

**THE EFFECTS OF SALINITY, NITROGEN, AND PHOSPHORUS ON SURVIVAL
AND WEIGHT GAIN OF *GRACILARIA sp.***

by

Hao Sun

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 2, 2014

Keywords: Gracilaria, Salinity, Nitrogen, Phosphorus

Copyright 2014 by Hao Sun

Approved by

Jesse A. Chappell, Chair, Associate Professor of Fisheries, Aquaculture and Aquatic Sciences
Claude E. Boyd, Professor of School of Fisheries, Aquaculture and Aquatic Sciences
Wheeler Foshee, Associate Professor of Horticulture

Abstract

Gracilaria is a genus of red marine algae, it is also an economically significant species of macroalgae. The aim of this study is try to use algae itself to reduce organic and inorganic pollution in the aquatic environment to achieve the effect of water quality improvement. Macroalgae is a kind of plant resource that can be utilized to obtain the growth a of food resource in nature, and thus address the problem of resource degradation and depletion brought by the increase in population, food safety, food security etc. This study used different concentrations in the solution, for salinity nitrogen, and phosphorus in culture of *Gracilaria*. In order to find the most suitable condition and nutrient profile, the growth rate, survival rate and ultimately weight gain of *Gracilaria* were evaluated in this study. In 20 ppt of salinity, the growth rate of *Gracilaria* was higher than that of 10 ppt and 30 ppt saliniy. However, in 30 ppt salinity, *Gracilaria* survival rate was higher than that observed at 10 ppt and 20 ppt. The concentrations of nitrogen and phosphorus did not significantly influence the growth rate or survival rates. The concentrations of all treatments of nitrogen and phosphorus were reduced in presence of *Gracilaria* during the culture period, so it can be concluded that *Gracilaria* has the ability to remove nitrogen and phosphorus from the water in which it is growing and thus reduce eutrophication and improve the water quality.

Acknowledgments

The author would like to express his deepest gratefulness to Dr. Jesse Chappell for his continuous help and advice from the start to the end of the whole experiment.

The author would like to take this chance to express his deepest gratitude to Dr. Jesse Chappell for his continuous advice and assistance throughout the course of this thesis.

He acknowledges his gratitude to Dr. Boyd and Dr. Foshee for serving as committee members. Special thanks to Jeremy Pickens and Robert Beauchamp for the help and support to make this experiment go well.

He is also grateful to their friends for their assistance to make his life in Auburn amazing. Thanks also to everybody in Dr. Chappell's lab for making his stay in Auburn a pleasant and memorable one.

Most importantly, he thanks his parents, Baoli Sun and Jiezi Lu for their selfless support.

Table of Contents

Abstract.....	ii
Acknowledgments.....	iii
Table of Contents.....	iv
List of Table.....	v
List of Figure.....	vi
List of Abbreviations	vii
Chapter 1.....	1
Introduction.....	1
Literature review.....	5
Material and Methods	8
Results and Discussion	12
Conclusion	20
References.....	21

List of Table

Table 1 Description of groups in different concentrations of nitrogen, phosphorus and salinity.	9
Table 2 Radom arrangement of <i>Gracilaria</i> samples.	10
Table 3 Dry weight of <i>Gracilaria</i> samples.	12
Table 4 Means of <i>Gracilaria</i> weight from 9 treatments during the culturing period.	13
Table 5 The survival situation of <i>Gracilaria</i> during the experiment.	18
Table 6 The coefficient of determination of all groups.	19

List of Figures

Figure 1 Growth and relative amount of chlorophyll present per cell at the various salinities in <i>Amphidinium carteri</i>	6
Figure 2 Growth and relative amount of chlorophyll per cell at the various salinities in <i>Platymonas</i> sp.	7
Figure 3 Linear regression lines of treatment 2.	13
Figure 4 Linear regression lines of treatment 3.	14
Figure 5 Linear regression lines of treatment 5.	14
Figure 6 Linear regression lines of treatment 8.	15
Figure 7 Duncan's pairwise comparison.....	19

List of Abbreviations

N	Nitrogen
P	Phosphorus

Chapter 1

Introduction

Gracilaria is a genus of red marine algae notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species for shellfish. Various species within the genus are cultivated among Asia, South America, Africa and Oceania.

