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Hubungan antara kondisi lingkungan dan pertumbuhan dua tahunan rumput laut *Saccharina japonica* var. *ochotensis*, Hokkaido utara, Jepang

Relationship between environmental conditions and biennial growth of kelp Saccharina japonica var. ochotensis*, northern Hokkaido, Japan*

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ABSTRAK

Hamparan rumput laut *Saccharina japonica* var. *ochotensis* muncul di laut utara Jepang. Survei penyelaman bulanan dilakukan di dua stasiun pengambilan sampel, Tsugaru di Pulau Rebun dan Hourai di distrik Wakkanai di utara Hokkaido, Jepang, dari Maret 2013 hingga November 2014. Kondisi oseanografi, suhu air, nitrat, dan radiasi dicatat, dan beberapa perbedaan dicatat, di antara Tsugaru dan Hourai. Radiasi yang lebih tinggi di Tsugaru selama Juli dan Agustus menyebabkan peningkatan fotosintesis oleh sporofit, dan suhu yang lebih rendah di area ini menyebabkan peningkatan penyimpanan manitol dari April hingga September, dengan lebih banyak polisakarida tersulfasi dalam sporofit dan ketebalan bilah yang lebih besar dibandingkan dengan spesimen rumput laut di Hourai. Suhu air yang lebih tinggi di Tsugaru selama bulan September dan Februari menyebabkan sporofit lebih besar, sedangkan suhu air musim dingin yang lebih rendah di Hourai menyebabkan sporofit lebih kecil. Suhu air yang lebih tinggi dari September hingga Februari dan konsentrasi nitrat yang lebih rendah sepanjang tahun kecuali pada April 2013 dan Maret 2014 di Tsugaru berkaitan dengan tingkat pembentukan sorus yang lebih tinggi dan tingkat regenerasi (transisi dari sporofit tahunan ke dua tahunan) yang lebih rendah daripada di Hourai. Penyinaran di Tsugaru lebih tinggi daripada di Hourai dari Juli hingga Agustus. Suhu yang lebih rendah dan radiasi yang lebih rendah menghasilkan laju pembentukan sorus yang lebih rendah; sedangkan konsentrasi nitrat yang lebih tinggi memunculkan laju regenerasi yang lebih tinggi.

Kata kunci: kepadatan, cahaya, nitrat, regenerasi, suhu air

The kelp Saccharina japonica *var.* ochotensis *occurs in the northern Sea of Japan. A monthly diving survey was conducted at two sampling stations, Tsugaru in Rebun Island and Hourai in Wakkanai district in northern Hokkaido, Japan, from March 2013 to November 2014. Oceanographic conditions, water temperature, nitrate, and irradiance were recorded, and several differences were observed between Tsugaru and Hourai. Higher irradiance at Tsugaru during July and August led to enhanced photosynthesis by sporophytes, and the lower temperature in this area led to increased storage of mannitol from April to September, with more sulfated polysaccharide in sporophytes and greater blade thickness compared to kelp specimens at Hourai. Higher water temperatures in Tsugaru during September and February led to larger sporophytes, whereas lower winter water temperatures in Hourai led to smaller sporophytes. Higher water temperatures from September to February and lower nitrate concentrations throughout the year except in April 2013 and March 2014 in Tsugaru were associated with higher sorus formation rates and lower regeneration (transition from annual to biennial sporophytes) rates than at Hourai. Irradiance at Tsugaru was higher than at Hourai from July to August. Lower temperatures and lower radiation result in a lower rate of sorus formation; whereas higher nitrate concentrations give rise to higher rates of regeneration.*

Keywords: *density, light, nitrate, regeneration, water temperature*

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1. Introduction

The kelp *Saccharina japonica* var. *ochotensis* (Miyabe) N. Yotsukura, S. Kawashima, T. Kawai, T. Abe *et* L.D. Druehl is distributed in the northern part of the Sea of Japan, between Hokkaido, Japan, southern Prymorie and southern Sakhalin in south-east Russia (Yotsukura *et al*. 2008). The species is one of the most important algal resources in northern Japan and south-east Russia, and both harvesting from aquaculture using ropes and collection of natural resources are established in northern Japan and south-east Russia (Kawai *et al*. 2014).

The life cycle of the species in northern Japan is as follows: zoospores are released from mature sporophytes (i.e. with sori occurring on their blades) during the autumn season, then zoospores attach to the substratum and grow as gametophytes during the winter season. Small (less than 1 cm) first year sporophytes occur during the early spring season and they grow quickly as large sporophytes, huge first year sporophyte kelp forests emerged before early summer season; then the terminal portions of the sporophytes are eroded and their length reduces during the summer season. Early in the autumn season, first year sporophytes have a holdfast, stipe and short blade (less than 30 cm), some sporophytes have sori to store zoospores for reproduction, but some sporophytes do not have sori and grow again as second year sporophytes (Sanbonsuga and Torii 1973; Akaike *et al*. 1998; Akaike and Tsuda 2005; Kawai *et al*. 2014).

Sporophytes of the species reach approximately 2 m in length and more than 30 cm in width, and plants can survive for 2 years (Yotsukura *et al*. 2008). Fishermen in this area harvest only second year individuals (Kawai *et al*. 2015). The kelp has been utilized mostly for the extract of "Dashi" stock (Ueda 1997), and in particular, traditional Kyoto dishes regard the kelp from Rebun Island, northern Hokkaido, to be of excellent quality, and it is therefore more expensive. However, the annual production of this alga from catch of natural resources of kelp in Rebun Island was recently been decreasing and investigation of the cause of the decrease is urgent for the sustainable utilization of this algal resource.

