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THE EFFECTS OF TEMPERATURE AND NUTRIENTS ON THE GROWTH OF SOME PEST SEAWEEDS IN FIJI

by

Harshna Kumari Charan

A thesis submitted in fulfillment of the requirements for the degree of
Masters of Science in Climate Change

Pacific Centre for Environment and Sustainable Development (PaCE-SD)
The University of the South Pacific

October, 2017

DECLARATION OF ORIGINALITY

Statement by Author

I, Harshna Kumari Charan declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with the material submitted for the award of any other degree at any institute, except where due acknowledgment is made in the text and reference.

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Statement by the Principal Supervisor

The research in this thesis was performed under my supervision and to my knowledge is the sole work of Harshna Kumari Charan.

Signature... Date.. 13 Nov 2017

Name. Antoine De Ramon N'Yeurt

Designation. Principal Supervisor

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ABSTRACT

Macroalgal blooms are becoming a problem in many countries worldwide. Climate change is further exacerbating this problem. This study looks at the individualistic effects of temperature, nutrients and the combined effects of temperature and nutrients on the growth of two pest macroalgae namely *Sargassum polycystum* and *Gracilaria edulis* under laboratory conditions. In the temperature phase of the experiment, the biomass and rhizoidal length of the seaweeds from Makaluva Island were measured. The macroalgae were subjected to different temperature regimes (28 ℃, 30 ℃, 32 ℃ and 34 ℃) and sodium nitrate nutrient concentrations (1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L). The optimum temperature for *G. edulis* Specific Growth Rate (SGR) was between the ranges of 28 ℃ - 32 ℃. The optimum temperature for *G. edulis* and *S. polycystum* Rhizoidal Length (RL) occurred between 32 ℃ - 34 ℃ and 28 ℃ - 34 ℃ respectively. The SGR and RL of *G. edulis* and RL of *S. polycytsum* had the optimum growth at 4 mg/L for the nutrient phase of the study. The % tissue nitrogen, phosphorus and potassium (% NPK) was determined for both seaweeds. The optimum % tissue nitrogen content for *G. edulis* was at 2 mg/L while for *S. polycystum* it was 1 - 4 mg/L. The optimum % P tissue content was at 2 - 4 mg/L for *G. edulis* while for *S. polycystum* it was 1 - 4 mg/L of sodium nitrate concentration. The optimum % K tissue content was at 3 - 4 mg/L for *G. edulis* while for *S. polycystum* it was 1 mg/L of sodium nitrate concentration. The synergistic effects of temperature and sodium nitrate nutrients derived from the temperature and nutrient phase of the study were also investigated; the % K was the highest in all the combined temperature and nutrients treatment. Contrary to expectation % tissue nitrogen was low even though sodium nitrate solution was administrated however % K was generally higher possibly due to seaweeds not taking in nitrogen.

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
SST	Sea Surface Temperature
PCCSP	Pacific Climate Change Science Programe
IPCC	Intergovernmental Panel on Climate Change
N	Nitrogen
P	Phosphorus
K	Potassium
FAO	Food and Agriculture Organization
NO_3^-	Nitrate ions
NH_4^+	Ammonium ions
PO_4^{3-}	Phosphate ions
PSII	Photosystem II
IMTA	Integrated Multi-Trophic Aquaculture
RCP	Representative Concentration Pathways
CMIP5	Coupled Model Intercomparison Project Phase 5
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
% NPK	Percentage Nitrogen, Phosphorus, Potassium
SGR	Specific growth rate
RL	Rhizoidal Length

UNITS OF MEASUREMENTS

Ls^{-1}	Liters per second (Unit for aeration volume)
ppm	parts per million
ppt	parts per trillion
μM	micro molar
mg/L	milligram per liter
g	grams
cm	centimeter
mm	milimeter
%	Percentage
°C	Degree Celsius
‰	Water Salinity
mol/L	moles per Liter
mg/g	milligrams per grams

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

Context

"Seaweeds are ecologically important primary producers, competitors, and ecosystem engineers that plays a central role in coastal habitats ranging from kelp forests to coral reefs….the impacts of ongoing and future anthropogenic climate change in seaweed-dominated ecosystems remain poorly understood" (Harley *et al*., 2012).

The current climate of the ocean varies and it is the interaction between the ocean and the atmosphere that drives the Earth's climate through interchanging heat, water and wind (Pacific Climate Change Science Programe, 2011). There are many components such as the oceanic and atmospheric circulation but one of the most important components is the Sea Surface Temperature or SST (PCCSP, 2011). Climate change has caused a ripple effect by affecting not only the lives of the people of the Earth but the entire ecological functioning and processes of the marine environment and organisms (Harley *et al*., 2012).

The fight for global power which was fueled by industrial growth and anthropogenic activities in the early 1800s, drastically resulted in a rapid increase in carbon dioxide gases by the early 20th century (Dressler, 2012; PCCSP, 2011; Intergovernmental Panel on Climate Change, 2013) (See Figure 1.0). The rates at which humans are contributing to the greenhouse gases suggest that by 2100, the surface temperature of the Earth would have risen by four degrees Celsius (IPCC, 2013). The current concentration of carbon dioxide in the atmosphere has reached the 400 mg/L threshold sealing the fate of the Earth to increasing temperatures in the future (IPCC, 2013). At the same time the ocean is absorbing the excess carbon dioxide present in the atmosphere causing an increase in oceanic acidity (Dressler, 2012; IPCC, 2013).

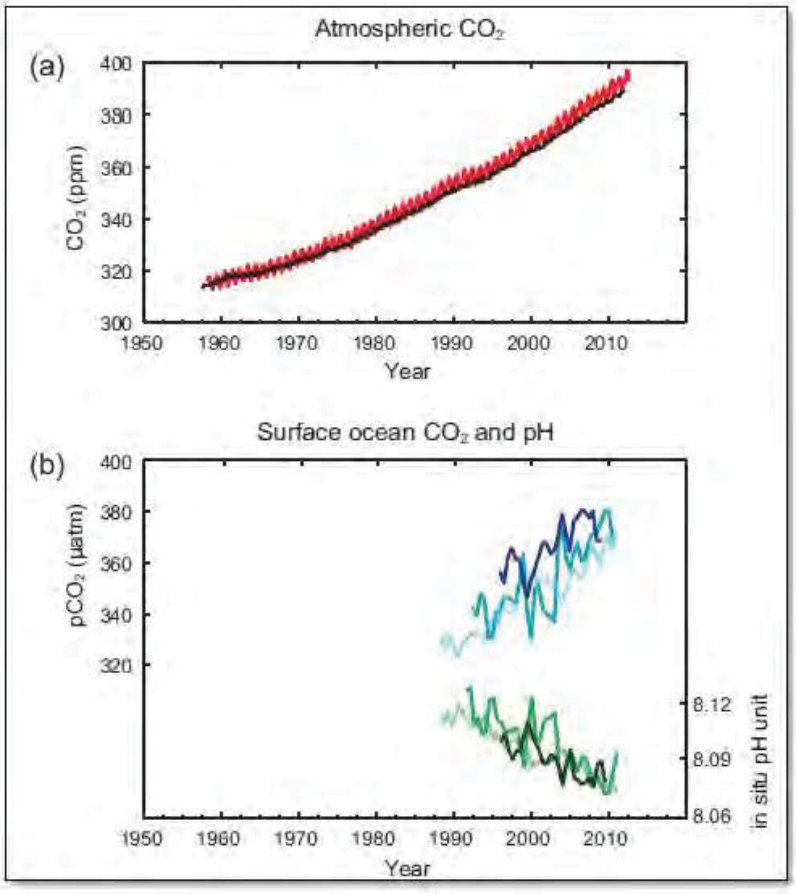

Figure 1.0 (a) Amount of Carbon dioxide in mg/L from Mauna Loa and South Pole (b) Partial pressure of dissolved carbon dioxide at the ocean surface.

Source: IPCC (2013)

The anthropogenic activity does not only include emissions of carbon dioxide into the atmosphere that are causing changes in the functioning of atmospheric, oceanic and ecological processes but other activities such as the excessive use of fertilizers that run off into rivers and streams (Mosley & Aalbersberg, 2003; Mosley & Aalbersberg, 2005; Hoey & Bellwood, 2010). Oceans are biologically rich and are home to many marine organisms (International Union for Conservation of Nature, 2009). As pristine marine resources are depleted or altered, the environment becomes habitable for invasive pest species and less inhabitable for native species (Loyolaa *et al.*, 2012). Pest species usually harm the environment by displacing the native species thus causing an ecological regime shift (McCook, 1999).

One such group of invasive pest are opportunistic seaweeds (Anderson, 2007). Seaweeds are important primary producers, competitors and photoautotrophs of the

marine environment where they are preyed upon by herbivores and humans alike (Harley *et al.*, 2012). According to McHugh (2005), seaweeds can be utilized in many different ways from being used as food to organic fertilizers. Macroalgae are multicelluar seaweeds that form a thallus (Food and Agriculture Organisation, 2003). There are some macroalgae that are regarded as opportunistic pest species in Fiji; two such macroalgae are the brown seaweed *Sargassum polycystum* and the red seaweed *Gracilaria edulis* (Johnson, 2007).

The occurrence of *S. polycystum* and *G. edulis* has become prevalent in the Pacific, especially Fiji recently (Johnson, 2007). For example, *S. polyyctsum* and *G. edulis* among other seaweeds are being washed ashore since 2009 at the Nasese foreshore in Fiji causing a foul smell (N'Yeurt & Iese, 2015; Andréfouët *et al*., 2017). The densely populated atoll of Funafuti in Tuvalu has been greatly affected by the invasion of *S. polycystum* into its lagoon waters (N'Yeurt & Iese, 2015). The abundance of *S. polycystum* resulted in multitudinal problems such as foul smell, unpleasant sight affecting tourism, loss of biodiversity and turbid waters (N'Yeurt & Iese, 2015). The invasive pest seaweed was found not to be a native species, rather an introduced invasive species which could have entered Tuvaluan waters through commercial shipping from Wallis Island (N'Yeurt & Iese, 2015). The abundance in nature has been mostly attributed to decreased herbivory and the fact that seaweeds have mechanisms to obtain, use, and accumulate a variety of forms of nitrate and phosphate found in the marine environment (Hanisak, 1990; Tomas *et al.*, 2011).

1.1. Justification of Current Research

Climate Change is a consequence of the greenhouse gas emissions which began in the mid-20th century (IPCC 2013). Almost 26% of produced carbon dioxide is absorbed by the oceans (PCCSP, 2011). However, what affects the Pacific Island Countries the most is sea level rise and food security issues (PCCSP, 2011). Sea level rise can further exacerbate food security issues as most crops cannot tolerate and grow in salty waters (FAO, 2007). *Sargassum polycystum* and *Gracilaria edulis* are two abundant seaweeds found in the Pacific which are marine pest species that can thrive in conditions that may prove to be harsh for other marine organisms (Johnson, 2007). *S. polycystum* proliferation in Tuvalu occurred in 2010 and since the abundance has become

prevalent becoming densely clustered by 2014 (Andréfouët *et al*., 2017). They are an edible form of seaweeds; however, their growth like any other macroalgae is mostly driven by increased nutrient concentrations in the waters (FAO, 2012). Consequently, impacts from future anthropogenic climate in the form of increased temperature and nutrients could have a significant impact positively or negatively on the growth of these two species of macroalgae. In addition, seaweed cultivation could provide a step closer to solving over abundance and possibly pave way for renewable energy resource. For instance, a Norwegian research group has been able to achieve bio-oil yields of 79% from *Laminaria saccharina,* mitigating macroalgal abundance as well (Bazilchuk, 2014). Thus it is imperative to analyze the growth of these seaweeds at different temperatures and also to determine the uptake of nutrients and at which uptake concentrations would be optimal. This would enable us to understand algal overabundance in the future and how it will affect food security if nutrient runoff is not curbed.

1.1.1. Hypotheses

- An increase in seawater temperature favors the growth of *S. polycystum* and *G. edulis* under controlled laboratory conditions.
- An increase in nutrient concentrations favors the growth of *S. polycystum* and *G. eduli* under controlled laboratory conditions.

1.1.2. Objectives

- To investigate the effect of temperature on the growth of *S. polycystum* and *G. edulis* under laboratory conditions.
- To examine the effect of nutrients on the growth of *S. polycystum* and *G. edulis* under laboratory conditions.
- To analyze the synergistic effects of temperature and nutrients on the growth and of *S. polycystum* and *G. edulis* under laboratory conditions.

1.2. Importance of This Study

The ability of macroalgae to absorb nutrients from the environment can have both negative and positive consequences. While increased nutrients lead to problematic algal blooms, a preliminary study done by N'Yeurt & Iese (2015) discovered that some

people on the island of Tuvalu were applying dried *S. polycystum* as a fertilizer which resulted in good growth of cucumber and water melons.

1.2.1.1. Bioremediation

The seepage of fertilizers from farms into rivers, streams and oceans has been largely blamed for the abundance of macroalgae. However according to Daudi *et al.* (2012) sewage discharges can also result in algae blooms. In the Pacific, sewage and fertilizer inputs are often unmanaged and both contribute to algal blooms and in many small island communities latrines and septic tanks are not available or leaking (N'Yeurt & Iese, 2015). Unsustainable development is also becoming a problem. For instance sewage from many hotels is being dumped directly into oceans without being safe for the environment (Harley *et al*., 2012; Mosley & Aalbersberg, 2003, 2005). However macroalgae have the immense ability to remove nutrients and carbon dioxide from the marine environment (Nian-Zhia *et al*., 2015). This ability makes them very useful as bioremedients. Moreover some opportunistic macroalgae such as *Enteromorpha intestinalis* have the ability to act as biological indicators for pollutants (Fong *et al*., 1998). From this study it was discovered that *S. polycystum* and *G. edulis* both grow well under nutrient enriched conditions. Both these species of macroalgae could be used as an indicator for marine pollution. Preliminary studies done on *S. polycystum* and *G. edulis* bloom along the coastal areas of Fiji has been associated with seepage of sewage and fertilizers which has been used as an indicator that waters are polluted (Mosley & Aalbersberg, 2003, 2005). According to Farley (2015), harvesting seaweeds will result in nitrogen being exported and at the same time remove excess nitrate from the environment. The procedure is often effective at driving nitrate levels below those detectable by most aquarium nitrate kits (about 0.5 ppm). This process also has the advantage of exporting phosphorus.

1.2.1.2. Integrated Multi - Trophic Aquaculture (IMTA)

The amount of excess nitrate in the water column ends up polluting the environment however there are many ways to mitigate pollution. It becomes the responsibility of the aquaculture industry to find solution to mitigate this effect (Chopin *et al*., 2001). Pioneering ideas are required to allow for better management of natural resources and

one of these is Integrated Multi - Trophic Aquaculture (IMTA). IMTA is a type of farming whereby organisms from different tropic levels with harmonizing ecosystem are grown together with the wastes from one organism serving as the nutrient source for others, and vice-versa in a recycle manner (Chopin *et al*., 2012; Clements & Chopin, 2016; Martinez-Espiñeira *et al*., 2016). Macroalgae serve as food for fishes, shrimps and other marine organisms which makes it ideal for seaweeds to be introduced into IMTA systems (Chopin & Neish, 2014a; Chopin *et al*., 1999b). According to Chopin *et al*. (1999a) seaweeds such as *Porphyra* require large amounts of nutrients which makes it ideal to be incorporated into IMTA. In another study Martinez-Espiñeira *et al.* (2016) discovered that the by-products from this system can be captured and converted into fertilizers. This system provides bio-mitigation services to the environment and enhanced cost-effective farm output through the co-cultivation of complementary species (Chopin *et al*., 2012; Chopin *et al.*, 1990).

In addition, seaweeds have the potential for nutrient and carbon trading (Chopin *et al*., 2012; Clements & Chopin, 2016; N'Yeurt & Iese, 2013). These unique features of macrolgae allow for mitigation of climate change. The two species studied in this research, *S. polycystum* and *G. edulis* could have the potential to be incorporated into an IMTA system due to being resilient to rising temperatures and increasing nutrient concentration, consequently allowing for a sustainable management of the environment through their controlled cultivation.

1.2.1.3. Pharmaceutical uses and active compounds from seaweeds

One of the most recently discovered uses of seaweeds is for pharmaceutical products and bio-active compounds (Gomez-Gutierrez *et al*., 2011). In a study done by Francavilla *et al.* (2013) on *G. gracilis*, the species contained high amounts of antioxidants. Secondary metabolites from red seaweeds are dominated by terpenes and halogenated phenols which show biological activities (Lin *et al*., 2010). According to Rasher & Hay (2010), seaweeds have allelochemicals that suppress coral recruitment. Red seaweed has been found to be effective against fungi and could have anti-malarial properties (Pennisi, 2014). In addition, seaweeds contain plant growth stimulating compounds however these bio-stimulating capabilities have not been fully explored (Khan *et al*., 2009).

1.2.1.4. Seaweeds as a Food Source

Seaweeds are high in nutritious proteins (FAO, 2016). They have been eaten as food in many countries and utilized as ingredients in many recipes (Mamat *et al*., 2013). Bread being the staple food for many people in the Pacific could also address food security issues through the utilization of seaweeds to make bread. There have been some studies done on the benefits of seaweeds. According to Hall *et al.* (2010) brown seaweeds are high in alginate which is a dietary fiber and can be utilized to make seaweed bread. *Gracilaria edulis* and *Sargassum polycystum* could be used as animal feeds (Ocean Feed, 2016). In addition, a total of seven seaweeds are harvested in Fiji for food, among which are *Caulerp*a spp., *Hypea* spp. and *Gracilaria* spp. which are the most consumed (South, 1993b). Seaweeds such as *Gracilaria verrucosa* are consumed by Fijians as a delicacy on feast days (South, 1993b). In other countries seaweeds have been used to feed animals. According to Ocean Feed (2016), seaweeds in the diet of horses and ponies promote a healthy gut by allowing for a greater level of absorption of nutrients and vitamins from the feeds given to them. As a result, animals are better able to perform, recover, behave and reproduce.

1.3. Organization of Thesis

This thesis is composed of six chapters. Chapter one is comprised of a brief introduction to climate change and the prevalence of pest seaweeds due to anthropogenic activities. Justification for the research is provided followed by hypotheses and objectives.

Chapter two consists of a literature review where seaweeds are generally described; seaweed importance is also discussed as well as how climate change has been having an impact on the abundance of seaweeds causing a phase shift.

Chapter three provides an overview of the methodology, sampling sites and the experiment treatments utilized in this thesis.

Chapter four entails the results while chapter five includes the discussion of the results.

The final chapter is Chapter six which summarizes the findings of this thesis, highlights the need for further research in the field of climate change affecting phycology.

CHAPTER 2

LITERATURE REVIEW

This chapter discusses seaweeds in general, their importance and benefits and how aspects of climate change has been affecting seaweeds in the past.

2.1.Seaweeds (Macroalgae)

Seaweeds are a useful resource used mainly as food for human consumption and for industrial, medical and cosmetic applications (McHugh, 2003; FAO, 2016). Global wet seaweed production amounts to about 8 billion kilograms, bringing US $5-6 billion in revenue (McHugh, 2003). Seaweeds are of different forms and can be large or attached forms of marine algae (Downes *et al.*, 2000). Common structures of seaweed include a thalloid body, holdfast, stipe, blade and reproductive structures (Anderson, 2007). There are three major groups of seaweeds namely Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae) (N'Yeurt *et al.*, 2012a). Green seaweeds are usually small and are a few centimeters to thirty centimeters long, some red seaweeds are upto 3 meters such as *Grateloupia turuturu*. Brown seaweeds are generally longer and, thicker growing to 2-4 meters in length (McHugh, 2003). There are many pigments associated with seaweeds based on their colour. Table 2.0 shows the kind of photosynthetic pigments present in the three major groups of seaweeds.

Table 2.0 The three different groups (green, red and brown) seaweeds with their respective photosynthetic pigments

Group	Photosynthetic pigment
Chlorophyta	chlorophylls
Rhodophyta	phycobilins (phycoerythrin)
Phaeophyta	carotenoids

Source: Trono (1968)

Seaweeds occur mostly in the intertidal to coastal regions of the marine environment in order to make use of light to photosynthesize (Vasuki *et al*., 2001). Macroalgae generally grow on reef flats or rocky shores (Downes *et al.*, 2000). Macroalgae which are multicellular are differentiated from microalgae which are unicellular and microscopic (McHugh, 2003). *Gracilaria edulis* and *Sargassum polycystum* are hence both known as macroalgae.

2.2. Macroalgal Flora of the Southern Pacific

The Southern Pacific islands are found between the longitudes 130°E and 125°W which consists of three diverse regions known as Micronesia, Melanesia and Polynesia (South, 1998). The overall total number of species of tropical benthic marine algae is not well-known due to the vast ocean that the Southern Pacific covers. The countries that are better accessible and closer to each other give a suggestion of the type of seaweed flora that can be found (South, 1998). Australia contains around 1185 species and since its neighboring country is Papua New Guinea, it can be expected that PNG has similar species of seaweed (South, 1998). This could be attributed to seaweeds being widely distributed and immigrating in nature (Lüining, 1990). Seaweed species richness is higher in bigger islands as opposed to smaller islands. According to N'Yeurt (1993), Fiji being the bigger island has 442 species while Rotuma being the smaller island has 88 taxa.

2.3. Noteworthy Commercial Macroalgae

According to FAO (2012), 98.9 percent of farmed algae produced in the world in 2010 were from Japanese kelp also known as *Saccharina*/*Laminaria japonica*, *Eucheuma* which is a mixture of *Kappaphycus alvarezi* and *Kappphycus cottonii* (known in the past as *Eucheuma cottonii*, and *Eucheuma* spp.).Other notable seaweeds include *Gracilaria* spp., nori/laver (*Porphyra* spp.), and wakame (*Undaria pinnatifida*) as can be seen in Table 2.1. *Sargassum fusiform* and *Caulerpa* spp. are also farmed in small quantities (FAO, 2012). *Kappaphycus* previously known as *Eucheuma* is the only commercially substantial seaweed in the South Pacific (South, 1998). However, all cultured stocks are imported from Philippines so naturally occurring species of *Eucheuma* are not viable for commercial production (South, 1993a). This species is a great source of carrageenan that is easily maintained (McHugh, 2006). On the other hand, cultivation can be quite difficult as countries such as Tonga, Solomon Islands, Federated States of Micronesia and Tuvalu have tried to cultivate and failed to do so (South, 1993a; Sulu *et al*., 2004). Production had been hindered in Fiji due to cyclones, political changes and competition for labor from other cash crops economies such as copra and bêche-de-mer (McHugh, 2006; Pickering & Mario, 1999).

Table 2.1 The Global Production of Framed Aquatic Plants in the World with *Sargassum spp*. and *Gracilaria spp*. become among the top six cultivated.

