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Seaweed seedling culture technique using LEDs and feeding behavior of herbivorous fish to suppress fouling by other seaweeds - in the case of "*hiziki*" *Sargassum fusiforme* culture -

Tsutomu NODA*[1,†], Tatsuru KADOTA*[1], Tomoyuki SHIKATA*[2], and Yuichiro FUJINAMI*[3]

Abstract: A brown seaweed *Sargassum fusiforme,* called "*hiziki*" in Japanese, is an important fishery resource in Japan. During the artificial seedling production of *hiziki*, fouling by other seaweeds (e.g. *Ulva* spp.) has become a great problem. In this study, we developed an efficient *hiziki* seedling culture technique using LEDs and the feeding behavior of herbivorous fishes: *Girella punctata* and *G. leonina*. *Hiziki* germlings (approximately 0.15 mm length) were cultured for 30 days under the LED light of four colors: green, blue, red, and white. At the end of the experiment, the respective mean length of the germlings in the green, blue, red, and white LEDs treatments were 1.95 mm, 1.44 mm, 0.25 mm, and 1.46 mm. The respective mean coverage of fouling by other seaweeds were 29.1%, 96.8%, 29.1%, and 87.2%. These findings indicate the green LED light is adequate for higher growth of *hiziki* and for growth suppression of fouling seaweeds. Furthermore, another experiment was conducted using herbivorous fish to remove other seaweeds. We placed *hiziki* germlings and other seaweeds propagating on a concrete block with 10 juveniles of *G. punctata* or *G. leonina* in a 100 L tank. After 4.5 h, the coverage of fouling by other seaweeds decreased from 23.5% to 0.5% in the *G. punctata* tank, and from 15.5% to 0.6% in the *G. leonina* tank. The lengths of *hiziki* on the block, those detached from the block, and those in the stomach contents of the fish were, respectively, 2.04 mm, 1.47 mm, and 0.86 mm in the *G. punctata* tank, and 2.37 mm, 1.54 mm and 1.25 mm in the *G. leonina* tank. These results demonstrate that larger *hiziki* seedlings are less susceptible to the browsing by *G. punctata* and *G. leonina*. Consequently, juvenile *G. punctata* and *G. leonina* were found to be effective for removing fouling seaweeds during the *hiziki* seedling production.

Key words: *Sargassum fusiforme*, LEDs, *Girella punctata*, *Girella leonina*, seaweed seedling production

Introduction

Seaweeds have lots of ecological functions such as absorption of dissolved nutrients and carbon, and some of them are fisheries-important species, too, such as human foods and industrial materials. Recently, their functions are attracting particular attention. A brown seaweed *Sargassum fusiforme* ("*hiziki*" in Japanese) is distributed on rocky shores in intertidal zones of Japan, Korea, and China (Yoshida 1998). In western Japan, *hiziki* grows up to 1-2 m in spring and early summer, and matures during May-June. *Hiziki* is regarded as resistant to high temperatures. Therefore, the *hiziki* habitat has extended from subarctic to subtropical areas. *Hiziki* is an important fishery resource in Japan, but wild *hiziki* production has been decreasing due to the changes in coastal environments and predation by herbivorous fish and benthos. In 1996, the

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*1 Coastal and Inland Fisheries Ecosystems Division, Environment and Fisheries Applied Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Nagasaki, Nagasaki 851-2213, Japan
*2 Environmental Conservation Division, Environment and Fisheries Applied Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Goto, Nagasaki 853-0508, Japan
*3 Management Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Goto, Nagasaki 853-0508, Japan
† Email: noda_tsutomu03 at fra.go.jp

production was about 5,000 tons in Nagasaki Prefecture, Japan. In recent years, it has decreased to less than 500 tons (Fig. 1, Nagasaki Prefecture, 1989, 2017). More than 90% of *hiziki* products recently distributed throughout Japan are imported from China and Korea. *Hiziki* has also been produced by aquaculture mainly using the seedlings collected from coastal areas, which has adversely affected the wild resources.