With the increasing population, natural resources are drying up and food issue has become a big challenge to humanity. The development of human civilization is causing bringing serious environmental degradation which also has a restrictive effect on global human development. However, the traditional method that is used to control the riverine or estuarine pollution often causes a secondary form of pollution, due to improper operations or technologies. So taking thoughtful biological approaches for restorative actions to initiate environmental restoration and create a healthy eco-system may be the best ways to address these problems..

Materials and energy have their own circulatory system in nature and this balance may be disabled when human factors are involved. For example, oil exploitation sometimes initiates problems brought about by sharply increasing content of carbon dioxide in atmosphere. The most effective solution that is widely accepted now is advocating the low-carbon lifestyle, and increasing the amount of vegetation cover rate. While phytoplankton and macro-algae contain chlorophyll and with light energy stimulates photosynthesis and is therefore autotrophic.

Pollution from a wide variety of sources also exists in some modern aquatic environments, and the concentrations of N and P in aquatic eco-system are significantly increased over that of a few years ago. As we know, the elevated high concentrations of nitrogen and phosphorus can negatively influence the water quality in natural environments and culture system, and cause fish, water plant, aquatic animal death. The impacts of high concentrations of nitrogen and phosphorus in natural and culture environments are obvious, so using water plants to remove the redundant nitrogen and phosphorus seems to be the best way to solve the problem.

Water quality issues are becoming the biggest challenge around the world, and have a big influence to aquaculture. Water pollution may cause many environment problems, such as destroying ecosystem balance, food safe, human health, and so on. In usual, In Ren é P. Schwarzenbach et al. (2010) pointed out that some pollution contains chemical compounds, particularly on inorganic and organic micropollutants including toxic metals and metalloids as well as a large variety of synthetic organic chemicals.

Aquatic is an autotrophic plant group species which contains chlorophyll and photosynthesis. They can live without rhizome of leaf differentiation, vascular bundle, or embryos like a thallus plant. Algae have been known as the original water plants and are usually found in the water.

Algae have two characteristics: (1) All kinds of algae, sometimes similar to higher plants in shape the structure of the root, stem, leaf, but can be different on the function for photosynthesis and release oxygen, algae without root leaf differentiation, and the algae body is actually a simple leaf, and therefore, algae system called thallus. (2) Their sexual

reproduction organs lack vegetative cells, commonly, some may be more cells surrounded by a layer of all cells are directly involved in reproductive function.

Salinity is considered as one of the most important factors in environment, particularly in wet land areas, which would affect the growth of algae.

Because of these two characteristics, culturing algae is the best way to improve the quality of water system in the river.

Only the surface water contamination from mining operation, mining activities world wide mobilize more than 50×10^9 metric tons of geological material per year, which is similar to the flux of particles transported by rivers from the continents to the sea (Schwarzenbach et al, 2000). And Bridge indicated (Bridge G, 2004) most mining operations trigger significant environmental and social problems as they result in large waste deposits, which are exposed to oxidation by air and weathering by precipitation, and subsequent pollution of water resources. The water pollutants also contains human and industrial wastewater and so on,

So the global wastewater issue in the whole world is enormous, and it brings will also cause a big influence to human's life.

Schwarzenbach (2010) reported the most direct and severe impact for human health is the lack of improving sanitation. Sanitation problem can be related to the lack of safe drinking water, which currently affects more than a third of the population in the world.

Water pollution includes inorganic pollutions (such as surface water contamination from mining operation) organic pollutions. In this experiment, the main purpose is to explore a means of biologically reducing organic pollution and thus address the objective of improving water quality and aquatic environment.

For the pollution within the ocean and/or riverine system, applying ecosystem to fix the environment by itself is always the best way to improve the water situation. Ammonia pollution is the most harmful factor for the water environment, waste water usually bring high concentration of nitrate to river and make fishes and aquatic livings dead. The best way to ameliorate the water environment is the utilization of algae.

During the experiment, we cultured algae at different concentrations of nitrogen and phosphorus and different salinities. The growth rate of algae was measured through the culturing period. And the optimum condition for *Gracilaria* to remove nitrogen and phosphorus in different areas was decided. The survival rate in different concentrations of salinity also determines whether *Gracilaria* is appropriate to be used for improving the quality of water.

Literature review

In a previous report, *Dunaliella* (McLachlan et al. 1960) was shown to have a broad salinity tolerance and range on chlorophyll synthesis, but content indicated that a narrower range of salinities was necessary for maximum synthesis of this pigment. Jack Mclachlan (1961) indicated that the purpose of the present study was to ascertain, in unicellular marine algae, a general relationship exists between the relative amount of chlorophyll per cell and growth at various salinities. Also, Kim (Kim et al. 1958) working with higher plants, showed that, in general, there was an inverse relationship between the chlorophyll content and the salt concentration of the culture solutions. Miller (Miller, et al., 1957) and Myers' (Myers, J., 1956) in a fresh-water study on planktonic algae, pigment changes have been noted with variations in the nutrients of the medium.

