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Original Article

Resource allocation of *Halimeda macroloba* Decaisne in relation to nitrogen and phosphorus enrichment

Jaruwan Mayakun[1*], Jeong Ha Kim[2], Brian E. Lapointe[3], and Anchana Prathep[1]

[1] *Seaweed and Seagrass Research Unit, Excellence Centre for Biodiversity of Peninsular Thailand, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand*

[2] *Department of Biological Science, Sungkyunkwan University, Suwon, 440-746 Republic of Korea*

[3] *Marine Ecosystem Health Program, Harbor Branch Oceanographic Institute, Florida Atlantic University, Ft. Pierce, Florida, 34946 United States of America*

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Abstract

Responses of *Halimeda macroloba* to growth and chemical concentrations when encountering elevated nutrient concentrations were experimentally tested in the subtidal zone. To determine the effect of nutrients, algae were fertilized using Osmocote® a slow-release fertilizer; the concentrations of nitrogen and phosphorus were enriched. A total of four treatments were manipulated: 1) enriched nitrogen that consisted of fertilizer (16-8-12 [N-P-K]); 2) enriched phosphorus (13-26-7 [N-P-K]); 3) enriched nitrogen and phosphorus (14-14-14 [N-K-P]); and 4) an ambient concentration. It was shown that only phosphorus had a positive effect on the growth and polyphenol concentrations of *Halimeda*. The dry weight and the length of thallus were increased after one week in enriched phosphorus plots but decreased at the third week. Enriched phosphorus resulted in increases in the polyphenol concentrations at the third week; this suggested that when phosphorus was added at the first week, *Halimeda* allocated more resources to growth than chemical concentrations, supporting predictions of the carbon/nutrient balance hypothesis (CNBH). Nitrogen had a negative effect on the growth and polyphenol concentrations of *H. macroloba*. For enriched both nutrients, the N+P had no effect on the dry weight, thallus length, and polyphenol concentrations. From the tissue nutrient, the C:N:P ratios indicated that the productivity of *H. macroloba* was phosphorus limited.

Keywords: resource allocation, chemical concentration, *Halimeda macroloba*, nitrogen, phosphorus

1. Introduction

In several the past decades, nutrient enrichment from natural and anthropogenic sources is a major threat to coral reefs community and has impact on coastal waters worldwide, especially on algal community (Thacker, Ginsburg, & Paul, 2001). Increased nutrient availability can stimulate macroalgal growth, biomass, primary productivity (Fong, Boyer, Kamer, & Boyle, 2003; Lapointe, 1987; Lapointe, Littler, & Littler, 1987; Thacker *et al.*, 2001) that can be a causal factor driving phase shifts on coral reefs (Lapointe, 1997; 1999). Nutrient enrichment may increase the nutritive value of algae for herbivores in terms of either reduced feeding-deterrent compounds or enhanced amounts of primary chemistry products such as carbohydrates and proteins (Honkanen & Jormalainen, 2002). Resource allocation among life history

* Corresponding author.
Email address: jaruwan.may@psu.ac.th

traits and the trade-off between growth and chemical defenses might be affected by nutrient enrichment (Bazzaz, Chiarello, Coley, & Pitelka, 1987; Mayakun, Kim, Lapointe, & Prathep, 2013). The carbon/nutrient balance hypothesis (CNBH) assumes that the plant growth has higher priority than the production of chemical compounds and that excess photosynthate is allocated to the carbon-based chemical compounds when the growth is nutrient limited (Pavia & Toth, 2000) and also states that the production of secondary metabolites is governed by the relative supply of photosynthetically fixed carbon and limiting nutrients.

However, little is known about how algae respond in term of resource allocation to nutrient enrichment and also it is still unclear about which nutrients between nitrogen and phosphorus can affect growth and chemical defense. Hence, in this study, a subtidal algal species, *Halimeda* was chosen according to their chemical defense and the most abundance and widely distribution in the tropical subtidal zones. *Halimeda* has become a dominant component of many reefs in the tropical regions of the Pacific Ocean, Indian Ocean (Beach *et al.*, 2003; Vroom *et al.*, 2003) and especially Thai waters. The experiments were carried out to test the CNBH hypotheses and get the results that give support to the relevance of the CNBH in explaining the effect of nutrient enrichment especially nitrogen and phosphorus on the tropical subtidal marine algae. In addition, the experiments were conducted to answer the following questions: Do elevated nitrogen and phosphorus concentrations influence the *Halimeda*'s resource allocation; insights into growth and chemical concentration and if how?

