Feeding, growth, and survival of post-larval abalone *Haliotis asinina* on different benthic diatoms

Emmanuel C. Capinpin Jr.
Marine Science Institute, College of Science
University of the Philippines
1101 Diliman, Quezon City
Pangasinan State University
Binmaley Campus, 2417 Binmaley, Pangasinan
Tel: (075) 543-3012
e-mail: manny_capinpin@yahoo.com
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ABSTRACT

The feeding behavior, digestive efficiency, growth, and survival of post-larval abalone *Haliotis asinina* fed with 5 species of locally isolated benthic diatom strains (*Navicula mollis*, *N. ramosissima*, *Stauroneis* sp., *Pleurosigma* sp., and *Cocconeis* sp.) were examined in the laboratory. Two 15-day feeding trials using 1 mm post-larvae were conducted. No significant differences were observed in sizes of post-larval abalone after 15 days in all diatom treatments ($P>0.05$). However, in both trials, *Cocconeis* sp. resulted in high survival rates (88.9±5.6% and 80.0±20.0% for Trials 1 and 2, respectively). *Cocconeis* sp. was efficiently digested by post-larval abalone, with most of the cells being ruptured during ingestion and/or passage through the gut. One diatom strain, *Pleurosigma* sp., resulted to a high survival but produced the slowest growth rate (<10 ìm.d^{-1} SL). It was probably not ingested easily during the experiment due to its large size or mobility. For the other diatom strains, *N. mollis* and *N. ramosissima*, most cells passed through the gut with the cells left intact. *Stauroneis* sp. is highly digestible, but did not result to high survival, although the remaining live post-larval abalone fed on this diatom as well as on *N. mollis* grew faster during the second week of both feeding trials. *N. ramosissima* resulted to poorest survival rate (<10%) due to its poor digestibility. Only *Cocconeis* sp. showed a fairly high growth rate, digestion efficiency, and survival rate. *N. mollis* which gave a fairly high survival rate and *Stauroneis* may be added towards the later stages of post-larval rearing as well as other large diatoms. The digestion efficiency of diatom strains is considered an important factor determining its dietary value, but other factors may also be important such as volume contents, biochemical composition, and other physical characteristics.

Key words: Digestive efficiency; Growth; Survival; Post-larval abalone; *Haliotis asinina*; Diatoms

**Corresponding author*

INTRODUCTION

Benthic diatom films are traditionally used to induce settlement and for use as initial food in abalone hatcheries (Hahn, 1989). A method commonly used is to establish a visible or dense film of diatoms to trigger settlement and to provide food for post-larvae. Older films (i.e. higher density) of some diatom strains are considered better in inducing settlement (Kawamura & Kikuchi, 1992; Moss & Tong, 1992; Daume et al., 1999) and for feeding (Searcy-Bernal et al., 2004; Day et al., 2004). However, without control over the type and density of diatoms, fast-growing naviculoids become dominant, which may or may not be good for settlement and consequently, feeding of post-larvae. These films develop rapidly in a short time, becoming dense and often peeling as sheets. They may interfere with settlement and feeding and may cause strong fluctuations in water quality in the diffusive boundary layer (Searcy-Bernal, 1996) such as oxygen supersaturation at high diatom densities when light is available and the opposite during dark conditions (Searcy-Bernal et al., 2004), which could explain the rapid decline in survival a few weeks after settlement. Also, the species combination on the settlement plate is unpredictable and varies with season which could lead to inconsistent and variable settlement rates and growth of post-larval abalone (Daume et al., 2000).

Several studies have examined post-larval feeding and growth using different diatom species (Kawamura et al., 1995, 1998a; Roberts et al., 1999a; Carbajal-Miranda et al., 2005; Gordon et al., 2006). Kawamura et al. (1998b) showed that post-larvae 0.8-2 mm in shell length grew faster on 'digestible' diatoms than on 'indigestible' ones. In addition, diatom cell size, attachment strength, frustule strength, condition of algal culture, aside from post-larval size can influence diatom digestibility (Kawamura et al., 1995, 1998b). Also the control of food supply is often considered an important factor in affecting growth and survival during the post-larval period as they increase feeding rate and as they grow (Roberts et al., 1999a; Martínez-Ponce & Searcy-Bernal, 1998). In fact, grazing and growth rates of older post-larvae increase linearly with diatom density (Searcy-Bernal et al., 2001) and under continuous dark conditions (Gorrostieta-Hurtado & Searcy-Bernal, 2004).

