Korean Journal of Malacology, Vol. 19(2): 95-106, December, 2003

Effects of Amino Acids on Larval Settlement and Metamorphosis in *Haliotis discus hannai*

Kyoung Ho Kang, Byoung-Hak Kim[1], Jae Min Kim and Young Hun Kim

Division of Aqua Life Science, Yosu National University, Yosu 550-749, Korea
[1]National Fisheries Research & Development Institute, Namhae-gun, Kyeongsangnam-do 668-800, Korea

ABSTRACT

The compositions of amino acid in 6 monocultured benthic diatoms used in aquaculture of *Haliotis discus hannai* were analyzed, and effects of 15 artificial synthetic amino acids on the settlement and metamorphosis of *H. discus hannai* larvae. Results showed that the content of L-aspartic acid was highest in all diatoms, and that of L-glutamic acid was second high. In experiment using settlement slat without benthic diatom attached, the highest settlement rate (33.3 ± 8.8%) was obtained with L-glutamic acid, and a higher value (16.7 ± 3.3%) was found with L-aspartic acid at 24 h after experimental commencement, compared to that of control (8.6 ± 5.1%). 80 h later the metamorphosis rates of L-glutamic acid (86.7 ± 10.7%) and L-aspartic acid (80.0 ± 3.3%) groups were higher than control group(0) and other amino acids significantly. The response rate of L-glutamic acid was the highest (62.0%), and those of L-aspartic acid (30.0%) and L-threonine (25.3%) groups were also significantly higher than control group. In the experiment using settlement slat with benthic diatom attached, the best effect of various amino acids on induction of larval settlement was obtained with L-glutamic (82.0 ± 6.9%) and L-aspartic acid (78.7 ± 5.1%) at 24 h after experimental commencement. The settlement rates of L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, and L-tyrosine groups were significantly lower than control group. The same differences in the metamorphosis rate at 56 h after experimental commencement and in the response rate were found. It should be noted that after 80 h the metamorphosis rates of L-histidine (74.0 ± 12.0%) and L-lysine (87.0 ± 8.8%) declined rapidly compared to those of 56 h (8.0 ± 12.0%; 7.7 ± 12.0%).

Keywords: Abalone, *Haliotis discus hannai*, Amino acid, Settlement, Metamorphosis.

INTRODUCTION

Abalone is an economically important marine gastropod commanding moderate to high prices. The culture of the abalone has been investigated for a number of decades in Japanese and Chinese (Ino, 1951; Chen *et al.*, 1977), and a rapid improvement in culture techniques in recent years has had a significant impact on abalone production, especially in Japan and Taiwan (Chen, 1989). Today, the techniques of abalone culture, especially, the seed production, such as the induction of spawning, fertilization and hatch, larval rearing and induction to settle, have been well established (Fleming, 1996). Most of these studies on abalone have been focused on the reproductive biology, characterizes of embryonic development, environmental conditions for larval rearing (Capinpin *et al.*, 1998; Chen *et al.*, 1977; Liu *et al.*, 1985; Lu *et al.*, 2001; Yang *et al.*, 1975), and nutritional requirements of various developmental stage (Kawamura *et al.*, 1996; Mai, 1998; Tan and Mai, 2002; Zhu *et al.*, 2002).

In general, the abalone larvae display the characteristics of attachment to substratum in the settlement stage after having passed through the pelagic stage. Following this, in a set period, metamorphosis occurs. During the whole lifespan of

Received September 8, 2003; Accepted December 6, 2003
Corresponding author: Kang, Kyoung Ho
Tel: (82) 61-659-3165 e-mail: mobidic@yosu.ac.kr
1225-3480/19203

theabalone, this stage plays an important role, which often leads to a high mortality. Therefore, the successful induction of settlement and metamorphosis is a key factor in seedling production for commercial aquaculture. Many researchers have investigated the effects of some chemical inducers on the settlement and metamorphosis. Among them, GABA, KCl and bromomethane could be often seen (Taniguchi *et al.,* 1994; Baloun *et al.,* 1984; Morse and Morse, 1984; Morse *et al.,* 1979, 1980; Akashige *et al,* 1981). Furthermore, benthic diatom films growing on plastic plates have traditionally been used as settlement substrata in abalone hatcheries worldwide (Seki, 1980; Ebert and Houk, 1984; Hahn, 1989).

Morse *et al.* (1979) found that settlement and metamorphosis of the larvae of the abalone (*Haliotis rufescens*) was induced with compounds available at the surface of crustose red algae. GABA and GABA-mimetic compounds form the crustose coralline algae induced settlement and metamorphosis of larvae of *H. rufescens* (Morse *et al.,* 1979). Larvae of *H. rufescens* also attach to the substratum and metamorphose in the presence of diatoms and conspecific mucus (Slattery, 1992).

