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Original

To what size can abalone, *Haliotis discus hannai*, grow on attached microalgae as a sole dietary source in deep seawater?

エゾアワビ *Haliotis discus hannai* は海洋深層水中で
付着性微細藻類のみの餌料でどこまで成長可能か?

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Abstract

The size to which abalone, *Haliotis discus hannai*, is able to grow on attached microalgae as a sole dietary source in deep seawater (DSW; seawater below the euphotic layer) using continuous and simultaneous culturing system was investigated in this study. A transparent 12 cm (diameter) ×50 cm (length) acrylic column containing many short-cut vinyl tubes of 3 cm (diam.) and 3 cm (length) as substrata, was used. DSW pumped from 320 m depth at Muroto City, in Kochi Prefecture, Japan, was supplied continuously and then incubated under natural light conditions (ca. 4,500 lx). After growing a sufficient number of attached microalgae in the columns, 1-year-old abalone *Haliotis discus hannai* juveniles with an average shell length of ca. 2.2 cm were placed into the column and reared simultaneously with attached food microalgae in the continuous DSW-flow system. During the 260-day rearing process, the juveniles grew up to 3.5–4.3 cm. Daily growth rates were around 60–80 μm d^{-1}, and sometimes over 100 μm d^{-1}. These results indicate that abalone *H. discus hannai* can grow up to at least 4 cm shell size on attached microalgae as the sole dietary source without any supply of seaweed or pellet food. This suggests that the continuous culturing of attached microalgae and simultaneous rearing of juvenile abalone using nutrient-rich DSW will be a possible culturing system without any feeding cost.

Key Words: Abalone growth, Attached microalgae, Continuous culture, Deep Seawater, *Haliotis discus hannai*

要　旨

海洋深層水（DSW）を連続的に通水して，自然光のもとで増殖してくる天然の付着性微細藻類群集のみで，エゾアワビ（*Haliotis discus hannai*）種苗がどれくらいの殻長まで成長するかを調べた．透明のアクリルの筒に切断した透明なビニールチューブを微細藻類の増殖付着基板として多数収容した容器に，室戸DSWを毎時40回転で連続的に通水し，平均殻長2.2 cmのエゾアワビ当歳稚貝を260日間飼育した．その結果，アワビは3.5–4.3 cmまで成長し，日間成長速度も60–80 μm d^{-1}，時には100 μm d^{-1}を超えることもあった．以上の結果から，エゾアワビはDSW由来の付着性微細藻類

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を唯一の餌料として，少なくとも約4 cmまで成長することが明らかとなった．このことは，無機栄養塩を豊富に含むDSWを自然光条件下で連続的に通水することで，従来のように給餌にかかるコストをかけないアワビの種苗生産が可能であることを示唆するものである．

キーワード：アワビの成長，エゾアワビ*Haliotis discus hannai*, 海洋深層水，付着性微細藻類，連続培養系

1. Introduction

Deep seawater (DSW) is defined as seawater below the euphotic layer of deeper than 200 m. It has several special properties, including low temperature throughout the year, and contains little organic matter, despite having abundant inorganic nutrients (Fukami *et al.*, 1997, 1998; Takahashi, 2000), we have been developing many effective utilizations of DSW in various fields to take advantage of these beneficial properties.

The Kochi Prefectural Deep Seawater Laboratory in Muroto City, Kochi, Japan, was established in 1989, and thereafter started to pump DSW from depths of 320 and 340 m. DSW is used particularly in fisheries and aquaculture. One of the most effective uses of DSW is the mass cultivation of planktonic and benthic microalgae, utilizing a high concentration of inorganic nutrients. It has already been reported that the culturing of a planktonic food diatom *Chaetoceros ceratosporum* (Fukami *et al.*, 1992), green algae *Chlorella* and *Dunaliella* (Matsubayashi *et al.*, 1994), dinoflagellate *Amphidinium* sp. (Akakabe *et al.*, 2016), and benthic diatom *Nitzschia* sp. (Fukami *et al.*, 1997) have been successful.