Previous studies have revealed that water temperature and nutrient concentrations influence growth and maturity of sea algae (Black and Dewar 1949; Atkinson and Smith 1983; Lobban and Harrison 1994; Nakata *et al*. 2001; Carney and Edwards 2010). This has also been suggested in saccharinian kelp species (Chapman and Craigie 1977; Anderson *et al*. 1981; Druehl *et al*. 1989; Egan and Yarish 1990). In particular, the relationship between growth of Saccharina japonica var. religiosa and water nutrient concentrations was studied in coastal areas of southwestern Hokkaido, finding that in general lower temperature (3–5 °C) with richer nutrient concentrations (more than 5 μM/L) promote richer kelp forests (Parke 1948; Larsen and Jensen 1957; Zimmerman 1985; Akaike *et al*. 1998; Akaike and Tsuda 2005; Agatsuma *et al*. 2014; Kuribayashi *et al*. 2016). This has also been confirmed under laboratory conditions in the gametophyte stage of *S. japonica* (Okada and Sanbonsuga 1980; Mizuta *et al*. 2001; Kawai *et al*. 2004) and sporophyte stage (Reed 1987; Nimura *et al*. 2002). Also, physiological and biochemical properties of laminarian species are different for each life stage (Hanelt *et al*. 1997; Wang *et al*. 2013; Bischof *et al*. 2019), and light irradiance has been shown to influence the sea algae (Lüning 1969; Round 1984; Dieck 1991; Kirk 1994), particularly ultraviolet radiation of light has been show to affect growth of laminariales species (Dring *et al*. 1996a, 1996b; Müller *et al*. 2008; Roleda *et al*. 2010). Although there are no scientific reports, aquaculture farmers along the coast of northern Hokkaido have stated that, based on their past experience, larger kelp individuals are not able to survive into their second year. With this knowledge, the aquaculture farmers suppress the growth of juvenile kelp sporophytes. Perhaps the first year sporophytes reach sexual maturity during summer season, after sporulation and release their zoospores during early in the autumn season before they die-off.

Relationships between oceanographic environmental conditions and the growth, maturity, and regeneration of *S. j.* var. *ochotensis* in northern Hokkaido, Sea of Japan, have not yet been studied. The present study compares the body size, sorus formation, and regeneration of the kelp *S. j.* var. *ochotensis* in a rich kelp forest (Hourai, Wakkanai district) and a poor kelp forest (Tsugaru, Rebun Island) in northern Hokkaido, and examines the difference in light irradiance, water temperature, and nutrient concentrations in between two areas. Also, a kelp tagging experiment using disc tags was carried out using larger (blade length more than 40 cm) kelp and smaller (less than 40 cm) kelp individuals, to clarify whether maturity and regeneration of kelp individuals is based on body size or not. The present study provides basic information for management of kelp fisheries and contributes to the sustainability of kelp resources in the northern area of the Sea of Japan, Hokkaido, Japan.

2. Methods

2.1. Time and location

Sampling stations were set up in Tsugaru, Rebun Island (45.311N, 141.053E), and Hourai, mainland Hokkaido (45.422N, 141.676E), at the northern end of the Japanese Archipelago (Figure 1). The Tsushima warm current flows directly past Tsugaru, Rebun Island, but a small branch of the Tsushima warm current often flows past Hourai. This provides sort of different oceanographic environmental conditions at the two sampling stations. These two areas have kelp fishery grounds where the bottom slopes down from 0 m to 8 m depth, and the major substratum is bedrock or large boulders over 1 m in diameter.

2.2. Tools and materials

Digital data loggers (HOBO Pendant Temperature/Light 64K Data Logger, Onset Computer Corporation Co. Ltd. Massachusetts, U.S.A.) were set on the bedrock at 1.5 m depth to record temperature and light irradiance at 1-h intervals in Tsugaru and Hourai from March 2013 to November 2014. Bottom seawater was sampled by scuba divers monthly from April to November 2013 at depths of 1.5-2.0 m and in 2014. The seawater samples were immediately frozen without filtering, and stored at -30 °C. The NO_3 concentration of the samples was determined using an Auto Analyzer (TRACCS 800, BL-TEC, Tokyo, Japan) by the naphthylethylene-diamine method (Armstrong *et al*. 1967).

2.3. Research Method

Kelp samples were collected every month using 25 cm X 25 cm quadrats. A quadrat was put randomly on the sea floor in the center of the kelp fisheries ground for a total of four times to cover 1 m^2, and kelp sporophytes in a

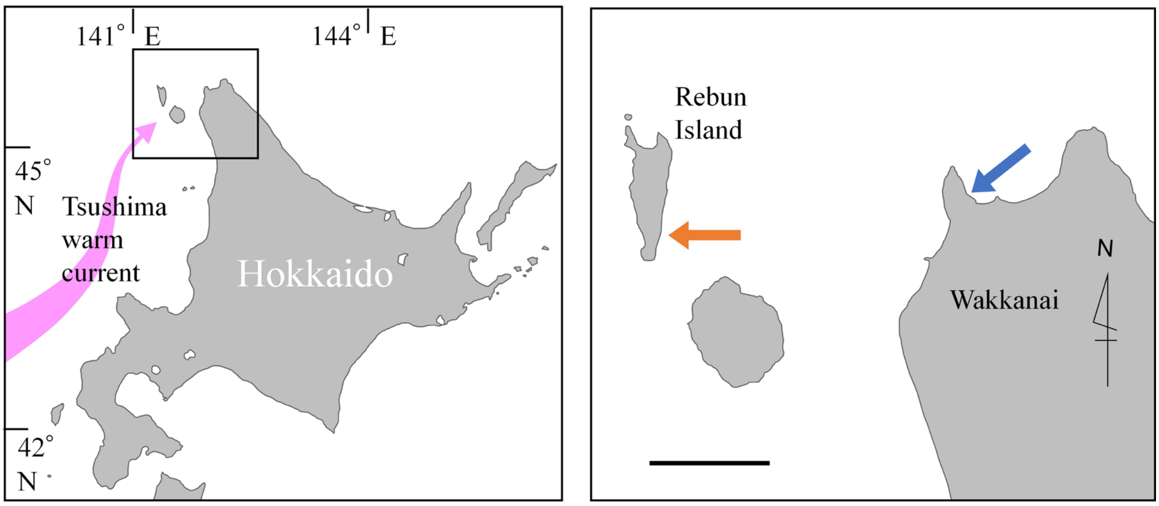

Figure 1. Map showing the sampling station.