Seaweed (Macroalgae)	2005	2010 (Thousand tonnes)	2013	2014
Kappaphycus alvarezii and *Eucheuma* spp.	2444	5629	10394	10992
Laminaria japonica	4371	5147	5247	7655
Gracilaria spp.	936	1696	3463	3752
Undaria pinnatifida	2440	1537	2079	2359
Porphyra spp.	1287	1637	1861	1806
Sargassum fusiforme	86	78	152	175
Spirulina spp.	48	97	82	86
Other aquatic plants	1892	3172	2895	482
Total	13504	18993	2895	482

Source: FAO (2016)

2.4. Edible Macroalgae

The Worldwide demand for seafood has improved steadily over the years, although for the past 20 years the amount of seafood harvested from wild populations has remained constant (Redmond *et al*., 2014). Seaweeds are edible in many parts of the world and their consumption dates back many centuries and has more prestige in the western world (Preisig & Andersen, 2005). Three countries are the largest consumers of seaweed-Japan, China and Korea (McHugh, 2003). Japan is very famous for the edible seaweed known as *Porphyra* (Nori) which is used in the making of Sushi (Preisig & Andersen, 2005). Likewise, according to McDermid & Stuercke (2003), the people of Hawaii were (and still are) regular consumers of limu (seaweed) both wild and cultivated (Abbott & Williamson, 1974). While not all seaweeds are edible, most are and some of the species that are widely consumed include *Caulerpa, Codium, Gracilaria, Hypnea* which can be found in Rotuma and Lord Howe Island (N'Yeurt, 1993). *Meristotheca procumbens* is an edible seaweed that is used to make puddings in Rotuma (N'Yeurt, 1993). There are many seaweeds in Fiji that are readily consumed (Pickering & Mario, 1999; South, 1993a). Some of the most common form of seaweeds found in Suva are *Gracilaria maramae, G. edulis, Hypnea pannosa*, *Caulerpa racemosa*, *Acanthophora spicifera, Laurencia* spp., *Gelidiella* spp., *Enteromorpha* spp., *Sargassum* spp., *Solieria*, and *Kappaphycus alvarezii* (Pickering & Mario, 1999). In Pickering & Mario (1999), the most popular seaweed sold in the

Suva municipal market that is traditionally eaten is *Gracilaria maramae,* locally known as lumi wawa. According to South (1993b), some preferred edible seaweeds in Fiji include *Caulerpa* spp., *Hypnea* spp. and *Gracilaria* spp. Migration of people all over the world has allowed seaweeds to be known and consumed worldwide (McHugh, 2003).

2.5. Health Benefits of Macroalgae

Seaweeds have great health benefits and have been used for generations to cure illness. *Gracilaria changgi* is an edible form of macroalgae in Malaysia that is very nutritional containing 74% of unsaturated fatty acids of which the majority consists of omega fatty acids (Norziah & Ching, 2000). Seaweeds such as *S. polycystum, Eucheuma cottonii* and *Caulerpa lentillifera* are high in antioxidants and could be an alternate to healthy food source (Matanjun *et al.*, 2009). Additionally, the brown seaweed *Turbinaria conoides* has been found to have enhanced antioxidant activity (Anantharaman *et al.,* 2014). Similarly, the brown edible seaweed *Fucus vesiculosus* has sulfated polysaccharides which could potentially be utilized as a natural antioxidant in the food industry (Rupérez *et al.*, 2002) and *S. polycystum* is known to curb weight gain by reducing plasma total cholesterol and triglycerides (Awang *et al*., 2013).

2.6. Important Uses of Macroalgae

Some macroalgae are invasive and pests in nature which could prove to be a problem for the marine environment; however, macroalgae can also be important to the marine environment and also to humans (Chopin, 2014a). Seaweeds can be incorporated with the integrated multi-trophic aquaculture (IMTA) to cultivate shrimps and fish where seaweeds act as food for the fish and at the same time excreta from fish in the form of ammonia acts as nutrient enrichment for the seaweeds (Barrington *et al.*, 2009; Chopin & Neish, 2014b). Another important use of seaweed is the production of alginate which is used to make agar. According to Calumpong *et al*. (1999), *Sargassum ilicifolium* produces high amounts of alginate yield. Similarly, *Gracilaria verrucosa* is an important source of agar with an agar yield of between 27-30% (Rath & Adhikary, 2004). Macroalgae are also very useful indicators for nutrient enrichment as Costanzo

et al., (2000) investigated with *G. edulis* by subjecting to 1000 times the ambient nutrient concentration that showed an increase in chlorophyll a , tissue nitrogen, and amino acid when incubated.

2.7. Invasive Pest Macroalgae

According to Pimentel *et al.* (2005), invasive species have caused $120 billion per year in environmental damage for the United States Government. Marine invasive species are a threat to biodiversity, fishing, tourism and human health and these problems will worsen in due time if not curbed (Bax *et al.*, 2003). Invasive species are mostly introduced from another region either intentionally or unintentionally (Rejmanek & Richardson, 1996). One of the modes of unintentional introduction for marine macroalgae could possibly be through commercial shipping (N'Yeurt & Iese, 2013). According to IUCN (2009), about 7,000 species are passed around the world in ballast water every day. An intentional vector is from using invasive species such as *Codium fragile* spp. and C*aulerpa* spp. for aquarium and mariculture (IUCN, 2009). One of most important invasive macroalgae in history was the killer seaweed *Undaria* that was intentionally introduced in France for commercial purposes in 1983 (IUCN, 2009). The native ecosystem was severely affected where the dominant seaweeds decreased its abundance while *Undaria* increased in abundance. According to Littler *et al*., 2010, cyanobacteria blooms prevented the colonization of crustose coralline algae when waters were enriched with dissolved inorganic nitrogen and soluble reactive phosphorus. *Sargassum* spp. are abundant in Coral Coast of Fiji waters which has elevated nutrient levels (Mosley and Aalbersberg, 2003 and 2005). According to Tamata & Morrison (2012), nutrient enrichment can cause abundance of invasive macroalgae such as *Sargassum* spp., while Kim *et al.* (2016) reported that *Gracilaria vermiculophylla* is an invasive species that displaces *Gracilaria tikvahiae,* a native species of Florida in the USA. Likewise, according to Johnson (2007), *Gracilaria edulis* has increased in abundance along the Fijian Resorts on the Coral Coasts in Fiji. There are certain attributes that make a macroalgae an invasive species as seen in Table 2.2.

Table 2.2 Important attributes for species to be regarded as an invasive species

Characteristics	Source
Tolerance for harsh environmental conditions	(Poorter *et al*., 2009 ; Bax *et al.*, 2003)
Large number of fast growing offspring	Pimentel *et al.* (2005),
Compete assertively for resources	(IUCN, 2009)
Displace native flora and fauna	(IUCN, 2009; Rejmanek & Richardson, 1996)

2.8. General Characteristics of *Sargassum* species

Sargassum C. Agardh is a brown macroalgae with about 400 species (Noiraksar & Ajisaki, 2008). In Fiji, *Sargassum* spp. have been found to be growing at a depth of 5-10 m (N'Yeurt, 2001). The taxonomy of *Sargassum* species has often been difficult to identify due to its complexity but recent research has been done on its morphology to identify and revise the taxonomy of *Sargassum* species (Mattio & Payri, 2011; Mattio *et al*., 2010; Mattio & Payri, 2009; Mattio *et al*., 2008). From the 9 listed taxa only 4 taxa were reported for Fiji in 2007 (Mattio, 2007). The genus *Sargassum* is branched into five sub genera known as *Phyllotricha, Schizophucus, Bactophycus, Arthtophycus* and *Eusargassum (Sargassum*) after deriving connections between blades and stems (Yoshida, 1983), seen in Figure 2.0. Conversely, the divisions did not follow botanical codes so confusion in nomenclature arose as result. Yoshida (1983) further divided the subgenera into sections based on morphological features such as compound auxillary fronds, simple lamina, vesicles arising from the distal section of the lamina, receptacles are smooth or armed, compound and found on modified axillary branches thus paving way for the subsections which were further divided into series. More recently Mattio & Payri (2009) revised the genus based on molecular evidence.

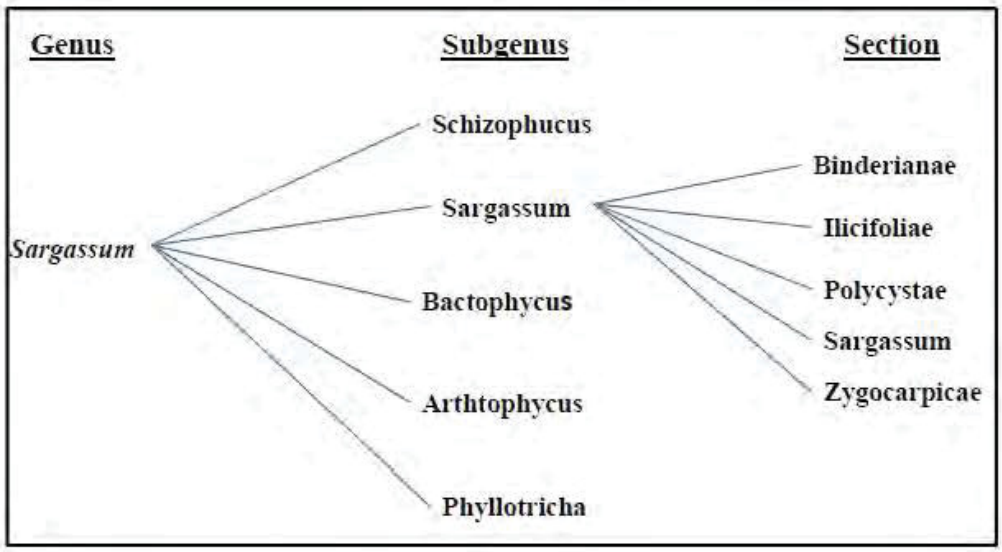

Figure 2.0 The Current taxonomic associations of genus *Sargassum* branching out into subgenus and sections.

Adapted from Mattio & Payri (2009)

2.8.1. Taxonomy of *S. polycystum*

The taxonomy of *S. polycystum* has been tortuous and it is often confused with other species due to some overlapping morphological characteristics (Noormohammadi *et al.*, 2011). General features include leaf-like blade, bulbous and obvious air bladders (Lobban & N'Yeurt, 2006) as seen in Figure 2.1. The main axes of *S .polycystum* are cylindrical, with warty processes which are 5-12 mm high, 1.2-1.8 mm in diameter, giving rise to several erect primary branches and descending to several elongated branched stolons. *S. polycystum* is similar than *Sargassum baccularia* but distinction between the two can be made based on the vesicles and female receptacles (Noiraksar & Ajisaki, 2008). *S. polycystum* has obovate vesicles and knobby stems with receptacles being terete while *S. baccularia* has spherical to elliptical vesicles which are triquetrous (Noiraksar & Ajisaki, 2008). In addition, *S. polycystum* has secondary holdfasts transformed into stolons (Wong *et al.*, 2004). Table 2.3 shows the general classification of *S. polycystum*.

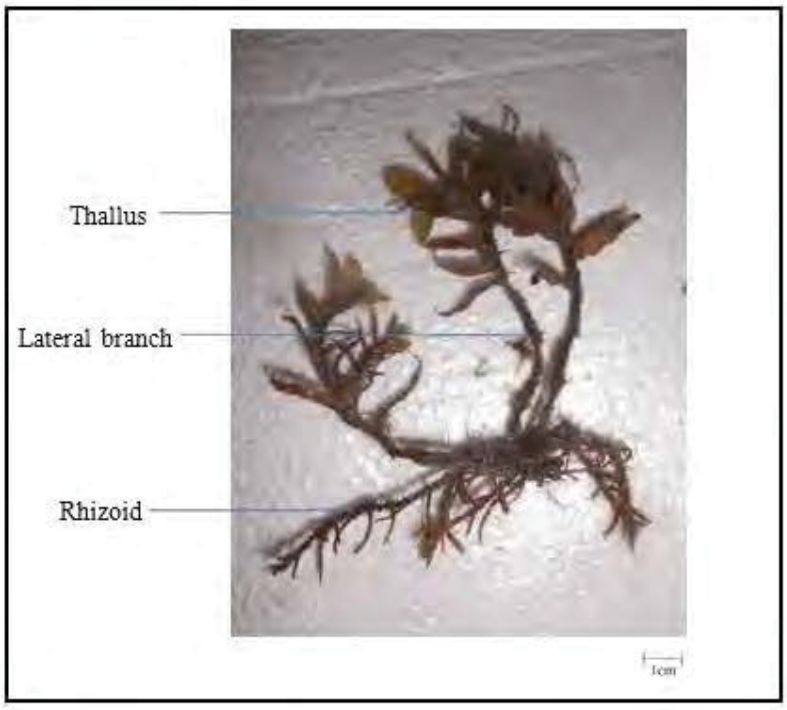

Figure 2.1 Habitat of *Sargassum polycystum* collected on 20/10/2015 from Makaluva Island with rhizoids, thallus and lateral branches.

Table 2.3 The General Classification of *S. polycystum* starting from Kingdom to species.

Kingdom	Chromista
Sub-kingdom	Harosa
Phylum	Ochrophyta
Sub-phylum	Phaeista
Class	Phaeophyceae
Order	Fucales
Family	Sargassaceae
Genus	*Sargassum*
Species	*polycystum*

Source from Cambie, *et al*. (2009) and Noiraksar & Ajisaki (2008)

2.9. General Characteristics of *Gracilaria* species

Gracilaria is one of the most easily recognizable genus of Rhodophyta. There are over 100 species of *Gracilaria* found around the world (Redmond *et al*., 2014). Many features of this genus include an erect or prostate thallus, terete branches which are laterally, secondly, radially or irregular, with a small discoid hold fast which is attached to the solid substratum (FAO, 2007). The main axes are indistinct, erect and

lax with long tapering branches, or the plants form massive stiff mats (Lobban & N'Yeurt, 2006), refer to Figure 2.2.

Figure 2.2 Habitat of *Gracilaria edulis* collected on 20/10/2015 from Makaluva Island

2.9.1. Taxonomy of *G. edulis*

The identification of *G. edulis* can be quite difficult. According to Iyer *et al*. (2004), the lack of knowledge in the taxonomy of *Gracilaria* has led to misidentification of species. Identification can be done based on morphology (Iyer *et al*., 2004) or molecular taxonomy (Gurgel *et al*., 2004; Kongkittayapun & Chirapart, 2011), refer to Table 2.4 for the general classification of *G. edulis*.

Table 2.4 The General Classification of *G. edulis* starting from kingdom to species.

Kingdom	Chromista
Sub- Kingdom	Biliphyta
Phylum	Rhodophyta
Sub- phylum	Eurhodophytina
Class	Florideophyceae
Order	Gracilariales
Family	Gracilariaceae
Genus	*Gracilaria*
Species	*edulis*

Source: Redmond *et al* (2014)

2.10. Climatic Drivers for Algal Dominance

Climate change has been a driving force for many changes in the environment and one of these changes is the increase in algal blooms. According to Ye *et al.* (2011), green tides have become prevalent worldwide due to increased nutrients present in the coastal waters. Likewise, Harley *et al.* (2012) attributed anthropogenic climate change to algae pervasiveness. The increase in carbon dioxide in the atmosphere has resulted in a change in important physical and chemical process not only in the terrestrial environment but also in the marine environment (Harley *et al.*, 2012). When the environment deviates from normal functioning and processes, invasive and pest species appear and thrive as the new environment becomes more suitable to their growth, in the process displacing natives species (Loyolaa *et al.*, 2012).

Climate change has often been associated with loss of habitat and this habitat loss increases the risk of biological invasion (Loyolaa *et al*., 2012). According to Meek *et al.* (2012), a small increase in temperature resulted in a large increase in growth of *Cordylophora caspia,* an invasive freshwater hydroid in the San Francisco Estuary, USA. However, macroalgae do grow naturally and their presence in the marine environment is required for the survival of herbivores (Harley *et al.*, 2012). Climate change in the future could affect the growth of some macroalgae negatively. According to Takao *et al*. (2015), future climatic scenarios such as the Representative Concentration Pathways (RCP) used in the Coupled Model Intercomparison Project Phase 5 (CMIP5) could result in a poleward shift of the seaweed *Ecklonia cava*. Increase in Sea-Surface Temperature (SST) for the past thirty years in the northern

part of Spain had caused kelp abundance to decline paving way for other warm temperate understory species to take their place in the ecosystem (Voerman *et al*., 2013).

2.10.1. Coral to Macroalgae Dominance (Phase Shift)

Coral reefs are important because they perform multitude functions such as protecting shores from tidal waves, sheltering marine organisms and acting as an indicator for a healthy functioning ecosystem. Global warming has always been a problem for corals since increasing SSTs have caused coral bleaching (Done *et al.,* 1997). The frequent occurrence of cyclones and lesser number of herbivores through overfishing has resulted in degraded coral ecosystems (McCook 1999). Macroalgal dominance over coral is becoming a serious issue which is threatening marine ecosystems throughout the Pacific region. On the Coral Coast of Fiji, the presence of excess nutrients in the water column and less grazing by herbivores have caused an abundance of macroalgae (Mosley & Aalbersberg, 2005). A model developed by McCook (1999) as shown in Figure 2.3, called "The Direct Effects of Nutrients on Algae" enlightens on nutrients being solely responsible for algal abundance.

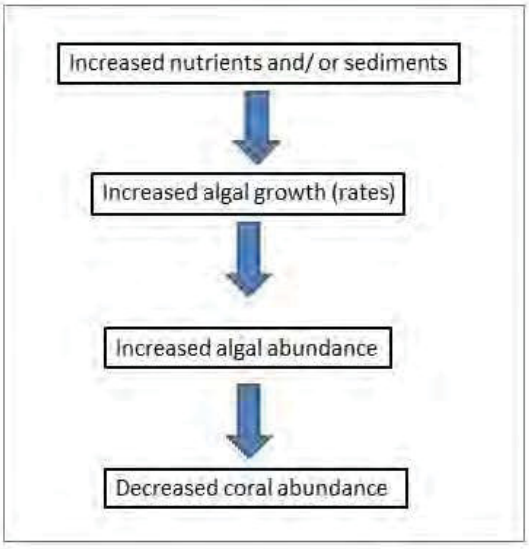

Figure 2.3 The Model of the Direct Effects of Nutrients on Algae that shows the sequence where increased nutrients/sediments leads to decreased coral abundance.
Source: McCook (1999)

According to McCook (1999), coral reef degradation mainly occurs due to a 'phase shift' from healthy corals to macroalgae when herbivory is controlled. This occurrence

is explained in Figure 2.3 where increased nutrients and sediments in the water column causes algae to grow fast and increase in biomass per time (rate) as opposed to corals whose habitat has been taken over by the rapidly increasing macroalgae biomass. Thus the coral to macroalgae ratio is lower and macroalgae dominance occurs (McCook, 1999)

2.11. A Combination of Impacts

It has been widely noted that macroalgal growth has been associated with the amount of nutrients present in the water column, but other variables such as temperature, salinity, irradiance, ocean acidification and seasonality can play a crucial role in macroalgal growth (Oliveira *et al.*, 2012; Raikar *et al.*, 2001; Shaffelke & Klumpp, 1998). The interaction between these variables creates an ideal growth environment for invasive pest species (Harley *et al.*, 2012; Meek *et al.*, 2012).

2.11.1. Effect of Nutrients on Macroalgae

For all life forms, nutrients are an essential component that is necessary for survival. Macroalgae, like any other primary producer, require nutrients from the ocean to grow and thrive, eventually being the foundation of the basic food web that supports the entire aquatic ecosystem (Mosley & Aalbersberg, 2003). Most of the nutrients present in the ocean are in ionic forms of nitrogen and phosphorus which are carried by the oceanic currents through the process of upwelling (Graneli *et al.*, 2004). Upwelling moves the cold nutrient rich waters in the deep oceans to displace the surface warm waters (Weisberg *et al.*, 2014). Surface tropical oceanic waters are oligotrophic due to rapid uptake of limiting nutrients (N, P, Si) by photosynthetic organisms such as phytoplankton (Graneli *et al.*, 2004).

Oceanic nutrients are mostly in the form of nitrate (NO_3^-) and phosphate (PO_4^{3-}) where major nutrient sources are from river run-off during heavy rain and seepage of sewage into coastal areas as a result of improper draining system (N'Yeurt, 2001). According to Mosley and Aalbersberg (2003), the waters of the coral coast on the island of Viti Levu had harmful mean levels of phosphate (0.21 μM) and nitrate (1.69 μM). The high levels of nitrate have been largely attributed to anthropogenic factors such

unsustainable development of hotels, resorts and villages which are located along the coast and these detrimental levels in the oceans can very well degrade marine organisms particularly causing damage to corals and causing macroalgae abundance (Mosley & Aalbersberg, 2005).

Similarly, studies carried out in other parts of the world such as Carrie Bow Cay, Belize found that macroalgae had 57% coverage with Dissolved Inorganic Nitrogen (DIN) at 2.06 μM while the Soluble Reactive Phosphorus (SRP) was at 0.12 μM when grazing was not taken into account, these levels being beyond the necessary levels of DIN at 1.0 μM and SRP at 0.1 μM (Littler *et al.*, 2010). *Sargassum* sp. such as *S. baccularia* have been found to be growing in abundance at the Great Barrier Reef (GBR), Australia due to short term nutrient pulse additions that consisted of ammonium, phosphate and the naturally present nutrients in the water (Shaffelke & Klumpp, 1998).

In India, Murthy & Rao (2002) investigated the effects of industrial wastes carried downstream on the growth and reproduction of nine different types of macroalgae including *S. polycystum,* whose growth was repressed when exposed to concentrations ranging from 0.001-15% of HZE-I, HZE-II and AFE effluents. Excess nutrients also have profound impacts on the physiology of macroalgae. According to Yu & Yang (2008), excess nitrogen and phosphorus can affect the physiological and biochemical processes of *Gracilariale maneiformis* to an extent where the growth rate decreases after the optimum growth rate has been reached. Nutrient influx in the water can also increase the abundance of bacteria (Graneli *et al*., 2004).

Macroalgae require a variety of elements for growth such as phosphorus, potassium, carbon, hydrogen, magnesium copper, zinc, silicon along with nitrogen is crucial (DeBoer, 1981; Fong *et al*., 1998; O'Kelley, 1974). A study by Kaliaperumal & Ramalingam (2005) found that when *G. edulis* was treated with calcium nitrate the biomass increased as the concentration increased. In another study by DeBoer *et al* (1978) on *Gracilaria foliifera,* growth increased with ammonium and nitrate addition. In addition, some seaweeds also have the ability to store unused nitrate in vacuoles and use all throughout summer when nutrient in the seawater is not sufficient to sustain them (Lobban & Wynne, 1981).

While nutrients are naturally present in the oceans, human influence has greatly increased their amount under certain situations. Many sources of nutrients come from a variety of natural or anthropogenic sources. Natural sources of nutrients include lightning; weathering of rocks, sedimentation and ocean upwelling. Examples of anthropogenic sources are runoff from fertilizer inputs in agriculture and poor rural and urban wastewater management systems (Pastorok & Bilyard, 1985). Sewage effluents are normally discharged erratically into coastal waters from hotels and highly populated settlements, and the concentration of nutrients is high compared to regions away from settlements (Tamata & Morrison, 2012). Untreated wastewater is one of the important sources of elevated nutrients in oceans followed by an increased use of household detergents, fertilizers and coastal developments such as hotels which cause sediments to deposit into oceans further contributing to nutrient pollution of the oceans (Mosley & Aalbersberg, 2005). According to Pastorok & Bilyard (1985) large surcharges of effluents into water columns have resulted in changes in species composition and increase in biomass of marine algae. Excess nutrient loading into ocean, rivers and streams are major causes of eutrophication (McLaughlin *et al.*, 2010).