Among these microalgal species, attached diatoms are important for post-larval and early juvenile abalones (Kawamura *et al.*, 1995; Takami *et al.*, 1997; Kawamura *et al.*, 1998; Onitsuka *et al.*, 2007; Correa-Reyes *et al.*, 2009; Courtois de Viçose *et al.*, 2012). They showed that the preferred species of diatoms changed depending on the growth stages of the post-larvae of abalone (Kawamura *et al.*, 1998), and the dietary values of the diatom species (Onitsuka *et al.*, 2007). However, most studies were on the post-larva of abalones with a shell length (SL) of less than 1 mm and juvenile abalones of no more than 8–10 mm SL, and little information is available on those of over 20 mm SL. It was also thought that pieces of seaweeds or artificial pellet food were necessary for abalones over such a size (Takami and Kawamura, 2003). They supposed that this was owing to the insufficient food supply, that is, the growth of attached diatoms did not compensate for the demand for abalone consumption (Kawamura, personal communication).

On the other hand, it has been reported that continuous culturing of attached food diatoms and simultaneous rearing of abalones, *Haliotis sieboldii* using DSW was successful owing to high concentration of inorganic nutrients (Fukami *et al.*, 1998). They used an incubation system, consisting of an acrylic pipe (7 cm diameter, 50 cm length) containing many short-cut vinyl tubes (2 cm diameter, 2 cm length), as substrata for attached diatoms. DSW was supplied continuously to the system and incubated under natural sunlight. Using this system, food diatoms grew actively, and the abalone in the system grew simultaneously, as large as ca. 20 mm. They supposed why juvenile abalones (20 mm) grew on attached diatoms without any supply of seaweeds nor pellet food in DSW was due to the high growth rate of diatoms, which in turn provided enough food for the growth of the abalones (Fukami *et al.*, 1998). After abalones grew to around 20 mm, however, their growth stopped as they were not able to move around in the culturing system (Fukami *et al.*, 1998).

These results suggest that juvenile abalones on diets of attached microalgae, including diatoms, can continue to grow to at least 20 mm SL, as long as the supply of microalgal food is sufficient. So far, we have no informa-

tion on how large abalones can grow on attached microalgae when a larger-scale system with enough space for them to move freely is used.

In this study, we will elucidate how large abalones can grow on attached microalgae as a sole dietary source. The results obtained in this study will provide valuable information for establishing an effective aquaculture system for juvenile abalones using DSW that can operate without any feeding cost.

2. Materials and Methods

2-1. Deep Seawater and Culturing System

The deep seawater (DSW) used in this study was collected from a depth of 320 m at the Kochi Prefectural Deep Seawater Laboratory in Muroto City, Japan (Fukami *et al.*, 1992). The systems for culturing abalones and attached microalgae were transparent acrylic columns (diameter, 12 cm; length, 50 cm) containing many transparent vinyl tubes (inner diameter 3 cm, outer diameter 3.6 cm) cut to 3 cm length as a substratum for microalgae (Fig. 1). The water volume of a column containing substrata was approximately 4.7 L. These columns were used for the systems to continuously and simultaneously culture attached food microalgae and juvenile abalones.

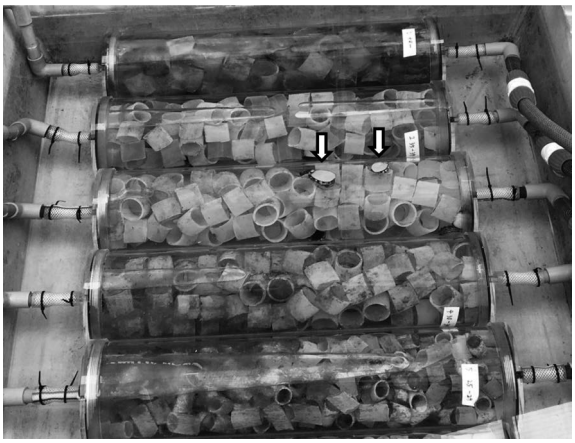

Fig. 1. Abalone culturing systems using transparent acrylic column (12×50 cm) containing many vinyl tubes with diameter of 3 cm. To these systems, DSW was supplied at a flow rate of 40 turnover times h^{-1}. High density of microalgae was grown in these systems (dark color part). Arrows show the growing abalones.

About 100 pieces of vinyl tubes were contained in a column. Therefore, the total surface area (sum of the inside wall of a column and in- and out-side surfaces of 100 vinyl tubes) of microalgal growth in one column was about 8000 cm^2.

