Terrestrial Nutrient Supply Contribution for Kelp in the Sea of Japan off Hokkaido, Japan

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Abstract. Nutrient supply for the kelp *Saccharina japonica* var. *religiosa* and *S. japonica* var. *ochotensis* was investigated on the northern and southwestern coasts of Hokkaido, Japan. For the investigation on the northern coasts, data from government official reports for four sampling points in Rishiri Island were used. The reports indicated that submarine discharged groundwater affects NO_3 -N concentrations in and concentrations at Shinminato were similar within distances of 0 to 500 m from the coast, and from 0 to 20 m in depth. Analyzing government reports for Tomari in southwestern Hokkaido, NO_3 -N concentrations increase from October to next March in river water. NO_3 -N concentrations on the coast of northern Hokkaido are higher than values of southwestern Hokkaido. Levels of $\delta^{15}N$ support values the saccharinian kelp assimilate terrestrial NO_3 -N in Oshoro, southwestern Hokkaido.

Keywords: freshwater spring, NO₃-N, PO₄-P, river, δ ¹⁵N

Introduction

The kelp *Saccharina japonica* var. *ochotensis* is distributed in northern Hokkaido, Sea of Japan and the known range of *S. j.* var. *religiosa* is along the southwestern coast of Hokkaido, Sea of Japan (Yotsukura *et al.*, 2008). These two species form one of the most important kelp resources in northern Japan and are used for human consumption as well as food for sea urchins (Kawashima, 1993). Since two decades ago, kelp resources have been decreasing sharply and sustainable utilization is urgently needed (Kawai *et al.*, 2014). However, the reasons for decreases in kelp have not yet been clarified.

Nutrient conditions in coastal waters certainly affect the structure of marine forests (Agatsuma *et al.*, 2014; Tada *et al.*, 2014; Zimmerman &

Robertson, 1985). Especially NO_3 -N accounted for more than 90% of DIN in southwestern Hokkaido, Japan and has been found to influence the growth of the kelp $S.\ j.$ var. religiosa there (Kuribayashi, 2016; Kuribayashi $et\ al.$, 2017). Dynamics of NO_3 -N in Oshoro Bay was reported (Nakata $et\ al.$, 2001) and experimental studies have confirmed the effect of NO_3 -N under laboratory conditions (Kawai $et\ al.$, 2004; Mizuta $et\ al.$, 1994, 2001).

This paper showed the possibility that terrestrial nutrient supply: freshwater springs (or submarine discharged ground water), rivers, and direct inflow from land areas (anthropogenic waste water) contributes to the maintenance of a rich kelp community in the Sea of Japan off Hokkaido, Japan, using previous data in government official reports,

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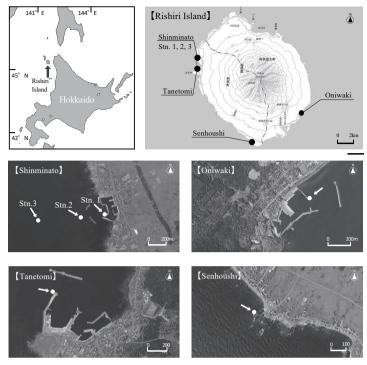


Fig. 1. Map showing the sampling stations in Rishiri Island, northwestern coast of Hokkaido, Japan. Aerial photography from Information Authority of Japan (https://mapps.gsi.go.jp/maplibSearch.do#1).

and investigated in southwestern Hokkaido, Japan. Moreover, algal species synthesize components in the tissue by assimilating nutrients from the surrounding water. This property indicates that the δ^{15} N in algal tissue is expected to provide integrated information on biological uptake of DIN (Costanzo *et al.*, 2001). δ^{15} N in sea algae have been often used as an indicator of DIN source (McClelland *et al.*, 1997). Authors have developed a technique involving analysis of the stable nitrogen isotope ratios signature (δ^{15} N) in algal tissue of *S. j.* var. *religiosa*.

These contribute to enable management proposals for sustainable conservation of the kelp forest on the south-western and northern coasts of Hokkaido, Sea of Japan.