As to the studies of *Haliotis discus hannai,* Kawanura and Kikuchi (1992) suggested that *C. scutellum* var. *parva* was very effective in inducing settlement, and other diatoms species forming flat, prostrate communities, such as *N. ramosissima,* also induced high settlement rates of *H. discus hannai.* Furthermore, some researchers investigated that relationship between the diatom density and the larval settlement, Kawanura and Kikuchi (1992) found highest settlement rates of the abalone *H. discus hannai* at highest diatom densities. Daume *et al.* (1999) reported that the larval settlement was higher on older diatom films. However, studies on the effective components of diatom to induce settlement are seldom performed. In the present study the compositions of amino acid in 6 monocultured benthic diatoms used in aquaculture of *H. discus hannai* were analyzed, and 15 artificial synthetic amino acids were chosen to induce the settlement and metamorphosis of larvae *H. discus hannai.*

MATERIALS AND METHODS

1. Diatom culture

Six diatom strains (*Achnanthes longipes, Amphora lineate, Navicula incerta, Nitzschia logissima, Navicula pelliculosa, Nitzschia* sp.) were obtained by routine isolation and purification from diatom film used in aquaculture of *H. discus hannai.* These diatom species were cultured in UV-treated f/2 medium (Guillard and Ryther, 1962) added with 1 ml Na_2SiO_3 solution (100 g $Na_2SiO_3 \square 6H_2O$ per liter of distilled water) per liter of medium and maintained in 1-liter beakers, at a constant temperature of 20-22℃, with a continuous illumination of 3,000-4,000 lux using fluorescent lighting. Three polyethylene plates (5 × 10 × 0.1 cm) were inserted in the beakers for the attachment of diatom. The monospecific diatom cultures were not axenic.

2. Compositional analyses of amino acids in six mono-cultured benthic diatoms

The cultured diatoms were scraped off from the polyethylene plates and centrifuged (10,000 rpm, 10 min). 0.1-0.5 g of diatom was hydrolyzed for 24 h with 1-5 ml of 6 mol/ml HCl at a constant temperature of 110℃ and vacuum condition. The hydrolyzed diatom was filtered with a decompressing filter at 40-50℃.The filtrate was added with 2 ml of sodium buffer (pH 2.2) to 10 ml. One ml of filtrate was filtered again through 0.2 μm filter-membrane, and the filtrate was used to analyze the composition of amino acids with an automatic analyzer (Pharmacia, Biochrom 20).

3. Observation and account of settlement and meta-morpho sis of the abalone larvae

The breeding conditions of the abalone larvae were as follows: water temperature: 18.0 ± 0.5℃ salinity: 30-31 psu pH: 8.1-8.2 dissolved oxygen: 5.2-6.5 mg/l, illuminance: < 500 lux (T-10W); aeration rate: 100 ml/min (LP-60A). The breeding seawater was exchanged in full each day and filtered through a 1 μm cartridge filter to maintain stable water quality. All the seawater was UV-treated to avoid contamination.

Larvae that had been setting for 86 h after

fertilization were used in following experiments. For the experiment on the number of *H. discus hannai* larvae settled, counting was done with a multi-functional projector (Nikon V-12). The settlement was confirmed by observing the floating larvae with the naked eye at the commencement of the experiment. Once the settlement was completed, an optical microscope was used to observe the process of metamorphosis in an interval of 2 hours. In order to make a judgement on the settlement and metamorphosis, the numbers of settled and metamorphosised larvae were investigated at 6, 24, 32, 56 and 80 h after experimental commencement, according to each settlement and metamorphosis stage. The methods of calculating the rates of settlement, metamorphosis and response were used the methods by Kawahara *et al.* (1995):

Rate of settlement (%) = (No. of larvae settled)/(No. of larvae inserted) X 100

Rate of metamorphosis (%) = (No. of larvae metamorphosized)/(No. of larvae settled) X 100

Rate of response (%) = [(No. of larvae settled + No. of larvae metamorphosized)/ No. of larvae inserted] X 100

4. Effects of amino acids on settlement and metamorphosis of abalone larvae on settlement slat without benthic diatom

According to results of compositional analyses of amino acids, 15 artificial synthetic amino acids, L-alanine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glycine, L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-pronine, L-serine, L-threonine, L-tyrosine, and L-valine were chosen to induce the settlement and metamorphosis of larvae of *H. discus hannai.* Thirty abalone larvae were put into the tanks containing 1,000 ml of breeding seawater treated as above methods. Fifteen amino acids were added to form 10^{-5} mol/l of final concentration, respectively. Three polyethylene plates (5 × 10 × 0.1 cm) without diatom attached were inserted in each tank for the settlement of abalone larvae. The breeding seawater without addition of any amino acid was used as control group.

