GROWTH RATES OF *GRACILARIOPSIS FUNICULARIS* (GRACILARIACEAE, RHODOPHYTA) IN LABORATORY CULTURE UNDER VARYING SALINITIES

BY:

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Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. Tjipute M. and Dr. Shuuluka D, and has not presented elsewhere for the award of the degree. All the sources have been duly and appropriately acknowledged.

Candidate Signature: …… Date:

……..

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Certification

This is to certify that this report has been examined and approved for the award of the degree of Bachelor of Science in Fisheries and Aquatic Science of the University of Namibia.

Internal examiner…Dr. Diina Shuuluka…….....signature……. 

External examiner…….signature…..……..

Head of Department…….....signature.....

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I am indebted to all SANUMARC staff members for their hospitality during my stay at the Centre and finally, my sincere thanks go to the University of Namibia for all the logistical support during my stay at SANUMARC.

Dedication

This project is dedicated to my mother Kupembona Nandjira, my late father Mr Ngandu Hamutenya Frans, my six siblings Christine Hamutenya, Maria Hamutenya, Norberth Hamutenya, Lucas Hamutenya, Martha Hamutenya and Silvester Hamutenya, and also Magreth Ndango and my great uncle Kashokora Haingura Pontianus for the love and encouragement they showed me throughout the project. Thanks for being there for me in all time of good or bad and my love to you is unlimited. I would also like to dedicate this to my friends and colleagues for their support. Finally, i would like to give thanks to the Lord Father God the creator of earth and heaven for giving me the strength and directions towards the project.

TABLE OF CONTENTS

ABSTRACT

Cultivation of seaweeds is being practiced in many countries in the world and for the successful cultivation of seaweeds, various environmental factors need to be considered and these factors are: temperature, nutrients, salinity, dissolved oxygen, and pH. Namibia has a rich and diverse seaweed flora. Increased commercial interest in these seaweed resources has been the stimulus for eco-physiology studies. Among seaweed species of commercial interest in Namibia is a red seaweed Gracilariopsis *funicularis* which has a huge potential for agar and other uses.

The current study investigated the effects of salinity levels (25ppt, 35ppt, and 40ppt) on the growth rate of *Gracilariopsis funicularis*. The seaweed samples were collected from Solitude Points in Henties Bay and cultured for a period of 30 days in the laboratory. The results showed that *Gracilariopsis funicularis* grew well in all salinities tested and all salinities provided a faster growth rate until day 12 and the growth started to decrease thereafter. The statistical analysis showed that there was no significant difference in the growth rate of *Gracilariopsis funicularis* grown at salinities of 25ppt, 35ppt and 40ppt ($P>0.05$). This study provided benchmark information for future study on the eco-physiology of this red seaweed species, *Gracilariopsis funicularis.*

Keywords: *Gracilariopsis funicularis*, salinity, growth, species

CHAPTER 1

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Namibia is a country rich in seaweeds species lying along the coastal area. But the most important seaweed harvested in Namibia are *Gracilaria gracilis, Laminaria schinzii, Ecklonia maxima* and *Gracilariopsi funicularis* (Burke, el., 1995). The gracilarioids species *(Gracilaria gracilis* and *Gracilariopsis funicularis*).are important for agars production. *Gracilariopsis funicularis* sp. nov. has a disjunct distribution along the coast of central Namibia, and in a discrete estuarine location in South Africa These plants display two morphotypes superficially resembling *Gracilariopsis tenuifrons* (in South Africa) and *Gs longissima* (in Namibia) (Iyer et al., 2005).

The red seaweed *Gracilaria* now account for more than 53% of all agar produced worldwide (McHugh, 1991) with a large portion of the 1000 tonnes agar produced each year being exported to the United States of America(Jensen,1993). Agar production is a very commercial profitable practice worldwide. Agar is a colloidal agent used for thickening, suspending and stabilizing. Agar can be prepared for the use of major application of pharmaceutical grade as culture medium for the growth of micro-organisms such as bacteria as well as fungus.

In cultivating of these seaweeds, it is important to maintain growth condition in optimal range to reduce the duration of cultivation and ensure the greatest yield. For a species acclimated to a particular set of environmental conditions, the optimal growth is always achieved at a specific combination of salinity and irradiance (Brinkhuis et al., 1984; Hanisak, 1987;

Lobban and Harrison, 1994). For large-scale outdoor operations, salinity and irradiance are two environmental factors that can be manipulated in inexpensive ways.

In Japan, China, Korea, and several other countries in East Asia, seaweeds are commercial exploited, and constitute multi-million dollar industries. Some of the seaweeds, example *Porphyra* species, *Laminaria* species, *Monostroma* spp., are in fact extensively farmed as cash crops. But in Africa, most of our marine plants have been neglected and few are been harvested (Keto, 2001).

Namibia has more than 1000km of coastline. Because of environmental conditions along coastlines and in the Atlantic Ocean, off the coast (including the cold Benguela upwelling and the nutrients it brings with it from the ocean floor) Namibia has one of the richest seaweed fields in the world. The waters off the coast of Namibia area very good environment for growing seaweeds, as there is a continuous upwelling of nutrients and the temperature of the water is relatively constant (Burke, et al.,1995).

These red seaweeds species contribute to more than a half the world agarophytes production and they are cultivated in countries such as Chile, China, Taiwan, South Africa and Namibia this because the most important attributes of almost all cultivated species reproduce solely through fragmentation, leading to high regenerative capacity (Dawes, 1995, Hurtado, Poace, 1990).

Table 1: Total (tonnes) macroalgae harvested in all fishing area of the world from 2000 to 2002 (Laura, B. Paolo, G. 2006).