Jack Mclachlan's work (1961) indicated different species of algae's toleration of salinity strongly influences growth rate. Compared with *Gracilaria*, *Monochrysis lutheri*, *Syracosphaera carterae*, *Cyclotella* and so on, these algae and their growth and survival are also strongly influenced by salinity level.

The micro-algae *Monochrysis lutheri*, *Syracosphaera carterae*, *Cyclotella* sp., *Thalassiosira decipiens*, and *Cryptomonas* sp. do not show a distinct salinity optimum with regard to growth, nor was there a significant difference in the concentration of chlorophyll per cell at the various salinities.

Growth of *Monochrysis* at 2.5‰ salinity was not as dense as at the higher salinities, and the chlorophyll concentration per cell was also low at this salinity. *Syracosphaera* failed to grow at all at 2.5‰, but growth and chlorophyll concentration per cell at the other salinities

were essentially the same. Growth and the chlorophyll concentration of *Cyclotella* were approximately the same from 2.5 to 3.5‰. *Thalassiosira* did not grow at salinities below 1.5‰, but growth and the chlorophyll concentration per cell were essentially the same as those found at the higher salinities. *Cryptomonas* grew about equally well at all salinities, and there was no significant change in the concentration of chlorophyll per cell throughout the salinity range.

Growth and relative amount (see text) of chlorophyll per cell at the various salinities in *Olisthodisus* sp.

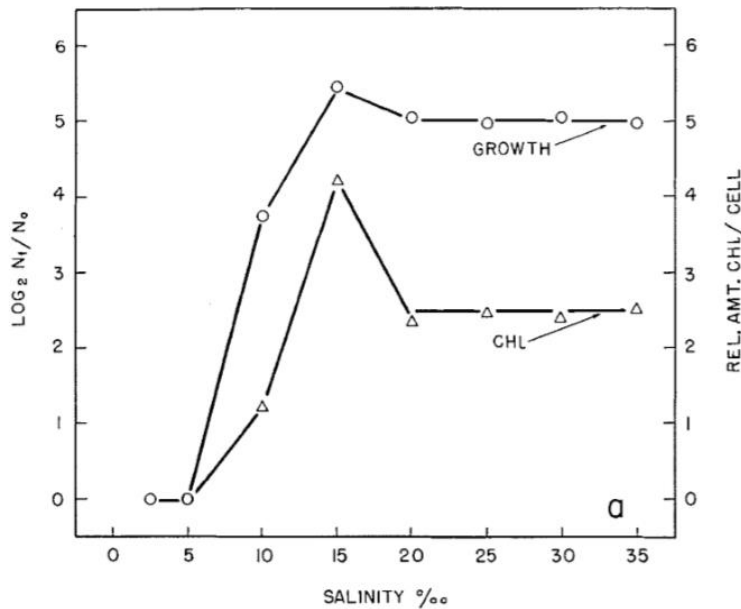


Fig 1 Growth and relative amount of chlorophyll per cell at the various salinities in *Amphidinium carteri*.

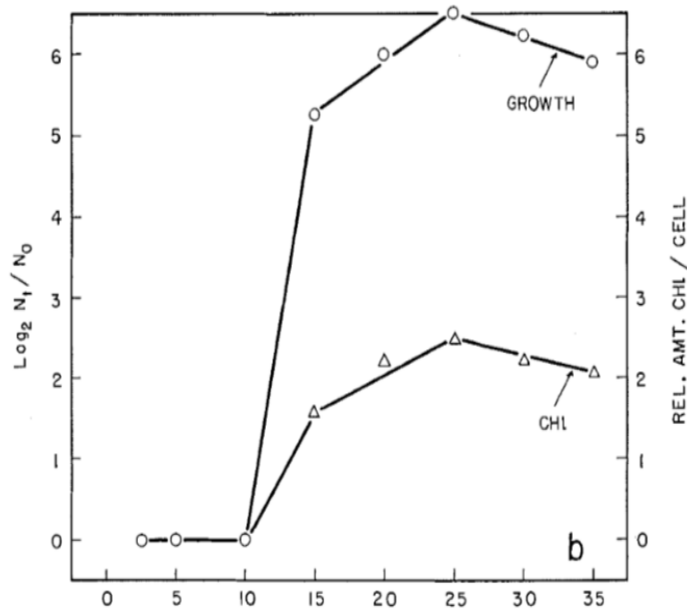


Fig2 Growth and relative amount of chlorophyll per cell at the various salinities in *Platymonas* sp

