

## Settlement and Early Growth of Abalone Larvae *Haliotis asinina* Linnaeus, in Response to the Presence of Diatoms

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**Abstract.** GABA (gamma-aminobutyric acid) of different concentrations ( $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$ ,  $10^{-3}$  M) was tested for its effects on the settlement response of abalone *Haliotis asinina* larvae of 48 hours old. The highest percentage (73.5%) of attachment was found in larvae reared in seawater containing  $10^{-6}$  M GABA. In addition, five benthic diatom species (*Navicula* sp. 1, *Navicula* sp. 2, *Navicula* sp. 3, *Nitzschia* sp. 1, and *Nitzschia* sp. 2) were isolated and maintained in culture. The species were grown in small bowls and tested in settlement experiments with *H. asinina* larvae. Settlement was very high in 2-day-old larvae fed five species of diatoms (89.8–94.3%). Survival rates declined when the larvae were older (6–7 days). The highest percentages of metamorphosis and shell growth were found in larvae fed *Navicula* sp. 1, *Nitzschia* sp. 2, and *Navicula* sp. 1. A flow-through system in large fiberglass tanks was developed to compare growth and survival of postlarvae reared on diatoms, *Navicula* sp. 1, *Navicula* sp. 2, and *Nitzschia* sp. 1 for 120 days. The shell length (SL) and weight (W) of postlarvae were measured once every 2 weeks. The best growth rate was obtained with postlarvae fed *Nitzschia* sp. 1 (SL 81.7  $\mu\text{m/day}$ , W 96.7  $\mu\text{g/day}$ ) and *Navicula* sp. 2 (SL 75.0  $\mu\text{m/day}$ , W 78.3  $\mu\text{g/day}$ ).

### INTRODUCTION

A critical stage in the life history of abalone larvae occurs during the termination of the planktonic stage. The transformation from larva to juvenile involves two distinct processes: settlement and metamorphosis (Crisp, 1974; Chia, 1978; Hadfield, 1984). Settlement has been described as a behavioral change typically characterized by the searching for, and orientation to, certain environmental factors, such as sediments, algae, conspecifics, and prey species (Crisp, 1974; Hadfield, 1984). Metamorphosis is a non-reversible process that involves anatomical and physiological changes in larvae yielding juvenile abalone (Bonar, 1976). Abalone larvae require highly specific cues to stimulate metamorphosis, and heavy mortalities are commonly reported during this period (Hooker & Morse, 1985; Searcy-Bernal et al., 1992a); usually less than 1% will complete metamorphosis and eventually survive (Morse, 1984). Abalone larvae settle and metamorphose in response to various substances including intact crustose coralline algae (CCA), extracts from CCA (Morse et al., 1980; Roberts & Nicholson, 1997) gamma-

aminobutyric acid (GABA) (Morse, 1984; Searcy-Bernal et al., 1992a; Morse, 1992; Yang & Wu, 1995; Roberts & Nicholson, 1997; Searcy-Bernal & Anguiano-Beltran, 1998; Moss, 1999), excess potassium ions (Yool et al., 1986; Yang & Wu, 1995; Roberts & Nicholson, 1997), cultures of benthic diatoms (Kawamura & Kikuchi, 1992; Roberts & Nicholson, 1997), and mucus (Seki & Kan-no, 1981; Slattery, 1992). Some variations in the settlement response of abalone larvae have been documented, such as GABA-induced attachment, but not metamorphosis in certain species of abalone. GABA is used in the commercial production to induce larval settlement in other species of *Haliotis*. To the best of our knowledge, there has been no study on the settlement of larvae of *Haliotis asinina* Linnaeus, 1758, a tropical abalone of potential economic importance in Thailand. Hence, the aim of this study was to determine the response of *H. asinina* larvae to GABA and various species of benthic diatoms.

Even though culture of abalone has been very successful in most parts of the world, the research on the feeding habits of abalone in the early life stages has been rather limited (Kawamura, 1996; Kawamura et al., 1998a) and control of the initial food is still one of the most critical problems in hatchery seed production (Hahn 1989a; Seki, 1997). Survival rates in many abalone hatch-

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eries are low and variable, especially in the first few months (Searcy-Bernal et al., 1992a). Benthic diatoms have traditionally been used as initial foods for postlarvae in abalone hatcheries until individuals are 7–8 mm, then switching to macroalgae (Seki, 1980; Ebert & Houk, 1984; Hahn, 1989a; Singhagraiwan & Doi, 1993). There are several species of benthic diatoms that occur naturally which have been used as food for juveniles such as *Nitzschia*, *Navicula*, *Amphora*, and *Cocconeis*. Recently, the dietary value of benthic diatoms for the growth of postlarval abalone has been investigated in detail (Kawamura & Takami, 1995; Kawamura et al., 1995). The results of these studies indicate that benthic diatoms growing on CCA play important roles as food sources for postlarval abalone both in the natural environment and in hatcheries (Kawamura et al., 1995; Kawamura, 1996; Takami et al., 1997a). In the present study, survival and growth rates of postlarval abalone *H. asinina* reared on different species of benthic diatoms were compared in order to determine the most suitable diatom species for postlarval growth.