All fishing area of the world	2000	2001	2002
Red macroalgae	2 275 141	2 472 253	2 791 006
Brown macroalgae	5 608 074	5 453 534	5 782 535
Green macroalgae	96 235	93 688	76 265

The current study will investigate the growth rate of the red seaweed species, *Gracilariopsis funicularis* under different level of salinities (25ppt, 35ppt, and 40ppt) in the laboratory. Along the Namibian coastal area, *Gracilariopsis funicularis* is found mainly along the coast of Walvis Bay, Swakopmund, and Henties Bay (Simwanza, 2009).

The University of Namibia's Sam Nujoma Marine and Coastal Resources Research Centre in Henties Bay will carry out research and development (R&D) on the cultivation of economical important seaweed. Research and development program is quite essential, and the results of which can be used as basis for the successful development of seaweed farming technology. Commercial cultivation and processing of seaweeds should be a national priority and taken up as a mission mode project and having agar extraction companies in Namibia can provide employment and this can able to reduce unemployment in our country.

The significant of this study is on seaweeds cultivation, and since the effects of salinity on growth rates of *Gracilariopsis funicularis* will be examined, such information will not only improves our understanding of the growth requirements of this commercial red alga, but will also be useful in selecting the suitable sites and developing a proper management protocol for successful cultivations.

1.2 Literature review

Algae can be defined as photosynthetic, non-vascular plants that common chlorophyll a and have simple reproductive structures (Trainor, 1973). Various algal species inhabit all known ranges of temperature and salinity in aquatic environments (Robert, 2000). Seaweeds are marine macroalgae are primary found in the Divisions Chlorophyta (green algae), Rhodophyta (red algae) and phaeophyta (brown algae) are commonly called seaweeds because of their size, multicellular construction, and attachment to firm substrata (Norton et al.,1996). According to Norton et al., 1996 stated that there are fewer marine macroalgal than microagal species

The growth rate of cultured seaweeds has also been shown to be regulated by environmental parameters such as irradiance, salinity, temperature and nutrient supply (Lüning, 1990; Lobban and Harrison, 1994). Salinity fluctuation has been considered to be the primary factor limiting the growth of macroalgae from rocky intertidal regions and estuaries (Lobban and Harrison, 1997). The tidal cycle is the main factor in controlling variation in salinity in estuarine environments. Changes in freshwater runoff, fluctuations in the currents, storms, winds and the solar cycle, also affect salinity (Yarish and Edwards, 1982). It is becoming increasingly important to understand how algae respond and adapt to salinity stress (Fan-Lu *et al*., 2006).

Salinity changes are important to marine algae in several ways. Salinity levels outside an individual species tolerance level may result in osmotic stress, unfavourable ionic balances, or a shortage of essential metabolites.

Salinity stress often occurs in tidal pools as they become extremely saline under hot, dry conditions and tend toward fresh water under rainy conditions. *Ulva*, which has adapted to this environment, has a high salinity tolerance ranging from as little as 3‰ to as much as

115‰ (Loban & Harrison, 1997). *Ulva* plants are able to regulate the amounts of dissolved internal salts, keeping their internal osmotic pressures somewhat higher than the surrounding medium. This process prevents loss of water to the surrounding saline environment allowing them to maintain a constant turgidity (Loban & Harrison, 1997).

1.3 PROBLEM STATEMENT

To date, there is no research conducted on the effect of environmental factors on growth rates of the Namibian valuable seaweeds such as *Gracilariopsis funicularis.* Seaweeds cultivation in Namibia is not been practiced and there is a need for public awareness on seaweeds and their benefits. Therefore this research was conducted to investigate the optimal salinity level for the cultivation of *Gracilariopsis funicularis.*

1.4 RESEARCH OBJECTIVES

The current research was aimed to determined:

(i) The fresh weights of the *Gracilariopsis funicularis* cultured under different salinity levels (25ppt, 35ppt, 40ppt) in a laboratory for a period of 30 days

(ii) The effects of salinity levels (25ppt, 35ppt, 40ppt) on the growth rate of the *Gracilariopsis funicularis* in the laboratory culture.

1.5 RESEARCH HYPOTHESIS

H_{01} There is no significant difference in specific growth rate percentage per day of the *Gracilariopsis funicularis* under culture of the three salinity levels.

$H_{02:}$ there is a significant difference in specific growth rate percentage per day of the *Gracilariopsis funicularis* under culture of the three salinity levels.

H_{11} there is no significant difference in mean fresh weights of *Gracilariopsis funicularis* cultured under the three levels of salinities (25ppt, 35ppt, and 40ppt) after 30 days period.

H_{12} there is a significant difference in mean fresh weights of *Gracilariopsis funicularis* cultured under the three levels of salinities (25ppt, 35ppt, and 40ppt) after 30 days period.

CHAPTER 2

2.0 METHODOLOGY

2.1. METHODS AND MATERIALS

2.1.1. Study area

The study was done at University of Namibia Sam Nujoma Marine Research Resources Centre (SANUMARC) laboratory from 09 December 2011 to 11 January 2012. SANUMARC is located on a 100 ha site and about 6 kilo metres (km) to the north of Henties Bay town, on dunes overlooking the ocean and the Omaruru Riverbed.

2.1.2 Sampling procedures

The seaweed samples were collected during low tide from Solitude Point (22°09'40S, 14°17'14E) which is about 11km south of Henties Bay town along the Namibian coastline.

