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Effects of Microalgal Species on the Settlement and Survival of *Haliotis discus hannai* Larvae

Su Keun Ko and Sung Bum Hur*

Department of Marine Bio-materials and Aquaculture, Pukyong National University, Busan 608-737, Korea

Abstract

Although culture techniques for the abalone *Haliotis discus hannai* are well known, mass culture of the benthic microalgae that are essential live food for the abalone larvae is still not practiced. This study was conducted to identify the microalgal species suitable for the growth of early larvae of *H. discus hannai*. The growth and attachment rates of 31 microalgal species were examined. Acrylic plates were used as the substrate. Among the 31 microalgal species, nine showing high growth and attachment rates were selected and tested for their dietary values via factors including settlement, metamorphosis, and survival rates of abalone larvae. *Tetraselmis hazeni* and *Rhaphoneis* sp. induced the highest settlement rate (65-69%) in abalone larvae. The metamorphosis rate was highest (57%) in larvae fed *Rhaphoneis* sp. and was also significantly higher in larvae fed *Oscillatoria splendida* (29%) and *T. hazeni* (22%) than in those fed other species. The highest survival rate of the larvae during the 15 days after metamorphosis was 67% in those fed *Rhaphoneis* sp., followed by *T. hazeni* (42%) and *O. splendida* (35%). In conclusion, *Rhaphoneis* sp. is the most suitable diatom for use as a live food for the culture of early larvae of *H. discus hannai*. In addition, *T. hazeni* and *O. splendida* are also potential species to be further developed and utilized in larval culture.

Key words: Abalone, *Haliotis discus hannai*, Larvae, Metamorphosis, Microalgae, Settlement

Introduction

Biofilm composed of mucus plays an important role in the settlement processes of invertebrate larvae such as *Haliotis discus hannai* (Pawlik, 1992; Keough and Raimondi, 1996; Roberts and Watts, 2010; de Viçose et al., 2010). In the artificial seed production of *H. discus hannai*, it is crucial for microalgae of sufficient quantity and quality to attach to a substrate such as acrylic plate.

Larval growth differs depending on the microalgal characteristics, and food resources for larvae may become insufficient when large amounts of microalgae fall off the acrylic plate due to their weight. When the microalgal membrane is flat, larval settlement onto the biofilm tends to be successful. However, if the membrane is three-dimensional, larvae show low settlement rates (Kawamura and Kikuchi, 1992; Searcy-Bernal, 1996; Roberts et al., 2007a). Furthermore, some diatoms with hard silica cell walls tend to have low digestion rates by the larvae. Therefore, it is necessary to obtain high-quality microalgae attached to acrylic plates to achieve the successful artificial seed production of *H. discus hannai*.

Studies on the cultivation of early and immediate post-settlement larval stages are extremely limited compared to those of later larval stages. Moreover, studies on the effectiveness of feed for the larvae of *H. discus hannai* have been limited to diatoms (Han and Hur, 2000; Parker et al., 2007; Roberts et al., 2007b). The aim of this study was to examine the growth and attachment rates of microalgae including the green algae *Tetraselmis* and blue-green algae. The settlement, metamorphosis, and survival rates of *H. discus hannai* larvae fed the aforementioned microalgae were also studied.

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***Corresponding Author**
E-mail: hurs@pknu.ac.kr

Materials and Methods

Growth and attachment rates of microalgae

The growth and attachment rates of 31 kinds of microalgae selected from the Korea Marine Microalgae Culture Center (KMMCC), including 24 diatoms, three green algae *Tetraselmis*, and four blue green algae were examined (Table 1). To examine the growth and attachment rates of microalgae, five acrylic plates (19 × 16 × 0.1 cm) were placed in a 14 L circular tank filled with 10 L of f/2 medium (Guillard and Ryther, 1962). Then, full-grown microalgae at the end of the log phase were inoculated at the 10% level. The total substratum area for microalgae attachment was 5,109 cm^2, with the areas of the walls and floor of the tank and the plates being 1,583 cm^2, 451 cm^2, and 3,075 cm^2, respectively. Microalgae were cultured for two weeks, with aeration at 23°C and 40 μmol $m^{-2}s^{-1}$, with continuous lighting.

