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## Nutrient environment in natural and cultivated grounds of *Laminaria japonica*\*

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### Abstract

The seasonal changes of major nutrient elements (nitrogen and phosphorus) in natural and cultivated grounds of Makombu (*Laminaria japonica* Areschoug) were investigated throughout the year. The study areas were divided into two periods: a) chemical environments that had a nutrient-rich period from winter to early spring, and b) environments with a nutrient-poor period from late spring to autumn. For the nutrient-rich period, nitrogen and phosphorus concentrations were lower in natural grounds compared to cultivated areas. The depletion of nutrients in late spring was caused by nutrient consumption that occurred during phytoplankton blooms. Moreover, the depletion of nitrogen during the same period was faster in natural grounds than in cultivated areas. These facts suggest that the supply of nutrients to natural grounds is limited by severe consumption of nitrogen by *Makombu* and phytoplankton in cultivated grounds. The scale and time of the blooming of phytoplankton may influence the growth pattern of *Makombu*.

### Introduction

Makombu (*Laminaria japonica* Areschoug) is one of many commercially important seaweeds. This seaweed has been used as food since ancient time and has been utilized in industrial materials (Tokuta, 1987). Recently, the cultivation system of seaweeds has developed and the production of *Makombu* has increased (Sanbonsuga, 1984a). However, the production of natural *Makombu* greatly fluctuated every year (Kawashima, 1984).

The production of seaweed is limited by several environmental factors. Nutrients are essential for the growth of seaweed and is often a limiting factor (DeBoer, 1981). Chapman and Craigie (1977, 1978) reported that nitrogen limited the growth of *L. longicruris* during the summer months. The limitations of growth caused by the absence of nitrogen were reported by several investigators (Conolly and Drew, 1985; Zimmerman and Robertson, 1985). Sanbonsuga (1984b) suggested that the growth of *L. japonica* was limited by two steps in summer, first the depletion of nitrate and second by water temperature. Although many studies exist on the importance of the nutrients above-stated, the nutrient environment in Kombu grounds has not been investigated in detail.

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The purpose of this study is to investigate the detailed environment of nutrients (nitrogen and phosphorous) in natural and cultivated *Makombu* grounds off Osatsube, Hokkaido. Moreover, the competition of nutrient consumption in *Kombu* grounds is discussed.

### Methods

Twelve sampling stations were chosen respectively from natural, cultivated grounds and outside of the cultivated apparatus in the coastal region off Osatsube, Hokkaido (Fig. 1). Field surveys were carried out once or twice a month from June 19, 1986 to June 30, 1987.

Water samples were collected from upper, middle and lower layers in the water column at twelve stations using a 5 l Van Dorn sampler. In natural *Kombu* grounds, the *Kombu* living layer corresponded to the middle (2.5 m) and lower (5.0 m) in natural *Kombu* grounds. Cultivated *Kombu* was hung in the middle layer (7.5 m) in cultivated grounds. On board, the water temperature was immediately measured and water samples were kept in each bottle for analysis of salinity, nutrients and chlorophyll *a*. In the shore laboratory, 500 ml of seawater was analyzed for chlorophyll *a* by being filtered with a glass fiber filter (Whatman GF/C) that was treated at 450°C. The loaded filter was stored at -20°C. A sample of 100 ml of seawater was also stored at -20°C for nutrient analysis.

Salinity was measured by a salinometer (Model 601 MKII, Watanabe Keiki Seisakusyo). Nutrients (ammonium-N, nitrate-N, nitrite-N, phosphate-P and silicate-Si) were analyzed by a Technicon Autoanalyzer II according to Strickland and Parsons (1972). Chlorophyll *a* was extracted from the filters with 90% acetone