The seaweeds were removed randomly from grow out habitats or substrata. Solitude Point is a rocky and sandy place that allows the seaweeds to attach themselves to the rocks that are buried in sand. The seaweeds were packed in plastics and were brought to SANUMARC's wet laboratory for cleaning and removal of epiphytes and animals attached to the seaweed thalli. Thalli are the vegetative body of seaweed. The seaweeds were rinsed with sterilised seawater, and cleaned with low-lint absorbent papers (Kimwipes, Kimberly-Clark) to remove any epiphyte. Epiphytes that were removed from the *Gracilariopsis funicularis* these were different seaweed species that are attached to *Gracilariopsis funicularis* seaweed, the mussels, limpets, and stones.

Figure 1: Figure 1: epiphytes being removed from the seaweed (*Gracilariopsis funicularis*)

Figure 2: (Removing animals such as mussels from the *Gracilariopsis funicularis* samples

Figure 3: well cleaned thalli of *Gracilariopsis funicularis* ready for incubation

2.1.3 Experimental design for salinity experiment

First of all before the seaweeds were cultured in the laboratory, the Walne's Medium for algal culture was prepared to enrich the sterilised and fine filtered natural seawater. The nine (9) 1000ml Erlenmeyer flasks were prepared with air supply which triplicate flasks of each targeted salinity levels (25ppt, 35ppt, 40ppt) were labelled (25ppt:A, 25ppt:B, 25ppt:C, 35ppt:A, 35ppt:B, 35ppt:C, 40ppt:A, and 40ppt:B, 40ppt:C). Therefore, the irradiance level of the cool-white fluorescent tubes light was measured using a light resistor meter in flux and it was converted to µmol photons m^{-2} s^{-1} and it was measured at 80 µmol m^{-2} s^{-1}. The formula used for converting light measurements from flux to µmol photons m^{-2} s^{-1} was the ratio of : 1000lux : 18 µmol photons m^{-2} s^{-1} .

Seawater was prepared in this way: the natural seawater was sterilised with UV-light and at the same time it was filtered with the fine filters. The three different salinities were achieved as follows: Salinity of 25ppt was prepared by mixing natural seawater with deionised freshwater, salinity of 35ppt is for natural seawater whilst salinity of 40 ppt was achieved by evaporating the natural seawater. All these different salinities were measured using a hand held optical refractometer. . The prepared seawater at different targeted salinity levels were then transferred in the 5000ml Erlenmeyer flasks and the well prepared stock solutions were added to enrich the seawater. 1ml of trace metal solution (TMS), vitamin solution, and nutrient solution were transferred into the 5000ml prepared seawater with a 1ml pipette and the pipette was rinsed with freshwater before the next 1ml of the stock solution was transferred. After the enriched seawaters were prepared, then it was transferred into the 1000ml Erlenmeyer flask about 800ml of enriched seawater in all triplicates of salinity levels.

The initial weight for *Gracilariopsis funicularis* thalli were weighed to about 3.3g on the analytic balance and it was transferred into the triplicate 1000ml Erlenmeyer flasks for incubation period.

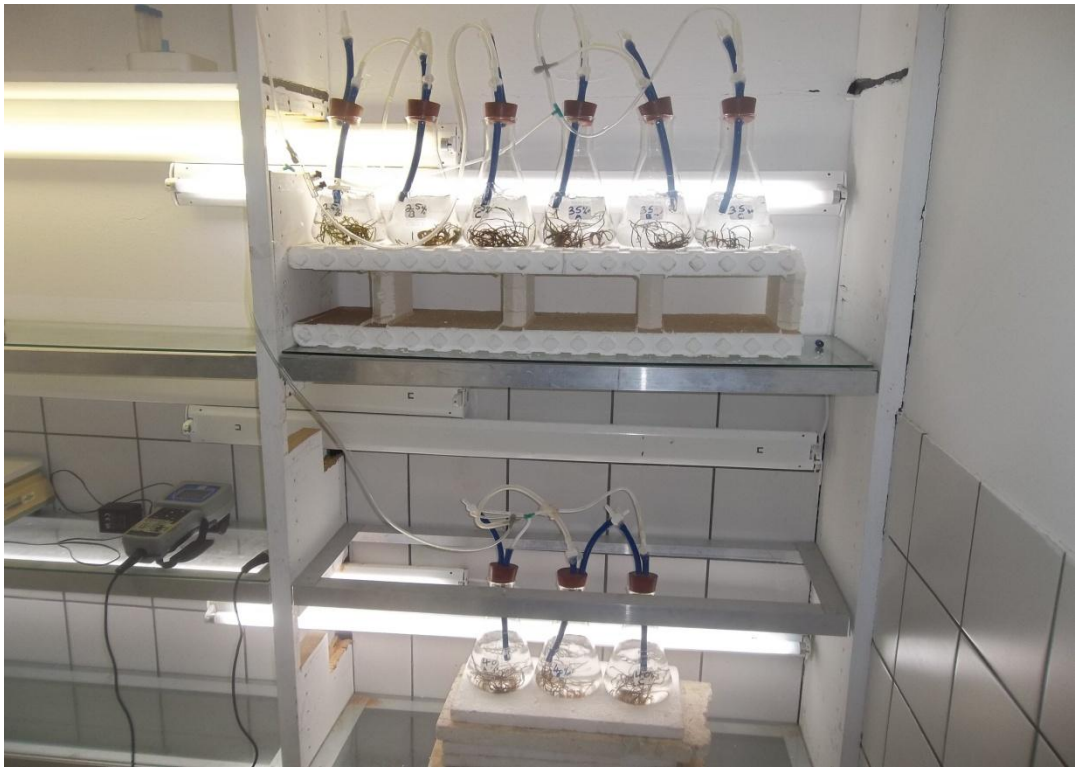

Figure 4: *Gracilariopsis funicularis* experimental set-up of the triplicates 1000ml flasks of the salinities replicates (25ppt, 35ppt, and 40ppt).