Orange arrow indicates Tsugaru, Rebun Island, and blue arrow indicates Hourai, Wakkanai. Scale bar, 20 km.

total area of 1 m^2 were sampled to determine the mean weight of sporophytes, density, and standing crop of annual (one year or first year) kelp. The largest 10 annual sporophytes of samples were measured for blade length, blade width, blade weight, and blade thickness, and occurrence of sori and presence or absence of regeneration from the first year to the biennial (two year or second year) kelp specimens were recorded. Age of sporophytes was discriminated by the shape of the holdfast (haptera) (Yotsukura *et al*. 2008), collected sporophytes have holdfasts that arise as one or two tiers and the second tier covers the proceeding the first tier, forming a conical and two tiers holdfasts which is defined as a biennial, single tier and only new holdfasts which are formed flat and plate-like holdfasts are defined as annual sporophytes. Presence or absence of sorus formation and regeneration were recorded, based on previous studies of the life cycle of the kelp (Sanbonsuga and Torii 1973; Akaike *et al*. 1998; Akaike and Tsuda 2005), if sori occur on one-side or both sides of blade, it was recorded as sorus occurrence. If the collected sporophyte has a new tiers which covers the proceeding tier and blade (lamina) has a narrow area by regrowing after erosion of the blade during summertime, the sporophyte is regeneration from first year sporophyte to second year sporophyte, if the sporophyte does not show those two features, then the first year sporophyte is not a regenerating sporophyte for the second year.

In the field, plastic 1 cm diameter disc tags were tied onto the stipe of kelp by cable ties (Gouda and Kawai 2012). Thirty first year kelp sporophytes were randomly labeled every diving survey in April 2013, and the occurrence of tagged sporophytes were recorded to calculate their survival rate. Survival rate in each survey month was calculated as the number of obtained tagged sporophytes in each survey /number of initial tagged sporophytes (30) X 100 (%).

By the chemical analysis, mannitol content of kelp sporophyte was found to fluctuate seasonally (Kodama and Kawai 2019). Thus, there is a possibility that the distribution of carbohydrate in the sporophyte also changed.

In this research, to examine this, a histochemical investigation was performed. Ten one-year individuals of the sporophytes from the collected samples by scuba diving survey sampling were processed to make tissue sections for histochemical observations. Tissue samples (approximately 1 cm×1 cm square) were obtained from around the growth point (meristem) of the basal portion of the blade of each kelp individual. The tissues were fixed in Formalin-acetic acid-alcohol (FAA) solution for approximately 24 hours; the solution was arranged as formalin 10 ml, acetic acid 5 ml, and 95 % ethanol 50 ml, diluted to 100 total with distilled water. FAA fixed samples were stored in 70 % ethanol until examination. The stored tissues were embedded in paraffin (Histprep 568, Wako Chemical Co Ltd, Tokyo Japan) through dehydration in a series 70–100 % ethanol and xylene. The embedded tissues were cut into sections of cross direction for blade by rotary microtome with thickness of 7 μm and mounted. The sections were treated with alcian blue pH 2.5 and periodic acid Schiff (PAS) double stain, method of classification that acid or natural polysaccharide and single carbohydrate distribution. Stained sections were observed by optical microscopy. These samples were also determined mannitol content by titration method (Cameron *et al*. 1948; Larsen 1978). These results have already been reported in another study (Kodama and Kawai 2019). To compare the survival rate of different length sporophytes, a total of 60 first year sporophytes were examined. The 30 larger (over 40 cm in blade length) and 30 smaller (less than 40 cm) sporophytes, were tagged with the 1 cm plastic disc tags (Gouda and Kawai 2012) in Tsugaru, Rebun Island, on 2nd September 2015. The labeled sporophytes were re-examined on 12th November 2015, and the blade length of the sporophytes were measured.

3. Result and Discussion

3.1. Result

Water temperatures at the two sampling sites, Tsugaru and Hourai, were recorded, and the difference in temperature was calculated

(Figure 2). In Tsugaru, water temperature was higher than that at Hourai during October, November, and February, and lower from March to September (Figure 2A-B). Irradiance at Tsugaru was higher than at Hourai from July to August (Figure 3). Nitrate concentrations at Tsugaru and Hourai were compared each month (Figure 4), values of nitrate concentration from May to November were lower than that at Hourai except for April in 2013, and the values of concentration in Hourai after September was more than twice that at Tsugau, 3.7 times (value of Hourai/Tsugaru) in September, 2.5 times in October, 2.3 times in November in 2013, the values from June to November were lower than that at Hourai except for April in 2013 and May in 2014.

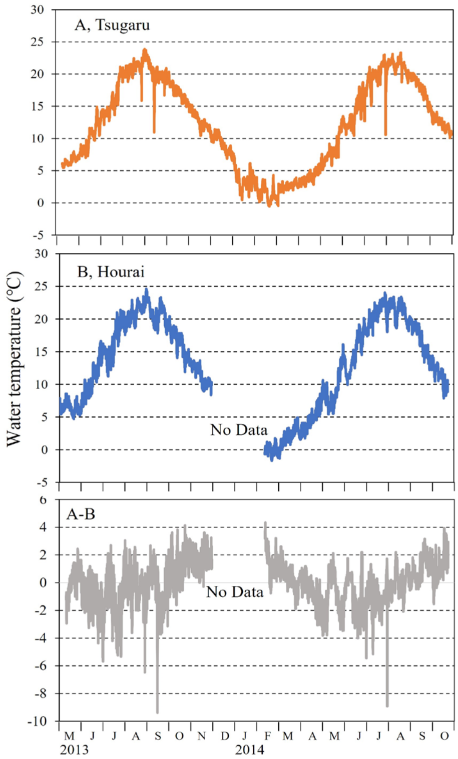

Figure 2. Seasonal changes of water temperature in Tsugaru, Rebun Island (orange color) and Hourai, Wakkanai (= blue color), northern Hokkaido, Japan and difference (A-B) between the two sampling stations.

