A method for land-based cultivation of *Undaria pinnatifida* seedlings using outdoor tanks

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Abstract. Grazing damage caused by herbivorous fish has been confirmed at various seaweed cultivation sites. In the case of *Undaria pinnatifida*, grazing damage during the nursery cultivation period in the sea is considered one of the main factors that decreases the production in Japan. To prevent grazing damage, we examined whether it is possible to produce *Undaria* seedlings (juvenile sporophytes) through nursery cultivation on land. Although the total length of the juvenile sporophytes grown by nursery cultivation in an outdoor tank was shorter than that of those grown by nursery cultivation in the sea, the juvenile sporophytes ultimately grew to adequate seedlings for main cultivation. Furthermore, we compared the growth of these sporophytes during the main cultivation period in the sea with that of the seedlings produced by nursery cultivation in both the outdoor tank and the sea. The total length and blade width of the sporophytes from seedlings produced in an outdoor tank on land were smaller than those of the sporophytes derived from seedlings produced in the sea until February 6, 2024, and the total weight of the former was also smaller than that of the latter until February 26. However, the results of further cultivation indicated that there was no difference in growth between the sporophytes from seedlings produced by the two methods. This result demonstrates that the *Undaria* seedlings used for cultivation can be produced by nursery cultivation using outdoor tanks on land to avoid grazing damage caused by herbivorous fish.
Key Words: juvenile sporophyte, nursery cultivation, outdoor tank, *Undaria pinnatifida*.

Introduction. Among the edible seaweed varieties cultivated in Japan, the production of *Undaria pinnatifida* (Harvey) Suringar is the second highest in value and totals approximately 47,000 tons, only after *Pyropia yezoensis* (nori), according to the 2021 statistics of the Japanese Fisheries Agency. *U*. *pinnatifida* is a commercially valuable seaweed species that is widely utilized in various products after processing stages such as boiling and drying (Yamanaka & Akiyama 1993; Dan et al 2015). Most products in Japan are produced from cultivated *U*. *pinnatifida* (Bai et al 2007). In Japan, approximately 80% of the *Undaria* production has been produced on the Sanriku Coast, Iwate Prefecture and Miyagi Prefecture, and on the Naruto Strait, Hyogo Prefecture and Tokushima Prefecture. For stable production of cultivated *U. pinnatifida*, development and improvement of the cultivation method have been conducted in the main cultivated areas as follows: development of seedling production methods (Dan et al 2015; Niwa 2015; Tanada et al 2015; Niwa 2016; Tanada 2016; Tanada et al 2020; Tada & Tanada 2022) and investigation to determine the optimal start of cultivation (Kurogi & Akiyama 1957).

In natural *U*. *pinnatifida*, microscopic zoospores (n) are released from sporophylls formed on mature sporophytes (2n) from spring to early summer in Japan and develop into male or female gametophytes (n). When the water temperature begins to decrease from late September to October, male and female gametophytes mature and fertilize. The fertilized eggs develop into juvenile sporophytes (Kurogi & Akiyama 1957; Niwa 2015; Tanada et al 2015; Niwa & Harada 2016).

Based on this life cycle, *U*. *pinnatifida* seedlings for cultivation are generally produced in the Seto Inland Sea (mainly in the Naruto Strait) using the following method. First, sporophylls that are collected from many sporophytes are placed into land-based tanks containing seawater from April to May to release zoospores, and the released zoospores attach to seed strings that are wrapped around seedling collectors. These collectors are maintained in tanks with water changes and light adjustments until early autumn, and during this period gametophytes are formed and maintained at vegetative phase. When the water temperature begins to decrease, water changes and light adjustments promote gametophyte maturation. After fertilization, many juvenile sporophytes develop from fertilized eggs (Ii 1964; Niwa 2015, 2016). In recent years, seedling production methods involving the preservation of gametophytes without attachment to the substrate (free-living gametophytes) have become widespread (Tanada et al 2015). In this method, male and female gametophytes that developed from zoospores released from a sporophyll are separately cultured in flasks or beakers. The isolated female and male gametophytes are put in an electric mixer and shredded to create a gametophyte mixture. By applying or spraying the gametophyte mixture onto the seed strings that are wrapped around the seedling collector, the gametophytes are attached to the seed strings. After maturation and fertilization in early autumn, the juvenile sporophytes grow on the seed strings that are wrapped around the seedling collectors in land-based tanks. Free-living gametophytes can proliferate in large numbers by regulating the temperature and day-length cycles in the incubator, and they can be preserved for long periods. Moreover, since free-living gametophytes promote maturation under laboratory culture, the starting time of seedling production can be adjusted (Niwa 2015; Tanada et al 2015). To take advantage of these features, several experiments using free-living gametophytes of this marine crop have been conducted, such as forced cultivation (Niwa & Harada 2016), double cropping (Niwa 2015), development of seedling production techniques using large indoor tanks (Niwa 2016) and attempts at large-scale seedling production (Tanada et al 2015, 2020).

In the Seto Inland Sea, from late October to early November, seedling collectors or iron frames (seed frames) that are wound with seed strings on which juvenile sporophytes of *Undaria* have settled are placed in the sea (nursery cultivation). In approximately one month, the juvenile sporophytes grow to 1-3 cm in length. Thereafter, the seed strings are cut into 2 to 3 cm lengths and inserted into culturing ropes for the main cultivation period. During the winter to spring period, sporophytes that have grown to more than 1 m in length are harvested (Tanada et al 2015; Niwa & Harada 2016). However, grazing damage caused by herbivorous fish has recently been recognized as a serious factor that decreases production during seaweed cultivation (Ganesan et al 2006; Kasim et al 2016). Furthermore, rising seawater temperatures due to global warming have caused serious grazing damage by fish at various cultivation sites, such as *P. yezoensis* (nori) and *U. pinnatifida* in Japan, because fish under high seawater temperatures actively graze cultivated seaweeds (Kiriyama et al 2018; Noda & Murase 2021; Takenaka et al 2021; Noda & Murase 2022; Kanamoto et al 2023; Takakura et al 2023; Tezuka et al 2023). In the case of *U. pinnatifida*, grazing damage frequently occurs during the nursery cultivation period, which usually continues for approximately one month in the Seto Inland Sea (Niwa & Harada 2016; Kajiwara et al 2024). Grazing damage significantly inhibits the growth of juvenile sporophytes and is considered a serious problem that leads to a decrease in *Undaria* production and lower profits (Kiriyama et al 2018; Noda & Murase 2021, 2022). Therefore, if it becomes possible to grow juvenile sporophytes in land-based tanks, it will be possible to secure a stable supply of *U. pinnatifida* seedlings without incurring grazing damage by fish.

Nursery cultivation using land tanks has been conducted in other kelps, such as *Lessonia corrugata* (Nardelli et al 2024) and *Nereocystis luetkeana* (Supratya & Martone 2024). However, there are no reports on the growing process of the *Undaria* seedlings in the sea, which were produced by nursery cultivation on land, after they are transferred to the sea. In addition, growth comparison during the main cultivation at sea between sporophytes derived from the seedlings produced in outdoor tanks and the sea has not been examined. In this study, using seed frames commonly used in the Seto Inland Sea, we examined whether the *Undaria* seedlings can be produced throughout nursery cultivation via an outdoor tank located on land, furthermore, to investigate whether the seedlings produced by nursery cultivation on land grow into large sporophytes in the sea, the growth of these sporophytes was monitored during the main cultivation period and compared to the growth of sporophytes produced by normal nursery cultivation practices on an *Undaria* farm.

