Botanica Marina
Vol. 33, pp. 375–383, 1990

Offprint

Effects of Nutrients on the Photosynthesis of *Sargassum thunbergii*

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(Accepted 7 April 1990)

Abstract

Sargassum thunbergii plants growing on a coast facing the open sea showed NO_3^-- and PO_4^{3-}-limited photosynthetic activity. NO_3^-- and PO_4^{3-}-limited plants showed little improvement in photosynthetic rate with single NO_3^-- or PO_4^{3-}-enrichment. The plants showed increased photosynthetic activity correlated with increased chlorophyll content on a fresh weight basis during incubation in NO_3^-- and PO_4^{3-}-enriched seawater for 3 to 7 days. Such increases in photosynthesis and chlorophyll content were related to increases in the size of the pools for NO_3^- and PO_4^{3-} in the tissues. *Sargassum thunbergii* plants from an inner bay, the coastal water of which is rich in nitrate and phosphate, showed no increase in photosynthetic capacity during incubation in NO_3^-- and PO_4^{3-}-enriched seawater.

Introduction

Seaweeds play an important role in the coastal ecosystem through their photosynthetic production. Estimations of the photosynthetic production of seaweeds are usually based on the relationships of photosynthesis and respiration to light and temperature. Little attention has been paid to nutrient conditions in the sea for estimation of the photosynthetic production or for comparison of photosynthetic capacity among different species of seaweeds. Chapman *et al.* (1978) reported that the photosynthetic capacity and chlorophyll content of *Laminaria saccharina* Lamour. sporophytes increased with the increase of external NO_3^- concentrations in laboratory culture. In the field, however, little is known (Zavodnik 1987) regarding specific species of seaweeds growing in coastal waters of different nutrient levels.

Nitrate and phosphate concentrations in the sea usually show seasonal variation (e. g. Chapman and Craigie 1977, Hanisak 1979, Wheeler and Srivastava 1984, Germann *et al.* 1987) and are usually higher in deeper water than in shallower water, and in coastal water of bays compared with coastal waters of the open sea. Different nutrient conditions in the sea can be expected to affect photosynthesis of a seaweed to different degrees. It is ecologically and physiologically important to know, to what degree, and how, photosynthesis of a seaweed may be affected by nutrients. Lapointe (1986) reported that the midday net photosynthetic rate of pelagic *Sargassum* species, *S. natans* (L.) J. Meyen and *S. fluitans* Børgensen, were two-fold higher with PO_4^{3-}-enrichment compared with NO_3^-, NH_4^+ and control treatments.

However, little is known about nutrient effects on photosynthesis in coastal *Sargassum* species. The present study examines the differences in photosynthesis observed in *Sargassum thunbergii* (Mertens *ex* Roth) O. Kuntze plants growing in a nutrient-rich bay and a nutrient-poor coastal water facing the open sea.

Material and Methods

Samples of *Sargassum thunbergii* (Mertens *ex* Roth) O. Kuntze were collected from the intertidal zone from two localities, Maizuru Bay and Takahama (Fig. 1), the coastal water of the former is rich in nitrate

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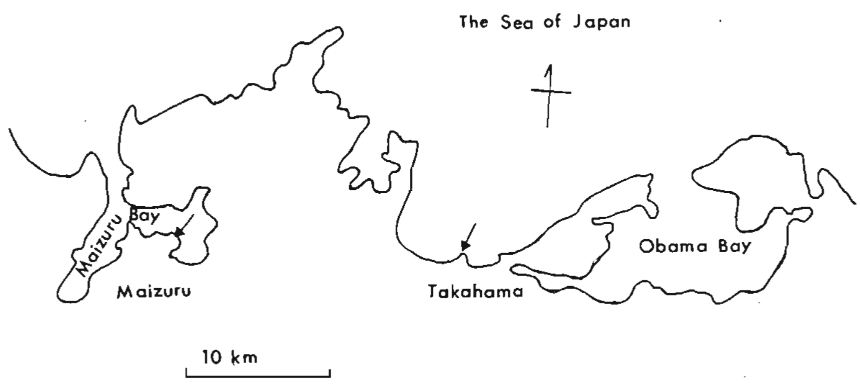

Fig. 1. Locations of sampling areas indicated by the arrows.

and phosphate (Nakahara 1978) and the latter is facing the open sea. Seawater samples were also collected at the same localities as the *S. thunbergii* plants, and stored at −20 °C for later nitrate and phosphate analyses. Following collection, the algae were cleaned of obvious epiphytes and the apical tips of main branches weighing 0.2 to 0.4 g were removed (Experiment II). Oceanic seawater with undetectable nitrate and phosphate was used to prepare the culture media. For the incubations, juveniles or apical tips were maintained in 5 L of medium in aerated 8 L glass jars kept in a flow-through seawater box under natural sunlight.