Table 1. List of microalgal species for the study

Group	KMMCC NO.	Species	Length (μm)
A	493	*Achnanthes* sp.	27 ± 3.9
	931	*Amphiprora gigantea* var. *sulcata*	42 ± 6.7
	714	*Amphora delicatissima*	18 ± 2.3
	935	*Amphora delicatissima*	25 ± 2.7
	716	*Amphora lineata*	22 ± 1.5
	773	*Amphora* sp.	23 ± 3.3
	823	*Amphora* sp.	12 ± 1.0
	939	*Amphora veneta* var. *coffeaeformis*	19 ± 3.0
B	941	*Navicula annexa*	21 ± 1.8
	905	*Navicula cancellata*	23 ± 2.2
	893	*Navicula elegans*	14 ± 1.2
	17	*Navicula incerta*	39 ± 1.7
	919	*Navicula viridis*	19 ± 1.9
	957	*Navicula* sp.	11 ± 1.2
	841	*Navicula* sp.	16 ± 4.2
C	900	*Caloneis schroder*	21 ± 2.5
	833	*Cocconeis californica*	24 ± 9.1
	267	*Cylindrotheca closterium*	34 ± 3.9
	26	*Nitzschia dissipata*	26 ± 1.0
	962	*Nitzschia* sp.	24 ± 1.0
	77	*Phaeodactylum tricornutum*	17 ± 1.8
	847	*Pleurosigma angulatum*	56 ± 8.6
	628	*Rhaphoneis* sp.	9 ± 1.7
	268	*Trachyneis aspera*	26 ± 3.3
D	62	*Tetraselmis hazeni*	15 ± 3.8
	109	*Tetraselmis* sp.	13 ± 2.6
	40	*Tetraselmis* sp.	13 ± 2.4
E	30	*Lyngbya taylorii*	4 ± 0.6*
	98	*Oscillatoria splendida*	2 ± 0.3*
	34	*Phormidium luridum*	2 ± 0.2*
	180	*Trichodesmium erythraeum*	5 ± 0.2*

KMMCC, Korea Marine Microalgae Culture Center.
*Width (μm).

Two weeks later, floating microalgae were collected using GF/C filters. Attached microalgae were detached from surfaces of the substratum using a soft brush and filtered by GF/C filters. The collected microalgae from each tank were separately dried at 60°C for 2 h and weighed to the nearest milligram. The following formulae were used for calculating the attachment and growth rates of the microalgae:

Attachment rate (%) = (Weight of attached microalgae/Total weight of microalgae) × 100

Growth rate (%) = $[(n_1 - n_0)/n_0 \times 100$ (n_1: final collection amount (mg), n_0: initial inoculation amount (mg)]

Settlement, metamorphosis, and survival rates of *Haliotis discus hannai*

Larvae of the veliger stage were examined 56 h after fertilization at 21°C and were used in the present study. Nine species among the 31 microalgae showed higher growth and adhesion rates. To culture these species, 10 mL of f/2 medium was placed inside a 6-well tissue culture chamber, and the full-grown microalgae were inoculated at the 10% level. They were cultured without aeration at 20°C and 40 μmol $m^{-2}s^{-1}$ of constant light for 10 days.

Each chamber in which the microalgae had been cultured was used for larval culture. Before larval culture, the chambers were cleaned several times with filtered seawater to remove the f/2 medium. Then, 30 larvae were placed into the chamber filled with 10 mL of filtered seawater. The cell density of the microalgae attached to the chamber was approximately 5-10 × 10^4 cells/mL. The larvae were reared at 20°C and 40 μmol $m^{-2}s^{-1}$ under a photoperiod of 8:16 h LD (light:dark) for 15 days. Upon the completion of larval settlement onto the microalgae, the culture water in the chamber was exchanged with 5 mL of filtered seawater daily. The control group contained no microalgae and was filled only with filtered seawater. All experiments were repeated six times for each treatment. The settlement and metamorphosis of the larvae were observed through a dissecting microscope. Larvae attaching to the microalgal film and having a foot were considered to be settled larvae and larvae with a shell molding were considered to be metamorphosed larvae. Larvae that showed no metamorphosis for 6 days were eliminated. The settlement rate measurement was repeated seven times. Measurements of metamorphosis rates and survival rates after metamorphosis were repeated twice. Dead larvae, immobile for more than 10 seconds with no heartbeat, were removed daily. Survival rates after metamorphosis were measured.

