水研センター研報，第32号，115－118，平成22年
Bull. Fish. Res. Agen. No. 32, 115-118, 2010

Seedling production in *Eisenia arborea* using Suruga Bay deep-sea water for restoration of kelp beds in isoyake area

Kazumi NIMURA [*1], Hiroyuki NODA [*2] and Kazutoshi OKAMOTO [*3]

Abstract; *Eisenia arborea* is distributed from central to southern Pacific coast of Japan. The wild sporophytes of the kelp extincted along the coasts of Omaezaki and Makinohara Cities in Shizuoka Prefecture after deforestation called isoyake (cf. non-urchin barren here) in 1980's. Fortunately, we have cultured the native sporophytes collected from Omaezaki Cape in our laboratory. Suruga Bay deep-sea water (DSW) is nutrient rich, cold and clean sea water pumped from two depths of 397 and 687m with pipelines. As nitrate and phosphate concentrations of DSW (ex. NO_3=21.1～30.7 μM, PO_4=1.7～2.8 μM in 2007) are higher than that of surface sea water (ex. NO_3=4.0～14.4 μM, PO_4=0.3～1.2 μM in 2007), growth rate of juvenile *Eisenia* sporophyte was higher in DSW than surface seawater. Therefore, we used DSW as culture medium for seedling production. Seedling production was started by the homogenization of the cultured gametophytes with the mixer. Culturing 1～2 weeks later, numerous number of the microscopic free living sporophytes were obtained. These sporophytes was settled on the strings within a day in batch culture and then cultured for about one month in DSW. This seedling of the *E. arborea* was transplanted to isoyake area for restoration of the kelp forests. The method of culture and transplantation on blocks were described here.
Key words: Deep-sea water, *Eisenia arborea*, Isoyake, Seedling, transplantation

Necessity of seedling production in *Eisenia arborea*

Eisenia arborea (Laminariales) is distributed from central to southern Pacific coast of Japan (Yoshida, 1998). The standard Japanese name of *E. arborea* is "Sagarame", which was named after "Sagara Town" (now incorporated in Makinohara City: Fig. 1) near the Omaezaki Cape in Shizuoka Prefecture. *E. arborea* has been utilized as a food in central and west areas in Shizuoka Prefecture; this kelp contains much mucus polysaccharide. Before mid 1980's, there had been a vast kelp forest of *E. arborea* and *Ecklonia cava* along the coast of Makinohara and Omaezaki Cities (Hasegawa *et al.*, 2003). Then, the kelp forests have decreased and replaced by barren ground (cf. non-urchin barren here). The phenomenon was a type of "Isoyake". There remained a few small kelp forests after mid 1990's, but in 2000's, no wild *Eisenia* sporophytes can be found on the coast. Therefore there is no

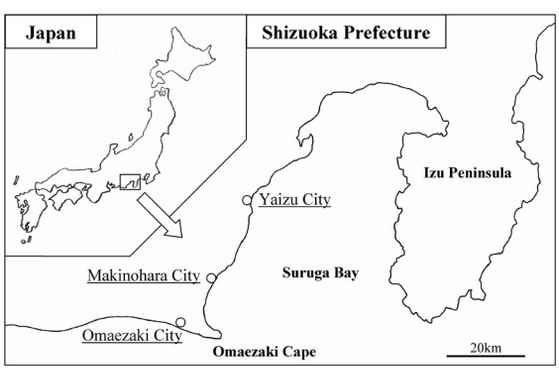

Fig. 1. Map indicates the location described in the text.

2010年7月1日受理（Received on July 1. 2010）
*1 Shizuoka Prefectural Research Institute of Fishery, 3690 Kogawa, Shizuoka 425-0033, Japan
*2 Suruga-Bay deep seawater aquaculture research center of Shizuoka, Prefectural Research Institute of Fishery, 136-24 Iwashigashima, Yaizu, Shizuoka 425-0032, Japan
*3 Office of Fishery management, Shizuoka Prefectural Office, 9-6 Otemachi, Aoi, Shizuoka, Shizuoka 420-8601, Japan

hope of recruitments in natural condition. However, fortunately, we have cultured the native *Eisenia* sporophytes collected from Omaezaki cape in our laboratory. Our purposes were the production of seedling in the laboratory and transplantation using this seedling for the restoration of the kelp beds. In this paper, the method of preparation of seedling using deep seawater and transplantation on to the blocks are described.