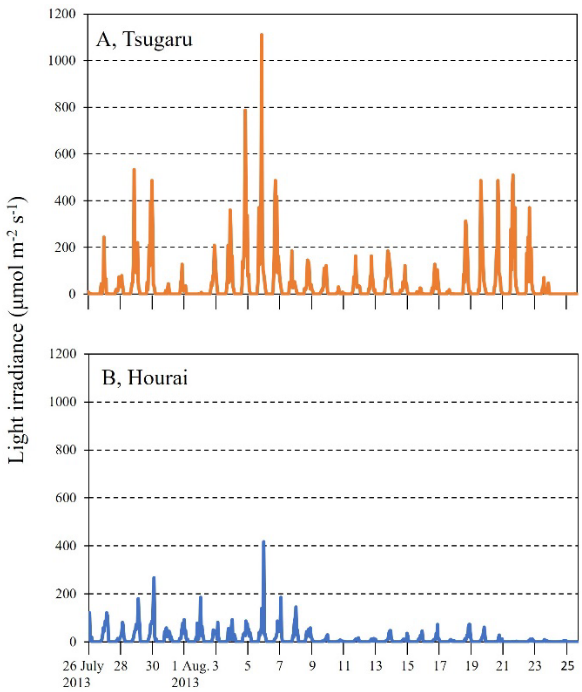

Figure 3. Change of light irradiance in Tsugaru, Rebun Island (orange) and Hourai, Wakkanai (blue), northern Hokkaido, Japan. Data logger was set in depth 1.5 m for both sites.

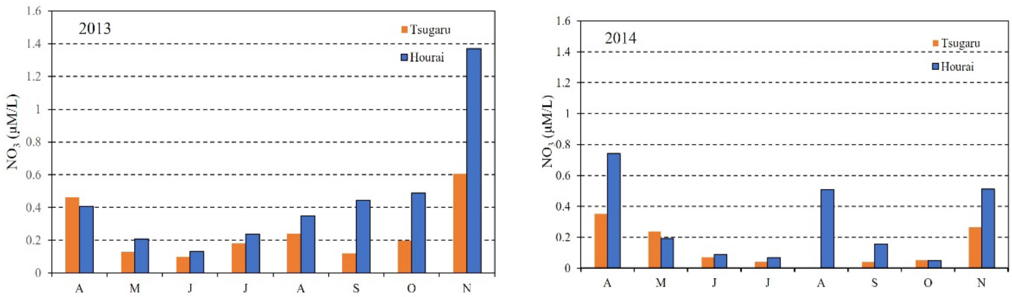

Figure 4. Nitrate concentration in Tsugaru, Rebun Island (orange) and Hourai, Wakkanai (blue), northern Hokkaido, Japan in 2013 and 2014.

Monthly changes in density and biomass (as determined by quadrat), and survival rate of sporophytes (as determined by tag markers) for 2013 are shown and compared between Tsugaru and Hourai in Figure 5. Density at Tsugaru decreased from April to June, then sharply increased in July before decreasing again after August. Density of kelp individuals at Hourai decreased from April to October. Biomass of kelp individuals in Tsugaru increase from April to July, then decreased. However, biomass of the kelp in Hourai increased from April to July then it decreased to November. Whereas, the change of biomass in Hourai was relatively constant throughout the research period. Survival rates of kelp individuals in Tsugaru decreased from July to November. However, in Hourai were over 90% in July, then decrease from July to August, after which survival rate was around 60%.

Monthly change of size of the kelp sporophyte individuals in Tsugaru and Hourai for 2013 and 2014 are shown in Figure 6. Blade length at the two sampling stations was similar throughout the research period in 2013 and the length in Tsugaru was longer except for November in 2014, in spite of blade weight and blade thickness of kelp at Tsugaru being larger than kelp individuals at Hourai except for November in 2014. Particularly, blade weight of kelp individuals at Tsugaru from June to July was heavier than that of kelp individuals at Hourai (Figure 6).

Sorus formation rate of kelp sporophyte individuals from Tsugaru and Hourai were markedly different (Figure 7). Samples from Tsugaru had formation rates over 86.7 % from September to November in 2013 and 93.3 % in November of 2014, but kelp individuals in Hourai had less than 10.0 % formation rate in 2013 and 2014 (Figure. 7). There was a large difference of regeneration rate of kelp individuals between Tsugaru (13.3 %) and Hourai (83.3 %) in 2013, between Tsugaru (6.6–40.0 %) and Hourai (3.3–93.3 %) in 2014.

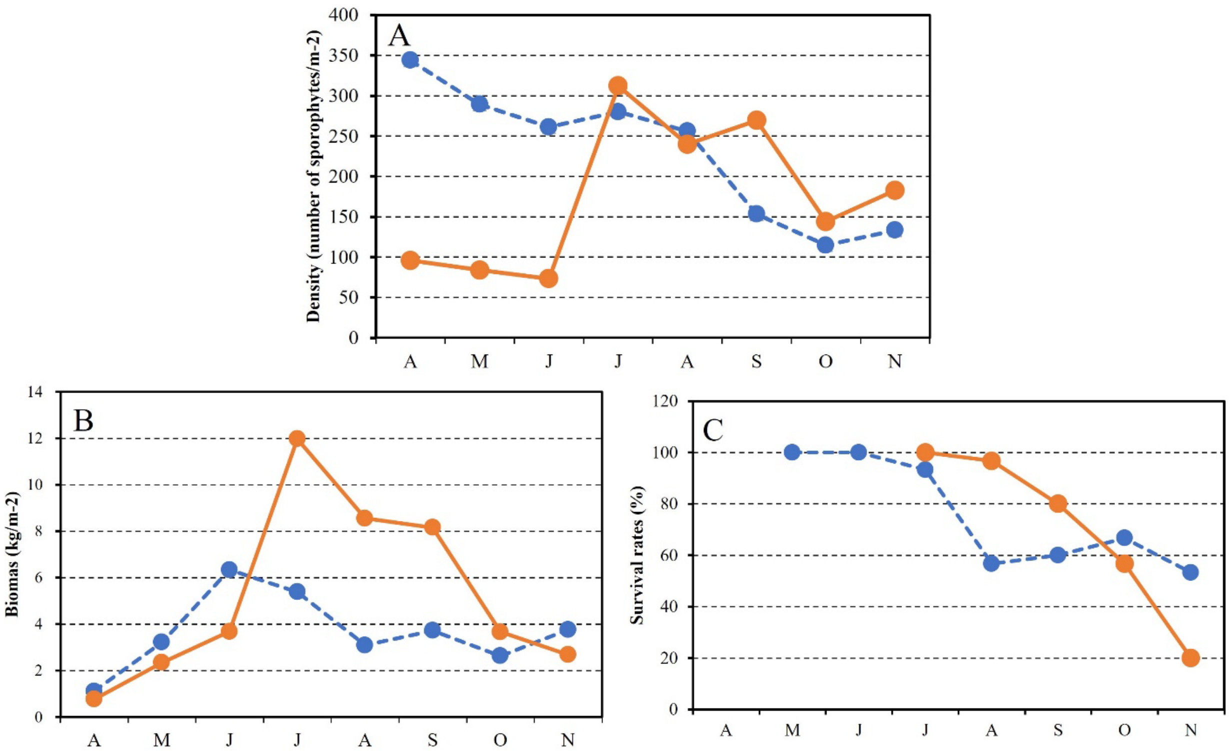

Figure 5. Monthly change of density, biomass, and survival rate of the kelp *Saccharina japonica* var. *ochotensis* in Tsugaru, Rebun Island and Hourai, Wakkanai, northern Hokkaido, Japan.