It is the aim of this study to isolate different local diatom strains and test their suitability as food source for post-larval *H. asinina*. By isolating and culturing benthic diatoms, there is a much better chance of achieving consistent larval settlement and good post-larval growth and survival. The diatoms to be used should practically be easy to culture and have fast growth in order to be a good candidate food species for the fast-growing post-larval abalone.

MATERIALS AND METHODS

Algal cultures

Several diatom species with prostrate growth forms were isolated from the acrylic settlement plates at the Integrated Mollusk and Echinoderm Hatchery at the Bolinao Marine Laboratory (BML) in Bolinao, Pangasinan. Kawamura & Kikuchi (1992) suggest that prostrate diatoms induce higher settlement success than three-dimensional diatom communities. Also, young post-larvae are known to actively select small, prostrate species as food source (Norman-Boudreau et al., 1986; Matthews & Cook, 1995; Siqueiros-Beltrones & Voltolina, 2000). Hence, efforts were geared towards isolation and culture of diatom strains forming flat communities.

Diatoms were isolated by picking single cells with a microcapillary using an inverted optical microscope as described by Brand (1990). Isolated cultures were grown in plastic culture plates (Costar, 12 wells) using modified Jørgensen's medium (Jørgensen, 1962), supplemented with 0.05 ìg.l^{-1} Vitamin B_{12} and maintained under a light intensity of 150 ìE.m^{-1}.s^{-1} at 12:12 L:D cycle in an air-conditioned room. The diatoms were grown and re-isolated for a number of times to make sure that a monospecific culture was attained.

Five isolated diatom strains were chosen to be used for the early feeding experiments (*Navicula mollis*, *N. ramosissima*, *Stauroneis* sp., *Pleurosigma* sp. and *Cocconeis* sp.). The diatom cultures were not axenic. The cell dimensions and density of diatoms used for the feeding experiments are shown in Table 1. The diatom density was counted using a square grid inserted into the eyepiece of the inverted microscope.

Diatom species	Cell length (µm, Mean±SE)	Cell width	Initial density cells.cm^{-2}, Mean±SE) Trial 1	Trial 2	Growth form[a] Type	Adhesive strength
Navicula mollis	36.45±0.70	7.18±0.17	(1.70±0.28) X10^4	(4.92±0.68) X10^4	A	+
Stauroneis sp.	21.36±0.45	5.36±0.17	(2.78±0.00) X10^5	(2.34±0.39) X10^5	A	+
Navicula ramosissima	18.63±0.59	7.00±0.23	(1.18±0.35) X10^5	(2.21±0.24) X10^5	A	+
Pleurosigma sp.	77.72±1.19	19.00±0.49	(4.07±1.36) X10^3	(1.63±0.09) X10^4	A	+
Cocconeis sp.	14.54±0.32	6.64±0.18	(2.37±0.50) X10^5	(3.46±0.04) X10^5	B	+++

[a]Growth forms based on classification by Kawamura (1996). They are classified into 7 types (A-G), based on mode of attachment, whether solitary or colonial, and by their motility and adhesive strengths.

Table 1. Mean cell sizes (length and width), initial density, and growth forms of 5 benthic diatom species used in post-larval feeding trials

Preservation and identification of diatoms

Light microscopy and scanning electron microscopy were used for the examination of whole cells to identify the diatom species (Round et al., 1990). The diatoms were scraped, washed, centrifuged, acid-cleaned (Hasle, 1978), fixed with 3% glutaraldehyde, washed with 0.1 M Phosphate buffer, post-fixed with 1% osmium tetroxide, buffer washed again, dehydrated in ascending alcohol series, stub mounted and coated with gold, and viewed up to 2,000 X using a scanning electron microscope (SEM Hitachi S-510).

Abalone post-larvae

Abalone larvae were obtained from controlled natural spawning and artificial fertilization at the BML hatchery. About 38-39 h after fertilization, larvae with creeping ability were placed in prepared settlement tanks with naturally occurring diatoms (Capinpin & Hosoya, 1995). After about a month, several post-larval abalone were dislodged from the settlement plates using fine needles. The needles were used to dislodge them at the posterior end of the shell. Only healthy and undamaged animals were used for the feeding trials.

Feeding Trials

Two feeding trials were carried out using Petri dishes (90-mm diameter) containing 30 ml filtered seawater (FSW).