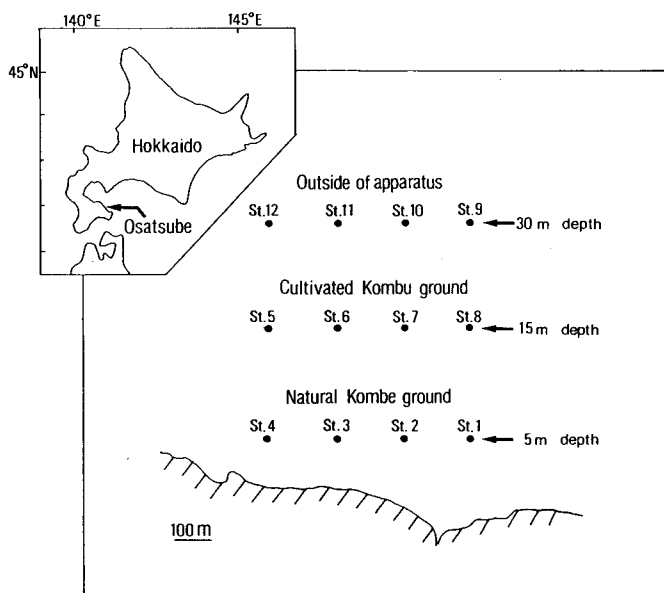


Fig. 1. Sampling stations off Osatsube, Hokkaido.

and measured by a fluorimetric method using a fluorometer (RF-540, Simazu Co.).

## Results

The oceanographic structures in natural and cultivated *Makombu* grounds off Osatsube are shown in the temperature-salinity (T-S) diagram (Fig. 2). Data in this figure were plotted as the average for three areas as referring to the water mass structure in Funka Bay (Ohtani, 1971). The structure in the study area was similar to that of Funka Bay. The *Makombu* grounds off Osatsube were covered with Oyashio waters (cold current) from middle December to early March in which the rapid growth of *Makombu* was observed.

Seasonal changes of total inorganic nitrogen (TIN) and phosphate-phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ) in natural and cultivated *Makombu* living layers are shown in Fig. 3. The TIN concentration was very low from April to October. In particular, this was less than  $1\ \mu\text{g at}\cdot\text{l}^{-1}$  during August and September. There was no difference between natural and cultivated *Kombu* grounds for TIN concentrations. The TIN concentration began to increase in November, and reached its maximum, 11–15  $\mu\text{g at}\cdot\text{l}^{-1}$ , in January and February. In winter, TIN concentrations in natural *Kombu* living layers were lower than those in cultivated. Particularly, the concentration of TIN in natural *Kombu* areas during the middle of March was about half that in cultivated areas. The composition on TIN is shown in Fig. 4. Nitrate ( $\text{NO}_3^-$ -N)

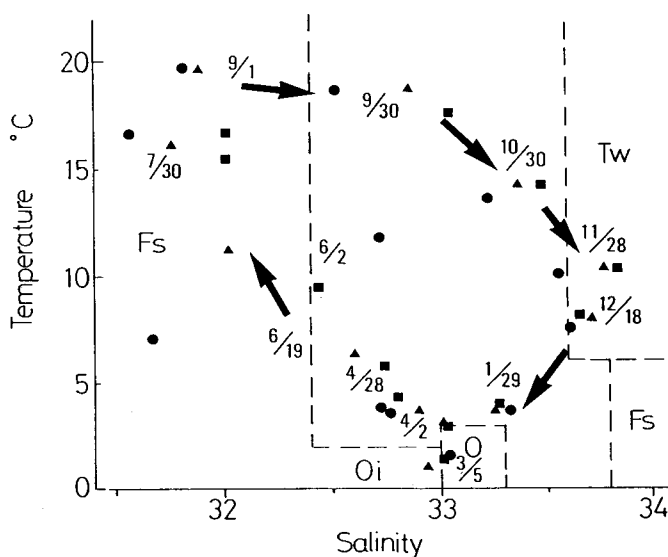


Fig. 2. Seasonal changes in T-S relations of natural *Kombu* grounds (●), cultivated *Kombu* grounds (▲) and outside of cultivated apparatus (■) off Osatsube. Closed circle, triangle and square indicate the mean value of each ground ( $N=4$ ). Numerals express the sampling dates. Tw, O, Oi and Fs denote Tsushima Warm Current, Oyashio Coastal Branch, melting Water of Oyashio and Funka Bay water formed in summer (after Ohtani, 1971), respectively.

