



## *Marine environmental factors influencing the rate of second-year transition of Rishiri Kombu in Rebus Island*

### Faktor lingkungan laut yang memengaruhi laju transisi tahun kedua Rishiri Kombu di Pulau Rebus

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#### ABSTRACT

The transition of *Saccharina japonica* var. *ochotensis* (Rishiri Kombu) from its first to second year of growth is crucial for commercial harvest. However, recent increases in seawater temperature and nutrient depletion are likely to influence this process. This study investigates the influence of autumn seawater temperature and nitrate concentrations on the second-year transition rate of Rishiri Kombu in northern Hokkaido. Field surveys were conducted from 2020 to 2024 at Ishikari and Rebus, where the population densities of first- and second-year individuals were quantified. Concurrently, in situ measurements of seawater temperature and nitrate concentrations were performed during autumn. The results indicate that higher seawater temperatures tend to be associated with lower second-year transition rates, whereas increased nitrate concentrations positively correlate with transition success. Statistical analyses suggest that nutrient availability has a stronger influence on transition rates than temperature changes. These findings suggest that nitrate depletion is a key limiting factor for the sustainability of Rishiri Kombu, highlighting the importance of monitoring climate-driven changes in nitrate availability in coastal ecosystems.

**Keywords:** Biennial growth cycle, Climate change impacts, Kelp population dynamics, Marine environmental impact, Nitrate availability

#### 1. Introduction

Rishiri Kombu (*Saccharina japonica* var. *ochotensis*) is a species of brown algae distributed along the northern coasts of the Sea of Japan and the Okhotsk Sea in Hokkaido (Yotsukura *et al.* 2008). Populations morphologically and ecologically similar to Rishiri Kombu, with closely related genetic structures, have also been identified near Ishikari City in regions influenced by the Ishikari River (Yotsukura *et al.* 2008). Along the Sea of Japan coast of Hokkaido, three taxa—*Saccharina religiosa* (Hosome Kombu), with a lifespan of one year; *Saccharina japonica* (Ma Kombu), which can survive up to two years; and *Saccharina ochotensis* (Rishiri Kombu), with a maximum

lifespan of two years—have traditionally been considered distinct due to differences in morphology and lifespan. However, Yotsukura *et al.* (2008) demonstrated through morphological observations, genetic analyses, and hybridization experiments that these three taxa belong to the same species.

Rishiri Kombu is an important fishery resource and serves as a fundamental ingredient in Japanese cuisine, especially as the base for dashi broth (Nagai and Kanetake 2001; Wada and Shiraishi 2002). Its economic value is significant, with only individuals that survive into their second year being targeted for harvest (Kawai *et al.* 2018). However, a sharp decline in harvest volumes has been observed in recent years (Nabata *et al.* 2003),

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and environmental changes affecting the transition to the second year of growth are considered a major contributing factor (Nabata *et al.* 1993). Previous studies have reported that water temperature is a key factor regulating growth and maturation (Kirihara *et al.* 2003), while nutrient availability strongly influences the early growth stages (Mizuta *et al.* 2001). Additionally, stable isotope analysis has shown that Rishiri Kombu incorporates nitrogen into its tissues (Kuribayashi *et al.* 2016).

Several studies have investigated the relationship between recent changes in the marine environment and Rishiri Kombu resource levels. Analysis of fishery statistics and mean seawater temperatures in northern Hokkaido revealed that years with higher autumn seawater temperatures correspond to lower harvest volumes in the following year (Kawai and Shinada 2020), suggesting that autumn seawater temperature may negatively impact the second-year transition rate. In coastal regions of Hokkaido and Sakhalin along the Sea of Japan, seawater temperatures tend to decrease northward. It has been reported that although first-year individuals of Rishiri Kombu tend to be smaller in northern regions, the total standing stock of first- and second-year individuals is higher there (Kawai *et al.*, 2014). Diving surveys around Rebun Island, northern Hokkaido, have confirmed that higher winter seawater temperatures significantly reduce the standing stock of Rishiri Kombu the following year (Kawai *et al.*

2024). Moreover, lower winter seawater temperatures correlate with higher nutrient concentrations, which lead to increased density and standing stock of first-year individuals (Shinada *et al.* 2014).