Orange color circles with line denotes Tsugaru, and blue color circles with dashed line denotes Hourai. Survival rate was calculated as (number of collected tagged sporophytes / collected first year 10 largest sporophytes x 100, %).

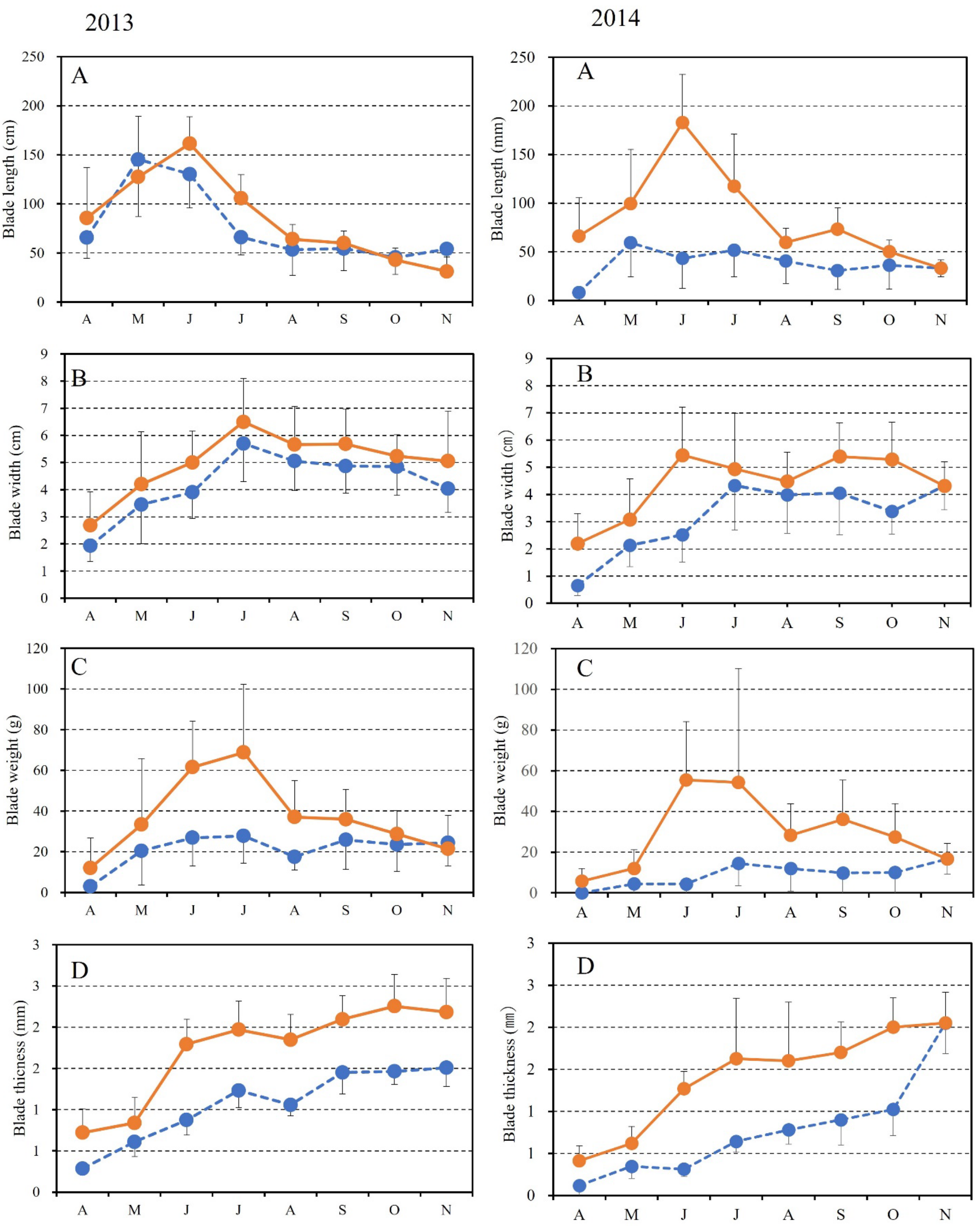

Figure 6. Monthly change of blade length, width, weight, and thickness of the kelp *Saccharina japonica* var. *ochotensis* in Tsugaru, Rebun Island and Hourai, Wakkanai, northern Hokkaido, Japan.

Orange color circle with line denotes Tsugaru, and blue color circle with dotted line denotes Hourai, vertical bars on the circles indicate standard deviation.

