Planned seedling production in the distribution area of *Saccharina japonica*: sorus formation through the induction of sporophyte maturation and the culture of the seedlings produced

Running title: Planned seedling production of *Saccharina*

Norishige Yotsukura[1], Shohei Seki[2], Sachiko Sasaki[3], Masanori Yoshida[3]

[1]Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0809, Japan
[2]Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0810, Japan
[3]Fisheries Institute, Aomori Prefectural Industrial Technology Research Center, Aomori 039-3381, Japan

Correspondence
Norishige Yotsukura, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0809, Japan

Abstract

Inconsistency in maturation of saccharinan kelp due to changes in the marine environment is a problem in terms of securing seedlings for aquaculture. In Hokkaido, the main producing area of *Saccharina japonica*, it has become a challenge to stably secure the parental algae of the species for aquaculture. When maturation was induced by short-day conditions in naturally collected *S. japonica* sporophytes as well as in the sporophytes collected from them and cultivated, sorus formation began in approximately three weeks and spread to 0.4–16.8% on the upper side area and 23.2–

41.1% on the underside area after about five weeks, with the highest rates, 23.2–59.5%, observed around the base part of the underside. Sorus formation began from the base and middle parts in the top-intact thalli and from the marginal part in the top-cut thalli. The released zoospore germlings grew steadily as aquaculture seedlings, and the blade width and wet weight of the seedlings collected in August and subjected to mariculture in November were significantly larger after approximately five and a half months compared to those of thalli cultivated using a conventional method (i.e., thalli collected just prior to the mariculture). Seedling production through maturation induction in sporophytes would be a feasible option for seedling production facilities and would be helpful for stably producing seedlings without the effect of the natural environment.

Keywords: aquaculture, maturation induction, *Saccharina japonica*, seedling production, sorus formation

INTRODUCTION

Saccharina japonica (Areschoug) C.E. Lane, C. Mays, Druehl & G.W. Saunders grows along the northern coasts of Japan and surrounding waters (Yotsukura et al., 2008); in Hokkaido, the main producing area of the species, it has been harvested mainly as foodstuff for more than 1000 years (Oishi, 1987). However, full-scale aquaculture of *S. japonica* in Hokkaido only began in 1966, and its yield, which has become increasingly stabilized thanks to advances in technology, has compensated for the progressive decline in wild catches of the species. Generally, this species is cultivated using a method in which zoospores released from the sporophytes (parental algae) are attached to seedling threads for seedling production in indoor tanks, and the seedling are then cultivated in coastal area (Kawashima, 1984; Su et al., 2017). In

Japan, to produce the seedlings, an important process for aquaculture, high-quality wild mature *S. japonica* sporophytes are collected from the periphery of the aquaculture sites in autumn as parental algae (Kawashima, 1984). However, in recent years, disruption in maturation of kelp sporophytes due to changes in the marine environment has become serious. For example, in the Oyasu district of Hakodate City, Hokkaido, the seedlings were formerly produced in mid-September but were only produced on September 29 in 2019 and October 11 in 2020, indicating the increasing difficulty in stable seedling production. This would be related to the slow decline in water temperature during that season in recent years.

Saccharina japonica has also been cultivated in China, Korea, and Russia (Sohn, 1998; Kawai et al., 2015; Shan et al., 2017; Hwang et al., 2019). Although not native to Chinese waters, *S. japonica* aquaculture began in the mid-1950s using local populations originated from Japan, and yield recently reached approximately 1.4 Mt (dry weight), well above Japan's yield (4319 t in 2018), making China the largest producer (Hwang et al., 2019). In China, a "summer sporeling technique" was developed in which seedling threads are made using farmed sporophytes that mature in early summer, and now more than 90% of the *S. japonica* products are produced using this technique (Tseng et al., 1955; Shan et al., 2011). This aquaculture technique, which allows seedling production in summer, has various advantages over autumn production techniques (Tseng et al., 1955; Shan et al., 2011) and is expected to increase the quality of cultivated kelp products, which are generally lower in industrial value compared to wild counterparts, since more time can be spent on the culture of seedlings, particularly in indoor tanks and the sea. This technique is expected to also become widespread in Japan. In China, the precocious sporophytes obtained from aquaculture are used as parental algae, which allows stable production of seedlings (Su et al., 2017). However, along the Japanese coasts, it is difficult to collect mature sporophytes of the species, which release zoospores most actively in

early summer (Kawashima, 2012). To obtain parental algae in this season, the zoospores need to be obtained by artificially inducing maturation in the sporophytes.