5. Effects of amino acids on settlement and metamorphosis of the abalone larvae on settlement slat with benthic diatom

In this experiment, polyethylene plates (5 × 10 ×

Table 1. Amino acid compositions (expressed as mg/100 g dry. matter, as is) of 6 benthic diatoms.

Component	*Achnanthes longipes*	*Amphora lineate*	*Navicula incerta*	*Navicula pelliculosa*	*Nitzschia logissima*	*Nitzschia* sp.	Mix.
Alanine	50.01	46.54	54.98	26.49	46.54	47.80	197.55
Arginine	49.03	38.64	48.40	24.61	38.64	40.42	185.37
Aspartic acid	117.94	96.96	113.42	60.73	96.96	98.24	345.86
Cysteine	7.60	14.07	15.08	7.79	14.07	6.26	39.25
Glutamic acid	99.23	90.76	102.93	55.15	90.76	92.58	457.23
Glycine	62.39	53.25	109.24	36.07	53.25	50.31	221.08
Histidine	21.97	15.20	19.08	10.83	15.20	12.47	58.38
Isoleucine	39.77	36.19	36.03	20.55	36.19	35.50	146.11
Leucine	59.44	59.38	59.13	32.09	59.38	55.10	266.89
Lysine	43.10	28.97	43.80	21.86	28.97	37.90	190.31
Methionine	1.24	16.80	16.91	7.30	16.80	10.64	80.49
Phenylalanine	36.87	40.55	40.08	22.25	40.55	38.90	161.90
Proline	34.51	30.84	30.36	20.69	30.84	32.70	117.36
Serine	58.99	48.75	129.12	32.99	48.75	48.06	174.26
Threonine	59.71	49.35	55.36	28.15	49.35	51.50	160.93
Tyrosine	31.09	23.66	33.64	15.34	23.66	21.74	92.38
Valine	53.29	47.24	46.44	27.32	47.24	51.34	167.06
Ammonia	163.93	83.05	110.16	53.54	83.05	76.78	689.94

Table 2. Rate of settlement, metamorphosis and response of *Haliotis discus hannai* larvae on 15 amino acids without benthic diatom.

Amino acids	Settlement rate (%)*		Metamorphosis rate (%)*			Response rate (%)*
	6 hr	24 hr	32 hr	56 hr	80 hr	
L-alanine	4 .3 ± 1.9^{efg}	4.3 ± 1.9^{ef}	-	75.0 ± 1.9^{ef}	75.0 ± 0.0^{ef}	7.7^{d}
L-aspartic acid	17.7 ± 1.9^{b}	16.7 ± 3.3^{b}	-	93.8 ± 1.9^{b}	80.0 ± 3.3^{b}	30.0^{b}
L-cysteine	10.0 ± 3.3^{cde}	10.0 ± 3.3^{cde}	-	88.9 ± 3.3^{cde}	56.7 ± 1.9^{cde}	15.7^{cd}
L-glutamic acid	30.0 ± 8.8^{a}	33.3 ± 8.8^{a}	-	93.3 ± 8.8^{a}	86.7 ± 10.7^{a}	62.0^{a}
L-glycine	8.7 ± 1.9^{def}	8.9 ± 1.9^{cdef}	-	87.5 ± 1.9^{cdef}	62.9 ± 1.9^{cdef}	14.7^{cd}
L-histidine	3.3 ± 1.9^{fg}	4.3 ± 0.0^{ef}	-	75.0 ± 0.0^{ef}	75.0 ± 1.9^{ef}	7.7^{d}
L-leucine	5.6 ± 1.9^{efg}	6.7 ± 1.9^{def}	-	83.3 ± 1.9^{def}	50.0 ± 1.9^{def}	10.0^{d}
L-lysine	4.3 ± 0.0^{efg}	7.8 ± 1.9^{def}	-	85.7 ± 1.9^{def}	72.5 ± 0.0^{def}	13.3^{cd}
L-methionine	2.3 ± 1.9^{g}	4.3 ± 1.9^{ef}	-	75.0 ± 1.9^{ef}	51.3 ± 1.9^{ef}	6.7^{d}
L-phenylalanine	2.3 ± 1.9^{g}	3.3 ± 1.9^{f}	-	66.7 ± 1.9^{f}	30.0 ± 1.9^{f}	4.3^{d}
L-proline	13.3 ± 1.9^{efg}	8.9 ± 1.9^{cdef}	-	92.3 ± 1.9^{cdef}	62.9 ± 1.9^{cdef}	14.7^{cd}
L-serine	13.3 ± 3.3^{bcd}	11.0 ± 1.9^{bcd}	-	90.9 ± 1.9^{bcd}	70.7 ± 3.9^{bcd}	18.7^{bcd}
L-threonine	14.3 ± 1.9^{bc}	14.3 ± 1.9^{bc}	-	92.3 ± 1.9^{bc}	77.5 ± 1.9^{bc}	25.3^{bc}
L-tyrosine	4.3 ± 1.9^{efg}	4.3 ± 3.3^{ef}	-	75.0 ± 3.3^{ef}	75.0 ± 3.3^{ef}	8.7^{cd}
L-valine	2.3 ± 1.9^{g}	5.6 ± 1.9^{ef}	-	80.0 ± 0.0^{ef}	58.8 ± 3.3^{ef}	9.0^{d}
control**	2.0 ± 1.9^{g}	8.6 ± 5.1^{cdef}	-	88.5 ± 5.1^{cde}	0	8.6^{d}