Incubations with various enrichments (Experiment I)

The effects of nitrate, phosphate and combinations of both on photosynthesis were first investigated with juvenile plants (about 3 cm long) collected from the coast of Takahama. The juveniles were incubated in NO_3^-- and PO_4^{3-}-enriched (N+P-enriched), NO_3^--(N-)enriched, PO_4^{3-}-(P-)enriched and non-enriched (0) seawater for 4 days (26−30 April). The concentrations of nitrate ($NaNO_3$) and phosphate (K_2HPO_4) in the enriched seawater were 150 μM and 15 μM, respectively. Water temperature in the jars varied with that in the sea within a range of 15 to 18 °C during incubation.

Net photosynthesis was measured every day for the same five samples in each jar. It took about 30 minutes to carry out the measurements. As soon as the measurements had been finished the samples were returned immediately to their jars under natural sunlight. Seawater (10 ml) was sampled from the glass jars every day in the morning (9:00) and in the evening (17:00) during incubation and stored at −20 °C for later analyses for nitrate and phosphate. Dark respiration and chlorophyll content were measured at the beginning and at the end of the incubation.

Simultaneous incubation of plants from Takahama and Maizuru Bay in NO_3^-- and PO_4^{3-}-enriched seawater (Experiment II)

Apical tips of plants from the coast of Takahama and Maizuru Bay were simultaneously incubated in separate glass jars in N+P-enriched (100 μM-NO_3^-, 10 μM-PO_4^{3-}) seawater. The jars were maintained in the flow-through seawater box under natural sunlight for 7 days (25 May−1 June). Water temperature in the jars varied with that in the sea within a range of 18 to 20 °C during the incubation. On the third day (28 May) of the incubation, photosynthetic, dark respiratory rates and chlorophyll contents were measured, and a number of samples were used. The remaining samples were continuously incubated in renewed enriched seawater. On the last day of the incubation period, photosynthesis, dark respiration and chlorophyll contents were measured again. Seawater (10 mL) was sampled from the glass jars every day in the morning (9:00) and in the evening (17:00) during incubation and stored at −20 °C for later analyses for nitrate and phosphate.

Photosynthesis and respiration measurements

Photosynthesis and respiration were measured in the laboratory as reported by Yokohama *et al.* (1986) with a differential gas-volumeter 'Productmeter' (Nikko Kagaku Ltd.), equipped with eight pairs of reaction and compensation vessels kept in a water bath fitted with a motor to shake the vessels. Light was supplied by incandescent lamps (National 'high beam balls', 110 V, 150 W). Dark respiration was measured by completely covering the reaction vessel with opaque plastic. The fresh weight of the samples was measured after blotting to remove water from the surface of the thallus with tissue paper. The photosynthetically active radiation (PAR, 400−700 nm) of the light sourse was measured with an underwater quantum sensor (LI-COR, LI-192S) linked with a recorder (Toa Electronics Ltd., FBR-253A). Water temperature in the water bath was controlled using a Taiyo Coonit (CL-30).

Photosynthetic measurements were carried out in the morning in order to avoid differences caused by diurnal photosynthetic variation under sunlight in *Sargassum thunbergii* (Gao and Umezaki 1989 b, c).

On a basis that photosynthesis of *Sargassum thunbergii* was saturated at about 300 $\mu E\ m^{-2}\ s^{-1}$ (Gao and Umezaki 1988, 1989 a), light-saturated photosynthesis was measured at 600 $\mu E\ m^{-2}\ s^{-1}$ in the present experiments.

Determination of chlorophyll content

Chlorophylls were determined by freezing samples at −20 °C, grinding in a mortar with quartz sand, extracting with 90% acetone, and filtering through absorbent cotton with 90% acetone. The absorbances of the acetone extract were measured at 750, 664, 630 nm with a spectrophotometer (Hitachi Ltd., Model 100-2). The concentrations of chlorphylls *a* and *c* were calculated using the formulae of Jeffrey and Humphrey (1975).

Nitrate and phosphate analysis

Nitrate and phosphate concentrations were determined with a Technicon AutoAnalyzer II (Technicon Ltd.).

For tissue nitrate and phosphate measurements, the materials were treated with 2 ml of 6% trichloroacetic acid solution and immediately ground with a tissue grinder. This extract was made up to 100 ml with filtered seawater (Whatman, GF/C) with no detectable nitrate and phosphate and then was neutralized with 4N NaOH to about pH 8. The neutralized extract was filtered through a glass microfibre filter (Whatman GF/B). These samples were stored at −20 °C for later analyses.

Growth rate calculation

Relative growth rate (RGR, %) was calculated using the following formula:

$$RGR = \frac{\ln W2 - \ln W1}{T} \times 100,$$

where W1 represents the initial fresh weight of the plant; W2, the fresh weight of the plant after T number of days.

Statistical analysis

Differences between means reported in 'Results' were compared using Student's *t*-test to ascertain statistical significance.

Results

Comparisons between plants from Takahama and Maizuru Bay

The plants from the two localities were different in colour, with the plants from the coast of Takahama being yellowish and those of Maizuru Bay being blackish. Tissue-NO_3^-, tissue-PO_4^{3-} and chlorophyll *a* and *c* contents of the plants from Takahama were much lower than in those from Maizuru Bay (Fig. 2).