Characteristics of Suruga Bay deep-sea water and juvenile growth

Suruga Bay deep-sea water (DSW) was pumped from Suruga Bay, Yaizu City, Shizuoka Prefecture, from depths of 397 m and 687 m using pipelines (Fig. 1). In this manuscript, the definition of "DSW" is the seawater existed under 200m in depth in the ocean (Nakashima 2002). The deep layer below 200 m in depth is mostly under compensation depth; degradation rate is higher than production rate by photosynthesis (Nakashima 2002). The characteristics of DSW are, 1) rich nutrients: nitrate, phosphate and silicate concentrations of DSW is higher than that of surface sea water (SSW), 2) cleanness: dissolved organic matter, sustained substances, almost no specific pathogens and artificial contaminants are included, 3) Coldness: water temperature of DSW is low below 10℃ and stable.

The fluctuations of nitrate concentration in DSW and SSW in 2007 were shown in Fig. 2. Nitrate concentration of DSW (21.1 ～ 30.7 μM) was higher than that of SSW (4.0～14.4 μM). Another nutrient, phosphate concentration of DSW was also higher concentration during the year (DSW: 1.7 ～ 2.8 μM; SSW: 0.3 ～ 1.2 μM). We investigated the effects of DSW and SSW as culture mediaum on the growth of juvenile *Eisenia* sporophytes. Juvenile sporophyte was larger in the length in dsw than in SSW at the end of culture (Fig. 3). Therefore we produced the seedling of *E. arborea* using DSW and transplanted to Isoyake area.

Seedling production using Deep seawater

Life cycle of *Eisenia* consists of macroscopic sporophyte and microscopic gametophyte (Hori, 1993), and both life stages are able to culture in our laboratory. Mother sporophytes was culturing in polycarbonate vessel (1kl volume) with DSW and aeration. Usually these sporophytes mature

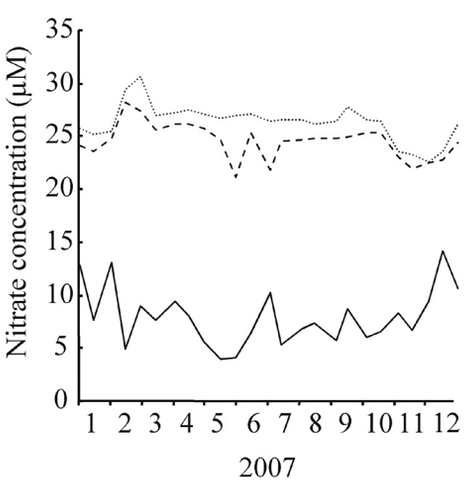

Fig. 2. Fluctuation of nitrate concentration in Suruga Bay deep-sea water (397m: broken line; 687m: dotted line) and surface sea water (solid line) in 2007.

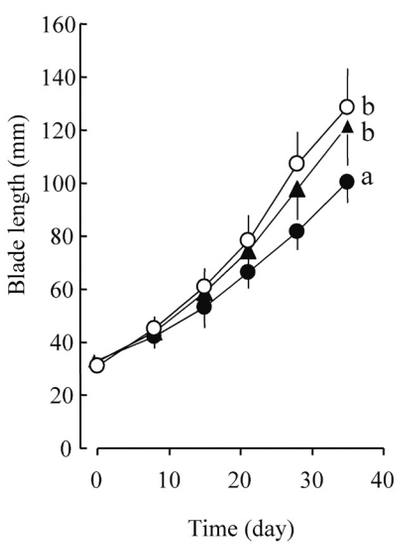

Fig. 3. Changes of the blade length of juvenile *E. arborea* sporophyte cultured in Suruga Bay deep-sea water (397m: ▲; 687m: ○) and surface sea water (●).