## MATERIALS AND METHODS

Experimental studies were conducted at the Coastal Aquaculture Development Center, Prachuap Khiri Khan Province, Thailand. Abalone *H. asinina* were spawned by the ultraviolet (UV) irradiated seawater technique (Kikuchi & Uki, 1974). The ova were fertilized, washed and raised to the trochophore stage and hatched out within 5 hours at 29–30°C in UV-treated filtered seawater (Singhagraiwan & Doi, 1993). The healthiest trochophores, as evidenced by their active swimming up to the surface of the water, were collected by siphon. They were incubated in nursing tanks with filtered seawater at the density of 10,000/L. The larvae metamorphosed to the veliger stage in the nursing tanks and acquired creeping ability within 24 hours. The creeping larval stage lasted for 1–3 days, during which time the larvae alternately crept and suspended, searching for a settlement place. The larvae in this experiment were 2 days old (44–48 hours).

### Experiment 1. Larval Settlement

**1.1 GABA Experiment.** In the GABA treatment, 100 competent 2-day-old *H. asinina* larvae were put into a small glass bowl (600 mL in volume) which contained various concentrations of GABA:  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$ , and  $10^{-3}$  M. Larvae put in seawater without GABA served as a control. Three replicates were performed for each treatment. Microscopic evaluation of larval attachment was performed 48 hours after the introduction. Larvae were not fed with diatoms and no aeration was provided. The GABA concentration which yielded the highest percentage of attachment was used in the diatom experiment.

**1.2 Diatom Experiment.** *Haliotis asinina* larvae were induced to settle using five species of benthic diatoms of various sizes: *Navicula* sp. 1 ( $38 \times 3 \mu\text{m}$ ) (length  $\times$

width), *Navicula* sp. 2 ( $13 \times 8 \mu\text{m}$ ), *Navicula* sp. 3 ( $18 \times 3 \mu\text{m}$ ), *Nitzschia* sp. 1 ( $75 \times 8 \mu\text{m}$ ), and *Nitzschia* sp. 2 ( $38 \times 12 \mu\text{m}$ ). These pure strains of benthic diatoms were isolated from seawater and cultured in the Guillard media (Guillard, 1975). Only *Nitzschia* sp. 1 was obtained from the culture at the Coastal Aquaculture Development Center; it was the diatom species used to feed abalone larvae.

For each treatment, 100 competent 2-day-old abalone larvae were introduced into a small glass bowl (600 mL in volume) which contained 200 mL filtered and UV-sterilized seawater with  $10^{-6}$  M GABA. Five milliliters of culture medium containing each species of diatom were dropped into the glass bowl. Larvae put in seawater with  $10^{-6}$  M GABA served as a control. Three replicates were performed for each species of diatom. The containers were kept under fluorescent illumination and no aeration was provided. Temperature ranges were 25–28°C. Seawater was changed 2 days after the introduction and daily up to 6 days. Larval settlement was quantified by inspecting the floor and walls of the container with a dissecting microscope every 2 days. We calculated the percentage of live larvae showing (a) shell growth (velum shed, peristomal shell visible), (b) metamorphosis (velum shed), (c) attachment (attached by foot, velum present) (Roberts & Nicholson, 1997). At the end of the experiment (6 days), all larvae were killed and counted.

### Experiment 2. Postlarval Growth

The purpose of this experiment was to study the survival and growth rate of postlarval abalone *H. asinina* reared on different species of diatom which gave a high percentage of settlement in experiment 1.2. These were *Navicula* sp. 1 and *Navicula* sp. 2. *Nitzschia* sp. 1 served as a control. Approximately 60,000 abalone 7–8 day old larvae were put in a fiberglass tank (50 L in volume) which contained different species of benthic diatoms, grown on culture plates ( $30 \times 30$  cm in size) 4–5 days prior to the introduction of larvae. Larvae were reared in the flow-through system at 28–30°C under natural daylight. Three replicates were performed for each treatment. No other food or diatoms were added to the experimental tanks for 1 month. After 1 month, 50 larvae from each replicate were sampled out, and the shell lengths were measured by an optical micrometer under a microscope, and the larval weights were measured using a precision balance. The measurements were taken biweekly for 16 weeks. At the end of 16 weeks, all larvae from each treatment were counted and their survival rates were calculated.

### Statistical Analysis

Differences in percentage of settlement, growth, and survival rates were determined in different treatments by one-way ANOVA. The multiple comparison, Duncan's