It has been reported that maturation in sporophytes of *Saccharina* kelp can be induced by removing the meristematic tissue followed by cultivation under favorable conditions (short-day and dark conditions, water temperature around 10°C, and high nutrient salt concentration) (Luning, 1988; Nimura and Mizuta, 2002a; Skriptsova and Titlyanov, 2003; Pang and Luning, 2004; Forbord et al., 2012; Boderskov et al., 2021). In *S. latissima*, a species widely distributed around the world, sorus formation and zoospore release were confirmed after a six-week cultivation of sporophytes, collected in August in Norway, under low-light and short-day conditions. When zoospores derived from the sori formed during the tank-cultivation of fertile sporophytes, obtained in December, under running seawater and low-light/short-day conditions were pre-cultured in a hatchery for 42 days, germlings grew notably well in subsequent deployment (Forbord et al., 2020). In regard to the sporophytes of *S. japonic*a, a species found in limited sea areas and is an important ingredient in Japanese food, it has found in a Chinese cultivar and a Chinese hybrid cultivar that sorus formation was induced under short-day conditions in sporophytes from which a blade part, not adjacent to the meristematic tissue, was removed and that in the latter, the release of zoospores was also confirmed (Su et al., 2020). In contrast, the only study conducted on maturation induction in sporophytes of Japanese *S. japonica* using wild thalli as parental algae for aquaculture, reported that sori were formed in young thalli (thalli not subjected to artificial removal of parts except for a marker bore) after approximately one month of cultivation at 5–20°C under a short-day condition (light 9 h: dark 15 h), and at 15°C under a day-neutral condition, in a tank through which filtered seawater flowed (Kirihara et al., 2003). However, the release of zoospores from sori was not confirmed. To incorporate seedling production technique in which maturation is artificially induced into

existing aquaculture production systems in the future, it will be necessary, using candidate thalli, to investigate their detailed maturation mechanisms and the growth of seedlings obtained from them. Therefore, in this study, in order to examine the possibility of producing seedlings by inducing maturation in the sporophytes and ultimately achieving stable seedling production and high-quality products, maturation was induced in an indoor tank in wild thalli and thalli cultivated from their zoospores. The formation process of sori was examined, and the germlings were grown from the zoospores obtained, thereby investigating the possibility of producing aquaculture seedlings through maturation induction.

MATERIALS AND METHODS

Materials used for this experiment are eight thalli of *Saccharina japonica*: seven sporophytes originated from 2-year-old wild kelp collected the year before and cultured in the sea and one wild sporophyte (Table 1). Within them, the four were collected in Hakodate City, on July, 2018 (Nos. 1 & 2, with sori formed on the top part; and Nos. 3 & 4, without sori) and immediately transported to the Fisheries Institute, Aomori Prefectural Industrial Technology Research Center in a refrigerated condition after seawater was wiped off their surfaces. The arrived sporophytes were bored using a cork borer 15 cm above the blade base and cultivated in a 1400-l tank (internal dimensions: 100 cm × 300 cm × 50 cm) inside a land facility, taking into consideration the results of a preceding study (Kirihara et al., 2003). The seawater used was pumped from the nearby sea, filtered, adjusted to 15 °C, and flushed at 500 l/h. The temperature of the seawater in the tank was recorded at 10-min intervals. The tank was covered with a blackout curtain, and a fluorescent light was placed above the tank so that the photon flux density below at the water surface was 117 μmol m^{-2} s^{-1} (short-day conditions of 9/15 h of light/dark). An ultra-miniature data recorder (MDS-MkV, Alec Electronics Co., Ltd.) was used to measure the water temperature and the light intensity. The cultivation was continued from July 25 to August 28,

2018, and blade length and the position of the marker hole were measured periodically during the period. In addition, the presence or absence of sori was investigated for the blade and three equal parts of the blade (i.e., top, middle, and base parts), and the percentage of sori area to blade area was calculated using the image measurement support tool TouchDeMeasure (Enomoto et al. 2017).

Two samples (Nos. 5 & 6) collected on June, 2019, in Hakodate City, with their top ends being cut so that their blade length became 600 cm; one sample (No. 7, an immature sporophyte collected from wild kelp the year before last and cultivated in the sea) collected on June, 2019, in Rishirifuji Town; and one sample (No. 8) collected on July, 2019, in Rausu Town (Table 1), were cultivated in the tank and measurements taken using the same method as in the previous year. The cultivation period was June 26 to August 16, 2019, for Nos. 5 and 6; June 26 to August 5, 2019, for No. 7; and July 8 to August 5, 2019, for No. 8. Six wild mature sporophytes (Nos. 9–14) collected on December 16, 2019, in the Oniwaki district of Rishirifuji Town were used for comparison. After the measurements were taken, the samples were turned upside-down, and cultivation continued.

Among the cultivated samples, No. 8 was taken out of the tank at the end of the cultivation period, and a piece (ca. 15 cm × ca. 30cm) was then cut from the mature portion. After being washed with filtered seawater, the piece was wrapped in a paper towel and stored overnight in a constant-temperature chamber at 10 °C. The next morning, the piece was placed gently in a 2-l Petri dish filled with sterile filtered seawater (10 °C) to induce the release of zoospores. The zoospores released in the dishes were then observed under a microscope (150×) after 15–20 min following the placement of the piece, and the piece was removed from the dish after confirming the release of more than 10 zoospores within the field of view. After being filtered using bleached cotton, the liquid in the dish was poured into a 12-l tank, and the seedlings were

collected using a seedling collector wrapped with 50 m of Cremona thread. This tank was then transferred into a constant-temperature chamber maintained at 10 °C, allowed to stand for 24 h in darkness, and then subjected to aeration culture under medium day conditions (12/12 h of light/dark) with a photon density of 25–65 μmol m^{-2} s^{-1}. The culture medium was PESI medium (Tatewaki, 1966), which was changed every week. Cultivated seedlings were subjected to mariculture at an aquaculture facility located at the shore where the original samples were collected (44°12′28.60″N, 145°20′ 28.09″E) from November 7, 2019, to June 29, 2020. The shape, length, and width of the blade, as well as the wet weight, were measured for the 10 samples for comparison with control samples attached to seedling threads just prior to the mariculture (i.e., samples cultivated using the conventional method), and the means of each trait were compared by t-test after confirming equal variance by F-test. Between November 19, 2019, and May 2, 2020, the aquaculture rope was dropped to near the seafloor (to a water depth of about 6 m in a 30-m depth area) to avoid flow-ice damage and the growth of sporophytes was suppressed.