2-2. Effects of Adding Attached Diatom Cultures

To obtain better food microalgae for rearing abalone and to evaluate the effects of adding these microalgal cultures to systems, we tried to isolate some fast-growing strains of attached diatoms from DSW. Algal lawn growing on the wall of an aquaculture tank, using DSW, was scraped and put into inorganic nutrient media (Sweeney, 1954). Growing microalgae, usually after several days, were inoculated on Sweeney agar plates, and the algal colonies were streaked on another plate. After repeating this procedure several times, two strains (N and C strains) of fast-growing attached diatoms were finally obtained.

Taxonomical identification of N and C strains was performed using the method of Nishimura *et al.* (2013, 2020). Finally, the C strain was identified as *Nitzschia laevis* and the N strain was tentatively identified as *Nitzschia lecointei* (data not illustrated).

2-3. Abalones and Culturing

The first experiment was carried out from December 13, 2017, to August 30, 2018. Prior to the rearing of abalones, four different culturing sets were prepared. One was a column without any algal inoculation (No. 1). The other three were inoculated as follows: No. 2 column was inoculated with a diatom assemblage isolated from DSW, No. 3 was inoculated with *Cylindrotheca closterium*, suitable as a diet for abalone post-larvae (Matsumoto *et al.*, 2018), and No. 4 was inoculated with a fast-growing diatom of the N strain as previously described. The replicate numbers of each culturing set were single (No. 1), duplicate (No. 2), and triplicate (No. 3 and No. 4) (Table 1). Diatom cultures preincubated on agar plates in the labo-

Table 1. Changes in numbers of living individuals of abalone in the culturing columns and survival rates at the end of culturing period. All living individuals in each replicate column were brought together in one column on May 8 and were cultured for 260 days until August 30, 2018. Explanation for each column No. is described in Fig. 2 (see Section 2-3 in Materials and Methods).

Column No.	Replication	Sampling Occasion (2017–2018)												Survival Rates (%)
		Dec. 12	Dec. 27	Jan. 10	Jan. 24	Mar. 13	Apr. 9	May. 8		May. 22	Jun. 19	Jul. 20	Aug. 30	
1	1	5	5	5	5	4	3	3	3	2	2	2	2	40
2	2	5	5	5	4	4	4	4	7	6	5	4	4	40
		5	5	5	5	4	4	3						
3	3	5	5	4	4	4	4	3	8	8	8	8	8	53
		5	5	5	5	4	4	3						
		5	5	5	5	4	2	2						
4	3	5	5	4	3	3	3	3	11	8	6	6	6	40
		5	5	5	5	5	4	4						
		5	5	5	5	4	4	4						

ratory and wiped by a piece of cotton were inoculated at the beginning of the experiment. Inoculation of diatom cultures was expected to accelerate the growth of food microalgae.

The second experiment was conducted between December 20, 2018, and January 25, 2019. Three incubation columns were prepared for each; either N or C strain was inoculated, or no diatom was inoculated as a control. In the second experiment, newly prepared cultures of the two diatom strains, preincubated in a similar way to the first experiment, were inoculated on every occasion of shell size determination.

After inoculating the attached diatom cultures, DSW was supplied continuously to all systems at a constant flow rate of ca. 40 turnover times h^{-1} (approximately 3 L min^{-1}). Culturing systems were incubated under the natural light conditions in the house.

Commercially available 1-year-old juveniles of *H. discus hannai* abalone were obtained in December 2017 (spawned on October 23, 2016) for the first experiment, and in September 2018 (spawned on October 30, 2017) for the second experiment from the Koyano Suisan, Ehime, Japan (https://www.koyanosuisan.com/). After a sufficient number of attached microalgae (diatom) had grown on the substrata in the culturing systems, usually after 10 days of inoculation, five juvenile abalone individuals were put into each system for the first experiment, and culturing commenced (Table 1). Abalone density was calculated as 6.3 individuals m^{-2}. The initial average shell length (SL) of abalone was approximately 2.2 (± SD 0.18) cm (n = 45). For the second experiment, the initial number of abalones in a column was 10 for all culturing systems (Table 2). Abalone density was calculated as 12.5 individuals m^{-2}. The initial average SL was 2.0 (± SD 0.10) cm (n = 30).

Table 2. Changes in numbers of living individuals of abalone in the columns during the culturing period of 36 days from December 20, 2018 to January 25, 2019. Explanation for each column was described in Fig. 3 (see Section 2-3 in Materials and Methods).