In the study by Jack McLachlan (1961). The growth of *Olisthodisczts* was completely suppressed at 2.5 and 5.0‰ (Fig. 1). Maximum growth occurred at 1.5‰, and from 2.0 to 3.5‰, growth was essentially the same. A maximum concentration of chlorophyll per cell was also found at 1.5‰. Therefore, both the cell concentration and chlorophyll per cell content indicate a growth optimum for salinity of 1.5‰ for this organism. Maximum growth and *chlorophyll* concentration per cell occurred at 2.5‰~0 for *Amphidinium* (Fig. 2).

Material and Methods

Sample collection

The sample *Gracilaria* were kept in bags without water from sourcing destination to laboratory. In order to prepare a single large one big tank up to 200+ gallons with 30 ppt of salt. The water was added into the tank to reach an estimated two hundred gallons and then salts were added slowly to reach a concentration of 30 ppt. The tank was mixed well after one night in room temperature. There were nine treatments in this experiment and each treatment required 20 gallons of saline water. In case of spillage, we have prepared 30 gallons of solution for each treatment. Two buckets were washed to prepare different concentrations of salt solutions. Salt solutions with concentration of 20 ppt and 10 ppt were obtained by diluting the 30 ppt salt solution with water.

Divide into groups

Set up 45 samples, and use three salinity 10ppt, 20ppt and 30ppt, three concentrations of Nitrogen and phosphorus, 10 N mg/L, 1 P mg/L; 50 N mg/L; 5 P mg/L and 100 N mg/L; 10 P mg/L. Group the sample with different salinities and concentration of Nitrogen and Phosphorus, 3*3, we can get 9 groups. Each group has 5 parallels and 85.6 grams *Gracilaria* were put in every bucket at the beginning of experiments.

The arrangement of creates 3 salinity numbers, and 3 concentrations of N, P. In treatment 1 to 9 is shown in Table 1.

Treatment No.	Salinity	Fertilizer N and P mg/l
1	10	100 N mg/l; 10 P mg/l
2	20	50 N mg/l; 5 P mg/l
3	30	10 N mg/l; 1 P mg/l
4	10	50 N mg/l; 5 P mg/l
5	20	10 N mg/l; 1 P mg/l
6	30	100 N mg/l; 10 P mg/l
7	10	10 N mg/l; 1 P mg/
8	20	100 N mg/l; 10 P mg/l
9	30	50 N mg/l; 5 P mg/l

Table 1 Description of groups in different concentrations of nitrogen, phosphorus and salinity.

Pretreatment

The greenhouse floor where buckets were cleaned sterilized by use of bleach. In algae experiments, wild seaweeds are the biggest hidden danger in the beginning of research. After the floor was bleached, it was thoroughly rinsed with treated city water to prevent bleach from influencing the experiment objectives, all the buckets were cleaned in the same way.

Samples of cultures

There were 9 treatments and each had 5 replicates in this experiment. So 45 buckets marked and the sequences of buckets were like table 2. The sequences of buckets were arranged randomly. From 1-1 to 9-5 are written in different 45 pieces of paper, mixed and pick them one by one. Finally we set up the bucket like table 2.

2-6	4-8	2-8	1-7	5-5	1-4	4-4	2-1	5-8
3-4	2-3	1-2	5-1	4-1	4-2	3-2	5-4	3-5
4-3	3-1	1-3	4-9	2-2	5-6	1-9	4-5	5-9
1-5	1-1	4-7	3-7	5-3	3-6	5-7	2-9	3-3
2-7	3-9	5-2	1-8	4-6	3-8	2-4	1-6	2-5

Table 2 Radom arrangement of *Gracilaria* samples.

Statistical analyses

All data were analyzed and assessed using standard statistical techniques and analyses.

All values were expressed as mean \pm standard deviation (s.d.). Statistical analyses were performed with the computer program (Excel and SAS), differences between the test and the control group were evaluated by Duncan's test. A difference with $P \leq 0.05$ was considered significant.