This work does not contain any experiments involving animals performed by any of the authors. And so, ethical approval is not required.

RESULTS

No noticeable increases in blade length and width were observed in the top-intact thalli during the cultivation period. The blades of Nos. 2 and 4 and No. 8 were shortened about one week and about two weeks after the start of cultivation, respectively, via the loss of the top part. However, growth (distance from the blade base to the marker hole) during the cultivation period was longest in No. 6 at 4 cm; followed by No. 7 at 3.5 cm; No. 3 at 1.5 cm; Nos. 1, 5 and 8 at 1 cm; No. 2 at 0.5 cm; and No. 4 at 0 cm.

As shown Table 2, among the first-year cultivated thalli where a small number of sori were

confirmed on both sides of their blades at the start of the experiment, in No. 1, noticeable expansion of sori occurred after the 19th day of the cultivation, especially in the base part on the upper side and the entire area on the underside (Figure 1-A). In No. 2, sori were observed in the whole area of both sides after the 13th day, and sori expansion afterward was remarkable on the underside, especially near the base. The percentages of sori area to blade area after the 19th, 26th, and 33rd days follow: for the upper side of No. 1, 12.2%, 14.3%, and 16.8%, respectively; and correspondingly for the underside of No. 1, 22.8%, 39.4%, and 41.1%; for the upper side of No. 2, 1.2%, 4.1%, and 4.9%; and the underside of No. 2, 22.8%, 32.0%, and 29.6%.

However, among the first-year cultivated samples that were immature at the beginning of the experiment, in No. 3, sori appeared clearly at the base part on the underside on the 19th day and also appeared slightly at the top part on the underside and the base part on the upper side. By the 26th day, sori had spread to the entire areas on both sides, except for the top and middle parts on the upper side. In No. 4, sorus formation started on the underside by the 19th day and spread to the upper side by the 26th day. The percentages of sori area to blade area after the 19th, 26th, and 33rd days follow: for the upper side of No. 3, 1.3%, 7.7%, and 8.3%, respectively; and correspondingly for the underside of No. 3, 4.4%, 37.7%, and 39.0%; for the upper side of No. 4, 0%, 4.2%, and 4.0%; and for the underside of No. 4, 11.2%, 26.9%, and 40.0%.

Among the first-year top-cut cultivated samples that were immature at the beginning of the experiment, in No. 5, on the 21st day, sori were confirmed near the cut site on the underside and also on the upper side to a lesser extent (Figure 1-B), and by the 28th day, spread to both sides. In No. 6, on the 21st day, sori were confirmed in the whole area on the underside and near the cut site on the upper side and, by the 28th day, spread to the whole area on the upper side. The percentages of sori area to blade area after the 21st, 28th, and 37th days follow: for the upper side of No. 5, 0.7%, 1.6%, and 0.8%, respectively; and correspondingly for the underside of No. 5,

4.1%, 20.8%, and 25.1%; for the upper side of No. 6, 1.7%, 2.9%, and 3.1%; and for the underside of No. 6, 7.7%, 16.1%, and 23.2%. In No. 7 (second-year cultivated thallus), sori, on the 28th day, were confirmed in the middle and base parts on the underside and the top and middle parts on the upper side and, by the 37th day, spread to the whole area on the underside and the top and middle parts on the upper side (Figure 1-C). The percentages of sori area to blade area after the 21st, 28th, and 37th days follow: for the upper side, 0%, 0.1%, and 0.4%, respectively; and for the underside, 0%, 21.6%, and 28.8%, respectively. In No. 8 (second-year wild thallus), sori were confirmed in the middle and base parts on the underside on the 16th day and also in the middle and base parts on the upper side on the 25th day (Figure 1-D, E). The percentages of sori area to blade area on the 16th and 25th days follow: for the upper side, 7.6% and 11.7%, respectively; and for the underside, 24.6% and 37.2%, respectively. Partial damage of blade, especially in the base part, occurred in most samples and a slight decrease in the percentages of sori are to blade area was seen. For the control samples (wild thalli), the percentages of sori area to blade area follow: for the upper side of Nos. 9–14, 0%, 0%, 0%, 3.6%, 6.6%, and 7.6%, respectively; and correspondingly for the underside, 50.0%, 50.7%, 77.7%, 31.3%, 31.7%, and 70.9%.

In the mariculture experiment, there were no significant differences in blade length between the experimental and control samples ($p = 0.05$), but there were significant differences in blade width and wet weight ($p = 0.002$ and $p = 0.003$, respectively) (Figure 2).