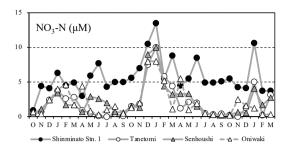
Materials and Methods

Reports from government and public research

institutions (Kawai & Goda, 2008-2011) analyzed were understand the nutrient dynamics on the northern and southwestern coasts of Hokkaido, Japan. Surface seawater from Om in depth from Shinminato, Tanetomi, Senhoushi. Oniwaki, on the coast of Rishiri Island, northern Hokkaido (Fig. 1) was sampled monthly from October 2008 to March 2011 using a Van Dorn water sampler (Rigo Co Ltd, 5026-A) to clarify influence of submarine discharged groundwater the beach shore environment. Seawater from each depth was sampled at Stn. 2 (0, 5, 10, 15, and 20 m in depth) and Stn. 3 (5 m in depth) in Shinminato

(Fig. 1) using the sampler. These two stations are of approximately 25–30 m depth and have an approximate 500 m interval distance. This sampling research was arranged to examine differences in the nutrient concentrations of various depth ranges and differences with distances from the beach shore.

Government reports (Hokkaido, 1998–2017) were used to analyze and clarify the influence of nutrients on seawater concentrations from inflowing river water. Surface seawater and river-water was sampled from 0 m in depth. Seawater was also sampled at Tomari, southwestern Hokkaido, Sea of Japan. The sampling points were a river (Horikappu River), Stn. 1 located near the estuary of the river, and at Stn. 2 and 3 located in coastal waters. There are no large out-flow river in this area, only the Horikappu River with a total length of 27.4 km and an outflow volume 1.62 m³/sec, area of river 262.5



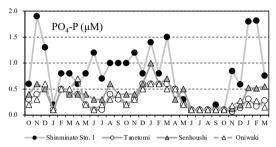


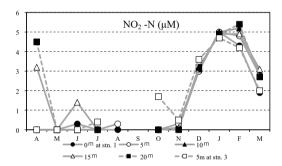
Fig. 2. Seasonal changes of nutrient concentrations at the four sampling stations on Rishiri Island, coast of Sea of Japan, Hokkaido, Japan based on reports from Hokkaido Government and Hokkaido Research Organization (Kawai and Goda, 2008–2011). Surface seawater (in 0m depth) was sampled from 2008 to 2011. Location of the four sampling stations are shown in Fig. 1.

km² (Wikipedia, unknown) (Fig. 4B). Seawater and river-water was sampled every three months from 1998 to 2017. The sampling protocol was the same as used for northern Hokkaido.

Seawater samples from the northern and southwestern coasts of Hokkaido, Japan were filtered and the $\mathrm{NO_3}$ -N and $\mathrm{PO_4}$ -P concentrations were determined using an autoanalyzer (QuAAtro2-HR, TRACCS 800: BL-TEC). Salinity in subsamples were determined using a salinometer (T.S-DIGI-AUTO MODEL-3G, T.S-DIGI-AUTO MODEL-5: Tsurumi-Seiki).

Sampling stations were arranged in Oshoro Bay, Otaru, Shiribeshi, southwestern Hokkaido, Sea of Japan (Fig. 4 A and C), sampling points were conducted in the mouth of the bay (Stn. 3), middle-bay (Stn. 2), and inner part of the bay (Stn. 1) to clarify the influence for absorbed nutrients from terrestrial sources of direct inflows

on the kelp. Sporophytes of Saccharina japonica var. religiosa were collected randomly by SCUBA diving from April to June 2017 when the blade length of S. j. var. religiosa was at a maximum in size (Abe et al., 1983, 1984) and three individuals were obtained from each sampling site. Levels of $\delta^{15}N$ in the algal tissue of the S. j. var. religiosa were analyzed following the methods in Kuribayashi et al. (2016). Samples of algal tissues with few cracks, tears, and attached organisms on the surface were selected. Any dirt attached to the sample surface was carefully removed, and rinsed with filtered sea water, followed by washing with distilled water. The samples were dried in an oven at 60°C and homogenized. Levels of $\delta^{15}N$ were determined



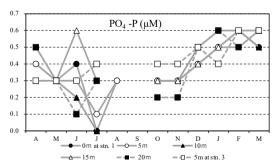
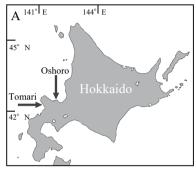
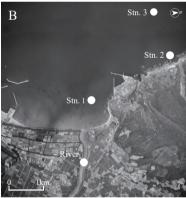