Taken together, these findings suggest that the transition of Rishiri Kombu from the first to the second year is highly influenced by autumn seawater temperature and nutrient conditions. However, direct field studies examining these relationships remain limited, particularly regarding the specific impact of autumn seawater temperature on the second-year transition rate. In this study, we hypothesize that “high autumn seawater temperatures and nitrate deficiency reduce the second-year transition rate of Rishiri Kombu.” To test this hypothesis, field surveys were conducted in northern Hokkaido, and the results are reported herein.

## 2. Methods

### 2.1. Rishiri Kombu Survey

The survey was conducted from 2020 to 2024. The survey sites were Ishikari (43°21'12.9"N 141°25'32.5"E, coordinates obtained from Google Maps: <https://www.google.co.jp/maps>) and Rebun (45°18'04.6"N 141°01'29.1"E) (Figure 1). The seabed in both areas was primarily composed of bedrock, with a gently sloping topography extending offshore (reaching a depth of approximately 10 m at 200 m from the shore).

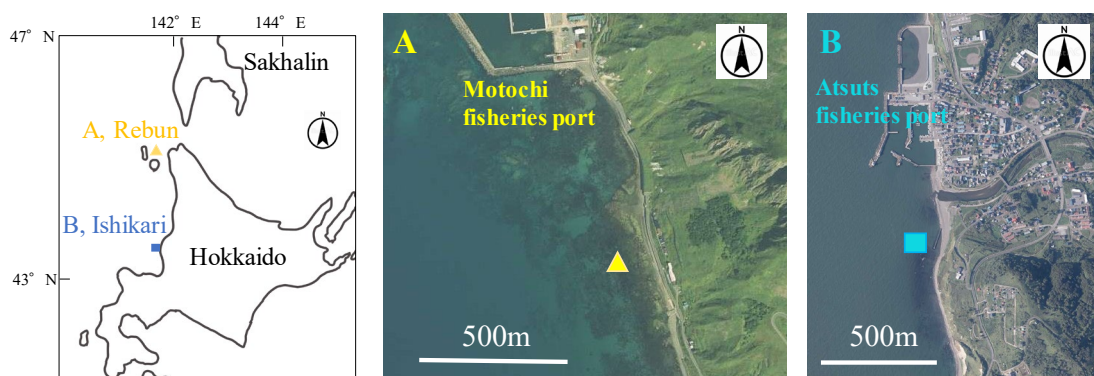


Figure 1. Map of the survey locations for *Saccharina japonica* var. *ochotensis* at Ishikari and Rebun, northern Hokkaido, Japan. Panel A and B are modified from the Geospatial Information Authority of Japan (<https://www.gsi.go.jp/tizu-kutyu.html>).

The survey was conducted annually in July, when the Rishiri Kombu population reaches its annual peak. At each survey site, we placed four quadrats of 0.5 m<sup>2</sup> at a depth of 2–3 m, the main growth zone of Rishiri Kombu. The total surveyed area was 1 m<sup>2</sup>, with four quadrats of 0.5 m<sup>2</sup> each. The collected specimens were classified by age following Yotsukura *et al.* (2008). Individuals whose holdfasts did not form concentric layers were classified as first-year individuals, while those with holdfasts exhibiting multiple layers formed in the previous year were classified as second-year individuals (Figure 2). The number of individuals in each category was recorded.



Figure 2. Morphological features of first- and second-year individuals of *Saccharina japonica* var. *ochotensis*. A, Sporophytes: the first-year individual is shown below and the second-year individual above; B, Holdfast of a second-year individual; C, Holdfast of a first-year individual.

## 2.2. Marine Environmental Survey

The marine environmental surveys were conducted from 2020 to 2023. In each survey year, a self-recording water temperature logger (Onset Corporation, Tidbit V2, UTBI-001) was deployed at a depth of 1.5 m at each survey site before September 1st. Temperature data were continuously recorded, and monthly average temperatures were calculated for the first and second halves of each month until December 15th.

To measure nitrate concentrations, water

samples were collected twice per month from September to early December at the same locations as the Rishiri Kombu survey. Nitrate concentration, a key limiting nutrient, was quantified using an autoanalyzer (TRACCS800, B+L Tech) following the cadmium reduction-naphthylethylenediamine spectrophotometric method.