DISCUSSION

In this study, under the short-day conditions set based on the results of a preceding study (Kirihara et al., 2003), in all experimental samples, sori were formed in approximately three weeks after the start of maturation induction and spread to the whole area in approximately five weeks, at which point the percentage of sori area to blade area did not significantly differ

between the experimental and wild thalli at the end of seedling production. A large number of zoospores were obtained from the experimental thalli on the 29th day as well as from the wild thalli, and their germlings grew healthily. Thus, the zoospores obtained using maturation induction could be used for stably supplying seedlings for aquaculture as they were not influenced by the natural environment. Note that in *S. japonica*, one study reported cultivation of immature sporophytes of a Chinese hybrid cultivar with their blade lengths cut to a uniform length of 1.5 m (using a recirculation system within a facility, which enabled the supply of nutrient salts, but otherwise with conditions similar to those of the present study) (Su et al., 2020). The blades were covered with sori after one month of cultivation and zoospores were released from the sori 44 days later. However, the authors did not discuss what percentages of the blade area were covered with sori one month after the start of cultivation or whether it was possible to produce seedlings at this stage. We used wild thalli for our study, which have maturation characteristics in the sea different to those of hybrid cultivars, and cultivated thalli derived from wild thalli (e.g. Kawashima, 2012; Su et al., 2020). Although the percentages of sori area to blade area varied among sample parts, the percentages around the base part of the underside – the part with the highest formation rate – on days 26–37 of cultivation were higher than about 50% in all thalli, showing that they were suitable as parental algae for cultivation. In this study, between the seedlings produced using maturation induction and the seedlings using a conventional method, significant differences were found in blade width and wet weight but not in blade length. It is suggested that growth of the experimental seedlings was suppressed because they were cultivated in the two-year aquaculture area, and the culture net was dropped to a greater depth during winter. Since it is one-year aquaculture that would enjoy the benefits of early marine input of seedlings, this technique needs to be verified in the future at southern sea areas in Hokkaido where this type of aquaculture is practiced.

As mentioned above, in China, precocious sporophytes obtained from aquaculture are used as parental algae, which allows the stable supply of seedlings (Su et al., 2017). However, as a result of repeated aquaculture, a decrease in productivity and quality of products resulting from the crossing of closely related seedlings may occur (Li et al., 2016; Zhao et al., 2016). Therefore, in areas where *S. japonica* naturally occurs, it is desirable that wild thalli be used to produce seedlings (in this case, the presence or absence of sori at the onset of maturation induction is not an issue). However, in Hokkaido, the adherence of Hydrozoa (e.g., *Plumularia*), Bryozoa (e.g., *Membranipora*), and Amphipoda (e.g., *Ceinina*) to kelp becomes more pronounced from late summer (cf. Figure 2-D), and their effects on seedling production have become serious (e.g., Akaike et al., 2002). We believe that systematically producing parental algae for aquaculture in indoor facilities under suitable conditions will protect them from these periphytons and would be very effective from the viewpoint of securing parental algae. Normally, second-year thalli are used as parental algae for aquaculture in Hokkaido. In this experiment, when the first-year and the second-year thalli were compared, sorus formation began earlier in the former but the extent of the spread of sori at the height of sorus formation was similar between the two. Since the second-year thalli are larger than the first-year thalli and have a larger sori area, which makes it easier to judge their useful characteristics as marine products, it would be more efficient to use the second-year thalli for maturation-induced seedling production.

At the seedling production facilities of the Oyasu branch of the Toi Fisheries Cooperation, Hakodate City, the main aquaculture area of this species in Hokkaido, the total length of seedling threads used for one production event is 23,400 m (Mr. N. Azuma, Toi Fisheries Cooperation, personal communication). Assuming 127 young thalli per centimeter of thread (set based on the authors' preliminary observations [N = 10]) at the onset of marine aquaculture, the

total number of young thalli is approximately 300 million, which is usually obtained from 40–50 parental algae. No more than 100 mature parental algae are needed to obtain 500 million young *S. japonica* seedlings for aquaculture (Zhao et al., 2016), and despite any differences in expectations toward aquaculture seedlings between the two countries, the seedlings needed would basically be the same. In this study, the maturation of four samples could be induced in one tank, which means that at the industrial level, one seedling production facility would need around 10 tanks. This scale is feasible for existing facilities – e.g., the aforementioned Oyasu facilities have 10 tanks.

In this study, in all experimental thalli and outdoor wild thalli, sorus formation started from the underside of the blade and spread to their upper side. Wild *S. japonica* has a distinct phase when sori are formed on one side of their blades during early maturation (Kawashima, 2012), whereas few maturation-induced thalli showed such a phase. In this species, both sides of blade pieces matured at the same time when both sides were cultivated under a uniform environment (Nimura and Mizuta, 2002b), and we suggest that the results of our study were influenced by the thalli being turned upside-down regularly.

In wild *S. japonica*, sorus formation starts from the base part (Kawashima, 2012). In this study, sorus formation began near the cut side in the top-cut thalli, whereas it predominantly occurred near the base and middle parts in the top-intact thalli. It has been speculated that in saccharinan kelp, auxin, when transported from the base part to the marginal part, suppresses sorus formation during the growth period (Buchholz and Lüning, 1999; Lüning et al., 2000; Pang and Lüning, 2004; Kai et al., 2006). The fact that sori are formed near the cut site when a part away from the base is removed supports this hypothesis (Su et al., 2020). However, there have been no reports in intact *S. japonica* plants that compare the sorus formation process between the top-cut and top-intact thalli. It is suggested that in this study, sorus formation

occurred as a result of the loss of the sorus formation inhibitor from the cut site, supporting the said hypothesis. For Nos. 3 and 4, during tank cultivation, the loss of the top part was negligible in the former but significant in the latter; however, there were no differences in sorus formation sites between the two samples. In Hokkaido, when this species is cultivated, sori are formed in the top parts of the blade during the growth period, and the margin of top parts are severely lost in these algae. Movement of resources associated with growth from the marginal part to the median and base parts has been suggested as well as the limitation of resources necessary for the maturation of the marginal parts (Mizuta et al., 1999), but knowledge on the effect of the loss of the marginal part on sorus formation is scarce. There is some indication that the localization of an abscisic acid-like substance, a sori induction promotor, is involved in the maturation of *S. japonica* (Nimura and Mizuta, 2002b, c). To be able to handle parental algae in an optimal manner, detailed studies on the loss and artificial removal of the top part are required from the viewpoints of both the inhibition and promotion of sorus formation.