Column	Sampling Occasion (2018–2019)				
	Dec. 20	Dec. 27	Jan. 8	Jan. 18	Jan. 25
N Strain	10	9	9	9	9
C strain	10	10	10	10	10
No Inoc.	10	10	10	10	10

During the rearing periods of 260 days (the first experiment) and 36 days (the second experiment), the SL of individual abalones was determined once or twice a month using a slide caliper. The growth of abalones was taken as an increase in the average SL of all living abalones in replicate columns at each sampling occasion. In addition, daily growth rates (DGRs) (μm d^{-1}) were also determined as follows:

$$DGR = (SL_{T2} - SL_{T1}) / T_2 - T_1$$

where SL_{T2} and SL_{T1} show the shell length at times T_2 and T_1, and T_2 and T_1 indicate two sampling occasions.

2-4. Water Temperature and Light Intensity

During the rearing period, the water temperatures of the DSW used for culturing abalones were obtained from the monitoring system of the Kochi Prefectural Deep Seawater Laboratory. Light intensities just above the rearing system in the rearing house were measured between July 5 and October 29, 2019, using HOBO UA-002-64 Data Logger (ONSET Computer Corporation, MS, USA). Both data were available every hour but were illustrated using values at 12:00 (noon time) every day.

2-5. Statistical Significance

The statistical significance of abalone SLs at the end of each rearing period was determined using the t-test. Differences were considered significant at $p < 0.05$.

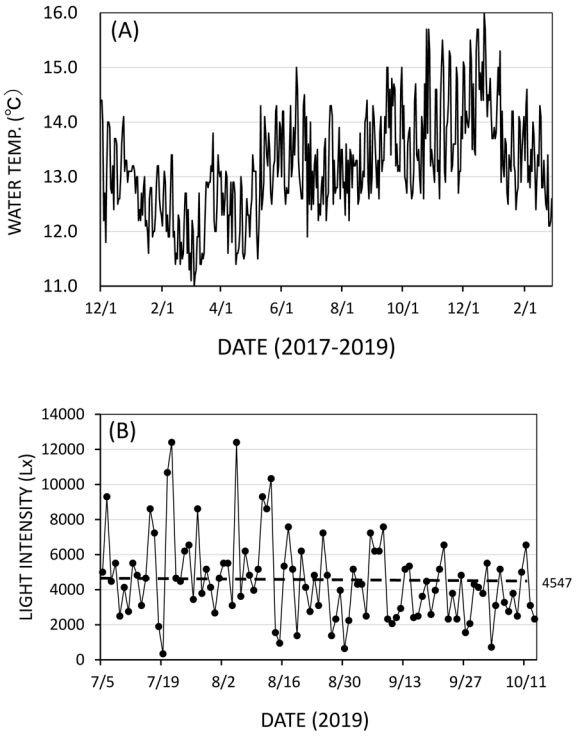

Fig. 2. Changes in water temperature of DSW pumped up to the laboratory (A), and light intensity in the rearing room (B). Both data were illustrated using values at 12:00 (noon time) every day. Average light intensity during the measuring period was 4547 lx.

3. Results

Figure 2A shows the changes in water temperature of DSW pumped up to the laboratory during the rearing period of December 1, 2017, to February 28, 2019. The water temperature of running DSW fluctuated between 11.0℃ (March) and 16.0℃ (December). It appears to increase in late autumn to early winter and to decrease in early spring. Changes in light intensity are illustrated in Fig. 2B. Unfortunately, we determined it only in 4 and a half months in 2019. It fluctuated between over 12,000 lx (sunny day) to 344 lx (rainy day). The average light intensity was 4547 lx (Fig. 2B).

Changes in the numbers of living abalones in each column in the first and second experiments are shown in Tables 1 and 2, respectively. For the 260 days rearing period of the first experiment, some abalone individuals died; therefore, the living individuals in each column set were combined and placed in one column on May 8, 2018. The final numbers of living abalone at the end of the experiment were 2 (No. 1), 4 (No. 2), 8 (No. 3), and 6 (No. 4) (Table 1). In the second experiment for 36 days rearing period, only one abalone individual died, while the others were very active (Table 2).