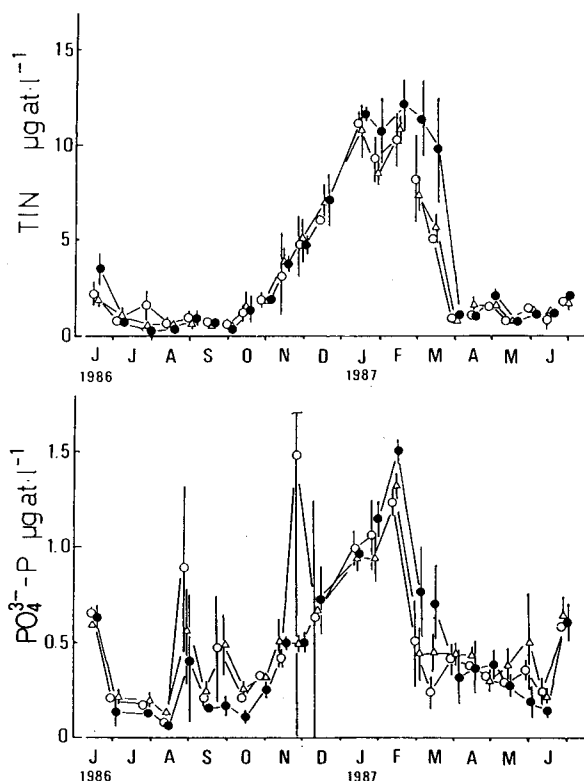


Fig. 3. Seasonal changes in the mean TIN and  $\text{PO}_4^{3-}\text{-P}$  ( $\bar{x} \pm \text{S.D.}$ ,  $n=4$ ) at natural *Kombu* living layers ( $\circ$ ; 2.5 m depth,  $\triangle$ ; 5.0 m depth) and cultivated *Kombu* living layers ( $\bullet$ ; 7.5 m depth) off Osatsube.

accounted for 90% of the TIN content during the high TIN period. This period corresponds to the elongation period of *Makombu*. Meanwhile,  $\text{NH}_4^+\text{-N}$  occupied 40–60% of TIN during the summer months. This tendency was more remarkable in the natural *Kombu* living layer than in the cultivated area. Nitrite ( $\text{NO}_2^-\text{-N}$ ) was not a major component of the TIN throughout the year. Phosphate-phosphorous (Phosphate-P) concentration fluctuated as well as TIN during the year and was low during the summer months (Fig. 3). Phosphate-P reached its maximum concentration (1.2–1.6  $\mu\text{g at.l}^{-1}$ ) in March. However, there were sharp peaks (0.8–1.5  $\mu\text{g at.l}^{-1}$ ) in September and late November. This tendency was more severe in the natural grounds than in cultivated grounds. It seems that the nutrients are supplied from land.

Seasonal changes of chlorophyll *a* in the natural and cultivated *Makombu* living layers are shown in Fig. 5. The peak of chlorophyll *a* was found in early April, ranging from 4.0 to 8.0  $\mu\text{g}\cdot\text{l}^{-1}$ . The peak is due to the spring bloom of phytoplankton. The maximum of chlorophyll *a* in the natural *Kombu* living layer was lower (4–5  $\mu\text{g}\cdot\text{l}^{-1}$ ) than that (7–8  $\mu\text{g}\cdot\text{l}^{-1}$ ) in the cultivated areas. During the other periods, chlorophyll *a* was below 1.0  $\mu\text{g}\cdot\text{l}^{-1}$ .