ACKNOWLEDGEMENTS

The authors thank Dr. K. Noro for the kind advice and useful comments. The authors also thank stuffs of Toi Fisheries Cooperation, Mr. K. Tenjin (Rausu Fisheries Cooperation), and Mr. R. Ueda (Rishiri Fisheries Cooperation) for collecting materials and cooperation with field experiment. Prof. M. Toda (Niigata University) and Dr. K. Enomoto (The University of Shiga Prefecture) are also thanked for consenting to use TouchDeMeasure. This research was supported by a research project grant-in-aid for Scientific Research (18K05774) from the Ministry of Education, Science, Sports, and Culture, Japan.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

Akaike, S., Takiya, A., Tsuda, F., Motoya ,A, & Takahashi, K. (2002). Seasonal occurrence of a kelp-boring amphipod, *Ceinia japonica* along the coasts of Hokkaido from 1997 to 2001. Scientific Reports of Hokkaido Fisheries Experimental Station, 61, 25-18. (in Japanese)

Boderskov, T., Rasmussen, M. B., Bruhn, A. (2021). Obtaining spores for the production of *Saccharina latissima*: seasonal limitations in nature, and induction of sporogenesis in darkness. Journal of Applied Phycology, 33: 1035–1046. https://doi.org/10.1007/s10811-020-02357-0.

Buchholz, C., & Lüning, K. (1999). Isolated, distal frond discs of the brown alga *Laminaria digitata* form sorus, but not discs near to the meristematic transition zone. Journal of Applied Phycology, 11, 579– 584.

Enomoto, K., Toda, M., Kawasaki, T., & Shimizu, Y. (2017). Image measurement support system "TouchDeMeasure" using touch operation. Image Laboratory, 28 (9), 1-7. (in Japanese)

Forboad, S., Skjermo, J., Arff, J., Handå, A., Reitan, K.I., Bjerregaard, R., & Lüning, K. (2012). Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. Journal of Applied Phycology, 24, 393–399. DOI 10.1007/s10811-011-9784-y.

Forboad, S., Steinhovden, K.B., Solvang, T., Handå, A., & Skjermo, J. (2020). Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study. Journal of Applied Phycology, 32, 2201-2212.

https://doi.org/10.1007/s10811-019-01936-0.

Hwang, E. K., Yotsukura, N., Pang, S. J., Su, L., & Shan, T. F. (2019). Seaweed breeding programs and progress in the Eastern Asian countries. Phycologia, 58, 484-495. https://doi.org/10.1080/00318884.2019.1639436.

Kai, T., Nimura, K., Yasui, H., and Mizuta, H. (2006). Regulation of sorus formation by auxin in Laminariales sporophyte. Journal of Applied Phycology, 18, 95–101. DOI: 10.1007/s10811-005-9020-8.

Kawai, T., Galanin, D., Krupnova, T., & Yotsukura, N. (2015). Harvest and cultivation of *Saccharina japonica* in northern Hokkaido, Japan, and southern Sakhalin and Primorye, Russia: A review. Algal Resources, 8, 155-163.

Kawashima, S. (1984). Kombu cultivation in Japan for human foodstuff. The Japanese Journal of Phycology, 32, 379-394.

Kawashima, S. (2012). Morphology and taxonomy of the laminariaceous algae in cold water area of Japan. Tokyo: Seibutsukenkyusha. (in Japanese)

Kirihara, S., Fujikawa, Y., & Notoya, M. (2003). Effect of temperature and day length on the zoosporangial sorus formation and growth of sporophyts *Laminaria japonica* Areschoug (Laminariales, Phaeophyceae) in tank culture. SUISANZOUSHOKU, 51, 385–390. (in Japanese)

Li, X., Zhang ,Z., Qu, S., Liang, G., Sun, J., Zhao, N., Cui, C., Cao, Z., Li, Y., Pan, J., Yu, S., Wang, Q., Li, X., Luo, S., Song, S., Guo, L., & Yang, G. (2016). Improving seedless kelp (*Saccharina japonica*) during its domestication by hybridizing gametophytes and seedling-raising from sporophytes. Scientific Reports, 6, 21 255. DOI 10.1038/srep21255.

Lüning, K. (1988). Photoperiodic control of sorus formation in the brown alga *Laminaria saccharina*. Marine Ecology Progress Series, 45, 137–144.

Lüning, K., Wagner, A., & Buchholz, C. (2000). Evidence for inhibitors of sporangium formation in *Laminaria digitate* (Phaeophyceae) during the season of rapid growth. Journal of Phycology, 36, 1 129-1 134. https://doi.org/10.1046/j.1529-8817.2000.00017.x.

Mizuta, H., Nimura, K., & Yamamoto, H. (1999). Sorus development on median and marginal parts of the sporophyte of *Laminaria japonica* Areschoug (Phaeophyceae). Journal of Applied Phycology, 11, *585-5*91.