In trial 1, six post-larval abalone (1,132.0±11.2 ìm SL) per dish were reared on each species of diatom at about 26-27°C under a light intensity of 120-150 ìE.m^{-2}.s^{-1} with a 12 h L:D photoperiod. Three replicates were made.

In trial 2, five slightly larger post-larvae (1,392.1±12.7 ìm SL) per dish were used. Two replicates were made under the same culture conditions.

In both trials, the culture medium for the diatoms in the dishes was changed with 0.2 ìm FSW prior to addition of the post-larval abalone. The feeding behavior of the post-larvae was observed and shell length measured individually using a micrometer inserted in the eyepiece of the optical microscope. Shell lengths were measured every 3 days over a period of 15 days. At the time of observations, the seawater in each of the dishes was also changed. The diatoms in all the dishes were abundant throughout the experiment. No antibiotics were used during the feeding experiments.

The daily growth rates were calculated for each individual for the whole period of 15 d and between 1-9 d and 10-15 days (Tables 3 and 4). Only post-larvae that are alive were used to calculate the mean daily growth rates. Post-larval survival was also observed throughout the experimental period.

Digestion efficiency

Digestion efficiency refers to the ability of post-larvae to digest diatoms which may result from disruption of cell wall during grazing or passage through the gut (Kawamura, 1996). To measure the digestion efficiency of post-larval abalone fed with the five diatom species, five abalone (1,300 ìm SL) post-larvae were reared on each diatom species in a Petri dish under the same

culture conditions. Before adding the post-larvae, the number of whole, live cells and broken ones in the Petri dishes were counted using the inverted optical microscope. After 3 days of sufficient feeding, the post-larvae were transferred to individual disposable plastic Petri dishes filled with 0.2 ìm FSW without diatoms. After 1-2 h, recently released feces excreted by the post-larval abalone in the individual Petri dishes were picked up using a microcapillary pipette, transferred onto a glass slide, covered with a cover slip, and the number of whole, live cells and that of broken ones counted under the light microscope. The digestive efficiency (Kawamura et al., 1995) was calculated using the formula below:

Digestion efficiency (%) = $(1-L/L_o)$ X 100
where L is the mean proportion of whole live cells to the total diatom cells in the fecal pellet and L_o is the mean proportion of whole live cells to the total diatom cells at the bottom surface of the Petri dish before grazing.

Data analysis

Data were analyzed using single classification Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. Data on survival rates were arcsine transformed prior to analysis (Gomez & Gomez, 1984). Some data on *N. ramosissima* were excluded from statistical analysis due to low survival.

RESULTS

Post-larval size

All abalone post-larvae reared on each diatom species showed active feeding behavior at the start of the experiments as evidenced by their grazing activity and by the ginger color of their digestive glands. The ingestion of diatom cells was confirmed through direct observations of feeding using the inverted optical microscope. In both trials 1 and 2, no significant differences in sizes (shell lengths) of post-larval abalone were observed in all diatom treatments (P>0.05) until day 15, except for *N. ramosissima* which was excluded in statistical analysis because of poor survival (Figure 1).