Results and Discussion

Dry weight

Each treatment received a mean weight of 85.6 grams of wet *Gracilaria* at the beginning of the experiment. Dry weights of four samples were determined and shown in Table 3.

Sample No.	1	2	3	4
Weight	10.23g	10.23g	10.23g	10.24g

Table 3 Dry weight of *Gracilaria* samples.

In the process of securing and culturing the sample, we needed to measure the dry weight of some random samples. *Gracilaria* must be held in water for survival during the process of transportation. Because the process of sampling live, wet algae will always include a small volume of water. The water in sampling and weighting of algae would not ensure the precision of the experiment, the dry weight of four samples with a drying period of seven days in the dryer were measured. The result error was less than 1 %. So we could ignore the influence which exists in the process of sampling and weighting within the experiment.

The changes weight of *Gracilaria* from 9 treatments.

No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
1	86.8332±2.49	87.68374±3.94	88.2764±5.35	90.1984±7.56	90.9286±9.51	93.7938±14.53	94.0122±15.11
2	90.9128±3.45	94.54266±3.64	100.7554±7.47	109.8856±9.50	116.5962±11.26	118.0066±11.68	119.616±11.74
3	94.1934±2.40	97.6128±1.95	102.8882±2.09	108.1644±2.84	112.5858±3.80	118.1844±4.41	120.7762±4.57
4	89.3724±1.88	92.9628±2.45	95.4208±1.22	96.7126±2.04	99.6768±2.42	102.0204±4.82	103.4136±6.30
5	93.044±3.41	95.5078±4.73	102.8466±10.06	110.61±16.34	115.1372±19.19	117.9374±20.59	119.8384±21.13
6	90.0188±1.42	91.7326±1.37	94.4572±1.86	96.9438±3.89	98.4508±5.21	100.6682±6.45	101.8094±7.42
7	88.5388±1.66	90.8942±3.36	92.9642±5.09	95.2892±6.36	96.4072±6.84	98.0338±8.21	99.0346±8.83
8	92.2096±3.56	96.849±3.80	103.952±8.97	109.128±10.59	113.738±13.38	116.8854±13.88	119.1034±14.17
9	89.4774±2.61	91.0128±3.05	92.2786±3.06	93.2558±3.56	94.2956±3.99	95.815±5.11	96.5934±5.33

Table 4 Means of *Gracilaria* weight from 9 treatments during the culturing period.

Linear regression analyses of the culturing period vs weight of algae

During the period of culturing of *Gracilaria*, because of the growth of *Gracilaria*, the various weight changes were occurred. Analysis of these changes may help to understanding the range of tolerance ability of *Gracilaria*. In recently research of the salinity stress of algae (Le Redulier et al. 1987), growth reflects the balance between photosynthesis and respiration. In different environment, growth rates of survival photosynthesis and respiration is totally different. Trowth of rates of survival, photosynthesis, and respiration have commonly been used to describe the range of tolerance (Krist, 1990). Using weight changes to reflect activity of the whole system of algae is a simple and convenient way.

Figures 3-6 are the linear regression lines of culturing time (week) vs the growth of *Gracilaria*. The dashed lines indicate the prediction interval (95%) and hatched area indicate the confidence interval (95%).

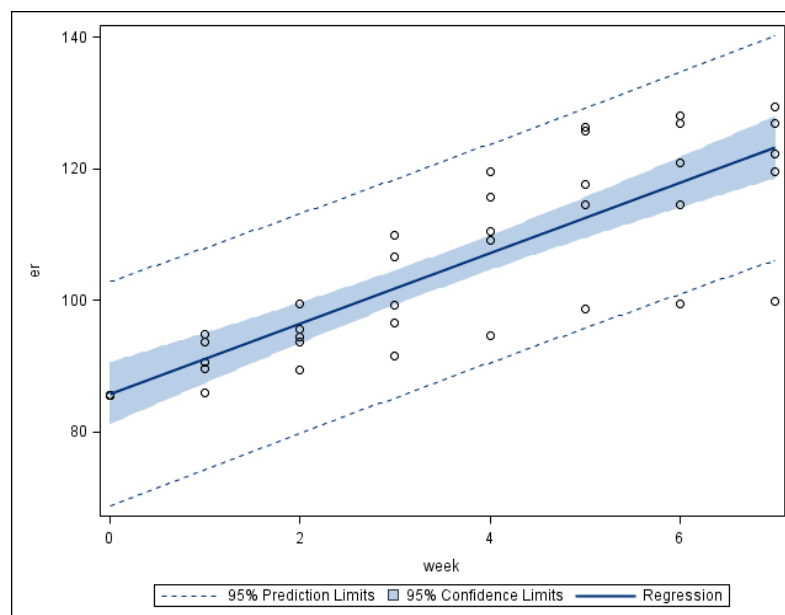


Fig 3. Linear regression lines of treatment 2.