Figure 5: seaweeds been blotted dry before they were weight

2.1.4 Growth measurements

The thalli were incubated and it was weight to obtain the fresh weight after every three days incubation period. But before the thalli were weight, the thalli were blotted dry on the towel papers (Kimberly-Clark). The same day of measuring the weight, enriched seawater was also changed in all the triplicate flasks to prevent nutrient limitation during the experiment. Other water parameters that were measured are temperature and dissolved oxygen with temperature/oxygen meter and the pH was measured with the pH indicator slits due to the absence of the pH meter. The light that was provided by the cool-white fluorescent tubes and the photoperiod was 16: 8, Light: Dark, the time of 17h00 – 09h00 lights and from 09h00am – 17h00 dark. The specific growth rates of the seaweed were calculated according to the following equation (Brinkhuis, 1985):

Growth rate (% d^{-1}) = 100 % ($\ln W_f - \ln W_i$)/ t

Were W_i and W_f are the initial and final fresh weight, respectively, and the t is the duration of the incubation period (d) from the initial incubation time.

2.2 STATISTICAL ANALYSIS

Graphpad statistical software package was used to analyse the data. One-way ANOVA was used for comparison of means of the SGR (% d^{-1}) and mean fresh weights to determine if there is a significant difference and of mean of the three replicates of salinities. And Tukey's Multiple Comparison Test was use to compare the significant difference between the three salinity replicates (25 ppt vs 35 ppt, 25 ppt vs 40 ppt, 35 ppt vs 40 ppt).

CHAPTER 3

3.1 RESULTS

During the experimental period, besides the growth measurements, three parameters (Dissolved oxygen, pH, and temperature) were also measured and the averages were recorded. As shown in Table 2, the temperature ranged from 18.9˚C – 23 ˚C whilst the dissolved oxygen ranged from 5 mg/l to 11.40 mg/l and on day 15, level lower than 4 mg/l was observed, see table 2 below.

Table 2: Average Temperature and dissolved oxygen that were measured after every three days incubation time. The pH value was constant at pH=8 for all days

Date	**Salinity levels (‰)**	**Average Temp. (˚C)**	**Average Dissolved O_2 (mg/l)**	**Date**	**Salinity levels (‰)**	**Average Temp. (˚C)**	**Average Dissolved O_2 (mg/l)**
	25	19.1	5.45		25	20.4	11.39
11/12	35	19.5	5.55	28/12/	35	20.9	9.92
/2011	40	18.9	5.78	2011	40	21.4	10.04
	25	19.8	5.89		25	21.3	6.26
13/12	35	19.6	5.47	31/12/	35	21.3	7.24
/2011	40	19.2	5.35	2011	40	22	5.53
	25	19.1	5.73		25	20.8	5.70

16/12 /2011	35	19.7	5.88	03/12/ 2011	35	21.1	6.72
	40	20.1	5.53		40	21.9	6.64
19/12 /2011	25	20.5	6.57	06/01/ 2012	25	20.5	9.44
	35	20.8	6.45		35	20.9	7.86
	40	21.2	6.39		40	21.7	7.35
22/12 /2011	25	20.4	6.28	09/01/ 2012	25	22.2	9.59
	35	20.7	6.55		35	22.3	8.21
	40	21.1	6.48		40	23.0	5.76
25/12 /2011	25	21.6	3.38				
	35	21.8	3.40				
	40	22.1	3.44				

Table 3: The average fresh weights (g) of *Gracilariopsis funicularis* that were measured during the experimental period of 30 days.

DAYS	Average values of the fresh weight(gram)					
	25‰	25‰ SE	35‰	35‰ SE	40‰	40‰ SE
0	3.3223±		3.3440±		3.3425±	
3	3.6949±	0.0156±	3.7822±	0.0610±	3.8663±	0.0277±
6	4.2163±	0.0504±	4.2579±	0.0283±	4.3905±	0.0502±

9	4.5042±	0.0147±	4.6076±	0.2209±	4.8344±	0.0982±
12	4.8318±	0.0443±	4.8973±	0.3528±	5.2275±	0.1154±
15	5.0046±	0.0438±	5.1882±	0.4807±	5.6909±	0.2188±
18	5.2593±	0.0421±	5.1199±	0.6229±	5.5518±	0.1501±
21	5.6581±	0.0856±	4.7838±	0.7943±	5.3613±	0.2226±
24	6.0406±	0.2050±	4.8038±	0.8467±	5.6129±	0.2494±
27	6.4360±	0.2904±	5.0324±	0.9147±	5.7637±	0.3180±
30	6.9863±	0.3480±	5.0211±	0.8570±	5.8015±	0.4002±

Table 4: The mean average SGR (% d^{-1}) with standard error

Days	**Average SGR(% d-1) with standard errors(SE)**					
	25‰	SE	35‰	SE	40 ‰	SE
0	0.0000		0.0000		0.0000	
3	3.5427±	0.0494	4.0964±	0.5673	4.8505±	0.2446
6	3.9693±	0.2486	4.0265±	0.0517	4.5431±	0.1538
9	3.3817±	0.0456	3.5357±	0.5258	4.0958±	0.2155
12	3.1207±	0.0591	3.1349±	0.6030	3.7226±	0.1808
15	2.7309±	0.0522	2.8726±	0.6050	3.5379±	0.2535
18	2.5516±	0.0347	2.2874±	0.6575	2.8149±	0.1480
21	2.5343±	0.0865	1.5814±	0.7571	2.2419±	0.1971

24	2.4864±	0.1528	1.3884±	0.6989	2.1518±	0.1846
27	2.4416±	0.1774	1.4003±	0.6376	2.0071±	0.2022
30	2.4696±	0.1733	1.2649±	0.5393	1.8226±	0.2283

The measurements of fresh weight of *Gracilariopsis funicularis* are shown in Table 3 and the results shows that the fresh weights at salinity level of 40ppt was increasing rapidly between day 3 to day 18 as compared to salinities of 25 ppt and 35 ppt. However from day 18, the fresh weights of *Gracilariopsis funicularis* was increasing rapidly at salinity of 25 ppt as compared to salinity of 35 ppt and 40 ppt. During day 21 and day 24, plants growing in salinity of 35 ppt seem to decrease in weight.