Settlement rate (%) = (No. of settled individuals/No. of inoculated individuals) × 100

Metamorphosis rate (%) = (No. of metamorphosed individuals /No. of settled individuals) × 100

Survival rate (%) = (No. of surviving individuals/No. of metamorphosed individuals) × 100

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), and Duncan's multiple range test (Duncan, 1955) was applied for the significance level ($P < 0.05$). SPSS version 17 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Growth and attachment rates of microalgae

The growth and attachment rates of each microalgal group were examined (Table 2). There was no significant difference in diatom biomass within group A, except for *Amphiprora gigantea* var. *sulcata* (18.1 mg) and *Amphora* sp. (KMMCC-823, 12.1 mg) ($P < 0.05$). However, the attachment rate of *Amphora* sp. (KMMCC-773) was the highest (97%) and that of *Achnanthes* sp. was the lowest (14%). The growth rates of *Amphora* sp. (KMMCC-823) and *Amphora veneta* var. *coffeaeformis*, 642% and 593%, were highest and that of *Achnanthes* sp., 150%, was lowest ($P < 0.05$).

The microalgal biomass of *Navicula* was 21.3-26.1 mg, and there was no significant difference in the biomasses of species in group B. However, the attachment rates of *N. incerta*, *N. viridis*, and *Navicula* sp. (KMMCC-841), 71-72%, were significantly higher than those of *N. cancellata* and *N. elegans* ($P < 0.05$). The growth rate of 1,826% for *Navicula* sp. (KMMCC-957) was significantly higher than those of other species ($P < 0.05$).

Table 2. Final total biomass (mg) and growth and attachment rate (%) of microalgal species

Group	Species	Final total biomass	Growth rate	Attachment rate
A	*Achnanthes* sp.	12.8 ± 5.4^{ab}	150 ± 106.9^{e}	14 ± 1.9^{e}
	Amphiprora gigantea var. *sulcata*	18.1 ± 1.4^{a}	363 ± 36.2^{cd}	44 ± 3.5^{c}
	Amphora delicatissima	17.0 ± 2.2^{ab}	254 ± 45.8^{de}	66 ± 6.1^{b}
	Amphora delicatissima	16.8 ± 0.4^{ab}	240 ± 7.2^{de}	39 ± 11.1^{cd}
	Amphora lineata	16.8 ± 0.4^{ab}	430 ± 11.2^{c}	31 ± 5.3^{cd}
	Amphora sp. (KMMCC-773)	17.3 ± 0.1^{ab}	498 ± 2.4^{bc}	97 ± 1.6^{a}
	Amphora sp. (KMMCC-823)	12.1 ± 1.6^{b}	642 ± 100.1^{a}	24 ± 10.9^{de}
	Amphora veneta var. *coffeaeformis*	15.9 ± 0.4^{ab}	593 ± 15.5^{ab}	42 ± 5.4^{c}
B	*Navicula annexa*	22.9 ± 1.9^{a}	487 ± 49.1^{c}	64 ± 7.5^{ab}
	Navicula cancellata	25.3 ± 4.4^{a}	359 ± 79.6^{c}	43 ± 2.6^{c}
	Navicula elegans	25.5 ± 6.0^{a}	843 ± 222.7^{b}	57 ± 4.9^{b}
	Navicula incerta	26.1 ± 4.5^{a}	592 ± 112.1^{bc}	71 ± 8.6^{a}
	Navicula viridis	23.2 ± 1.1^{a}	606 ± 32.4^{bc}	71 ± 5.1^{a}
	Navicula sp. (KMMCC-957)	21.3 ± 0.1^{a}	$1,826 \pm 6.4^{a}$	69 ± 5.1^{ab}
	Navicula sp. (KMMCC-841)	23.2 ± 1.1^{a}	838 ± 45.7^{b}	72 ± 0.2^{a}
C	*Caloneis schroder*	15.1 ± 0.4^{a}	262 ± 30.1^{def}	72 ± 4.8^{bc}
	Cocconeis californica	8.0 ± 0.0^{c}	217 ± 25.4^{ef}	69 ± 1.8^{bc}
	Cylindrotheca closterium	13.7 ± 0.7^{b}	$2,302 \pm 128.5^{a}$	24 ± 3.6^{e}
	Nitzschia dissipata	8.4 ± 0.5^{c}	161 ± 3.6^{f}	67 ± 2.8^{c}
	Nitzschia sp.	16.1 ± 0.5^{a}	$1,057 \pm 2.0^{b}$	30 ± 1.3^{de}
	Phaeodactylum tricornutum	6.7 ± 0.0^{d}	901 ± 53.9^{c}	37 ± 5.3^{d}
	Pleurosigma angulatum	6.2 ± 0.3^{d}	257 ± 43.0^{def}	77 ± 3.9^{ab}
	Rhaphoneis sp.	8.4 ± 0.1^{c}	389 ± 67.0^{d}	80 ± 2.4^{a}
	Trachyneis aspera	7.2 ± 1.0^{cd}	360 ± 76.7^{de}	36 ± 0.9^{d}
D	*Tetraselmis hazeni*	13.6 ± 1.8^{a}	275 ± 42.5^{b}	69 ± 12.1^{a}
	Tetraselmis sp. (KMMCC-109)	12.5 ± 1.3^{a}	593 ± 149.0^{a}	17 ± 1.0^{b}
	Tetraselmis sp. (KMMCC-40)	15.2 ± 3.7^{a}	432 ± 44.6^{ab}	87 ± 0.1^{a}
E	*Lyngbya taylorii*	10.1 ± 0.7^{bc}	676 ± 52.6^{a}	83 ± 3.7^{a}
	Oscillatoria splendida	14.5 ± 0.2^{a}	863 ± 10.9^{a}	90 ± 1.2^{a}
	Phormidium luridum	7.7 ± 2.8^{c}	$1,378 \pm 539.4^{a}$	11 ± 4.8^{b}
	Trichodesmium erythraeum	12.3 ± 0.4^{ab}	996 ± 31.6^{a}	6 ± 1.0^{b}