An artificial seedling production method of *hiziki* has been studied. Growth inhibition of *hiziki* occurs mainly due to green algae contamination during the seedling production. Noda *et al*. (2022) reported a method of removing fouling seaweeds using the feeding behaviors of juvenile herbivorous fish. Their study was performed with approximately 1.0 cm *hiziki* because smaller *hiziki* germlings might be consumed by the herbivorous fish. Thus, other methods are needed to remove the fouling seaweeds for smaller *hiziki* germlings. Brown algae (including *hiziki*) and green algae (including fouling seaweeds, e. g. *Ulva* spp.) have photosynthetic pigments of different types. The growth of both algae can be controlled by changing the light wavelength as demonstrated by Murase *et al*. (2018) and others. Both brown algae and green algae have chlorophyll a, which absorbs blue and red light. Green algae have chlorophyll b, and brown algae have chlorophyll c, both of which absorb blue light. By contrast, fucoxanthin in brown algae absorbs blue and green light, as described in Bricaud *et al*. (2004) and others. Thus, an experiment was planned to ascertain whether green light selectively promote the *hiziki* germling growth. As described in this report, we introduce an efficient *hiziki* seedling culture technique using LEDs. A suitable size of *hiziki* germlings that could be applied to the removal of fouling seaweeds by juvenile herbivorous fish *Girella punctata* (largescale blackfish) and *G. leonina* (smallscale blackfish) was also elucidated.

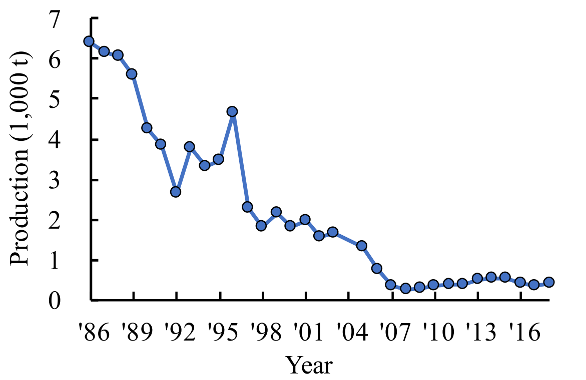

Fig. 1 Change of *hiziki* production in Nagasaki Prefecture (Nagasaki Prefecture 1989, 2017)

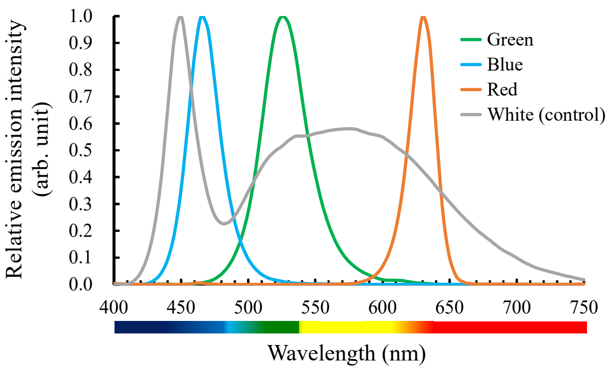

Fig. 2 Relative emission intensity of LEDs used for the experimentation

Green, NCSG119 (Nichia); Blue, #182521 (Stanley Electric); Red, NCSR119 (Nichia); White, NVSW119F (Nichia). The relative emission intensity (arb. unit) was measured using a Spectromaster C-7000 (Sekonic).

Materials and Methods

Test I

Hiziki germling culture experimentation using LEDs was performed at Goto Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency (FRA), Nagasaki, Japan. After adult stage *hiziki* were collected from the coastal areas of Goto city during May and June in 2021, they were stocked in tanks filled with sand-filtrated seawater until maturation. On June 9, 2021, we collected the germlings (approximately 0.15 mm) from mature *hiziki* in the tanks and cultured them on eight concrete blocks (about 10,000 germlings per block, 39 × 19 × 10 cm). These germlings were cultured for 30 days, meanwhile they were exposed (100 μmol/m^2/s; 12L:12D light regime) to four colors of LED light: green, 526 nm central wavelength; blue, 466 nm; red, 631 nm; and white, complex light as a control (Fig. 2). The product numbers of green, red, and white LEDs were NCSG119, NCSR119, and NVSW119F (Nichia, Tokushima, Japan), and that of blue LEDs was #182521 (Stanley Electric, Tokyo, Japan), respectively. The relative radiant intensity (arb. unit) was measured using a Spectromaster C-7000 (Sekonic, Tokyo, Japan). We used black 100 L polyethylene tanks (2 tanks for each color). The test tanks were covered with a lid to prevent outside light from entering, and sand-filtrated seawater was supplied (50 L/h). The water temperature was 20.6–25.2°C (no water temperature control). On June 16 (during cultivation) and July