Statistical analyses were performed using Python software, and hypothesis testing and statistical analyses were conducted based on methods described by Virtanen *et al.* (2020).

## 3. Result and Discussion

### 3.1. Result

#### 3.1.1. Rishiri Kombu Survei

The second-year transition rate of Rishiri Kombu was calculated using the following formula: (Number of second-year individuals in the survey year/Number of first-year individuals in the previous year)×100(%). The survey results for transition rates are shown in Figure 3. In Ishikari in 2023, the seawater temperature remained abnormally high from late summer to early autumn, and as a result, no first-year Rishiri Kombu individuals were observed in 2024. Therefore, the second-year transition rates for Ishikari and Rebun in 2024 were excluded from the analysis.

The average transition rate was 1.38 in Ishikari and 0.80 in Rebun. To evaluate differences between the two regions, Welch's *t*-test (Welch 1947) was applied. The calculated *t*-value was 1.83, and the *p*-value was 0.079. Since the significance level was above 0.05, the difference was not statistically significant; however, as the *p*-value was below 0.1, a marginal trend toward significance was observed.

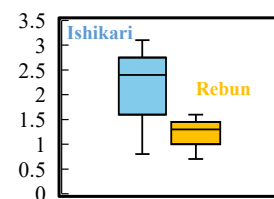


Figure 3. Box-and-whisker plot comparing the second-year transition rates of *Saccharina japonica* var. *ochotensis* between Ishikari and Rebun.

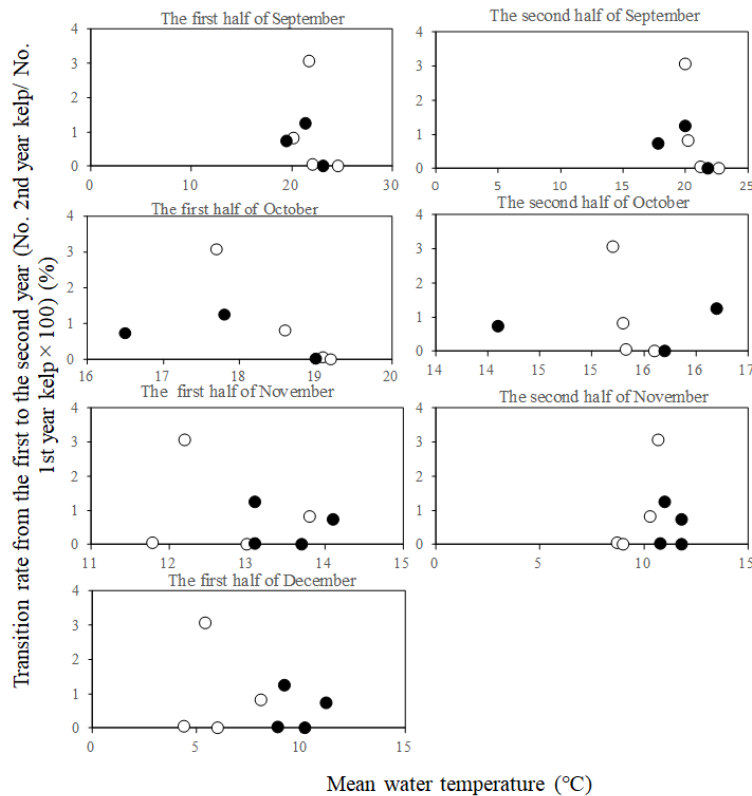
3.1.2. Marine Environmental Survei

The relationship between transition rates and seawater temperature is shown in Figure 4. The statistical analysis was conducted by combining the water temperature data from surveys performed annually at Ishikari and Rebun, and correlations were analyzed using Pearson’s correlation coefficient. The correlation coefficient was -0.33, with a *p*-value of 0.523, indicating a weak negative correlation that was not statistically significant. This suggested a weak negative tendency between transition rates and seawater temperature; however, the relationship was not statistically significant ( $r = -0.33, p = 0.523$ ).

The relationship between transition rates and nitrate concentrations is shown in Figure 5. The combined nitrate concentration data from Ishikari and Rebun were analyzed in relation to transition rates. The correlation coefficient was 0.83, with a *p*-value of 0.042, indicating a statistically significant positive correlation. This result suggests that transition rates tend to increase as nitrate concentrations rise.