KMMCC, Korea Marine Microalgae Culture Center : Different superscripts in the same column by each microalgal group mean significant difference ($P < 0.05$).

The biomasses of the diatoms *Nitzschia* sp. and *Caloneis schroder* in group C were significantly higher than those of other species, at 15-16 mg. *Phaeodactylum tricornutum* and *Pleurosigma angulatum* had the lowest biomasses at 6 mg ($P < 0.05$). The attachment rates of *Rhaphoneis* sp. and *P. angulatum* were highest at 77-80%. *Cylindrotheca closterium*, which had relatively high biomass, had the lowest attachment rate at 24%. However, the growth rate of *C. closterium* was the highest, at 2,302% ($P < 0.05$).

The biomasses of three kinds of *Tetraselmis* in group D were 12-15 mg, and were not significantly different from each other. In comparison with *Tetraselmis* sp. (KMMCC-109, 13%), the attachment and growth rates of *Tetraselmis* sp. (KMMCC-40), were significantly high, at 87% and 593%, respectively.

The biomasses of *Oscillatoria splendida* (14.5 mg) *and Phormidium luridum* (7.7 mg) in group E were the highest and lowest, respectively. The attachment rates of *O. splendida* (90%) and *Lyngbya taylorii* (83%) were significantly high, and that of *Trichodesmium erytheaeum* (6%) was significantly low. The growth rates of the four species of cyanophyceae, however, showed no significant differences, ranging from 676-1,378% ($P < 0.05$).

Settlement, metamorphosis, and survival rates of *Haliotis discus hannai* larvae

Nine microalgal species showing high growth and attachment rates were examined for their effectiveness as feed for *H. discus hannai* larvae. The larvae reacted to the substrate an hour after their introduction and began settling. The settlement rates of all experimental groups began to rise after 12 h and reached their maxima after another 12 h. After 24 h, the larvae showed a tendency to constantly perish (Table 3).

After 24 h, the settlement rates in all experimental groups except for *P. tricornutum* were higher than that of the control group. *Rhaphoneis* sp. and *Nitzschia* sp. showed the highest rates of 98% and 91%, respectively ($P < 0.05$). *Tetraselmis hazeni* also indicated a high settlement rate of 80%. The settlement rates after 96 hours in *T. hazeni* and *Rhaphoneis* sp. were 69% and 65%, respectively, which were significantly high. However, *P. tricornutum* and *C. closterium* showed lower rates than for the control group ($P < 0.05$). The experimental group of *Nitzschia* sp. at 24 h showed a settlement rate of 91%, which was as high as that of *Rhaphoneis* sp. The rate, however, rapidly decreased to 50% by 96 h.

The metamorphosis rates of the larvae that fed on *Rhaphoneis* sp. in the experimental group were 39% on the fourth day and 57% on the sixth day. This was significantly the highest rate, with metamorphosis rates in the *O. splendida* and *T. hazeni* groups being second highest ($P < 0.05$). The *C. closterium* group showed a significantly lower metamorphosis rate than did the control group, of 0.5% and 1.5% on the fourth and sixth days, respectively (Fig. 1).

The experimental group fed on *Rhaphoneis* sp. had a significantly higher larval survival rate on day 15 after metamorphosis than did other experimental groups, at 67% ($P < 0.05$) (Fig. 2). All experimental groups except the *Rhaphoneis* sp., *T. hazeni* (42%), and *O. splendida* (35%) groups showed lower survival rates than the control group ($P < 0.05$). On the second and fourth days, all larvae in the *C. closterium* and *P. tricornutum* groups had perished. Such low survival rates meant that it was not possible to conduct an analysis of growth difference according to microalgal species.