Increases in the shell length (SL) of abalones and the daily growth rate (DGR) in each culturing column for the first experiment are illustrated in Fig. 3A and 3B, respectively. As living individuals of each replicate column were combined on May 8, 2018, the results were treated separately before (first stage) and after (second stage) this date. At the first stage of the first experiment for 146-day rearing period (from Dec. 13, 2017 to May 8, 2018), juvenile abalones with initial average SL of 2.2 (±SD 0.18) cm grew up to 3.2 (±0.19) cm (No. 1 column), 3.0 (±0.20) cm (No. 2), 2.9 (±0.11) cm (No. 3), and 2.8 (±0.27) cm (No. 4), as the average SLs, respectively (Fig. 3A, left half). There were no clear differences among 4 columns. During the second stage, which lasted for 114 days (from May 8 to Aug. 30), the abalones in the No. 1 column grew constantly, with a final average SL of 4.3 (±0.05) cm, which

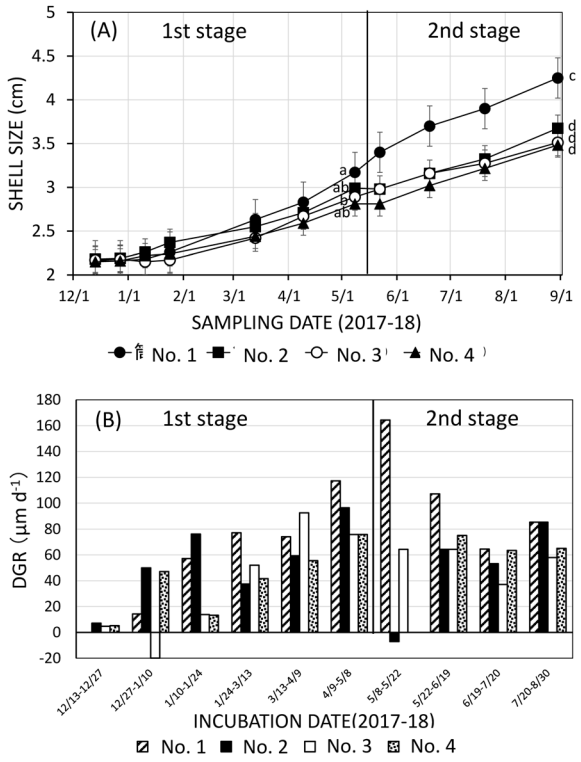

Fig. 3. Changes in shell size (A) and the daily growth rate (DGR) (B) of 1-year-old abalone, *Haliotis discus hannai*, cultured under continuous DSW flow for 260 days from December 13, 2017 to August 30, 2018. Column No. 1: without any algal inoculation, No. 2: inoculated with diatom assemblage isolated from DSW, No. 3: inoculated with *Cylindrotheca closterium*, No. 4: inoculated with a fast-growing diatom of the N strain (see Section 2-3 in Materials and Methods). As living individuals of each replicate column were combined on May 8, 2018, the results were treated separately before (first stage) and after (second stage) this date. Small letters in (A) show the significant differences at $p<0.05$.

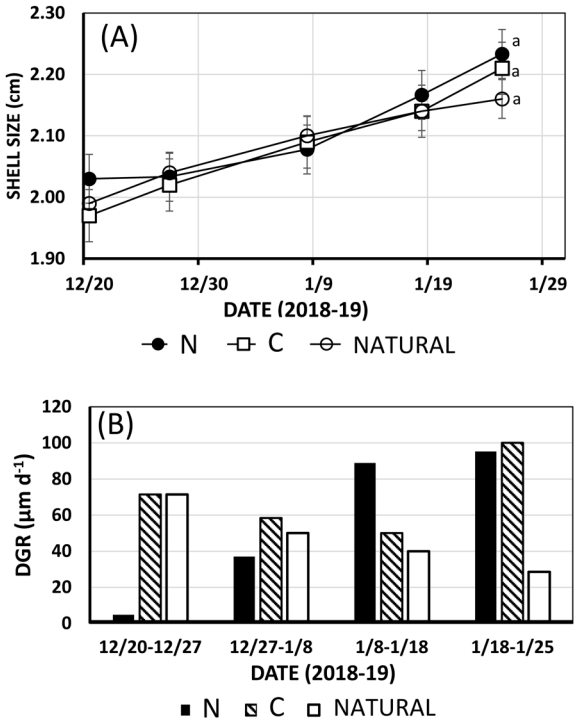

Fig. 4. Changes in shell size (A) and the daily growth rate (DGR) (B) of 1-year-old abalone, *Haliotis discus hannai*, cultured under continuous DSW flow for 36 days from December 20, 2018 to January 25, 2019. N: inoculated with N strain diatom at every sampling occasion, C: inoculated with C strain diatom at every sampling occasion, NATURAL: without any algal inoculation (see Section 2-3 in Materials and Methods). Small letters in (A) show the significant differences at $p<0.05$.

was significantly greater than those in the other three columns (Fig. 3A, right half). The final average SLs in No. 2–4 columns, were 3.7 (±0.08) cm, 3.5 (±0.18) cm, and 3.5 (±0.34) cm, respectively, with no significant difference among them.