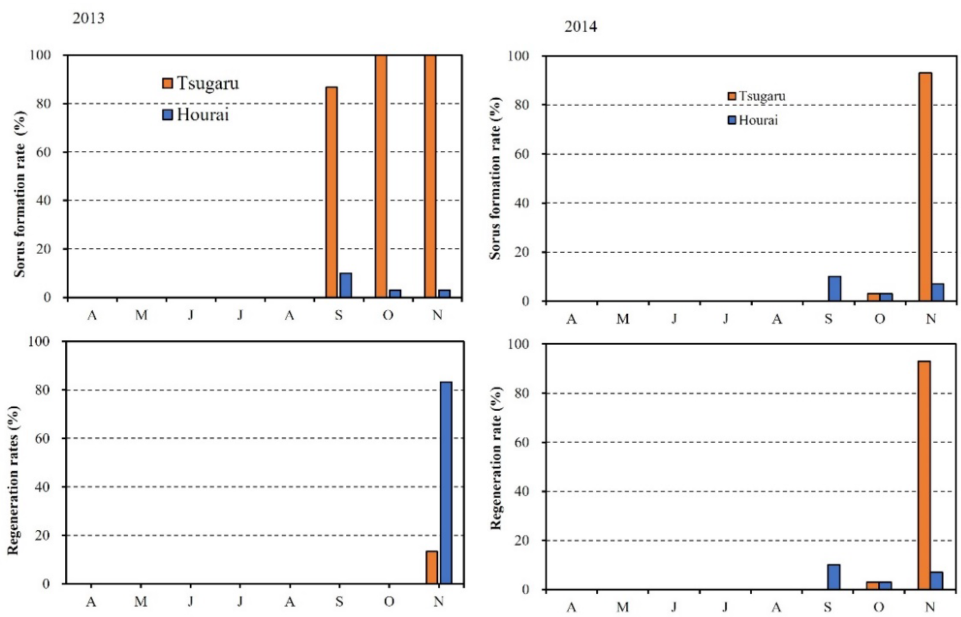

Figure 7. Monthly change of sorus formation and regeneration from the first year to the second year of the kelp *Saccharina japonica* var. *ochotensis* in Tsugaru, Rebun Island (= orange color) and Hourai, Wakkanai (= blue color), northern Hokkaido, Japan.

Sorus formation rate was calculated as (number of sorus occurred sporophytes / number of collected first year sporophytes (10) x 100, %). Regeneration rate was calculated as (number of regenerated sporophytes from first year to second year / number of collected first year sporophytes (10) x 100, %).

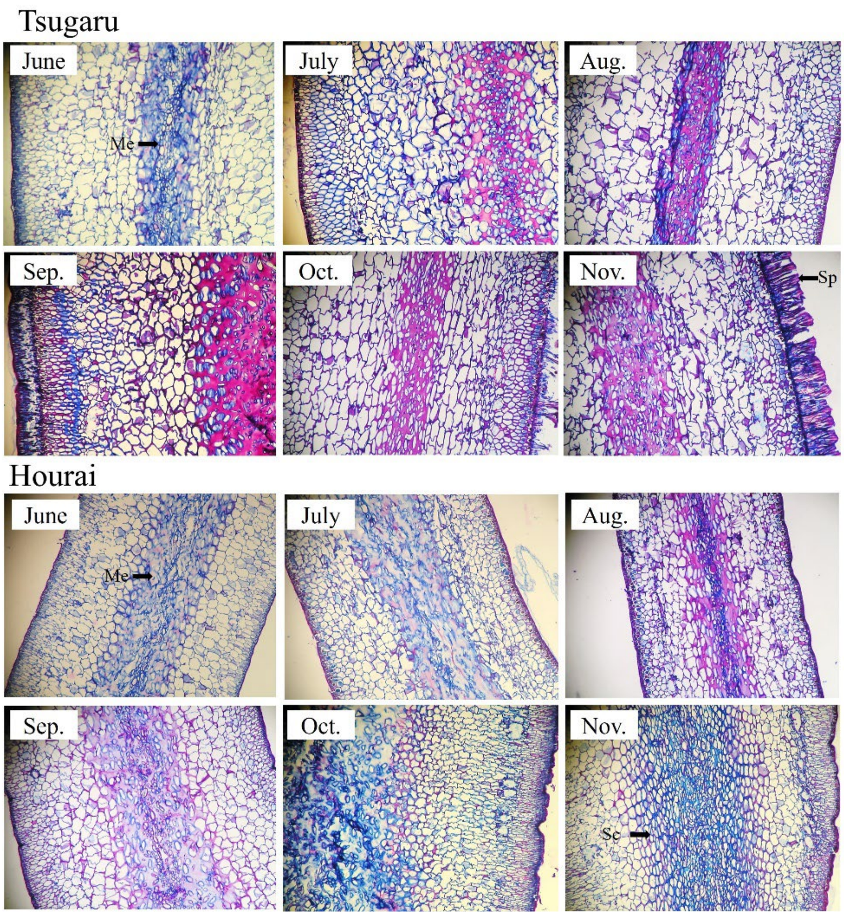

Figure 8. Monthly change of tissue sections of the kelp *Saccharina japonica* var. *ochotensis* in Tsugaru, Rebun Island and Hourai, Wakkanai, northern Hokkaido, Japan. (Me), medulla; (Sc), small cell; (Sp), sporangium.

In the cross sections, a cell like constitution was observed in sporophytes. These cytoskeletons (cell walls) were positive for alcian blue pH 2.5. Though the stainability of the cell wall did not particularly change, the medulla part of the sporophyte showed a significant difference ("Me" in Figure 8). Reddish purple color of fuchsine as PAS (periodic acid Schiff) reaction positive were observed in the medulla, surface, and sporangium of samples (Figure 8). In Tsugaru and Hourai, PAS positive reaction of the medulla was clearer from June to August at both sampling points. PAS positive reaction of the medulla in Tsugaru was clearest in September, and PAS reaction positive remained in October and November. In Hourai, the clearest PAS positive reaction was observed in August and then the pinky red color gradually reduced from September to November, while large numbers of smaller cells occurred in the medulla (Figure 8 in Hourai, November).

Survival rate between larger kelp sporophytes and smaller sporophytes using tag marking in Tsugaru were compared, revealing a remarkable difference in survival rate of the two different sizes (Figure 9). Survival with regeneration occurred only in smaller kelp individuals (Figure 9). Blade length of larger kelp sporophytes was mean 77.6±12.1 SD; mean blade length of smaller kelp sporophytes was 18.0±5.5.

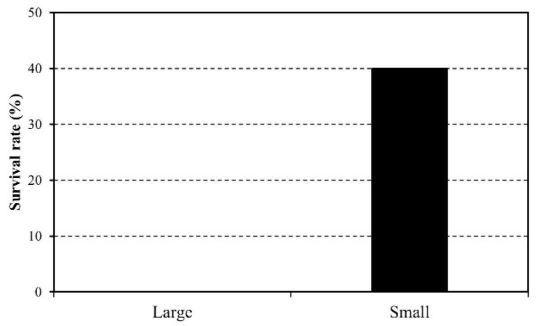

Figure 9. Comparison of the occurrence of survived sporophytes from the first year to the second year.

Larger (40 cm<) kelp *Saccharina japonica* var. *ochotensis* sporophyte in Tsugaru, Rebun Island northern Hokkaido, Japan and smaller (39 cm>) were compared.