Fig. 3. Seasonal changes of nutrient concentrations at each depth of Stn. 2 and Stn. 3 in Shinminato based on reports from Hokkaido Government and Hokkaido Research Organization (Kawai and Goda, 2008-2011). In Shinminato, seawater was sampled at 0m, 5m, 10m, 15m and 20 m in depth at Stn. 2, and at only 5m in depth at Stn. 3 from 2007 to 2008. Location of the two sampling stations are shown in Fig. 1.

using an elemental analyzer equipped with an isotope ratio mass spectrometer (Fisons NA 1500-Finnigan MAT 252). $\delta^{15}N$ was expressed as per mille (‰) deviation from the standard (atmospheric N2) as defined by the following equation: $\delta^{15}N = \left[\left(^{15}N / ^{14}N\right)_{\text{sample}} / \left(^{15}N / ^{14}N\right)_{\text{standard}} - 1 \right] \times 1000$ (‰). The analytical error was within \pm 0.2‰.





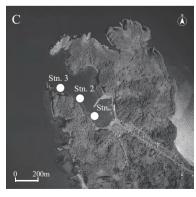


Fig. 4. Map showing sampling stations in Tomari (B) and Oshoro, Otaru (C), off the coast of Hokkaido, Japan. Aerial photography from Information Authority of Japan (https://mapps.gsi.go.jp/maplibSearch.do#1).

Results

Seasonal changes of nutrient concentrations at the four sampling stations on Rishiri Island are shown in Fig. 2. NO_3 -N and PO_4 -P concentrations have a similar tendency: increasing in winter from December to February, and decreasing from March to October. They were relatively high only at Shinminato Stn. 1 compared to values at the other sampling stations. The NO_3 -N concentration except Shinminato Stn. 1 reached 5μ M in winter and did not exceed 0.5 μ M in summer of all years. Similarly, PO_4 -P concentration reached 0.5 μ M in winter and did not exceed 0.1 μ M in summer for all years. On the other hand, the NO_3 -N and PO_4 -P concentrations at Shinminato Stn. 1 were higher throughout the survey period.

Seasonal changes of nutrient concentrations at each depth of Stn. 2 and Stn. 3 in Shinminato (Fig. 1) are shown in Fig. 3. NO₃-N and PO₄-P concentrations among each depth of Stn. 2 and Stn. 3 are similar without any exceptions.

Seasonal change of $\mathrm{NO_3}$ -N concentration at the four sampling stations in Tomari (Fig. 4A and 4B) are detailed in Table 1. Maximum and minimum concentrations were observed from January to March and from July to September respectively. Values from April to June were lower than those of October to December. The values among the four stations show spatial variations, values in the river (Stn. 1) are ten times higher than those of Stn. 2 and Stn. 3, and mean values of Stn. 1 are higher than those of Stn. 2 and Stn. 3.

The relationship between $\mathrm{NO_3}\text{-N}$ and salinity near the estuary, Stn. 1, is shown in Fig. 5. Autumn season (from October to December) and winter season (from January to March) have negative correlations whereas spring season (from April to June) and summer season (from July to September) do not have any correlations. Especially, the autumn season exhibits the clearest negative correlation,

Table 1. Comparison of NO₃-N concentration (mean value with standard deviation) in each sampling station in Tomari, southwestern coast of Hokkaido and Horikappu river, Japan, based on Hokkaido Government Reports (Hokkaido, 1998–2017). Location of each sampling station is shown in Fig. 4B.

	Horikappu River	Stn. 1	Stn. 2	Stn. 3
Apr.– June	25.6 ± 15.4	1.8 ± 1.5	1.2 ± 1.7	0.6 ± 0.7
July–Sep.	23.6 ± 6.1	0.8 ± 1.3	0.5 ± 0.8	0.2 ± 0.4
OctDec.	30.4 ± 10.2	3.4 ± 2.7	1.6 ± 1.6	1.6 ± 1.4
Jan.–Mar.	51.0 ± 20.7	5.8 ± 2.0	4.4 ± 1.3	5.0 ± 1.3

Table 2. Comparison of salinity (mean value with standard deviation) in each sampling station in Tomari, southwestern coast of Hokkaido and Horikappu river, Japan, based on using Hokkaido Government Reports (Hokkaido, 1998–2017). Location of each sampling station is shown in Fig. 4C.

