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Phosphorus requirement of the sporophyte of *Laminaria japonica* (Phaeophyceae)

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Abstract

The relationship between phosphorus uptake and internal phosphorus status was studied on the kelp species, *Laminaria japonica* Areshoug. Elongation of the cultivated sporophyte ceased after June when phosphorus content was less than 1.3 μg P $(g\,DW)^{-1}$, indicating that this is the critical phosphorus content required for growth. The uptake rates of inorganic phosphorus, organic phosphorus, and extracellular alkaline phosphatase rapidly increased when the phosphorus content decreased to the critical level. These results suggest that phosphorus acquisition by the sporophyte is closely linked to the internal phosphorus level. Moreover, nucleic acid content correlated to the phosphorus content when the level of phosphorus was greater than the critical value, suggesting that the critical phosphorus content is a good indicator of phosphorus requirement and utilization within the plant.

Keywords: Critical content; *Laminaria japonica*; Nucleic acids; Phosphorus; Uptake

1. Introduction

Phosphorus is an important major element in seaweeds, in the form of ATP, ADP, nucleic acids and phospholipids. Phosphorus is a limiting factor on the growth of seaweeds, with the ambient phosphorus concentration varying throughout the year and among habitats (Lapointe, 1986, 1987; Chopin et al., 1995; Harrison et al., 1990). Many benthic plants have a high C:N:P ratio, indicating that they are more depleted in phosphorus, relative to carbon, than phytoplankton (Atkinson and Smith, 1983; Duarte, 1992). Seaweeds principally utilize orthophosphate as a phosphorus source from surrounding seawater. The parameters of orthophosphate uptake kinetics, such as maximum uptake rate and half saturation

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concentration, have been reported in *Agardhiella subulata* (DeBoer, 1981), *Chaetomorpha linum* and *Ulva regida* (Lavery and McComb, 1991), *Gracilaria tikvahiae* (Friedlander and Dawes, 1985), and *Macrocystis pyrifera* (Manley, 1985). The phosphorus uptake rate is influenced by environmental factors such as water temperature, light intensity and ambient phosphorus concentration, and internal factors such as phosphorus content, age, and region of the plant. In addition, some seaweeds can utilize some organic forms of phosphate, such as glycerophosphate, by producing extracellular alkaline phosphatase (Walther and Fries, 1976; Weich and Graéli, 1989; Hernández et al., 1992, 1993). The availability of dissolved organic phosphate increases after external inorganic phosphate has been exhausted. Some seaweeds can also store phosphorus (Lundberg et al., 1989).

Laminaria japonica is an important kelp species used for foods and industrial materials, and is widely cultivated in southern Hokkaido and Aomori prefecture, Japan. Accordingly, it is important to study the phosphorus requirement of *L. japonica* to ensure appropriate management of the species. This study determines how the phosphorus status of *L. japonica* changes seasonally, and how the sporophytes acquire dissolved inorganic phosphorus and dissolved organic phosphorus from the surrounding seawater in the natural environment.

2. Materials and methods

2.1. Field cultivation

To prepare for cultivation of *L. japonica* in the field, we obtained zoospores from fertile plants in sterilized seawater after cooling them at 4 °C in the dark. The zoospores were attached to a rope (1.5 mm in diameter and 1 m in length). After culturing of zoospores at 10 °C under 35 μmol photons $m^{-2}\,s^{-1}$ (light:dark = 12 h:12 h) for approximately 40 days, the rope with young sporophytes attached (grown to ca. 1 cm in length) was attached to another rope (1.5 cm in diameter). The ropes were cultivated at a depth of 2–3 m in the coastal area off Usujiri Fisheries Station of North Biosphere, Hokkaido University from November 2000 to August 2001. In January 2001, eight sporophytes were marked with plastic tags, and they were punched at a point 50 cm from the stipe-blade transition by a cork borer (1.3 cm in diameter). New holes were added once a month at the same point. Mean elongation of the sporophyte was estimated by subtracting the net blade length from the gross blade length without apical erosion. Apical erosion rate was estimated by measuring the distance from the hole to the apex. Discs removed by a cork borer were used to measure the phosphorus and nucleic acid contents. Water temperature was measured at each sample by collecting 500 ml of the surface seawater. The seawater was filtered with a Whatman GF/C glass fiber filter, and used to analyze the dissolved inorganic and organic phosphorus.