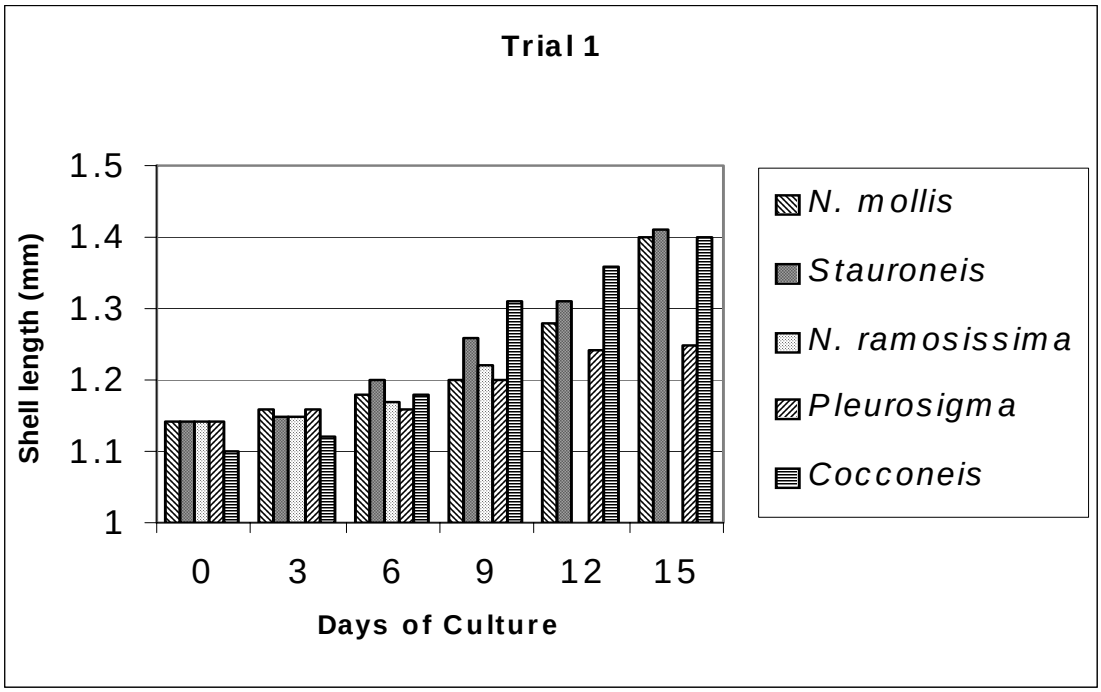
Post-larval survival

In terms of survival of post-larval abalone, not all the diatoms offered resulted in high survival rates (Tables 3 and 4, Figure 2). In trial 1, significant differences were observed in survival rates beginning day 9, with both *Cocconeis* sp. and *N. mollis* giving the highest survival rates of 100%, followed by *Pleurosigma* sp. (88.89%), *Stauroneis* sp. (72.22%), and *N. ramosissima* (27.78%). At the end of the culture trial (day 15), highest survival rates were consistently obtained from *Cocconeis* sp. (88.89%) followed by *Navicula mollis* (66.67%) and *Pleurosigma* (66.67%), with no significant differences among the three species (P>0.05). Significantly low survival was attained from *Stauroneis* sp. (33.33%) and *N. ramosissima* (5.56%). In trial 2, closely similar results were observed with the highest survival rates on *Cocconeis* sp. and *Pleurosigma* sp. (both 80%) followed by *N. mollis* (40%) at the end of the 15-day feeding trial (P<0.05; Figure 2, Table 4). Significantly low survival was attained on *N. ramosissima* (0%) on days 12-15.

Daily growth rates

In trial 1, no significant differences in daily growth rates of post-larval abalone were observed on all diatoms *N. mollis*, *Stauroneis* sp., *Cocconeis* sp. and *Pleurosigma* sp. during the entire culture period, except *N. ramosissima* which was excluded in statistical analysis due to lack of data (i.e., poor survival). However, significantly faster growth rates were observed on *N. mollis* and *Stauroneis* sp. during the second week than the first week (P<0.05).

In trial 2, no significant differences in DGRs were observed on *H. asinina* post-larvae fed *Cocconeis* sp. (26.20 ìm.d^{-1}), *N. mollis* (22.95 ìm.d^{-1}), and *Stauroneis* sp. (21.30 ìm.d^{-1}) during the entire culture period (P>0.05). However, the daily growth rates observed on the 3 diatom strains were significantly different from *Pleurosigma* sp. (9.05 ìm.d^{-1}). Significantly faster growth rates were also observed on *N. mollis* and *Stauroneis* sp. during the second week than the first week (P<0.05).