Table 3. Settlement rate (%) of *Haliotis discus hannai* larvae on different microalgal species

Microalgae	Elapsed times after inoculation (h)						
	1	6	12	24	48	72	96
Control	4.4 ± 1.7^{ab}	19.8 ± 5.3^{cd}	35.8 ± 7.5^{e}	36.4 ± 9.2^{f}	35.0 ± 3.6^{d}	38.8 ± 4.7^{e}	39.3 ± 4.8^{de}
Rhaphoneis sp.	4.9 ± 2.1^{a}	21.7 ± 5.9^{bcd}	51.1 ± 11.5^{bc}	98.3 ± 1.9^{a}	95.3 ± 3.1^{a}	86.8 ± 3.6^{a}	65.3 ± 9.7^{ab}
Phaeodactylum tricornutum	6.4 ± 2.0^{a}	5.7 ± 2.1^{e}	31.1 ± 7.4^{e}	42.2 ± 14.9^{ef}	34.8 ± 14.3^{d}	23.0 ± 12.5^{d}	21.2 ± 9.3^{f}
Navicula sp. (KMMCC-841)	5.3 ± 3.0^{a}	34.5 ± 4.8^{a}	53.3 ± 7.0^{abc}	65.7 ± 5.9^{cd}	64.5 ± 6.6^{bc}	64.4 ± 6.4^{b}	52.6 ± 12.1^{c}
Nitzschia sp.	4.9 ± 1.7^{a}	28.2 ± 7.2^{ab}	61.1 ± 5.4^{ab}	91.2 ± 2.6^{a}	87.3 ± 5.6^{a}	45.1 ± 5.9^{c}	49.7 ± 7.0^{cd}
Amphora sp. (KMMCC-773)	2.1 ± 2.5^{bcd}	27.7 ± 7.5^{b}	33.2 ± 8.5^{e}	64.8 ± 5.5^{cd}	65.8 ± 7.1^{bc}	59.4 ± 4.0^{b}	39.2 ± 6.3^{de}
Cylindrotheca closterium	1.6 ± 1.8^{cd}	16.1 ± 3.1^{d}	20.8 ± 7.7^{f}	$46.2 \pm 3.2e$	30.8 ± 10.5^{d}	29.4 ± 9.2^{d}	23.9 ± 7.8^{f}
Tetraselmis hazeni	4.3 ± 1.8^{ab}	25.2 ± 8.6^{bc}	48.6 ± 13.3^{cd}	79.6 ± 7.1^{b}	58.1 ± 9.1^{c}	64.8 ± 10.6^{b}	68.6 ± 10.7^{a}
Tetraselmis sp. (KMMCC-40)	0.6 ± 1.3^{d}	4.0 ± 1.4^{e}	38.7 ± 9.5^{de}	57.3 ± 6.9^{d}	37.6 ± 10.5^{d}	40.2 ± 9.0^{c}	34.6 ± 10.5^{e}
Oscillatoria splendida	3.9 ± 1.4^{abc}	17.2 ± 4.4^{d}	63.2 ± 5.9^{a}	69.0 ± 3.5^{c}	69.8 ± 5.9^{b}	66.3 ± 7.3^{b}	56.8 ± 5.7^{bc}

KMMCC, Korea Marine Mocroalgae Culture Center : Different superscripts in the same column mean significant difference ($P < 0.05$).