2.2. Phosphorus and nucleic acids measurements

Inorganic phosphorus concentration in seawater was measured according to Parsons et al. (1984) after filtration (Whatman GF/C). Dissolved organic phosphorus was measured according to Menzel and Corwin (1965). Five milliliter of seawater was autoclaved with 800 μl of 5% (w/w) peroxythosulfate solution at 121 °C under 1.055 kg cm^{-2} pressure. Reactive

phosphate produced by decomposing dissolved organic phosphorus was measured in a similar manner to the measurement of dissolved inorganic phosphate (Parsons et al., 1984). To measure the phosphorus content of plant segments, each segment was dried at 60 °C, and then powdered with a mill. Dry materials were autoclaved with 3 ml of peroxythosulfate solution in a similar manner used to measure the dissolved organic phosphorus in seawater (Menzel and Corwin, 1965). DNA and RNA content were measured according to a modified method of Clemmesen (1993) where CTAB is used instead of SDS to extract nucleic acids.

2.3. Measurements of phosphorus uptake rate and extracellular alkaline phosphatase activity

Sporophytes of *L. japonica* were collected between August 2001 and December 2001. Discs (1.8–3.0 cm in diameter) were collected from the marginal parts of the middle area along the thallus of the sporophyte, and precultured in 500 ml of seawater at 15 °C under 100 μmol photons m^{-2} s^{-1} (light:dark = 14L:10D). After 24 h, discs were placed in 100 ml of artificial seawater, ASP_{12} (Provasoli, 1963) without vitamins, and were incubated with a stirring bar for 2 h at 20 °C under 200 μmol photons m^{-2} s^{-1} to measure the maximum phosphate uptake rate. The phosphorus source was NaH_2PO_4 as dissolved inorganic phosphorus, and β-glycerophosphate as dissolved organic phosphorus. The initial phosphorus concentration was adjusted to 10.0 μmole l^{-1}. Uptake rates of dissolved inorganic and organic phosphorus by sporophyte discs were determined by measuring from the decrease of those concentrations in seawater, and expressed as μgP h^{-1} cm^{-2}.

Extracellular alkaline phosphatase activity (APA) was measured according to Reichardt et al. (1967). Discs were incubated in 40 ml ASP_{12} using *p*-nitrophenyl phosphate (*p*NPP) as an organic phosphorus source at 15 °C under 100 μmol photons m^{-2} s^{-1} for 3 h. The phosphorus concentration was adjusted to 50.0 μmole l^{-1}. After incubation, the amount of *p*-nitorophenol (*p*NP) produced was calorimetrically measured by a spectrophotometer (V530, Nihonbunko Co. Ltd.).

3. Results

3.1. Phosphorus environment

Monthly changes of ambient dissolved inorganic and organic phosphorus concentration, and seasonal changes of water temperature are shown in Fig. 1. Minimum water temperature (1.0 °C) occurred in early February, increasing until September when it reached a maximum (19.8 °C) (Fig. 1a). The water temperature gradually decreased after September onwards. Dissolved inorganic phosphorus was high during December 2000 and March 2001. The maximum concentrations of inorganic phosphorus (1.5 μmole l^{-1}), and dissolved organic phosphorus (1.3 μmole l^{-1}) were observed in March (Fig. 1b). The percentage of dissolved inorganic phosphorus to dissolved total phosphorus ranged from 54.5 to 76.2% during December 2000 and March 2001. Dissolved phosphorus was lower than 0.3 μmole l^{-1} from April to September. The percentage of dissolved organic phosphorus to total dissolved phosphorus concentration (53.0–60.3%) was greater than 50% from April to September.