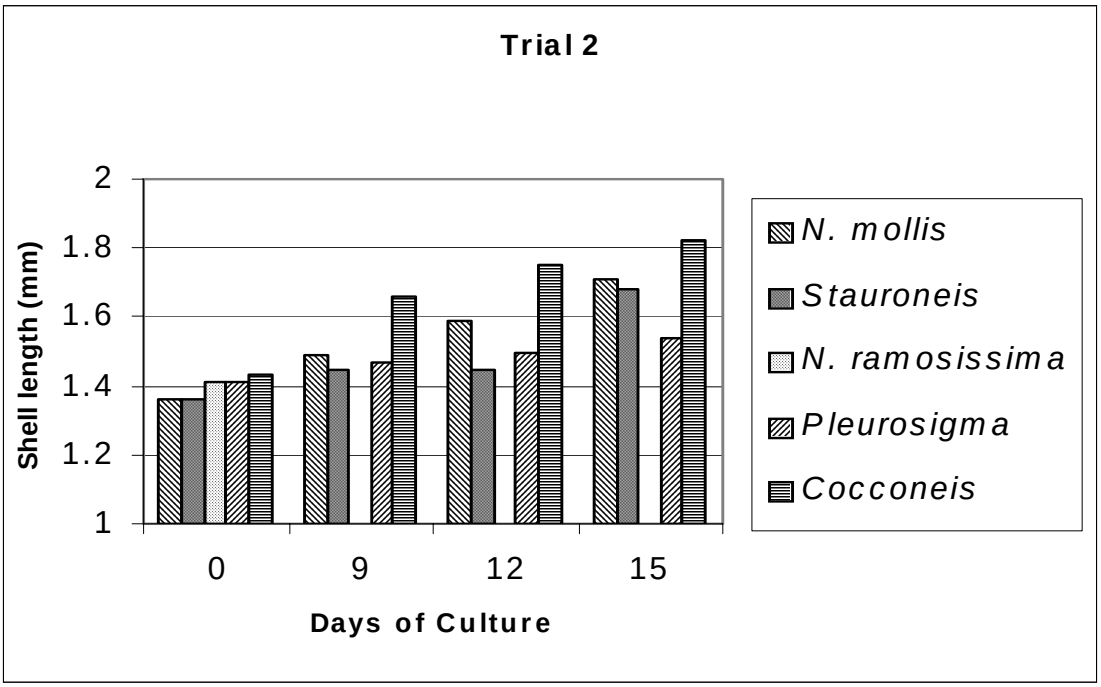


Figure 1. Shell lengths of post-larval abalone H. asinina cultured using different diatom strains for 15 days.

Digestion efficiency

In terms of digestion efficiency, the most digestible species were *Cocconeis* (97.4%) and *Stauroneis* (100%) with no significant differences between the two species (P>0.05, Table 2), followed by *Pleurosigma* (47.11%), *N. mollis* (28.65%), and *N. ramosissima* (7.81%).

The digestion efficiencies of post-larval abalone fed on *Stauroneis* sp. and *Cocconeis* sp. were high compared to other diatoms (Table 2). However, of the 2 species, only *Cocconeis* sp. supported high survival throughout the culture trials (Figure 2, Tables 3 and 4). *Stauroneis* sp. resulted to a high digestion efficiency, which was also confirmed by SEM when most cells were destroyed during scraping and acid cleaning, but it did not necessarily supported a high survival rate. Although it supported good growth rates during the second week, these growth data were taken only from few surviving ones.

Diatom species	Percentage intact cells (%, Mean±SE)		Digestion efficiency*
	Before grazing (*Lo*)	In the fecal material (*L*)	
N. mollis	100.00±0.00	71.35±3.60	28.66±3.60^{c}
Stauroneis sp.	100.00±0.00	0.00±0.00	100.00±0.00^{a}
N. ramosissima	100.00±0.00	92.19±1.62	7.81±1.62^{d}
Pleurosigma sp.	94.63±2.47	52.93±5.46	44.35±4.80^{b}
Cocconeis sp.	100.00±0.00	2.60±1.26	97.40±1.26^{a}

*Digestion efficiency (%)=(1-*L*/*Lo*)X100

Table 2. Digestibility (digestive efficiency) of 5 benthic diatom species used in post-larval feeding trials

The formation of first respiratory pore (notch stage) was observed at a shell length of 1.70-1.80 mm, whereas the closed respiratory pore (juvenile stage) was observed at 1.85-1.90 mm. At the end of trial 2, most of the animals reared on *Cocconeis* sp. were in the notch stage or have closed respiratory pores, whereas only a few reared on *N. mollis* and *Pleurosigma* sp. are in the notch stage. Post-larval *H. asinina* fed on *Cocconeis* sp. were the largest, although no significant differences were observed in size compared to other diatom treatments probably because of the short observation period (i.e., 15 days). Highest survival was also attained using *Cocconeis* and *Pleurosigma* as compared to the other treatments.

DISCUSSION

A method commonly used in abalone hatcheries is to establish a visible (=dense) film of diatoms to trigger settlement and provide food for post-larvae. Without control over the type of diatoms, fast growing naviculoids are usually dominant. These may or may not be good for settlement and may or may not produce good post-larval growth and survival. These films develop rapidly, becoming very dense and dark, and often peeling as sheets. These cause strong fluctuations in boundary layer water quality (Searcy-Bernal, 1996; Gorrostieta-Hurtado & Searcy-Bernal, 2004), and this could explain the sudden mortality episodes that hatcheries often experience 2-8 weeks after settlement.

