Phosphorus dynamics during growth and maturation periods of a brown alga *Sargassum macrocarpum*

褐藻ノコギリモクの伸長期から成熟期におけるリンの利用動態

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SYNOPSIS

Perennial canopy-forming temperate macroalgae can experience the effects of limited phosphorus availability during seasonal phosphorus depletion periods. When nutrients are sufficient, they are stored in algal tissues after luxury uptake and are available to support growth during phosphorus-depleted conditions. In this doctoral thesis, the phosphorus dynamics during the growth and maturation of a brown alga *Sargassum macrocarpum* were evaluated as follows: (1) annual variations of the *in-situ* uptake rate and demand for phosphorus, and (2) storage capacities for phosphorus. In study 1, the monthly investigations to estimate the variations of *in-situ* uptake and growth-related demand for phosphorus were conducted between the growth and maturation periods from December 2017 to January 2019. To evaluate the nutrient-uptake characteristics, the algal individuals were incubated for up to 6 hrs under multiple concentrations of nutrients. Thereafter, the photosynthetic rates were measured as oxygen production for up to 60 min under multiple-light conditions. Seasonal phosphorus demands were estimated by multiplying the photosynthetic-based growth rates by the phosphorus-tissue contents. The uptake rate exceeded the demand during winter, suggesting the accumulation of phosphorus into the tissues. In contrast, the uptake rates between March 2018 and November 2018 were 0.1–0.8 times lower than the demand, resulting in the use of phosphorus-tissue contents to sustain growth during this phosphate-depleted period. In study 2, the pre-cultivations during the growth period in March 2019 and maturation period in August 2019 under different nutrient conditions provided individuals with different nutrient-tissue contents. The maximum photosynthetic rates were subsequently examined under nutrient-depleted conditions to evaluate “storage capacity”, which is defined as the amount of phosphorus-tissue contents that can support maximum growth. The growth rates of juveniles increased when stored phosphorus content was high. Conversely, no tendency of the relationship in mature individuals was found. The storage capacities for the growth and maturation periods were 19 and more than 16 weeks, respectively, suggesting that this alga can endure several months of phosphorus depletion to utilize their phosphorus contents in tissue. In conclusion, this alga can be well adapted to phosphorus depleted-environment.

Keywords: demand, life stage, oligotrophication, Sargassaceae, storage capacity, uptake rate

INTRODUCTION

Nutrient in coastal waters are the key contributor to the growth of marine macroalgae. However, oligotrophication of European and Japanese coastal waters has occurred recently due to the decreases in anthropogenic nutrient loading (Le Fur et al. 2019) and the nutrient supply from deep water (Tadokoro et al. 2009). In response to each environmental stress, the area covered by seaweed beds has clearly declined in California (Edwards & Estes 2006) and Japan (Tsuchiya et al. 2018). Though less common than N limitation, restrictions in the amount of available phosphorus (P) also limit the growth of temperate seaweeds. Therefore, an understanding of the physiological responses of temperate seaweeds to such oligotrophication is essential.

The genus *Sargassum* can form extensive seaweed beds from temperate to subarctic regions. *S. macrocarpum* is one of the large perennial brown seaweeds found throughout the temperate regions of Japan (Murase 2001). Since *S. macrocarpum* widely distributes in Japanese coastal areas, it surely has superior tolerance to changes of environmental factors (i.e., temperature and nutrient concentration) in coastal environments.

Pedersen et al. (2010) examined that the annual changes of *in situ* P-uptake rate and growth-related P-demands based on seasonal variations of external nutrient concentration and light intensity in six macroalgal species among the different growth strategies. Fast-growing algae (i.e., *Ceramium rubrum* and *Ulva lactuca*) were potentially suffered from P limitation during summer due to high P-demands derived from the fast growth, whereas slow-growing algae (i.e., *Ascophyllum nodosm* and *Laminaria digitata*) were able to acquire more sufficient external P than their demands throughout the year. Seaweeds are capable of storing a large quantity of external nutrients when the uptake is higher than that required to support growth (Hanisak 1990). The nutrients stored in tissues can be subsequently used to support growth during late spring and summer, when nutrient demands cannot be met by absorbing external nutrients in the environment (Gordon et al. 1981). The storage capacity is defined as the period that nutrient-tissue contents can be utilized for supporting the growth. This parameter heavily depends on growth rate. These results suggested that P dynamics are different among seaweeds with different growth strategies. Hypothetically, since growth rate of seaweeds are also different with the life stages such as growth and maturation periods (Murase 2001), the uptake rate, demand and storage capacity for P would change with the life stage. It is necessary to evaluate annual changes in P