*Values (mean ± s.d. of three replicate groups) in the same column not sharing a common superscript are significantly different ($p < 0.05$).
**Control: Non-diatom on the polyethylene plates.

0.1 cm) with diatom attached were used as settlement slats. These slats were pre-cultured with mixed diatom that were used to analyze compositions of amino acids, for 3 days to let the diatom form a thin layer of algal film on the slat. The settlement slats were dipped three times in UV-treated seawater to remove excess diatoms and debris prior to their use in the experiment. Thirty abalone larvae were cultured in seawater containing different amino acids described as above. The control group used settlement slats with mixed diatom and breeding seawater without amino acids.

6. Data analysis

Each experiment was carried out in triplicate. Data were analyzed with an ANOVA using SAS program (SAS Inc., 1999) and administered with Duncan's multiple range test (Duncan, 1955), in order to officially verify the significance of the average value within a 95% confidence limit. ANOVA has been used to analyze the correlating elements of settlement and metamorphosis.

RESULTS

1. Amino acid compositions of 6 monocultured benthic diatoms

Amino acid compositions of 6 monocultured benthic diatoms were showed in Table 1. The content of L-aspartic acid was the highest in all diatoms, and that of L-glutamic acid showed the second high.

2. Effects of amino acids on settlement and metamorphosis of the abalone larvae on settlement slat without benthic diatom

The rates of settlement, metamorphosis and response of abalone larvae were showed in Table 2. At 6 h after experimental commencement, the highest settlement rate (30.0 ± 8.8%) was obtained with L-glutamic acid, a higher value (17.7 ± 1.9%) was found with L-aspartic acid, and that of L-cysteine, L-glycine, L-serine, L-threonine also was higher than control significantly. There was no significant difference in settlement rate between control and other amino acids. After 24 h only the settlement rates of L-glutamic and L-aspartic acid were significantly higher than that of control group ($p <$

0.05; Fig. 1).

Fifty six hours the higher metamorphosis rates of abalone larvae were found in L-glutamic (93.3 ± 8.8%) and L-aspartic acid (93.8 ± 1.9%) groups, that

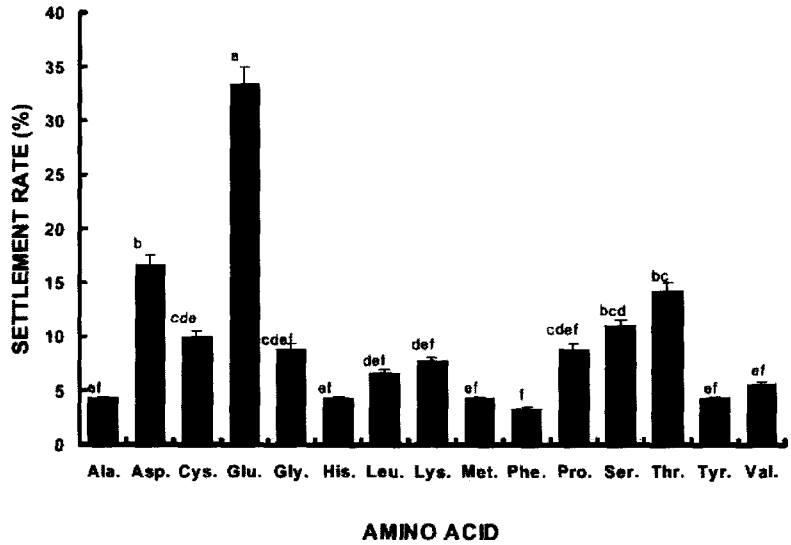

Fig. 1. Settlement rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and non-benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