During the rearing period at the first stage, DGR values in the first 2–3 months were relatively lower in all the columns; however, they increased gradually, and the maximum DGR values for each incubation set were 117 μm d^{-1} (No. 1), 97 μm d^{-1} (No. 2), 92 μm d^{-1} (No. 3), and 76 μm d^{-1} (No. 4), respectively (Fig. 3B, left half). In the second stage, the maximum DGRs for each incubation set were 164 μm d^{-1} (No. 1), 85 μm d^{-1} (No. 2), 64 μm d^{-1} (No. 3), and 75 μm d^{-1} (No. 4), respectively (Fig. 3B, right half).

In the second experiment which lasted for 36 days, juvenile abalones with the initial average shell length of 2.0 (±0.10) cm grew to the average of 2.2 (±0.16) cm with N strain, 2.2 (±0.09) with C strain, and 2.2 (±0.11) with no diatom inoculation, respectively (Fig. 4A). There were no statistically significant differences among the 3 sets. The DGRs of the column inoculated with the N strain was very low at the beginning of the experiment; however, it increased gradually and the maximum DGR was 95 μm d^{-1} at the end of the experiment (Fig. 4B). The DGR values of the C strain-inoculated column were relatively constant, being within 58–100 μm d^{-1} throughout the experiment, while those of the column without

any diatom inoculation gradually decreased from 71 to 29 μm d^{-1} (Fig. 4B).

4. Discussion

In the present study, we developed a large-scale culturing system containing vinyl tubes of larger diameter (Fig. 1). DSW was supplied at a high flow rate of 40 turnover times h^{-1} to cancel the seasonal changes in inorganic nutrient concentration in DSW for the growth of attached microalgae (Fukami *et al.*, 1997, 2000).

As a result, the juvenile abalones grew up to 4.0 cm on the attached microalgae as a sole dietary source (Fig. 3A). Previous reports showed that food supply as pieces of seaweed or pellet was necessary for rearing juvenile abalones of more than 10 mm shell size (Uki *et al.*, 1986). Kikuchi *et al.* (1967) reported the growth of abalone, *Haliotis discus hannai*, reared on 20 different seaweed foods and showed that they grew 0–3.62 mm in 30 days and the daily growth rates calculated were 0–121 μm d^{-1}. The results of the present study indicate that it is possible to rear juvenile abalones to 4 cm SL without any artificial food supply when the appropriate culturing system is prepared and inorganic nutrient-rich DSW is used for culturing (Fig. 3A). These sizes are already sufficient for a release (Yamazaki 1991).

At the first stage of the first experiment of 146-day rearing period, the SLs of abalones showed no clear differences among all the four column sets (Fig. 3A, left half). Although diatom cultures inoculated into the columns were different among the four culture sets, we added them only at the beginning of the experiment. During the rearing, the apparent community structure of attached microalgae in each column no longer showed any distinct differences among all four systems under the microscope (data not shown). Predominated communities growing on substrates of vinyl tubes were mostly several species of the diatom, in addition to some blue-green algae. This result indicates that there are many "seeds" of microalgae (resting cells or cysts) in DSW, and they start to grow under light conditions using sufficient inorganic nutrients in the DSW (Yoshimoto and Fukami, 2009). Inoculation of fast-growing diatom culture only at the beginning of the rearing period was not adequate for maintaining the dominance of the fast-growing diatom.

In the second stage after May 8, as there were no clear differences in the attached microalgal communities in the four column sets, the difference in abalone growth depended on the number of individuals in a culturing column. We found that the significantly higher growth of the abalones in the No. 1 column (Fig. 3A, right half) could be attributed to the performance of a few individuals (low density) compared with the other three columns (Table 1).

In the second experiment, we supplied newly prepared diatom cultures (either N or C strains) to the column at every sampling occasion (see Materials and Methods). As a result, the N or C strain continued to coexist with natural community throughout the rearing period, as observed under the microscope although we did not have quantitative data. Nevertheless, there were no significant differences in the growth of the abalones between those with or without diatom inoculation nor between the N and C strains (Fig. 3A). This is probably owing to the short rearing period of 36 days.