Post-larval abalone begins feeding soon after metamorphosis and eats small diatoms cells. However, most of the diatoms that post-larvae eat are not ruptured during feeding, and will pass through the gut alive. Some strains of diatoms are readily ruptured while others are not. Young post-larvae grow at similar rates on digestible and indigestible diatoms, but older post-larvae (>0.6-0.8 mm shell length) grow much more rapidly when fed breakable diatoms. *Stauroneis* sp. is easily broken because they have very weak cell walls and appears to be a good candidate species for early feeding of abalone. However, this species is very fast-growing, usually becoming dense in a few days, and often observed to be peeling as sheets. This could cause large fluctuations in boundary layer water quality (e.g. dissolved oxygen), which may not be beneficial for young post-larvae. Because of their fast growth, they are observed to be growing on the shells of post-larvae. They may also interfere with proper settlement and metamorphosis as the larvae become entangled (personal observations) and as mentioned by Gorrostieta-Hurtado & Searcy-Bernal (2004). Hence, they are best added during the later stages of post-larval period. Indeed, ungrazed diatom plates induce

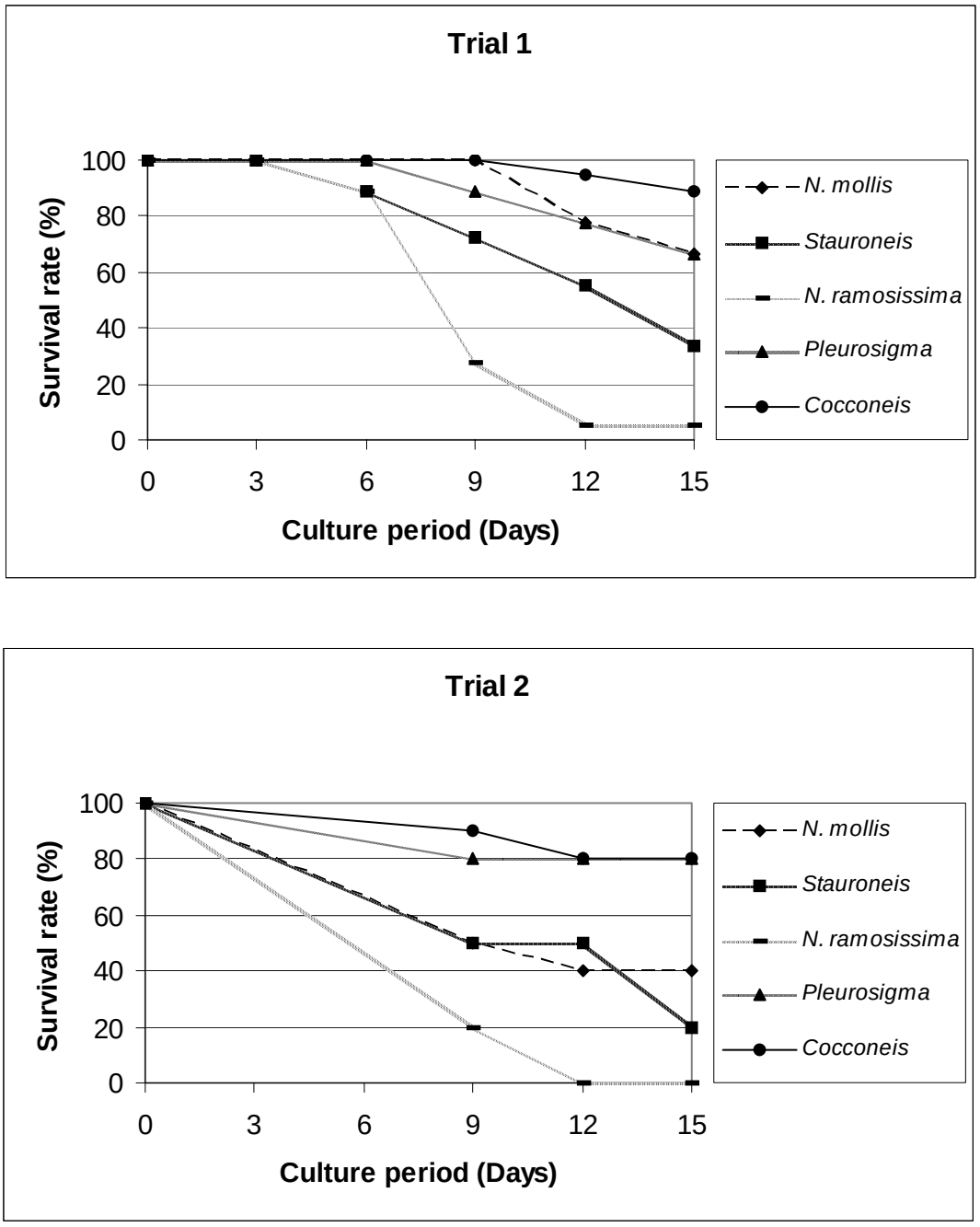


Figure 2. Survival rates of post-larval abalone H. asinina fed various diatom strains for 15 days.

lower attachment than pre-grazed diatom plates (Gallardo & Buen, 2003).

Cocconeis sp. has high adhesive strength and cells are easily broken during ingestion. On the other hand, all the other diatoms are mobile with low adhesive strengths and easily ingested by post-larval abalone without destruction of siliceous frustules, with exception of *Stauroneis* sp. Kawamura et al. (1995; 1998a) suggested that it is important for larger post-larvae (similar size to that used in the present study) to split open the cell walls by action of radula during ingestion or passage through gut to enable them to utilize the contents. As shown in the present study, *Cocconeis* sp. a highly digestible diatom resulted to good growth and high survival rates especially in actively feeding post-larvae, but there may be exceptions (e.g. *Stauroneis* sp.). These results are in conformity with earlier studies on temperate abalone (Kawamura et al., 1995, 1998a).

The results of this study verified that the physical characteristics of a diatom species, whether it is ruptured or not during grazing, is one of the most important factors determining its dietary value for growth of 1-2 mm post-larval abalone. Various other factors may also influence the nutritional value of diatoms. These include the nature and quantity of their extracellular substances and secretions, the health (stage) of diatom culture, associated microbial flora (Kawamura et al., 1998b) and biochemical composition (Brown & Jeffrey, 1995; Carbajal-Miranda et al., 2005; Gordon et al., 2006).