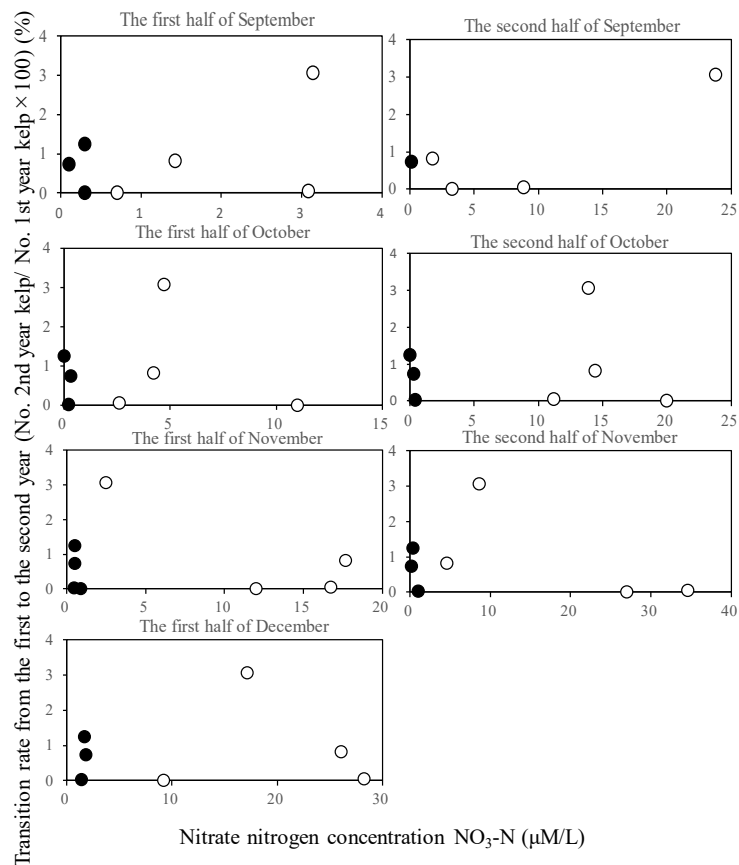
To further investigate these relationships, regression analysis was performed. In a simple regression model, the regression coefficient for seawater temperature and transition rates was  $\beta = -0.20$ , with an  $R^2$  value of 0.109 and a *p*-value of 0.523. This indicates a tendency for seawater temperature to negatively affect transition rates, but the effect was not statistically significant.

For the simple regression model of nitrate concentration and transition rates, the regression coefficient was  $\beta = 0.81$ , with an  $R^2$  value of 0.684 and a *p*-value of 0.042, demonstrating a significant positive effect of nitrate concentration on transition rates. In a multiple regression model incorporating both seawater temperature and nitrate concentration, the coefficient of determination ( $R^2$ ) was 0.779, indicating a good fit. The *p*-value for seawater temperature was 0.340, whereas for nitrate concentration, it was 0.057. These results suggest that the influence of nitrate concentration was stronger, while the effect of seawater temperature was nearly negligible.



Notes: Black circles represent Rebun, and white circles represent Ishikari.

Figure 4. Relationship between the second-year transition rates of *Saccharina japonica* var. *ochotensis* and autumn mean seawater temperature.



Black circles represent Rebun, and white circles represent Ishikari.

Figure 5. Relationship between the second-year transition rates of *Saccharina japonica* var. *ochotensis* and autumn nitrate concentration.

### 3.2. Discussion

In this study, we examined the relationship between the second-year transition rate of Rishiri Kombu and autumnal marine environmental factors, specifically seawater temperature and nitrate concentration (Figure 6). The results showed a trend where higher autumn seawater temperatures were associated with lower second-year transition rates, whereas higher nitrate concentrations correlated with increased transition rates. These findings support our initial hypothesis, which stated that high autumn seawater temperatures and nitrate deficiency reduce the second-year transition rate of Rishiri Kombu.