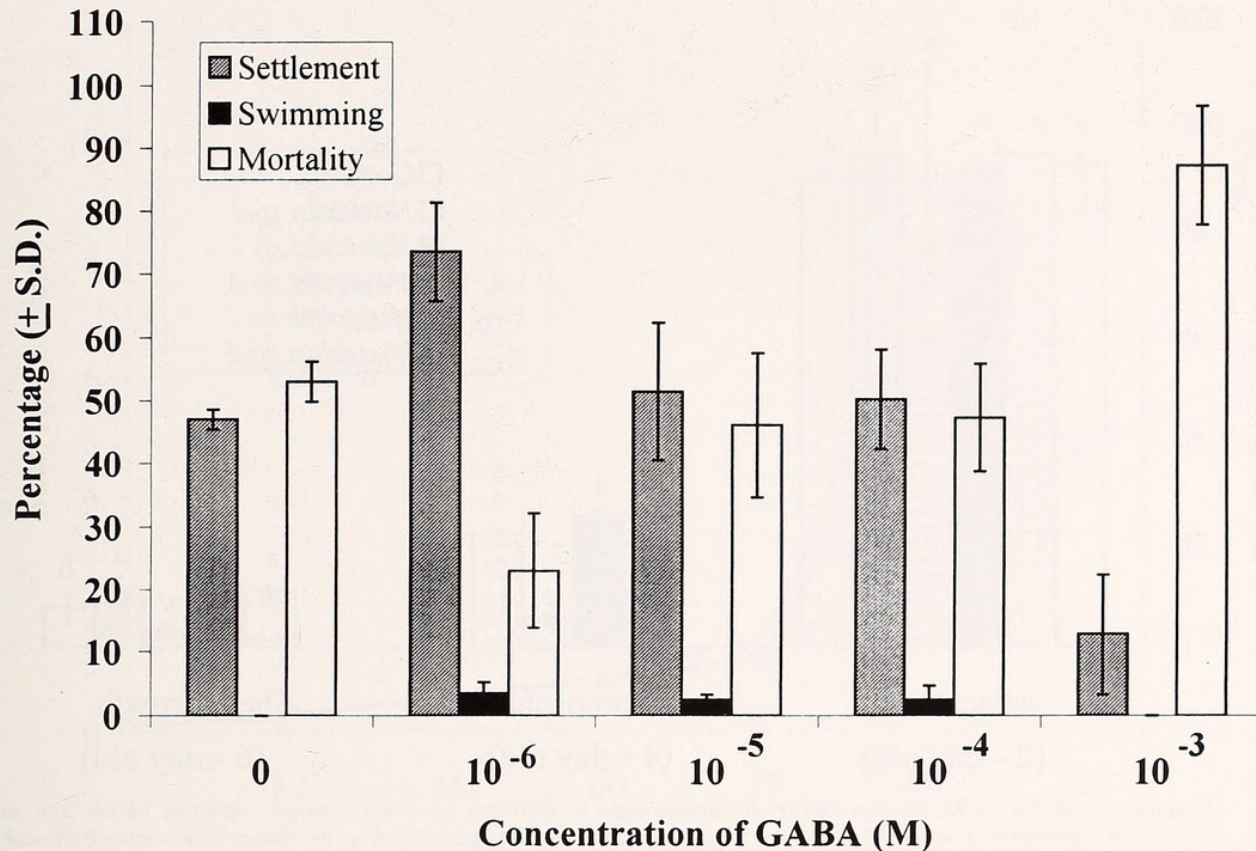


Figure 1. Settlement response of *H. asinina* larvae to different concentrations of GABA after 48 hours of exposure.

multiple range test, was further used to determine significant differences between each treatment. SPSS for Windows, Version 6.0 was the statistical software used for all statistical analysis.  $P < 0.05$  was used as the significance level.

## RESULTS

### 1. Larval Settlement

**1.1 GABA.** Figure 1 shows the induction of settlement of *H. asinina* larvae after 48 hours of exposure to different concentrations of GABA. The percentage of attachment was highest (73.5%) in *H. asinina* larvae reared in seawater containing  $10^{-6}$  M of GABA. There was no difference in attachment percentage between  $10^{-5}$  M and  $10^{-4}$  M GABA treatments ( $P > 0.05$ ) (51.4 and 50.2%, respectively). The lowest attachment percentage (12.8%) and highest mortality (87.2%) were found in the  $10^{-3}$  M GABA treatment.

**1.2 Diatoms.** Figure 2 compares settlement responses of abalone larvae *H. asinina* to five species of benthic diatoms. Rapid attachment was induced by  $10^{-6}$  M GABA and various species of diatoms tested. The percentages of attachment were not significantly different ( $P > 0.05$ ) among the species of diatoms tested and the control. Almost all abalone larvae were attached to the bot-

tom or walls of the glass bowls after 2 days. However, the percentages of metamorphosis were significantly different ( $P > 0.05$ ) among treatment groups. Abalone larvae in the control (without diatoms) all died 3 days after induction. There was no significant difference in metamorphosis percentage among larvae exposed to *Navicula* sp. 1 (20%), *Navicula* sp. 2 (25.5%), and *Nitzschia* sp. 1 (21.8%) ( $P > 0.05$ ) while those exposed to *Nitzschia* sp. 2 (10.2%) showed a significantly lower metamorphosis percentage ( $P < 0.05$ ). Larvae exposed to *Navicula* sp. 3 had the lowest percentage of metamorphosis (5.6%). Similar results were obtained in shell growth response. There was no significant difference in percentage shell growth among larvae fed *Navicula* sp. 1 (11%), *Navicula* sp. 2 (13.5%), and *Nitzschia* sp. 1 (12%;  $P > 0.05$ ). However, both the *Nitzschia* sp. 2 (7.8% shell growth) and *Navicula* sp. 3 (3.3%) groups showed a significantly lower percentage of shell growth ( $P < 0.05$ ).