Material and Method

Seedling production by free-living gametophytes. The *Undaria* seedlings used in this study were produced from free-living male and female gametophytes that were isolated from parent sporophytes cultivated in the Sano area of Awaji city, Hyogo Prefecture, and collected in April 2023 (Figure 1). The isolation procedure for gametophyte strains that were developed from single zoospores was as described by Niwa (2015) and Niwa et al (2017). These isolated gametophytes were separately cultured with aeration in an incubator at the Fisheries Technology Institute, Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries (hereafter referred to as the "center") at 20°C, 70 μmol photons m^{-2} s^{-1}, and a light/dark cycle of 14 hL:10 hD.

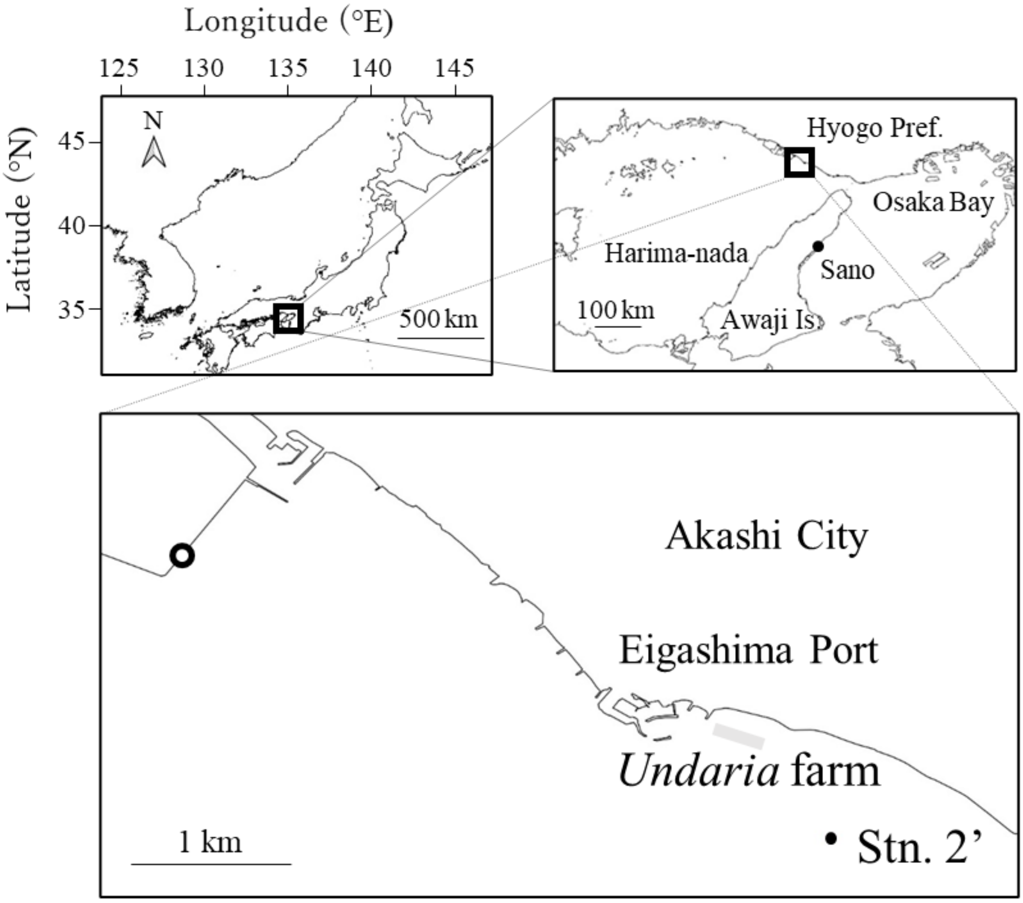

Figure 1. Maps showing the Eigashima cultivation site in the Seto Inland Sea and other locations mentioned in the study. The open circle shows the measurement site for the seawater temperature. The closed circle (Stn. 2') shows the sampling site used for determining the dissolved inorganic nitrogen (DIN) concentrations in seawater.

For seedling production, a seedling collector (Figure 2a) was made by wrapping a 4-m long vinylon string (cross-sectional diameter, 2 mm: hereafter referred to as the "seed string"), which had been cleaned of oil and feathers by using the method of Niwa (2015), around a stainless frame (7.3 × 5.5 cm: cross-sectional diameter, 3 mm). On October 18, 2023, 0.05 g (wet weight) the isolated female and male gametophytes were put in an electric mixer with 100 mL of NPM medium (Niwa & Aruga 2003) and shredded for 1 min to make a gametophyte mixture (Figure 2b). The gametophyte mixture was left in the mixer for some time to precipitate the cut fragments of the gametophytes. Then, 80 mL of the supernatant was removed, and the remaining solution (20 mL) was divided and placed into two 10 mL screw-cap vials (Figure 2c). These vials were placed in an incubator at 20°C to precipitate the cut gametophytes. The next day (October 19), the supernatant of the gametophyte mixture was removed, the remaining volume was adjusted to a total of 15 mL and shaken well. The gametophyte mixtures in the two screw-cap vials were injected into a 30-mL spray bottle and sprayed evenly onto the front and back of the seedling collectors (Figure 2d). After confirming under a stereomicroscope that the gametophytes attached to the two seedling collectors, the seedling collectors were hung in a 500-mL beaker containing NPM medium and stored in an incubator. To promote gametophyte maturation and fertilization, the temperature and light intensity were set to 20°C and 100 µmol photons m^{-2} s^{-1}, and the light/dark cycle was set to a short-day cycle (10 hL:14 hD) in the incubator (Figure 2e). The seedling collectors were observed under a stereomicroscope approximately once each week to check for gametophyte fertilization and the development of juvenile sporophytes (Figure 2f). Aeration culture was started 7 days after the seeding collectors were hung in the 500-mL beaker (October 26, 2023), and the aeration level was gradually increased with the growth of the juvenile sporophytes.

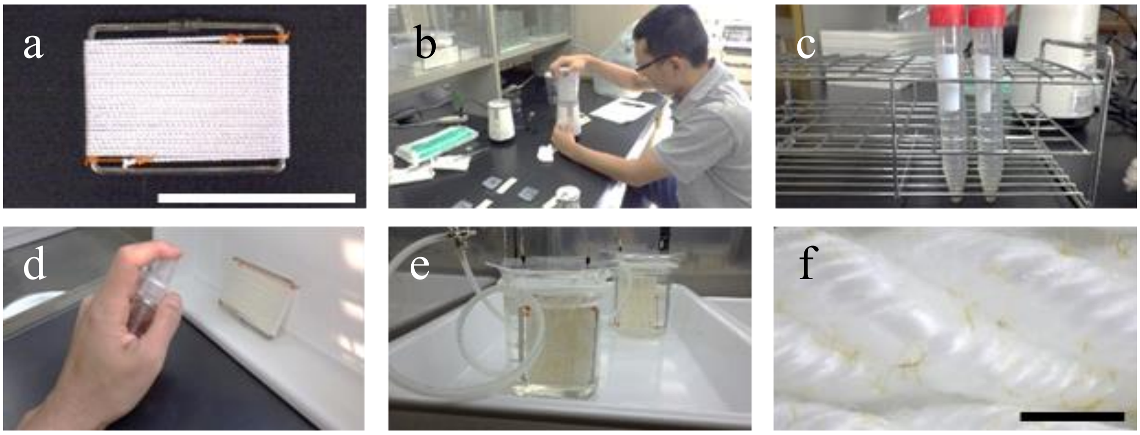

Figure 2. Procedure for the production of *Undaria pinnatifida* seedlings. (a) The seedling collector used for seedling production. Scale bar, 10 cm; (b) Shredding of free-living gametophytes via an electric mixer; (c) Screw-cap vials containing shredded gametophyte mixtures; (d) Spraying the seedling collector with the gametophyte mixture; (e) Seedling collectors placed in beakers; (f) Male and female gametophytes on the seed string observed under a stereomicroscope. Scale bar, 1 mm.