Table 1 Concentrations of nutrients in the *hiziki* culturing seawater in Test I

Data (2021)	NO_3-N (μmol/L)	NO_2-N (μmol/L)	NH_4-N (μmol/L)	PO_4-P (μmol/L)	SiO_2-Si (μmol/L)	Dissolved Inorganic Nitrogen (μmol/L)
June 16	2.801	0.119	0.119	0.280	6.410	3.039
July 9	4.649	0.124	0.194	0.377	6.074	4.967

9 (at the end of cultivation) in 2021, nutrient concentrations in the culturing water in the tanks were analyzed using an autoanalyzer QuAAtro (BLTEC, Osaka, Japan) (Table 1). Since the seawater had a possibility of including other seaweeds embryos, the experiment was designed to mimic the conditions of mass seedling production in natural environment. After 30 days of culture, we measured the germling growth (from tip to root, over 30 individuals), the remaining rate of *hiziki*, and the coverage of fouling by other seaweeds. The remaining rates were calculated using the number of individuals at the start and that survived at the end of the examination by photographing the center of each block using a digital camera TG-5 (Olympus, Tokyo, Japan). The coverage of fouling by other seaweeds was analyzed for the photographs of concrete block surfaces using image analysis software ImageJ (US National Institutes of Health, Schneider *et al*. 2012).

Test II

The experiment of removing fouling seaweeds using juveniles of herbivorous *G. punctata* and *G. leonina* was performed at Goto Field Station, FRA. These fish were collected from the coastal area of Goto city during February and June in 2021 (Ito *et al*. 2018) and reared in sand-filtrated seawater until experimentation. Then, on July 19, 2021, a tank experiment was conducted. The water temperature was 25.0 °C. The *hiziki* germlings and other seaweeds propagated on two concrete blocks, which had been cultivated under the green LEDs, were used for the experiment. The coverage proportions of fouling by other seaweeds (*Ectocarpus* spp.) on the two blocks were estimated as 23.5% and 15.5% using ImageJ as described above. We placed each block in a black 100 L polyethylene tank with 10 juveniles of *G. punctata* (60.4 ± 9.0 mm TL) or *G. leonina* (62.1 ± 5.9 mm TL). After 4.5 h, the fish were euthanized in 100 ppm eugenol to examine the fish stomach contents. Then, the lengths of *hiziki* on the block, those detached from the block by browsing of the fish, and those found in the fish stomach contents were measured using a profile projector V-12 (Nikon, Tokyo, Japan).

Results and Discussion

Test I

After 30 days of cultivation under LEDs, the respective mean length of the germlings used in the green, blue, red, and white LED light treatments were 1.95 mm, 1.44 mm, 0.25 mm and 1.46 mm. The respective rates of remaining *hiziki* were 33.9%, 28.8%, 16.6%, and 15.7%. *Hiziki* cultivated under green LEDs grew largest with the highest rate of remaining *hiziki*. The respective coverages of fouling by other seaweeds in the green, blue, red, and white LED light treatments were 29.1%, 96.8%, 29.1%, and 87.2%. The appearances of the blocks showed no difference until 10 days. After 20 days, the fouling by other seaweeds had begun to propagate under blue and white LEDs. After 30 days, the blocks under blue and white LEDs were covered by other seaweeds. Green algae *Ulva* spp. increased mainly under the blue LED light, and brown algae *Ectocarpus* spp. increased mainly under the white LED light. By contrast, fouling by other seaweeds was slow on the blocks under green or red LEDs. These results demonstrate that the green LED light is adequate for higher growth of *hiziki* and for growth suppression of fouling by other seaweeds (Fig.3). In addition, the propagation of *Ectocarpus* spp. started to increase later during the 30 days of cultivation even under green LEDs. These results suggest that the green light cannot completely suppress the seaweed fouling.

Test II

In the fouling seaweeds removal experiment using juvenile herbivorous fish, the coverage of fouling by other seaweeds decreased from 23.5% to 0.5% in the *G. punctata* tank, and from 15.5% to 0.6% in the *G. leonina* tank. The mean length of *hiziki* on the block was longer than that detached from the block or that found in the fish stomach contents. These trends were similar between both fish species: more than 80% of *hiziki* detached or eaten by fish were smaller than 2.0 mm (Fig.4). These results suggest that larger *hiziki* were less susceptible to the browsing by the fish. Therefore, *G. punctata* and *G. leonina* were found to be effective for removing the fouling seaweeds during the production of *hiziki* seedling that had grown larger than 2 mm. Noda *et al*. (2022) had previously reported that the use of *G. punctata* had a sufficient effect on the removal of other seaweeds without *hiziki* predation by the fish. The present results suggest that *G. leonina*, which is phylogenetically similar to *G. punctata*, can also be used similarly in *hiziki* seedling production.