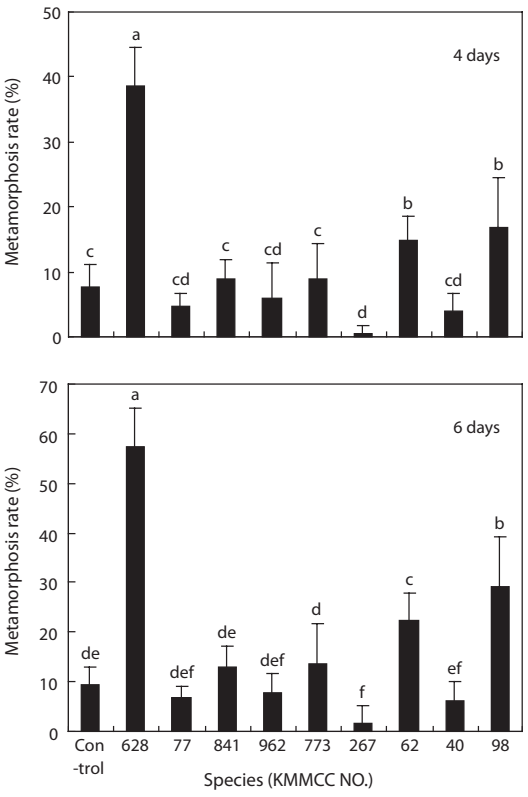

Fig. 1. Metamorphosis rate (%) of *Haliotis discus hannai* larvae reared with different microalgal species on 4 days (up) and 6 days (bottom) after inoculation KMMCC, Korea Marine Microalgae Culture Center ; (KMMCC-628, *Rhaphoneis* sp.; KMMCC-77, *Phaeodactylum tricornutum*; KMMCC-841, *Navicula* sp.; KMMCC-962, *Nitzschia* sp.; KMMCC-773, *Amphora* sp.; KMMCC-267, *Cylindrotheca closterium*; KMMCC-62, *Tetraselmis hazeni*; KMMCC-40, *Tetraselmis* sp.; KMMCC-98, *Oscillatoria splendida*). Different superscripts on the bar mean significant difference ($P < 0.05$).

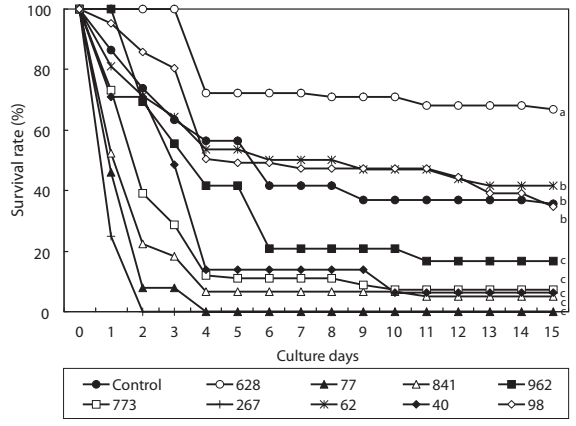

Fig. 2. Survival (%) of *Haliotis discus hannai* larvae after metamorphosis KMMCC, Korea Marine Microalgae Culture Center ; (KMMCC-628, *Rhaphoneis* sp.; KMMCC-77, *Phaeodactylum tricornutum*; KMMCC-841, *Navicula* sp.; KMMCC-962, *Nitzschia* sp.; KMMCC-773, *Amphora* sp.; KMMCC-267, *Cylindrotheca closterium*; KMMCC-62, *Tetraselmis hazeni*; KMMCC-40, *Tetraselmis* sp.; KMMCC-98, *Oscillatoria splendida*). Different superscripts on 15 days mean significant difference ($P < 0.05$).

Discussion

The larvae of *H. discus hannai* settle to substrate within 96 h after hatching and begin metamorphosis (Morse, 1985; Searcy-Bernal, 1999; Roberts and Lapworth, 2001). Metamorphosed larvae begin feeding on attached diatoms (Seki and Kanno, 1981; Kawamura and Takami, 1995; Roberts et al., 2007b). As prospective feed for *H. discus hannai* larvae, microalgae showing fast growth and high attachment rates were examined in this study.

Diatoms generally have high attachment rates compared to other microalgae, but most microalgae can adhere to substrate to a certain degree. In terms of nutritional value, diatoms, green algae, and blue-green algae generally have high contents of lipid, protein, and minerals, respectively (Borowitzka and Borowitzka, 1988). Diatoms can be divided into eight types in terms of the existence of mucous, their attachment form, and mobility. Among them, the best types as feed for the larvae of *H. discus hannai* are phlegmatic with plane form and slow mobility (Kawamura, 1994; Roberts et al., 2007a). Considering these features, this study aimed to find the best kinds of microalgae to be used as feed among diatoms, green algae such as *Tetraselmis*, and blue-green algae.

In this study, diatoms generally showed higher attachment rates than did other microalgae. However, *Tetraselmis* sp. (KMMCC-40) and the blue-green algae *O. splendida* and *L. taylorii* also had high attachment rates in the range of 83-90%. Among the diatoms, *Navicula* and *Amphora* showed higher biomass and attachment rates. The results of this study on the growth and attachment rates of microalgae can be used as a foundational resource for the development of feed for adhesive larvae and the mass culture of adhesive microalgae.