Anthropogenic sources of nutrient input into fresh and marine waters have slowly increased in the past two centurties (Smith *et al.*, 1999). Runoff of nitrogen and phophorus from agricultural land has decreased the water quality and at the same time poses a threat to the health of marine organisms (Withers & Lord, 2002). Eutrophication has been a major cause of algal blooms and the death of other marine organisms (McLaughlin *et al*., 2010). In addtion, eutrophication can also result in extreme production of autotrophs, especially algae and cyanobacteria (Correll, 1996).This process can result in oxygen depletion causing hypoxia or anoxia resulting in an extremely stressful environment for other marine organisms such as corals (McLaughlin *et al.*, 2010). According to Naldi & Viaroli (2002), as the nitrate in the water column increases so does the biomass of *Ulva rigida* at the eutrophic lagoon of the Po River Delta in Italy. Eutrophication also distresses wetland plants and triggers the early onset of natural succession at a relatively faster rate (Khan & Ansari, 2005).

The elemental composition of marine organic matter (C: N: P) is constant across all of the regions with the atomic ratio of 106: 16: 1 also known as the Redfield ratio (Geider & La Roche, 2002). Nitrogen and phosphorus are the most important nutrients that

limit the growth of seaweeds in the marine environment. However, this is not the same for all species of seaweed. Silicon is also a limiting element that is given less attention. According to Howarth & Marino (2006), a reduction of P to upstream freshwater ecosystems can also benefit coastal marine ecosystems through mechanisms such as increased Si fluxes. However, most of the time it is either nitrogen that is limiting or phosphorus, while at other times it could be both. According to Fong *et al.* (1994), different species of algae have complex mechanisms to absorb varying amounts of nitrogen and phosphorus for optimum growth. A study on *Enteromorpha intestinalis* showed that *E. intestinalis* prefers nitrogen but this species is more inclined towards ammonium NH_4^+ than to nitrate NO_3^- (Fong *et al*., 1994). In the open ocean, seawater contains nitrogen and phosphorus in an atomic ratio of 15N:1P (Mosley & Aalbersberg, 2005). Table 2.5 shows a summary of limiting nutrients for different species.

Table 2.5 Limiting nutrients in enrichment experiments for various species of seaweeds at different seasons.

Sites	Species	Limiting Nutrient	Seasons	Source
Coastal lagoons of southern California, USA	*Enteromorpha* sp.	N	-	(Fong *et al.*, 1994)
Jamaica	*Codium isthmocladum*	P	-	(Lapointe, 1997)
Southeast Florida, USA	*Codium isthmocladum*	N	-	(Lapointe, 1997)
Florida Keys, USA	*Gracilaria tikvahiae*	P	Summer	(Lapointe, 1987)
		N and P	Winter	
Rhode Island, USA	*Codium fragile* ssp. *tomentosoides*	N	Summer	(Hanisak, 1979)

Invasive species can be alien, non-indigenous or exotic species which spread and become abundant in an area where they were not previously found (Grosholz *et al.*, 2015; Sodhi *et al*., 2007). In 2013, *S. polycystum* had shown characteristics that identified it as being an alien invasive species which had become abundant in the waters of Tuvalu (N'Yeurt & Iese, 2013). According to N'Yeurt & Iese (2013), the preliminary evidence suggests that *S. polycystum* was an introduced invasive species

which may have invaded the Tuvaluan waters through the international shipping from Wallis and Futuna. According to the McCook (1999), increased nutrient concentration causes increased macroalgal dominance provided herbivory is negligible. For this study, herbivory was negligible thus McCook's model (Figure 2.4) complemented this study. However, according to Cormaci & Furnari (1999), sewage, agricultural and industrial pollution can cause a decrease in flora especially for a subtle species like *Sargassum* spp. due to other opportunistic species.

Nutrients present naturally in oceans act as fertilizers for seaweeds and other marine organisms but if present in large amounts could cause macroalgal abundance and associated changes in the palatability of macroalgae; for instance, *Acanthophora spicifera* enriched with nitrogen and phosphorus was preferred by 91% of herbivores (Boyer *et al*., 2004). Likewise, a study done by Endo *et al.* (2015) discovered that nutrient enrichment increased nitrogen concentration and a positive correlation was found between nitrogen concentration and the consumption rate by herbivores of *Sargassum yezoense.*

Nitrogen is a macronutrient that exists in various forms such as amino acids, purines, pyrimidines, amino sugars and amines in the algae which are required in major metabolic functions (Lobban & Harrison, 1997; Korpinen *et al*., 2010). Macroalgae generally use up the elemental inorganic nitrogen that is already present in the water in the form of nitrate, nitrite and ammonium. The concentrations of nitrate, nitrite and ammonium ranging from 1-500 ppm, 0.1-50 ppm and 1-50 ppm respectively (Lobban & Wynne, 1981).

Phosphorus is a nutrient that is necessary in the development of algae (DeBoer, 1981; Lobban & Harrison, 1994; O'Kelley, 1974). Phosphorus is required for structure and transfer of energy and some common examples of this include ATP, GTP, phospholipids, co-enzymes and nucleic acids (DeBoer, 1981; Lobban & Harrison, 1994). Phosphate exists in seawater as HPO_4^{2-}, PO_4^{3-} and $H_2PO_4^-$ (Riley & Chester, 1971). Phosphate ions occur in seawater as a result of detergents, fertilizers, sewage being washed away into the coastal waters. These ions are then absorbed by the macroalgae and which thrive on phosphate. In macroalgae such as *Acetabularia mediterranea,* phosphate accumulation increases the polyphosphate granules which

allows the macroalgae to thrive in conditions which could be phosphate deprived (Riley & Chester, 1971).

Potassium is an element that is required for osmostic regulation, pH control conformation and stability of proteins and has to be in an ionic form for these process to occur (DeBoer, 1981). Potassium deficiency can impair protein synthesis in seaweeds (Stephenson, 1968). According to Gomez-Gutierrez *et al.* (2011), potassium is essential to regulate the growth of seaweeds. Cytosol is a component of the cytoplasm of a cell, within which various organelles and particles are suspended and K^+ ions provide an ionic environment for cell metabolism to occur (Riley & Chester, 1971). There is about 9 mg/g of potassium present in kelp seaweed (Stephenson, 1968). According to Ipatenco (2017), 99223 mg serving of raw kelp contains 233 mg of potassium.

2.11.2. Effects of Herbivory on Macroalgae

Macroalgae sustain many marine organisms including fish which use them as a food source. Consumption by marine organisms regulates the growth of macroalgae. Herbivory is a key component that drives and controls the structure of benthic communities (Hoey & Bellwood, 2010). Grazing intensities of *Sargassum swartzii and S. cristaefolium* at different location, depth and wave exposure was observed at the Great Barrier Reef, Australia and it was discovered that shallow regions underwent more herbivory than deeper regions (Hoey & Bellwood, 2010). However, the relationship between the removal of macroalgae and the biomass of herbivores is complex and other factors such as palatability of macroalgae or nutrient enrichment could play a role (Armitage *et al*., 2009).

According to Engelen *et al.* (2011), herbivory affects the distribution and abundance of most macroalgae but *Sargassum muticum* was not preferred by herbivores as native species were more desired which resulted in the abundance of the invasive *S. muticum*. Monteiro *et al* (2009) determined that the algal invader *S. muticum* had a higher growth rate when subjected to low grazing pressure. On the other hand, for coastal algal turf communities exposed to heavy sediment loads of over 2 mm, Clausing *et al.* (2014) reported that a reduction in algal growth occurred which was independent of grazing pressure. Fish herbivory keeps the production of macroalgae under control

(FishChaneel.com, 2015). According to Endo *et al.* (2015), temperature is not correlated to phlorotannin concentration, a defensive compound in *S. yezoense*, nitrogen concentration, or the consumption rate by *Hemicetrotus pulcherrimus,* a sea urchin. Thus temperature may not be a factor that affects the palatability of macroalgae to herbivores.

2.11.3. Effect of Temperature on Macroalgae

Temperature plays a crucial role in the marine environment and is closely linked to atmospheric processes (PCCSP, 2011). It is estimated that the ocean has absorbed 17 x 10^{22} Joules of heat in the last 30 years due to global warming (IPCC, 2013). This increase will have a significant impact on the marine organisms that are not adapted to increasing temperature changes (Harley *et al.*, 2012). On the other hand, there are many marine organisms such as macroalgae that can tolerate wide fluctuations in temperature. These macroalgae can be invasive and/or of pest proportions. Temperature is one of the factors governing the growth of seaweeds as changing temperatures is an inevitable component of the marine environment (Goodwin *et al*., 2013; Kailasam & Sivakami, 2004; Raikar *et al*., 2001; Wienckel & Dieck, 1989). An increase in temperature could cause an abundance of pest macroalgae because temperature could have an impact on the distribution of many species (Hiscock *et al*., 2004). For instance, a change in temperature can severely impact the breeding cycles of salmons (Handeland *et al*., 2008). When this happens, herbivory is reduced thus leading to increased abundance of macroalgae. However, this may not always be the case as Van den Hoek (1982) reported that algae that spent their entire life in warmer waters would grow better as the summer increased temperatures while others that were better suited for colder waters and would become better adapted for even colder waters.

Likewise, seaweed such as *Devaleraea ramentacea* found in one part of the world such as North America would have a different tolerance limit when compared to the same species of seaweed found in Europe (Hiscock *et al.*, 2004). According to Hiscock *et al* (2004), climate change in the form of increased SST has provided an ideal environment for distribution of macroalgae by enhancing survival of spores. A study has been done on several *Gracilaria* spp. by Raikar *et al.* (2001) who reported that plants collected from the temperate zone showed retarded growth after being subjected to temperatures

above 25 ℃. On the other hand, *Gracilaria* spp. sampled from the tropical zones were found to be growing well at 24 -30 ℃. In another study, *Sargassum* spp. collected from the subtropical zone in Shantou China were found to be growing at temperatures ranging from 15-25 ℃ but growth slowed down above 30 ℃ (Chena & Zouab, 2014). Temperature does not always have the same effect on different species of the same genus as Rao & Rao (2002) discovered that temperature is a fundamental factor for affecting seasonality. According to Kokubu *et al.* (2015), *S. fusiforme* from Japan was dominant from March to June growing at 16.0-22.8 ℃ but from July to August only the holdfast was present at higher temperatures of 27 ℃ and 29 ℃.

According to Jansi & Ramadhas (2009), *Chaetomorpha aerea, Enteromorpha compressa, Gracilaria verrucosa and Hypnea musciformis* were found to be growing in euryhaline salinity and temperatures ranging between 23.57 - 31.70 ℃ on the South Western - Coast of India. The oceanic temperatures of the mid-latitudinal zones of the planet are also affected by global warming and this has impacted the growth of seaweeds found in these regions. According to Wilson *et al.* (2015), in Nova Scotia, seaweeds such as *Laminaria digitata* and *Chondrus crispu*s had maximum growth at 12 °C which was the lowest temperature treatment, *Fucus vesiculosus* and *Codium fragile* spp. *Tomentosoides* which is an invasive species survived at 16 °C while *Ascophyllum nodosum* survived at 20 °C. However, *codium* and the invasive *C. crispus* survived at high temperatures due to acclimation to high temperatures. In another study isolates of *Microdictyon boergesenii, M. calodictyon and Valonia utricularis* all survived at 30 °C but they died at a higher temperature of 35 °C (Pakker & Breeman, 1996). Table 2.6 shows that optimum growth temperatures for the genus *G. edulis* which is one of the seaweeds being studied in this project.

Table 2.6 The optimum growth temperatures for various species of seaweed

Species	Optimum growth Temperature	Source
Kappaphycus alvarezii	25°C	(Mandal *et al.*, 2014)
Gracilaria	25°C	(Raikar *et al.*, 2001)
vermiculophylla	25°C	(Raikar *et al.*, 2001)
G. incurvata	25°C	(Raikar *et al.*, 2001)
G. foliifera	25°C	(Raikar *et al.*, 2001)
G. corticata		
G. edulis	30°C	(Raikar *et al.*, 2001)
G. lichenoides	30°C	(Raikar *et al.*, 2001)

Fluctuations in the oceanic temperatures have long been a contributing factor to changes in the marine ecology (Meek *et al*., 2012; Nian-Zhia *et al*., 2015). Macroalgae communities are becoming dominant worldwide due to increasing temperature (Harley *et al*., 2012; Wernberg *et al.*, 2012). Increasing temperature has been known to cause a shift from coral dominated ecosystems to macroalgae dominated (Fong *et al*., 2006). Furthermore, Cook *et al.* (2001) discovered that coral degradation due to increasing temperature results in increased abundance of opportunistic macroalgae. According to Raikar *et al.* (2001), when the optimum temperature is 30 ℃ then the species can make a transition from growing in temperate regions to tropical regions. In this study *S. polycystum* and *G. edulis* are researched upon because they are considered opportunistic.

2.11.4. Sea Surface Temperature of Fiji

Fiji is found in the Southern Pacific ocean, located at 18°00'S and 175°00'E. A total of 322 islands make up the Fiji Islands (Stanley, 1996). According to the Fiji Meteorological Services (2016), the warmest water in Fiji is in Rotuma where water on average was 30 °C throughout the year of 2016. On the contrary, the lowest SST temperature was in Nausori where water was 28 °C on average throughout the year of 2016. This means the difference between the warmest (Rotuma) and coldest (Nausori) place in Fiji is 2 °C. Figure 2.4, depicts the overall average SST in Fiji for the year 2016.

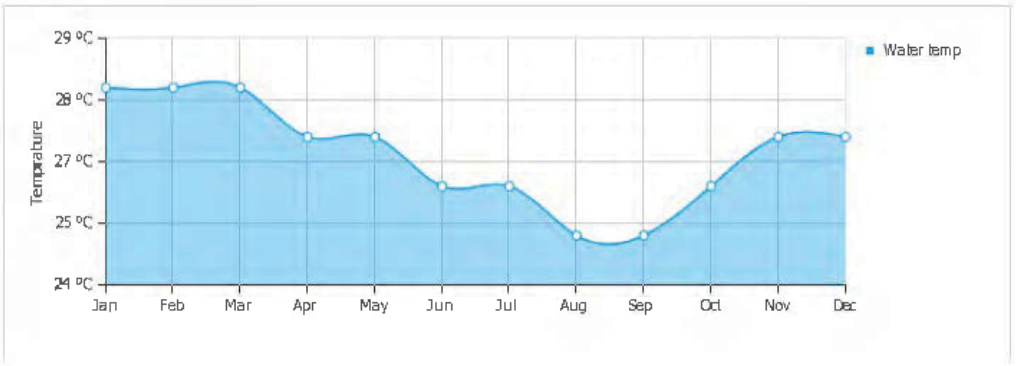

Figure 2.4 The Average Sea Surface Temperatures (SST) in Fiji for the year 2016 where there was a fluctuation in the temperatures between August and September reaching a lower 25 °C.

Source: Fiji Meteorological Services, 2016.

2.11.5. Geographical Distribution

The geographical distribution of macroalgae can be attributed to the seawater temperature tolerated by the macroalgae (Breeman, 1988; Lobban & Harrison, 1997; Sarojini *et al*., 2013). In addition, macroalgae of the same species could have different tolerance to temperature thus have different geographical boundaries based on those temperature tolerance regimes (Breeman, 1988; McLachlan & Bird, 1984). According to Breeman (1988) high and low temperature ranges avert the microthalli growth which is the most resilient part in the life cycle of macroalgae. Invasive species are distributed widely because they can tolerate a range of temperature (Breeman, 1988; Dick, 2015; Harley *et al*., 2012; Occhipinti-Ambrogi & Savini, 2003; Voerman *et al.*, 2013). *Sargassum* spp. and *G. edulis* are seaweeds that are regarded as invasive and thus can be widely distributed (Lobban & Wynne, 1981; Johnson, 2007).

2.11.6. Seasonality

Seasonality is one of the factors that governs the growth of macroalgae and is difficult to mimic in a laboratory. There are four seasons, spring, autumn, summer and winter. Changes in the seasonal cycle can result in phenologic changes in the seaweed. *Sargassum* spp. occurs mostly in warm temperate regions (Kantachumpoo *et al*., 2013). In this study, *S. polycystum* and *G. edulis* were present on Makaluva waters from October to December but in March 2016, the abundance decreased which is attributed to Cyclone Winston that occurred in February 2016. Another visit in May 2016 showed that macroalgae abundance was evident suggesting that *S. polycystum*

and *G. edulis* occur annually. *S. polycytsum* undergoes an annual reproduction cycle but changes in the environmental conditions can cause a bimodal cycle in order to adapt to a harsh environment (Akira & Masafumi, 1999; Chopin & Floc'h, 1987). *Gracilaria* spp. grows well in spring and starts decreasing in abundance from June to October (Givernaud *et al*., 1999). One of the seasonal changes in the environment is El Niño Southern Oscillation (ENSO). On the other hand, ENSO is just not a seasonal event it is a cyclical event that varies over a period of 5-7 years.

Seasonality and nutrient enrichment have profound impacts in determining the abundance of macroalgae (May-Lin & Ching-Lee, 2013). According to Shaffelke & Klumpp (1998), during the summer wet seasons more rain is carried downstream into the oceans and rivers, increasing nutrient levels in the water column leading to an abundance of *S. baccularia* on the Great barrier reef. Similarly, the biomass of *Salicornia virginica*, a common seaweed found on the western coast of USA doubled in abundance during winter (Boyer *et al*., 2001). In addition, *S. polycystum* collected during winter time from the east coast of India had maximum growth during this time period (Rao & Rao, 2002). In another study, red and brown macroalgae from the east coast of India had maximum biomass during winter, November to December (Sarojini *et al.*, 2013). Similarly, Rao (1975) discovered that harvesting of *Gracilaria cortica,* a species endemic to the Indian subcontinent, is favorable during August to September and December to February.

2.11.7. El Niño Southern Oscillation (ENSO)

The El Niño Southern Oscillation is a naturally occurring phenomenon. However, this event is intensifying due to increasing SST (PCCSP, 2011). The ENSO event is governed by the Walker Circulation which weakens over the Pacific Ocean around the equator causing warming and cooling of water (Nian-Zhia *et al*., 2015). The weakening of trade winds coming from the east to the west causes the warm surface water to be loaded in the west (PCCSP, 2011). There is less upwelling which is generated from weakening winds resulting in El Niño. On the other hand, the strengthening of trade winds allows for more upwelling to occur in the east causing the La Niña effect (Collins *et al*., 2010). Pacific Island countries feel the brunt of increasing SST even more with the El Niño event. According to Takao *et al.* (2015),

increasing SST and ENSO can result in a shift of seaweed diversity towards the poles. A similar result was discovered by Voerman e*t al.* (2013) where rising SST resulted in decreased kelp abundance Northern Spain. For the same event, the kelp population recovered in the deeper parts of ocean that were rich in nutrients.

2.11.8. Effect of Salinity on Macroalgae

The salinity of oceans play a crucial role in the growth of many marine organisms including seaweeds since 97 % of surface water present on our planet is salt water while 3 % is freshwater (IPCC, 2013). The five oceans of the world; Atlantic, Pacific, Indian, Arctic and the Southern (Antarctic) oceans all have different salinities because salinity is governed by many factors such as weathering, creation of ice in the oceans, precipitation, freshwater as well as nutrient runoff into oceans and melting ice (IPCC, 2013). The average salinity of seawater is 35 g/L or 35 ‰. According to Jayasankar (2005), the salinity that was best suited for *G. edulis* and *G. crassa* growth was 35 g/L. Likewise, most species of *Gracilaria* obtained had favorable growth at 35‰ (Raikar *et al*., 2001).

Many species of macroalgae grow well in this salinity but optimum growth of any species could occur at a lower or higher salinity (Ding *et al.,* 2013; Jansi & Ramadhas, 2009; Jayasankar, 2005). Ding *et al.* (2013) looked at the effect of salinity on the growth of the red seaweed *Hypnea cervicornis* and found that growth was enhanced in salinity range of 25-30 ‰ but at a high salinity of 50 ‰, growth decreased. At higher salinities the process of osmosis which occurs in cells where water moves in and out of the cells until equilibrium is obtained, becomes difficult. Seaweeds are osmo-conformers whereby there are no mechanisms through which osmosis can be controlled (Jansi & Ramadhas, 2009). This results in seaweeds having the same amount of salt content in their cells as out of the cells. When seaweed from a less saline environment is placed in a more saline environment, through osmosis the cells will become dehydrated and the seaweed desiccates as the water is moving out of the cells (Jansi & Ramadhas, 2009).

Similarly, low salinities can also retard growth of seaweeds. According to laboratory studies by Raikar *et al.* (2001), salinities of 10 ‰ and 15 ‰ were detrimental to the

health of *Gracilaria* spp. collected from India and Malaysia. In a similar study, seaweeds tend to prefer euryhaline salinity ranging from 11.15 ± 0.48 mg/L to 27.18 ± 0.88 mg/L (Jansi & Ramadhas, 2009). However, this may not be true for all seaweeds as geography and distributions can also impact their growth based on species adaptability. Raikar *et al.* (2001) also found that seaweeds from the tropics had a better response when compared to seaweeds from the temperate regions as plants were able to survive at 10 ‰. Salinity can have a profound impact on the photosynthetic performance of seaweeds. The effects of salinity on the photosynthetic rate of two seaweeds *Ulva lactuca* and *Sargassum stenophyllum* showed that *U. lactuca* photosystem II (PSII) remained nearly unchanged by low salinities while the photosynthetic parameter of *S. stenophyllum* was reduced (Scherner *et al.,* 2013). This decline was attributed to regulation abilities of energy distribution at the PSII of the two species therefore could possibly to lead to seaweed community shifts.

2.11.9. Effect of Ocean Acidification on Macroalgae

Ocean acidification is a huge problem for calcifying marine organisms such as corals, crustaceans and coralline algae. However, those organisms that do not have the calcifying mechanisms are not affected. Non calcifying seaweeds and seagrasses instead have the ability to sequester carbon dioxide from the oceans and help in mitigating the effects of ocean acidification (N'Yeurt *et al.*, 2012). Elevated levels of carbon dioxide can increase the germination of some seaweeds such as *Sargassum henslowianum* (Chena & Zouab, 2014).

2.11.10. Effect of Irradiance on Macroalgae

Similar to terrestrial plants; marine algae require sunlight to grow, thrive and sustain the complex foodchain. However, obtaining sunlight is difficult in the marine environment due to the depth at which marine macroalgae grow. A study done on *S. fusiforme* showed that continuous irradiance of 1000 µmol photons m^{-2} s^{-2} decreased the response of effective quantum yield in the PSII (Kokubu *et al.*, 2015). However, the response of effective quantum yield in PSII is also affected by temperature and varies with different species (Kokubu *et al.*, 2015). According to Vasuki *et al.* (2001), optimum growth varies between species where *Sargassum cynosum* and *S. filipendula*

had optimum germling growth at low light intensity of 10-20 $\mu E/m^2/s$ while *S. patens, S. tortile* and *S. muticum* grew better at 200 $\mu E/m^2/s$. Moreover, *Sargassum* spp. has the potential to be developed into novel organic light emitting material (Jung *et al*., 2015).