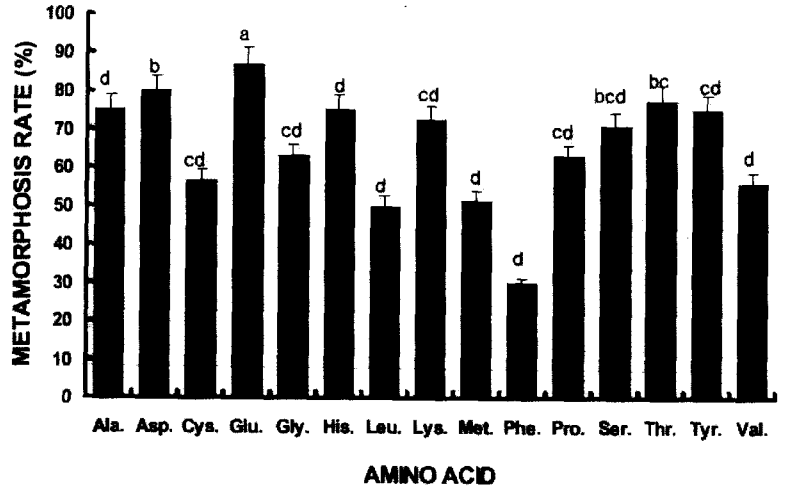

Fig. 2. Metamorphosis rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and non-benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

of L-phenylalanine was significantly lower than control, and there was no significant difference between control and other amino acids. 80 h later the metamorphosis rates of control group and most amino acids declined obviously except L-alanine, L-histidine, and L-tyrosine, and those of L-glutamic (86.7 ± 10.7%) and L-aspartic acid (80.0 ± 3.3%) groups were still higher than others significantly ($p < 0.05$; Fig. 2). The highest response rate (62.0%) was obtained with L-glutamic acid, and those of L-aspartic acid (30.0%) and L-threonine (25.3%) was also significantly higher than control group. There was no significant difference between control and other amino acids group ($p < 0.05$; Fig. 3).

3. Effects of amino acids on settlement and metamorphosis of the abalone larvae on settlement slat with benthic diatom

In the experiment using settlement slat with benthic diatom attached, the best effect of various amino acids on induction of larval settlement was obtained with L-glutamic (82.0 ± 6.9%) and L-aspartic acid (78.7 ± 5.1%) at 24 h after experimental commencement. The settlement rates of L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, and L-tyrosine groups were significantly lower than control. There was no significant difference between control group and other amino acids group (Table 3, Fig. 4). The same difference in metamorphosis rate at 56h after experimental commencement and in response rate were found (Table 3, Fig. 5, 6). The metamorphosis rates and response rates of L-glutamic (96.3 ± 6.9%; 155.0%) and L-aspartic acid groups (96.2 ± 5.1%; 153.0%) were significantly higher than others, and those of L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, and L-tyrosinegroups were significantly lower than control. It should be noted that after 80 h the metamorphosis rates of L-histidine (8.0 ± 12.0%) and L-lysine (7.7 ± 12.0%) declined rapidly compared to those of 56 h (74.0 ± 12.0%; 87.0 ± 8.8%; $p < 0.05$).

DISCUSSION

Most larvae of invertebrate must pass through the period of settlement and metamorphosis, which makes

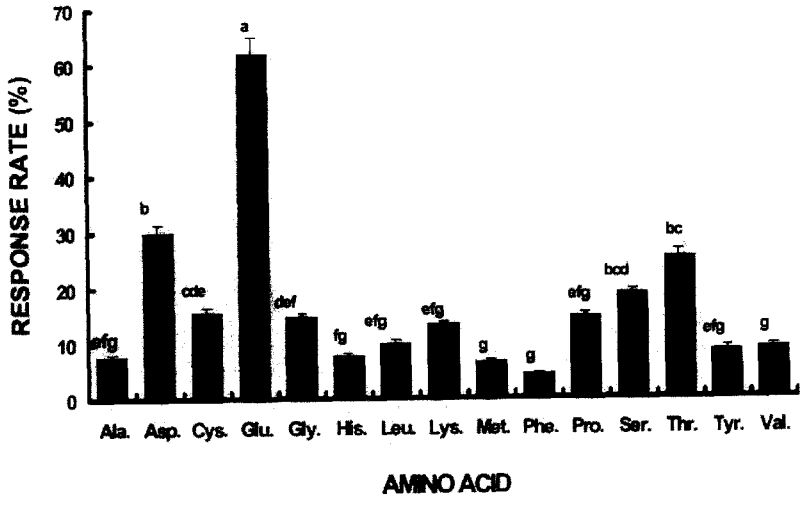

Fig. 3. Response rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and non-benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

Table 3. Rate of settlement, metamorphosis and response of *Haliotis discus hannai* larvae on 15 amino acids with benthic diatom.