Nimura K, Mizuta H, Yamamoto H (2002a) Critical contents of nitrogen and phosphorus for sorus formation in four *Laminaria* species. Botanica Marina, 45, 184–188.

Nimura, K., & Mizuta, H. (2002b). Pattern of sori on the blade segments of *Laminaria japonica* Areschoug (Laminariales, Phaeophyceae). *S*UISANZOUSHOKU, 50, 157-162. (in Japanese)

Nimura, K., & Mizuta, H. (2002c). Inducible effects of abscisic acid on sporophyte discs from *Laminaria japonica* Areschoug (Laminariales, Phaeophyceae). Journal of Applied Phycology, 14, 159-163.

Oishi, K. (1987). Kombu no michi. Tokyo: Daiichi shobo. (in Japanese)

Pang, S. J., & Lüning, K. (2004). Breaking seasonal limitation: year-round sporogenesis in the brown alga *Laminaria saccharina* by blocking the transport of putative sporulation inhibitors. Aquaculture, 240, 531-541. https://doi.org/10.1016/j.aquaculture.2004.06.034.

Shan, T., Liu, F., Liu, Q., & Pang, S. (2011). Review and prospect of “summer sporeling” technique of *Saccharina japonica* in China. Journal of Agricultural Science and Technology, 13: 129–134. (in Chinese)

Shan ,.T, Yotsukura, N., & Pang, S. (2017). Novel implications on the genetic structure of representative populations of *Saccharina japonica* (Phaeophyceae) in the Northwest Pacific as revealed by highly polymorphic microsatellite markers. Journal of Applied Phycology, 29, 631-638. DOI 10.1007/s10811-016-0888-2.

Skriptsova, A.V., and Titlyanov E.A. (2003). Effect of the meristem on sporification of *Laminaria cichorioides*. Russian Journal of Marine Biology, 29, 372-377.

Su, L., Pang, S. J., Shan, T. F., & Li, X. (2017). Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. Journal of Applied Phycology, 29, 3 003-3 013. DOI 10.1007/s10811-017-1154-y.

Su, L., Shan,,T. F., Jing, L., Pang, S. J., Leng, X. F., Zhang, Y., & Gao, H. T. (2020). Aquaculture of the hybrid cultivars of *Saccharina japonica*: Removing the obstacle of sori production by photoperiodic control. Aquaculture, 519, 734 917. https://doi.org/10.1016/j.aquaculture.2019.734917.

Sohn, C. H. (1998). The seaweed resources of Korea. In A.T. Critchley & M. Ohno (Eds.), Seaweed resources of the world (pp. 15-33). Tokyo: JICA.

Tatewaki, M. (1966). Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. Phycologia, 6, 62-66.

Tseng, C. K., Sun, K. Y., & Wu, C. Y. (1955). On the cultivation of Haidai (Laminaria japonica Aresch) by summering young sporophytes at low temperature. Acta Botanica Sinica, 4, 255–264. (in Chinese)

Yotsukura, N., Kawashima, S., Kawai, T., Abe, T., & Druehl, L. D. (2008). A systematic re-examination of four *Laminaria* species: *L. japonica, L. religiosa, L. ochotensis and L. diabolica*. Journal of Japanese Botany, 83, 165-176.

Zhao, X. B., Pang, S. J., Liu, F., Shan, T. F., Li, J., Gao, S. Q., & Kim, H. G. (2016). Intraspecific crossing of *Saccharina japonica* using distantly related unialgal gametophytes benefits kelp farming by improving blade quality and productivity at Sanggou Bay, China. Journal of Applied Phycology, 28, 449-455. DOI 10.1007/s10811-015-0597-2.

Table

Table 1 The sporophytes of *Saccharina japonica* that was conducted maturation induction in this study.

Sample No.	Collection date	Collection site	Age	Maturity at the time of collection	Artificial cutting of balade	note
No. 1	July 24. 2018	Oyasu, Hakodate City	1st year	*		originated from 2-year-old wild kelp collected the year before and cultured in the sea
No. 2	July 24. 2018	Oyasu, Hakodate City	1st year	*		
No. 3	July 24. 2018	Oyasu, Hakodate City	1st year			
No. 4	July 24. 2018	Oyasu, Hakodate City	1st year			
No. 5	June 24. 2019	Oyasu, Hakodate City	1st year		*	
No. 6	June 24. 2019	Oyasu, Hakodate City	1st year		*	
No. 7	June 24. 2019	Oniwaki, Rishirifuji Town	2nd year			
No. 8	July 6. 2019	Kuzurehama, Rausu Town	2nd year			wild

Table 2 The percentages of sori area to blade area on each part of blade of experimental thallus (No. 1-8) with maturation induction.

