern European Coastal Lagoons. *Hydrobiologia*, 550: 57-71.

Wahbeh, M.I. (1997). Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. *Aquaculture*, 159: 101-109.

Ward, S. and Walsh, V. (2010). Energy for Large Cities Report, World Energy Congress. Energy and Climate Change Branch Environmental Department City of Cape Town Resource Management City of Cape Town. South Africa.

Wassef, E.A. (2005). Alternative protein sources for fish feeds in Egypt. In Montero, D., Basurco, B., Nengas, I., Alexis, M., Izquierdo, M. (eds.). Mediterranean fish nutrition. Zaragoza: CIHEAM, *Cahiers Options Méditerranéennes*, 63: 127-141.

Wassef, E.A., El-Sayed, A.F.M., Kandeel, K.M. and Sakr, E.M. (2005). Evaluation of Pterocladia (Rhodophyta) and *Ulva* (Chlorophyta) Meals as additives to Gilthead Seabream Sparus Aurata diets. *Egyptian Journal of Aquatic Research*, 31: 321-332.

Wassef, E.A., El-Masry, M.H. and Mickhail, F.R. (2001). Growth enhancement and muscle structure of stripped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research*, 32(Suppl.1): 315-322.

Weich, R.G. and Graneli, E. (1989). Extracellular alkaline phosphatase activity in *Ulva lactuca* L. *Journal of Experimental Marine Biology and Ecology*, 129: 33-44.

Wijkström, U.N. (2009). The use of wild fish as aquaculture feed and its effects on income and food for the poor and the undernourished. In M.R. Hasan and M. Halwart (eds). Fish as feed inputs for aquaculture: practices, sustainability and implications. Fisheries and Aquaculture Technical Paper. No.518. Rome, FAO. pp. 371-407.

Williamson, P. and Turley, C. (2012). Ocean acidification in a geoengineering context. *Philosophical Transaction of the Royal Society*, 370: 4317-4342.

Winter, C.J. (2009). Hydrogen energy - Abundant, efficient, clean: A debate over the energy-system-of-change. *International Journal of hydrogen energy*, 4: 51-52.

Wong, K.H. and Peter, C.K. (2000). Nutritional evaluation of some subtropical red and green seaweeds: Part I — proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry*, 71: 475-482.

World Energy Outlook (2015). Special report on energy and climate change. Retrieved from http//www.iea.org/publications.

World Bank (2008). World Development Report 2008: Agriculture for Development. World Bank, Washington, DC.

World Population Prospects (2009). The 2008 Revision". Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat. June 2009. Retrieved from http//: www.un.org/esa/population/publications/wpp.2008.

Wosnitza, T.M.A. and Barrates, J. G. (2006). Utilization of seaweed *Ulva* sp. In Paracas bay Peru), experimenting wth compost. *Journal of Applied phycology*, 18(1): 27-31.

Wu, R.S.S. (1995). The environmental impacts of marine fish cul-ture: towards a sustainable future. *Marine Pollution Bulletin*, 31: 159-166.

Wu, H., Zou, D. and Gao, K. (2008). Impact of increased atmospheric CO_2 concentration on photosynthesis and growth of micro and macroalgae. *Science in China Series C, Life Science*, 51(12): 1144-1150.

Xu, Z., Zou, D. and Gao, K. (2010). Effects of elevated CO_2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina*, 53: 123-129.

Yang, Y. (2013). Effects of Temperature, Light Intensity and Quality, Carbon Dioxide, and Culture Medium Nutrients on Growth and Lipid Production of *Ettlia oleoabundans.* PhD Dissertation. Worcester Polytechnic Institute. U.S.

Yokoyama, H. and Ishihi, Y. (2010). Bioindicator and biofilter function of *Ulva lactuca* sp. (Chlorophyta) for dissolved inorganic nitrogen discharged from a coastal fish farm, potential role in integrated multitrophic aquaculture. *Aquaculture*, 310(1): 74-83.

Yong, J., An, L., Wang, Q., Wang, H., Su, Q. and King, X. (2000). Application of RAPD in *Ulva* and *Enteromorpha*. *OceanoLimnol- Sin, Hayang – Yu - Huzhao*, 31(4): 408-413.

Zhao, Y., Hao, Y. and Ramelow, G.J. (1994). Evaluation of treatment techniques for increasing the uptake of metal ions from solution by nonliving seaweed algal biomass. *Environmental Monitoring and Assessment*, 33(1): 61-70.