Amino acids	Settlement rate (%)*		Metamorphosis rate (%)*			Response rate (%)*
	6 hr	24 hr	32 hr	56 hr	80 hr	
L-alanine	47.7 ± 10.7^{b}	47.7 ± 10.7^{bc}	-	93.0 ± 10.7^{bc}	90.9 ± 11.6^{b}	91.0^{bc}
L-aspartic acid	78.7 ± 8.4^{a}	78.7 ± 5.1^{a}	-	96.2 ± 5.1^{a}	94.5 ± 5.1^{a}	153.0a
L-cysteine	46.7 ± 6.7^{b}	46.7 ± 6.7^{bc}	-	92.7 ± 6.7^{bc}	87.9 ± 8.4^{b}	87.7bc
L-glutamic acid	84.3 ± 10.7^{a}	82.0 ± 6.9^{a}	-	96.3 ± 6.9^{a}	89.4 ± 5.8^{a}	155.0^{a}
L-glycine	48.7 ± 11.7^{b}	51.0 ± 5.1^{bc}	-	93.5 ± 5.1^{bc}	88.9 ± 3.9^{b}	96.3^{bc}
L-histidine	7.6 ± 1.9^{c}	16.7 ± 12.0^{e}	-	74.0 ± 12.0^{c}	8.0 ± 12.0^{cd}	30.0^{e}
L-leucine	22.0 ± 6.8^{c}	24.3 ± 5.8^{de}	-	86.3 ± 5.8^{de}	86.3 ± 10.0^{cde}	45.3de
L-lysine	6.7 ± 1.9^{c}	15.3 ± 8.8^{e}	-	87.0 ± 8.8^{e}	7.7 ± 12.0^{e}	26.3^{e}
L-methionine	16.7 ± 3.3^{c}	22.0 ± 8.4^{de}	-	86.4 ± 8.4^{de}	84.9 ± 8.4^{cde}	40.7de
L-phenylalanine	7.6 ± 6.7^{c}	20.0 ± 8.8^{de}	-	83.3 ± 8.8^{de}	83.3 ± 8.8^{cde}	36.7^{de}
L-proline	37.7 ± 15.0^{b}	35.3 ± 11.7^{cd}	-	90.6 ± 11.7^{cd}	87.7 ± 13.5^{bc}	66.3^{cd}
L-serine	54.3 ± 5.1^{b}	53.3 ± 3.3^{b}	-	95.6 ± 3.3^{b}	87.5 ± 3.4^{b}	100.0b
L-threonine	46.7 ± 16.7^{b}	47.7 ± 11.7^{bc}	-	93.0 ± 11.7^{bc}	90.9 ± 14.5^{b}	91.0bc
L-tyrosine	20.0 ± 5.1^{c}	30.0 ± 1.9^{de}	-	88.9 ± 1.9^{de}	88.9 ± 1.9^{cd}	56.7^{de}
L-valine	50.0 ± 7.8^{b}	50.0 ± 8.8^{bc}	-	93.3 ± 8.8^{bc}	90.7 ± 18.7b	95.3^{bc}
control**	4.3 ± 3.3	43.5 ± 8.4^{b}	-	92.3 ± 8.4^{b}	80.8 ± 6.9	82.0^{b}

*Values (*mean* ± s.d. *of three replicate groups*) in the same column not sharing a common superscript are significantly different ($p < 0.05$).
**Control: Non-diatom on the polyethylene plates.

the pelagic larvae turn to the benthic lifestyle and grow to juveniles. During the whole lifespan, this stage plays an important role, which often leads to a high mortality. In aquaculture, this is a key factor to obtain the high yield and production. Larvae of many marine gastropods settle and metamorphose in response to pharmaceutical agents, such as gamma-aminobutyric acid (GABA) (Morse *et al.*, 1979), choline (Hirata and Hadfield, 1986; Tod, *et al.*, 1991), excess potassium (Yool *et al.*, 1986; Pechenik and Heyman, 1987; Pechenik and Gee, 1993). Natural settlement cues of larvae of benthic marine invertebrates may originate from conspecific adult (Seki and Kan-no, 1981; Burke, 1984; Pawlik, 1986 Bonar *et al.*, 1990; Pearce and Scheibling, 1990; Slattery, 1992; Zimmerfaust and Tamburri, 1994), diatom and bacterial films (Kirchman *et al.*, 1982; Maki *et al.*, 1989; Fitt *et al.*, 1990; Zimmerfaust and Tamburri, 1994), and prey items (Steneck, 1982; Morse and Morse; 1984; Todd, 1985; Hadfield and Pennington, 1990; Pearce and Scheibling, 1991).