Nursery cultivation. After confirming that the juvenile sporophytes grew densely to approximately 1 cm in total length on the seed string, nursery cultivation was started both in an outdoor tank and in the sea on November 22, 2023. Before nursery cultivation, the seed strings were detached from the seedling collectors and were immersed in a 200-L square polyethylene tank RL-200 L (Dailite Co., Ltd., Japan) that was filled with sand-filtered seawater. While the seed strings were immersed in the square tank, an iron seed frame (51.3 × 35.3 cm) was slowly rotated around the water surface to rewrap the seed string around the seed frame (Figure 3a). For the nursery cultivation using the outdoor tank, 75-g weights were attached to both ends of the seed frame, which was hung in a 200-L Artemia hatching tank SBF-200 (Nihon Sacas Co., Ltd., Japan, hereafter referred to as the "outdoor tank") located on the center's premises (Figure 3b). Since the outdoor tank has a drainage hole in the center of the bottom, a 25-mm (inner diameter) polyvinyl chloride (PVC) pipe was inserted into the hole to adjust the water depth to 70 cm in this study (Figure 4). The outdoor tank was placed in a roofed experimental facility adjacent to a building in the center, where the tank was less exposed to direct sunlight and relatively less influenced by wind and rain (Figure 3c). During nursery cultivation in the outdoor tank, it was aerated, and sand-filtered seawater was poured through a hose at a rate of 353.4 mL s^{-1}. The seed frame was visually inspected daily to check for attached dirt, blade shape, and coloration of juvenile sporophytes. On November 27 (5 days after the start of nursery cultivation), we wiped the outdoor tank with Kim Towel paper towels (Nippon Paper Crecia

Co., Ltd., Japan) and washed the seed string wrapped around the seed frame with sand-filtered seawater sprayed through a hose (Figure 3d) because diatoms had attached to the juvenile sporophytes. At the *Undaria* farm (4–5 m depth) in the Eigashima area of Akashi city (Figure 1), nursery cultivation in the sea also began on November 22, 2023 (Figure 3e), the same day as the start of nursery cultivation in the outdoor tank, according to previous studies (Niwa 2015; Niwa & Harada 2016; Niwa & Kobiyama 2019). Seed frames with attached seed strings and weights were hung from a rope at a depth of approximately 50 cm in a floating cultivation facility (Figure 3f). Seed frames were set in areas of the *Undaria* farm where grazing damage by fish was empirically known to be minimal. During the nursery cultivation period in the sea, the seed strings that were wrapped around the seed frames were washed as necessary by a water pump to prevent the attachment of dirt and diatoms according to Niwa (2016).

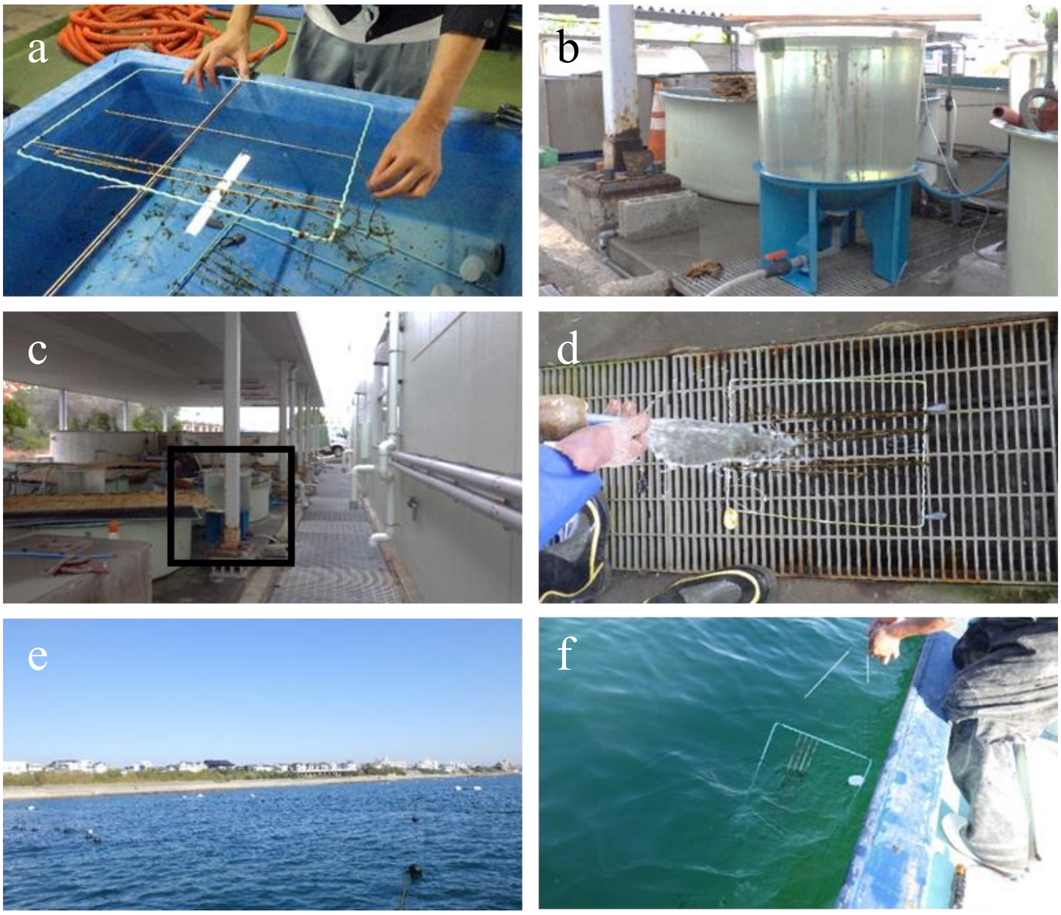

Figure 3. Procedures for the nursery cultivation of *Undaria pinnatifida* in an outdoor tank and in the sea. (a) Wrapping the seed string around the seed frame; (b) Nursery cultivation using an outdoor tank; (c) Location of the outdoor tank (open rectangle); (d) Sprinkling with seawater using a hose; (e) A view of the *Undaria* farm at Eigashima; (f) The rewound seed frame was placed at a depth of approximately 50 cm for nursery cultivation in the sea.