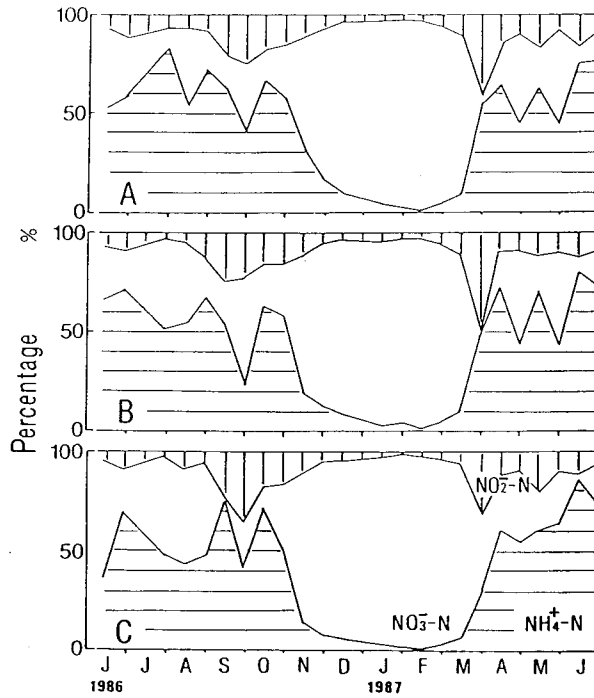


Fig. 4. Seasonal changes of relative abundance of inorganic nitrogen compounds to TIN at natural *Kombu* living layers (A: 2.5 m depth, B: 5.0 m depth) and cultivated *Kombu* living layers (C: 7.5 m depth) off Osatsube, expressed as mean value ( $n=4$ ) of each ground.

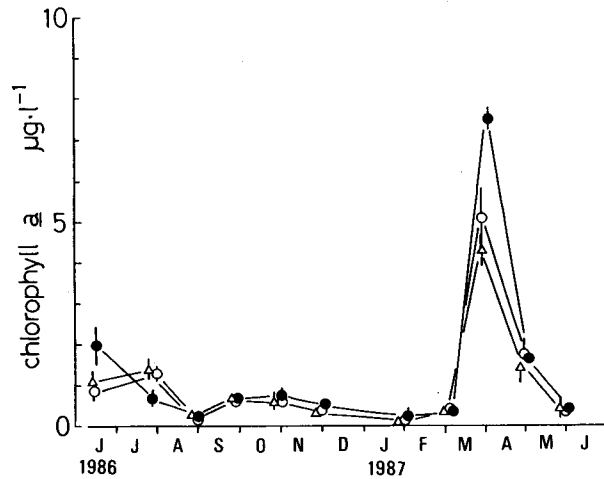


Fig. 5. Seasonal changes in the mean chlorophyll *a* ( $\bar{x} \pm S.D.$ ,  $n=4$ ) at natural *Kombu* living layers ( $\circ$ ; 2.5 m depth,  $\triangle$ ; 5.0 m depth) and cultivated *Kombu* living layers ( $\bullet$ ; 7.5 m depth) off Osatsube.

### Discussion

The chemical environment in these study areas was divided into two periods for nitrogen and phosphorous concentrations; nutrient-rich periods and nutrient-poor periods (Fig. 3). The nutrient-rich period started in October when the Tsushima warm current flowed into *Kombu* grounds and the condition was exchanged by the Oyashio cold current which has high nutrient concentration until early spring (Fig. 2). The end of the nutrient-rich periods corresponded to the spring bloom of phytoplankton (Fig. 5). These environmental conditions are in accord with a general description (Sanbonsuga, 1984b) that the growth of *L. japonica* was divided into two phases, the enlargement phase and the substantiatilization phase. Furthermore, it has been found that the chemical composition in the blade of *Laminaria japonica* was changed during these two periods (Yokoyama and Sanbonsuga, 1979; Yokoyama et al., 1980). The composition of TIN in seawater was drastically changed between two periods (Fig. 4). The main nitrogen source was  $\text{NH}_4^+\text{-N}$  in the nutrient-poor period and  $\text{NO}_3^-\text{-N}$  in the nutrient-rich period. This indicates that the available nitrogen for *Makombu* is different between these two periods. Sato et al. (1959) reported that nitrogenous components in the blade of *Porphyra tenera* were remarkably changed by the difference of the nitrogen source. This suggests that the nitrogen metabolism in the blade may be greatly changed between these two phases.

The nutrient-poor period is the critical season for the growth of *Makombu*. The spikes of  $\text{PO}_4^{3-}\text{-P}$  in *Kombu* grounds were often observed in the nutrient-poor period (Fig. 3). The spikes seem to be input from coastal runoff. Zimmerman and Rosenberg (1985) pointed out that the episodic and periodic intrusions of nutrient rich water may be necessary for survival of seaweeds during the summer. Rumas and Venable (1987) reported that the growth of *Ulva* increased with pulse frequency of  $\text{NH}_4^+\text{-N}$ . These suggest that the input of  $\text{PO}_4^{3-}\text{-P}$  is effective for the growth of *Makombu*. However, such a remarkable spike was not found for inorganic nitrogen. In the nutrient-rich period, TIN and  $\text{PO}_4^{3-}\text{-P}$  tended to be lower in the natural *Kombu* grounds than cultivated areas (Fig. 1). In particular, TIN was significantly lower in natural grounds during March. The decrease of TIN in late March was faster in natural *Makombu* grounds than in the cultivated areas. This suggests that the change of nutrient composition is a good indicator for understanding the area's environmental condition.