3.2. Discussion

Sanbonsuga and Torii (1973) and Nabata *et al*. (1993) reported, based on transplant experiments of the first-year sporophytes of kelp, that growth and survival of the sporophytes are remarkably affected by oceanographic condition in winter season. Nabata *et al*. (2003) and Shinada *et al*. (2014) mentioned that biomass of the first-year sporophytes of *Saccharina japonica* var. *ochotensis* in northern Hokkaido have been influenced by concentration of nutrient and water temperature in winter season. Higher temperature at Tsugaru, Rebun Island during the colder season (October, November, and February) were associated with lower nitrate concentration at the sampling point, whereas lower temperature at Hourai, Wakkanai district during the colder season was associated with higher nitrate concentration (Figures 1–2). Water temperature, light irradiance, and nitrate concentration would influence the growth, survival, maturity, regeneration, and metabolism of the kelp *Saccharina japonica* var. *ochotensis* in northern Hokkaido, Japan. The two sampling stations are geographically close and have a distance between the two stations of approximately 40 km, however Tsushima warm current directly affects Rebun Island whereas a small side current of Tsushima warm current reaches Hourai, Wakkanai (Figure 1), this can lead to differences in the oceanographic conditions (water temperature, transparency, and nutrient) between the two sampling stations. Higher water temperature at Tsugaru was also associated with lower nitrate concentration, the difference of environmental conditions between Tsugaru and Hourai correlate with the distinction of the biological features of kelp forest between the two sampling points. Its synergistic effect could have resulted in greater sizes of sporophytes in Tsugaru, on one hand, the opposite was observed in Hourai. It is suggested that higher water temperature in Tsugaru during the colder season (October, November, and February) produces larger sporophytes, whereas lower water temperature in Hourai, Wakkanai during the colder season produces smaller sporophytes.

In Figure 5 indicates that the decreasing survival rate at Tsugaru after July led to the decrease of biomass and density of the kelp at the sampling areas from July to November, whereas the survival rate at Hourai remained roughly unchanged after August, resulting in a steady biomass at Hourai. As Shinada *et al*. (2014) mentioned, for production of kelp *S. j.* var. *ochotensis* in Rebun Island, northern Hokkaido, Japan there are remarkable yearly fluctuations, relatively low temperature during the winter season (from January to March) with higher nitrate concentrations produces a rich kelp community with a high individual density and small sized first year sporophytes. Although lower temperature with higher nitrate concentrations at Hourai, Wakkanai during the colder season (October, November, and February) lead to a higher individual density and small sized annual sporophytes, quadrat sampling in Hourai from April to June could not obtain a high number of the small sized annual sporophytes and density was maintained at less than 100 sporophytes/ m^2 (Figure 5A). It seems that growth of sporophytes may have slowed down during the spring season (from April to June) and the size of annual sporophytes was too small for quadrat sampling in this season of the present study.

Alcian blue positive reaction at pH 2.5 indicated that a sulfated acidic polysaccharide or another acidic polysaccharide was present. By this stain, sulfated acidic polysaccharides are stained blue black, against other acidic polysaccharides which are stained light blue. In contrast, PAS reaction indicated neutral polysaccharides or simple carbohydrates that are oxidized by 1 % periodic acid as a reddish-purple color of *p* fuchsine (Kiernan 1999).

In general, brown algae include sulfated acidic polysaccharides (e.g., fucoidan) and carboxylic acidic polysaccharides (e.g., alginic acid), and other polysaccharides or monosaccharides (e.g., cellulose, mannitol) (Hurd *et al*. 2014). In the histochemical observations, the cytoskeleton of kelp was clearly stained a blue-black color in Tsugaru only in June, while for samples from Hourai in June, July, October, and November (Figure 8), this shows that sulfated acidic polysaccharides were accumulated in the cell wall. On the other hand, medulla of Tsugaru in June to September, and Hourai in June to November were stained a light blue color, showing that non-sulfated acidic polysaccharides were accumulated in the medulla (Figure 8). It is presumed that they were fucoidan, and alginic acid, respectively.

Mannitol is a photosynthetic primary products and is stored in kelp sporophytes (Hurd *et al*. 2014). It is suggested that the seasonal change of alginic acid in kelp sporophytes in Aomori, Japan, is relatively small, but the amount of mannitol in kelp can change depending on the season (Odagiri *et al*. 2014). PAS stain will be positive reaction for carbohydrates, this were oxidized with 1 % periodic acid, and then occur aldehyde. In general, sugar alcohols like mannitol are easily oxidized by 1 % periodic acid. Because of this, the quantity of mannitol in the section, was higher, stainability of PAS positive reaction in tissue image was stronger. Mannitol is easily stained by PAS reaction; it is presumed that seasonal difference of PAS reaction in tissue image reflects the quantity of mannitol. The pinky red color in medulla in section of kelp sporophytes reflects the quantity of mannitol. It is indicated that the samples from Tsugaru could contain more abundant mannitol from July to November (Figure 8); a more exact quantitative analysis of mannitol using the method of Cameron *et al*. (1948) and Larsen (1978) confirmed the present observation of tissue sections (Wakkanai Fisheries Research Institution 2015). Actually, by the chemical analysis, same tendency was also discovered with mannitol content in sporophyte that collected from Tsugaru and Hourai (Kodama and Kawai 2019).

Size of cells in medulla in the sample from Hourai, in November are smaller and more abundant (Figure 8). It is suggested that newly formed cells were created for regeneration of sporophytes. Namely, the affinity of alcian-blue and PAS reaction, and cell size of medulla in Hourai would relate to the that re-growth, after active growing of blade length from April to May, increasement of length of sporophytes stopped due to higher water temperature with lower nutrient during July to October, increasing their length resumed the water temperature is lower with higher nutrient after November growth (Figure 6A).