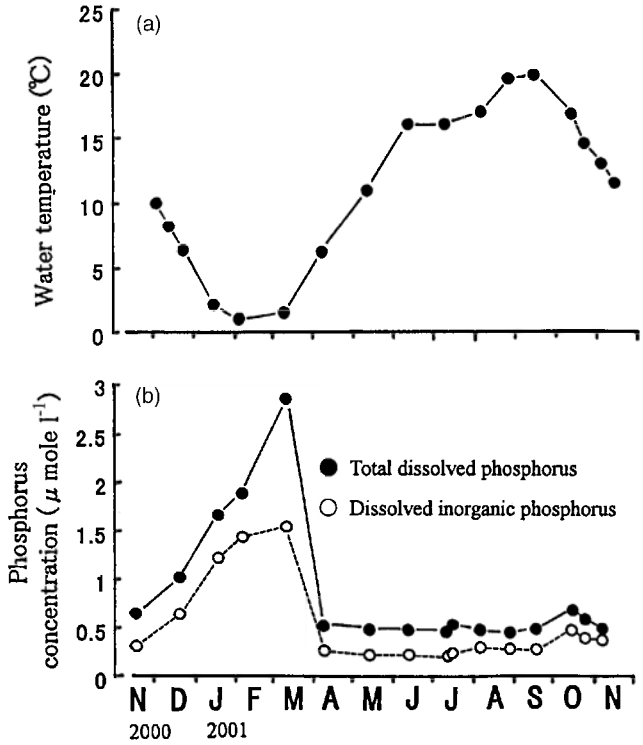

Fig. 1. Monthly changes in seawater temperature (a) and dissolved phosphorus concentrations (b) in the surface water of the *L. japonica* cultivated grounds at Minamikayabe, Hokkaido.

3.2. Growth of *L. japonica* sporophyte

Monthly changes of blade length and elongation rate of the sporophytes are shown in Fig. 2. The blade length increased from January to June with some sporophytes reaching more than 6 m in length in June (Fig. 2a). Blade length decreased from June onwards. Fresh weight increased with blade length. Elongation rate of the blade length was 4.1 ± 0.4 cm per day in early March, and gradually decreased (Fig. 2b). During June and July, the mean elongation rate (<1.0 cm per day) was less than the erosion rate. In particular, the mean erosion rate remained at approximately 2.0 cm per day during June and August.

3.3. Phosphorus and nucleic acids contents

Phosphorus content in the blade was higher in winter than in summer (Fig. 3a). In February, phosphorus content was 5.3 ± 0.8 μg P $(\text{g DW})^{-1}$ and then decreased, with the lowest content (1.1 ± 0.3 μg P $(\text{g DW})^{-1}$) being observed in August. Nucleic acid content showed similar seasonal changes to phosphorus content (Fig. 3b). The maximum DNA and RNA content (1.45 ± 0.20 and 2.49 ± 0.20 μg DNA $(\text{mg DW})^{-1}$, respectively) occurred

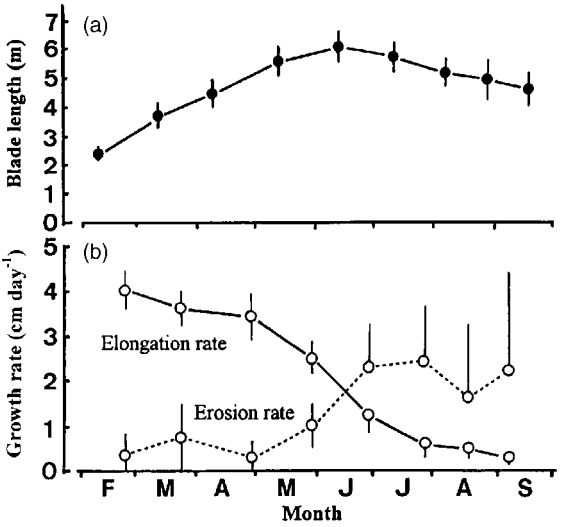

Fig. 2. Monthly fluctuations in the elongation of the sporophyte of *L. japonica* (a) and the mean elongation and apical erosion rates (b). Vertical bars indicate standard deviations ($n = 7$–8).

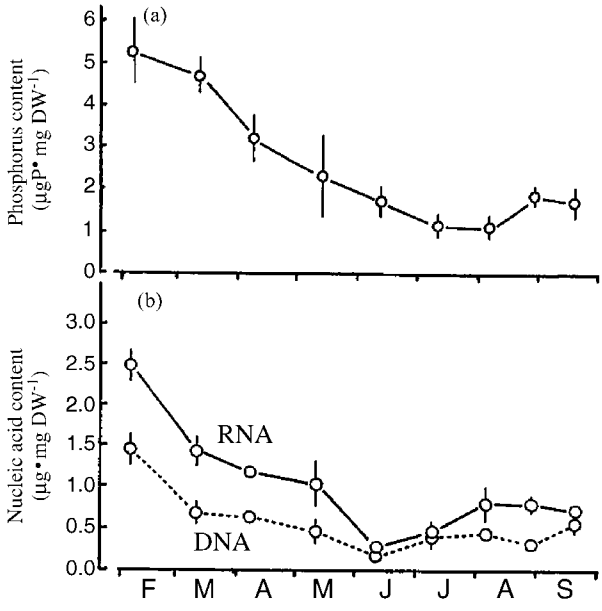

Fig. 3. Monthly fluctuations in phosphorus content (a) and nucleic acid content (b) of the basal part of *L. japonica* sporophytes collected at Minamikayabe, Hokkaido. Samples were collected from the basal area at a point 50 cm from the stipe-blade transition. Vertical bars indicate standard deviations ($n = 3$–8).