In treatment 2, the equation of culturing period vs. weight was $y=5.34x+85.78$, the slope of 5.34 reflect the growth rate.

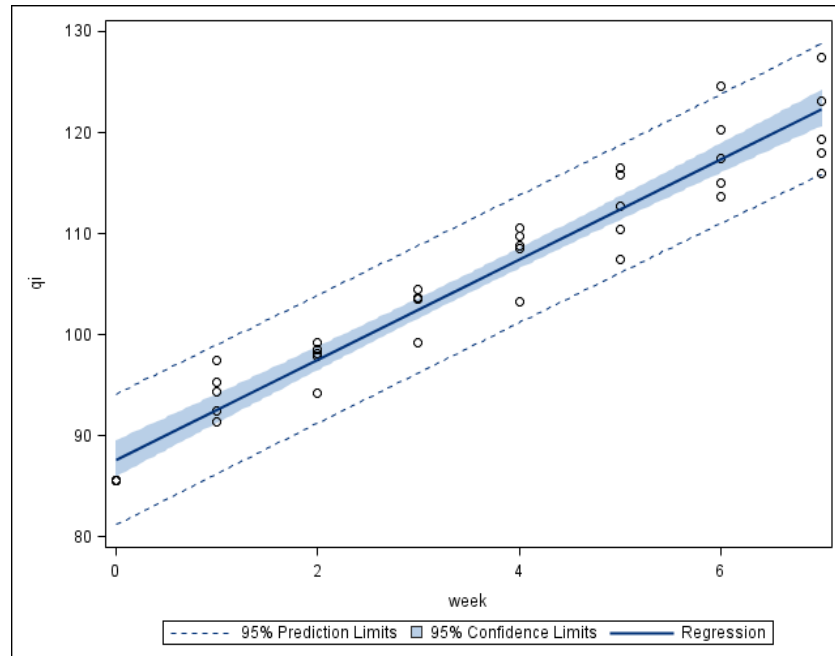


Fig 4. Linear regression lines of treatment 3.

In treatment 3, the equation $y=4.95x+87.56$, $R^2=0.93$ was the most stable one out of all nine groups. Because the concentration of N and P is the 10 mg/L and 1 mg/L, and the salinity is also the lowest, in this environment the growth rate of *gracilaria* is in a real low speed.

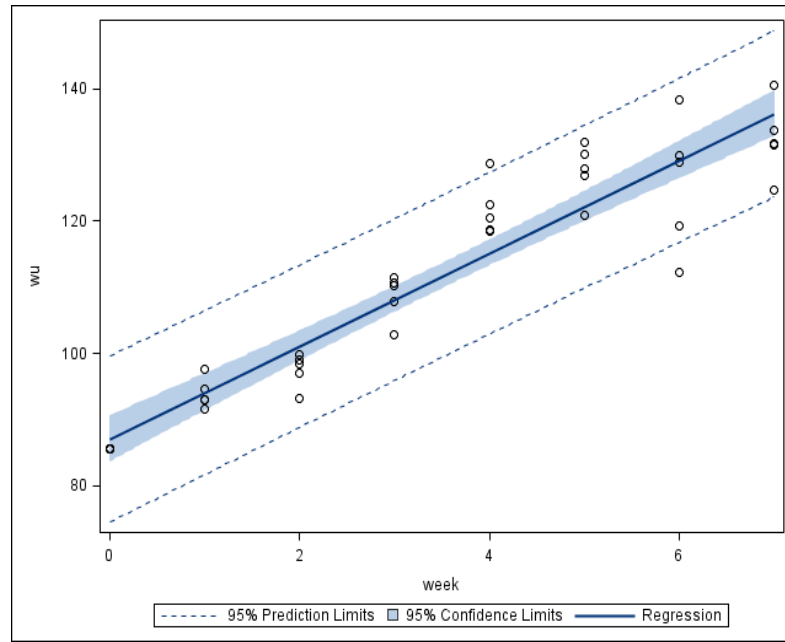


Fig 5. Linear regression lines of treatment 5.

In treatment 5, the equation was $y=7.02x+87.06$, the slope of 7.02 reflect the growth rate.