2.12. Summary of Key Points from the Literature Review Relevant to This Study

Environmental factors and interactions within the ecosystem play a key role in governing of macroalgae abundance in the marine environment. Climate change is becoming an important issue in that the increase in SST temperature is disrupting the proper functioning of the ecosystem. Increase in SST and anthropogenic activities could possibly be the cause of macroalgae abundance. *S. polycytsum* and *G. edulis* are two invasive pest macroalgae found in Fiji. They carry with them the attributes of being invasive species (refer to Table 2.2). They can survive harsh environments and are fast growing when provided with nutrient. In this study, the individualistic effect of temperature and sodium nitrate on the growth of these macroalgae was studied. Increase in SSTs and nutrient runoff into ocean could be a major catalyst for their abundance. Nitrogen and phosphorus are both limiting elements. However, for the practical purpose of this study, nitrogen was studied instead of phosphorus because a preliminary study was done by the author and included in the results sections that showed that percentage tissue nitrogen content was in higher amounts than phosphorus. The results from this study is presented and discussed in Chapter 4 and 5. According to Howarth & Marino (1998; 2006), nitrogen represents the largest pollution problem in the coastal areas since nitrogen is the element most limiting to production in most coastal marine ecosystems in the temperate zone.

CHAPTER 3

RESEARCH METHODOLOGY

This Chapter presents the methods used to determine the effects of temperature and nutrients on the growth of *S. polycystum* and *G. edulis* as well as the synergistic effects that temperature and nutrients have on the growth of *S. polycystum* and *G. edulis.* The general description of the sampling site is given followed by the method of collection, then application of treatments at varying sodium nitrate concentrations and temperatures and also for the combined effects, sodium nitrate was used. An overview of the method for analyzing and determining of % nitrogen, phosphorus and potassium in macroalgae is also provided.

3.1. Description of the Sampling Site

Sampling for *S. polycystum* and *G. edulis* took place From October, 2016 to March, 2017 at Makuluva Island in Fiji which is located at 18°11'00''S and 178°31'00''E (Figure 3.0).

Figure 3.0 Map of the five collecting sites at Makuluva Island which were sampled from 20/10/2015 to 22/03/2016.

Source: Google maps

3.2. Measurement of Seawater Parameters at Makaluva Island and Method of Collection of *S. polycystum* and *G. edulis*

The seawater parameters; nitrate, nitrite, ammonia and phosphate were measured n=15 at Makaluva Island using the API marine Seawater Master Test Kit™. The API Saltwater Master Test Kit™ is a highly-accurate complete kit for testing saltwater aquariums to protect marine fish and invertebrates from unsafe water conditions.

The temperature, salinity and pH were measured using a YSI model 85 meter prior to the collection of the samples. The samples were collected from the wild using the quadrat method where a 0.25 m × 0.25 m random quadrat was placed in the intertidal region. The wet weight of the *S. polycytsum* samples collected ranged between 4.00 - 9.00 g. For *G. edulis,* wet weight varied between 2.00 - 4.00 g. The samples collected were then cleaned by removing off sediments and other particles and placed in dark polyethylene bags containing sterile seawater to prevent any further metabolism of the seaweeds. Ten samples of *S. polycystum* and *G. edulis* were oven dried at 60 ℃ and sent to the Koronivia Agricultural Research Station for analysis of tissue % NPK present in the pre- treatment samples. The samples were then transferred to a tank to be treated for 1 week to allow the seaweeds to use-up the remaining nutrients present in the tissues with LED lamps at 6000 LUX allowing for 12 hours day and 12 hours night inside the laboratory as described in Raikar *et al.* (2001).

3.3. Experimental design

The first part of the experiment was based on the individualistic effect of temperature and nutrient on *S. polycystum* and *G. edulis*. The second part of the experiment was based on the synergistic effect of temperature and nutrients on the growth of *S. polycystum* and *G. edulis*.

For practical reasons, a quasi – experimental design with pre-post testing was used. The control and treatment groups differed not only in terms of the experimental treatment they were assigned but also in that they may lack true control. Hence, in this study for the temperature phase of the experiment, the following arbitrarily chosen values 28 ℃, 30 ℃, 32 ℃ and 34 ℃ were studied. For the nutrient phase of the experiment, the following arbitrarily chosen values 1.00 mg/L (11.7µM), 2 mg/L (23.5µM), 3 mg/L (35.3 µM) and 4.00 mg/L (47.06 µM) were studied (Tamata, 2007). A split plot experiment design was used as shown in Figure 3.1. There were eight tanks where four tanks were for *S. polycystum* and the remaining for *G. edulis*. Each tank contained three replicates with the sample size being twelve. The samples were places in 600 ml plastic jars as seen in Figure 3.2.

Filtered seawater was sourced from the coast (Nasese) adjacent to the University and pumped directly into the laboratory. The salinity of the sourced water was 34 ‰ and temperature was 29 ℃, which was deemed close to the natural conditions of the Makaluva Island lagoons (Fong *et al.,* 1994). The concentration of nutrients present in the sourced water was measured using the API Saltwater Master Test Kit™ before and after changing the water every three days for a month.

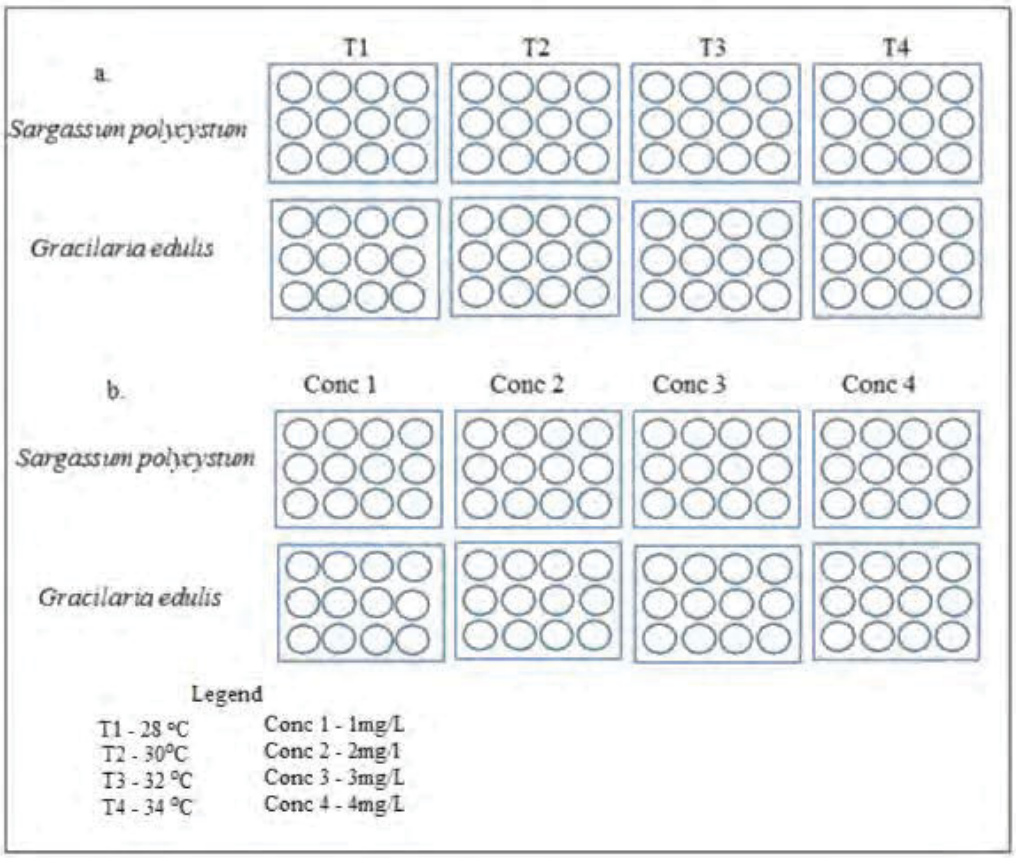

Figure 3.1 Split plot design consisting of experimental plastic jars used for experiments in different temperatures and (a) and at different sodium nitrate nutrient concentrations (b).

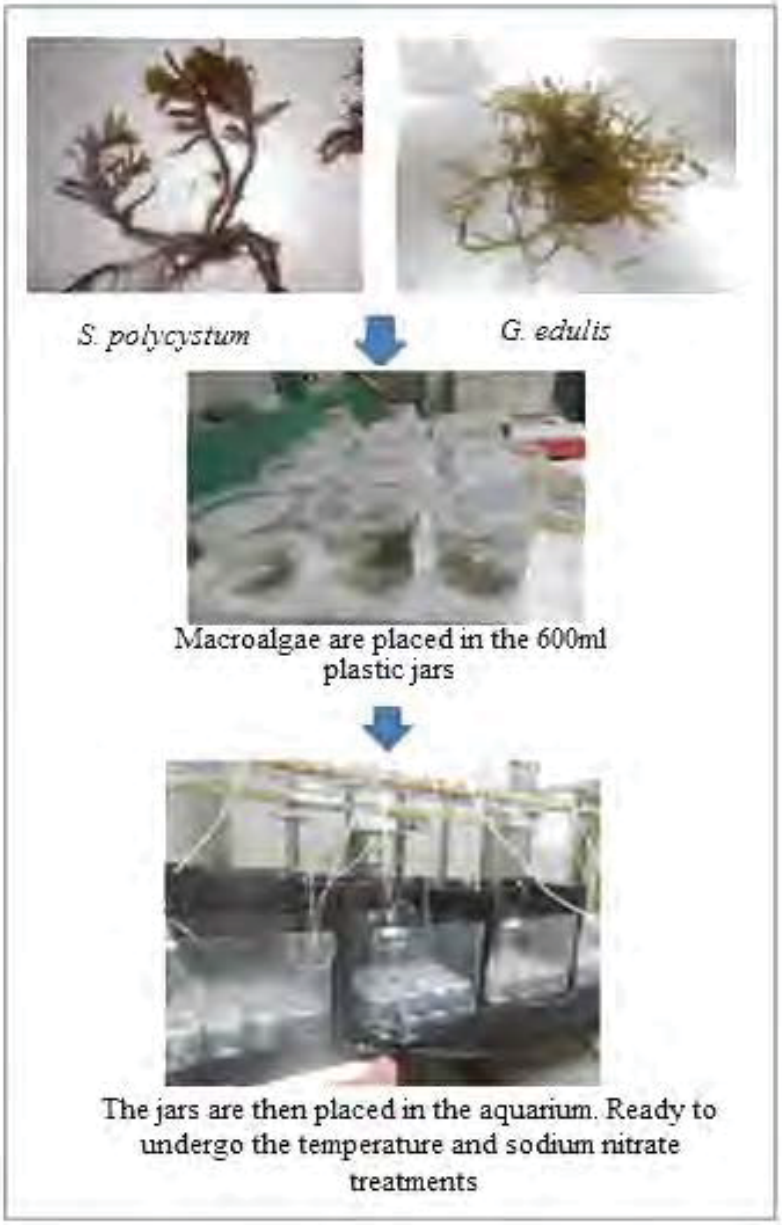

Figure 3.2 Experimental setup of *S. polycystum* (top left) and *G. edulis* (top right). Note* For the sodium nitrate treatment, the different concentration of 1 mg/L, 2 mg/L 3 mg/L and 4 mg/L of sodium nitrate were added to the 600 ml jars prior to being placed in the aquarium.

3.4. Treatment at Varying Temperatures

Pre-testing was done on the samples in the laboratory. Ten samples each of *S. polycystum* and *G. edulis* were obtained from Makaluva island, cleaned and then acclimatized to the laboratory conditions at the arbitrarily temperatures of 28 ℃, 30 ℃, 32 ℃ and 34 ℃ following the methods of Kaliaperumal *et al.* (2001) for acclimatization. However, it was found that after being nutrient starved for one week, the plants died, hence the samples were nutrient-starved for only three days. The wet biomass of *S. polycystum* and *G. edulis* were measured on a top pan balance scale after pat-drying the samples. Four tanks were used, with the fixed parameters being photoperiod, salinity and dissolved oxygen while temperature varied. Aeration was applied at 0.5 Ls^{-1} continuously. Seawater temperatures in Fiji range between 23 and 32℃ (ReefsTEMPS Fiji, 2017) and the temperature of the planet is expected to increase by 2℃ by the year 2100 (IPCC, 2013), the temperatures of the experimental tanks were set at 28 ℃, 30 ℃, 32 ℃ and 34 ℃ accordingly. The temperature and salinity were measured using a YSI model 85 meter. Temperature was kept constant by using an Aqua One™ 55Watts glass heater which was calibrated with an automatic thermostat control.

The aquarium tanks were covered with clear polyethylene plastic sheeting to prevent the entry of foreign matter and also to prevent heat from escaping, helping to keep temperature constant. Salinity was kept constant by changing the water every three days and replacing it with filtered seawater with 34 ‰. A regular diurnal pattern of 12 hours daylight/12 hours darkness was maintained using a 24 Hour Slimline™ Timer (model D810SLIM).

Each experimental plant was pat-dried using paper towels and weighed on a top pan balance scale every three days over a period of one month. The growth in terms of biomass (SGR) and rhizoid length (RL) were compared between treatments through the use of statistical analysis and determined if temperature had a significant effect on the growth of *S. polycystum* and *G. edulis.* The Specific Growth Rate (SGR) was calculated using the following formula below (Luhan and Sollesto, 2010).

$$\text{SGR} = \frac{ln\,Wf - ln\,Wo}{\text{t}} \times 100\%$$

Where:

ln Wf = final wet weight

ln Wo = initial wet weight

t = days of cultures

3.5. Addition of Nutrient: Sodium Nitrate

Since the seaweeds in this study were cultured in a laboratory setting, there was no herbivory present. Usually in the natural environment even though seaweeds grow well under a variety of conditions, unless in enclosure, macrocosm herbivory prevents the accurate study of nutrient enrichment experiments. Under ideal conditions and high nutrients, macroalgae have the perfect conditions for growth away from herbivory in a laboratory setting (Hoey & Bellwood, 2010).

The sole nutrient that was added to the treatment was nitrate in the form of the solution of sodium nitrate. The sodium nitrate solution was prepared by weighing an appropriate mass of the respective scientific grade reagent and dissolving it in filtered seawater to get the required concentrations of 1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L.

3.5.1. Treatments at varying Sodium Nitrate Concentration

After allowing the seaweed samples to use up the remaining nutrients for three days, the wet biomass of *S. polycystum* and *G. edulis* was measured after pat-drying the samples. There were four tanks for *S. polycystum* and four tanks for *G. edulis.* The tanks were set at 1.00 mg/L (11.7 µM), 2 mg/L (23.5 µM), 3mg/L (35.3 µM) and 4.00 mg/L (47.0 µM). These concentration regimes were taken into account because pristine waters contain nitrate that are < 1 µM (Mosley & Aalbersberg, 2003; 2005). In order to understand nutrient enrichment, these concentrations were used. The sodium nitrate ($NaNO_3$) solution was administered into the tanks every third day before taking the biomass for one month (Fong *et al.*, 1994). The water in the tank was changed every

three days and the amounts of nitrate present in the water before and after administration of sodium nitrate was measured using an API Saltwater Master Test Kit™ (API FishCare, USA).

Samples were pat-dried and the biomass and rhizoidal length were measured every three days over a period of one month. The samples of both species were oven-dried at 60 °C and the ash product tissue nutrient contents was analyzed at the Koronivia Agricultural Research Station in Fiji using the micro-Kjeldhal digestion method (Blakemore *et al*.,1987) and distillation apparatus to determine the Nitrogen content (percentage). The percentage of Phosphorus was determined using a Lambda 25 Perkin Elmer Ultraviolet/ Visible light Spectrophotometer (UV-vis Spectrophometry) (Chapman & Pratt, 1961) and percentage Potassium using a Perkin Elmer AAnalyst 400 Atomic Absorption Spectrophotometry (AAS) located at Koronivia Agricultural Research Station who also performed all the analysis. The results were estimated directly as % NPK (Antia *et al.,* 2006*)*.

3.5.2. Kjeldahl Method

The organic and inorganic components of nitrogen and potassium present in the algae samples were determined using the Kjeldahl method. The samples were oven dried to a constant weight for dry matter. Nitrogen, phosphorus and potassium were measured after Kjeldahl digestion method for plant samples as described by Blakemore *et al.* (1987) and Daly *et al.* (1984). The Kjeldhal method was used to determine the organic and inorganic nitrogen contents in the seaweeds. The determination of nitrogen using the Kjeldhal method involves the transformation of organic nitrogen to ammonia (NH_3-N) by digesting the samples with concentrated H_2SO_4 and measuring the amount of NH_3. According to Blakemore *et al.* (1987), copper, selenium or mercury catalyst is commonly added as Kjeldhal catalyst during digestion to increase the boiling point and thus increase the conversion rate. The NH_3 produced during digestion is basically measured by making the digest strongly alkaline and collecting it into a boric acid indicator solution by steam distillation followed by titrimetric determination of the ammonia trapped as ammonium borate. The equation below was used to determine the %N.

$$\% N = (g N / g S) \times 100$$

Where:

% N= Percentage nitrogen

g N = grams of nitrogen in the sample

g S = grams of samples

3.5.3. Atomic Absorption Spectrophotometry (AAS)

AAS was employed to determine tissue % K by the Koronivia Agricultural Research Station In this technique the atoms of elements were vaporized and atomized in the flame. The atoms then absorbed the light at a characteristic wavelength. The source of the light was a hollow cathode lamp, which was made up of same element which was to be determined. The lamp produces radiation of an appropriate wavelength which while passing through the flame was absorbed by the free atoms of the sample. The absorbed energy was measured by a photodetector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample which was calculated using the following equation (Antia *et al.,* 2006*)*.

3.5.4. UV- vis spectrophotometry

Phosporous was determined using the UV-vis spectrophometry by the Koronivia Agricultural Research Station. To prepare a colour developing solution, 20 mL of acidified solution of ammonium molybdate and 10 mL of ascorbic acid stock solution are added to 800 mL of distilled de-ionized water and diluted to 1L with distilled de-ionized water. The standards and samples are diluted. The diluted samples are left for at least 30 minutes for the colour development. The absorbance was measured on a UV-visible spectrophotometer at 660 nm (Chapman & Pratt, 1961). The equation below determined the amount of P and K.

$$\text{Available phosphorus /potassium mg/kg} = \frac{C \times 14}{ODW}$$

Where:

C = phosphorus/potassium concentration

ODW = Oven-dry sample weight (g)

14 = Dilution factor

Quality assurance and control was checked by the Koronivia Agricultural Research Station. The quantifications were performed using standard solutions. The competency of the methodology was carried by Koronivia Agricultural Research Station using known (reference) standards for % NPK.

3.6. Synergistic effect of temperature and nutrient

This part of the experiment followed similar protocol as the above except that the temperature and nutrient concentration varied simultaneously. The temperature and sodium nitrate concentration which produced the higher biomass were taken from the previous experiments and applied together for a period of one month. For *G. edulis*, the biomass was highest at the temperature of 30 °C and a sodium nitrate concentration of 4 mg/L when compared with the other temperature and nutrient concentration treatments. The Rhizoidal length (RL) growth was highest at 32 °C and sodium nitrate concentration of 4 mg/L. For *S. polycystum*, the RL showed highest growth at 28°C and sodium nitrate concentration of 4 mg/L. These combinations were taken into account for the synergistic effect of temperature and nutrient. The biomass of *S. polycystum* started to degrade therefore the biomass of *S. polycstum* was not taken into account and instead the RL was taken as a unit of measurement for growth.

There were four tanks where the parameters of photoperiod, salinity and pH, dissolved oxygen were constant; however, the temperature and nutrient concentration varied. The seaweeds *S. polycystum* and *G. edulis* were pat-dried and the biomass was measured on a top pan balance scale. Since temperature and nutrient variables are combined, the optimum temperature and nutrient concentration which was determined from the first part of the temperature and nutrients concentration experiments. The biomass of both the macroalgae was measured every three days for a further month.

At the end of the one month period, the samples of both species were oven-dried at 60 °C and the ash product was analyzed for nutrient contents within the tissues at the Koronivia Research Station. The change in biomass was analyzed, and statistically tested.

3.7. Statistical Analysis of the Results

The IBM Statistical Packaging for Social Sciences (SPSS) was utilized for the analysis of difference in biomass and rhizoid length at different temperatures (28 ℃, 30 ℃, 32 ℃ and 34 ℃) and at varying sodium nitrate nutrient concentrations (1 mg/L, 2 mg/L , 3 mg/L and 4 mg/L). The homogeneity of variance test was performed to ascertain whether the data was normally distributed. If the data was normally distributed, parametric test (A Paired Samples t- test Independent t- test, one-way ANOVA and Tukey *post hoc*) was performed and if it was not normally distributed, a non- parametric test (Kruskal-Wallis H) was done.

Paired Samples t-test was used to evaluate the differences before and after measures. For this experiment the before and after measured of the amount of nitrates present in the water which can be seen in Results Chapter in Figure 4.7 of the present study.

The independent t- test compared the means between two unrelated groups on the same continuous, dependent variable. It was used when cases were randomly assigned to one or two groups of samples that were not paired.

A one-way ANOVA was utilized to established if there were significant differences between the four temperature and nutrient regimes. Square root transformation was used for data that were transformed. Not all data were transformed but only the data on the tissue % NPK found naturally in the macroalgae without any treatments was. Square root transformation was applied to zero values and has moderate effect on distribution and shape. The following equation was used for the transformation of data.

$$x \text{ to } x^{(1/2)} = \text{sqrt}(x)$$

x being the value that is being transformed

Post Hoc test (Tukey's test) of multiple comparisons was carried out to determine which temperature and nutrient treatments significantly differed from each other. Post Hoc test is usually done when there is a significant difference in the one way ANOVA test since one way ANOVA does not determine the significant difference between each treatments of temperature and nutrient concentrations separately. A Tukey's HSD

(honest significant difference) test, or the Tukey–Kramer method, is a single-step multiple comparison procedure and statistical test. The Kruskal-Wallis H test was used to rank the temperatures and nutrient concentrations and determine if there were statistically significant differences between two or more treatments of temperature and nutrient concentration. Kruskal-Wallis H test is the non parametric counterpart of the Post Hoc test.

CHAPTER 4

RESULTS

This Chapter dwells on the results and data obtained from this study. There are four parts to the results. The first part shows the results from the seawater quality parameters of Makaluva Island and the % tissue NPK content that is present in *S. polycstum* and *G. edulis* without any treatments. The second part delves into the temperature phase of the experiment. The third part details on the results obtained from the sodium nitrate concentration (nutrient) phase of the experiment. The fourth and final part of the experiment provides the findings from the combined effects of temperature and sodium nitrate nutrient concentrations on the growth of *S. polycystum* and *G. edulis.*

4.1.Water quality tests for Makaluva Island

The quality of seawater at the Makaluva Islands collecting sites were tested using the API marine Seawater Master Test Kit™ (API FishCare, USA) which measured nitrate, nitrite, ammonia and phosphate where sample size was fifteen refer to Figure 4.0. The average salinity of Makaluva Waters was found to be 34.4 ‰ with an average temperature of 29.9 ℃ where n=15. The measurement of pH was 8.28 using a YSI model 85 meter. Figure 4.1 shows the seawater quality parameters.

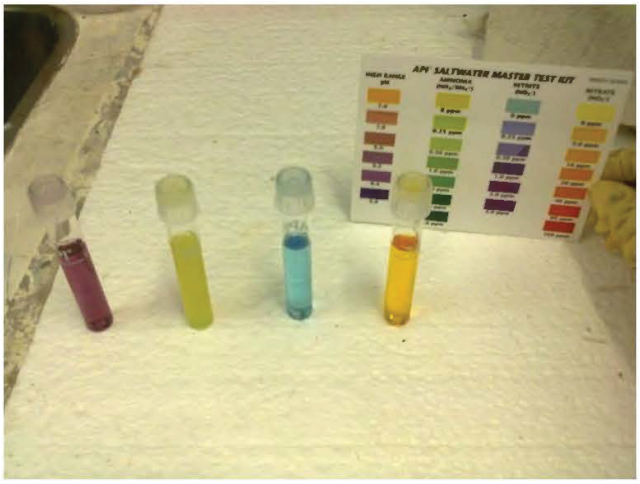

Figure 4.0 Testing the seawater water parameters form Makaluva Island using the API marine Seawater Master Test Kit™.