GABA, which is contained in *Coralline* algae in high concentration (10^{-2} M), activates larval settlement by 98% at the concentration level of 10^{-6} M (Morse *et al.*, 1979). In addition, it has been reported that in L-α,β-diaminopropionic acid, the settlement inducement effect becomes acknowledged in 10^{-5} M under the existence of GABA of 2.5×10^{-7}, while L-lysine, *etc.* perform similar effects to those of L-α and ω-diamino acid (Trapido-Rosenthal and Morse, 1985). The chemical substances mentioned above are perceived to be harmful to the larvae in high concentration level. According to Akashige *et al.* (1981), it has been reported that with the GABA in *Haliotis discus hannai,* the ciliation movement of larvae velum is suspended and after a temporary falling off, rotation occurs and they continue to die. In one of the recent documentations on abalone, the investigation on H. diversicolor reports that GABA does not induce the metamorphosis (Lin *et al.*, 1986).

In addition, Kawahara *et al.* (1995) has reported that the metamorphosis inducement for *Strongylocentrotus intermedius* larvae is provided by Potassium chloride and that the performance of potassium chloride in promoting metamorphosis for a variety of marine invertebrate larvae has been

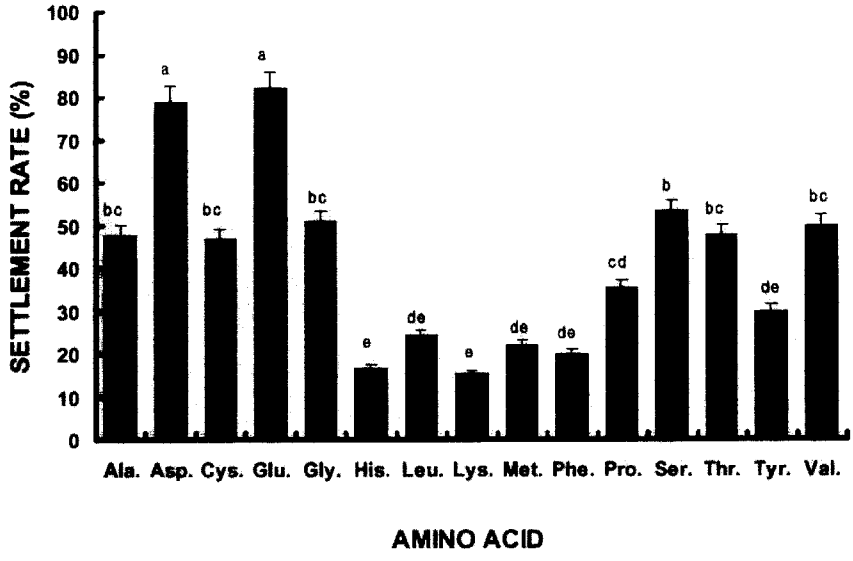

Fig. 4. Settlement rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

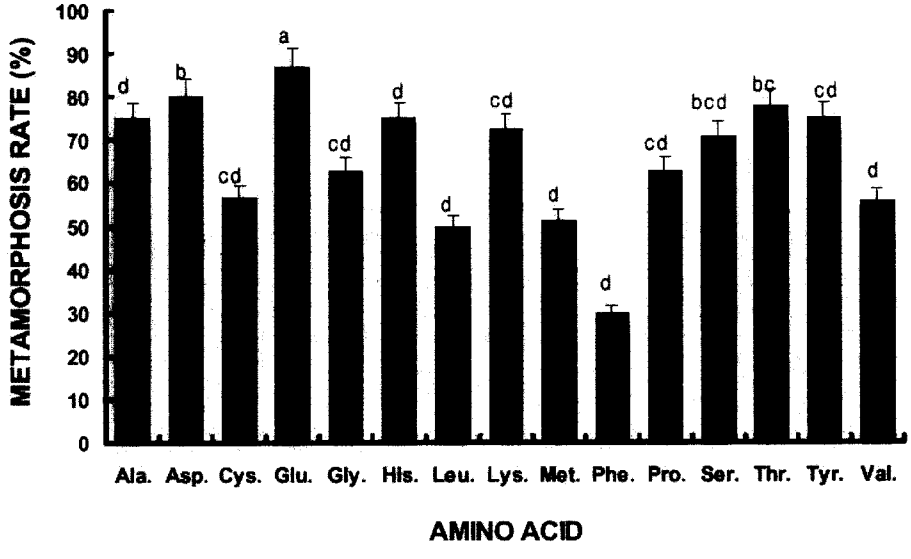

Fig. 5. Metamorphosis rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

acknowledged. A report shows that the metamorphosis of *Strongylocentrotus intermedius* had been completed by 100% in the duration of 60 minutes at the concentration level of 18 mM while the settlement and metamorphosis of *Haliotis rufescens* larvae are known to be induced by the increase in the concentration level of K^+ (Yool *et al.*, 1986). Settlement, as well as metamorphosis, according to K^+ is considered to differ according to each particularity of the species. Presumably it is because the increase of K^+ applies the K^+ ion on the excitability membrane of the larvae, resulting in the depolarization of this membrane (Baloun and Morse, 1984; Morse, 1985).