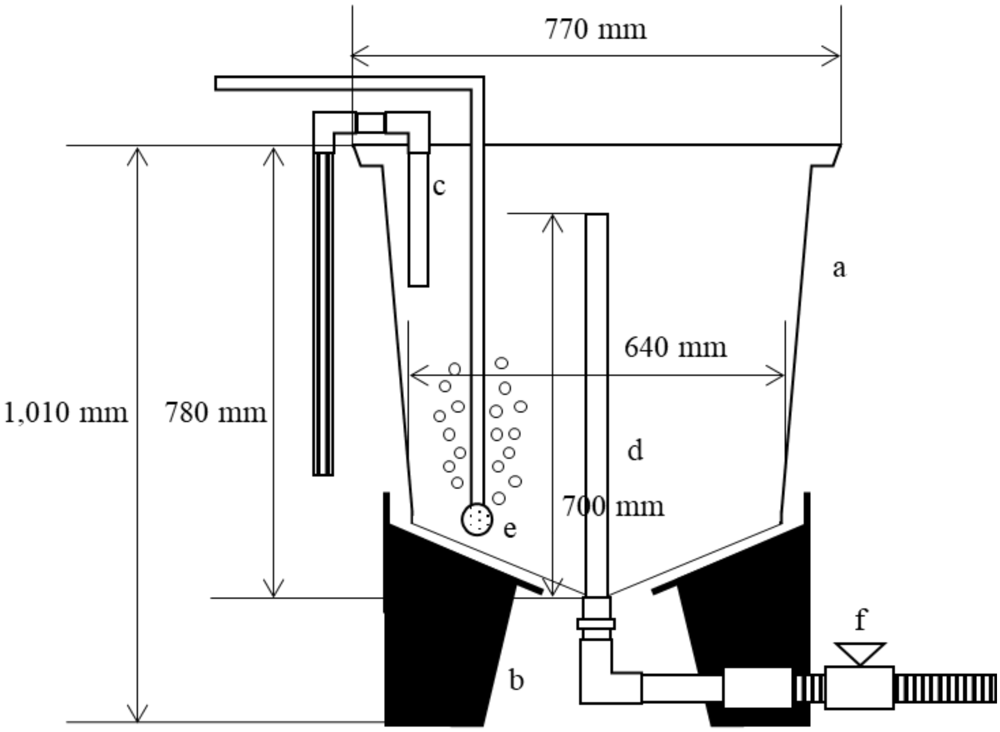

Figure 4. Schematic view of the outdoor tank used for land-based nursery cultivation. (a) polycarbonate tank; (b) fiber reinforced plastic (FRP) base; (c) polyvinyl chloride (PVC) pipe for the seawater supply; (d) PVC pipe for seawater drainage; (e) aeration stone; and (f) pole valve.

Main cultivation. After nursery cultivation, the main cultivation period started at the *Undaria* farm on December 11, 2023. For the main cultivation, seed strings with juvenile sporophytes that grew during both the nursery cultivations in the outdoor tank and the sea were detached from the seed frames and cut to approximately 15 cm in length. Both ends of the cut seed strings were inserted into a polyethylene rope (cross-sectional diameter: approximately 1.4 cm) at 50 cm intervals (Figure 5a, b). Approximately 15 seed strings per nursery cultivation method were inserted into the rope. The density of the sporophytes from one string section that was inserted into the rope was approximately 15–20 individuals. To conduct cultivation under the same conditions, all the seed strings were inserted into a single rope.

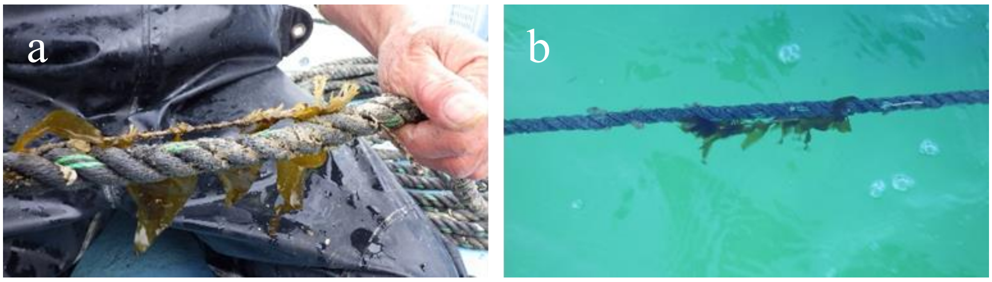

Figure 5. Main cultivation of *Undaria pinnatifida* sporophytes. (a) The seed string produced by nursery cultivation was cut, and the cut string was inserted into the rope for the main cultivation; (b) *Undaria pinnatifida* sporophytes just after the start of the main cultivation.

Environmental measurement. In the outdoor tank, a UA-002-64 data logger (Onset Computer Corporation, USA) was installed to record the water temperatures and light intensities during the nursery cultivation period. The water temperatures were measured hourly, and the mean daily values were calculated. The light intensities were measured hourly during the day (8:00–16:00), and the mean daily values were calculated. Any undesirable substances, such as diatoms, adhering to the sensor part of the data logger were wiped off during the seed frame observations. The water velocities were measured every 6 s via an electromagnetic current meter INFINITY-EM (JFE Advantech Co., Ltd., Japan), and the average values of the composite velocities over 7 days were calculated. The water velocities in the outdoor tank were measured at an upper position located 5 cm below the surface of the seawater. The water velocities were calculated as the totals of the values for the horizontal X-axis and Y-axis. Additionally, water samples were collected from the tank 2 times a month during the nursery cultivation period and analyzed for dissolved inorganic nitrogen (DIN: NO_3-N, NO_2-N, and NH_4-N) concentrations via a QuAAtro 2-HR nutrient analyzer (BL TEC K. K., Japan).

The water temperatures at the *Undaria* farm were measured via an automatic monitoring device fixed near the center of the tank (Figure 1), and the daily average values were calculated. The DIN concentrations were determined via measurements obtained from the Stn. 2' sampling site (Figure 1), on the basis of the environmental information on *Pyropia* farms published by the Hyogo Prefectural Federation of Fishery Cooperative Associations (http://www.hggyoren.jf-net.ne.jp/, accessed on June 8, 2024).

Measurements and analyses of juvenile sporophytes and sporophytes. To conduct the measurements, the juvenile sporophytes on the seed strings were collected from the seed frame on November 22, November 30, December 5, and December 11, 2023, during both nursery cultivations methods in the outdoor tank and in the sea. The 30 longest juvenile sporophytes on the seed strings per each nursery cultivation method were collected, photographed, and measured to determine the total lengths and blade widths (Figure 6). When juvenile sporophytes are grazed by fish, the tip of a juvenile sporophyte is bitten, and the total length is extremely short (Kiriyama et al 2000). Therefore, we observed whether the tips of the juvenile sporophytes growing in the sea were grazed by the fish.

During the main cultivation period, the 10 longest sporophytes from each nursery cultivation method were collected evenly from all the aggregates (all the seed string sections were inserted into the rope) on January 16, February 6, February 26, and March 11, 2024 and were used as specimens for measurement. The total weight (wet weight) of each sporophyte was measured and each was photographed. The blade shapes and colorations were observed before the total lengths and blade widths were measured (Figure 6).

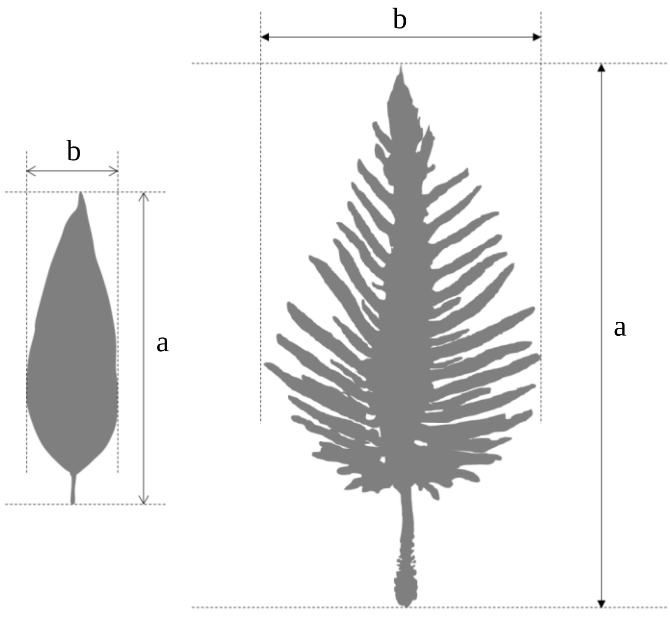

Figure 6. Illustration of a juvenile *Undaria pinnatifida* sporophyte (left) and large sporophyte (right) showing the total lengths (a) and blade widths (b).