	Horikappu River	Stn. 1	Stn. 2	Stn. 3
Apr.– June	0.02 ± 0.05	30.14 ± 2.78	30.28 ± 2.62	31.68 ± 1.21
July–Sep.	0.09 ± 0.17	31.82 ± 2.23	32.18 ± 2.16	32.26 ± 2.17
OctDec.	0.02 ± 0.04	31.46 ± 1.40	32.63 ± 0.84	33.28 ± 0.68
Jan.–Mar.	0.02 ± 0.04	32.53 ± 1.10	33.22 ± 0.59	33.38 ± 0.72

Y=-1.4655X+49.526, correlation is -0.76738.

In Oshoro (Fig. 4A and 4C), the δ^{15} N value in the tissues of the kelp *S. j.* var. *religiosa* at Stn. 1 (inner part of bay) is near 7 ‰, which is higher than that of 4–5 ‰ at Stn. 2 (middle-bay) and Stn. 3 (mouth of the bay) (Fig. 6).

Discussion

There are no large rivers on Rishiri Island in northern Hokkaido Japan (Fig. 1), especially there is no river or brook flowing along the coast of Shinminato (Fig. 1). However, Shinminato Stn. 1 (Rishiri Island in Fig. 1) showed higher concentration of NO₃-N and PO₄-P throughout the years of the research (Fig. 2). Abundant submarine groundwater discharges flow out along the coast near Shinminato (Hayashi *et al.*, 1999; Marui *et al.*, 1999; Yamaguchi & Ohara 1971; 1972). Nutrient concentrations at Shinminato Stn. 1 were certainly influenced by terrestrial sources, freshwater springs (submarine groundwater discharge). Nutrient concentrations

in Tanetomi, Senhoushi, and Oniwaki could represent values in the coastal waters of northern Hokkaido, the maximum value of NO3-N ranged from 5 to 10 μ M in the winter season (from December to February, 1992-1998) (Fig. 2). NO₃-N concentration Oshoro, Otaru, southwestern Hokkaido Japan, Sea of Japan showed generally 3–6 μ M in the winter season. The NO₃-N concentration in coastal waters of northern Hokkaido could be higher than that in Oshoro Bay in southwestern Hokkaido. Seasonal changes of nutrient concentrations at each depth (0-20 m depth) of sampling station in Shinminato, Rishiri, were similar (Fig. 3).

A large amount of NO₃-N is included in river water. Therefore, it is possible that river inflowing influx cause high NO3-N concentrations near estuarine and coastal areas in Tomari, Hokkaido, and is especially higher during the autumn season and lower during the winter season (Fig. 5A and 5B, Tables 1 and 2). The Hokkaido Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism, is monitoring volumetric flow rate of Horikappu River in Tomari (Fig. 4B), this can be downloaded from website (http://www.pref.hokkaido.lg.jp/ kn/kss/ksn/H22F209.pdf downloaded on 1st April 2019). The data indicate seasonal change with much water flow during April to August. The higher concentration of NO₃-N can contribute to development of rich kelp forest near estuarine areas, and broad saccharinian kelp forest are sustainably maintained near the estuary of the larger Shiribetsu River in southwestern Hokkaido Japan, while other coastal areas have been affected by the "Isoyake" phenomena (Kawai,

1997). We should focus on this function to supply nutrients in order to maintain kelp forests in Isoyake areas.