Furthermore, as shown in Table 4, the calculated specific growth rate per day (SGR (% d^{-1})) showed that the plants grew well in all salinities tested. All salinities provided a faster growth rate until day 12 and the growth started to decrease thereafter (Figure 8). It worth noting that salinity of 35 ppt had the lowest growth rate as from day 21 until the end of the experimental period, with growth rate ranging from 1.58 % d^{-1} to 1.26 % d^{-1}.

From observation during the experimental period, after day 18, *Ulva* started to appear in the flasks that were incubated at 25 ppt and 35 ppt, however , *Ulva* didnt appear in the flask that were incubated at 40 ppt. Its clearly illustrated on the graph with an arrow to show the point where *Ulva* appeared.

The statistical analysis showed that there was no significant difference in the growth rate of *Gracilariopsis funicularis* grown at salinities of 25 ppt, 35 ppt and 40 ppt ($P>0.05$).

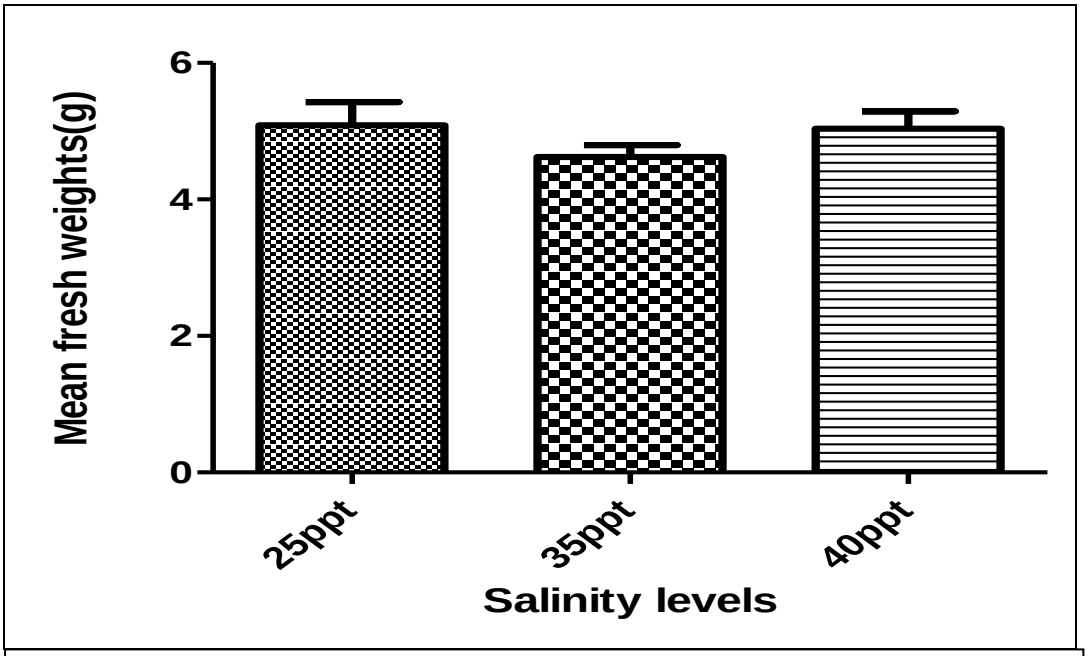

Figure 6: Mean fresh weights of *Gracilariopsis funicularis* cultured in three salinity levels, Vertical bars indicate Standard Errors.

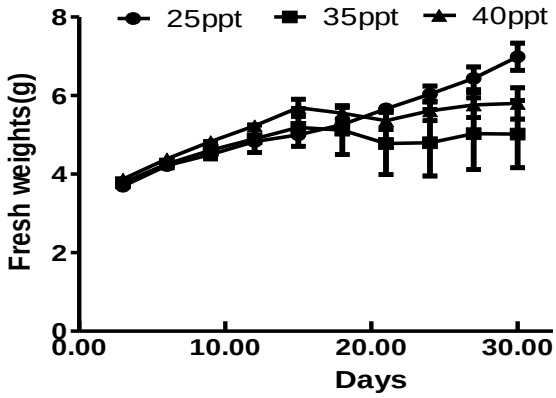

Figure 7: Mean fresh weights of *Gracilariopsis funicularis* cultured in three salinity levels, Vertical bars indicate Standard