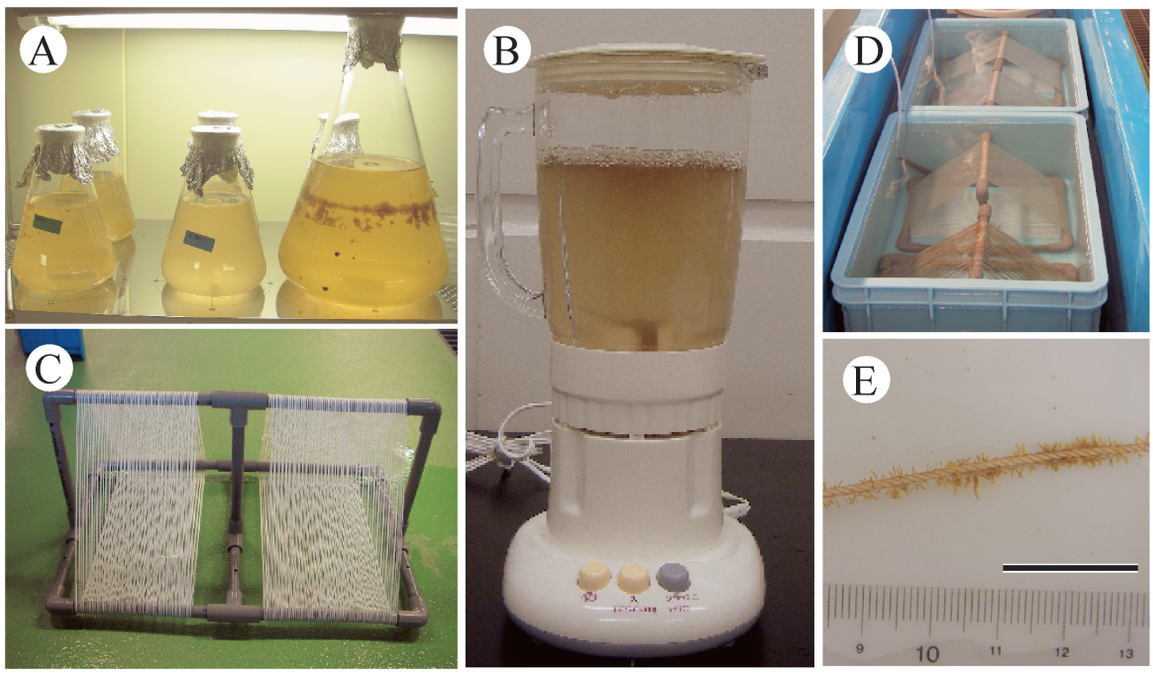

Fig. 4. Photographs of seedling production. A: Propagation of the gametophytes, B: Homogenate of gametophytes, C: Strings wound on the triangular frame, D: Cultivation of seed strings, E: Microscopic sporophytes on seed string (scale bar indicates 2cm).

in autumn, and we obtained zoospores from sori formed on the blades. The zoospores were cultured under 40 ～ 60 μmol m^{-2} s^{-1} (light: dark cycle=12hr: 12hr) at 18℃ in filtered and autoclaved seawater. After the zoospores differentiated to male and female gametophytes. The gametophyte were cultured under 20 ～ 80 μmol m^{-2} s^{-1}(light: dark cycle=12hr: 12hr) at 18℃ with gently stirring or batch culture in modified Provasoli's enriched seawater (PESI) without iron (Fig. 4 A). Maturation of the gametophyte is needed iron above 2.0 mg l^{-1} in *Saccharina* (*Laminaria*) *angustata* (Motomura *et al.*, 1981), and we observed same phenomenon in *E. arborea* (Nimura *et al.*, 2005). We could stock the gametophytes for several years in this condition.

Seedling production was started by the homogenization of the cultured gametophytes. The gametophytes were homogenized with the mixer to cut into pieces of the filamentous gametophytes (Fig. 4 B). These gametophytes were cultured in PESI with gently stirrer or aeration under 40 ～ 60 μmol m^{-2} s^{-1} (light: dark cycle=12hr: 12hr) at 18℃. After 1 ～ 2 weeks later, we could obtain numerous numbers of the microscopic free living sporophytes. These sporophytes were scattered on the strings (approximately 1 mm in diameter) wound on the triangular frame made from vinyl chloride pipe (Fig. 4 C) in the same vessel filled with DSW. DSW was filtered with cartridge filter (pore size: 0.45 μm) to prevent propagation of contaminated algae and organisms because DSW contains a little number of the microphytes. After the sporophytes were settled on the strings, they were cultured in filtered DSW at approximately 80 μmol m^{-2} s^{-1}(light: dark cycle=12hr: 12hr) at 12 ～ 17℃ for about 1 month (Fig. 4 D). As a result, we obtained a large number of the macroscopic sporophytes on the strings (Fig. 4 E). Seeds on strings were cultured for acclimation in the sea under natural condition (Fig. 5 A). One or two weeks later, seed strings were wound to a rope and attached to the concrete reef (Fig. 5 B, C). The concrete reef was set into Isoyake area (Fig. 5 D).

We produced the *Eisenia* seedlings using DSW, and other species, for example, *E. bicyclis, Ecklonia cava, Saccharina* (*Laminaria*) *japonica, Undaria pinnatifida.* These seedlings can be also used for the cultivation.