The atomic ratio of total inorganic nitrogen to phosphorous (TIN/P) for the nutrient poor period was 1-9. This ratio is lower than the atomic ratio of TIN/P (ca. 9) of *Makombu* (Johnston, 1971). It has been pointed out by several authors (Chapman and Craigie, 1977, 1978; Sanbonsuga, 1984) that nitrogen is the limiting nutrient in the growth of *Laminaria* during in this period. Fig. 6 shows the monthly changes of the atomic ratio ( $\text{NO}_3\text{-N/P}$ ,  $\text{Si/NO}_3\text{-N}$  and  $\text{Si/P}$ ) in the nutrient-rich period. The  $\text{NO}_3\text{-N/P}$  varied from 7-16 except during the period of the phytoplankton spring bloom. There was no difference between natural and cultivated grounds. The  $\text{Si/NO}_3\text{-N}$  increased before the bloom of phytoplankton. The  $\text{Si/P}$  formed a peak in the middle of March and decreased after that. The increase of  $\text{Si/NO}_3\text{-N}$  and  $\text{Si/P}$  were faster in the following increasing order; natural cultivated grounds, and the area outside of the cultivated apparatus. This indicates that in natural grounds the depletions of  $\text{NO}_3\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  are rapid when

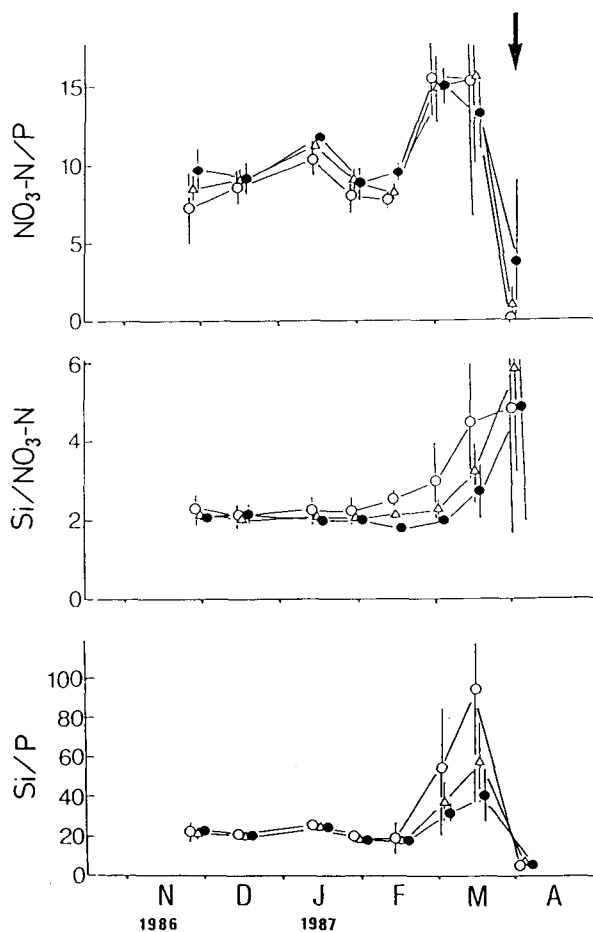


Fig. 6. Seasonal changes in atomic ratios ( $\bar{x} \pm S.D.$ ,  $n=12$ ) of  $NO_3-N/P$ ,  $Si/NO_3-N$  and  $Si/P$  at natural *Kombu* grounds ( $\circ$ ), cultivated *Kombu* grounds ( $\triangle$ ) and outside of cultivated apparatus ( $\bullet$ ) from Dec. 1986 to Apr. 1987. Arrow indicates the period of maximum chlorophyll *a* production.

compared with Si. Jackson and Winant (1983) reported that the ocean current in a kelp forest was weakened by the kelp communities. Wheeler (1980) found that the decrease of water speed resulted in the suppression of the uptake of inorganic carbon. These reports demonstrate that the consumption of nutrient is stronger along the boundary area of *Kombu* grounds, but not inside the *Kombu* grounds. Therefore, it is suggested that the severe consumption in cultivated *Kombu* system off Osatsube lead to a limit on the nutrient supply to the natural grounds.