The total lengths and blade widths of the sporophytes were measured via the image analysis software ImageJ (National Institutes of Health, USA) after each photograph was imported into a computer. A two-way ANOVA was used to determine differences in total length, blade width, and total weights by cultivation method and sampling date. If a significant interaction was detected, a Welch's *t* test was applied to compare differences between cultivation methods at each sample collection date. The analysis was conducted using data analysis toolpak in Excel 2016 (Microsoft Corp., USA) with a significance level (p) of 0.05.

Results

Nursery cultivation. In both nursery cultivations in the outdoor tank and in the sea, few seedlings were observed that had dropped from the seed frame. Furthermore, no abnormal morphologies or bleaching was observed in the juvenile sporophytes. Additionally, no grazing damage by fish was observed in juvenile sporophytes during nursery cultivation at sea.

Growth of the juvenile sporophytes during cultivation in both nurseries are shown in Figure 7. At the start of nursery cultivation (November 22, 2023), the total lengths and blade widths of the juvenile sporophytes were 1.11 cm and 0.30 cm, respectively. At the end of the nursery cultivation period (December 11, 2023), the total lengths and blade widths of the juvenile sporophytes increased to 5.32 cm and 1.54 cm in the outdoor tank, whereas in the sea, the lengths and widths increased to 9.09 cm and 2.79 cm, respectively (Table 1). An interaction was found between nursery cultivation method and sampling date during the nursery cultivation period ($p < 0.05$). Both the total lengths and blade widths of the juvenile sporophytes that were cultivated in the sea were significantly greater than those cultivated in the outdoor tank throughout the nursery cultivation period ($p < 0.05$), indicating that juvenile sporophytes cultivated in the sea grew faster than those cultivated in the outdoor tank did (Figure 8).

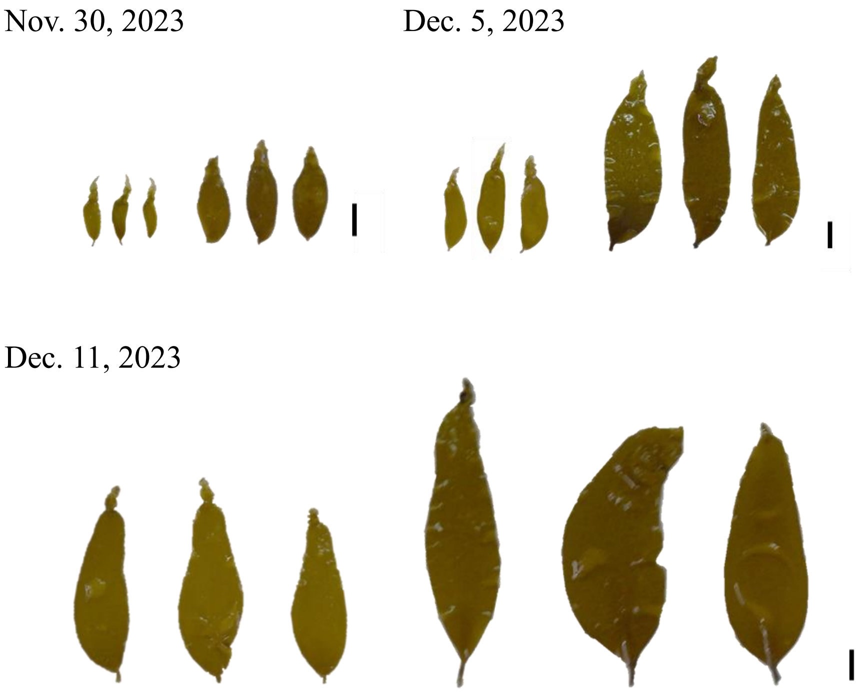

Figure 7. Juvenile sporophyte samples of *Undaria pinnatifida* that were produced by nursery cultivation in an outdoor tank (three sporophytes on the left) and in the sea (three sporophytes on the right). Scale bars, 1 cm.

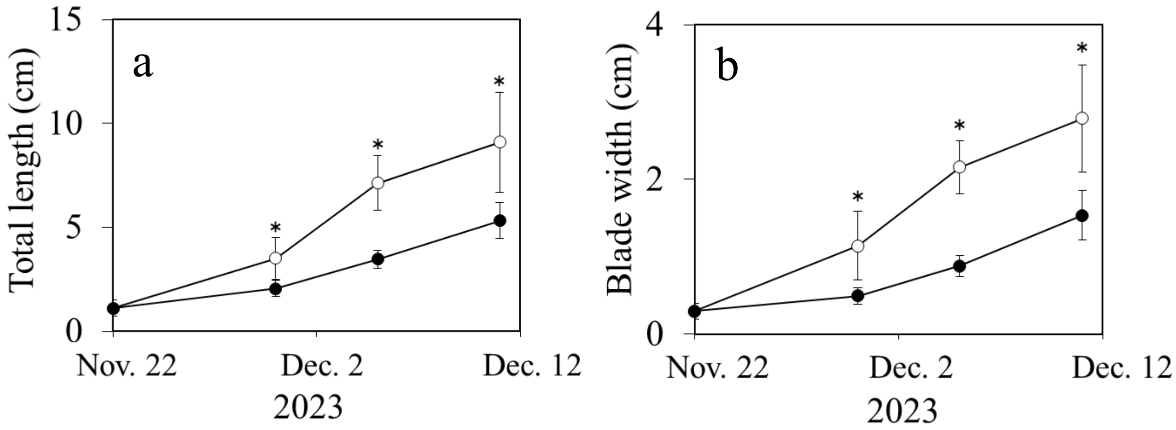

Figure 8. Growth of juvenile *Undaria pinnatifida* sporophytes during the nursery cultivation period in an outdoor tank (closed circle) and in the sea (open circle). (a) Total length; (b) Blade width. Average±SD (n = 30). The asterisk indicates a significant difference ($p < 0.05$).

Table 1

Growth of juvenile *Undaria pinnatifida* sporophytes during nursery cultivation in an outdoor tank and in the sea

	November 30, 2023		*December 5, 2023*		*December 11, 2023*	
	Tank	*Sea*	*Tank*	*Sea*	*Tank*	*Sea*
TL	2.04±0.39*	3.51±1.01*	3.46±0.44*	7.13±1.34*	5.32±0.86*	9.09±2.41*
BW	0.49±0.10*	1.14±0.45*	0.88±0.13*	2.16±0.34*	1.54±0.32*	2.79±0.69*

The data are presented as the averages±SDs (n = 30). The asterisks indicate a significant differences ($p < 0.05$). TL total length (cm) and BW blade width (cm).

The water temperature at the start of nursery cultivation was 17.5°C in the outdoor tank and 17.8°C in the sea. During these nursery cultivations, the temperatures for each location gradually decreased, and the temperatures in the outdoor tank and the sea were 15.3°C and 15.1°C, respectively, at the end of the nursery cultivation period (Figure 9a). There was little difference in water temperature between the outdoor tank and the sea during the nursery cultivation period. From mid-November to early December, the DIN concentrations were approximately 10-13 μM in the outdoor tank, whereas they were approximately 2-8 μM in the sea. During the nursery cultivation period, the DIN concentrations in the outdoor tank were always higher than those in the sea (Figure 9b). The light intensities during the daytime in the outdoor tank were 30.9 μmol photons m^{-2} s^{-1}, and the water velocities were 5.8±1.4 cm s^{-1} (mean±S.D.).