dynamics, while comparing how the *in-situ* uptake rate, growth-related demands and storage capacity for P change during the growth and maturation periods.

In this study, the variation of *in situ* uptake rate and growth-related demands for P in *S. macrocarpum* during the growth and maturation periods was investigated to compare how these variations may change during growth and maturation periods (Study 1). The storage capacities for P in growth and maturation of *S. macrocarpum* were evaluated to reveal the availability of P-tissue contents to support the growth under P-depleted condition (Study 2). Finally, we discussed the year-round P-dynamics of *S. macrocarpum* to be adaptable to P-depleted coastal waters.

MATERIALS AND METHODS

Study 1. Phosphorus uptake rate and demand of S. macrocarpum *during growth and maturation periods*

To investigate the annual variation of the *in situ* uptake rate and demand for P in *S. macrocarpum*, we conducted two cultivations to evaluate the nutrient uptake kinetics and photosynthetic parameters.

This study selected Arikawa Bay as *Sargassum* beds, located in Goto Islands, Nagasaki, Japan (32°59'17.0"N; 129°07'07.3"E) for the collection of algal samples and measurement of environmental factors in every month from December 2017 to January 2019 in except January 2018. The algal collections were conducted in accordance with the life cycle and consisted of samples from two periods between the growth and maturation. According to reports of the life cycle (Murase and Kito 1998), the length of new branches on the perennial stems increased gradually in autumn and winter and then increased rapidly in spring onward. The productions of the receptacles and the release of oospores and embryos were found from late spring to summer. Since receptacles were observed after May in 2018, the maturation period in this study was defined from May to August. Two growth periods of this alga were defined as follows: first growth period was from December 2017 to April 2018, and second growth period was from September 2018 to January 2019.

A cosine-corrected PAR (photosynthetically active radiation) sensor was used to monitor light intensity inside the *Sargassum* beds. Water samples for nutrient analysis were collected once a month, just below the water surface and within the *Sargassum* beds. Collected samples were transported back to the institute and the nitrate and phosphorous concentrations were determined with a nutrient-autoanalyzer.

Phosphorus uptake rate

To evaluate the nutrient uptake characteristics of *S. macrocarpum*, the thalli were cultivated with multiple concentrations of nutrients. The seawater tank used for the experiments was indoors at the institute and was illuminated by a LED lamp. Three buckets were placed inside the tank and set just below the LED to maintain a constant light intensity. The upper part of the bucket was closed by a transparent acrylic board, and the buckets were immersed in the seawater to maintain a constant temperature during cultivation. Three algal samples were incubated in each 13-L plastic bucket filled with freshly filtered seawater (< 0.5 μm). The seawater in the buckets was enriched with $NaNO_3$ and NH_4Cl as N sources and NaH_2PO_4 as a P source to provide different nutrient conditions. The starting nutrient concentrations for each condition were adjusted as follows: 40, 80, 160, 320 and 500 μM ammonium and nitrate and 2, 4, 8, 16 and 25 μM phosphate. The thalli were pre-cultured using filtered seawater without nutrient enrichment for one day. Cultivation was carried out under each nutrient condition over a period of 1–2 h for matured thalli (July and August 2018) and 4–6 h for juveniles (in the other months mentioned above). Seawater samples (10 mL) were collected from each bucket at the start and end of the cultivation period to measure the nutrient concentrations. The nutrient uptake rates were determined by the disappearance of the nutrients from the culture medium over a given period. The nutrient uptake rates were plotted against the average concentration in time interval, and the Michaelis–Menten function was fitted to the data by least-square regression. The *In-situ* uptake rate of *S. macrocarpum* was evaluated as a result of assigning *in-situ* nutrient concentration to the function curve in every month.