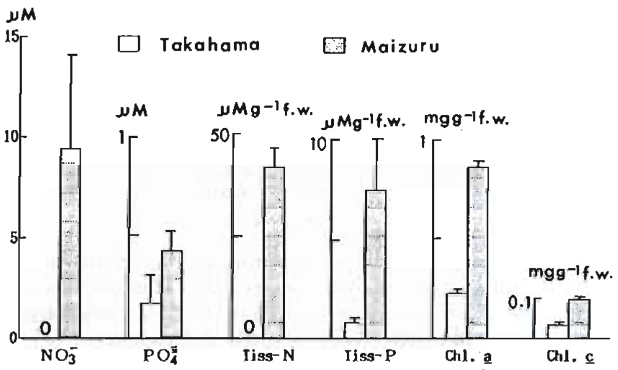

Fig. 2. Comparisons of NO_3^- and PO_4^{3-} concentrations of the coastal water, tissue NO_3^- (Tiss-N), tissue PO_4^{3-} (Tiss-P), chlorophyll (chl.) *a* and *c* contents of *Sargassum thunbergii* plants from Maizuru Bay and Takahama. Means ± SD of 3 to 5 samples.

The chlorophyll *c* to *a* ratio was bout 30% higher in the plants from Takahama than those from Maizuru Bay. *i.e.* chlorophyll *c* is more concentrated in the plants with lower chlorophyll contents. Nitrate concentration was nearly zero (undetectable) in seawater from Takahama and about 9.5 μM in seawater from Maizuru Bay; and phosphate concentrations in the seawater was about 0.2 μM at the former and about 0.4 μM at the latter (Fig. 2). The different nitrate and phosphate concentrations in seawater probably contributed to the differences in tissue-NO_3^-, tissue-PO_4^{3-} and chlorphyll *a* and *c* contents in the plants from the two localities. These differences would be responsible for the differences in photosynthetic capacity of the plants from the two sites.

Figure 3 shows comparisons of the photosynthetic and dark respiratory rates of *Sargassum thunbergii* plants from Takahama and Maizuru Bay. The net photosynthetic rates at 600 and 100 $\mu E\ m^{-2}\ s^{-1}$ of the plants from Maizuru Bay were respectively about 2.7 and 2.3 times higher than those from Takahama

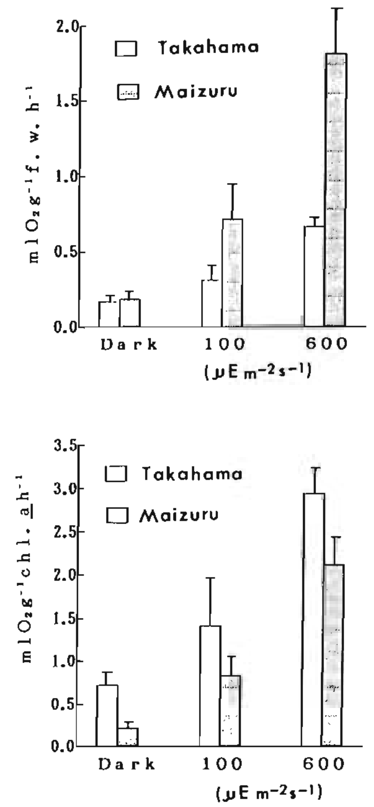

Fig. 3. Comparisons of dark respiratory and net photosynthetic rates in *Sargassum thunbergii* plants from Maizuru Bay and Takahama. A, on a fresh weight basis; B, on a chlorophyll *a* basis. Measured at 20 °C. Mean ± SD of 5 samples.

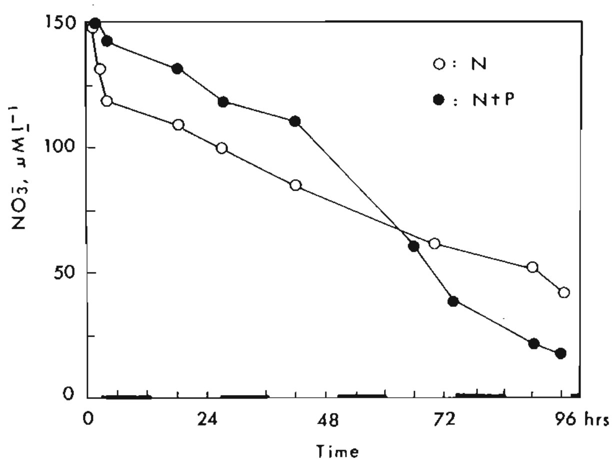

Fig. 4. Changes of NO_3^- in seawater during incubation of *Sargassum thunbergii* plants from Takahama in NO_3^--enriched, and NO_3^-- and PO_4^{3-}-enriched seawater. Black bars indicate night time.

on a fresh weight basis (Fig. 3A). However, dark respiration rates on a fresh weight basis were similar in the plants from Maizuru Bay and Takahama (Fig. 3A). On a chlorophyll *a* basis (Fig. 3B), net photosynthetic rates at 600 and 100 μE m^{-2} s^{-1} and dark respiratory rates of the plants from Takahama were about 1.4, 1.7 and 2.3 times higher than those from Maizuru Bay, respectively. The differences in net photosynthesis both on a chlorophyll *a* basis and on a fresh weight basis were significant ($P < 0.01$).