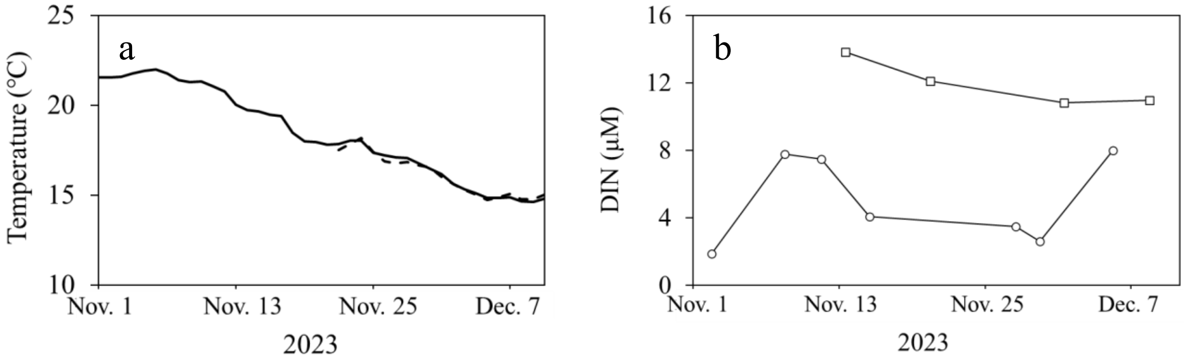

Figure 9. Changes in water temperatures and dissolved inorganic nitrogen (DIN) concentrations during the nursery cultivation period. (a) Record of seawater temperatures in the outdoor tank (dotted line) and in the sea (solid line); (b) Record of the DIN concentrations in the outdoor tank (open squares) and in the sea (open circles).

Main cultivation. Regardless of the two nursery cultivation methods, the sporophytes growing in this main cultivation period showed no deformities in blade shape and no blade bleaching on any sampling date (Figure 10). The changes in the growth of sporophytes during the main cultivation period are shown in Figure 11. An interaction was found between nursery cultivation method and sampling date during the main cultivation period ($p < 0.05$). On January 16 and February 6, 2024, the total lengths and blade widths of the sporophytes that developed from the seedlings from the nursery cultivation in the sea were significantly greater than those of the sporophytes that were derived from the nursery cultivation in the outdoor tank ($p < 0.05$). However, on February 26 and March 11, no significant differences in total lengths or blade widths were detected between the sporophytes that were derived from the two nursery cultivation methods (Figure 11a, b). The total weights of the sporophytes derived from nursery cultivation in the sea were significantly greater on January 16, February 6, and February 26 ($p < 0.05$). However, no significant differences in total weight were detected between the sporophytes derived from the two nursery cultivation methods on March 11 (Figure 11c).

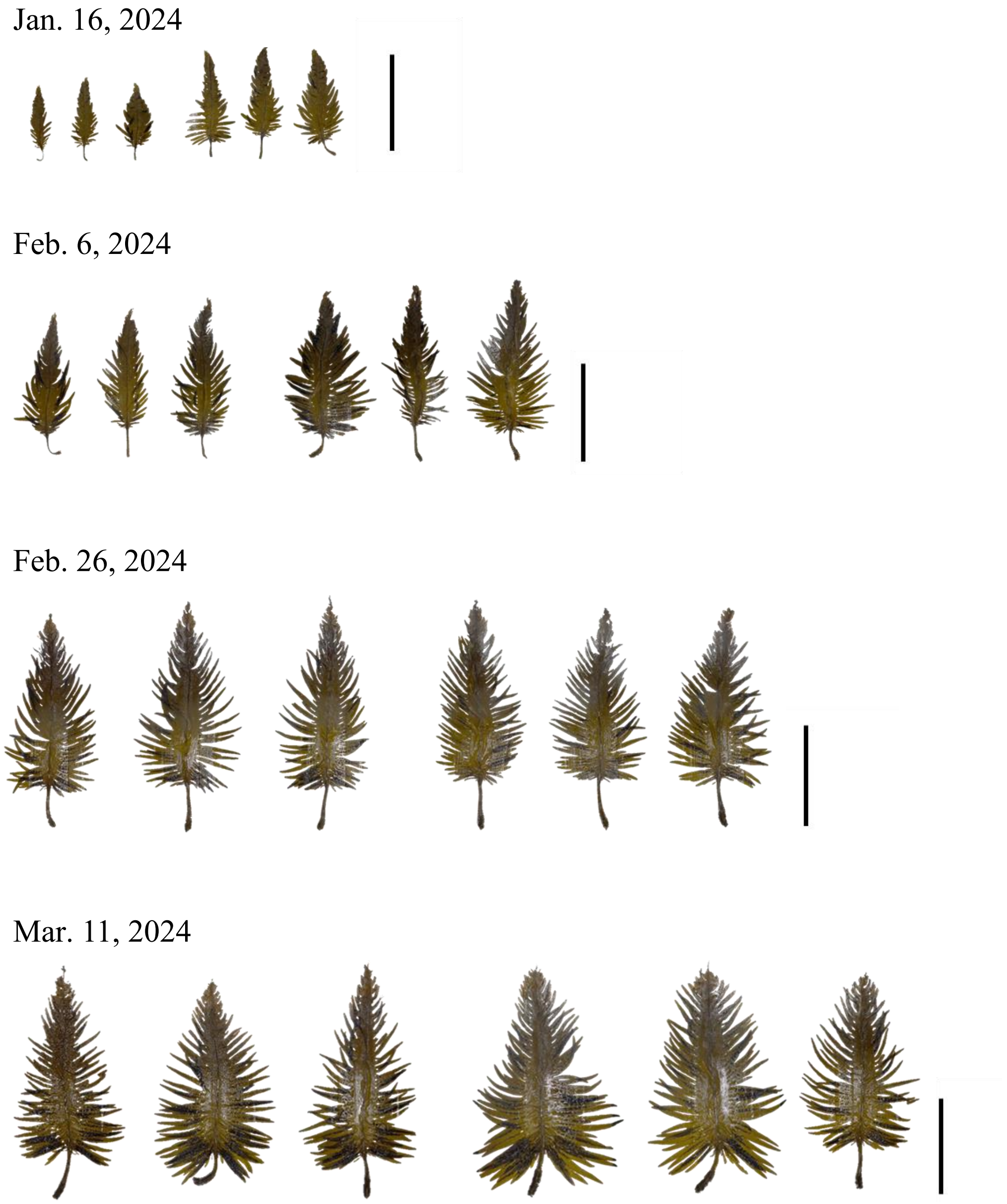

Figure 10. Sporophyte samples of *Undaria pinnatifida*, which are derived from both nursery cultivations in an outdoor tank (three sporophytes on the left) and in the sea (three sporophytes on the right), were collected during the main cultivation period. Scale bars, 1 m.