At present benthic diatom films growing on plastic plates have traditionally been used as settlement substrata in abalone hatcheries worldwide (Seki, 1980; Ebert and Houk, 1984; Hahn, 1989). Wenresti and Shelah (2003) reported the important contribution and effectiveness of *Navicula* in larval settlement of the tropical abalone *Haliotis asinine*. *Navicula* is also effective in the larval settlement of temperate species *Haliotis laevigata* (Daume *et al.*, 1999). In the work of Daume *et al.* (1999), the abalone *H. laevigata* settled particularly well to films of *N. ramosissima*. Kawamura and Kikuchi (1992) tested 18 species of diatoms and all had the effect in inducing the settlement of *H. discus hannai* larvae.

In the present study, the compositional analyses of amino acids in 6 monocultured benthic diatoms used in aquaculture of *H. discus hannai* showed the contents of L-aspartic and L-glutamic acid were higher in all diatoms. In experiment using settlement slat without benthic diatom attached, the rates of settlement, metamorphosis and response in L-glutamic and L-aspartic acid groups were higher significantly than those of control. In addition, the settlement rate of L-cysteine, L-glycine, L-serine, L-threonine also was higher than control significantly. This indicated that these amino acids had an obvious effect of inducing the settlement and metamorphosis of *H. discus hannai* larvae. However, the inducing effects of L-aspartic and L-glutamic acid were still worse than that of benthic

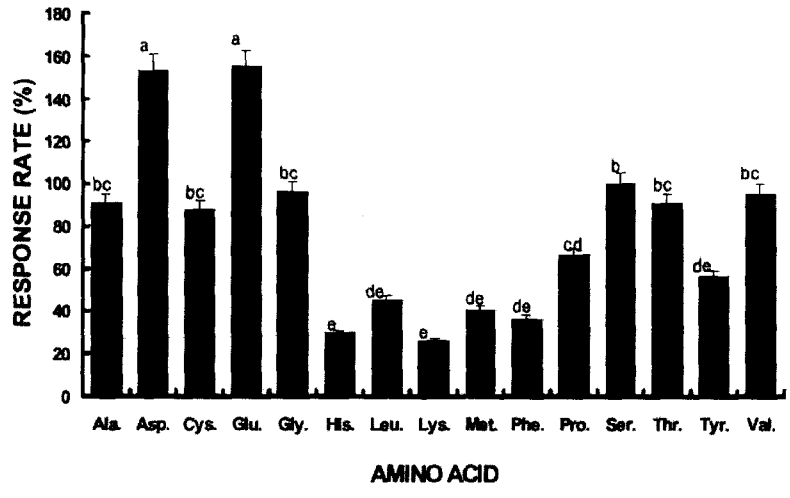

Fig. 6. Response rate of larval abalone on various amino acids (Ala.: L-alanine, Asp.: L-aspartic acid, Cys.: L-cysteine, Glu.: L-glutamic acid, Gly.: L-glycine, His.: L-histidine, Leu.: L-leucine, Lys.: L-lysine, Met.: L-methionine, Phe.: L-phenylalanine, Pro.: L-proline, Ser.: L-serine, The.: L-threonine, Tyr.: L-tyrosine, Val.: L-valine) and benthic diatom. Different superscripts on the bars within a figure are significantly different ($p < 0.05$).

diatom. The diatom is necessary for larval rearing of abalone since it also served as diet to provide nutrition for larval development and growth.

Results of experiment using settlement slat with benthic diatom attached suggested that the inducing effects of L-glutamic and L-aspartic acid were cumulative with benthic diatom. However, the rates of settlement, metamorphosis and response of L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, and L-tyrosine groups were significantly lower than control, especially after 80 h the metamorphosis rates of L-histidine (8.0 ± 12.0%) and L-lysine (7.7 ± 12.0%) declined rapidly compared to those of 56 h (74.0 ± 12.0%; 87.0 ± 8.8%). This may be due to the harm caused by high concentrations of these amino acids.

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