Side	Part of blade	Elapsed days	Sample No.			
			No. 1	No. 2	No. 3	No. 4
Upper side	Top part	13		1.4		
		19	6.3	10.1		
		26	16.7	4.5	8.9	3.2
		33	21.0	4.5	9.3	3.5
	Middle part	13		0.1		
		19	5.3	0.1		0.1
		26	7.4	4.5	4.0	1.4
		33	8.2	5.4	2.8	1.2
	Base part	13		0.7		
		19	16.2	1.2	0.3	
		26	19.9	1.7	7.4	6.6
		33	21.3	1.3	9.9	4.9
Underside	Top part	13		8.1		
		19	36.0	12.1	0.4	0.5
		26	46.2	18.3	26.0	20.1
		33	53.8	19.1	25.4	18.6
	Middle part	13		4.7		
		19	16.4	10.2		0.8
		26	24.4	23.8	26.4	12.0
		33	23.3	23.2	27.2	14.0
	Base part	13		18.1		
		19	51.6	44.3	14.5	24.3
		26	55.8	52.6	59.5	47.1
		33	54.3	49.6	59.5	48.2

Side	Part of blade	Elapsed days	Sample No.			
			No. 5	No. 6	No. 7	No. 8
Upper side	Top part	16	-	-	-	
		21	2.0	2.0		-
		25	-	-	-	
		28	3.2	2.9	0.8	-
		37	0.1	3.1	5.0	-
	Middle part	16	-	-	-	0.6
		21				-
		25	-	-	-	1.2
		28	1.4	3.9	0.2	-
		37	2.0	2.9	0.4	-
	Base part	16	-	-	-	17.6
		21				-
		25	-	-	-	34.4
		28	0.5	0.8		-
		37	0.2	2.9		-
Underside	Top part	16	-	-	-	
		21	11.8	5.4		-
		25	-	-	-	1.6
		28	14.4	8.7		-
		37	14.2	7.7	11.5	-
	Middle part	16	-	-	-	11.9
		21		8.2		-
		25	-	-	-	19
		28	17.4	19.0	13.2	-
		37	25.2	24.1	18.1	-
	Base part	16	-	-	-	37.7
		21		8.7		-
		25	-	-	-	67.7
		28	31.1	23.1	37.8	-
		37	50.7	44.4	46.5	-

Figure legends

Figure 1 Sori formed on the underside of experimental thalli (A-E) and of a wild thallus (F) of *Saccharina japonica*. The sori were processed green with TouchDeMeasure. A: No. 1 after 19th day of the cultivation, B: No. 5 after 21th day of the cultivation, C: No. 7 after 37th day of the cultivation, D: The underside of No. 8 after 16th day of the cultivation, E: No. 8 after 25th day of the cultvation, F: Wild thallus collected on December 16, 2019, in the Oniwaki district of Rishirifuji Town. Scale=10cm.

Figure 2 Averages of total length (A), blade length (B), blade width (C), and wet weight of thalli of *Saccharina japonica* brought up in the sea. a: Experimental thalli derived from seedlings obtained using maturation induction, b: Thalli derived from seedlings obtained by the conventional method. **: $p<0.01$.