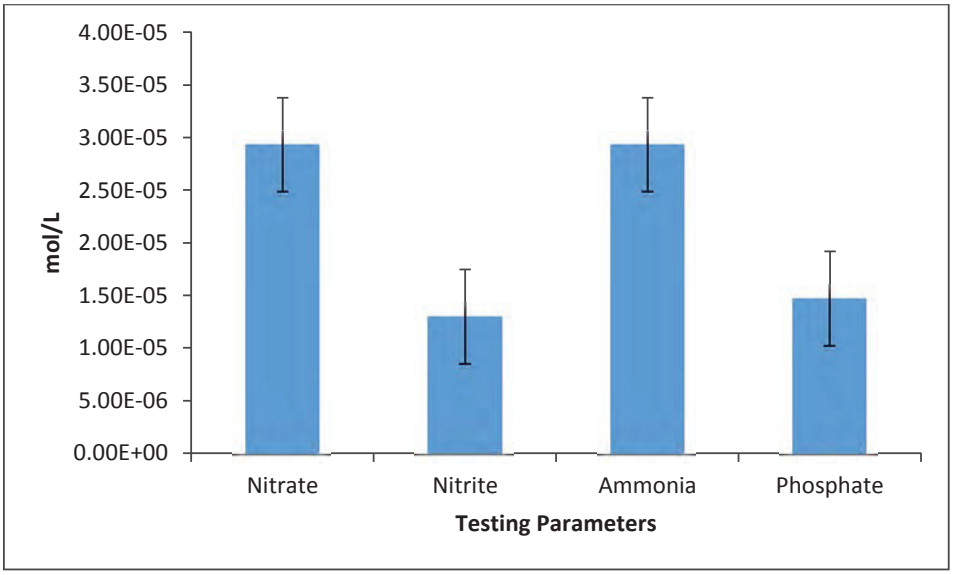

Figure 4.1 Seawater quality parameters of Makaluva Island for nitrates, nitrites, ammonia and phosphate (Mean ± S.E.) over a five month period from 20/10/2015 to 22/03/2016 with n=15 for each parameter.

Ammonia was at a higher amount of 2.93×10^{-5} mol/L when compared to nitrate, nitrite and phosphate. Nitrate was the second highest in amounts followed by phosphate then nitrite. This could indicate that Makaluva Island waters are not pristine as the Rewa river effluents are in its path which could be a contributing factor towards higher amounts of ammonia.

4.1.1. Assessment of % Tissue Nitrogen, Phosphorus and Potassium in Macroalgae collected from the waters of Makuluva Island

The total tissue % Nitrogen, Phosphorus and Potassium (% NPK) was analyzed by the Koronivia Agricultural Research Station. It was found that from the 10 samples for each species, *G. edulis* and *S. polycystum* both had significantly higher amounts of potassium (K) when compared to nitrogen (N) and phosphorous (P) as seen in Figure 4.2.

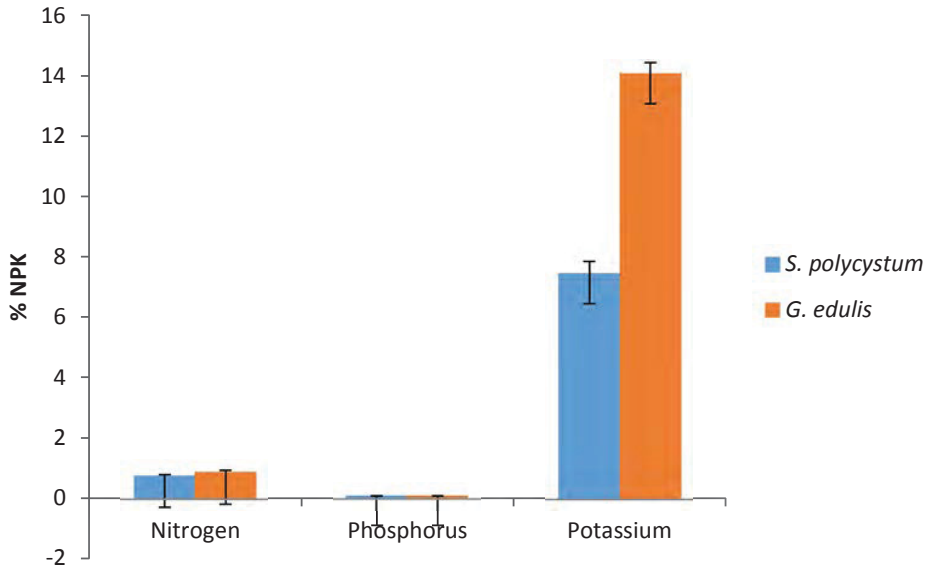

Figure 4.2 Percentage (%) NPK content (Mean ± S.E.) of *S. polycystum* and *G. edulis* from Makaluva Island. *Note n=10

The data for Figure 4.2 was further analyzed by using the IBM SPSS software version 21. A one-way ANOVA was carried out after the data was transformed to square root to achieve normality. The results from the one-way ANOVA took into account nitrogen and potassium while phosphorus was the same in both the samples of seaweed. There was no significant difference between the % N in both the seaweeds ($F(1, 18) = 1.641$, $p = 0.216$) however there was a significant difference in % K present in both the seaweeds ($F(1, 18) = 137.502$, $p = 0.00$). The % P data was constant in both seaweeds. In this study it was discovered that potassium was in very high amounts in *S. polycystum* while nitrogen was highest in *G. edulis* followed by phosphorus which was in the least amount and equal in both.

4.2. Effect of Temperature on the Growth of Macroalgae

The results of experiments on the effects of temperature on the growth of *G. edulis* are shown as SGR in Figure 4.3, and the Rhizoidal Length (RL) for both algae in Figure 4.4. The statistical data for the Tukey's test is summarized in Table 8.3 and Table 8.5, Appendix II.

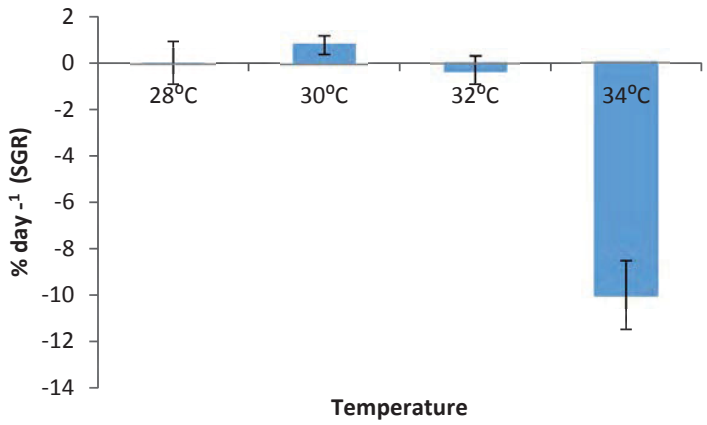

Figure 4.3 Specific Growth Rate in % day^{-1} (SGR) as Mean ± S.E. for *G. edulis* at various temperatures. *Note n=12

A one-way ANOVA was performed after data was found to be normally distributed. No transformation of data was required. There was a significant difference between the four temperatures ($F(3, 44) = 28.926$, $p = 0.00$). A Tukey *post-hoc* test confirmed that there was a significant between 28 ℃ - 34 ℃, 30 ℃ - 34 ℃, and 32 ℃ - 34 ℃, shown in Table 8.3, Appendix II. The optimum temperature for the growth of *Gracilaria edulis* is hence between the ranges of 28 ℃ - 32 ℃ as shown in Figure 4.3. There was a clear loss of biomass at 34 ℃. The SGR for *G. edulis* was the greatest at approximately (0.79 % day^{-1} ± 0.39) at the temperature of 30 ℃ as seen in Figure 4.3. The other three temperature regimes showed a decreased in biomass in the following trend 28 ℃ > 32 ℃ > 34 ℃.

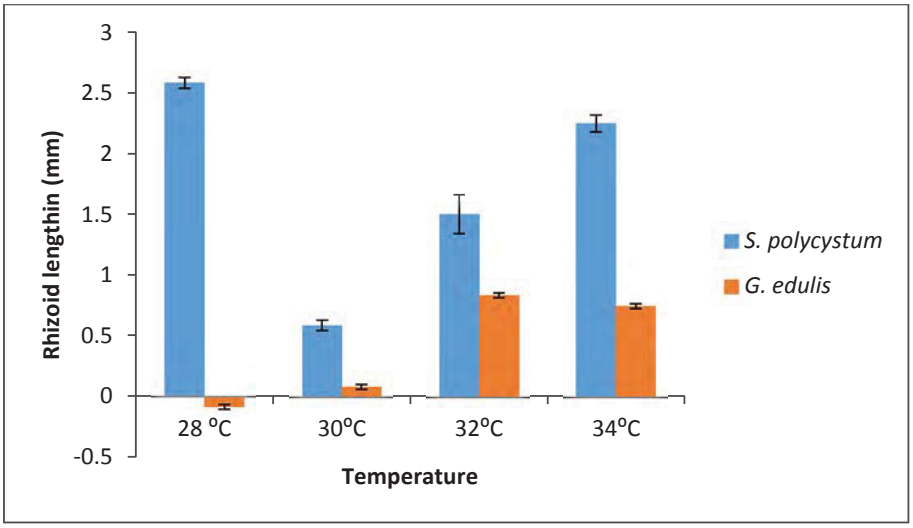

Figure 4.4 Rhizoidal Length (RL) in mm (Mean ± S.E.) of *S. polycystum* and *G. edulis* at varying temperatures regimes.
*Note n=12

For *S. polycystum*, a one-way ANOVA was performed since the data (Figure 4.4) was normally distributed. There was no significant difference between the four temperature regimes in *S. polycystum* ($F(3, 44) = 0.936$, $p = 0.432$). The Tukey's test also showed no significant difference. The data for *G. edulis* was not normally distributed so a Kruskal-Wallis H test was performed. The data could not be transformed since square root transformation is applied to zero value and there were no zero values. There was a significant difference of RL between the temperatures ($H(3) = 13.034$, $p = 0.05$) for *G. edulis*. The multicomparison of RL of *G. edulis* between 28 ℃ - 32 ℃, 28 ℃ - 34 ℃, 30 ℃ - 32 ℃ and 30 ℃ - 34 ℃ showed significant difference, shown in Table 8.5, Appendix II, all had the $p < 0.05$. The maximum growth of RL for *G. edulis* occurred at 32 ℃ with the optimum RL growth occurring at 32 ℃ - 34 ℃. The trends for *G. edulis* rhizoidal length were as follows 32 ℃ > 34 ℃ > 30 ℃ > 28 ℃ with the highest RL growth at 32℃. The trends for *S. polycystum* rhizoidal length were as follows 28 ℃ > 34 ℃ > 32 ℃ > 30 ℃ as depicted in Figure 4.4, the optimum RL is therefore between 28-34 ℃ for *S. polycystum*.

4.3. The Effect of Sodium Nitrate on the Growth of Macroalgae

The results on the individualistic effects of sodium nitrate on the SGR of *G. edulis* are shown in Figure 4.5 and the rhizoidal length of *G. edulis* and *S. polycystum* are shown in Figure 4.6. The amount of nitrate present in the water was monitored by measuring the amounts of nitrate present using the API marine Seawater Master Test Kit™ (API FishCare, USA) before and after the administration of $NaNO_3$ for the various sodium nitrate concentrations administered as shown in Figure 4.7.

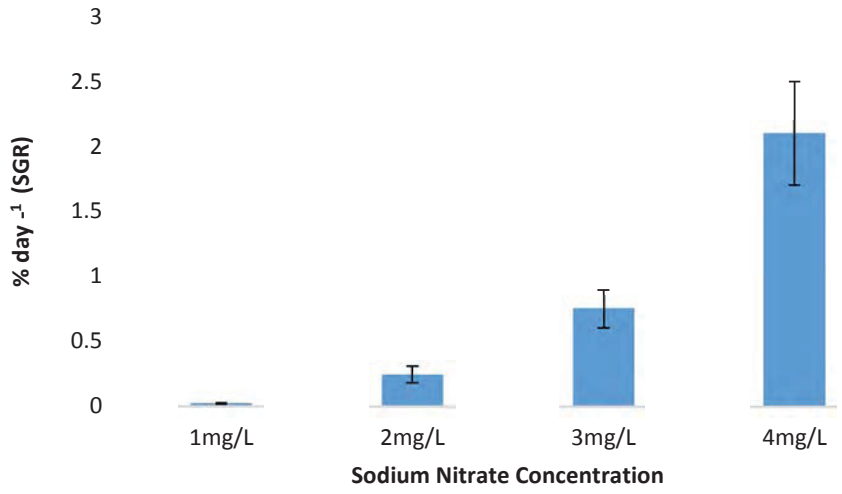

Figure 4.5 Specific Growth Rate in % day^{-1} (SGR) as Mean ± S.E. for *G. edulis* at various sodium nitrate concentrations.
*Note n=12

The data for Figure 4.5 was not normally distributed therefore the Kruskal-Wallis H test was performed and there was a significant difference between concentrations of sodium nitrate ($H(3)= 41.140$, $p=0.00$) refer to Table 8.7, Appendix II. The SGR for *G. edulis* (2.10 % $day^{-1} \pm 0.40$) was optimum at the administered sodium nitrate nutrient concentration of 4 mg/L (see to Figure 4.5). The other three nutrient regimes showed an increase in biomass in the following trend 4 mg/L > 3 mg/L > 2 mg/L > 1 mg/L. Table 8.7, Appendix II shows the multicomparison between each sodium nitrate concentration where $p < 0.05$.

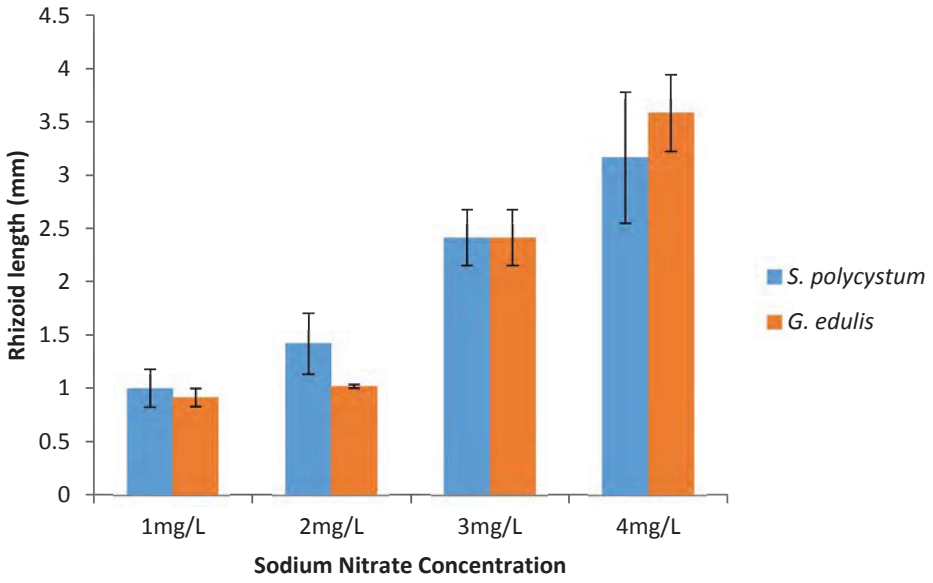

Figure 4.6 Rhizoidal Length (RL) in mm (Mean ± S.E.) of *S. polycystum* and *G. edulis* at varying concentrations of sodium nitrate.
*Note n=12

The above data for Figure 4.6 was not normally distributed for both the species therefore the non parametric Kruskal-Wallis H test was performed. There was a significant difference between sodium nitrate concentrations for both species with *G. edulis* RL ($H(3)= 39.945$, $p=0.00$) and for *S. polycystum* ($H(3)= 24.304$, $p=0.00$), refer to Table 8.9, Appendix II for the multicomparison of sodium nitrate concentrations. From the multicomparison of the different sodium nitrate concentration for both the species, the optimum concentration for the rhizoidal growth for *S. polycystum* and *G. edulis* was at 4 mg/L. The following trends of 4 mg/L > 3 mg/L > 2 mg/L > 1 mg/L showed increased RL in *S. polycystum* however there was no significant difference between the 1 mg/ L and 2 mg/L. Likewise, for *G. edulis* the trend was as follows 4 mg/L > 3 mg/L = 2 mg/L > 1 mg/L as seen in Figure 4.6 but there was no significant difference between 1 mg/L and 2 mg/L.

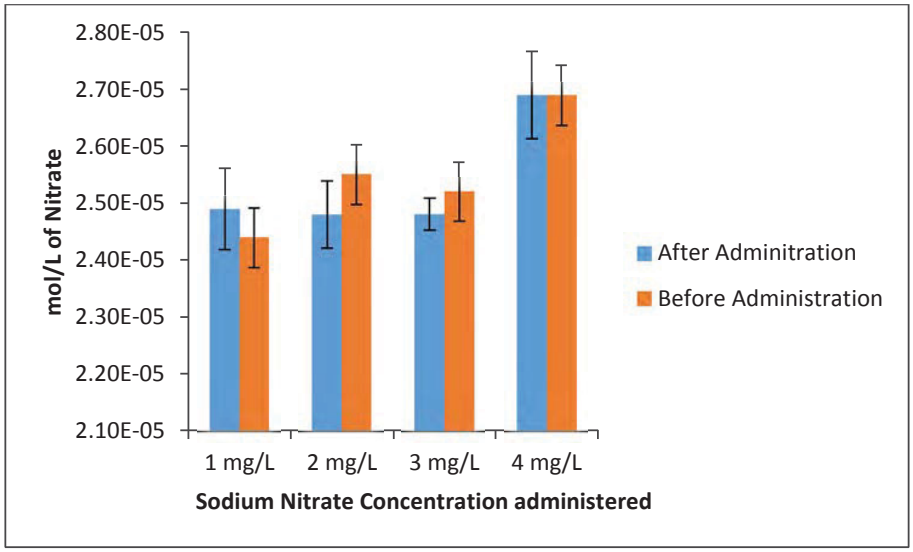

Figure 4.7 Nitrate concentrations mol/L (Mean ± S.E.) present in the water before and after the administration of sodium nitrate for the various treatments. Note* n=15 for each treatment.

There was no statistically significant difference in the amount of nitrate present in the water before and after the administration of sodium nitrate (see Figure 4.7 and Table 8.10, Appendix II) as determined by Paired Sampled t- test ($t = -0.628$, df 19, $p < 0.538$). Nitrate that were present before changing the water were in similar amounts as after addition of sodium nitrate. However, before administration of sodium nitrate shows a gradual increase from 1 mg/L to 2 mg/l then a decrease at 3 mg/L followed by an increase. However, after administration of nitrates there was not much variation between 1 mg/L and 3 mg/L in both the before and after administration of $NaNO_3$ but at 4mg/L there was an increase in both the before and after administration $NaNO_3$.

4.3.1. Nitrogen Analysis of *G. edulis* and *S. polycytsum*

The results of the total nitrogen % tissue content of *G. edulis* and *S. polycystum* at various sodium nitrate concentrations are shown in Figure 4.8 and Table 8.12, Appendix II for the multicomparison at different sodium nitrate concentrations.

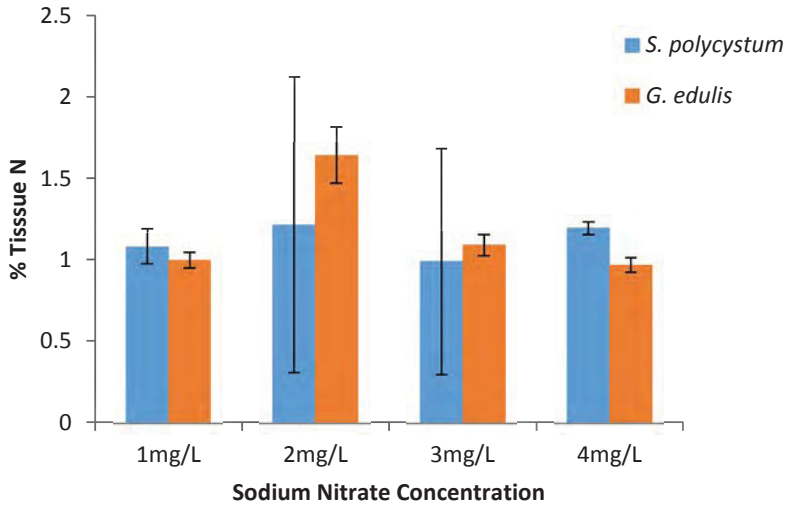

Figure 4.8 Tissue Nitrogen content (%) (Mean ± S.E.) of *G. edulis* and *S. polycystum* at varying sodium nitrate concentration.
*Note n=12

Figure 4.8 data was not normally distributed therefore the Kruskal-Wallis H test was performed and it was discovered that there was a significant difference between the different concentrations for *G. edulis* (H(3) = 17.95, p = 0.000) while there was no significant difference for *S. polycystum* (H(3) = 3.855, p = 0.278). In this study, the percentage tissue nitrogen content for *G. edulis* and *S. polycystum* were highest at 2 mg/L as depicted in Figure 4.8 with the trend for % tissue nitrogen content as follows for *G. edulis*; 2 mg/L > 3 mg > 1 mg/L > 4 mg/L. There was significant difference between 1 mg/L- 2 mg/L, 2 mg/L - 3 mg/L and 2 mg/L - 4 mg/L, refer to Table 8.12, Appendix II. For *S. polycystum* the trend were as follows; 2 mg/L > 4 mg/L > 1 mg/L > 3 mg/L refer to Figure 4.8. The optimum % tissue nitrogen content for *G. edulis* was at 2 mg/L while for *S. polycystum* it was 1 - 4 mg/L.

4.3.2. Phosphorus Analysis of *G. edulis* and *S. polycytsum*

The results of the total phosphorus % tissue content of *G. edulis* and *S. polycystum* at various sodium nitrate concentrations are shown in Figure 4.9 and Table 8.13, Appendix II for the multicomparison at different sodium nitrate concentrations.