Maximum photosynthetic rate and growth rate

Algal photosynthetic rate at saturated light condition was evaluated as a proxy for algal growth potential. The experimental equipment was the same as the nutrient uptake experiment. The incubation period was from 30 to 60 minutes. The incubation at dark condition (0 μmol photons m^{-2} s^{-1}) aimed to measure an algal respiration rate while those at multiple light conditions (40–600 μmol photons m^{-2} s^{-1}) were to evaluate the net photosynthetic rate. The variation of dissolved oxygen (DO) was measured with a DO logger inside each bucket and was monitored at every minute. The net photosynthetic rates (P_n) and respiration rates (R_d) in each condition were calculated and the relationship between light intensity and these rates was decided by using the specific equation. The maximum growth rate was evaluated by using values obtained by subtracting R_d from P_{max}, assuming maximum growth rate of *S. macrocarpum* throughout the life stage. Algal growth rates were calculated from photosynthetic quotient (PQ) ratios reported in previous studies (e.g., Mercado et al. 2003). The growth rates were estimated assuming daily light exposure (ca. 12 h) from measurements during December 2017 to January 2019, obtaining daily growth rates of units of g C g^{-1} tissue C d^{-1}. Finally, the exponential growth rate (ln unit day^{-1}) was calculated. Separately from the evaluation of maximum growth rate, based on *in-situ* PAR at one meter above the bottom of Arikawa Bay, the *in-situ* growth rates were calculated by using photosynthetic rate as a result of assigning PAR to photosynthesis-irradiance (PI) curve in every month.

Phosphorus demand

To measure total C and P contents of the algae, the algal samples collected after the cultivation were dried at 60°C. Tissue P contents were measured based on Parsons et al. (2014) methods. Maximum and *in-situ* demands for P were evaluated based on each growth rates and total P contents in every month.

Study 2. Phosphorus storage capacity of* S. macrocarpum *during growth and maturation stages

Growth rate and total P contents

To evaluate the storage capacity for P during growth and maturation period of *S. macrocarpum*, two-phase cultivation composed of nutrient enriched phase and starvation phase was conducted.

Algal collections of *S. macrocarpum* were conducted at Arikawa Bay during the growth period in March 2019 and the maturation period in August 2019. Collected algae were cultivated in multiple conditions of nutrient concentration for 9 days (water exchanges in every 3 days). Each individual into a glass beaker (500 mL) filled with filtered sea water (<0.45 μm) was cultivated using an incubator to keep low light (ca. 80 μmol photons m^{-2} s^{-1}) and ambient temperature (March: 20°C, August: 25°C). The starting nutrient concentrations were adjusted at five levels (n=3) as follows: 8, 60, 120, 240, 320 and 400 μM ammonium and nitrate and 0.4, 3, 6, 12 and 20 μM phosphate. Thereafter, the incubation under starvation condition was conducted for 9 days after exposed to nutrient enriched conditions. Photosynthetic rates of individuals (n=15) under light-saturated condition (ca. 500 μmol photons m^{-2} s^{-1}) were measured by a DO logger for 30 minutes in three times. The growth rates were evaluated based on measured photosynthetic rate and PQ ratios mentioned above. The algal samples after starvation phase were dried at 60°C and then were analyzed total tissue P contents.

Storage capacity for P under starvation

The storage capacity, here defined as the time by which P-tissue contents could sustain algal growth without taking up external nutrient, was determined by plotting growth rate against total P content. The absolute amount of P that could be accumulated in the algae was estimated from the difference between the highest observed P-tissue content and the critical P content (P_c). The capacity was evaluated based on the amount of P-tissue content and calculated maximum growth rate (μ) derived from a non-linear model (i.e., Droop model). The μ for the growth and maturation periods were defined as 67% of the estimated highest growth rate.