Moreover, our results are generally consistent with previous studies. This study is particularly significant because it empirically verified the negative impact of high autumn seawater temperatures and low nitrate availability on second-year transition rates using field-based diving surveys and actual

marine environmental measurements. Additionally, statistical analyses clarified that nitrate deficiency had a more significant impact on the decline in transition rates than seawater temperature.

Previous studies have reported that areas near river inflows tend to maintain higher nutrient concentrations compared to other marine areas (Kawai and Kuribayashi 2021), and correspondingly, the occurrence of second-year Rishiri Kombu tends to be higher in such locations (Kawai and Kuribayashi 2024). In this study, the transition rate in Ishikari was higher than in Rebun, which is consistent with previous findings. While this study demonstrated the influence of autumn seawater temperature on the second-year transition rate of Rishiri Kombu, several unresolved issues remain. In addition to seawater temperature, factors such as light conditions and ocean currents may also impact transition rates and should be considered in

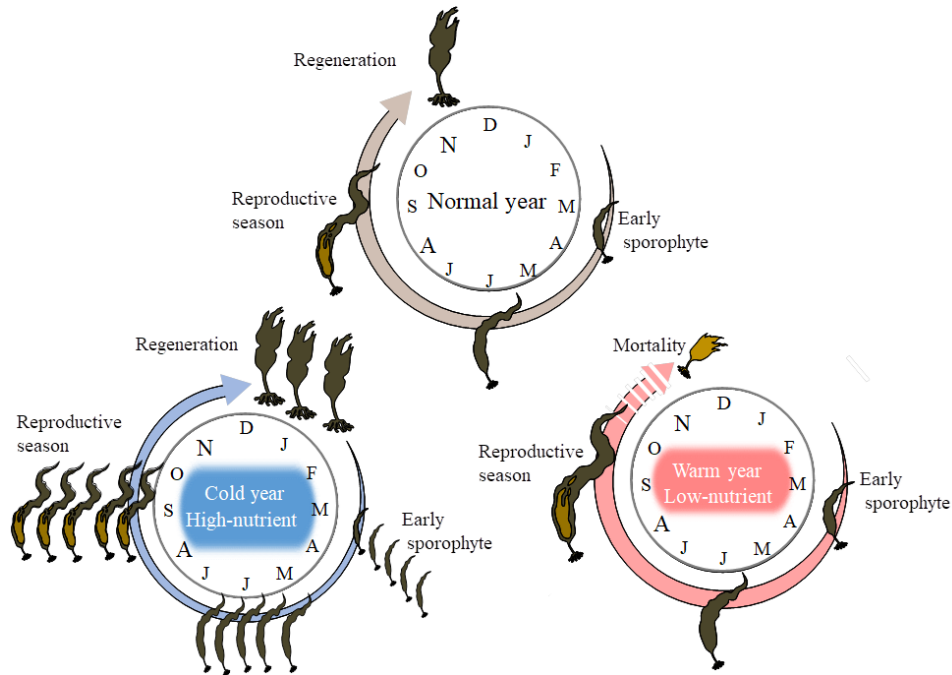


Figure 6. Schematic diagram of the relationship between the second-year transition rates of *Saccharina japonica* var. *ochotensis* and marine environmental factors. The diagram was created based on this study as well as previous studies (Kawai *et al.* 2024; Kawai and Shinada 2020).

future research. Moreover, further analysis using long-term data spanning several decades would provide deeper insights into the relationship between warming trends and the population dynamics of Rishiri Kombu. Additionally, controlled laboratory experiments and aquaculture trials, where seawater temperature and nitrate concentrations are precisely controlled, would help complement the findings obtained through field observations.

It has also been reported that lower winter seawater temperatures correlate with higher nutrient concentrations, leading to increased density and standing stock of first-year Rishiri Kombu (Shinada *et al.* 2014).

#### 4. Conclusion

This study demonstrated that the second-year transition rate of Rishiri Kombu is influenced by autumn seawater temperature and nitrate availability. The results indicated that years with higher seawater temperatures and lower nitrate concentrations tend to exhibit lower transition rates. This suggests that the increase in autumn seawater temperatures and

the decline in nitrate concentrations due to global warming may contribute to the decline in Rishiri Kombu populations. These results are consistent with previous studies, reinforcing the hypothesis that the decrease in nutrient availability plays a more significant role in reducing transition rates than seawater temperature.

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