The plants growing on the coast of Takahama showed lower values in net photosynthesis and chlorophyll contents on a fresh weight basis but higher net photosynthetic rates on a chlorophyll *a* basis than those growing on the coast of Maizuru Bay. The differences in net photosynthetic rate on a fresh weight basis were much greater than those on a chlorophyll *a* basis.

Effects of nitrate, phosphate and the combination of both on photosynthesis in plants from Takahama

Nitrate in seawater was absorbed by juvenile plants from Takahama in N-enriched and N+P-enriched seawater, and exhausted after 4 days treatment (Fig. 4). The weight of the samples increased 2.2% to 3.0% per day during incubation in N-, P-, N+P- and non-enriched seawater. No statistically significant difference ($P > 0.1$) in the relative growth rate of *S. thunbergii* juvenile plants was found between the incubations (Table I). The average relative growth rate was highest with N+P-enrichment compared with other treatments.

Table I. Relative growth rates (RGR, %day^{-1}) of *Sargassum thunbergii* juvenile plants from Takahama during incubation in NO_3^-- (N), PO_4^{3-}- (P) and $NO_3^- + PO_4^{3-}$- (N+P) enriched and non-enriched (0) seawater.

(n = 5)	0	N	P	N+P
X̄	2.48	2.21	3.16	3.00
± SD	± 0.34	± 0.47	± 1.29	± 0.76

The light-saturated net photosynthetic rate showed no statistically significant changes during incubation in N-enriched or P-enriched seawater compared with that in non-enriched seawater (Table II). The light-saturated net photosynthetic rate increased from the third day with N+P-enrichment, and showed significant ($P < 0.05$) increase on third and fourth days compared to that without enrichment. Comparing average values, the light-saturated net photosynthetic rate at the end of the incubation was in the following rank order from the highest to the lowest: N+P-enriched, N-enriched, P-enriched and non-enriched seawater incubations. The net photosynthetic rates were finally reduced in non-enriched and P-enriched seawater ($P < 0.05$) but not in N-enriched seawater ($P > 0.05$) compared with the rates at the beginning of incubation.

Table II. Comparisons of net photosynthesis and dark respiratory rates (ml $O_2 g^{-1}$ f. w. h^{-1}) of *Sargassum thunbergii* juvenile plants from Takahama before and after incubation in NO_3^-- (N), PO_4^{3-}- (P) and $NO_3^- + PO_4^{3-}$- (N+P) enriched, and non-enriched (0) seawater. Photosynthesis was measured at 15 °C every day during incubation (26—30 April 1988). The data are the means ± SD of 5 samples.

Date	Before incubation	0	N	P	N+P
26	0.429 ± 0.061 (0.083 ± 0.038)*				
27		0.342 ± 0.099	0.373 ± 0.133	0.357 ± 0.104	0.358 ± 0.055
28		0.314 ± 0.102	0.369 ± 0.090	0.362 ± 0.096	0.385 ± 0.076
29		0.336 ± 0.106	0.404 ± 0.111	0.398 ± 0.063	0.504 ± 0.074
30		0.294 ± 0.078 (0.097 ± 0.029)*	0.407 ± 0.148 (0.084 ± 0.022)*	0.331 ± 0.054 (0.091 ± 0.017)*	0.527 ± 0.077 (0.175 ± 0.039)*

* Dark respiratory rates.

Table III. Comparisons of chlorophyll content (mg^{-1} f. w.) of *Sargassum thunbergii* juvenile plants from Takahama before and after incubation in NO_3^-- (N), PO_4^{3-}- (P) and $NO_3^- + PO_4^{3-}$- (N+P) enriched, and non-enriched (0) seawater. The data are the means ± SD of 4 samples.

	Before incubation	After incubation of 4 days			
		0	N	P	N+P
Chl. *a*	0.240 ± 0.039	0.162 ± 0.001	0.233 ± 0.059	0.213 ± 0.016	0.353 ± 0.038
Chl. *c*	0.036 ± 0.003	0.026 ± 0.003	0.034 ± 0.006	0.027 ± 0.002	0.051 ± 0.001
Chl. *c*:*a*	0.150 ± 0.013	0.158 ± 0.021	0.148 ± 0.013	0.125 ± 0.001	0.144 ± 0.014

Little difference ($P > 0.5$) was found in the dark respiration at the beginning and at the end of the incubation in non-enriched, N-enriched and P-enriched seawater. However, the dark respiratory rate was more than twice ($P < 0.01$) as high at the end as at the beginning of the incubation in N+P-enriched seawater (Table II).

Chlorophyll *a* and *c* contents at the end of the incubation were much higher ($P < 0.01$) in N+P-enriched seawater, but lower ($P < 0.05$) in non-enriched seawater, as compared with those at the start of incubation (Table III). Chlorophyll *a* contents at the end of incubations in P-enriched and N-enriched seawater were little different ($P > 0.2$) from those at the beginning of incubation. The chlorophyll *c* to *a* ratio was the lowest in P-enriched seawater and highest in non-enriched seawater at the end of incubation.