Figure 1

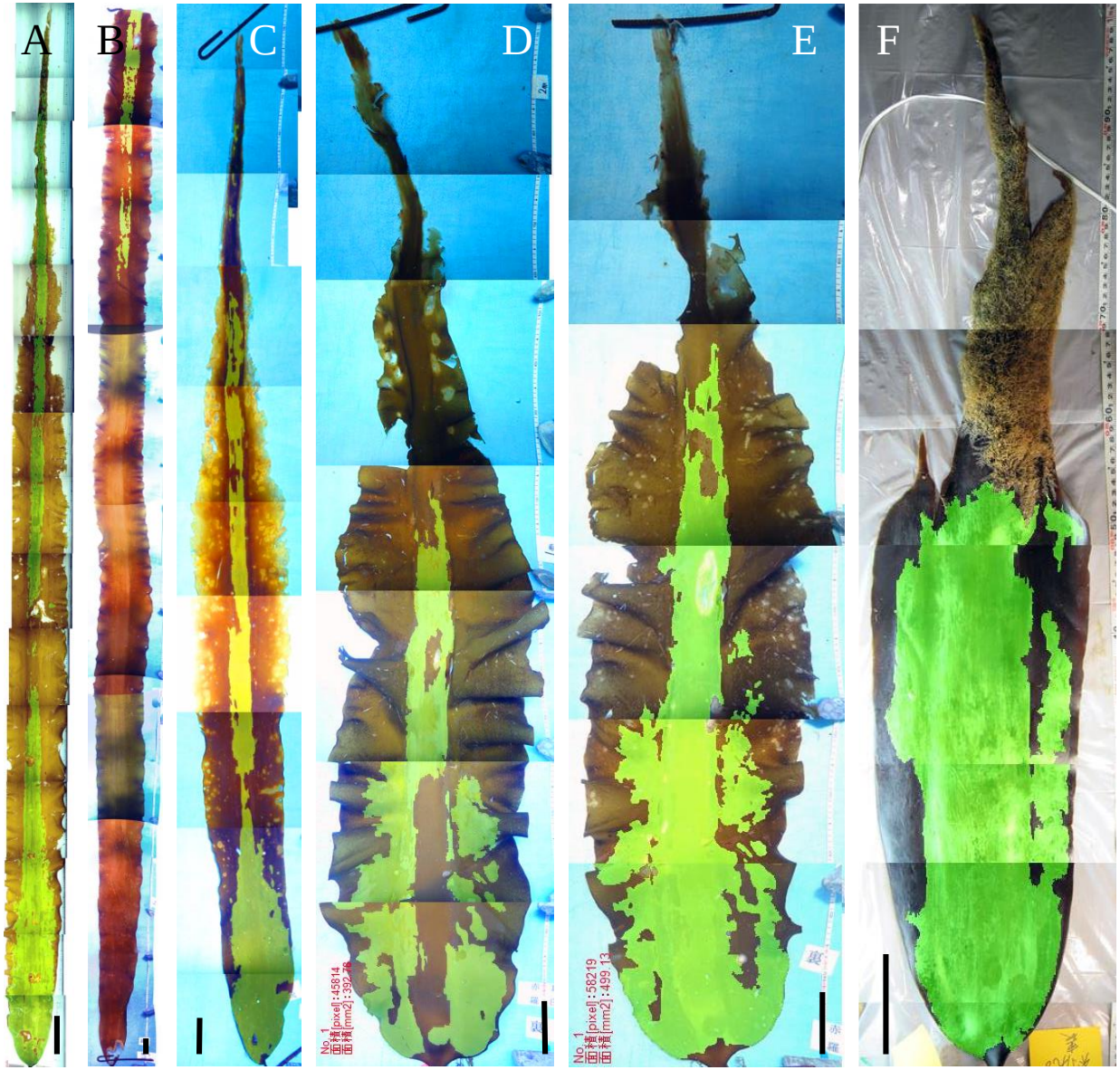
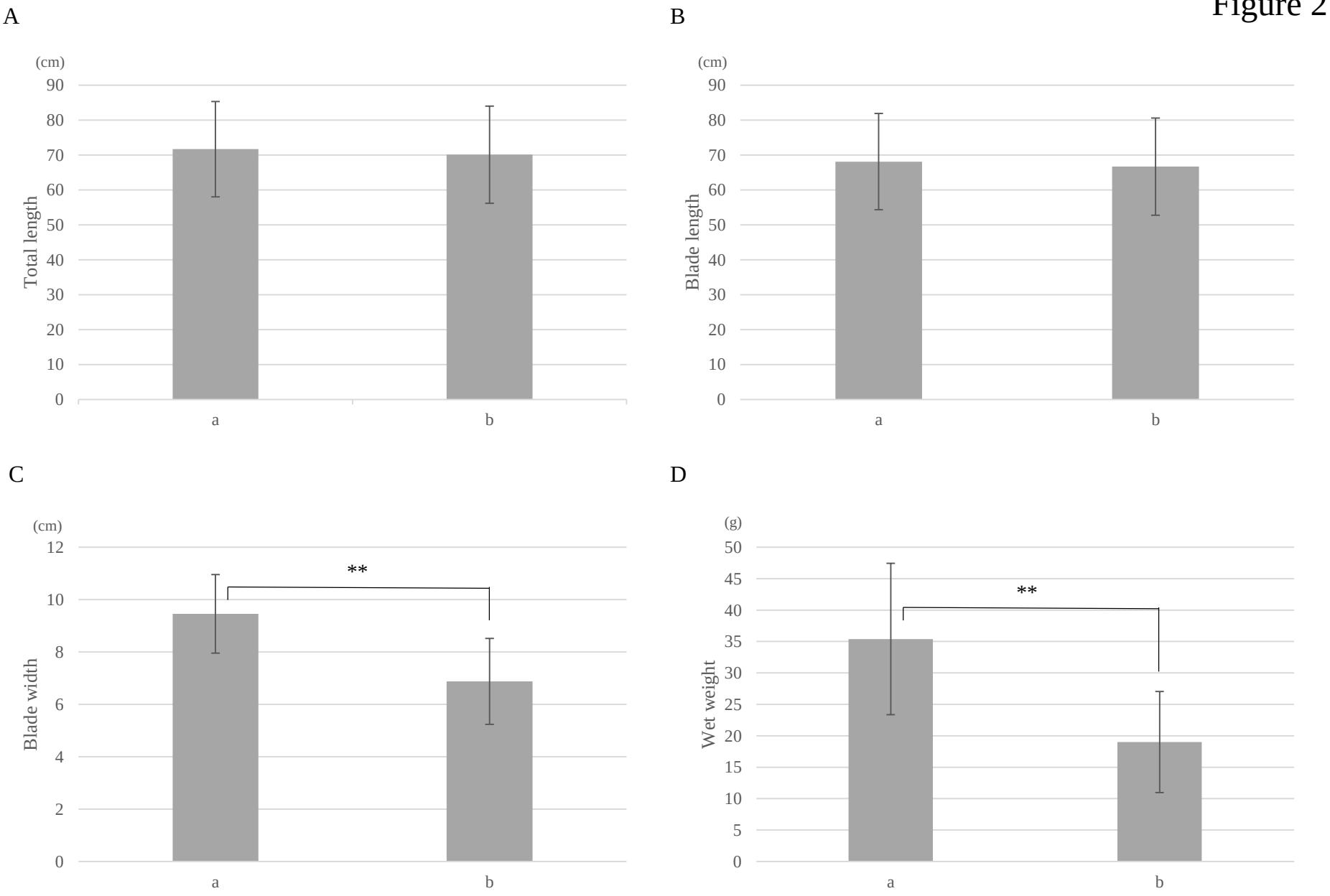

A
B
C
D
No. 1
面積[pixel]:45814
面積[mm2]:392.76
E
No. 1
面積[pixel]:58219
面積[mm2]:499.13
F

Figure 2


A
(cm)
Total length
90
80
70
60
50
40
30
20
10
0
a
b
B
(cm)
Blade length
90
80
70
60
50
40
30
20
10
0
a
b
C
(cm)
Blade width
12
10
8
6
4
2
0
**
a
b
D
(g)
Wet weight
50
45
40
35
30
25
20
15
10
5
0
**
a
b