### 2. Postlarval Growth

Figure 3 shows growth in shell length of *H. asinina* postlarvae reared on different species of diatoms for 120 days. The postlarvae grew well on *Nitzschia* sp. 1 (control) during the experimental period (81.7  $\mu\text{m}/\text{day}$ ) and reached 9.9 mm in shell length at the end of 120 days (Figure 3, Table 1). *Navicula* sp. 2 yielded a moderately



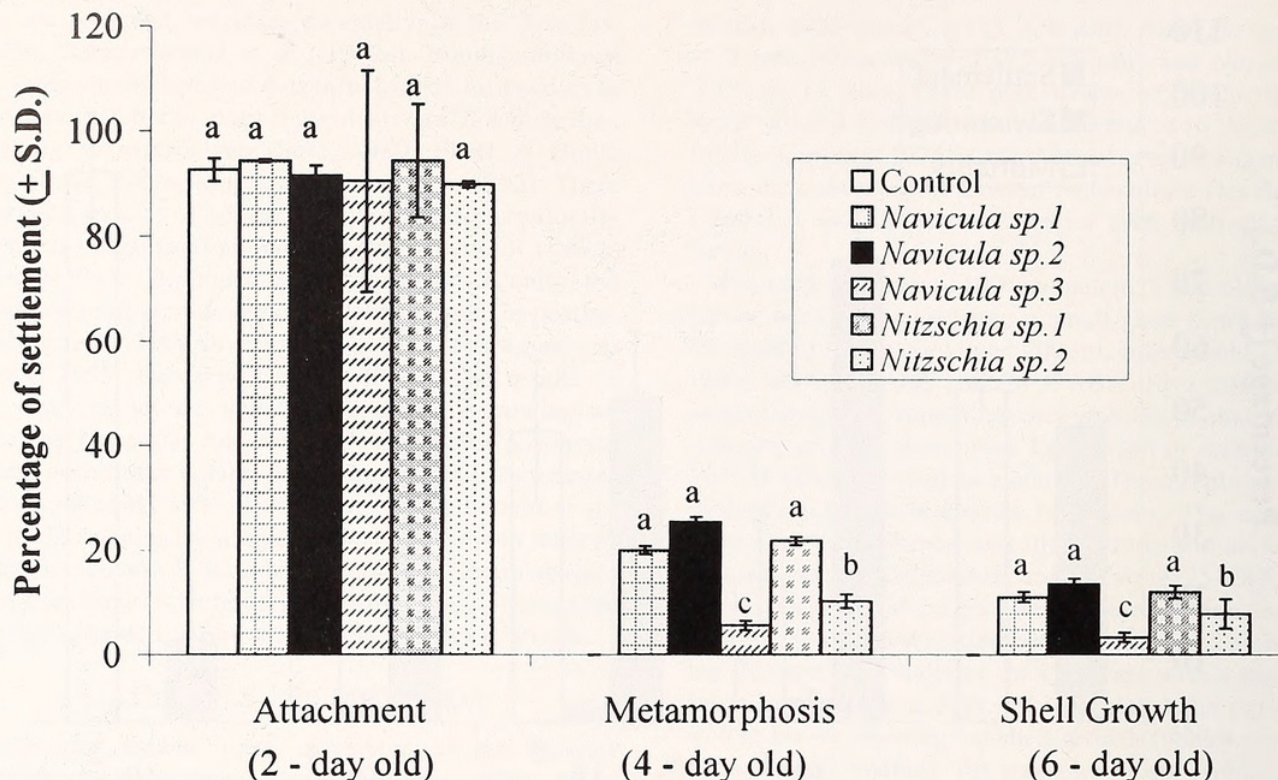


Figure 2. Settlement response of *H. asinina* larvae of various ages to different species of benthic diatoms. Means and standard deviations are presented. Analysis of variance of Duncan's multiple range test was performed on the means of percentage of settlement; the same letter identifies the values that are not significantly different ( $P > 0.05$ ).

good growth with  $78.3 \mu\text{m/day}$ , and the postlarvae reached  $9.1$  in shell length after 120 days (Figure 3, Table 1). In contrast, the mean growth rate of postlarvae reared on *Navicula* sp. 1 ( $64.2 \mu\text{m/day}$ ) was significantly lower ( $P < 0.05$ ) than those reared on *Nitzschia* sp. 1 and *Navicula* sp. 2. They attained the shell length of only  $6.0$  mm after 120 days (Figure 3, Table 1).

Figure 4 shows growth in weight of *H. asinina* postlarvae reared on different species of diatoms for 120 days. The highest growth in weight was found in postlarvae reared on control, *Nitzschia* sp. 1 ( $96.7 \mu\text{g/day}$ ) which was significantly different ( $P < 0.05$ ) from those reared on *Navicula* sp. 2 ( $78.3 \mu\text{g/day}$ ) and *Navicula* sp. 1 ( $64.2 \mu\text{g/day}$ ) (Table 1). They also reached a heavier weight ( $12.8$  mg) compared to those reared on *Navicula* sp. 2 ( $10.5$  mg) and *Navicula* sp. 4 ( $8.9$  mg) (Figure 4, Table 1).

The survival rate of *H. asinina* was not observed until the end of the experiment (120 days) because these postlarvae were so fragile, and they attached very tightly to the diatom plates. The greatest survival rate was found in postlarvae reared on *Navicula* sp. 1 ( $75.8\%$ ) which was significantly higher ( $P < 0.05$ ) than those reared on *Navicula* sp. 2 ( $55.8\%$ ) and *Nitzschia* sp. 1 ( $20.6\%$ ) (Table 1).

## DISCUSSION

### Larval Settlement

Abalone larvae require highly specific cues to stimulate metamorphosis, and heavy mortalities are commonly reported during this period (Hooker & Morse, 1985; Searcy-Bernal et al., 1992a). Three general methods, diatom film, mucus, and GABA have been used for inducing abalone metamorphosis in commercial-scale operations (Hooker & Morse, 1985; Hahn, 1989b). The diatom-film method was originally developed in Japan and is also practiced in Taiwan, USA, New Zealand, Mexico (Hahn, 1989b), and Thailand (Singhagraiwan & Doi, 1993). The mucus method, also developed by the Japanese (Seki & Kan-no, 1981), is currently used in Japan, Korea, and Australia. The GABA method was proposed after the discovery in the USA that certain coralline algae provide the natural cues for metamorphosis in haliotids and that GABA, an inexpensive amino acid related to the natural inducer molecule, also elicits rapid, synchronous, and complete metamorphosis in small-scale laboratory experiments with antibiotics and excellent environmental quality (Morse et al., 1979).