Significantly faster growth rates during the second week for *N. mollis* and *Stauroneis* sp. could be due to their increased capability to digest diatoms as they grow.

The digestion efficiency of *Stauroneis* sp. was high, although it had relatively low attachment strength, which could be due to structurally weak silica frustules, similar to that observed by Kawamura et al. (1995) for *Cylindrotheca closterium*. The others, *N. mollis* and *N. ramosissima*, passed through the gut intact. Both were easily ingested due to low adhesive strengths. The low digestive efficiencies of these diatoms as expected resulted to slow growth rates particularly during the first week.

Roberts et al. (1999b) and Onitsuka et al. (2004) found several progressive changes in the radula as post-larval abalone grow. Small post-larvae (*H. iris*, <1 mm SL and *H. diversicolor aquatilis*, <2 mm SL) had highly curved teeth and low clearance angles, meaning that their teeth function as "scoops" which slide across the surface capable of collecting small diatoms and other fine loose particles such as extracellular mucus of diatoms. On the other hand, larger post-larvae had higher clearance angles, which are more suitable for grazing substrata to efficiently remove strongly attached diatoms. Post-larvae also develop digestive enzymes as they grow (Takami et al., 1998).

Cocconeis sp. is a very tightly attached, prostrate species. They become probably energetically inadequate as post-larvae grow as shown by slowing of growth during the second week (Tables 3 and 4). The relatively higher settlement rate (unpublished data) and food value of this diatom make it a favorable candidate in the initial stages of abalone culture. Three dimensional diatom communities, larger species, as well as fast-growing diatoms (e.g. *Stauroneis* sp.) provide a much higher biomass per unit area than low volume prostrate cells. Hence, they are also needed as they grow and best added during the later stages of post-larval rearing. A combination of selected species may also be beneficial (Gordon et al., 2006).

The ability to maintain a suitable quantity of food is also important in abalone hatcheries. Ingestion rates increase as they grow (Martínez-Ponce & Searcy-Bernal, 1998; Roberts et al., 1999a; Searcy-Bernal et al., 2001), so rapid clearing of diatom films is common. Post-larvae are also observed to feed less and grow slower at lower diatom densities (Day et al., 2004). It is interesting to note that post-larvae are relatively tolerant of starvation, but should not be starved for period longer than a week (Roberts et al., 2000).