The main consumers in both natural and cultivated *Kombu* grounds are *Makombu* and phytoplankton. To discuss the relationship between nutrient consumption and the decrease of available nutrients, the nutrient-rich period was divided into two stages; from March 5 to 18 and from March 18 to April 2. The

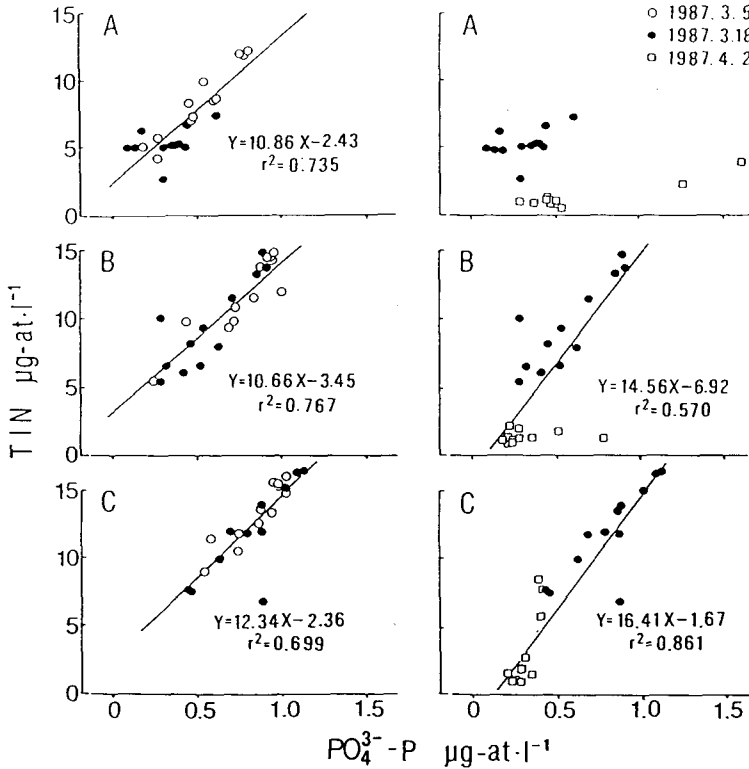


Fig. 7. Relationships between TIN and  $\text{PO}_4^{3-}\text{-P}$  in natural *Kombu* grounds (A), cultivated *Kombu* grounds (B) and outside of cultivated apparatus (C) in two stage of nutrient depletion, first from Mar. 5 to Mar. 18, 1987 (left side) and second from Mar. 18 to Apr. 2, 1987 (right side).

consumption ratios of TIN/P in these two stages for the natural grounds, cultivated areas and outside the cultivated apparatus are shown in Fig. 7. During the former stage, TIN/P kept around 11 in the natural and cultivated grounds. This value is approximately similar to the composition ratio ( $\text{N/P}=9$ ) of *Laminaria japonica* (Johnston, 1971). On the other hand, TIN/P during the later stage remained around 14-16 except to in the natural grounds. This value is similar to RKR ratio ( $\text{N/P}=15-16$ ) of composition of phytoplankton. In the natural grounds, a significant relationship between TIN and  $\text{PO}_4^{3-}\text{-P}$  was not shown, because of the depletion of TIN before April 2, 1987. These facts suggest that the consumption of nutrients is qualitatively reflected by *Kombu* during the former stage of decreasing nutrients and by phytoplankton in the later stage. The depletion of nutrients in *Kombu* grounds is caused mainly by the consumption of phytoplankton and was severely changed from a nutrient-rich condition to a nutrient-poor condition. This suggests that the blooming of phytoplankton suddenly changes the nutrient environment in *Makombu* grounds and influences the growth of the *Makombu* blade.

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\*<sup>1</sup> English title translated from Japanese by authors.