The present study demonstrates that GABA at  $10^{-6}$  M or  $1 \mu\text{M}$  induced the highest percentage of attachment ( $76.5\%$ ) over the other concentrations ( $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$



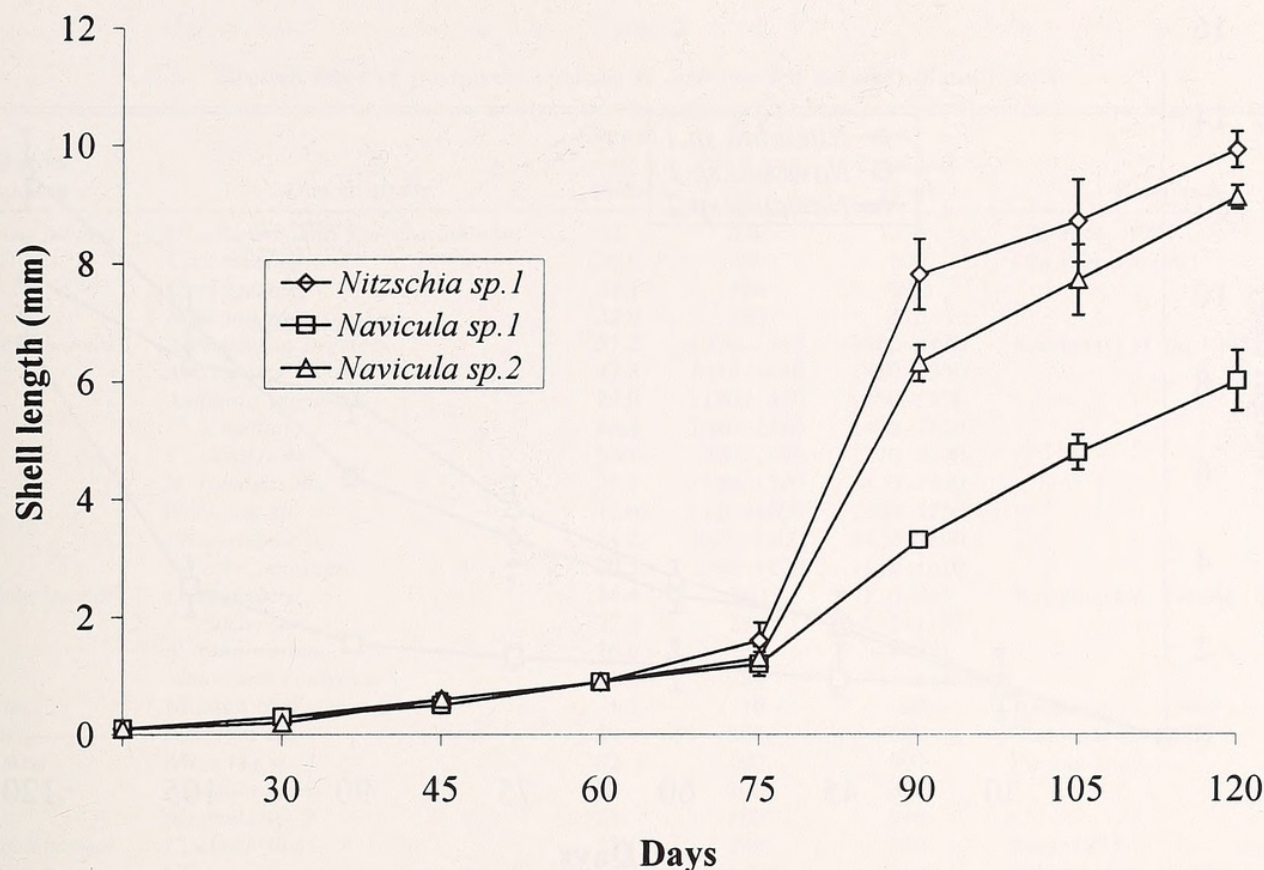


Figure 3. Growth in shell length of *H. asinina* larvae fed different species of diatoms up to 120 days after settlement.

M). High mortality occurred in the highest concentration of GABA ( $10^{-3}$  M). However, GABA could not induce metamorphosis in *H. asinina* larvae because all the larvae died after 3 days of induction. GABA's performance varies among abalone species, and in some species, including *H. asinina*, it triggers attachment without metamorphosis (Roberts, 2001; present study). The attachment response with GABA has been reported by many workers, such as Morse et al. (1979) and Slattery (1992) in *H. rufescens* Swainson, 1822, and Akashige et al. (1981) in *H. discus hannai* Ino, 1953. However, Morse (1990) proved that GABA could induce metamorphosis in *H.*

*discus hannai*. Furthermore, Roberts & Nicholson (1997) reported different settlement responses in *H. iris* Gmelin, 1791, and *H. virginea* Gmelin, 1791, larvae. In *H. iris*, 1  $\mu$ m GABA induced 90–100% attachment and 20–60% metamorphosis within 2 days while in *H. virginea*, GABA induced 65–100% attachment and 0.5% metamorphosis.