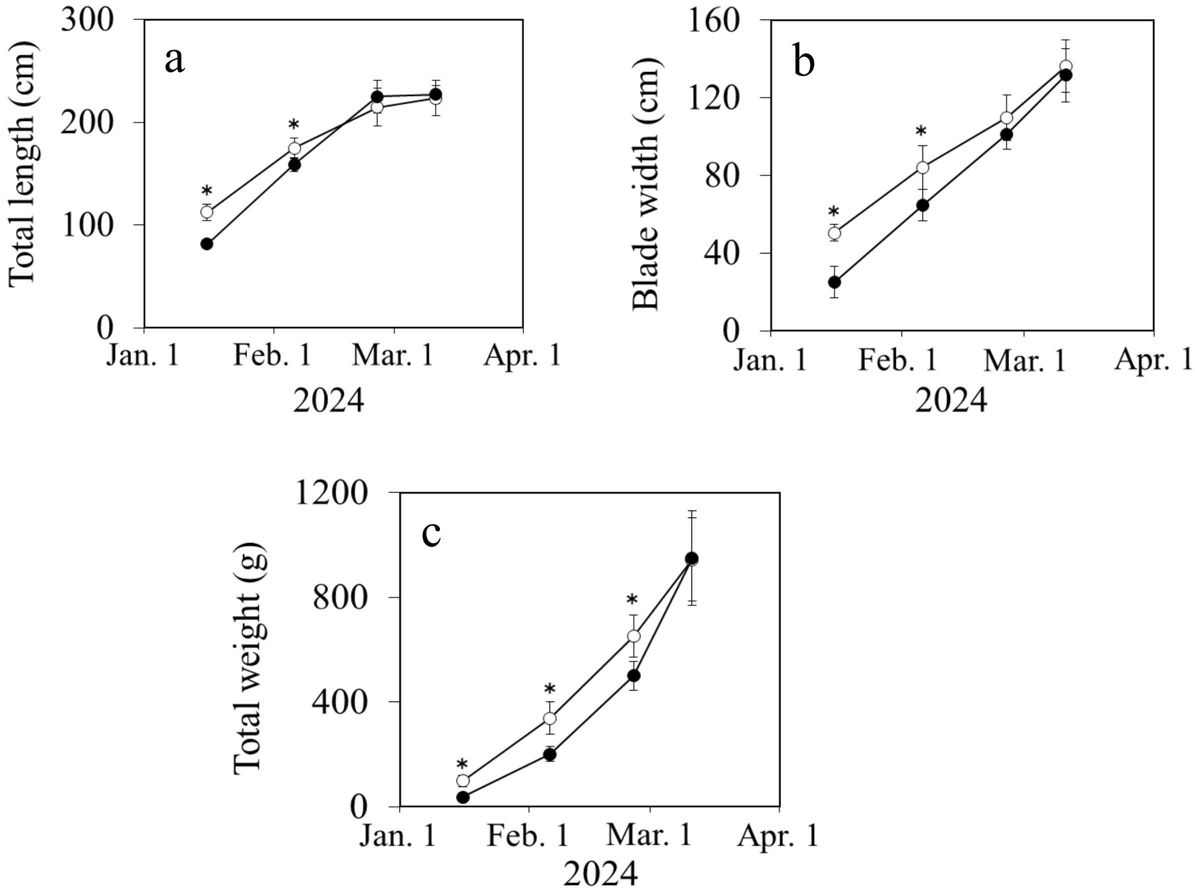

Figure 11. Growth of *Undaria pinnatifida* sporophytes, which are derived from nursery cultivation in an outdoor tank (closed circle) and in the sea (open circle), during the main cultivation period. (a) Total lengths; (b) Blade widths; (c) Total weights. Averages±SDs (n = 10). The asterisk indicates a significant difference ($p < 0.05$).

During the main cultivation period (mid-December to January 25), the water temperature at the *Undaria* farm gradually decreased from 15.0 to 9.2°C. Afterward, it slowly increased, reaching 10.0°C on the last sampling day (March 11, Figure 12a). The DIN concentrations fluctuated within the range of 1-3 μM, except for the value of 6.8 μM observed on January 5, 2024 (Figure 12b).

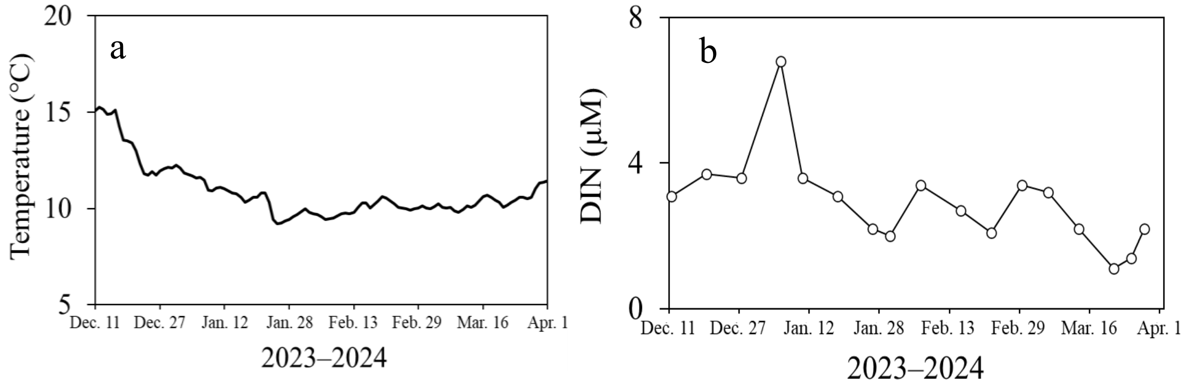

Figure 12. Changes in water temperature and dissolved inorganic nitrogen (DIN) concentrations during the main cultivation period. (a) Record of seawater temperatures in the sea; (b) Record of DIN concentrations in the sea.

Discussion. In this study, it was demonstrated that juvenile of *U. pinnatifida* sporophytes grew to a total length of 5.32 cm throughout the nursery cultivation period in the outdoor tank, reaching a size suitable for the main cultivation (approximately 1 to 5 cm in total length; Dan et al 2015; Tanada et al 2015; Niwa & Harada 2016). This result indicates that the seedlings used for the main cultivation period can be successfully produced on land. Herbivorous fish usually graze from the upper portion of the juvenile sporophytes (Kajiwara et al 2024). If the *Undaria* seedlings are small, it is easier for the fish to graze the growth point at the lower portion of the blade. Thus, the *Undaria* seedlings cannot grow further and become unsuitable for main cultivation. However, as the seedlings grow larger, the length and thickness of the sporophytes increase. As a result, it is less likely to graze the growth point, located at the lower portion of the blade (Kanamoto et al 2023). Therefore, it is expected that the grazing damage can be reduced by producing larger seedlings via nursery cultivation on land. The growth of *U. pinnatifida* seedlings is significantly influenced by environmental conditions during nursery cultivation. For example, juvenile sporophytes grow well at a water temperature of 20°C, but their growth slows at relatively high temperatures (Morita et al 2003; Baba 2008). Excessively high water temperatures cause juvenile sporophytes to fall off the seed strings, and sporophyte bleaching occurs above 28°C (Murase et al 2021). Gao et al (2013) reported that juvenile sporophytes cultured in nutrient-poor media exhibit poor growth at temperatures ranging from 15 to 27°C. Furthermore, the growth of juvenile sporophytes is faster under higher light intensities (Oka 2006; Niwa 2016), and adhered matter such as mud and diatoms inhibit the growth of juvenile sporophytes (Niwa 2016; Kajiwara et al 2024). Moreover, it is empirically known that juvenile sporophytes fall off seed strings because of adhered matter (Niwa 2016). In this study, juvenile sporophytes grew normally during nursery cultivation in the outdoor tank, and abnormal morphologies or color fading were not observed in the juvenile sporophytes. Additionally, limited sporophyte detachment was observed. Therefore, the outdoor tank environment in this study met certain conditions for producing normal *U. pinnatifida* seedlings. However, this nursery cultivation in the outdoor tank was conducted without controlling environmental factors, such as seawater temperature, light intensity and DIN concentration. If these environmental factors are optimally controlled in the tank, it is expected that the land-nursed seedlings can be produced more consistently in a shorter period.