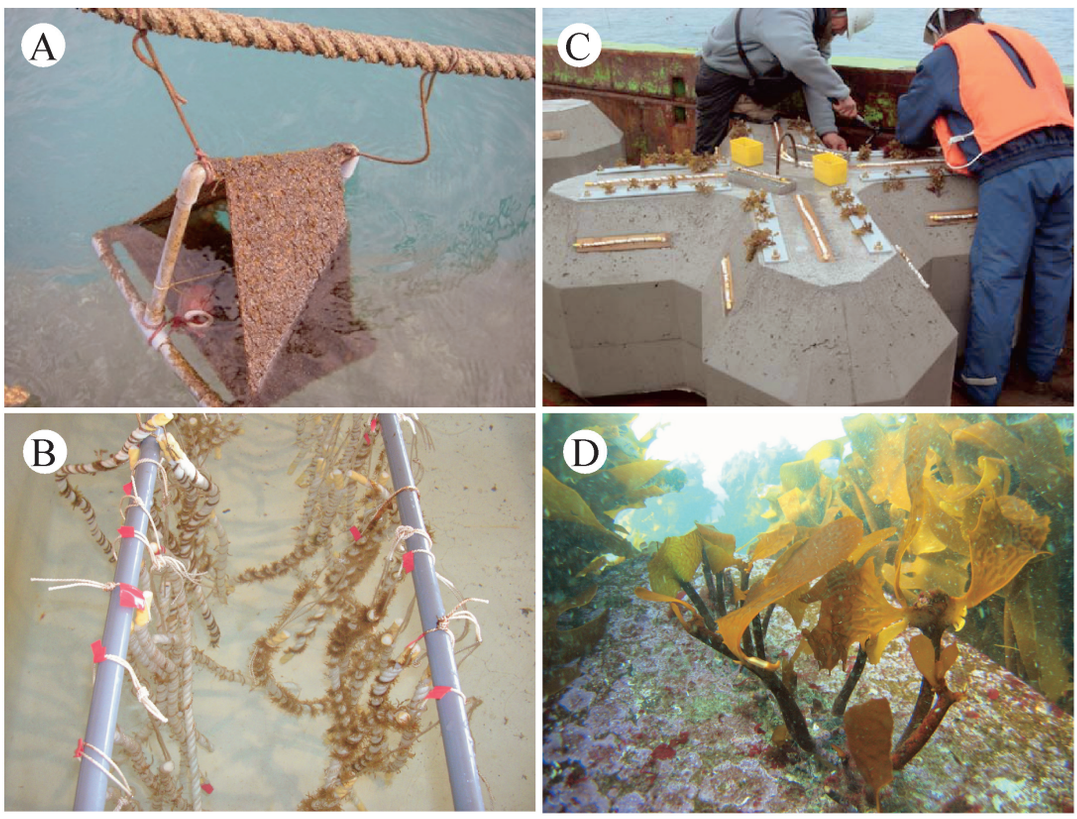

Fig. 5 Photographs of transplanting, A: Cultivation of seed strings in the sea under natural condition, B: Rope wound with seed strings, C: Concrete reef attached with the rope, D: *E. arborea* sporophytes derived from the seed strings on the concrete reef.

Acknowledgment

This study was partially supported by "Isoyake Recovery Emergency Project", Fisheries Agency. We would like to thank stuff of Suruga Bay deep-sea water aquaculture research center of Shizuoka prefectural research institute of fishery.

References

Hasegawa M., Koizumi K., Konagaya T., and Noda. M. 2003: Grazing of herbivorous fish as a continuous factor of Isoyake off the coast of Hainan, Shizuoka Prefecture. *Bull. Shizuoka Pref. Fish. Exp. Stn.* **38**, 19–25 (in Japanese).

Motomura T., Sakai Y., 1981: Effect of chelated iron in culture mediua on oogenesis in *Laminaria angustata*. *Bull. Jap. Soc. Sci. Fish.*, **47**, 1535–1540.

Nakashima T. 2002: What is deep sea water? in "Utilization of Deep-sea water". Midori Shobo, Tokyo. pp. 68–91 (in Japanese).

Nimura K., Okamoto K., Takase S., 2005: Effect of Suruga Bay deep-sea water on the growth and maturation of gametophytes of *Eisenia arborea* Areschoug (Phaeophyceae). *Deep Ocean Wat. Res.* **6(1)**, 35–40 (in Japanese)

Terawaki, T. 1993: Eisenia bicyclis in "An Illustration Atlas of the life History of Algae, volume 2" (ed. By Hori T.) Uchida Rokakuho, Tokyo, pp. 132-133 (in Japanese).

Yoshida T. 1998: Sagarame, in "Marine Algae in Japan" (ed. By Yoshida T.), Uchida Rokakuho, Tokyo, pp.346 (in Japanese).