Zou, D. and Gao, K. (2002). Photosynthetic responses to Inorganic carbon in *Ulva lactuca* under aquatic and aerial states. *Acta Botanica Sinica*, 44: 1291-1296.

Zou, D., Gao, K. and Run, Z.X. (2007). Daily timing of emersion and elevated atmospheric CO2 concentration affect photosynthetic performance of the intertidal macroalga *Ulva lactuca* (Chorophyta) in sunlight. *Botanical Marina*, 50: 275-279.

Zou, D., Gao, K., Xia, J., Xu, Z., Zhang, X. and Liu, S. (2007). Responses of dark respiration in the light to desiccation and temperature in the intertidal macroalga, *Ulva lactuca* (Chorophyta) during emersion. *Phycologia*, 46: 363-370.

Zou, D. (2005). Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, Hizikia fusiforme (Sargassaceae, Phaeophyta). *Aquaculture*, 250: 726-735.

Chapter 10

Acknowledgements

First and foremost, praise goes to God, for blessing me with the wellbeing, courage, strength, and endurance to finally finish this work. I am very fortunate to have conducted my study under able and enthusiastic supervisors, Prof. Gavin W. Maneveldt: Thank you for accepting me as a PhD student and bringing me into the world of modern science. Your constructive ideas, critical approaches to data interpretation, and tireless guidance were invaluable to the accomplishment of this work. To my co-supervisors, Dr. Deborah V. Robertson-Andersson, You readily answered my integrated aquaculture questions, provided constant and spontaneous discussion, helped put ideas into my head, and for promptly correcting my drafts with thoroughness and preciseness. I can't forget the regular long drive to the farms (I & J Cape Cultured Abalone and Benguela Holdings) at West coast and working in the pool of smelly seaweed with you. Prof. John J. Bolton, Thank you for the thorough criticisms and introducing me to the world of algae eco-physiology. Through you I found myself in the cold room conducting research! I appreciate your support. Thank you all for sharing your world class experiences and indepth knowledge in the area of seaweed aquaculture, ecology and physiology and indeed for granting me the chance of being your student.

Many thanks to TETFUND and the managements of Adeniran Ogunsanya College of Education (AOCOED), Lagos, Nigeria, especially the Provost, Mr. Olalekan W. Bashorun for granting me the study leave and for his financial support. With this support I was able to learn new things and met new people in my life. Without your support, I could not have managed.

To my colleagues and students at the Department of Agricultural Science (AOCOED), Ekiti State University (AOCOED): Thank you. You have made my journey very

enjoyable and colourful. To members of my research group, Bahai, Courtney and Elizabeth in the marine biology/ coralline algae / integrated seaweed aquaculture and the entire staff of the BCB department: Thank you very much for sharing all the wonderful moments that finally I accomplish this study. The working environment was very pleasant because of you.

Outside of academia, I wish to extend my sincere gratitude to my family, especially my dad Emmanuel Omoyele Amosu and to my siblings - Oluwabukola, Olawunmi, Oluwafunmilayo, Seun, Funke, Femi, Tosin and Oluwadamilola, aunts, uncles, and church brethrens for your love, prayers, and encouragements. You were always there for me. My loving mum Mrs S. O Adenekan-Williams (Omidare) thanks for your care. To my friends, Dr Ayofe Hammed, Mr. Paul Williams, Isaiah Ajose, Barr. ‘Remi Ojeyomi, Barr. ‘Femi Egbeyemi, Barr. Toyin Oloyede, Femi Zubar, Sunday Viho and Femi Hunga, I say: ‘Thank You’.

To the Nigerian community in UWC, UCT, US, CPUT and DUA group in Cape Town, South Africa, Dr ‘Seun Babalola, Jeremiah Arowosegbe, Kehinde Agbele, Sunday Vodah, James Ayuk, Kemi Tovide, Jelil Badmus, Femi Alamu, John Alegbe, Wale Olowu, Seun Fadipe, Femi Alamu, Paul Thovoethin, Ayodele Toyin, Ademola Rabiu, Mukadas Akindele, Habeeb Bankole etc. I really appreciate your cohort.

My sincere appreciation goes to Mr.‘Niyi Togunde for all his support and elderly advice in making this journey a reality. Also special thanks go to my kids: you guys’re the most important and unique gift in my life. It is impossible to mention every person who contributed to the successful accomplishment of this study. But I extend my sincere appreciation to every person (in and out of academia) who in one way or another facilitated the successful completion of this programme. God bless you all!