With respect to the settlement and metamorphosis of the abalone larvae, *Cocconeis scutellum* (Roberts and Nicholson, 1997; Parker et al., 2007) and *Navicula ramosissima* (Kawamura and Kikuchi, 1992; Roberts and Watts, 2010) are reported to induce high settlement and metamorphosis rates in *H. virginea* and *H. discus hannai* larvae. In the present study, larvae fed *Rhaphoneis* sp. showed the highest settlement rate of 98% at 24 h, and *T. hazeni* also showed a high settlement rate of 80%. At hour 96, these two microalgae showed no significant difference in their settlement rates of over 65%. Thus, it can be concluded that *T. hazeni* is also suitable as a settlement substrate for *H. discus hannai* larvae ($P < 0.05$). The metamorphosis of the larvae attached to *Rhaphoneis* sp. was the highest at 57%, and *T. hazeni* and *O. splendida* showed higher metamorphosis rates (20-30%) than did other diatoms. The growth rates of *P. tricornutum* and *C. closterium* were high, but the settlement and metamorphosis rates of the larvae fed these diatoms were low. The larvae fed *P. tricornutum* and *C. closterium* perished after growing to sizes of 344 μm and 305 μm, respectively. Thus, these two species are considered to be unsuitable as feed for *H. discus hannai* larvae.

Within two days after metamorphosis, the larvae of *H.*

discus hannai begin consuming their feed (Seki and Kanno, 1981; Martinez-Ponce and Searcy-Bernal, 1998; Roberts et al., 1999). They can consume bacteria or substances other than microalgae (Garland et al., 1985; Kawamua, 1996; Kitting and Morse, 1997); however, they tend to grow faster by consuming microalgae such as diatoms (Garland et al., 1985; Takami et al., 1997; Roberts et al., 1999). Furthermore, before growing to 800 μm in shell length, the larvae of *H. discus hannai* are known to utilize cell secretions as their source of nutrition (Kawamura and Takami, 1995; Roberts et al., 2007b).

However, immediately following metamorphosis, the larvae receive nutritional substances from yolk. Thus, it is difficult to observe differences in their growth according to feed type during such early developmental stages (Kawamura et al., 1998). Moreover, the premature development of the digestive system of the larvae at early stages makes it hard to examine the effectiveness of feed (Roberts et al., 1999). For the larvae of *H. discus hannai*, yolk is the main source of nutrition for 10 days after metamorphosis, and they grow up to 400 μm without any external source of nutrition (Takami et al., 2000).

In this study, larvae which fed on microalgae except for *Rhaphoneis* sp. and the control group that fed on no microalgae showed no difference in growth immediately following metamorphosis. Such results are explained by the fact that, in the early developmental stage, larvae feed on yolk more than on microalgae. The control group, which did not feed on microalgae, had 500 μm larvae, and 36% of its survival rate could be explained by the utilization of bacteria and organic substances in the seawater as nutrition sources.

The diameter of the larval mouth after metamorphosis measures only 10 μm; thus, the size of microalgae is also important as a condition of suitable feed for abalone larvae (Kawamura et al., 1998). The sizes of microalgae used in this study, except for *Rhaphoneis* sp., were over 10 μm. *Rhaphoneis* sp., being smaller than 10 μm, was a suitable feed for the larvae after the metamorphosis phase. Considering these results, the size, form, and nutrition and the attachment form, rate, and strength of each microalgae species should influence the settlement and growth of the larvae (Matthews and Cook, 1995; Roberts et al., 1999; de Viçose et al., 2010).

In this study, green algae and blue-green algae other than diatoms were also found to be prospective feeds for the larvae of *H. discus hannai.* The high content of protein in green algae and rich minerals in blue-green algae are inferred to have a positive influence on the formation of the larval shell of *H. discus hannai*. In conclusion, the most suitable microalgae for rearing *H. discus hannai* larvae was the diatom *Rhaphoneis* sp. The green alga *T. hazeni* and the blue-green alga *O. splendida* may also be useful when combined with *Rhaphoneis* sp.

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References

Borowitzka MA and Borowitzka LJ. 1988. Micro-algal Biotechnology. Cambridge University Press, Cambridge, GB.

de Viçose GC, Viera M, Bilbao A and Izquierdo M. 2010. Larval settlement of *Haliotis tuberculata coccinea* in response to different inductive cues and the effect of larval density on settlement, early growth, and survival. J Shellfish Res 29, 587-591.

Duncan DB. 1955. Multiple-range and multiple *F* tests. Biometrics 11, 1-42.

Garland CD, Cooke SL, Grant JF and McMeekin TA. 1985. Ingestion of the bacteria on and the cuticle of crustose (non-articulated) coralline algae by post-larval juvenile abalone (*Haliotis ruber* Leach) from Tasmanian waters. J Exp Mar Biol Ecol 91, 137-149.

Guillard RRL and Ryther JH. 1962. Studies of marine plankton diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8, 229-239.