2. Materials and Methods

The effect of nutrient enrichment was assessed in *Halimeda macroloba* in the shallow waters with 2-3 m depths at Ko Rab, Mu Ko Thale Tai National Park (9° 19′ 20″ N, 99° 46′ 80″ E), Gulf of Thailand, Southern Thailand. The climate of this area is under monsoonal influence as described in Mayakun *et al.* (2013). Each 50 cm x 50 cm plot was permanently marked and placed a maximum of 1 or 2 m apart. 30 young mature plants of *H. macroloba* with 9.4±0.87 cm of mean length were left in each plot.

Nitrogen and phosphorus concentrations were enriched to determine the effects of nutrients. The enrichment could have potentially increased nutrient concentrations about 2 times comparing with the ambient treatment (Mayakun *et al.*, 2013). A total of 12 different treatments were manipulated (n=3 for each treatment): 1) enriched nitrogen that consisted of fertilizer (16-8-12 [N-P-K]); 2) enriched phosphorus (13-26-7 [N-P-K]); 3) enriched nitrogen and phosphorus (14-14-14 [N-K-P]); 4) an ambient concentration. In the enriched plots, the plots were fertilized using 60 g of Osmocote® a slow-release fertilizer; fertilizer were enclosed in three mesh bags and placed in the center and at two diagonal corners as described in Mayakun *et al.* (2013).

Thalli of *H. macroloba* were collected at two fixed sampling times, after 7 and 21 days of each experiment, the samples were returned to the laboratory for the tissue nutrient (C:N:P ratio) analysis and the growth measurements including biomass, the maximum length, the width of the base of the stipe, number of blades or segments, holdfast and stipe diameter, and the chemical concentrations. To measure the nutrient uptake ability, the tissue nutrient concentrations (carbon, nitrogen, and phosphorus content) in the vegetative tissues of algae were measured. The assessment has been described in Mayakun *et al.* (2013). The dried samples were analyzed for C:N:P ratios at the Central Analytical Center, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand.

To determine polyphenol concentrations, the algae were collected from each plot and returned to the laboratory under dark and cold conditions. Algae were frozen in -85°C until used. The polyphenol concentrations were measured using a modified Folin-Ciocalteu Method (Folin & Ciocalteu, 1927) for quantification of the total polyphenol concentration and the assay was described in Mayakun *et al.* (2013). The absorbance of the samples was read at 725 nm. Phloroglucinol (1, 3, 5-trihydroxybenzene) was used as a standard.

3. Statistical Analyses

A 2-way ANOVA was used to examine the effects of the fixed factors: nutrient treatments and sampling times on the growth and chemical defense concentration. Cochran's *C*-test was used to determine the homogeneity of variances. In the case of a violation, a log (x+1) transformation was made prior to analysis. Differences among the mean effects of nutrients at each sampling time were tested with *t*-test for the independent samples. All statistical analyses were made using the computer program SPSS for Windows version 11.5.

4. Results

Enriched phosphorus had significantly affected the thallus length and dry weight of *Halimeda macroloba* (p= 0.032, p=0.029; Table 1) whereas enriched nitrogen and enriched both nitrogen and phosphorus did not (p>0.05). At 1 week (wk) of phosphorus enrichment, *Halimeda* increased the biomass and had longer length, 1.33±0.06 g and 10.0± 0.41 cm, respectively, compared to the ambient, 1.16±0.20 g and 8.87±0.67 cm, respectively (*t*-test; p=0.008, p=0.014, respectively, Figure 1 A-B). However, this trend continued at the 1 wk of the measurement but the trend reversed at the 3 wk. When nitrogen was enriched, the dry weight and the thallus length did decrease through time. For enriched both nutrients, the N+P had no or negative effect on the dry weight and thallus length (Figure 1 A-B). The tissue levels (% dry weight) of *H. macroloba*, the initial value of the C:P and N:P increased. C:N, C:P, and N:P ratios of all treatments varied through time. However, only C:N:P of *Halimeda* in

Table 1. Effect of nutrient on the thallus length and dry weight of *Halimeda macroloba*.