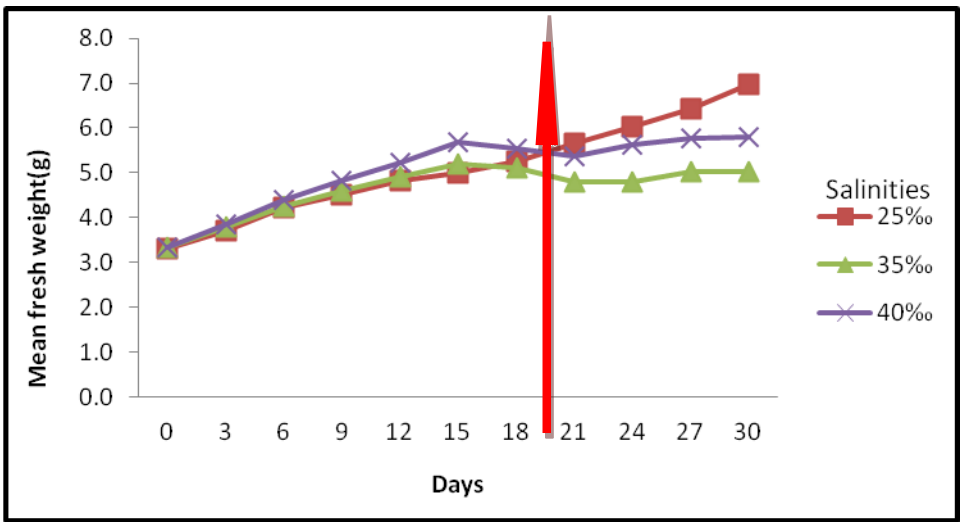

Figure 8: The average fresh weights(g) of the *Gracilariopsis funicularis* during the experimental period of 30 days. Red arrow shows the days when the *Ulva* spp started appearing in 25ppt and 35ppt.

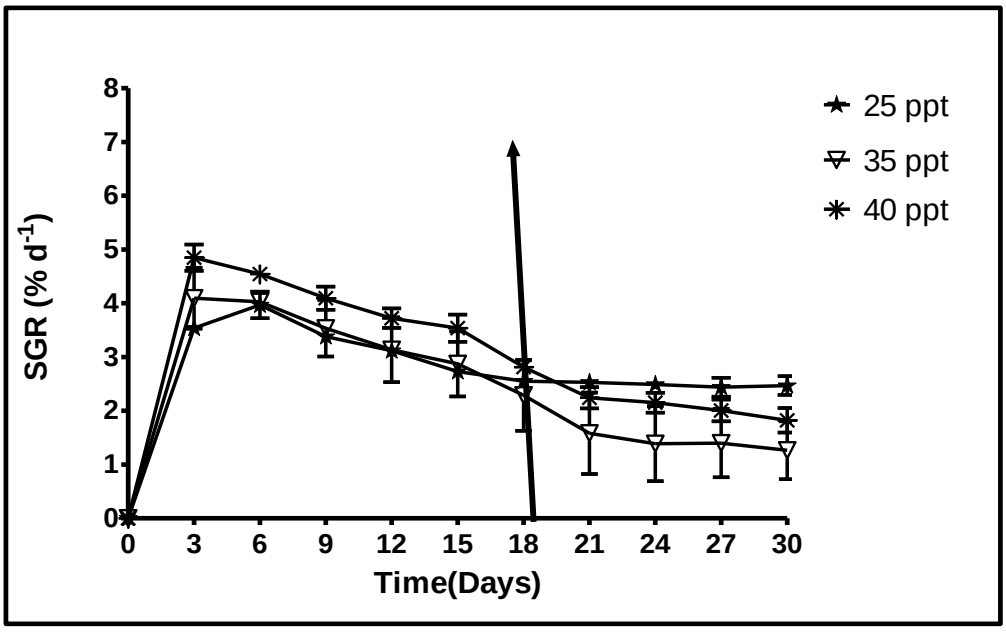

Figure 9: SGR (% d^{-1}) with standard error of *Gracilariopsis funicularis* during the experimental period of 30days.

CHAPTER 4

4.1 DISCUSSION

In recent decades, research on the physiology and ecology of marine macroalgae has increased due to potential commercial usage (Bird and Benson, 1987). In the wild, the growth of seaweeds is affected by various chemical, physical and biological factors (Pedersen *et al*., 2004). The growth rate of cultured seaweeds has also been shown to be regulated by environmental parameters such as irradiance, salinity, temperature and nutrient supply (Lüning, 1990; Lobban and Harrison, 1994).

The results obtained from the current study showed that there is no significant difference in fresh weight increment of plants cultured between under different salinities; it was observed that salinity of 40ppt provided a rapid grow at the beginning of the experiment until day 18. This indicates that seaweed *Gracilariopsis funicularis* can tolerate salinity level above the natural salinity of seawater which is 35ppt. Salinity level of 25ppt all provided significant growth, indicating that *Gracilariopsis funicularis adapt* to salinity levels lower than 35 ppt.

The results of the specific growth rates showed that the *Gracilariopsis funicularis* grew well at all salinities tested and there was no significant difference on the mean specific growth rate ($P>0.05$).

As it's stated on above that, there is no significant difference in mean fresh weights and mean SGR (%d^{-1}), it was also observed that the vegetative growth mechanisms were not the same. Gracilariopsis funicularis cultured less than 25ppt and 35ppt were growing well

on vegetative growths. The small thalli were observed more in 25ppt and 35ppt. Since *Gracilariopsis funicularis* is mainly found in South Africa's estuaries (Iyer et al.;2005) it can grow best vegetative in the seawater salinity below 35ppt. Enough nutrients supplied to the seaweeds and with moderate salinity levels boosted the growth of new thalli on seaweeds original thalli. Some of the seaweed thalli were seen breaking up from the original thalli but they were observed growing vegetative individual. 40ppt salinity levels, thalli were increasing without vegetative yield. The increases in fresh weights were gained from the growth of the original thalli. Due to high salinity level, *Gracilariopsis funicularis* can struggle to grow vegetative in this level of salinity. 25ppt was increasing faster in fresh weights continuously and linearly due to adaption to the seawater lower than 35ppt. Acclimation of seaweeds to the new environments makes some of the seaweeds so special.

After day 18, the *Ulva* species, were also found growing on the thalli of the cultured seaweed species. This was only seen in salinity level 25ppt and 35ppt. *Ulva* species find their ways in the cultured spp due to the water used contains some *Ulva* spores and sterilization of water with Uv-lights might be that was not enough, but the situation was handled property with proper control. And in 40ppt was not found because the water was completely sterilized with high temperature when boiled to evaporate water for salinity water above 35ppt could be achieved, and this process enabled most of the *Ulva* spores to be sterilized.