Conclusion

Our study suggests that the use of LEDs and feeding behaviors of juvenile herbivorous fish is important for effective production of *hiziki* seedlings. During an early period of *hiziki* seedling production, green LEDs should be used for their growth promotion and the growth suppression of fouling

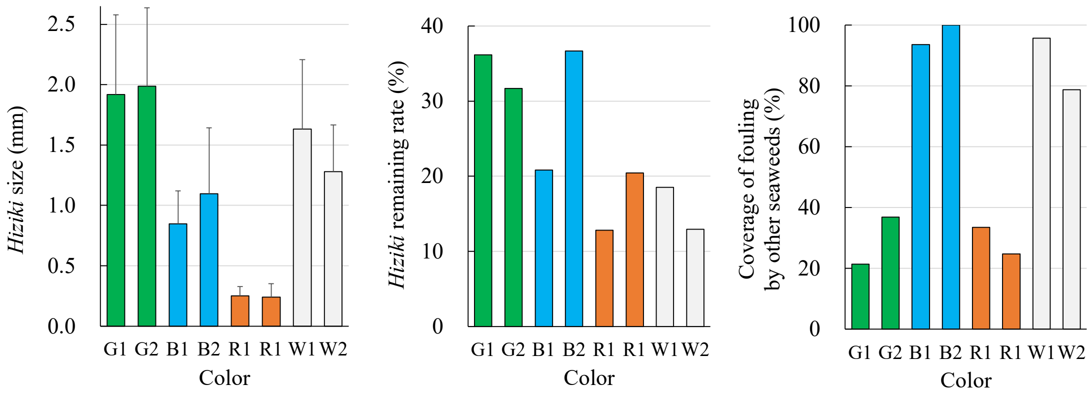

Fig.3 *Hiziki* size, remaining rate, and coverage of fouling by other seaweeds after the 30 days of culture

Vertical bars represent the standard deviations.

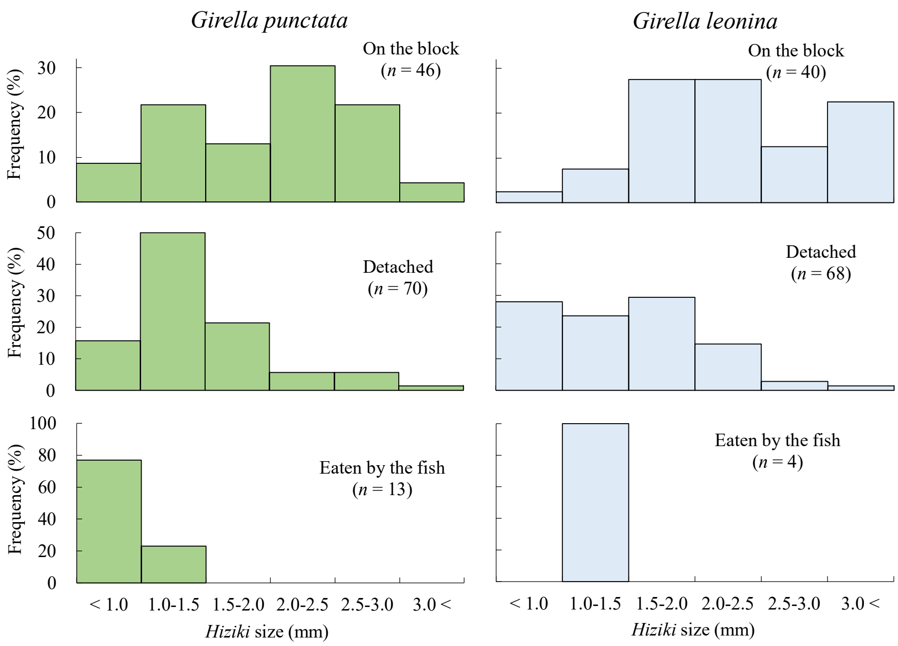

Fig.4 Histograms of the size of *hiziki* on the block, detached from the block, and eaten by *Girella punctata* and *G. leonina*

Fig.5 Appearances of concrete blocks before and after the introduction of juvenile *Girella punctata* and *G. leonina* during *hiziki* seedling production

by other seaweeds. After *hiziki* grew to 2 mm length, the feeding behavior of *G. punctata* and *G. leonina* can be applied to the removal of fouling seaweeds. Our method could be applied during practical *hiziki* seedling production (Fig.5). We are performing a test practice for artificial seedling production using this method. Further examinations must be conducted to assess the appropriate light intensity for *hiziki* growth, suitable sizes of *G. punctata* and *G. leonina* for fouling seaweeds removal, and potential use of other herbivorous fish species.