Comparisons in plants from Takahama and Maizuru Bay during incubation in N+P-enriched seawater

Figure 5 shows the decreases of NO_3^- in seawater with the samples from Takahama and Maizuru Bay. In the first 3-day incubation, NO_3^- was exhausted about 30 and 60 hours after the start of incubation with the samples from Takahama and Maizuru Bay, respectively. In the second 3-day incubation, NO_3^- was exhausted about 50 hours after the change of enriched seawater. The PO_4^{3-} also decreased with time in incubations with the samples from Takahama and Maizuru Bay (Fig. 6). Both the first and second 3-day incubations, PO_4^{3-} was exhausted about 50 hours after

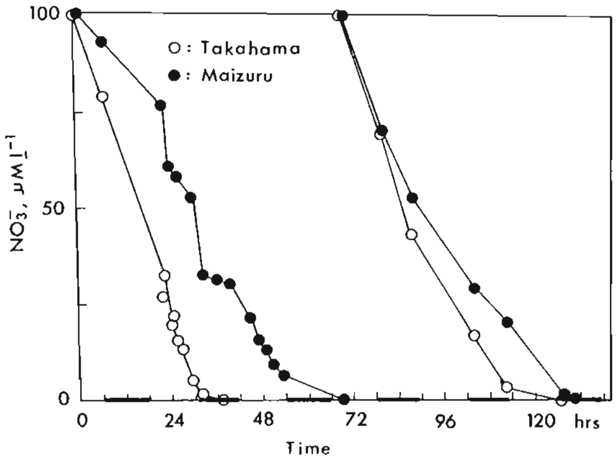

Fig. 5. Changes of NO_3^- in seawater during incubation of *Sargassum thunbergii* plants from Takahama and Maizuru Bay in NO_3^-- and PO_4^{3-}-enriched seawater. The enriched seawater was changed on the third day. Black bars indicate night time.

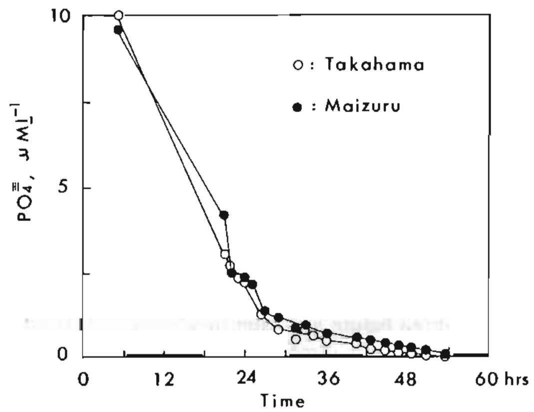

Fig. 6. Changes of PO_4^{3-} in seawater during incubation of *Sargassum thunbergii* plants from Takahama and Maizuru Bay in NO_3^-- and PO_4^{3-}-enriched seawater. Black bars indicate night time.

Table IV. Comparisons of relative growth rates (RGR) of *Sargassum thunbergii* plants from Takahama and Maizuru Bay during the incubation.

	RGR (%day^{-1})	Sample (n)
Takahama	3.2 ± 2.0	11
Maizuru Bay	11.1 ± 1.7	13

the beginning of the incubation with the samples from Takahama or Maizuru Bay.

Table IV indicates the relative growth rates (RGR) of the samples from Takahama and Maizuru Bay when incubated in P+N-enriched seawater. The RGR of the samples from Maizuru Bay was more than 3 times ($P < 0.01$) higher than that of the samples from Takahama.

Light-saturated net photosynthetic rates on a fresh weight basis of the plants from Takahama increased ($P < 0.05$) during incubation (Fig. 7A). However, little difference ($P > 0.5$) was found in the dark respiration rate at the start and at the end of the incubation. The light-saturated net photosynthetic rate, on a chlorophyll *a* basis, did not change significantly ($P > 0.2$) during the incubation.

For the plants from Maizuru Bay (Fig. 7B), the light-saturated net photosynthetic rate on a fresh weight basis decreased to about 60% and 50% of that at the beginning of the incubation after 3 days and 7 days incubation, respectively. Such decrease was significant ($P < 0.001$). Little difference ($P > 0.1$) was found in the dark respiratory rate before and after incubation. Light-saturated net photosynthetic rate on a chlorophyll *a* basis decreased to 67% of that at the beginning of incubation after 3 days and 7 days of incubation. Such a decrease was also significant ($P < 0.05$).

Chlorophyll contents of the samples from Takahama increased significantly ($P < 0.01$) at the end of 7 days incubation (Table V). Chlorophyll *a* and *c* contents were respectively about 32% and 29% higher at the end than at the beginning of the incubation. Little difference ($P > 0.5$) was found in chlorophyll *c* to *a* ratios at the beginning and at the end of the incubation.