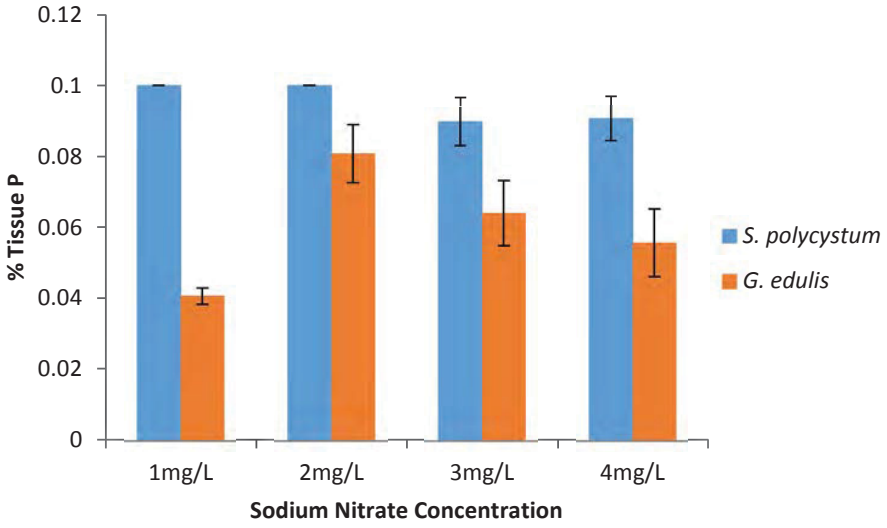

Figure 4.9 Tissue Phosphorus content (%) (Mean ± S.E.) of *G. edulis* and *S. polycystum* at varying sodium nitrate concentration.
*Note n=12

The data was not normally distributed therefore the Kruskal-Wallis H test was carried out to determine that there was a significant difference between the concentration of *G. edulis* (H(3) = 17.951, p = 0.000). However, at the concentrations of 1 mg/L and 2 mg/L, the % P of *S. polycystum* was found to be constant and is included in the results where the overall result of % P for *S. polycystum* was non-significant (H(1) = 0.008, p = 0.929). The highest % P tissue content for *S. polycystum* was at 1 mg/L with the trends as follows 1 mg/L = 2 mg/L > 3 mg/L = 4 mg/L as seen in Figure 4.9 while for *G. edulis* at 2 mg/L with the following trends 2 mg/L > 3 mg/L > 4 mg/L > 1 mg/L. There was a significant difference between 1 mg/L – 2 mg/L and 3 mg/L – 4mg/L for *G. edulis* refer to Table 8.13, Appendix II. The optimum % P tissue content was at 2-4 mg/L for *G. edulis* while for *S. polycystum* it was 1- 4 mg/L of sodium nitrate concentration.

4.3.3. Potassium Analysis of *G. edulis* and *S. polycytsum*

The results of the total potassium % tissue content of *G. edulis* and *S. polycystum* at various sodium nitrate concentrations are shown in Figure 4.10 and Table 8.14, Appendix II for the multicomparison at difference sodium nitrate concentrations.

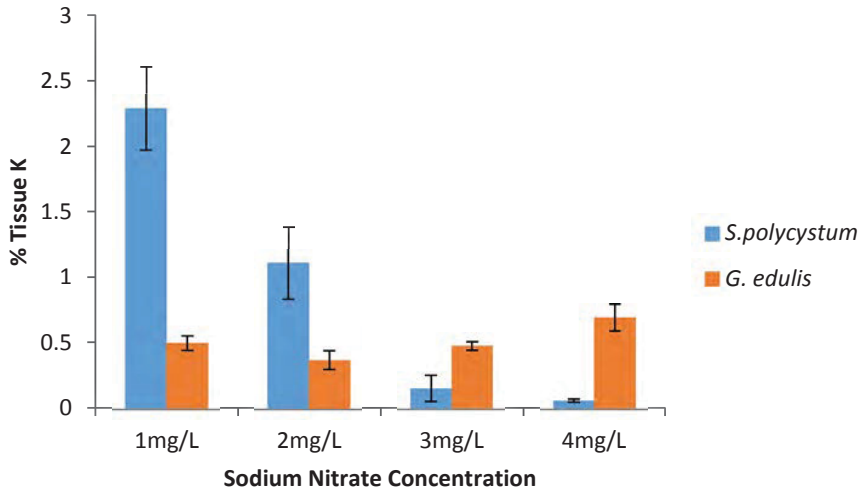

Figure 4.10 Tissue Potassium content (%) (Mean ± S.E) of *G. edulis* and *S. polycystum* at varying sodium nitrate concentration.
*Note n=12

The data for Figure 4.10 was not normally distributed therefore the Kruskal-Wallis H test was carried out to determine whether that there was a significant difference in the % tissue K content at different concentration in *G. edulis* (H (3) = 10.716, p = 0.013). Likewise, for *S. polycystum* there was a significant difference (H (3) = 35.449, p = 0.000). The % K tissue content was highest at 1mg/L concentration of sodium nitrate for *S. polycystum* with the following trend; 1 mg/L > 2 mg/L > 3 mg/L > 4 mg/L as shown in Figure 4.10. However, the % K was highest when sodium nitrate concentration was 4 mg/L for *G. edulis* with the following trend 4 mg/L > 1 mg/L > 3 mg/L > 2 mg/L. For the *G. edulis*, there was a significant difference between 1 mg/L – 2 mg/L, 2 mg/L – 3mg/L and 2 mg/L - 4 mg/L however, for *S. polycystum,* apart from comparison between 1 mg/L – 2 mg/L and 3 mg/L – 4 mg/L, the remaining multicomparisons between the sodium nitrate concentrations had the p = 0.000 as shown in Table 8.14, Appendix II. The optimum % K tissue content was at 3 - 4 mg/L for *G. edulis* while for *S. polycystum* it was 1 mg/L of sodium nitrate concentration.

4.4. The Synergistic Effects of Temperature and $NaNO_3$ concentration on the Growth of *Gracilaria edulis* and *Sargassum polycystum*

Specific Growth Rate (SGR)

From sections 4.2 and 4.3 of the Results, the optimum temperature for *G. edulis* SGR between 28 - 32 ℃ and the optimum $NaNO_3$concentration was 4 mg/L for *G. edulis*. Hence for this study the synergistic combination adopted for the *G. edulis* SGR experiment was 30℃ and 4 mg/L $NaNO_3$.

Rhizoidal Growth (RL)

For *Sargassum polycystum*, the range of temperature at which rhizoids grew best was between 28℃- 34℃, however, for the purpose of this study the highest RL was at 28℃ therefore, 28℃ was taken together with 4 mg/L NaNO3 for the combination effect. The rhizoidal length for *G. edulis* was optimum at 30℃- 32℃ however, growth was highest at 32℃ which was combined with the optimum $NaNO_3$ concentration of 4 mg/L. Hence, for the purpose of this study, the combined temperature and $NaNO_3$ concentrations for the RL of *G. edulis* were studied at 32℃ and 4 mg/L. The control temperature was 29℃ and without the administration of $NaNO_3$.

4.4.1. Specific Growth Rate (SGR) and tissue NPK content (%) of *Gracilaria edulis* at the temperature of 30 ℃ and sodium nitrate concentration of 4 mg/L

The results of the experiment on the combined effects at the temperature of 30 ℃ and 4 mg/L of sodium nitrate concentrations on the SGR of *G. edulis* are shown in Figure 4.11 and % NPK of *G. edulis* in Figure 4.12.

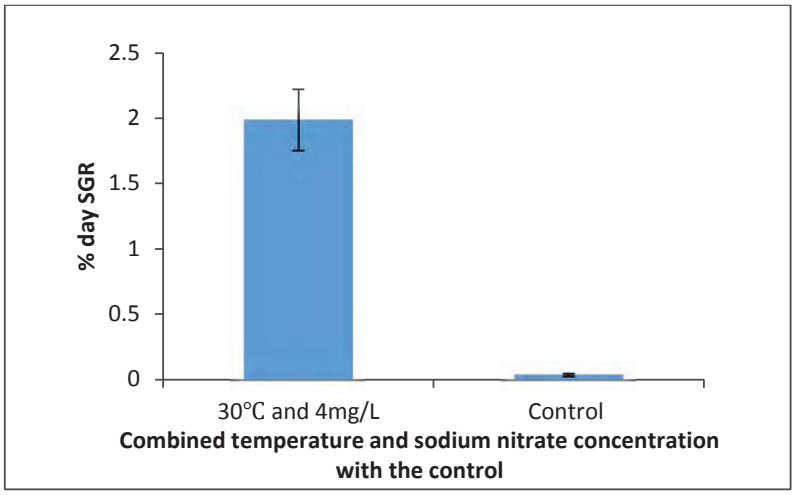

Figure 4.11 Specific Growth Rate in % day^{-1} (SGR) (Mean ± S.E.) for *G. edulis* for the combined temperature and sodium nitrate nutrient concentration of 30 ℃ and 4 mg/L. Note* n=12

For the data of Figure 4.11, an Independent Samples T test was performed and there was a statistically significant difference in the SGR of *G. edulis* at the combined temperature and nutrient of 30 ℃ and 4 mg/L when compared with the control which was 29 ℃ with no administration of nutrients ($t = 8.235$, $df = 22$, $p < 0.000$). As shown in Figure 4.11, SGR was highest at the combined temperature and nutrients of 30 ℃ and 4 mg/L as compared to control.

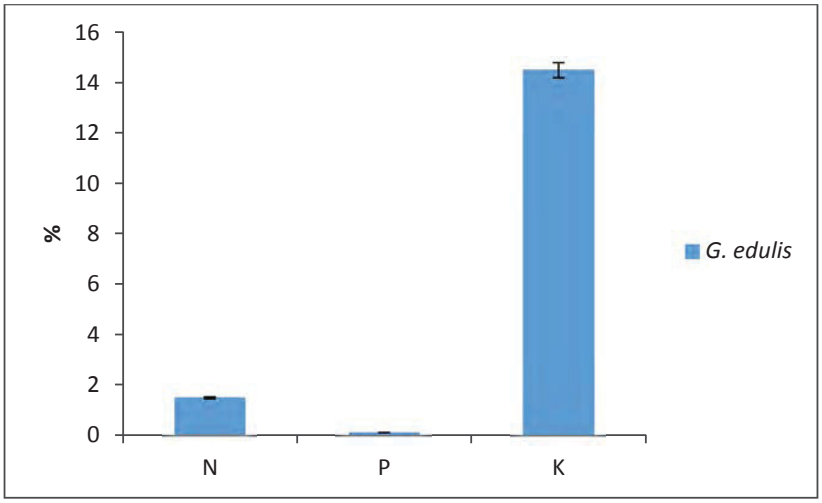

Figure 4.12 Tissue NPK content (%) (Mean ± S.E.) of *G. edulis* at the combined temperature of 30 ℃ and 4 mg/L of sodium nitrate concentration.
Note * n=12. The % NPK of control could not be carried out due to low biomass.

For the data of Figure 4.12, a One way ANOVA was performed ($F(2, 33) = 2069.434$, $p= 0.000$). A Tukey's *post- hoc* test also revealed a significant difference between, P and K where p=0.00. The % K was higher than the % N and % P with the following trend % K > % N > % P.

4.4.2. Rhizoidal length (RL) and tissue NPK content (%) of *Gracilaria edulis* at the temperature of 32 ℃ and sodium nitrate concentration of 4 mg/L

The results of the experiment on the combined effects at the temperature of 32 ℃ and 4 mg/L of sodium nitrate concentration on the RL of *G. edulis* are shown in Figure 4.13 and % NPK of *G. edulis* in Figure 4.14.

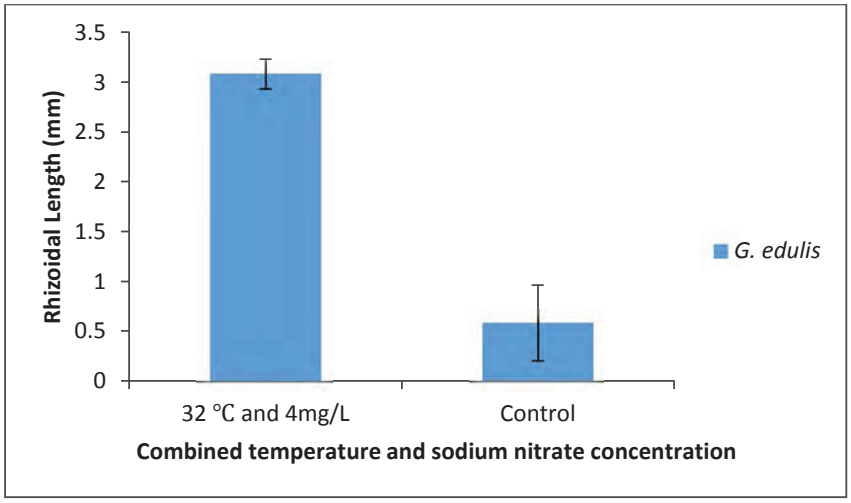

Figure 4.13 Rhizoidal length in mm (Mean ± S.E) of *G. edulis* for the combined temperature and sodium nitrate nutrient concentration of 32 ℃ and 4 mg/L.
Note * n=12. The % NPK of control could not be carried out due to low biomass.

The data in Figure 4.13 was not normally distributed thus a Kruskal-Wallis H Test was performed and it was found that there was a significant difference between the means of *G. edulis* and the control ($H(1) = 19.045$, $p = 0.000$). As shown in Figure 4.13, the combined temperature of 32℃ and $NaNO_3$ concentration of 4 mg/L had the highest rhizoidal length compared to control.

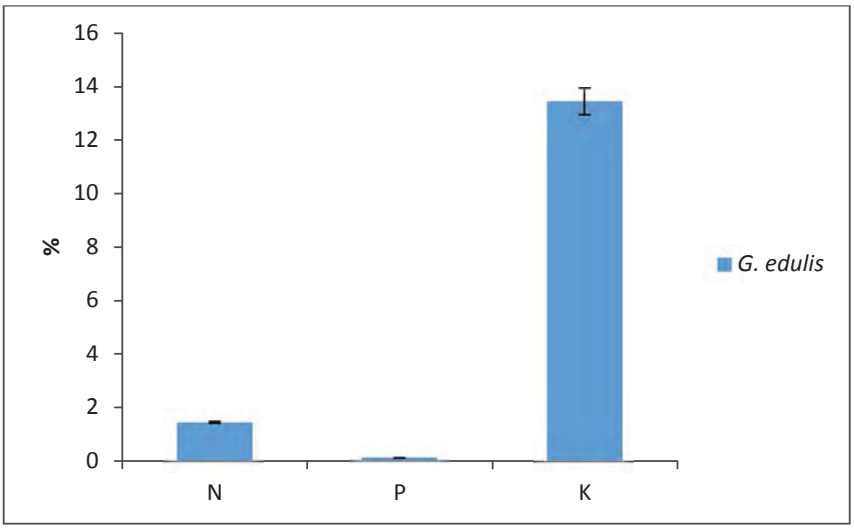

Figure 4.14 Tissue NPK content (%) (Mean ± S.E.) of *G. edulis* for the combined temperature and sodium nitrate nutrient concentration of 32 ℃ and 4 mg/L. Note * n=12

A one way ANOVA was performed on the data for Figure 4.14 ($F(2, 33) = 668.764$, $p = 0.000$). A Tukey's *post- hoc* test also revealed a significant difference between, P and K where $p < 0.05$. The % K was in greater amounts than % N which was more

than % P. The % NPK at control could not be determined because seaweeds had deteriorated due to experimental parameters as there was no administration of nutrients to help with the growth.

4.4.3. Rhizoidal length (RL) and tissue NPK content (%) of *Sargassum polycystum* at the temperature of 28 ℃ and sodium nitrate concentration of 4 mg/L

The results of the experiment on the combined effects at the temperature of 28 ℃ and 4 mg/L of sodium nitrate concentrations on the RL of *S. polycytsum* are shown in Figure 4.15 and % NPK of *G. edulis* in Figure 4.16

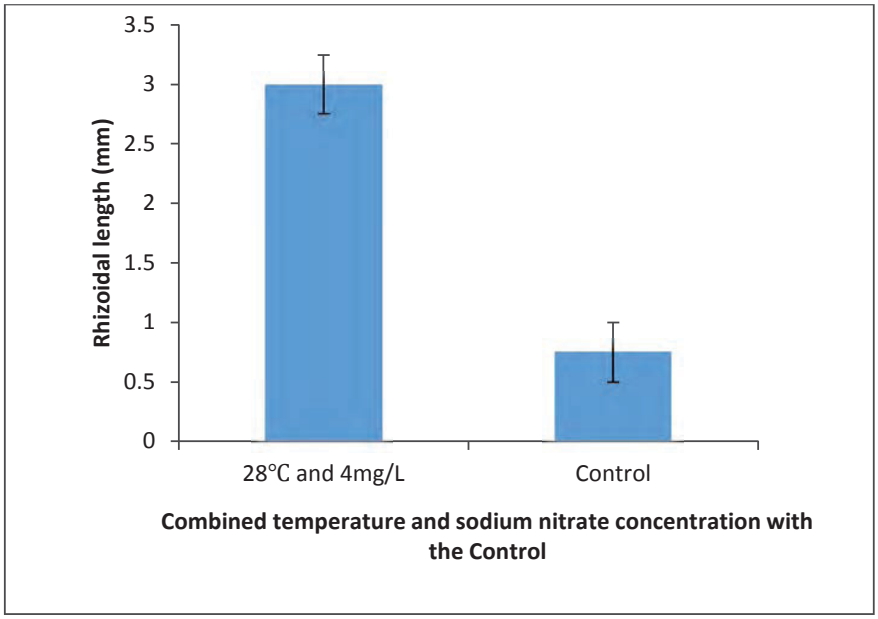

Figure 4.15 Rhizoidal length in mm (Mean ± S.E.) of *S. polycystum* for the combined temperature and $NaNO_3$ concentration of 28 ℃ and 4 mg/L. Note * n=12

A Kruskal-Wallis H Test was performed in Figure 4.15 and found that there was a significant difference between the means of (H (1) = 19.675, p = 0.000). The rhizoidal length was greater at the temperature and $NaNO_3$ combination of 28 ℃ and 4 mg/L than the control temperature of 29 ℃ and with no administration of $NaNO_3$.

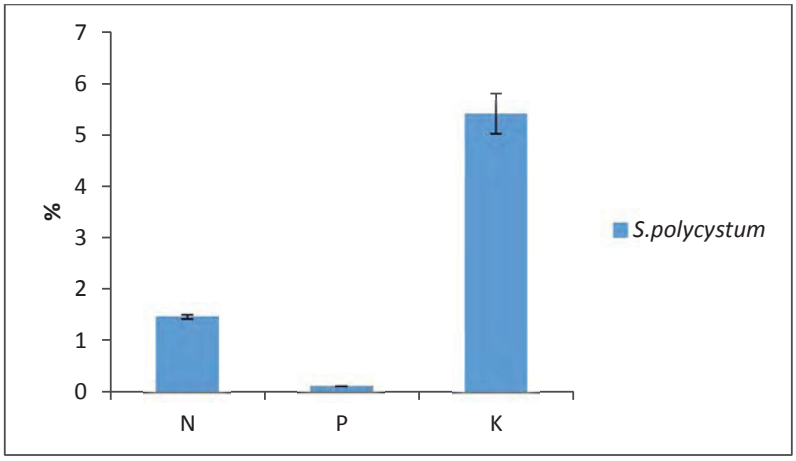

Figure 4.16 Tissue NPK content (%) (Mean ± S.E.) of *S. polycystum* for the combined temperature and $NaNO_3$ nutrient concentration of 28 ℃ and 4 mg/L.
Note * n=12. The % NPK of control could not be carried out due to low biomass due to control samples deteriorating.

For Figure 4.16, a one-way ANOVA was performed ($F(2, 33) = 151.031$, $p= 0.000$). A Tukey's *post- hoc* test also revealed a significant difference between, P and K where $p = 0.00$. The % K was more than % N which was more than % P. The % NPK at control could not be attained because seaweeds had deteriorated as there was no administration of nutrients in the control. Seaweeds usually require a lot of nutrients when cultured experimentally however this was a control so there was no administration of nutrients.

CHAPTER 5

DISCUSSION

The first part of this chapter deals with seawater parameters with the evaluation of the % NPK that is naturally present in *S. polycystum* and *G. edulis*. The second part discusses the individualistic effect of temperature on *S. polycystum* and *G. edulis*. The third part discusses the individualistic effects of nutrients on the growth of *S. polycystum* and *G. edulis* with an evaluation of the % NPK and the fourth and final part discusses the combination effects of temperature and nutrients on growth of *S. polycystum* and *G. edulis* with evaluation of the % NPK.

5.1. General Discussion on Seawater Quality and % NPK found in *S. polycystum* and *G. edulis*

Major nutrients present in the marine environment consist of nitrate, nitrite, ammonia and phosphate. In this study, ammonia is in higher amounts (2.93×10^{-5} mol/L) when compared to nitrate, nitrite and phosphate over a five month period from 20/10/2015 to 22/03/2016. Ammonia concentrations of less than 0.01 mg/L (5.87×10^{-7} mol/L) is considered not harmful to the marine environment (Mosley & Aalbersberg, 2003). Polluted waters usually have high amounts of nitrate or phosphate; however this was not the case for Makaluva waters. According to Mosley & Aalbersberg (2003), in order for waters to be considered pristine the amount of nitrate should be < 1µM/L (1×10^{-6} mol/L) and phosphate should be < 0.1µM/L (1×10^{-7} mol/L). Makaluva waters had nitrate and phosphate at 2.63 10^{-5} mol/L and 1.47×10^{-7} mol/L respectively which are higher than the harmful levels. The Rewa river is polluted and merges into the Makaluva waters which could be a contributing factor to its pollution (Mosley & Aalbersberg, 2005). Hence, the waters of Makaluva island cannot be considered pristine which could explain the high abundance of the opportunistic seaweeds *G. edulis* and *S. polycystum*.

The % NPK tissue content of *S. polycystum* and *G. edulis* found in Makaluva waters was determined in this study because the % NPK tissue content can be an indication of water enrichment (Bjoernsaeter & Wheeler, 1990; Jeff, 1999). In this study, it was discovered that potassium was in very high amounts in both *S. polycystum* and *G.*

edulis while nitrogen was the second highest followed by phosphorus which was the least in amount, as seen in Figure 4.2 above in Results. There is scare information available on the % NPK tissue content analysis of *S. polycystum* and *G. edulis*, however, a study by Haq *et al* (2011) who analyzed the combined biomass of seaweeds such as *Ulva fasiata*, *Chondria tennussima*, *Sargassum* spp., and *Valoniopsis pachyema* for % NPK tissue content. It was found that the seaweeds had an overall content of 2.3% Nitrogen, 0.86% Phosphate and 1.8% Potassium, with nitrogen being in the highest amount. In the present study, phosphate was in the highest amount for both *G. edulis* (14.07 % ± 0.36) and *S. polycytstum* (7.47 % ± 0.39), however, these two species were not investigated by Haq *et al* (2011). Better comparison could be made if *Sargassum* spp. from Haq *et al* (2011) was analyzed separately for tissue % NPK rather than combined biomass; generally seaweeds are low in Nitrogen content. On the other hand, Rizvi & Shameel (2003) found that red seaweed *Gracilaria corticata* contained on average high amounts of potassium ranging from 2411.38 ppm – 76714.25 ppm. The present study demonstrated that potassium is naturally present in high amounts as opposed to nitrogen, which could be attributed to the two macroalgae under study having different physiological properties (Chopin *et al*., 1996; Edding *et al*., 1987; Fong *et al*., 1998; Goodwin *et al*., 2013; Lobban & Harrison, 1994; Naldi & Viaroli, 2002). According to Valiela *et al* (1997), macroalgae store nutrients based on the environmental conditions subjected upon them, hence environmental parameters appear to govern the growth of *S. polycystum* and *G. edulis* in Fijian waters that were investigated.