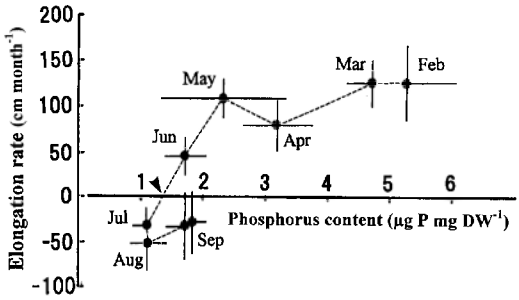

Fig. 4. Relationship between the elongation rate of *L. japonica* sporophytes and the phosphorus content in the basal part. Vertical bars indicate standard deviations ($n = 3$–8). An arrow indicates the phosphorus content when the apparent elongation rate is zero.

in February (Fig. 3b). The content of RNA was always higher than DNA content, and RNA/DNA ratio ranged from 1.2 to 2.4. There was no significant difference in the ratio among months (*t*-test, $P > 0.05$).

3.4. Relationship between growth, nucleic acid content and phosphorus content

The relationship between elongation and phosphorus content in *L. japonica* sporophytes is shown in Fig. 4. When phosphorus content was high in winter months, elongation

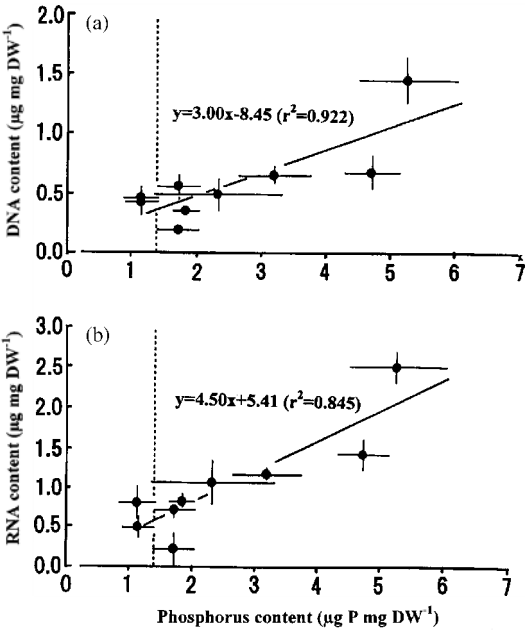

Fig. 5. Relationship between phosphorus content and DNA (a), and RNA (b) phosphorus content in the basal part of *L. japonica* sporophytes. Vertical bars indicate standard deviations ($n = 3$–8).

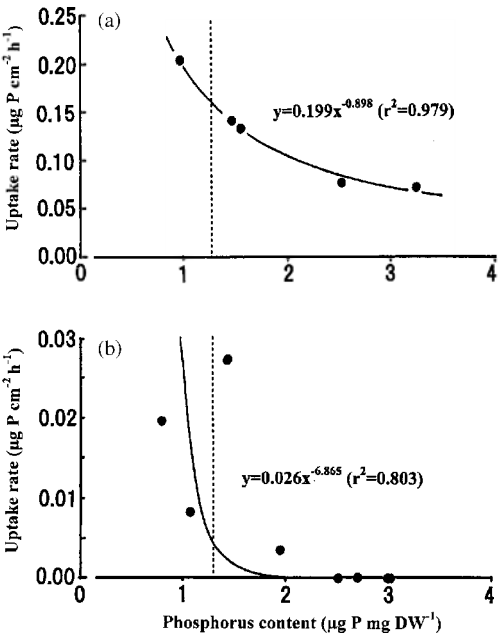

Fig. 6. Relationship between phosphorus content and uptake rates of inorganic phosphate (a), and organic phosphate (b) in *L. japonica* sporophytes.

was positive. However, elongation ceased when the phosphorus content was less than 1.3 μg P (g DW)$^{-1}$, indicating that the content is critical for growth.

The relationship between nucleic acid content and phosphorus content is shown in Fig. 5. Both DNA (Fig. 5a) and RNA content (Fig. 5b) are significantly correlated to phosphorus content (t-test, $P < 0.01$).