As for the samples from Maizuru Bay, chlorophyll *a* and *c* contents after 3 days of incubation were not significantly ($P > 0.3$) different from those before incubation, although the average was a little lower (Table V). Chlorophyll *a* and *c* contents after 7 days incubation decreased to about 74% and 93% of those at the beginning of incubation, respectively. The decreases in chlorophyll *a* and *c* contents were significant ($P < 0.05$). The chlorophyll *c* to *a* ratio increased significantly ($P < 0.01$) after 7 days of incubation.

It is likely that increased chlorophyll contents in the plants from Takahama were contributing to the increase in the net photosynthetic rate on a fresh weight basis, when incubated in N+P-enriched seawater. The N+P-enriched seawater did not bring about any increase in photosynthesis of the plants from Maizuru Bay.

The NO_3^- and PO_4^{3-} contents in tissues of *S. thunbergii* from Takahama and Maizuru Bay are shown in Table VI. At the beginning of the incubation, both PO_4^{3-} and NO_3^- contents in the plants from Maizuru Bay were much higher than those from Takahama. The NO_3^- and PO_4^{3-} contents in the plants from Takahama increased remarkably during incubation, and came closer to those of the plants from Maizuru Bay. The PO_4^{3-} contents increased but, in spite of absorption of NO_3^- in the media, NO_3^- contents decreased during incubation in the plants from Maizuru Bay.

Table V. Chlorophyll contents (mg g^{-1} f. w.) of the apical tips of *Sargassum thunbergii* plants from Takahama and Maizuru Bay before and after incubation in NO_3^-- and PO_4^{3-}-enriched seawater for 3 (25–28 May 1988) and 7 days (25 May–1 June 1988). The data are the means ± SD of 5 samples.

	Before incubation			After 3 days			After 7 days		
	Chl. *a*	Chl. *c*	Chl. *c*:*a*	Chl. *a*	Chl. *c*	Chl. *c*:*a*	Chl. *a*	Chl. *c*	Chl. *c*:*a*
Takahama	0.227 ± 0.021	0.035 ± 0.004	0.154 ± 0.010	0.240 ± 0.041	0.037 ± 0.008	0.152 ± 0.008	0.299 ± 0.014	0.045 ± 0.003	0.151 ± 0.008
Maizuru Bay	0.855 ± 0.032	0.099 ± 0.006	0.116 ± 0.004	0.795 ± 0.112	0.099 ± 0.017	0.124 ± 0.008	0.635 ± 0.048	0.088 ± 0.007	0.135 ± 0.006

Table VI. NO_3^- (N) and PO_4^{3-} (P) contents (μM g^{-1} f. w.) in tissues of *Sargassum thunbergii* plants from Maizuru Bay and Takahama. Measured before and after incubation (25 May–1 June 1988) in NO_3^- and PO_4^{3-}-enriched seawater.

	P		N	
	Maizuru	Takahama	Maizuru	Takahama
Before	7.42 ± 2.65 (n = 3)	0.79 ± 0.37 (n = 5)	42.75 ± 4.45 (n = 2)	0.00 ± 0.00 (n = 4)
After	13.36 ± 5.34 (n = 6)	7.63 ± 2.13 (n = 7)	26.43 ± 3.66 (6 = 6)	11.78 ± 6.71 (n = 7)