On the other hand, in *H. rufescens*, Searcy-Bernal et al. (1992b) and Searcy-Bernal & Anguiano-Beltran (1998) obtained very good settlement from GABA in conjunction with diatoms over conventional diatom film and mucus. However, they suggested that high postlarval

Table 1

Growth and survival rates of *H. asinina* postlarvae fed different species of diatoms for 120 days. Means and standard deviations are presented. Analysis of variance of Duncan's multiple range test were performed on the means of shell length and body weight increases; the same letter identifies the values that are not significantly different ( $P > 0.05$ ).

Diatom	Survival rate			Shell length (mm)		Body wet weight (mg)		Growth	
	Initial	Final	%	Initial	Final	Initial	Final	Length ( $\mu$ m/day)	Weight ( $\mu$ g/day)
<i>Nitzschia</i> sp. 1	80,000	16,500	20.6 <sup>c</sup>	0.1 $\pm$ 0	9.9 $\pm$ 3.3 <sup>a</sup>	1.2 $\pm$ 5.8	12.8 $\pm$ 9.1 <sup>a</sup>	81.7 $\pm$ 6.6 <sup>a</sup>	96.7 $\pm$ 3.6 <sup>a</sup>
<i>Navicula</i> sp. 1	80,000	60,600	75.8 <sup>a</sup>	0.1 $\pm$ 0.1	6.0 $\pm$ 4.7 <sup>b</sup>	1.2 $\pm$ 4.6	8.9 $\pm$ 11.7 <sup>c</sup>	49.2 $\pm$ 7.8 <sup>c</sup>	64.2 $\pm$ 6.8 <sup>c</sup>
<i>Navicula</i> sp. 2	80,000	44,700	55.8 <sup>b</sup>	0.1 $\pm$ 0	9.1 $\pm$ 3.3 <sup>a</sup>	1.1 $\pm$ 3.2	10.5 $\pm$ 8.2 <sup>b</sup>	75.0 $\pm$ 8.8 <sup>b</sup>	78.3 $\pm$ 5.7 <sup>b</sup>



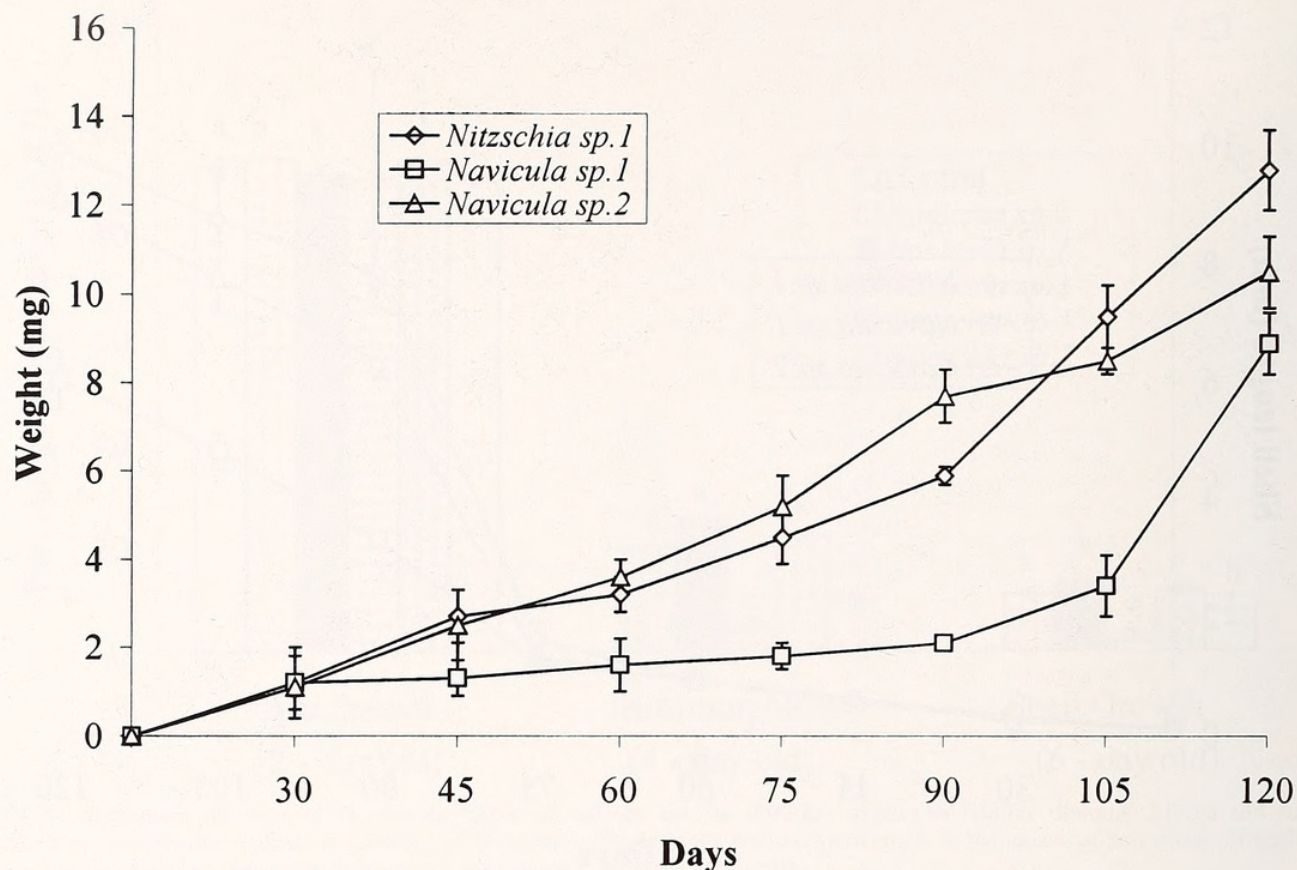


Figure 4. Growth in weight of *H. asinina* larvae fed different species of diatoms up to 120 days after settlement.