Han HK and Hur SB. 2000. Dietary value of benthic diatoms for newly settled abalone, *Haliotis discus hannai* Ino. J Aquac 13, 153-161.

Kawamura T. 1994. Taxonomy and ecology of marine benthic diatoms. Mar Fouling 10, 7-25.

Kawamura T. 1996. The role of benthic diatoms in the early life stages of the Japanese abalone (*Haliotis discus hannai*). In: Survival Strategies in Early Life Stages of Marine Resources. Watanabe Y, Yamashita Y and Oozeki Y, eds. Balkema, Rotterdam, NL, pp. 355-367.

Kawamura T and Kikuchi S. 1992. Effects of benthic diatoms on settlement and metamorphosis of abalone larvae. Suisanzoshoku 40, 403-409.

Kawamura T and Takami H. 1995. Analysis of feeding and growth rate of newly metamorphosed abalone *Haliotis discus hannai* fed on four species of benthic diatoms. Fish Sci 61, 357-358.

Kawamura T, Roberts RD and Nicholson CM. 1998. Factors affecting the food value of diatom strains for post-larval abalone *Haliotis iris*. Aquaculture 160, 81-88.

Keough MJ and Raimondi PT. 1996. Responses of settling invertebrate larvae to bioorganic films: effects of large-scale variation in films. J Exp Mar Biol Ecol 207, 59-78.

Kitting KL and Morse DE. 1997. Feeding effects of postlarval red abalone, *Haliotis rufescens* (Mollusca: Gastropoda) on encrusting coralline algae. Molluscan Res 18, 183-196.

Martinez-Ponce D and Searcy-Bernal R. 1998. Grazing rates of red abalone (*Haliotis rufescens*) postlarvae feeding on the benthic diatom *Navicula incerta*. J Shellfish Res 17, 627-630.

Matthews I and Cook PA. 1995. Diatom diet of abalone post-larvae (*Haliotis midae)* and the effect of pre-grazing the diatom overstorey. Mar Freshw Res 46, 545-548.

Morse DE. 1985. Neurotransmitter-mimetic inducers of larval settle-

ment and metamorphosis. Bull Mar Sci 37, 697-706.

Parker F, Davidson M, Freeman K, Hair S and Daume S. 2007. Investigation of optimal temperature and light conditions for three benthic diatoms and their suitability to commercial scale nursery culture of abalone (*Haliotis laevigata*). J Shellfish Res 26, 751-761.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr Mar Biol Annu Rev 30, 273-335.

Roberts RD and Lapworth C. 2001. Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. J Exp Mar Biol Ecol 258, 1-13.

Roberts RD and Nicholson CM. 1997. Variable response from abalone larvae (*Haliotis iris*, *H. virginea*) to a range of settlement cues. Molluscan Res 18, 131-142.

Roberts RD and Watts E. 2010. Settlement of *Haliotis austrralis* larvae : role of cues and orientation of the substratum. J Shellfish Res 29, 663-670.

Roberts RD, Kawamura T and Nicholson CM. 1999. Growth and survival of postlarval abalone (*Haliotis iris*) in relation to development and diatom diet. J Shellfish Res 18, 243-250.

Roberts RD, Kawamura T and Handley CM. 2007a. Factors affecting settlement of abalone (*Haliotis iris*) larvae on benthic diatom films. J Shellfish Res 26, 323-334.

Roberts RD, Revsbech NP and Damgaard LR. 2007b. Effect of water velocity and benthic diatom morphology on the water chemistry experienced by postlarval abalone. J Shellfish Res 26, 745-750.

Searcy-Bernal R. 1996. Boundary layers and abalone postlarval culture: preliminary studies. Aquaculture 140, 129-137.

Searcy-Bernal R. 1999. Settlement and postlarval ecology of the red abalone *Haliotis rufescens* in culture systems. Ph.D. Disseratation, University of California, Davis and San Diego State University, San Diego, CA, US.

Seki T and Kanno H. 1981. Induced settlement of the Japanese abalone, *Haliotis discus hannai*, veliger by the mucous trails of the juvenile and adult abalones. Bull Tohoku Reg Fish Res Lab 473, 29-36.

Takami H, Kawamura T and Yamashita Y. 1997. Contribution of diatoms as food sources for post-larval abalone *Haliotis discus hannai* on a crustose coralline alga. Molluscan Res 18, 143-151.

Takami H, Kawamura T and Yamashita Y. 2000. Starvation tolerance of newly metamorphosed abalone *Haliotis discus hannai*. Fish Sci 66, 1180-1182.