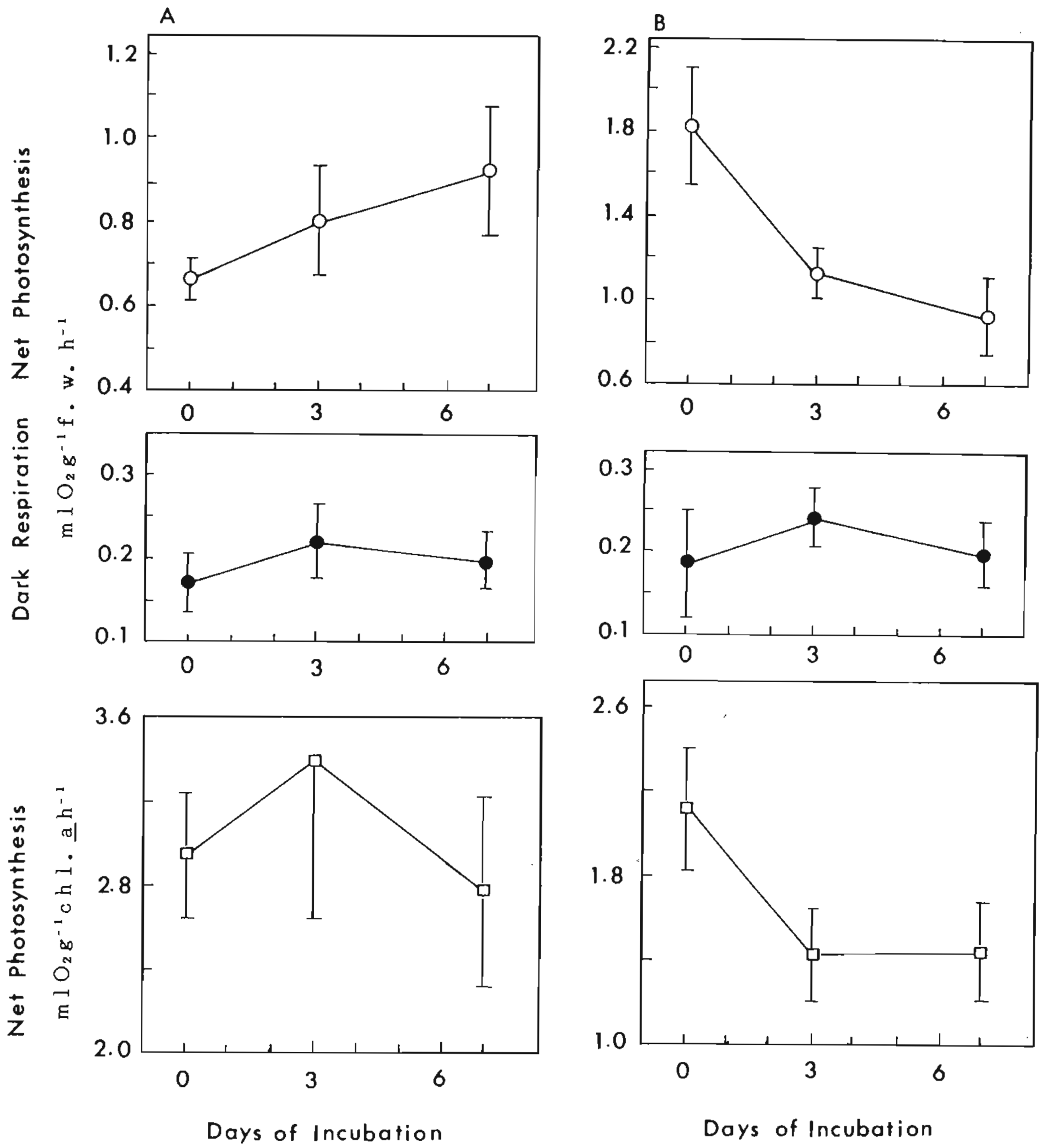

Fig. 7. Changes with time in net photosynthesis and dark respiration of *Sargassum thunbergii* plants from Takahama (A) and Maizuru Bay (B) during incubation in NO_3^-- and PO_4^{3-}-enriched seawater. Measured at 20 °C and 600 μE m^{-2} s^{-1}. Means ± SD of 5 samples.

Discussion

In the sea, nitrate and phosphate concentrations differ in different coastal waters. Coastal water with more runoff from land can hold more nitrogen and phosphorus (Nakahara 1978, Wallentinus 1988). Zavodnik (1987) reported that *Ulva rigida* C. Ag. and *Porphyra leucosticta* Thuret exhibited higher photosynthetic rates and pigment content in the plants from a nutrient-rich coastal water compared to those from nutrient-poor places. The photosynthetic capacity of *Sargassum thunbergii* has proved to be dependent on the availability of nitrate and phosphate. Consequently, coastal waters, according to their levels of nitrate and phosphate concentrations, may give significant differences in photosynthetic productivity of seaweeds.

Nitrate and phosphate concentrations in the sea usually vary according seasons being lowest in summer and highest in winter. Chapman and Craigie (1977) with *Laminaria longicruris* De la Pylaie, and Wheeler and Srivastava (1984) with *Macrocystis integrifolia* Bory, reported that the NO_3^- concentration in tissues varied with fluctuations in nitrate concentration in the sea throughout the year. In studies on *Codium* *fragile* ssp. *tomentosoides* (van Goor) Silva, Hanisak (1979) reported that the internal nitrogen content was minimal in summer and maximal in winter. The NO_3^- or PO_4^{3-} content in tissues of *Sargassum thunbergii* can be expected to vary with seasonal variations in nitrate or phosphate concentrations in the sea. Therefore, seasonal variation of photosynthetic capacity of this species can be expected to be correlated with the seasonal variation of nutrient conditions in the sea.

Chapman *et al.* (1978) reported the dependence of photosynthetic capacity and chlorophyll content of *Laminaria saccharina* Lamour. on the external NO_3^- concentrations in laboratory experiments. They used a modified medium based on the ES of Provasoli (1968) to carry out their cultures in a flow-through system in the laboratory and showed that light-saturated gross photosynthesis of *L. saccharina* increased by about 50% and 65% at NO_3^- concentrations of 10 μM and 500 μM NO_3^-, respectively, as compared with that with no NO_3^- in culture for 6 days. The present study indicated the significant dependence of the photosynthetic capacity of *Sargassum thunbergii* on the nitrate and phosphate concentrations in seawater. Light-saturated gross photosynthesis of *S. thunbergii* plants growing in NO_3^-- and PO_4^{3-}-limited coastal water of Takahama increased by 20–40% after incubation under natural sunlight in NO_3^-- and PO_4^{3-}-enriched seawater for 3–7 days. Although the culture conditions of the present study were different from those of Chapman *et al.* (1978), the dependence of photosynthetic capacity and chlorophyll content of *S. thunbergii* on the external NO_3^- (with PO_4^{3-} supply) is in agreement with that found for *L. saccharina*.