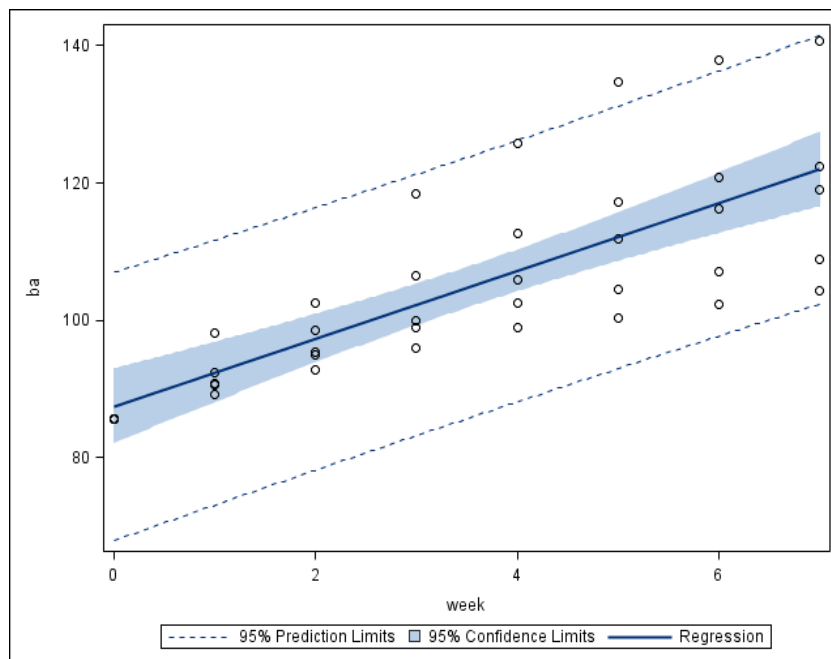


Fig 6. Linear regression lines of treatment 8.

In treatment 8, the equation is $y=4.95x+87.44$, the slope of 4.95 reflect the growth rate.

As a word, the growth rate of treatments 2, 5 and 8 were much faster than other treatments. The salinities in 2, 5 and 8 were 20 ppt salinity, indicating that the *Gracilaria* grow fastest in 20 ppt salinity , with compare to those in 10 ppt salinity and 30 ppt salinity.

In the whole experiment the algae in same groups were not healthy by week 4, but the weight was still gaining, so the data in these groups were not stable and regular. In the living groups, we can see that the growth rate in group 3 was the highest. In treatment No. 3, the concentration fertilizer N and P was 10mg/l and 1mg/l.

In table 4, the data and analysis indicate the growth rate and law curve are changed a lot from the forth week. In a new environment, the period time of the *Gracilaria* for adapting was about 4 weeks.

In treatments 2, 5, and 8, growth rate were more rapid than other groups, their concentration of salinity, N, and P were 20salinity, 50N mg/l and 5 P mg/l; 20salinity, 50 N mg/l and 5 P mg/l and 20salinity, 100 N mg/l and 10 P mg/l.

Coefficient of Determination:

The coefficients of determination (R^2), slopes, and intercepts for each equation were determined by the statistical functions of Microsoft Excel (Microsoft Corporation 1999). The R^2 reflected the stability of increasing, in treatment 3, the stability was the highest.

Treatment No.	1	2	3	4	5	6	7	8	9
R^2	0.11	0.706	0.93	0.78	0.88	0.63	0.40	0.60	0.47

Table 6 The coefficient of determination of all groups.

Survival of Plant Materials

Within the whole experiment, the algae in some groups began to decline after four weeks. The survival observed is shown in table 5.

Treatment No.	1	2	3	4	5	6	7	8	9
1-4 week	alive	alive	alive	alive	alive	alive	alive	alive	alive
5-6week	death	alive	alive	death	alive	alive	alive	alive	alive
7 week	death	death	alive	death	alive	alive	death	alive	alive

Table 5 The survival situation of *Gracilaria* during the experiment.

Base on table 5, in 30 salinity, the survival rate was highest, followed by 20 salinity , and the lowest was 10 salinity. The concentration of N and P did not influence the survival rate significantly.

In order to find out the best salinity for the growth of *Gracilaria*, ten samples with different salinities from 21-30 ppt were set up in the end of the experiment. Eight weeks later, the samples from 26 ppt to 30 ppt were still alive, but the samples from 21-25 ppt began to decline. The growth rate of *Gracilaria* was fastest in 20 ppt. In nature environment, the seawater salinity is always bigger than 30 ppt (Hajer, 2006). So the ability of *Gracilaria* to remove N and P could not reach the top in nature environment.