5.2. Effect of Temperature on *G. edulis* and *S. polycystum* Thallus Growth and Rhizoidal Extension

The optimum temperature for *G. edulis* SGR was within the range of 28 - 32 ℃. A study done by Raikar *et al* (2001) discovered that *G. edulis* from India and *G. lichenoides* from Malaysia showed a high daily growth rate of 11.69 % and 12.26 % respectively at 30 ℃. The seaweed *G. edulis* was found to be abundant at Makaluva Island where the seawater temperatures were measured over a five month period from 20/10/2015 to 22/04/2016 with n=15 for each parameter and found to be an average of 29.9 ℃ ±0.2. However, according to Raikar *et al* (2001), *Gracilaria* species were

found to be abundant when the seawater temperatures fluctuated from 20-27 °C but they became adapted to higher temperatures as *Gracilaria* spp. from the tropical regions are found to be growing well at 28 ℃ (Raikar *et al*., 2001). Seawater temperatures in the Arabian Sea, range from 26 to 30 °C, Bay of Bengal has 24 to 30 °C and Malaysia has 26 to 28.5 °C (Raikar *et al*., 2001). Temperatures below this range would hinder the growth and lower temperatures became lethal. It thus suggests that lower winter temperatures towards the temperate regions act as barriers to the distribution of species from tropical regions. The SGR of *G. edulis* decreases at 34 ℃; this loss in biomass has been attributed to *G. edulis* being not able to cope with too high temperatures (Raikar *et al*., 2001). The experimental results of the present study concur with the experimental results from Raikar *et al* (2001) in that *G. edulis* was found to grow optimally at 28 - 32 ℃. Macroalgae will grow naturally in the environment in which they are adapted and temperatures above or below their optimum temperature range would deter growth (Annigeri, 1979; Mojumdar, 1979; Toh, 1996). According to Kim *et al* (2016), *Gracilaria vermiculophylla* is able to tolerate high temperatures and has high growth capacity which makes it ideal for successful invasion which is why it is able to grow both in temperate regions and tropical regions having various temperature regimes. The SGR of *S. polycystum* could not be ascertained in the present study because *S. polycystum* lost its biomass over the course of the experiment during acclimatization (similar to what was reported by Zhao *et al* (2008) for *Sargassum thungerbii*), thus RL was taken as a unit of measurement for growth as rhizoids are better adapted than the thallus at higher temperatures of 31 ℃ (Kokubu *et al.,* 2015). Also rhizoids are present all throughout the year thus measurements can be conducted throughout the year (Kokubu *et al*., 2015).

In the present study, the optimum temperature for the RL for *G edulis* and *S. polycytsum* were 32 - 34 ℃ and 28 - 34 ℃ respectively. For *G. edulis,* the rhizoidal length grew optimally at 32 – 34 ℃ because of its ability to tolerate high temperatures approximately between 35 – 30 ℃ (Iyer *et al.*, 2004; Raikar *et al*., 2001). From the results of this current study, it can be indicated that *S. polycystum* has a wide tolerance for a broad range of temperatures similar to what Zhao *et al*.(2008) had found. In the latter study, the RL increased to 1.5 mm as temperature increased from 10 - 25 ℃ in *Sargassum fulvellum* under 12 hour photoperiod. However, in the present study, at

28 ℃, *S. polycystum* had the highest rhizoidal length of 0.26 mm ± 0.05 indicating that the species prefers cooler temperatures. This correlates to a study done by Hwang *et al.* (2006) where *Sargassum fulvellum* preferred fall and winter temperature of 5- 20 ℃ in Jeonna, Korea. The RL of *S. polycystum* also gradually increased between 30 ℃ and 34 ℃ with the rhizoids remaining intact at the highest temperature, which could be attributed to *S. muticum* rhizoids becoming resilient to higher temperatures to increase survivability, as reported by Brawley & Johnson (1992). Another reason for having such wide optimum temperature ranges is that *Sargassum* spp. grow in a highly heterogeneous spatial environment (Wright *et al.*, 2004).

5.3. Effect of Nutrients on Macroalgae and the Evaluation of % NPK

SGR

Nutrient uptake rate varies considerably based on various physical, biological and chemical factors. For the purpose of this study, physical and biological factors were kept constant while the concentration of sodium nitrate nutrient was varied. Nutrients are naturally present in the marine environment in various forms such as nitrate, nitrite, ammonia, phosphate to name a few. These nutrients are utilized up by many marine organisms. However, in coastal populated areas there is often an influx of nutrients due to anthropogenic effluents sewage seepage from villages and hotels, fertilizer run offs from agriculture and household wastes waters containing detergents high in phosphates (Mosley & Aalbersberg, 2005; McLaughlin *et al*., 2010).

In this study the optimum $NaNO_3$ concentration for *G. edulis* SGR was at 4 mg/L. The biomass gradually increased as sodium nitrate concentration was increased. According to Dawes *et al.* (1993), enriched sodium nitrate of 10 mol/L increases the number of branches of *Gracilaria tikvahiae* when compared to sterile seawater. The main nutrient component from sodium nitrate is nitrate, this is an essential element which is required for algal metabolism (Lobban & Harrison, 1997; Korpinen, 2010). Increase in SGR of *G. edulis* with increasing sodium nitrate concentration could allow for increased metabolism leading to bloom of the species. According to Martins *et al.* (2011), seaweeds such as *Hypnea musciformis* have the ability to store nitrogen mainly in the

form of proteins, but since in our case the Nitrogen content of *G. edulis* and *S. polycystum* was low, this adaptation mechanism may not apply in these species.

RL

In the present study, the rhizoidal length of both seaweeds increased gradually as the sodium nitrate concentration increased and the optimum sodium nitrate concentration was found to be at 4 mg/l for the RL of both seaweeds however, *G. edulis* had greater extension in rhizoid length compared to *S. polycystum.* This could be attributed to *G. edulis* thallus remaining intact and so having the ability to store more nutrients than *S. polycystum* which lost thallus mass during the oncourse of the experiment (Kaliaperumal & Ramalingam, 2005; Murthy & Rao, 2002). According to Hay (1986), thallus loss is attributed to seaweeds growing close together in the marine environment where being clustered together reduces mortality however, in a laboratory setting growing seaweeds separately causes mortality as observed in the present study. Moreover, macroalgae that have multilayered, flat and narrow thallus such as *Sargassum* spp. with flaccid, drooping leaves that could induce thalli loss (Hay, 1986). *Sargassum* spp. is also abundant in waters near hotels which are enriched with nutrients because of their rapid intake of nutrients (Mosley & Aalbersberg, 2005; Murthy & Rao, 2002). The difference in the extension of rhizoids in *S. polycystum* and *G. edulis* could be attributed to their different nutritional requirements.

The amount of nitrate was measured using the API marine Seawater Master Test Kit™ (API FishCare, USA) before and after changing the waters in the experimental jars. There was not a significant difference in the amount of nitrate before and after addition of sodium nitrate (refer to Table 8.10, Appendix II) because macroalgae have the ability to take up nutrients quickly from the environment which makes it difficult to ascertain the amount of nutrients already present (Mosley and Aalbersberg, 2003; 2005). According to Tamata (2012). *Sargassum* spp. responds to nutrient enrichment (sodium nitrate and sodium phosphate) by immediate rapid uptake of nutrients. Likewise, nitrogen-starved *Gracilaria tikvahiae* plants were reported to assimilate ammonium-nitrogen very quickly (McLaughlin *et al*., 2010).

The % tissue NPK content for both seaweeds was determined in order to verify earlier studies on sodium nitrate enrichment experiment on seaweeds by Fong *et al.* (1998). Nutrients are rapidly taken up by macroalgae which makes it difficult to use water column concentrations as nutritive enrichment indicators (Fong *et al*., 1998; Hanisak, 1979). The % tissue nitrogen content provides a better indication than biomass of nitrogen supply in the environment (Edwards *et al*., 2006; Fong *et al*., 1998). According to Bird *et al.* (1982), total tissue N and amino acid concentrations of macroalgae are subjected to the availability of dissolved nutrients in the water column. Tissue % nitrogen content is best suited for discerning nutrient availability for any month (Fong *et al*., 1998).

The highest % tissue Nitrogen content for *G. edulis* and *S .polycystum* were at 2 mg/L and 1- 4 mg/L of sodium nitrate respectively. For *G. edulis*, highest growth at a lower calcium nitrate concentration of 10 mg/L as compared to 50 mg/L has previously been reported (Kaliaperumal & Ramalingam, 2005; Rao, 1975). Seaweeds react to nitrate enrichment by taking up the nitrates and utilizing it for growth while storing surplus nitrate for future growth or when the surrounding environment is nutrient starved (Fong *et al*., 1998). According to Todd *et al*. (2009), nitrogen is present in seaweeds days after enrichment making it ideal to for the % tissue Nitrogen content present in *G. edulis* to be studied in enrichment experiments. *Gracilaria edulis* has the ability to absorb 100 times the ambient nitrates present in the water column which increases the tissue nitrogen content to 1.6 % (Constanzo *et al*., 2000). The variability in the % tissue Nitrogen of *G. edulis* in this study is indicative that nitrogen could be limiting and does not get stored up in the storage vacuoles (Todd *et al*., 2009). On the other hand, nitrogen may not be the only limiting nutrient. There could be co-limitation of nitrogen with other nutrients such as phosphorus (Todd *et al*., 2009; Fong & Zedler, 1994). Moreover according to Todd *et al.* (2009), a large natural influx of water adequately increases the delivery of nitrogen to algal cells but this was not in our case as this study was performed in a laboratory setting. Red macroalgae have the ability to store more tissue nitrogen compared to brown macrolagae (Todd *et al*., 2009). According to Kim *et al.* (2014), the tissue N content of the brown macroalga *Sargassum* spp. was relatively unchanged when compared to red macroalgae. This was evident in our results as *G. edulis* (a red seaweed) which had a higher % N content then *S. polycystum*, a brown seaweed. According to Gantt (1990), the higher nitrogen content of the red

alga *G. edulis* may be due in part to the presence of phycoerithrin and phycobilisomes which are light sensitive pigments. Table 5.0 shows the % N content present in the some seaweed based on the amounts of nitrate administered.

Table 5.0 Total tissue nitrogen (%) for different species of macroalgae under conditions of high nitrate enrichment.

Species	Amount of Enriched Nitrate in Experiment	Total Tissue Nitrogen %	Source
Ulva. Expansa	> 300 µM	25	(Kennison *et al.*, 2011)
Enteromorpha intestinalis	1000 µM	73-93	(Fong *et al.*, 2003)
Enteromorpha sp.	780 µM	0.7-3.5	(Fong, 1994)
Sargassum polyphyllum	3.62 mg/L	2.4	(Reef *et al.*, 2012)
Laurencia intricata	3.62 mg/L	1.8	(Reef *et al.*, 2012)

As seen from Table 5.0, total % tissue nitrogen content increases with increased administration of nitrates in similar experimental conditions. This makes % tissue N an excellent measure of determining the amount of nitrates present in the samples due to nutrient enrichment.

The optimum % tissue phosphorus content for *G edulis* and *S .polycystum* were at 2-4 mg/L an 1- 4 mg/L of sodium nitrate respectively. Overall there was no variation between the % P content in *S. polycytsum.* However, *G. edulis* had the lowest % P content (0.041% $\pm$ 0.002 at 1 mg/L, % P peaked at 2 mg/L and gradually decreased from 3 mg/L to 4 mg/L. There was not any administration of phosphorus based nutrient however there is a variation in the phosphorus content in *G. edulis*. The low amount of phosphorus in the tissue of macroalgae is attributed to macroalgae not storing sufficient phosphorus. According to Hwang *et al.* (2004), *S. polycystum* is P limited in its early growth period and then N limited when matured. In our study, the % phosphorus content was less than nitrogen. This could mean that the *S. polycystum* samples were not matured. The % tissue P was also reported in low amounts (0.27% ± 0.02) when compared with % tissue N (1.75% ± 0.03) in *Sargassum filipendula*

(Diniz *et al*., 2009). Phosphorus along with nitrogen can be a limiting nutrient which could result in low % tissue phosphorus (Todd *et al*., 2009; Fong & Zedler, 1994). Phosphorus is required by macroalgae to perform several important functions such as transfer of energy and is one of the important nutrient component the governs the growth of macroalgae (Haq *et al*., 2011).

The optimum % tissue potassium content for *G edulis* and *S .polycystum* were at 3- 4 mg/L an 1 mg/L of sodium nitrate respectively. Potassium is naturally present in high amounts in many plants and macroalgae (Lobban, 1994). The potassium based nutrients were not administered however results showed a variation in % tissue potassium content. The high amount of % tissue potassium in *Sargassum polycystum* was at 1 mg/L which could be attributed to potassium being stored in the tissues when nutrient starved (Juneja *et al*., 2013). However, *Gracilaria edulis* had higher % tissue potassium at 4 mg/L due to its ability to store excess nutrients for future growth (Hanisak, 1979). Therefore, both species of macroalgae store high amounts of potassium.

5.4. Combination Effect of Temperature and Nutrient and the Evaluation of % NPK

For this current research, based on the individualistic effects of temperature and sodium nitrate nutrient concentrations, the combined effects at 30 ℃ and 4 mg/L on SGR of *G. edulis,* was higher (1.99% day^{-1} ± 0.24) than the control (0.04% day^{-1} ± 0.01). For *G. edulis,* 30 ℃ is an ideal temperature for growth and administration of nutrients had a positive effect on growth. The combined temperature and sodium nitrate concentrations for the RL of *G. edulis* was (0.30 mm ± 0.01) at 32 ℃ and 4 mg/L which was much more than the control RL of (0.07 mm ±0.03). The combined temperature and sodium nitrate concentrations for the RL of *S. polycystum* was (0.30 mm ±0.02) at 32 ℃ and 4 mg/L which was much higher than the control RL of (0.08 mm ±0.03), indicating that under conditions which are optimum for growth the opportunistic seaweed would bloom and become invasive.

The tissue % NPK showed that potassium was in high amounts under all three temperatures and sodium nitrate combinations. It was expected that % tissue N would be higher than % P and % K, but this was not the case. According to Boderskov *et al.*

(2016), at temperature of 28- 35 ℃ and high nutrient availability of 70 µM effluents, the amount of nitrogen, fucoxanthin, and chlorophyll *a* increased by 50.1–60.1 %, 21.7–53.7 %, and 47.0–73.5 %. However, % tissue K was more than % P and % N. A study by Nazni & Deepa (2015), found potassium to be naturally high at 6 % in *Gracilaria verrucosa*. According to Boderskov *et al.* (2016), under low nutrient availability, there is a net loss of biomass (8.1–9.5 %) and tissue nitrogen (10.7–44.1 %) in seaweeds. Thus the control biomass loss is attributed to lack of nutrients in the *ex-situ* culture Tamata (2012).

In general, from the results of this study it could be clear that under high conditions of nutrient availability as occurs in populated coastal areas of mainland Fiji, both *Gracilaria edulis* and *Sargassum polycystum* would be expected to bloom and become invasive pest species, which is indeed the observed case.

CHAPTER 6
CONCLUSIONS

In the present study, the individualistic effects of temperature and sodium nitrate nutrients on the growth of *S. polycystum* and *G. edulis* were evaluated. From the above experiments the optimum temperature and nutrients concentrations were determined. The synergistic effects of temperature and nutrients were studied by combining the optimum temperatures with the optimum sodium nitrate nutrient concentration which were determined. Overall The SGR of *G. edulis* can tolerate temperatures ranging from 28 ℃ - 32 ℃ with 4 mg/L of sodium nitrate concentration solutions. For *S. polycytsum* and *G. edulis* RL, the optimum temperature ranges were 28 ℃ - 34 ℃ and 32 ℃ - 34 ℃ respectively with 4 mg/L of sodium nitrate concentration solutions. In the % NPK studies, % K was in higher amounts in both the seaweeds.

S. polycystum and *G. edulis* are pest species that can displace native species biodiversity. This study showed that they can grow within a relatively wide range of temperature and nutrient regimes. Global warming will hence not be a problem to their growth, which in turn could exacerbate tropical algal blooms. Moreover, pollution from sewage and domestic runoffs could cause an increase in macroalgal abundance since the water column around the coastal areas in Fiji is becoming overloaded with nutrients. However, further research needs to be conducted *in situ* at coastal areas since the present study was performed in a laboratory setting. A socio-economical study could also be undertaken to understand how communities can better utilize the abundance of these two pest seaweed using traditional knowledge and suggestions such as made by N' Yeurt & Iese (2015) for value-added products from pest seaweeds (as fertilizers, biofuel and animal feeds).

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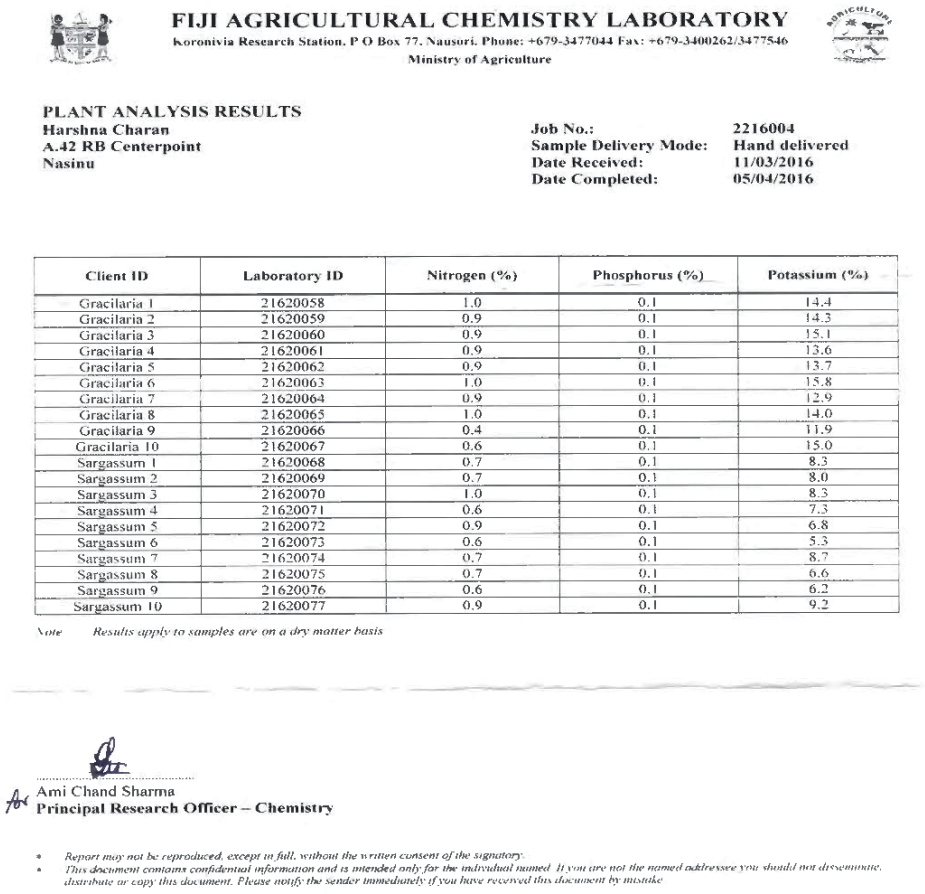
APPENDICES

Appendix I: % NPK analysis from Koronivia Research Station, Fiji.



FIJI AGRICULTURAL CHEMISTRY LABORATORY
Koronivia Research Station, P O Box 77, Nausori. Phone: +679-3477044 Fax: +679-3400262/3477546
Ministry of Agriculture

PLANT ANALYSIS RESULTS
Harshna Charan
A.42 RB Centerpoint
Nasinu

Job No.: 2216004
Sample Delivery Mode: Hand delivered
Date Received: 11/03/2016
Date Completed: 05/04/2016

Client ID	Laboratory ID	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Gracilaria 1	21620058	1.0	0.1	14.4
Gracilaria 2	21620059	0.9	0.1	14.3
Gracilaria 3	21620060	0.9	0.1	15.1
Gracilaria 4	21620061	0.9	0.1	13.6
Gracilaria 5	21620062	0.9	0.1	13.7
Gracilaria 6	21620063	1.0	0.1	15.8
Gracilaria 7	21620064	0.9	0.1	12.9
Gracilaria 8	21620065	1.0	0.1	14.0
Gracilaria 9	21620066	0.4	0.1	11.9
Gracilaria 10	21620067	0.6	0.1	15.0
Sargassum 1	21620068	0.7	0.1	8.3
Sargassum 2	21620069	0.7	0.1	8.0
Sargassum 3	21620070	1.0	0.1	8.3
Sargassum 4	21620071	0.6	0.1	7.3
Sargassum 5	21620072	0.9	0.1	6.8
Sargassum 6	21620073	0.6	0.1	5.3
Sargassum 7	21620074	0.7	0.1	8.7
Sargassum 8	21620075	0.7	0.1	6.6
Sargassum 9	21620076	0.6	0.1	6.2
Sargassum 10	21620077	0.9	0.1	9.2

Note *Results apply to samples are on a dry matter basis*

Ami Chand Sharma
Principal Research Officer – Chemistry

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Figure 8.0 The analysis of the % NPK tissue content naturally present in *G. edulis* and *S. polycystum* received by the Koronivia Agricultural Research Station.