During the two experiments, the DGRs were usually 60–80 μm d^{-1} and sometimes over 100 μm d^{-1} (Figs. 3B and 4B). The values obtained in this study were comparable to those of previous reports, where artificial food was supplied in sufficient quantity by hand (Kikuchi *et al.*, 1967; Uki *et al.*, 1986; Yamazaki, 1991; Kawamura and Takami, 1995).

The results obtained in this study strongly suggest that abalone can grow up to 30–40 mm in SL on indigenous attached microalgae as the sole dietary source when microalgae grow actively on sufficient nutrients in DSW, and compensate for consumption by abalone. Inoculation of diatom cultures is not practical in aquaculture and it may not be necessary (Figs. 3 and 4).

One remaining consideration is the water temperature (WT) of the DSW. The WT of DSW pumped up to the aquarium was between 11 and 16℃ (Fig. 2A). This is slightly too low for most abalones. The growth of abalones at the first stage of the first experiment (Fig. 3A, left half) appeared to be relatively lower, which may be owing to the low WT during this period (Fig. 2A). There is a need to exmine how the WT increases during culturing.

Another viewpoint to be considered is the density of the juvenile abalone. As mentioned in Section 2–3 in Materials and Methods, culturing densities of abalones were 6–13 individuals m^{-2}. Based on our experience, besides the "areal" density, the "spatial" density is also important, and an appropriate abalone density is around 10 kg m^{-3}. As the weight of a 4 cm SL abalone is around 10 g, 10 kg m^{-3} corresponds to 1 individual L^{-1}. In the present study, as the water volume of a culturing column was about 4.7 L (see Section 2–1 in Materials and Methods), 4–5 individuals are appropriate for a column. From this point of view, our culturing systems do not pose serious problems for the culturing density of abalones.

Although the problem of low temperature of DSW should be solved, this system remains promising because it can help to avoid the feeding cost and the intensive labor involved in providing artificial food of pieces of seaweed or pellets. In establishing an abalone aquaculture system, the high productivity of DSW can be positively utilized for continuous culturing of attached food microalgae and the simultaneous rearing of juvenile abalone.

Acknowledgments

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References

Akakabe, M., K. Kumagai, M. Tsuda, Y. Konishi, A. Tominaga, D. Kaneno, E. Fukushi, J. Kawabata, A. Masuda and M. Tsuda (2016) Iriomoteolides-10a and 12a, cytotoxic macrolides from marine dinoflagellate *Amphidinium* species. Chem. Pharm. Bull., 64, 1019–1023.

Correa-Reyes, J. G., M. del P. S.-Saavedra, M. T. Viana, N. F.-Acevedo and C. V.-Peláez (2009) Effect of eight benthic diatoms as feed on the growth of red abalone *(Haliotis rufescens*) postlarvae. J. Appl. Phycol., 21, 387–393.

Courtois de Viçose, G., M. P. Viera, S. Huchette and M. S. Izquierdo (2012) Improving nursery performances of *Haliotis tuberculate coccinea*: Nutritional value of four species of benthic diatoms and green macroalgae germlings. Aquaculture, 334–337, 124–131.

Fukami, K., T. Nishijima and Y. Hata (1992) Availability of deep seawater and effects of bacteria isolated from deep seawater on the mass culture of food microalga *Chaetoceros ceratosporum*. Nippon Suisan Gakkaishi 58, 931–936.

Fukami, K., S. Nishimura, M. Ogusa, M. Asada and T. Nishijima (1997) Continuous culture with deep seawater of a benthic food diatom *Nitzschia* sp. Hydrobiologia, 358, 245–249.

Fukami, K., A. Kawai, M. Asada, M. Okabe, T. Hotta, T. Moriyama, S. Doi, T. Nishijima, M. Yamaguchi and M. Taniguchi (1998) Continuous and simultaneous cultivation of benthic food diatom *Nitzschia* sp. and abalone *Haliotis sieboldii* by using deep seawater. J. Mar. Biotechnol., 6, 237–240.

Fukami, K., J. Matsumoto, T. Kadota, Y. Nakano and T. Nishijima (2000) Seasonal fluctuations of the water quality of deep seawater and the relationship to the

growth potential of a microalga. Deep Ocean Wat. Res., 1, 27–31 (in Japanese with English abstract).