3.5. Relationship between phosphate uptake and phosphorus content

The relationship between phosphorus uptake rate and phosphorus content in *L. japonica* is shown in Fig. 6. The uptake rate of inorganic phosphate increased with decreasing phosphorus content (t-test, $P < 0.05$) (Fig. 6a). The dissolved organic phosphorus uptake showed the same relationship (Fig. 6b). In particular, the organic phosphate uptake rate drastically increased when the phosphorus content decreased to less than 1.3 μg P (g DW)$^{-1}$.

Extracellular alkaline phosphatase activity at two different substrate concentrations is shown in Fig. 7. APA is also negatively correlated to phosphorus content.

4. Discussion

The phosphorus environment at the study site was divided into two periods: low water temperature and high phosphorus period from December to April, and high water temper-

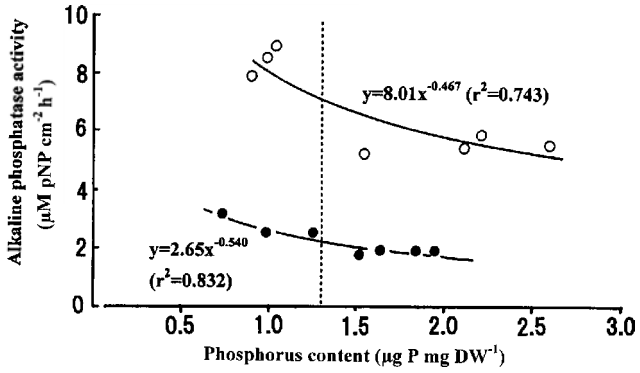

Fig. 7. Relationship between phosphorus content and the extracellular alkaline phosphatase activity of segment ($n = 7$) in *L. japonica* sporophytes. The segments were cultured in ASP_{12} added 10 μmole l^{-1} *p*NPP (closed symbol) and 50 μmole l^{-1} *p*NPP (open symbol).

ature and low phosphorus period from May to November. In the low water temperature and high phosphorus period, the sporophyte of *L. japonica* were considered to use mainly inorganic phosphorus because they comprised more than 50% of total available dissolved phosphorus. On the contrary, dissolved organic phosphorus was considered to be the main phosphorus source for the sporophytes because they comprised more than 50% of total dissolved phosphorus in high water temperature and low phosphorus period. The increase of water temperature is advantageous for the utilization of dissolved organic phosphate due to the increase in uptake rate with increasing water temperature (Hernández et al., 1992).

Nutrient contents, and the ratios such as C:P and N:P, have been useful for judging the nutritional status of seaweeds (DeBoer et al., 1978; Duarte, 1992; Chopin et al., 1996). In *L. japonica* sporophytes, elongation ceased when phosphorus content was less than the critical content of 1.3 μg P (g DW)$^{-1}$, indicating an absolute demand for growth. Moreover, the uptake rate of inorganic phosphate increased markedly with decreasing phosphorus content to the critical content. In particular, the uptake rate of dissolved organic phosphate drastically increased when the phosphorus content decreased to less than the critical content. The increase in the uptake rate is supported by the extracellular alkaline phosphatase activity. These results support the hypothesis that the sporophyte activates dissolved organic phosphorus uptake via the extracellular alkaline phosphatase in phosphorus-poor conditions.

Absorbed phosphorus is divided into two processes: storage and incorporation. Lundberg et al. (1989) reported that the brown algae *Pilayella* stores phosphorus mainly as phosphate in its vacuole. It is most likely that *L. japonica* stores phosphate during the phosphorus-rich period in winter. Phosphorus is also incorporated into many macromolecules, such as nucleic acids, proteins, and phospholipids. In this study, nucleic acid content is significantly correlated to phosphorus content (*t*-test, $P > 0.01$), indicating that the synthesis of nucleic acids is carried out according to the internal amount of phosphorus. It is concluded from these results that phosphorus metabolism is closely linked to the environment. In this case, the critical phosphorus content is good indicator for elucidating the phosphorus status of the sporophyte.

Sporophytes of *L. japonica* require 1.3% DW of nitrogen as the absolute demand for growth (Mizuta et al., 1994). Considering the phosphorus demand, it is suggested that the optimal N:P atomic ratio is about 22.1. Accordingly, the growth is limited by nitrogen when the atomic ratio is less than the optimal ratio, and conversely, when the supply of a higher N:P ratio is more than the optimal ratio, the result is phosphorus limitation. In the case of cultivation management of *L. japonica*, the optimal N:P ratio is also an indicator for judging the nutrient conditions in the natural and cultivated grounds.

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