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Ministry of Agriculture

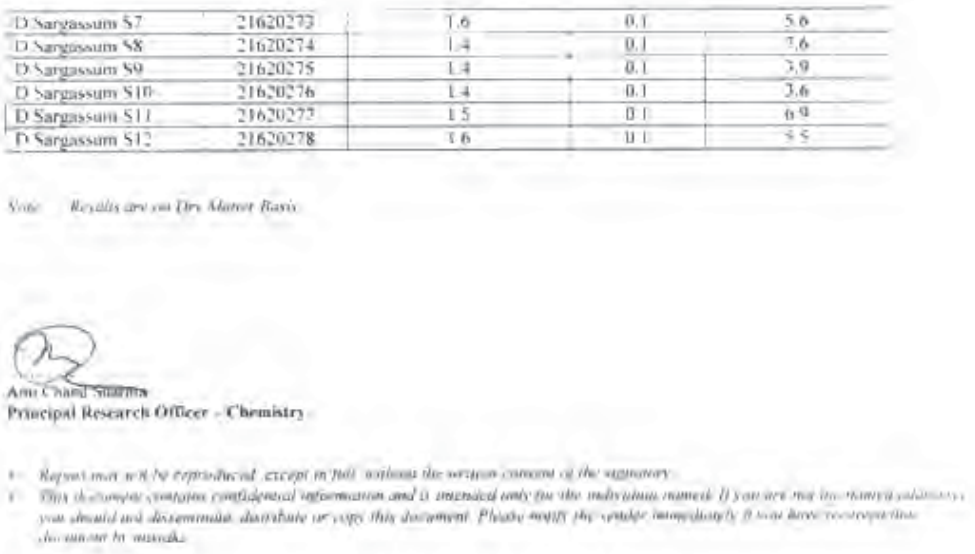
SEAWEED ANALYSIS RESULTS

Harshna Charan
P.O.Box A 42
RB Centrepoint

Job No.: 2216008
Sample Delivery Mode: Hand Delivered
Date Received: 10/05/16
Date Completed: 03/06/16

Client ID	Laboratory ID	Nitrogen (%)	Phosphorus (%)	Potassium (%)
A Gracilaria S1	21620183	1.3	0.1	13.4
A Gracilaria S2	21620184	1.5	0.1	14.7
A Gracilaria S3	21620185	1.3	0.1	15.9
A Gracilaria S4	21620186	1.6	0.1	14.2
A Gracilaria S5	21620187	1.6	0.1	15.8
A Gracilaria S6	21620188	1.7	0.1	14.6
A Gracilaria S7	21620189	1.4	0.1	13.3
A Gracilaria S8	21620190	1.5	0.1	15.0
A Gracilaria S9	21620191	1.5	0.1	15.3
A Gracilaria S10	21620192	1.3	0.1	12.5
A Gracilaria S11	21620193	1.3	0.1	14.1
A Gracilaria S12	21620194	1.6	0.1	15.1
B Gracilaria S1	21620195	1.5	0.1	12.0
B Gracilaria S2	21620196	1.3	0.1	14.7
B Gracilaria S3	21620197	1.6	0.1	10.2
B Gracilaria S4	21620198	1.4	0.1	11.7
B Gracilaria S5	21620199	1.4	0.1	13.7
B Gracilaria S6	21620200	1.6	0.1	13.8
B Gracilaria S7	21620201	1.6	0.1	12.5
B Gracilaria S8	21620202	1.5	0.1	14.1
B Gracilaria S9	21620203	1.3	0.1	14.7
B Gracilaria S10	21620204	1.3	0.1	12.5
B Gracilaria S11	21620205	1.3	0.1	15.7
B Gracilaria S12	21620206	1.3	0.1	15.8
C Gracilaria S1	21620207	1.4	0.1	15.9
C Gracilaria S2	21620208	1.3	0.1	14.0
C Gracilaria S3	21620209	1.2	0.1	10.8
C Gracilaria S4	21620210	1.5	0.1	13.3
C Gracilaria S5	21620211	1.2	0.1	14.9
C Gracilaria S6	21620212	1.3	0.1	14.6
C Gracilaria S7	21620213	1.5	0.1	14.0
C Gracilaria S8	21620214	1.3	0.1	13.2
C Gracilaria S9	21620215	1.2	0.1	11.6
C Gracilaria S10	21620216	1.2	0.1	13.8
C Gracilaria S11	21620217	1.4	0.1	13.8
C Gracilaria S12	21620218	1.3	0.1	11.7
D Gracilaria S1	21620219	1.3	0.1	13.8

D Gracilaria S2	21620220	1.2	0.1	12.6
D Gracilaria S3	21620221	1.2	0.1	10.7
D Gracilaria S4	21620222	1.3	0.1	11.6
D Gracilaria S5	21620223	1.5	0.1	10.4
D Gracilaria S6	21620224	1.4	0.1	12.7
D Gracilaria S7	21620225	1.5	0.1	13.0
D Gracilaria S8	21620226	1.5	0.1	15.4
D Gracilaria S9	21620227	1.6	0.1	12.3
D Gracilaria S10	21620228	1.6	0.1	11.9
D Gracilaria S11	21620229	1.3	0.1	8.8
D Gracilaria S12	21620230	1.5	0.1	13.9
A Sargassum S1	21620231	1.5	0.1	5.3
A Sargassum S2	21620232	1.4	0.1	8.9
A Sargassum S3	21620233	1.9	0.1	3.0
A Sargassum S4	21620234	1.7	0.1	3.4
A Sargassum S5	21620235	1.7	0.1	3.3
A Sargassum S6	21620236	1.6	0.2	5.2
A Sargassum S7	21620237	1.5	0.1	5.1
A Sargassum S8	21620238	1.2	0.1	4.6
A Sargassum S9	21620239	1.2	0.1	3.9
A Sargassum S10	21620240	1.6	0.1	5.4
A Sargassum S11	21620241	1.6	0.1	3.2
A Sargassum S12	21620242	1.6	0.1	4.8
B Sargassum S1	21620243	1.6	0.1	3.7
B Sargassum S2	21620244	1.5	0.1	2.5
B Sargassum S3	21620245	1.2	0.1	3.4
B Sargassum S4	21620246	1.7	0.1	6.4
B Sargassum S5	21620247	1.7	0.1	5.4
B Sargassum S6	21620248	1.2	0.1	3.8
B Sargassum S7	21620249	1.6	0.1	4.9
B Sargassum S8	21620250	1.4	0.1	2.7
B Sargassum S9	21620251	1.3	0.1	3.6
B Sargassum S10	21620252	1.3	0.1	2.6
B Sargassum S11	21620253	1.2	0.1	4.2
B Sargassum S12	21620254	1.2	0.1	2.3
C Sargassum S1	21620255	1.3	0.1	4.9
C Sargassum S2	21620256	1.2	0.1	4.4
C Sargassum S3	21620257	1.2	0.1	3.8
C Sargassum S4	21620258	1.6	0.1	4.2
C Sargassum S5	21620259	1.3	0.1	6.5
C Sargassum S6	21620260	1.3	0.1	4.8
C Sargassum S7	21620261	1.7	0.1	6.8
C Sargassum S8	26120262	1.3	0.1	3.7
C Sargassum S9	26120263	1.3	0.1	4.0
C Sargassum S10	26130264	1.3	0.1	4.6
C Sargassum S11	26120265	1.2	0.1	2.6
C Sargassum S12	21620266	1.7	0.1	5.0
D Sargassum S1	21620267	1.6	0.1	4.2
D Sargassum S2	21620268	1.3	0.1	4.4
D Sargassum S3	21620269	1.4	0.1	5.1
D Sargassum S4	21620270	1.3	0.1	7.0
D Sargassum S5	12620271	1.3	0.1	4.6
D Sargassum S6	21620272	1.7	0.1	6.6



D Sargassum S7	21620273	1.6	0.1	5.6
D Sargassum S8	21620274	1.4	0.1	7.6
D Sargassum S9	21620275	1.4	0.1	3.9
D Sargassum S10	21620276	1.4	0.1	3.6
D Sargassum S11	21620277	1.5	0.1	6.9
D Sargassum S12	21620278	1.6	0.1	5.5

Note: Results are on Dry Matter Basis

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Principal Research Officer - Chemistry

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Figure 8.1 The analysis of the % NPK tissue present in *G. edulis* and *S. polycystum* after treatment with sodium nitrate concentrations received by the Koronivia Agricultural Research Station.

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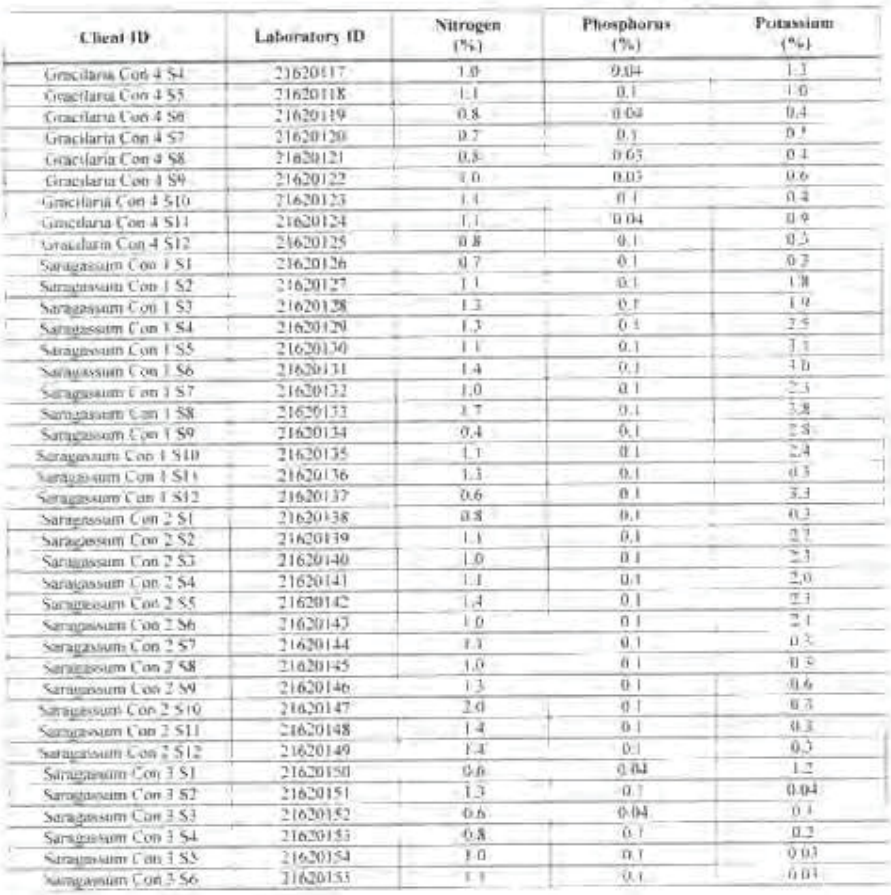
Ministry of Agriculture

PLANT ANALYSIS RESULTS

Harshna Charan
A 42
R.B Centre Point
Nasinu

Job No: 2216005
Sample Delivery Mode: Hand delivered
Date Received: 21/03/2016
Date Completed: 28/04/2016

Client ID	Laboratory ID	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Gracilaria Con 1 S1	21620078	1.1	0.03	0.3
Gracilaria Con 1 S2	21620079	0.8	0.04	0.5
Gracilaria Con 1 S3	21620080	1.1	0.05	0.5
Gracilaria Con 1 S4	21620081	0.8	0.04	0.9
Gracilaria Con 1 S5	21620082	0.8	0.04	0.3
Gracilaria Con 1 S6	21620083	1.0	0.05	0.4
Gracilaria Con 1 S7	21620084	0.8	0.03	0.4
Gracilaria Con 1 S8	21620085	1.1	0.05	0.5
Gracilaria Con 1 S9	21620086	1.1	0.04	0.7
Gracilaria Con 1 S10	21620087	1.0	0.03	0.4
Gracilaria Con 1 S11	21620088	1.3	0.05	0.8
Gracilaria Con 1 S12	21620089	1.1	0.04	0.4
Gracilaria Con 2 S1	21620090	1.0	0.04	0.4
Gracilaria Con 2 S2	21620091	1.1	0.1	0.4
Gracilaria Con 2 S3	21620092	1.8	0.1	0.2
Gracilaria Con 2 S4	21620093	2.0	0.1	0.2
Gracilaria Con 2 S5	21620094	1.5	0.04	0.2
Gracilaria Con 2 S6	21620095	2.2	0.05	0.2
Gracilaria Con 2 S7	21620096	3.1	0.04	0.2
Gracilaria Con 2 S8	21620097	1.1	0.1	0.2
Gracilaria Con 2 S9	21620098	1.8	0.1	0.4
Gracilaria Con 2 S10	21620099	1.3	0.1	0.5
Gracilaria Con 2 S11	21620100	1.5	0.1	1.1
Gracilaria Con 2 S12	21620101	1.3	0.1	0.3
Gracilaria Con 3 S1	21620102	1.1	0.04	0.4
Gracilaria Con 3 S2	21620103	1.4	0.05	0.5
Gracilaria Con 3 S3	21620104	1.3	0.1	0.5
Gracilaria Con 3 S4	21620105	1.0	0.1	0.4
Gracilaria Con 3 S5	21620106	0.7	0.04	0.4
Gracilaria Con 3 S6	21620107	1.1	0.03	0.4
Gracilaria Con 3 S7	21620108	1.0	0.04	0.4
Gracilaria Con 3 S8	21620109	1.3	0.1	0.5
Gracilaria Con 3 S9	21620110	1.3	0.1	0.4
Gracilaria Con 3 S10	21620111	1.1	0.04	0.8
Gracilaria Con 3 S11	21620112	1.1	0.1	0.5
Gracilaria Con 3 S12	21620113	0.7	0.03	0.5
Gracilaria Con 4 S1	21620114	1.1	0.03	0.7
Gracilaria Con 4 S2	21620115	1.0	0.03	0.5
Gracilaria Con 4 S3	21620116	1.1	0.03	1.3



Client ID	Laboratory ID	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Gracilaria Con 4 S4	21620117	1.0	0.04	1.3
Gracilaria Con 4 S5	21620118	1.1	0.1	1.0
Gracilaria Con 4 S6	21620119	0.8	0.04	0.4
Gracilaria Con 4 S7	21620120	0.7	0.1	0.7
Gracilaria Con 4 S8	21620121	0.8	0.05	0.4
Gracilaria Con 4 S9	21620122	1.0	0.03	0.6
Gracilaria Con 4 S10	21620123	1.1	0.1	0.4
Gracilaria Con 4 S11	21620124	1.1	0.04	0.9
Gracilaria Con 4 S12	21620125	0.8	0.1	0.5
Saragassum Con 1 S1	21620126	0.7	0.1	0.2
Saragassum Con 1 S2	21620127	1.1	0.1	1.8
Saragassum Con 1 S3	21620128	1.3	0.1	1.9
Saragassum Con 1 S4	21620129	1.3	0.1	2.5
Saragassum Con 1 S5	21620130	1.1	0.1	2.1
Saragassum Con 1 S6	21620131	1.4	0.1	1.0
Saragassum Con 1 S7	21620132	1.0	0.1	2.3
Saragassum Con 1 S8	21620133	1.7	0.1	3.8
Saragassum Con 1 S9	21620134	0.4	0.1	2.8
Saragassum Con 1 S10	21620135	1.1	0.1	2.4
Saragassum Con 1 S11	21620136	1.3	0.1	0.3
Saragassum Con 1 S12	21620137	0.6	0.1	3.3
Saragassum Con 2 S1	21620138	0.8	0.1	0.3
Saragassum Con 2 S2	21620139	1.1	0.1	2.2
Saragassum Con 2 S3	21620140	1.0	0.1	2.3
Saragassum Con 2 S4	21620141	1.1	0.1	2.0
Saragassum Con 2 S5	21620142	1.4	0.1	2.3
Saragassum Con 2 S6	21620143	1.0	0.1	2.1
Saragassum Con 2 S7	21620144	1.1	0.1	0.3
Saragassum Con 2 S8	21620145	1.0	0.1	0.5
Saragassum Con 2 S9	21620146	1.3	0.1	0.6
Saragassum Con 2 S10	21620147	2.0	0.1	0.5
Saragassum Con 2 S11	21620148	1.4	0.1	0.3
Saragassum Con 2 S12	21620149	1.4	0.1	0.3
Saragassum Con 3 S1	21620150	0.6	0.03	1.2
Saragassum Con 3 S2	21620151	1.3	0.1	0.04
Saragassum Con 3 S3	21620152	0.6	0.04	0.1
Saragassum Con 3 S4	21620153	0.8	0.1	0.2
Saragassum Con 3 S5	21620154	1.0	0.1	0.03
Saragassum Con 3 S6	21620155	1.1	0.1	0.03

Figure 8.2 The analysis of the % NPK tissue present in *G. edulis* and *S. polycystum* after treatment with combination of temperature and sodium nitrate concentrations received by the Koronivia Agricultural Research Station.

Appendix II

Table 8.0 Mean and Standard Error for seawater parameters in mol/L for the five month period.

Seawater Parameters (n=15)	Mean (mol/L) ± S.E.
Nitrate	2.63E-05± 0.04E-05
Nitrite	1.30E-05± 0.02E-05
Ammonia	2.93E-05± 0.02E-05
Phosphate	1.47E-05 ± 0.03E-05

Table 8.1 Mean and Standard Error in % NPK content for *G. edulis* and *S. polycystum*

Macroalage n= 10	%	Mean ± SE
G. edulis	N	0.85 ±0.06
	P	0.10 ±4.63E-18
	K	14.07±0.36
S. polycystum	N	0.74±0.05
	P	0.10±4.63E-18
	K	7.47±0.39

Table 8.2 Mean and Standard Error of Specific Growth rate (SGR) for *G. edulis* at different temperatures

Macroalgae	Temperature (°C)	Mean (%day^{-1}) ± SE
G. edulis	28	0.004± 0.913
	30	0.79± 0.39
	32	-0.30±0.61
	34	-9.99±1.49

Table 8.3 Summary of PostHoc Tukey's Test between different temperatures for *G. edulis* SGR in % day^{-1}.

Post Hoc (Tukey's) Test between Temperatures(℃)	P-value
28 and 30	0.937
28 and 32	0.996
28 and 34	0.000
30 and 32	0.849
30 and 34	0.000
32 and 34	0.000

Table 8.4 Summary for the Mean and Standard Error in Rhizoidal length (RL) of *G. edulis* and *S. polycystum* at varying temperatures

Macroalage	Temperature (℃)	Mean (mm)± SE
G. edulis	28	-0.01±0.01
	30	0.01±0.01
	32	0.08±0.02
	34	0.07±0.01
S. polycystum	28	0.26±0.05
	30	0.06±0.04
	32	0.15±0.15
	34	0.23±0.07

Table 8.5 Summary of Kruskal -Wallis Test between different temperatures for *G. edulis* RL in mm.

Kruskal-Wallis H Test Between Temperature (℃)	Chi- square value	P-value
28 and 30	0.383	0.536
28 and 32	7.455	0.006
28 and 34	7.247	0.007
30 and 32	5.360	0.021
30 and 34	5.048	0.025
32 and 34	0.066	0.797

Note* Multicomparison test could not be performed for *S. polycystum* since there was no significant difference between the temperatures for *S. polycystum*

Table 8.6 Mean and Standard Error of Specific Growth Rate (SGR) for *G. edulis* at different concentrations (mg/L) of Sodium Nitrate

Macroalgae		Concentration (mg/L)	Mean (%day^{-1})± SE
G. edulis	Sodium Nitrate	1	0.02±0.01
		2	0.24±0.06
		3	0.75±0.15
		4	2.10±0.40

Table 8.7 Summary of Kruskal-Wallis Test between different concentrations of sodium nitrate for *G. edulis* SGR in % day^{-1}.

Kruskal-Wallis H Test Between sodium nitrate Concentrations (mg/L)	Chi- square value	P-value
1 and 2	16.825	0.00
1 and 3	17.303	0.00
1 and 4	17.303	0.00
2 and 3	12.000	0.01
2 and 4	17.280	0.00
3 and 4	12.000	0.01

Note* There is a significant difference in all the multicomparison sodium nitrate concentration since the $p < 0.05$.

Table 8.8 Mean and Standard Error of Rhizoidal length (RL) for *G. edulis* and *S. polycystum* at varying Sodium Nitrate concentration (mg/L)

Macroalgae	Concentration (mg/L)	Mean (mm) ± SE
G. edulis	1	0.09±0.01
	2	0.10 ±4.18E-18
	3	0.24±0.03
	4	0.36±0.04
S. polycystum	1	0.10±0.02
	2	0.14±0.03
	3	0.24±0.03
	4	0.32±0.06

Table 8.9 Summary of Kruskal- Wallis Test for Rhizoidal Length (RL) of *G. edulis* and *S. polycystum* at different sodium nitrate concentrations (mg/L).

Macroalgae	Kruskal Wallis Test Between concentrations (mg/L)	Chi-square value	P-value
G. edulis	1 and 2	1.0000	0.317
	1 and 3	17.333	0.000
	1 and 4	19.530	0.000
	2 and 3	17.783	0.000
	2 and 4	20.185	0.000
	3 and 4	6.6040	0.010
S. polycystum	1 and 2	3.2800	0.070
	1 and 3	11.744	0.001
	1 and 4	12.208	0.000
	2 and 3	8.1140	0.004
	2 and 4	11.162	0.001
	3 and 4	6.0050	0.014

Table 8.10 Nitrate concentrations mol/L (Mean ± S.E.) present in the water before and after the administration of sodium nitrate for the various treatments. Note: n=15 for each treatment.

Nitrate mg/L	Before (mol/L) ± S.E.	After (mol/L) ± S.E.
1	2.44E-05±0.07E-05	2.49E-05±0.08E-05
2	2.55E-05±0.03E-05	2.48E-05±0.05E-05
3	2.52E-05±0.04E-05	2.48E-05±0.04E-05
4	2.69E-05±0.02E-05	2.69E-05±0.04E-05

Table 8.11 Mean and Standard Error in NPK content (%) for *G. edulis* and *S. polycystum* at varying concentrations of Sodium Nitrate

Macroalgae	%	Concentrations (mg/L)	Mean (%)± SE
G. edulis	N	1	1.00 ±0.05
		2	1.64±0.17
		3	1.09±0.06
		4	0.97±0.04
	P	1	0.04±0.01
		2	0.08±0.01
		3	0.06±0.01
		4	0.06±0.01
	K	1	0.51±0.06
		2	0.38±0.07
		3	0.48±0.03
		4	0.69±0.10
S. polycystum	N	1	1.08±0.11
		2	1.22±0.90
		3	0.99±0.69
		4	1.19±0.06
	P	1	0.10 ±4.18E-18
		2	0.10 ±4.18E-18
		3	0.09±0.01
		4	0.09±0.01
	K	1	2.29±0.31
		2	1.11±0.28
		3	0.16±0.09
		4	0.07±0.01

Table 8.12 Summary of tissue nitrogen content (%) for Kruskal-Wallis H Test between different sodium nitrate concentrations (mg/L) for *G. edulis*.

Kruskal-Wallis H Test Between Concentrations (mg/L)	Chi- square value	P-value
1 and 2	11.200	0.001
1 and 3	1.2210	0.269
1 and 4	0.2690	0.604
2 and 3	7.2490	0.007
2 and 4	12.601	0.000
3 and 4	2.4130	0.120

Note* Multicomparison test could not be performed for *S. polycystum* since there was no significant difference between the tissue nitrogen content (%) at different sodium nitrate concentrations.

Table 8.13 Summary of tissue phosphorus content (%) for Kruskal-Wallis H Test between different sodium nitrate concentrations (mg/L) for *G. edulis*.

Kruskal-Wallis H Test Between Concentrations (mg/L)	Chi-square value	P-value
1 and 2	9.275	0.002
1 and 3	1.913	0.167
1 and 4	0.000	1.000
2 and 3	2.067	0.151
2 and 4	4.932	0.026
3 and 4	1.071	0.301

Note* Multicomparison test could not be performed for *S. polycystum* since there was no significant difference between the tissue phosphorus content (%) of *S. polycystum* at varying sodium nitrate concentrations.

Table 8.14 Summary of tissue potassium content (%) for Kruskal-Wallis H Test between different sodium nitrate concentrations (mg/L) for *G. edulis.*

Macroalge	Kruskal Wallis Test Between concentrations (mg/L)	Chi-square value	P-value
G. edulis	1 and 2	4.428	0.035
	1 and 3	0.000	1.000
	1 and 4	1.674	0.196
	2 and 3	5.751	0.016
	2 and 4	7.474	0.006
	3 and 4	2.080	0.149
S. polycystum	1 and 2	6.409	0.011
	1 and 3	16.448	0.000
	1 and 4	17.711	0.000
	2 and 3	14.403	0.000
	2 and 4	17.992	0.000
	3 and 4	0.0010	0.976

Table 8.15 Mean and Standard Error of *G. edulis* and *S. polycystum* at synergistic temperature and Sodium Nitrate concentration

Macroalgae		Synergism	Mean ± SE
G. edulis	SGR	30 ℃ and 4mg/L	1.99±0.24
Control	SGR	Ambient Conditions	0.04± 0.01
S. polycystum	RL	28℃ and 4mg/L	0.30±0.02
Control	RL	Ambient Conditions	0.08±0.03
G. edulis	RL	32 ℃ and 4mg/L	0.30±0.01
Control	RL	Ambient Conditions	0.07±0.03

*Note RL= Rhizoidal length (mm), SGR- Specific Growth Rate (%day^{-1})

Table 8.16 Mean and Standard Error of % NPK of *G. edulis* and *S. polycystum* at synergistic temperature and Sodium Nitrate concentration

Macroalgae		Synergism		Mean (%) ± SE
G. edulis	SGR	30 ℃ and 4 mg/L	N	1.47±0.04
			P	0.10 ±4.18431E-18
			K	14.49±0.29
S. polycystum	RL	28 ℃ and 4 mg/L	N	1.46±0.039
			P	0.10 ±4.18E-18
			K	5.42±0.39
G. edulis	RL	32 ℃ and 4 mg/L	N	1.43±0.04
			P	0.10 ± 4.18E-18
			K	13.45± 0.49

*Note the % NPK for the control could not be computed due to sample being small; however the %NPK were compared with each other.