Duncan's pairwise comparison:

The GLM Procedure
Least Squares Means

treatment*week Effect Sliced by week for weight

week	DF	Sum of Squares	Mean Square	F Value	Pr > F
1	8	215.228146	26.903518	0.39	0.9247
2	8	413.279449	51.659931	0.75	0.6459
3	8	1274.244385	159.280548	2.32	0.0205
4	8	2663.186128	332.898266	4.84	<.0001
5	8	4115.253714	514.406714	7.48	<.0001
6	8	4540.341780	567.542723	8.26	<.0001
7	8	5132.828008	641.603501	9.33	<.0001

Fig 7 Duncan's pairwise comparison

Least squares means analysis for the interaction parameter.

For week 1 and 2, the p value >0.05 means the treatments have no significant effect to the weight of algae. But from week 3, the p value <0.05 means the treatments have significant effect to the change of weight of algae during the culturing period.

Conclusion

In 20 ppt salinity, the growth rate of *Gracilaria* was higher than those of 10 ppt and 30 ppt salinity. In treatment 5 with 10 N mg/l, and 5 P mg/l, the growth rate was the highest across all the treatments.

In 30 ppt salinity, the survival rate of *Gracilaria* was highest. When the salinity was lower than 26 ppt salinity, the *Gracilaria* begins to decline and die.

The concentration of N and P could also influence the growth rate, but not significantly. In conclusion, culturing *Gracilaria* in the aquatic environment could be used as an effective method to improve the water quality.

References

- Bridge G. Contested terrain: mining and the environment[J]. *Annu. Rev. Environ. Resour.*, 2004, 29: 205-259.
- Cairns J, Pratt J R. Ecological restoration through behavioral change[J]. *Restoration ecology*, 1995, 3(1): 51-53.
- Diamond J. Reflections on goals and on the relationship between theory and practice[J]. *Restoration ecology: a synthetic approach to ecological research*, 1987: 329-336.
- Douglas I, Lawson N. Material flows due to mining and urbanization[J]. *RU Ayres y LW: Ayres*, eds. *Handbook of Industrial Ecology*, 2002: 351-364.
- Hajer A S, Malibari A A, Al-Zahrani H S, et al. Responses of three tomato cultivars to sea water salinity 1. Effect of salinity on the seedling growth[J]. *African Journal of Biotechnology*, 2006, 5(10).
- Juren J. The points and consideration of ecological remediation [J]. *Chinese water-soil interaction*, 2003 (2): 1-2.
- Kim C M. Effect of saline and alkaline salts on the growth and internal components of selected vegetable plants[J]. *Physiologia Plantarum*, 1958, 11(3): 441-450.
- Kirst G O. Salinity tolerance of eukaryotic marine algae[J]. *Annual review of plant biology*, 1990, 41(1): 21-53.

- McLachlan J. The effect of salinity on growth and chlorophyll content in representative classes of unicellular marine algae[J]. *Canadian Journal of Microbiology*, 1961, 7(3): 399-406.
- Meijer E G M, Brown D C W. Role of exogenous reduced nitrogen and sucrose in rapid high frequency somatic embryogenesis in *Medicago sativa*[J]. *Plant cell, tissue and organ culture*, 1987, 10(1): 11-19.
- Miller J D A, Fogg G E. Studies on the growth of Xanthophyceae in pure culture[J]. *Archives of Microbiology*, 1957, 28(1): 1-17.
- Myers J, Kratz W A. Relations between pigment content and photosynthetic characteristics in a blue-green alga[J]. *The Journal of General Physiology*, 1955, 39(1): 11-22.
- O'riordan T, Jordan A. The precautionary principle in contemporary environmental politics[J]. *Environmental Values*, 1995, 4(3): 191-212.
- Santelices B, Doty M S. A review of *Gracilaria* farming[J]. *Aquaculture*, 1989, 78(2): 95-133.
- Schwarzenbach R P, Egli T, Hofstetter T B, et al. Global water pollution and human health[J]. *Annual Review of Environment and Resources*, 2010, 35: 109-136.
- Stentoft, M. and Farham, W.F. 1997. Northern distribution boundaries and thermal requirements of *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta) in Atlantic Europe and Scandinavia. *Nord. J. Bot.* **5**: 87–93