mortality may occur afterward (Searcy-Bernal et al., 1992b). This can be avoided by using antibiotics, which yield excellent survival (93%) 1 month after GABA induction (Morse, 1984). Searcy-Bernal et al. (1992b) suggested that the postlarvae died because of the bacterial growth in the culture stimulated by utilization of the amino acid in the GABA itself.

GABA arrests swimming activity so that the larvae sink, then attach where they land on non-vertical substrates (Roberts, 2001). Increasing concentrations of GABA increase the number of larvae settling and decrease the time of settlement (Hahn, 1989b), for example,  $10^{-3}$  M GABA causes 10% of the larvae to settle within 7 minutes and  $10^{-6}$  M GABA induces settlement in only 26% after 38 hours (Morse et al., 1980). The minimum concentration causing detectable settlement is  $10^{-7}$  M GABA (Hahn, 1989b). However, prolonged exposure to higher concentrations is toxic (Morse et al., 1979). At concentrations higher than  $10^{-6}$  M, settlement and attachment are induced but metamorphosis is inhibited and the juveniles eventually die (Morse, 1984).

Benthic diatom films growing on plastic plates have traditionally been used as settlement substrata in abalone hatcheries worldwide (Seki, 1980; Ebert & Houk, 1984; Hahn, 1989b; Singhagrainwan & Doi, 1993). In the present study, five species of diatoms (*Navicula* sp. 1-3, *Nitzschia*

sp. 1-2) were used to induce settlement in *H. asinina* larvae. The result showed no significant difference in percentage of attachment in the diatoms and GABA until 2 days after induction. The highest percentages of metamorphosis were found in *Navicula* sp. 1, sp. 2, and *Nitzschia* sp. 1 treatments. Kawamura & Kikuchi (1992) tested the settlement of *H. discus hannai* larvae on film of 18 species of diatoms grown at different densities, and found marked differences both in the time of completion of settlement and settlement rate. Larvae generally responded better to films with higher diatom density. The diatoms which resulted in high settlement success were species which formed flat, prostrate communities, while most species forming three-dimensional colonies resulted in low settlement of larvae (Kawamura & Kikuchi, 1992). This could possibly account for the different settlement percentages in *H. asinina*.

In addition, preferential settlement of different abalone larvae in the presence of various diatom species was found in *H. rubra* Leach, 1814, and *H. laevigata* Donovan, 1808 (Daume et al., 1999). *Haliotis rubra* did not respond to films of any diatom species tested, but settled on the natural settlement surface, a non-geniculate coralline red algae (NCA), *Phymatolithon repandum*. *Haliotis laevigata*, however, settled particularly well on films of *Navicula ramosissima* and the NCA *Sporolithon du-*



Table 2  
Growth rates of postlarval abalone *H. asinina* fed unialgal diatom diets.

Abalone species	Diatom strain	Growth rate ( $\mu\text{m/day}$ )	Initial size ( $\mu\text{m}$ )	Final size ( $\mu\text{m}$ )	Reference
<i>H. discus hannai</i>	<i>Ulvelia lens</i> and <i>Synedra tabulata</i>	32	330	1350	Uki et al., 1981
	<i>Cocconeis</i> sp.	30.6	280	800	Ohgai et al., 1991
	<i>C. closterium</i>	24.1	280	690	
	<i>Navicula ramosissima</i>	22.9	280	670	
<i>H. discus hannai</i>	<i>Actinocyclus brevipes</i>	57.2	1230–1540	1960–2470	Kawamura et al., 1995
	<i>A. longipes</i>	47.8	1420–1540	1860–2360	
	<i>Amphora augusta</i>	29.0	1120–1420	1450–1770	
	<i>C. scutellum</i>	46.4	1050–1310	1460–1950	
	<i>C. closterium</i>	50.1	920–1300	1270–2190	
	<i>N. ramosissima</i>	21.1	1120–1590	1470–1790	
	<i>Nitzschia</i> sp.	13.6	1120–1600	1260–1750	
	<i>Pleurosigma</i> sp.	24.2	1330–1600	1420–2100	
	<i>Synedra investiens</i>	20.5	980–1270	1140–1610	
	<i>C. scutellum</i>	14.4	280	510–865	
	<i>C. closterium</i>	27.2	280	735–1177	
<i>H. discus hannai</i>	<i>N. ramosissima</i>	16.6	280	548–841	Kawamura & Takami, 1995
	<i>Stauroneis constricta</i>	21.6	280	724–865	
	<i>H. midae</i>	1.7	10	40	
	Mixture of diatoms	1.7	10	40	
<i>H. rubra</i>	<i>Navicula</i> sp.	39	800	3000	Knauer et al., 1996
<i>H. asinina</i>	<i>Nitzschia</i> sp. 1	82	100	990	Daume et al., 2000
	<i>Navicula</i> sp. 1	49	100	600	
	<i>Navicula</i> sp. 2	75	100	910	
<i>H. discus hannai</i>	<i>C. closterium</i>	27	280	820	Seki, 1997
<i>H. discus hannai</i>	CCA + diatoms ( <i>Amphora</i> spp. + <i>Cylindrotheca closterium</i> )	53.4	500	2000	Takami et al., 1997b
	Diatoms only	24.4	500	1000	
<i>H. gigantea</i>	<i>A. tenuissimus</i>	12.8	321	552	Ishida et al., 1995
	<i>Cocconeis</i> sp.	32.6	340	927	
	<i>Melosira moniliformis</i>	12.8	352	518	
	<i>N. ramosissima</i>	28.0	333	836	
<i>H. iris</i>	<i>A. longipes</i> 1	33.7	570	1245	Kawamura et al., 1998a
	<i>A. longipes</i> 2	11.3	549	788	
	<i>C. pseudomarginata</i>	11.8	570	830	
	<i>Navicula britannica</i>	16.4	555	896	
	<i>N. ramosissima</i>	17.0	606	938	
	<i>Navicula</i> sp.	15.1	603	902	
	<i>Nitzschia ovalis</i>	15.4	568	886	
	<i>Nitzschia</i> sp.	35.3	553	1280	