Schmitt and Adams (1981) reported that the photosynthesis (at 1000 μE m^{-2} s^{-1}) of the freshwater macrophyte *Myriophyllum spicatum* L. increased with increase in the internal phosphorus concentration, and a Michealis-Menten relationship was observed between internal phosphorus concentration and apparent photosynthesis in plants grown both in the laboratory and field. Smith (1983) reported that light-saturated photosynthesis of the fresh water phytoplankton, *Scenedesmus quadricauda* (Turp.) de Breb. increased hyperbolically with increasing cellular phosphorus quata. Lapointe (1986) reported that both growth rate and midday net photosynthetic rate in pelagic *Sargassum* species, *S. natans* and *S. fluitans* were two-fold higher with PO_4^{3-} enrichment but did not differ significantly with NO_3^- or NH_4^+ single enrichment compared to control treatment. The occurrence of a N-fixing blue-green algal flora on the surface of pelagic *Sargassum* species is well known (Carpenter 1972, Hanson 1977). Lapointe (1986) therefore suggested that enhancement of growth and photosynthesis in the pelagic *Sargassum* species by P enrichment might be due to in part to its effects on N fixation, as Redfield (1958) pointed out that N compounds could accumulate until the available P was utilized. In the present study the photosynthetic capacity and chlorophyll content of *Sargassum thunbergii* plants from Takahama were elevated in NO_3^-- and PO_4^{3-}-enriched seawater but not in NO_3^-- or PO_4^{3-}-enriched media. Our results support the dependence of photosynthesis on NO_3^- or PO_4^{3-} as shown by the above authors, but strongly suggest the interacting effects of NO_3^- and PO_4^{3-} on photosynthetic capacity of both N- and P-limited plants.

In simultaneous incubations of the plants from Takahama and Maizuru Bay in NO_3^- and PO_4^{3-}-enriched seawater the light-saturated net photosynthetic rate on a fresh weight basis increased with time in the plants from Takahama but decreased with time in the plants from Maizuru Bay. Levels of NO_3^- and PO_4^{3-} in the seawater were depleted almost equally during incubation by the samples from Takahama and Maizuru Bay. The PO_4^{3-} contents in tissues at the end of the incubation increased in the plants from both localities, NO_3^- contents in tissues increased in the plants from Takahama but decreased in those from Maizuru Bay. The reduced NO_3^- content in the samples from Maizuru Bay at the end of incubation might have partially contributed to the decreased net photosynthesis. Relationships of light-saturated net photosynthesis and chlorophyll content to the tissue NO_3^- content of *S. thunbergii* indicated that both the net photosynthetic rate and chlorophyll *a* content increased linearly ($P < 0.05$) with the increase in NO_3^- content of the tissue (Fig. 8). The relative growth rate was more than 3 times higher in the samples

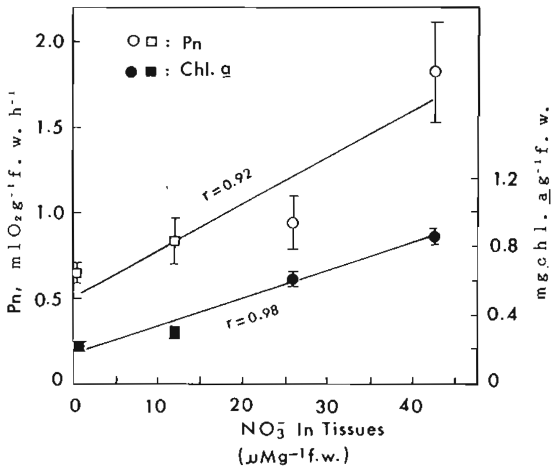

Fig. 8. Relationships of net photosynthesis (Pn) and chlorophyll *a* content in *Sargassum thunbergii* plants from Takahama (□ ■) and Maizuru Bay (○ ●) to NO_3^- content in tissues. Photosynthesis was measured at 20 °C and 600 μE m^{-2} s^{-1}. Means ± SD of 5 samples.

from Maizuru Bay than in the samples from Takahama during the incubation. It can be expected that faster growth needed more NO_3^-, which therefore resulted in reduced NO_3^- content in tissues at the end of the incubation. However, net photosynthesis on a chlorophyll *a* basis was also reduced in the plants from Maizuru Bay, which might be related to reduced enzymatic activity.

In culture studies, plants of *Laminaria longicruris* grown from spores obtained from parents in nitrate-rich and nitrate-poor bays showed higher maximum specific growth rate and maximum nitrate uptake rate for the nitrate-poor bay compared to the nitrate-rich bay (Espinoza and Chapman 1983). In the present study, the relative growth rate was much higher in *Sargassum thunbergii* plants from nitrate- and phosphate-rich Maizuru Bay compared to those from Takahama with nitrate- and phosphate-poor coastal water. The higher photosynthetic rate resulted in a higher relative growth rate in the plants from Maizuru Bay.

In conclusion, nitrate and phosphate concentrations in coastal water should be considered in order to determine the photosynthetic capacity and productivity of *Sargassum thunbergii*. Attention should be paid to the nutrient conditions in the sea and nutrient contents in tissue for the estimation of photosynthetic production of seaweeds.

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