Species	*Halimeda macroloba*							
		Thallus length				Dry weight of thallus		
Source	df	MS	*F*	*P*	df	MS	*F*	*P*
Time	2	0.276	7.264	0.01	2	1.824	1.767	0.167
Nutrient	3	0.132	3.483	0.032	3	0.604	1.452	0.029
Time x nutrient	6	0.080	2.102	0.227	6	0.571	1.373	0.377
Error	23	0.038			23	0.416		
Total	35				35			

Table 2. Tissue levels (% dry wt.) of C:N, C:P, and N:P of *Halimeda macroloba* (Values in parentheses = SE, n=3).

Species	*Halimeda macroloba*											
Tissue nutrient	C:N				C:P				N:P			
Times/treatments	Am	N	P	N+P	Am	N	P	N+P	Am	N	P	N+P
Week 1	17.4	18.0	17.4	17.5	509	629	599	580	29.2	34.7	34.4	32.8
	(0.86)	(1.08)	(0.86)	(0.52)	(104)	(138)	(198)	(169)	(5.35)	(6.97)	(9.21)	(11.4)
Week 3	16.6	17.9	18.5	16.6	774	594	643	516	46.5	32.1	34.8	31.2
	(0.59)	(2.66)	(0.29)	(1.03)	(96.1)	(162)	(114)	(106)	(4.35)	(3.97)	(5.65)	(6.46)

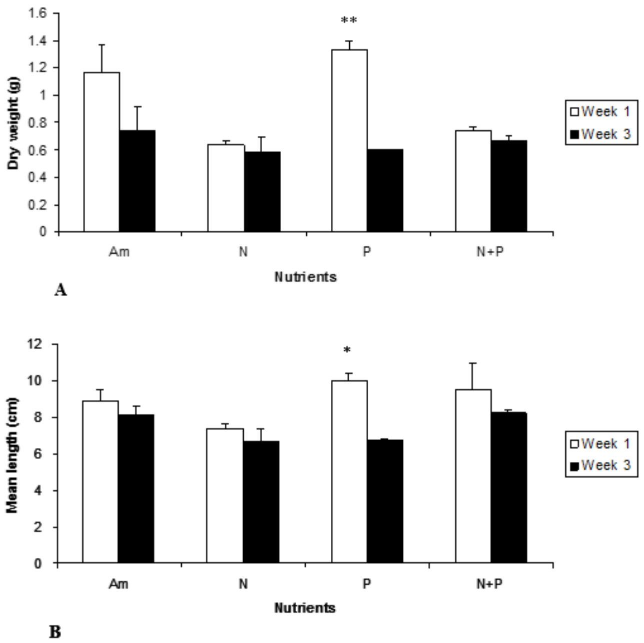

Figure 1. Effect of N, P, and N+P enrichment on dry weight (A) and length of thallus (B) of *Halimeda macroloba* compared to ambient. Data are means ± SE of three replicates of each treatment and sampling time. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

the enriched phosphorus plots increased (Table 2).

For the polyphenol concentrations, nutrients had effect on polyphenols ($p=0.001$) and varied through time ($p=0.000$), showing the interaction between time x nutrient ($p=0.023$; Table 3). The trend of polyphenol concentrations remained the same compared to ambient at 1 wk, ranging from 16.0±1.44 to 19.9±2.69 µg/ml but polyphenols decreased in enriched N, 23.0±0.92 µg/ml and N+P, 24.9±0.55 µg/ml, treatments at the 3 wk compared to the ambient treatment,

Table 3. Effect of nutrient on polyphenol concentrations of *Halimeda macroloba*.