RESULTS

***Study 1. Relationship between phosphate uptake rate and demand of* S. macrocarpum**

The *in situ* phosphate concentration was high (ca. 0.2-0.3) during winter, and low (ca. 0.1 μM) during summer. Phosphate uptake rates of *S. macrocarpum* increased with increasing substrate concentrations in a hyperbolic fashion described by Michaelis-Menten. The ratio of V_{max} to K_m (V_{max}/K_m) for phosphate was high in winter and constantly low in summer. The estimated *in situ* uptake rate for P was low (ca. 0.2 μmol P g^{-1}-DW d^{-1}) during spring to late summer, and high (ca. 0.4 to 1.7 μmol P g^{-1}-DW d^{-1}) during winter (Fig. 1).

Maximum growth rate and *in situ* growth rate of *S. macrocarpum* based on *in situ* PAR in the growth period were relatively higher than those in the maturation period. Total P contents peaked in March 2018 and then maintained low corresponding to low phosphate concentration in Arikawa Bay.

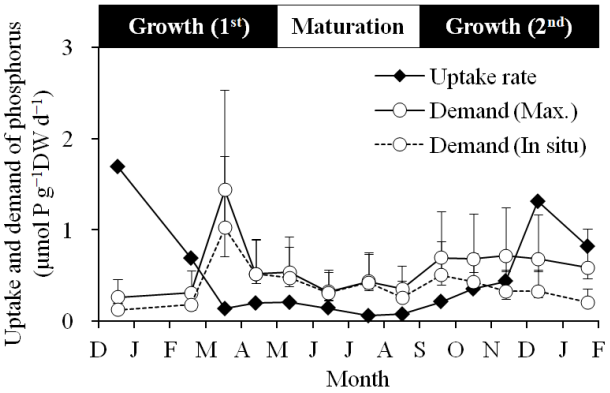

Fig. 1 Uptake rate, maximum and *in-situ* demands for P in *S. macrocarpum* during the growth (Dec. 2017 to Apr. 2018 and Sep. 2018 to Jan. 2019) and maturation (May 2018 to Aug. 2018) periods.

The estimated maximum demand for P was low (~0.3 μmol P g^{-1}DW d^{-1}) in December 2017 to February 2018 and the highest (1.44 μmol P g^{-1}DW d^{-1}) in March 2018 because maximum total P content and growth rate were observed. Thereafter, the P demand during the maturation period during May to August 2018 (~0.5 μmol g^{-1}DW d^{-1}) was relatively lower than that in the second growth period during September 2018 to January 2019 (~0.7 μmol g^{-1}DW d^{-1}). In addition, the *in-situ* demand for P showed a single peak (1.0 μmol P g^{-1}DW d^{-1}) in March 2018, and then was approximately constant (0.2–0.5 μmol P g^{-1}DW d^{-1}) from April 2018 to January 2019 (Fig. 1).

***Study 2. Storage capacity for phosphorus during growth and maturation in* S. macrocarpum**

In the growth period, the total P contents after high nutrient supply phase significantly increased with raising phosphate concentration of medium, which ranged from 0.39 to 0.52%-DW. The trend in total P content during the maturation period was also similar (0.39 to 0.52 %-DW), indicating that the ability to accumulate the P contents in the tissue both periods was observed.

The photosynthesis-based growth rates during the growth period of *S. macrocarpum* tended to significantly correlate with total P content (Fig. 2), fitting to the Droop model (R^2 = 0.556, p <0.001). Contrarily, no tendency during the maturation period was found. This result in the growth period indicates that *S. macrocarpum* is able to maintain growth rate even under an algal status in which the nutrient reserves largely vary for a certain period. The P_c for the maturation stage was defined as less than the lowest obtained value of total P content in this study. The P_c during the growth and maturation periods was 119.5 μmol P g^{-1} DW and less than 108.1 μmol P g^{-1} DW, respectively. Based on these parameters, P-storage capacities that can support growth rates during growth and maturation in *S. macrocarpum* were approximately 19 weeks and more than 16 weeks, respectively.