4.2 CONCLUSION

Salinity is one of the important environmental parameter for the growth rates of the *Gracilariopsis funicularis*. It does only affect the adaptation habits of the seaweeds especial on the vegetative growth of the seaweed. Light intensity also affected the colour

of the new thalli formation, due to more light supplied to the seaweeds, it increased the rate of photosynthesis and this caused green pigments to dominant the other pigments. Sufficient sterilization of the used water can prevent the growth of other seaweeds species that are not targeted or aimed to be cultured. The correct supply of nutrients to the seaweeds in the cultured environments is very important because every plant need supply of nutrients. In the natural environments, the seaweeds are supplied with nutrients by the waves and water current; therefore, this was supplemented in the cultured environment.

4.3 RECOMMENDATION

The growth rates of the seaweeds are been affected by many factors, such as: temperature, nutrients, lights, pH, dissolved oxygen, and investigating one environmental factor will not provide adequate information on the optimal growth conditions for the cultivation of *Gracilariopsis funicularis*. This was an interesting study and more studies need to be carried out to investigate the effect of environmental conditions on the growth of *Gracilariopsis funicularis*. This will provide sufficient information on the optimal growth conditions of this valuable species which has a huge potential for mariculture.

4.4 CONTRIBUTION TO KNOWLEDGE

The research project has contributed to the knowledge on the importance of seaweeds and how to cultivate some of the economic importance seaweed species. The project has also

created courage and passions within me about seaweeds and willing to do more research on seaweeds. This project has helped me to differentiate between *Gracilaria gracilis* from *Gracilariopsis funicularis* since they are difficult to be differentiated from each other. This research project will also help seaweed farmers conduct a good practice on cultivation of *Gracilariopsis funicularis* as well as other economical important seaweeds species. Finally, the project has improved on the knowledge of better statistical analysis and interpretation of the statistically results, research designs, and data collections

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APPENDIX

Appendix 1

1.1 Tables of Fresh weights, SGR (% d^{-1}), standard error, of the three salinity levels

	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**	**Day 24**	**Day 27**	**Day 30**
Flasks	Initial weight	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰
A	3.30	3.666	4.302	4.496	4.771	4.971	5.198	5.812	6.451	6.975	7.680
B	3.33	3.700	4.220	4.533	4.806	4.952	5.340	5.645	5.846	6.355	6.684
C	3.33	3.719	4.127	4.484	4.918	5.092	5.240	5.517	5.825	5.978	6.595
Mean		**3.695**	**4.216**	**4.504**	**4.832**	**5.005**	**5.259**	**5.658**	**6.041**	**6.436**	**6.986**
StDev		**0.027**	**0.087**	**0.025**	**0.077**	**0.076**	**0.073**	**0.148**	**0.355**	**0.503**	**0.603**
Std error		**0.016**	**0.050**	**0.015**	**0.044**	**0.044**	**0.042**	**0.086**	**0.205**	**0.290**	**0.348**
sqrt		**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**

	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30
	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰	25‰
	0.0350	0.0441	0.0343	0.0307	0.0273	0.0252	0.0269	0.0279	0.0277	0.0282
	0.0349	0.0394	0.0342	0.0305	0.0264	0.0262	0.0251	0.0234	0.0239	0.0232
	0.0364	0.0355	0.0329	0.0324	0.0282	0.0251	0.0240	0.0232	0.0216	0.0227
Mean	**3.5427**	**3.9693**	**3.3817**	**3.1207**	**2.7309**	**2.5516**	**2.5343**	**2.4864**	**2.4416**	**2.4696**
St Dev	**0.0856**	**0.4305**	**0.0790**	**0.1024**	**0.0903**	**0.0600**	**0.1498**	**0.2646**	**0.3073**	**0.3001**
Std error	**0.0494**	**0.2486**	**0.0456**	**0.0591**	**0.0522**	**0.0347**	**0.0865**	**0.1528**	**0.1774**	**0.1733**
sqrt	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**	**1.7321**

		Day 3	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**	**Day 24**	**Day 27**	**Day 30**
	Initial weight	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰
A	3.336	3.673	4.247	4.919	5.446	6.109	6.322	6.343	6.477	6.848	6.726
B	3.368	3.790	4.312	4.723	5.008	4.966	4.801	4.266	4.196	4.319	4.319
C	3.328	3.884	4.215	4.181	4.238	4.489	4.237	3.742	3.739	3.931	4.018
Mean	3.344	**3.782**	**4.258**	**4.608**	**4.897**	**5.188**	**5.120**	**4.784**	**4.804**	**5.032**	**5.021**
StDev		**0.106**	**0.049**	**0.383**	**0.611**	**0.833**	**1.079**	**1.376**	**1.467**	**1.584**	**1.484**
Std error		**0.061**	**0.028**	**0.221**	**0.353**	**0.481**	**0.623**	**0.794**	**0.847**	**0.915**	**0.857**
sqrt		**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**