Higher light irradiance in Tsugaru, Rebun (Figure 2) led to enhanced photosynthesis of S. j. var. ochotensis sporophytes in that area and also lower temperature led to increased storage compounds in the sporophytes (Figure 8). In general mannitol represents the metabolic compounds from photosynthesis (Hurd *et al*. 2014), and this led to more blade thickness compared to Hourai, Wakkanai (Figure 6D). Lower water temperature brings higher nutrient from deeper depth due to increase vertical mixing in off coast of Hokkaido, Sea of Japan during the winter season, whereas higher water temperature leads to lower nutrient concentrations due to the decrease in vertical mixing during the summer season, relatively higher water temperature makes relatively lower nitrate throughout year (Nakata *et al*. 2001). Tsugaru has experiences relatively higher water temperature with relatively lower nutrient compared to Hourai, this oceanographic condition of Tsugaru bring lower density and biomass of sporophytes in spring season from April to June, longer blade length except May and November in 2013 but lower growth (narrower width, lighter weight, and thinner thickness of blade) of sporophytes from April to November (Figure 6), lower survival of the sporophytes in autumn season from October and November (Figure 5), higher sorus formation rate and lower regeneration rate from September to November (Figure 7). Annual and biennial sporophytes (Figure 10) show similar relationship between oceanographic environmental conditions and metabolism of mannitol. Plenty of mannitol is contained in sporophytes, creating the more desirable taste of Dashi stock produced from kelp at Rebun Island, which continues to provide more expensive, excellent grade kelp products; their grade certainly depends on the concentration of mannitol in products (Kawai and Tazono, personal information). Mannitol is an the most important factor for taste in such Japanese foods (Ueda 1997). Higher light irradiance and lower water temperature in Rebun may contribute to the superior taste and subsequently more expensive value of the kelp products from that area.

Higher temperature with lower nitrate concentration in Tsugaru, Rebun from September to November (Figures 2 and 4) relates to a higher sorus formation rate and lower regeneration rate, more of the energy obtained from photosynthesis is utilized for reproduction than growth at summer and autumn season. and indeed, sporulation takes during from September to November (Figure 7). In contrast, lower temperature with higher nitrate concentration in Hourai, Wakkanai from September to November have a lower sorus formation rate with higher regeneration rate. First year sporophytes in Tsugaru have longer length in summer, and are wider, heavier, and thicker in blade (Figure 6). First year sporophytes in Tsugaru are larger than at Horai. Survival rate of the larger sporophytes in Tsugaru during the autumn season (from October to December) is lower than that at Hourai (Figure 5). There was consistency between the field observations and results of the tagged experiment at Tsugaru, Rebun, that smaller first year sporophytes have higher survival rates (Figure 9).

Based on the present study, the authors suggest the following scenario for the fluctuation of catch of kelp in northern Hokkaido, Japan: Several decades ago, greater numbers of kelp individuals could regenerate from the first year to the second year, which led to rich forests. Life cycle of the species in northern Japan is follows: zoospores were released from sori on mature sporophytes during the autumn season, small sporophytes occur early in the spring season, then large sporophytes grow during the summer season, some sporophytes form sori and then die off during early winter season, but some sporophytes do not have sori and grow again as second year sporophytes (Kawai *et al*., 2014).

4. Conclusion

The study suggests that in recent years, if the colder water temperature occurred during the winter season, the oceanographic

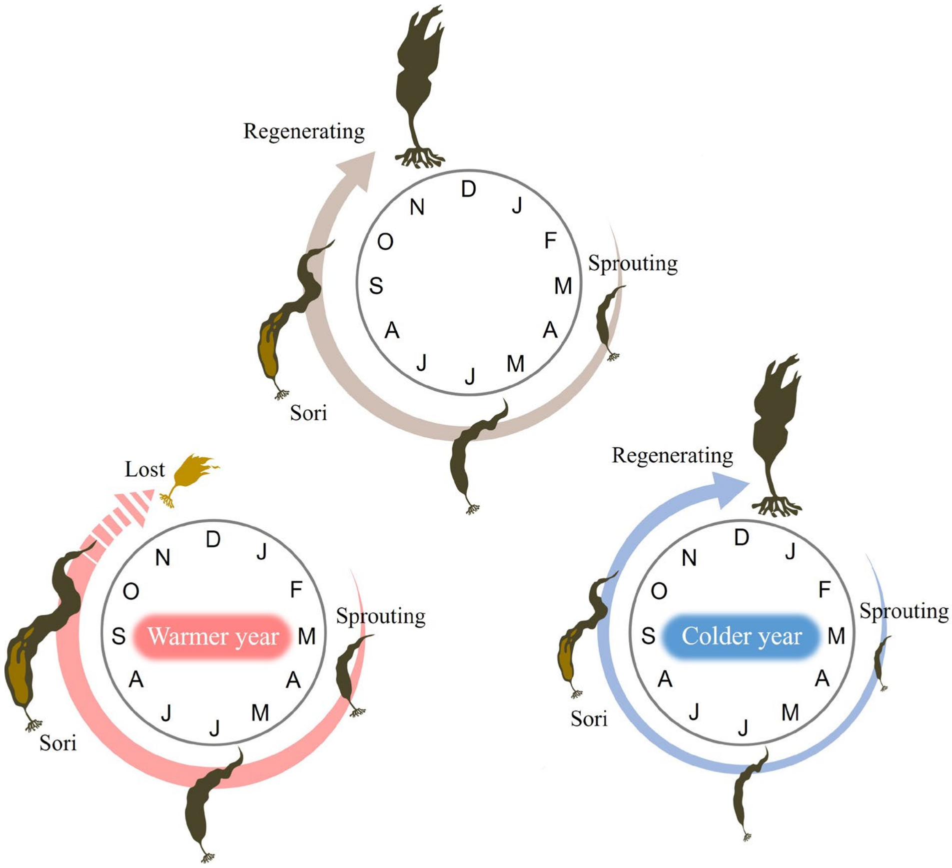

Figure 10. Schematic diagram of life cycle of *Saccharina japonica* var. *ochotensis* in northern Hokkaido, Japan.

environment would restrain the growth of the first-year sporophytes; and this would lead to induce smaller-sized sporophytes in the spring season and a higher regeneration rate of sporophytes, with higher transition from first year sporophytes to second year sporophytes, eventually producing a rich kelp forest. In contrast, warmer water temperatures promote the growth of first year sporophyte, and this results in larger sporophytes in the spring season (from April to June) with lower survival rates in the autumn season (from October to December) (Figure 10), leading to a poor kelp forest. Oceanographic conditions, especially water temperature and nitrate concentrations, influence the resource of kelp in northern Hokkaido.

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