Nakata et al. (2001) reported that NO₃-N

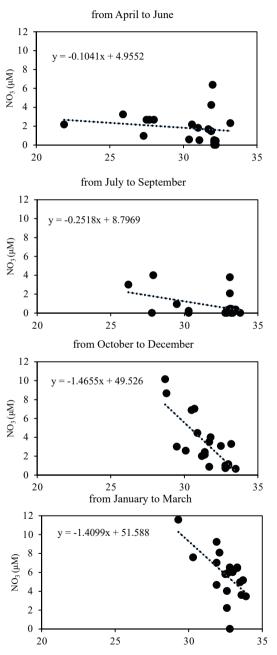
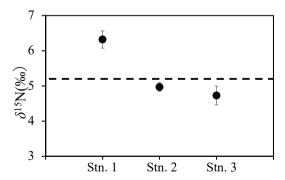


Fig. 5. Relationship between NO_3 -N concentration and salinity at Stn. 1, near the estuary at Tomari, southwestern coast of Hokkaido, Japan. Location of sampling station Stn. 1 is shown in Fig. 4B.

concentration in Oshoro Bay is highest in the inner part of the bay, next in the middle-bay, and the lowest at the mouth of a bay in the Appendix in the paper. Furthermore, Kawai et al. (2014) noted that the higher concentration of NO₃-N at Stn. 1 in Oshoro Bay had not been affected by the large river inflow and freshwater springs near the shore, but the bay has been affected by geographical features of subsidence and household discharge from landfill sites from several houses in the near shore in the bay (Fig. 4C). Therefore, it seems that some terrestrial waters that include a large amount of nutrients are inflowing into Oshoro Bay and remains in the bay, the terrestrial NO₃-N would be expected to be used by the kelp in the bay. $\delta^{15}N$ values in the tissue of saccharinian kelp at Stn. 1 (inner part of the bay) in Oshoro, Otaru, off the coast of Hokkaido, Japan is higher, near 7 ‰ than the δ^{15} N value of NO₃-N (5.3 ± 0.7 ‰) in the open sea off Hokkaido, Sea of Japan (Kuribayashi et al., 2017) (Fig. 5). All consumers show stepwise enrichment of ¹⁵N with the increasing trophic level (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). Humans which form a high trophic



level organism in food chains also shows high levels of δ^{15} N in body components. Moreover, NH₃ volatilization selectively removes isotopically lighter ¹⁴N to the atmosphere, with heavier ¹⁵N remaining at relatively higher levels. These properties promote high δ^{15} N of anthropogenic DIN in human activities. The δ^{15} N of DIN at Stn. 1 may be affected by input of anthropogenic DIN and would be expected to increase (McClelland & Valiela 1998; Jones *et al.*, 2001). This result indicates that the saccharinian kelp assimilates anthropogenic DIN from land areas.

DIN of the Sea of Japan, Hokkaido is lower than that of Pacific side of Hokkaido (Nakata et al., 2001; Hokkaido Reserch Organization, unknown; Nishihama & Kawamata 1979), initial growth of the kelp is during autumn to winter (Abe et al., 1983; Akaike et al., 1998; Shinada et al., 2014), concentration of NO_3 -N in the autumn and winter seasons influence remarkably the initial growth of the kelp (Kawai et al., 2018; Mizuta et al., 2001; Okada & Sanbonsuga 1980). Hence, inflow water (river, submarine discharged groundwater, terrestrial sources) containing DIN into coastal sea areas have an effect to provide NO₃-N for growth of the kelp. Knowledge of relationship between inflow land water and growth of the kelp will contribute for the study of Isoyake and sustainable exploitation of the kelp forest resource near river estuaries on the southwestern coast of Hokkaido.

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北海道日本海利尻島におけるコンブへの 陸からの栄養塩供給の寄与

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2008~2011年に、北海道日本海北部に位置する 利尻島におけるリシリコンブへの栄養塩供給を調査し た. 新湊, 種富, 仙法志, 鬼脇地区沿岸では, 新湊 地区でのみ硝酸塩濃度とリン酸塩濃度が比較的高く推 移し、湧水の寄与が示唆された。新湊地区では岸から 離れた場所の栄養塩濃度も各深度において冬に高く夏 に低くなる傾向が見られた。利尻島との比較目的で調 査した同じ北海道日本海の南西部に位置する泊村沿岸 では、10~3月に河川水の流入により河口域の硝酸 塩濃度が上昇していた。同じく北海道日本海南西部に 位置する小樽市忍路地区では、生活排水水由来と考え られる窒素がコンブ藻体に取り込まれていることが窒素 安定同位体比の分析により確かめられた。 これらの結 果から、利尻島沿岸では外海以外にも陸域がリシリコ ンブへの栄養供給源として重要であることが明らかにさ れた.