	Day 3	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**	**Day 24**	**Day 27**	**Day 30**
	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰	35‰
	0.032	0.040	0.043	0.041	0.040	0.036	0.031	0.028	0.027	0.023
	0.039	0.041	0.038	0.033	0.026	0.020	0.011	0.009	0.009	0.008
	0.051	0.039	0.025	0.020	0.020	0.013	0.006	0.005	0.006	0.006
Mean	**4.096**	**4.026**	**3.536**	**3.135**	**2.873**	**2.287**	**1.581**	**1.388**	**1.400**	**1.265**
StDev	**0.983**	**0.090**	**0.911**	**1.044**	**1.048**	**1.139**	**1.311**	**1.210**	**1.104**	**0.934**
Std error	**0.567**	**0.052**	**0.526**	**0.603**	**0.605**	**0.658**	**0.757**	**0.699**	**0.638**	**0.539**

sqrt	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**

	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**	**Day 24**	**Day 27**	**Day 30**
	initial weights	40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰
A	3.331	3.823	4.301	4.645	5.034	5.369	5.304	5.129	5.371	5.464	5.544
B	3.358	3.858	4.475	4.886	5.215	5.595	5.530	5.149	5.356	5.428	5.274
C	3.338	3.918	4.396	4.973	5.433	6.109	5.822	5.806	6.112	6.399	6.587
Mean	3.343	**3.866**	**4.391**	**4.834**	**5.227**	**5.691**	**5.552**	**5.361**	**5.613**	**5.764**	**5.802**
StDev		**0.048**	**0.087**	**0.170**	**0.200**	**0.379**	**0.260**	**0.386**	**0.432**	**0.551**	**0.693**
Std error		**0.028**	**0.050**	**0.098**	**0.115**	**0.219**	**0.150**	**0.223**	**0.249**	**0.318**	**0.400**
sqrt		**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**

		Day 3	**Day 6**	**Day 9**	**Day 12**	Day 15	**Day 18**	Day 21	**Day 24**	**Day 27**	**Day 30**
		40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰	40‰
		0.046	0.04	0.04	0.034	0.032	0.026	0.021	0.02	0.018	0.017
		0.046	0.05	0.04	0.037	0.034	0.028	0.02	0.019	0.018	0.015
		0.053	0.05	0.04	0.041	0.04	0.031	0.026	0.025	0.024	0.023
Mean		**4.85**	**4.54**	**4.1**	**3.723**	**3.538**	**2.815**	**2.242**	**2.152**	**2.007**	**1.823**
StDev		**0.424**	**0.27**	**0.37**	**0.313**	**0.439**	**0.256**	**0.341**	**0.32**	**0.35**	**0.395**

Std error		**0.245**	**0.15**	**0.22**	**0.181**	**0.254**	**0.148**	**0.197**	**0.185**	**0.202**	**0.228**
sqrt		**1.732**	**1.73**	**1.73**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**	**1.732**

Appendix 2

2.1 TABLE OF ANOVA

2.1.1 ANOVA TABLE FOR FRESH WEIGHTS

Table Analyzed for average f. Weights	Data 1				
One-way analysis of variance					
P value	0.2553				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.437				
R square	0.09619				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	6.066				
P value	0.0482				
P value summary	*				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1.597	2	0.7984		
Residual (within columns)	15	27	0.5557		
Total	16.6	29			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
25ppt vs 35ppt	0.5138	2.18	No	ns	-0.3134 to 1.341
25ppt vs 40ppt	0.05312	0.225	No	ns	-0.7741 to 0.8803
35ppt vs 40ppt	-0.4607	1.954	No	ns	-1.288 to 0.3665

2.1.2 ANOVA table for SGR (% d^{-1})

Table Analyzed	Data 1				
One-way analysis of variance					
P value	0.5825				
P value summary	Ns				
Are means signif. different? (P<0.05)	No				
Number of groups	3				
F	0.5502				
R square	0.03538				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	1.093				
P value	0.5789				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1.764	2	0.8822		
Residual (within columns)	48.1	30	1.603		
Total	49.86	32			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P <0.05?	Summary	95% CI of diff
25 ppt vs 35 ppt	0.3301	0.865	No	ns	-1.001 to 1.661
25 ppt vs 40 ppt	-0.2335	0.612	No	ns	-1.564 to 1.097
35 ppt vs 40 ppt	-0.5636	1.476	No	ns	-1.895 to 0.7672

Appendix 3

3.1 Table of data collection of the *Gracilariopsis funicularis* for 30 days of experiment

Date	Flask	Measurements(weights in grams) from the three level of Salinities				Date	Flask	Measurements(weights in grams) from the three level of Salinities		
		25‰	**35‰**	**40‰**				**25‰**	**35‰**	**40‰**
11/12/2011	A	3.3007	3.3363	3.3314		28/12/2011	A	5.1980	6.3224	5.3038
	B	3.3318	3.3676	3.3581			B	5.3400	4.8007	5.5295
	C	3.3344	3.3280	3.3381			C	5.2400	4.2366	5.8222
13/12/2011	A	3.6658	3.6730	3.8230		31/12/2011	A	5.8123	6.3432	5.1286
	B	3.6995	3.7895	3.8578			B	5.6453	4.2663	5.1489
	C	3.7193	3.8840	3.9180			C	5.5167	3.7418	5.8063
16/12/2011	A	4.3015	4.2469	4.3009		03/01/2012	A	6.4505	6.4765	5.3707
	B	4.2203	4.3115	4.4747			B	5.8461	4.1959	5.3564
	C	4.1270	4.2153	4.3959			C	5.8252	3.7390	6.1117
19/12/2011	A	4.4961	4.9191	4.6447		06/01/2012	A	6.9745	6.8480	5.4636
	B	4.5327	4.7233	4.8856			B	6.3551	4.3186	5.4282
	C	4.4838	4.1805	4.9730			C	5.9783	3.9306	6.3994
22/12/2011	A	4.7713	5.446	5.0339		09/01/2O12	A	7.6804	6.7262	5.5435
	B	4.8060	5.008	5.2154			B	6.6840	4.3193	5.2743
	C	4.9181	4.238	5.4331			C	6.5945	4.0178	6.5868
25/12/2011	A	4.9706	6.1093	5.3690						
	B	4.9516	4.9659	5.5952						
	C	5.0916	4.4893	6.1085						