It is very important to provide constant supply of appropriate diatoms during various stages of post-larval rearing. This issue is important for hatchery management in order to plan activities such as the type of diatoms to be used for settlement, type of food suitable at a particular stage, and the time at which it should be added. The control of diatoms used for settlement and early feeding in abalone hatcheries

Diatom strain	Shell length (µm, Mean±SE)		Daily growth rate (µm.day-1, Mean±SE)			Survival rate (%)		
	Initial	Final	Whole period (15 days)	Week 1 (Day 1-9)	Week 2 (Day 10-15)	Day 3	Day 9	Day 15
N. mollis	1,143.6±29.4	1,403.2±15.5	17.3±3.0[a]	6.3±0.7[a]	33.8±8.0[a]	100.0±0.0[a]	100.0± 0.0[a]	66.7± 9.6[a, b]
Stauroneis sp.	1,136.0±19.0	1,408.3±38.6	18.2±3.7[a]	13.8±1.9[a]	24.7±7.6[a, b]	100.0±0.0[a]	72.2± 5.6[c]	33.3± 9.6[b, c]
N. ramosissima	1,143.2±17.8	1,317.1*	10.1*	9.0±4.8	7.7*	100.0±0.0[a]	27.8± 5.6[d]	5.6± 5.6[c]
Pleurosigma sp.	1,139.7±28.7	1,249.1±14.9	7.3±2.8[a]	6.4±2.2[a]	8.6±6.1[b]	100.0±0.0[a]	88.9± 5.6[b]	66.7±16.7[a, b]
Cocconeis sp.	1,097.5±35.2	1,401.8±63.5	20.3±6.6[a]	23.2±9.5[a]	16.0±2.2[a, b]	100.0±0.0[a]	100.0± 0.0[a]	88.9± 5.6[a]

Trial 1 = 3 replicates
Means with the same superscript are not significantly different from each other (P>0.05)
*For 1 replicate only

Table 3. Shell length, daily growth rate, and survival rate of post-larval H. asinina fed on 5 diatom strains (Trial 1)

Diatom strain	Shell length (µm, Mean±SE)		Daily growth rate (µm.day-1, Mean±SE)			Survival rate (%)		
	Initial	Final	Whole period (15 days)	Week 1 (Day 1-9)	Week 2 (Day 10-15)	Day 3	Day 9	Day 15
N. mollis	1,363.2±53.7	1,707.22±26.1	22.9±1.8[a]	14.6±1.7[b]	35.4±2.1[a]	50.0±30.0[a]	40.0±20.0[a, b]	40.0±20.0[a, b]
Stauroneis sp.	1,358.2± 7.2	1,677.5±61.3	21.3±4.6[a]	10.0±3.5[b]	38.3±6.2[a]	50.0±10.0[a]	50.0±10.0[a, b]	20.0± 0.0[b]
N. ramosissima	1,405.9± 0.9			8.4*		20.0±20.0[a]	0.0±0.0[b]	0.0± 0.0[b]
Pleurosigma sp.	1,405.2±27.2	1,540.9±85.0	9.0±3.9[b]	7.2±3.4[b]	11.9±4.5[b]	80.0±20.0[a]	80.0±20.0[a]	80.0±20.0a
Cocconeis sp.	1,427.7± 2.5	1,820.8±66.2	26.2±4.6a	25.6±2.0[a]	27.1±8.5[a, b]	90.0±10.0[a]	80.0±20.0[a]	80.0±20.0[a]

Trial 2 = 2 replicates
Means with the same superscript are not significantly different from each other (P>0.05)
*For 1 replicate only

Table 4. Shell length, daily growth rate, and survival rate of post-larval H. asinina fed on 5 diatom strains (Trial 2)

should improve juvenile production. The use of diatoms as early food source for abalone still remains as the most viable method, although a new system utilizing artificial diet bound with agar/alginate solution to the plastic plates have shown promising results (Stott et al., 2003, 2004).

Cocconeis is easy to culture and has very fast growth rates. It should be provided during the initial stages of the culture period particularly during settlement and early feeding. As post-larval abalone grows, the addition of other diatom species (*Stauroneis* sp., *N. mollis* and other larger diatoms) should be applied in order to support fast growth rates.

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