DISCUSSION

The uncoupled variations between the uptake rate and demand for P have been reported throughout life cycle of *S. macrocarpum*. Considering the relation between the

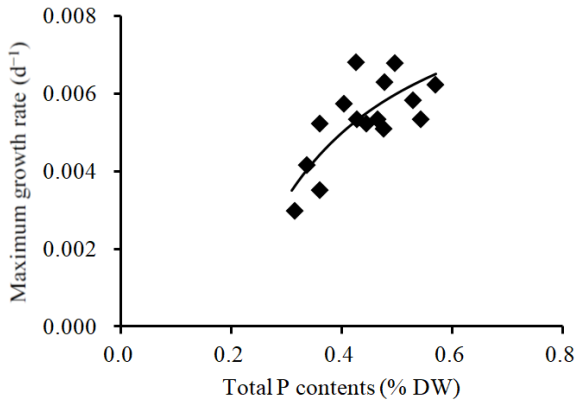

Fig. 2 The relationship between total P content and photosynthesis-based growth rate during the growth stage of *S. macrocarpum* cultivated after 9 days during nutrient enriched phase.

uptake rate and demand, the uptake rates during winter (December 2017 to February 2018, December 2018 to January 2019) were exceeded 4.0-13.6 times as compared with the *in situ* demand, indicating P accumulation in the thallus. In contrast, the uptake rates during spring, summer and autumn (March 2018 to October 2018) were 0.1-0.8 times less than the *in situ* demand, suggesting the use of P-tissue contents for growth. The period where the uptake rate would be inadequate to meet demand was long. This result means that phosphate uptake could not satisfy their demand in oligotrophic seasons (i.e., summer) in Arikawa Bay. *S. macrocarpum* were probably able to meet their P demand by means of the following characteristics: (1) a sufficient uptake of external P supplied by the physical disturbances such as wind-induced upwelling and vertical mixing (Zheng & Tang 2007), and (2) an effective use of P contents in tissues for growth (Schaffelke & Klumpp 1998).

The P-tissue contents of slow-growing algae can support maximum growth for more than 10 weeks, while those of fast-growing algae are available only less than two weeks due to rapid dilution of P reserves by fast growth (Pedersen et al. 2010). Comparing the capacity for P, *S. macrocarpum* could be categorized as slow-growing algae. However, the period from the storage capacity was shorter than the period during which the uptake rate would be inadequate to meet the demand. Indeed, the N and P reserves obtained through winter and spring can obviously not sustain maximum growth of *Gracilaria vermiculophylla* throughout extended periods of low nutrient concentration (i.e., mid-May to mid-September) (Pedersen & Johnsen 2017). For *S. macrocarpum* in this study, the estimated variation in P uptake between March and November 2018 ranged from 10 to 50% of the P demand, suggesting that P-tissue content was available 1.1 to 2 times longer when compared with no uptake from external P. Therefore, the combined effects of continuous nutrient uptake and slower growth (i.e., lower nutrient demands per unit biomass and time) lead to an increase of the storage capacity.

SUMMARY

In Study 1, the variations between *in-situ* uptake and growth-related demands for P examined in *S. macrocarpum* were revealed. These variations were uncoupled different trends and therefore the P dynamics probably varied with season. The primary growth period of *S. macrocarpum* during autumn was a response to a balance between P-uptake rate and demand. Thereafter, P accumulation in late growth period during winter because P uptake exceeded P demand, and the use of P-tissue contents in maturation period during summer are clarified.

In Study 2, the storage capacity of *S. macrocarpum* was evaluated as an indicator to sustain growth rate under nutrient depleted environment. The storage capacities during growth and maturation in *S. macrocarpum* were 19 weeks and more than 16 weeks, respectively. This result indicate that *S. macrocarpum* have high capacity to sustain growth during nutrient depleted seasons from spring to late summer by using P reserves obtained during winter, when P availability is high.

This species may be able to adapt to oligotrophic environments due to P accumulation during winter and the effective use of P-tissue contents during spring to late summer. The effective availability of P results in a year-round availability of P that is sufficient to meet demands throughout the growth and maturation periods of the *S. macrocarpum* life cycle.

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