rum. Hence, settlement of abalone larvae in response to diatom films depends on the abalone species, the diatom species tested, and the density of the diatoms on the plate (Daume et al., 1999).

#### Postlarval Growth

Three major transitions in postlarval feeding are recognized (Kawamura et al., 1998a). The first is the transition from lecithotrophy to particle feeding. There appears to be overlap between lecithotrophy and early ingestion. A second transition is evident at around 600–800  $\mu\text{m}$  shell length (SL). Postlarvae below this size grow at similar rates regardless of the diatom strain to which they

are exposed. In contrast, larger postlarvae grow much more rapidly on certain diets, such as highly digestible diatoms. The final transition is from a diatom diet to a macroalgae diet (5–10 mm SL).

The results of the present study on postlarval growth (100  $\mu\text{m}$  SL) reared on different unialgal diatom strains showed significantly different growth rates. The highest growth rate (81.7  $\mu\text{m/day}$ ) was found in postlarvae reared on the control (*Nitzschia* sp. 1). *Navicula* sp. 2 yielded a moderate growth rate (75.0  $\mu\text{m/day}$ ) and *Navicula* sp. 1 showed the lowest growth rate (49.2  $\mu\text{m/day}$ ). Only a few studies have examined the growth of abalone postlarvae (~300–1500  $\mu\text{m}$  SL) reared on unialgal diatom strains



(Table 2). In comparison, *H. asinina* postlarvae reared on *Nitzschia* and *Navicula* species seemed to have higher growth rates than those of other abalone species (Table 2). *Nitzschia* spp. and *Navicula* spp. are the usual diatom species given to postlarvae for food (Ebert & Houk, 1984; Hooker & Morse, 1985; Hahn, 1989a). The difference in growth rates of *H. asinina* and other *Haliotis* postlarvae could be due to the fact that *H. asinina* is a tropical species which usually grows in water temperatures 25–28°C, whereas the other *Haliotis* (Table 2) are temperate species which have the optimum temperature around 15°C for growth. Normally, the growth of tropical abalone is faster than that of temperate species. *Haliotis asinina* becomes sexually mature when it is 7–8 months old while it usually takes 2–3 years for temperate abalone to reach maturity (Hahn, 1989a; Singhagrain & Doi, 1993). In addition, diatoms multiply faster in warmer temperatures and the density of diatoms is another factor that contributes to the higher growth rates. Uki et al. (1981) found that the postlarval growth rate of *H. discus hannai* fed certain benthic diatoms under various temperatures generally increased with higher temperature up to an optimal temperature (22.5°C for postlarvae of *H. discus hannai*). Growth rates at temperatures above this optimum declined.

Several possible causes can be inferred for the different growth rates of *H. asinina* postlarvae reared on benthic diatoms, such as digestibility, and biochemical composition of diatoms (Kawamura et al., 1998b). Digestibility refers to the proportion of diatom cells that lose cell contents when ingested and pass through the postlarval gut. Diatom cell size and growth form can limit digestibility by precluding ingestion. Two strains of *Actinocyclus longipes* with different cell sizes and stalk lengths produced different growth and survival rates of *H. iris* postlarvae (Kawamura et al., 1998a). The attachment strength of the diatom strain is another important factor. Very tightly attached diatoms such as *Cocconeis* spp. and *Achnanthes* sp. (Kawamura et al., 1995, 1998a), require considerable force to be detached from substrata and are usually ruptured if dislodged. In contrast, many diatom strains with low adhesive strength are ingested without cell rupture, and the majority of ingested cells pass through the gut alive and unbroken (Kawamura et al., 1995, 1998b). The biochemical composition of diatom cells may affect growth rates (Kawamura et al., 1998b). Fatty acid profiles are a critical component in aquaculture feeds and are known to vary among diatom species.

Survival rate is another important factor that has to be considered in aquaculture of abalone. Even though the *Nitzschia* sp. 1 treatment showed the highest growth rate in shell length, the survival rate was quite low (20.6%). On the other hand, the *Navicula* sp. 1 treatment which had the lowest growth rate, showed the highest survival rate (75.8%). This may be explained in term of density of abalone. Postlarvae with high survival rates, and there-

fore high densities, competed for food more strongly and exhibited lower growth rates than did those with lower survival rates.

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