The growth of juvenile sporophytes during the nursery cultivation period in the outdoor tank was slower than that during cultivation in the sea, and the total length of the former sporophytes at the end of nursery cultivation period was approximately half the length of the latter sporophytes. The water temperatures during the nursery cultivation period ranged from 15 to 18°C, with few differences between the outdoor tank and the sea. The water temperatures decreased below 23°C, which is the standard for the start of nursery cultivation in the Seto Inland Sea area (Dan et al 2015; Niwa & Harada 2016). The DIN concentrations were higher in the outdoor tank than in the sea. These data suggest that the delayed growth in the outdoor tank was not due to the water temperatures or DIN concentrations. On the other hand, the light intensity in the outdoor tank was 30 µmol photons m^{-2} s^{-1}, which is slightly lower than the optimal range of 50-100 µmol photons m^{-2} s^{-1} for the growth of juvenile sporophytes (Baba 2008). Thus, the low light intensity could be the reason for the delayed growth compared with the sporophyte growth in the sea. Furthermore, water velocities also significantly affect seaweed growth (Baba et al 2006; Nanba et al 2011; Peteiro & Freire 2011; Peteiro et al 2019; Nardelli et al 2024). Peteiro et al (2019) reported that the daily growth rate of juvenile *U. pinnatifida* sporophytes was highest when the water velocity was 15.8-17.1 cm s^{-1}, at both 28 and 56 days after the start of the experiment. Baba et al (2006) showed that the growth of juvenile sporophytes was favorable at water velocities of 10 and 20 cm s^{-1} on the basis of experiments in an outdoor tank system. In this study, the water velocity in the outdoor tank was 5.8 cm s^{-1}, suggesting that it did not reach the optimal level for adequate growth. In addition, ultraviolet (UV) rays influence the growth rate of *U. pinnatifida* seedlings, resulting in poor growth under a UV intensity of 1.84 W m^{-2} (Morita et al 2009). Since the UV intensities were not measured in this study, further study on the influence of UV intensity is needed to investigate the environmental conditions to obtain better growth.

Baba et al (2006) and Baba (2008) reported that the optimal conditions for the water velocities and light intensities for the growth of juvenile sporophytes vary depending on changes in water temperature during the experiment. Previous studies on other kelps have also showed that growth of juvenile sporophytes is determined by the interplay of various environmental factors, including temperature, light intensity, nutrient concentration and water velocity (Dean & Jacobsen 1984; Gao et al 2016; Mabin et al 2019; Peteiro et al 2019). Therefore, seedling growth may be affected by complex interactions among various environmental factors that determine the optimal environmental conditions for growth.

After the *U. pinnatifida* seedlings were produced via nursery cultivation in the outdoor tank, they were transplanted to the sea for the main cultivation period. The sporophytes grew to sizes suitable for sale as fresh produce (approximately 80 cm in total length) in mid-January, 2024. During the peak harvesting season (late February to March) in the Seto Inland Sea (Dan et al 2015; Niwa & Harada 2016), the sporophytes that were derived from the nursery cultivation in the outdoor tank grew to a sufficient size for use as processed products (over 2 m in total length). The size of the sporophytes was the same as that of the sporophytes that were cultivated via the normal method. Therefore, this study demonstrated that *U. pinnatifida* seedlings that are produced by nursery cultivation on land can grow to a sufficient size for use as commercial products until the peak harvesting season. Since the seedlings produced by nursery cultivation in the sea were larger than those produced in the outdoor tank, it seems that the growth saturation of the sporophytes was also faster in the former than in the latter. Thus, it was inferred that the sporophytes derived from nursery cultivation in the outdoor tank grew to the same size in the late cultivation period. In addition, the water temperature at the start of the main cultivation in this study was 15.3°C, which was significantly lower than the starting temperatures of 21-23°C recommended by Ii (1964) and Dan et al (2015). Afterward, the changes in water temperature during this experimental cultivation period generally followed the same trend as those reported in previous studies conducted at the same *Undaria* farm (Niwa 2015; Niwa & Harada 2016). Additionally, although the DIN concentrations during this cultivation period were very low (approximately 3 μM), bleaching of the sporophytes was not confirmed. This result indicates that *U. pinnatifida* is more tolerant to low nutrition than *P. yezoensis* is, as reported by Niwa (2015).

In recent years, grazing damage by fish to cultivated seaweeds, such as *U. pinnatifida* and *Saccharina latissima* (Peteiro & Freire 2011), *Eucheuma denticulatum* (Kasim et al 2016), *Gracilaria lemaneiformis* (Yang et al 2006, 2015), *Sargassum fulvellum* (Hwang & Park 2020), and *Undaria undarioides* (Kimura et al 2007), has been reported worldwide. Kasim et al (2016) reported that more than 60% of seaweed production was reduced by the feeding activities of herbivorous fish. For the *Undaria* farms in Japan, grazing damage frequently occurs during the nursery cultivation period (Niwa & Harada 2016; Kajiwara et al 2024). Herbivorous or omnivorous fishes, such as *Siganus fuscescens* (Kiriyama et al 2000), *Thamnaconus modestus* (Noda & Murase 2022; Kajiwara et al 2024), *Acanthopagrus schlegelii* (Noda & Murase 2021; Kajiwara et al 2024) and *Kyphosus bigibbus* (Kiriyama et al 2018), graze on *U. pinnatifida* seedlings. Grazing damage during the cultivation period has caused a decrease in *Undaria* production and severe financial harm to cultivators (Kiriyama et al 2018). Consequently, *Undaria* cultivators in Japan sometimes need to purchase seed frames from other cultivators. However, owing to the shortage of seed frames after nursery cultivation, it is becoming more difficult for cultivators to obtain seedlings. To prevent herbivorous or omnivorous fish from grazing on seaweed in seaweed farms, several experiments have been conducted using various methods, such as nets (Ganesan et al 2006, 2019), floating cages (Kasim et al 2016), and net cages (Kimura et al 2007). An experiment in which polyethylene protective nets were wrapped around cultivation ropes growing juvenile sporophytes of *U. pinnatifida* demonstrated a certain degree of protection against grazing damage (Kiriyama et al 2000). However, it is difficult to apply this method to seed frames that are widely used for the nursery cultivation of *U. pinnatifida* in the Seto Inland Sea. If the land-based nursery cultivation method in this study is applied, it will be possible to reliably produce seedlings for the main cultivation period without grazing damage by fish occurring.

In the case of nursery cultivation in the sea, it is necessary to frequently visit *Undaria* farms to check the growth conditions of juvenile sporophytes on the seed frames and to clean off dirt adhering to the seed strings via a pump used to spray seawater (Niwa 2016). Moreover, there is a risk of losing seed frames due to strong waves caused by low pressure during typhoons. On the other hand, in the case of nursery cultivation in a water tank, there were small amounts of dirts adhering to the seed strings, and cleaning the seed frames and tanks once every three weeks was sufficient to maintain cleanliness. Furthermore, the seed frames are not lost because they are put into the tank on land. Therefore, the land-based nursery cultivation method using outdoor tanks is more efficient and safer than the sea-based method. It is therefore necessary to conduct further studies on the nursery cultivation method using many seed frames for stable *Undaria* cultivation.

Conclusions. This study examined whether the *Undaria* seedlings for main cultivation can be produced throughout nursery cultivation via an outdoor tank located on land. The result indicated that the juvenile sporophytes grew to a size suitable for the main cultiviation, although the size of the seedlings was smaller than that of the seedlings produced in the sea. Furthermore, the sporophytes that were derived from the nursery cultivation in the outdoor tank grew to a sufficient size for use as commercial products until the peak harvesting season. These results demonstrated that the *Undaria* seedlings for main cultivation can be produced by nursery cultivation using outdoor tanks on land to avoid grazing damage caused by herbivorous fish. Thus, this method has potential for the stable seedling production under the recent marine environment where grazing damage by herbivorous fish is constantly occurring.

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