Source	df	*F*	*P*
time	1	78.241	0.000
nutrient	3	6.029	0.001
time x nutrient	3	3.402	0.023
Error	64		
Total	72		

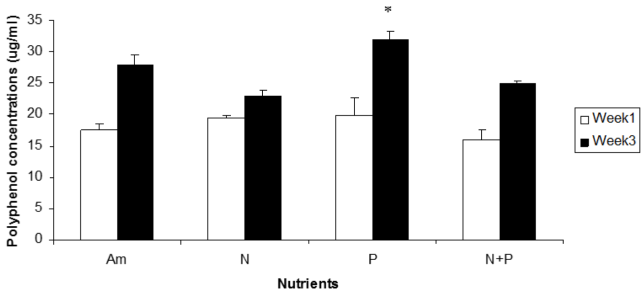

Figure 2. Effect of N, P, and N+P enrichment on polyphenol concentrations of *Halimeda macroloba* compared to ambient. Data are means ± SE of three replicates of each treatment and sampling time. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

28.0±1.41μg/ml (Figure 2). Enriched phosphorus did affect the polyphenol concentrations, indicating the concentrations at 3 wk, 31.9±1.31 μg/ml had greatly higher than the concentrations in ambient, 28.0±1.41μg/ml (p=0.016; Figure 2).

5. Discussion

Of the three nutrient enrichment conditions, only phosphorus had a positive effect on the growth and polyphenol concentrations of *Halimeda*. Enriched phosphorus increased the dry weight and length of thallus at the 1 wk and decreased at the 3 wk. Enriched phosphorus resulted in increases in polyphenol concentrations at the 3 wk; this suggested that when phosphorus was added at the 1 wk, *Halimeda* allocated more resources to the growth than chemical concentrations, supporting the predictions of the carbon/nutrient balance hypothesis (CNBH). Nutrients affected the resource allocation among the important life-history traits such as growth, reproduction and defenses (Pfister & Van Alstyne, 2003) and modify the trade-off between growth and defenses. When nutrient are enriched, the allocation to defense will be cost in terms of growth thus growth increased and defense decreased (Jormalainen & Honkanen, 2004; Yates & Peckol, 1993).

In this study, the growth is enhanced by only phosphorus addition and N:P ratio was higher than 24, so it suggested that phosphorus is nutrient limiting the productivity of *H. macroloba* (Menéndez, Herrera, & Comín, 2002). This result was similar to the work in Bahamas where phosphorus was nutrient limitation for psammophytic species, *H. tuna* and *H. simulans* (Littler, Littler, & Lapointe, 1988).

However, in this study, nitrogen had no positive effect on the growth and polyphenol concentrations of *H. macroloba*. It contrasts to the studies of Van Alstyne and Pelletreau (2000), testing the nutrient enrichment on the growth and phlorotannin production in *Fucus gardneri* embryos. They found that the nitrogen enrichment significantly increased the embryo growth rates and decreased embryo phlorotannin concentrations. For the phosphorus enrichment, phosphorus had no effect on growth but decreased phlorotannin concentrations (Van Alstyne & Pelletreau, 2000). Also, Delgado and Lapointe (1994) indicated that nitrogen was the nutrient limiting the productivity of calcareous alga, *H. opuntia*. Thus, in this study site, nitrogen is not the nutrient limiting factor of productivity of *H. macroloba*. Enriched both N+P had no or negative effect on dry weight, length of thallus, and polyphenol concentrations. This result is similar to Littler *et al.* (1988), indicating that the treatment of $N+P_i$ pulses inhibited net maximum photosynthesis of the rock growers, *H. lacrimosa* and *H. copiosa*. However, the effects of nitrogen and the combination of nitrogen and phosphorus on growth and secondary metabolites were difficult to explain and not clear.

From the previous studies of nutrient effects on *Halimeda*, there are still controversies about which nutrient limitation is; there are different nutrient limitation among two form groups of *Halimeda*, psammophytic and epilithic groups. Littler *et al.* (1988) suggested that phosphorus is nutrient limiting productivity of psammophytic *Halimeda* species, *H. tuna* and *H. simulans* whereas the productivity of epilithic species, *H. lacrimosa* and *H. copiosa*, is nitrogen limited.

In conclusion, only phosphorus had positively effect on the growth and polyphenol concentrations of *H. macroloba* and the productivity of *H. macroloba* is phosphorus limited in this study. When phosphorus was added, *Halimeda* allocated more resources to growth than secondary metabolites, supporting some predictions of the carbon/nutrient balance hypothesis (CNBH).

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