Kawamura. T., T. Saido, H. Takami and Y. Yamashita (1995) Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. J. Exp. Mar. Biol. Ecol., 194, 189–199.

Kawamura, T. and H. Takami (1995) Analysis of feeding and growth rate of newly metamorphosed abalone *Haliotis discus hannai* fed on four species of benthic diatom. Fisheries Sci., 61, 357–358.

Kawamura, T., R. D. Roberts and C. M. Nicholson (1998) Factors affecting the food value of diatom strains for post-larval abalone *Haliotis iris*. Aquaculture, 160, 81–88.

Kikuchi, S., Y. Sakurai, M. Sasaki and T. Ito (1967) Food values of certain marine algae for the growth of the young abalone, *Haliotis discus hannai*. Bull. Tohoku Natl. Fish. Res. Inst., 27, 93–100 (in Japanese with English abstract).

Matsubayashi, T., I. Maruyama, S. Kido, Y. Ando, T. Nakashima and T. Toyota (1994) Effects of deep seawater on the growth of several species of marine micro-algae. J. Appl. Phycol., 6, 75–77.

Matsumoto, Y., S. Houki, A. Nakatsubo, T. Saido, T. Noro, T. Kishi, H. Takami and T. Kawamura (2018) The optimum culturing conditions for benthic diatom *Cylindrotheca dosterium* and a developmental study of a mass culturing method for commercial application. Nippon Suisan Gakkaishi, 84, 233–240 (in Japanese with English abstract) .

Nishimura, T., S. Sato, W. Tawong, H. Sakanari, K. Uehara, Md M. R. Shah, S. Suda, T. Yasumoto, Y. Taira, H. Yamaguchi and M. Adachi (2013) Genetic diversity and distribution of the ciguatera-causing dinoflagellate *Gambierdiscu*s spp. (Dinophyceae) in coastal areas of Japan. PLoS ONE, 8 (4), e60882. doi: 10.1371/journal.pone.0060882

Nishimura, T., H. Uchida, R. Noguchi, H. Oikawa, T. Suzuki, H. Funaki, C. Ihara, K. Hagino, S. Arimitsu, Y. Tanii, S. Abe, K. Hashimoto, K. Miura, K. Tanaka, I. Yanagida and M. Adachi (2020) Abundance of the benthic dinoflagellate *Prorocentrum* and the diversity, distribution, and diarrhetic shellfish toxin production of *Prorocentrum lima* complex and *P. caipirignum* in Japan. Harmful Algae, 96, 101687. https://doi.org./10.1016/j.hal.2019.101687

Onitsuka, T., T. Kawamura, S. Ohashi, T. Hori and Y. Watanabe (2007) Dietary value of benthic diatoms for post-larval abalone *Haliotis diversicolor* associated with feeding transitions. Fisheries Sci., 73, 295–302.

Sweeney, B. M. (1954) *Gymnodinium splendens*, a marine dinoflagellate requiring vitamin B_{12}. Am. J. Bot., 41, 821–824.

Takahashi, M. M. (2000) DOW: Deep Ocean Water as Our Next Natural Resource, Terra Scientific Publishing Company, Tokyo, 99p.

Takami, H. and T. Kawamura (2003) Dietary changes in the abalone, *Haliotis discus hannai*, and relationship with the development of the digestive organ. Jpn. Agricul. Res. Quart. (JARQ), 37, 89–98.

Takami, H., T. Kawamura and Y. Yamashita (1997) Survival and growth rates of post-larval abalone *Haliotis discus hannai* fed conspecific trail mucus and/or benthic diatom *Cocconeis scutellum var. parva*. Aquaculture, 152, 129–138.

Uki, N., M. Sugiura and T. Watanabe (1986) Dietary value of seaweeds occurring on the Pacific coast of Tohoku for growth of the abalone *Haliotis discus hannai*. Bull. Japan. Soc. Sci. Fish., 52, 257–266.

Yamazaki, M. (1991) Food consumption of juvenile Haliotids in laboratory experiments. Nippon Suisan Gakkaishi, 57, 865–867 (in Japanese with English abstract) .

Yoshimoto, N. and K. Fukami (2009) Growth of harmful phytoplankton, *Heterosigma akashiwo*, in the mixture of surface and deep seawater. Deep Ocean Wat. Res., 10, 1–8.