Acknowledgments

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Ito T, Iino Y, Nakai S, Itoi S, Sugita H, Takai N (2018) Distribution patterns of settlement-stage juveniles of *Girella punctata* and *Girella leonina* on the rocky coast of the Kanto–Izu region, Japan. *Fish. Sci*., **84**, 627-640.

Murase N, Abe M, Noda M (2018) Growth and maturation of gametophyte in *Undaria pinnatifida* under different light quality from light emitting diodes (LEDs). *J. Natl. Fish. Univ.*, **67 (2)**, 91-97 (in Japanese with English abstract).

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Noda T, Kadota T, Shimaoka K, Fujinami Y (2022) Removal of fouling seaweed on artificial substrate for *Sargassum fusiforme* seedling culture by juvenile *Girella punctata* grazing. *Aquacult. Sci.*, **70 (1)**, 113-117 (in Japanese with English abstract).

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Yoshida T (1998) Marine algae of Japan. Uchida Rokakuho Publishing, Tokyo, pp. 367-368 (in Japanese).

Annotated Bibliography of Key Works

(1) Noda T, Kadota T, Shimaoka K, Fujinami Y (2022) Removal of fouling seaweed on artificial substrate for *Sargassum fusiforme* seedling culture by juvenile *Girella punctata* grazing. *Aquacult. Sci*., **70 (1)**, 113-117 (in Japanese with English abstract).

Tank experiments were conducted to develop a technique to remove fouling seaweeds from seedling production of *Sargassum fusiforme*. They set a substrate with *S. fusiforme* and fouling seaweeds (*Ulva australis* and *U. intestinalis*) and 12 juvenile *Girella punctata* in replicate tanks (n = 4). The coverage and length of the fouling seaweeds decreased as time proceeded, while those of *S. fusiforme* did not decrease. In addition, *S. fusiforme* were not observed in the stomach contents of *G. punctata*. These results suggest that *G. punctata* can remove fouling seaweeds without apparent damage to *S. fusiforme* in its seedling production.

(2) Murase N, Abe M, Noda M (2018) Growth and maturation of gametophyte in *Undaria pinnatifida* under different light

quality from light emitting diodes (LEDs). *J. Natl. Fish. Univ.* ,**67 (2)**, 91-97 (in Japanese with English abstract).

The effects of light quality on growth and maturation of gametophyte in *Undaria pinnatifida* were examined in an indoor culture at 20°C under the lighting condition of 12h light-12h dark cycle and 50 μmol/m^2/s using four different light emitting diodes (LEDs) and a fluorescent light. The relative growth rates of the male and female gametophytes under green LEDs showed higher values, but those under red LEDs showed lower values. The female gametophyte matured quickly under blue LEDs and the fluorescent light. On the other hand, they matured more slowly under white and green LEDs. Under the red LEDs condition, the maturation of female gametophytes was not observed at all.

(3) Ito T, Iino Y, Nakai S, Itoi S, Sugita H, Takai N (2018) Distribution patterns of settlement-stage juveniles of *Girella punctata* and *Girella leonina* on the rocky coast of the Kanto–Izu region, Japan. *Fish. Sci.*, **84**, 627-640.

The early life history of girellid fishes in Japanese waters is unclear, and little is known about their species-specific reproductive strategies. They examined seasonal changes of the distribution patterns for settlement-stage juveniles of *Girella punctata* and *G. leonina* on the rocky shore in the regions of Kanto and Izu, Japan, to infer the influence of the Kuroshio Current on their reproduction. They collected 813 settlement-stage juveniles mainly in Sagami Bay and genetically identified the species.

The juveniles of *G. punctata* were collected on the rocky shore in Sagami Bay from April to August, with abundant catches in May and June. Thus, they inferred that juvenile *G. punctata* ubiquitously inhabited the rocky shore in the area in spring and summer. By contrast, juveniles of *G. leonina* were rarely collected in Sagami Bay, with a total catch of only 66. Notably, no juveniles were collected during the wintertime in Sagami Bay, although an abundant catch of *G. leonina* had been previously reported in Sagami Nada off Sagami Bay during January to March. These clear-cut differences between the habitats of two species likely reflect the differences in their proximity to the path of the Kuroshio Current. They expected that the Kuroshio Current strongly influenced the reproductive success of *G. leonina*.