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Settlement of the tropical abalone *Haliotis asinina* on different diatoms

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Abstract
The settlement of abalone *Haliotis asinina* larvae on 5 species of locally isolated diatom strains (*Navicula mollis, Stauroneis* sp., *N. ramosissima, Pleurosigma* sp., and *Cocconeis* sp.) were examined in the laboratory. Attachment and metamorphosis of *H. asinina* were 2 distinctly different responses. Attachment was observed as the larval foot becomes firmly attached to the surface of the substratum. In the experiment, high attachment on all treatments, including control, was observed at 6 h after start of the experiment (about 45 h after fertilization) at 26.5-27.5 °C. Larvae were observed to detach and swim off the substratum for over 3 d after beginning of the experiment. The metamorphosis was non-reversible and could occur in larvae at 4.6 d after fertilization. All the diatom films stimulated attachment behavior but only a few induced low levels of normal metamorphosis over several days. Completion of metamorphosis as evidenced by shell growth was observed at low levels (4-16%) 5 days after experiment began (6 d after fertilization) only on *Cocconeis* sp., *N. mollis*, and *Pleurosigma* sp.

Keywords: Diatoms, Early feeding, *Haliotis asinina*, Post-larvae, Settlement, Tropical abalone

1. Introduction
The transition from a swimming abalone larva to a crawling, feeding post-larva collectively termed "settlement" is considered one of the most critical stages in abalone hatcheries which may be divided into 2 distinct stages: attachment and metamorphosis [1, 2]. During attachment, the larvae stop swimming, sink to the bottom, and attach to the substratum by their foot. It is a behavioral change and reversible because the velum has not yet been shed and can resume swimming. This stage is attained in *H. asinina* at 26-30 h after fertilization and may last 2-3 d [3, 4]. On the other hand, metamorphosis is a non-reversible process, which consists of 2 sub-stages: initiation and completion of metamorphosis. Initiation of metamorphosis is indicated by the loss of velum. If the post-larva continues to develop normally after initiation of metamorphosis, then feeding commences and peristomal shell growth occurs, indicating completion of metamorphosis. Settlement often does not progress rapidly through attachment, metamorphosis, and shell growth [1]. The onset and completion of metamorphosis in *H. asinina* is unknown and previously not differentiated carefully with attachment. Larval attachment is not a useful end point for an abalone farmer so it is important to distinguish between stages of settlement [1].
Benthic diatom films are traditionally used to induce settlement in abalone hatcheries [5]. A method commonly used is to establish a visible or dense film of diatoms to trigger settlement and provide food for post-larvae. Older films (higher density) of some diatom strains are considered better in inducing settlement [6-8]. However, without control over the type and density of diatoms, fast-growing naviculoids become dominant, which may or may not be good for settlement. These films develop rapidly in a short time, becoming dense and often peeling as sheets. They may interfere with settlement and cause strong fluctuations in water quality in the diffusive boundary layer [9], which could explain the precipitous decline in survival a few weeks after settlement. Also, the species combination on the settlement plate is unpredictable and varies among plates which could lead to inconsistent and variable settlement rates [10].
Kawamura and Kikuchi (1992) [6] suggested that diatom species good for settlement are those that form prostrate communities, especially adhesive prostrate (Type B) species, although not all are favorable for settlement.

Different abalone species settle at varying rates in response to different diatom films ranging from 0-100% [6, 8, 10-12]. These results indicate that more experiments are needed to establish which diatom species induce consistent and high settlement for each abalone species [8]. Culture protocols for *H. asinina* are available [13, 14], but refinements are needed specifically on improving settlement and post-larval survival [15].

It is the aim of this study to isolate different local diatom strains and test their suitability as settlement substrate 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 have fast growth in order to be a good candidate food species for fast-growing post-larval abalone.

2. Materials and methods

2.1 Algal Cultures

Several diatom species with prostrate growth forms were isolated from acrylic settlement plates at the integrated mollusk and echinoderm hatchery at the Bolinao Marine Laboratory (BML) in Bolinao, Pangasinan. It was suggested that prostrate diatoms induce higher settlement success than three-dimensional diatom communities [6]. Also, young post-larvae are known to actively select small, prostrate species as a food source [16-18]. Hence, efforts were geared towards isolation of diatom strains forming flat communities.

Diatoms were isolated by picking single cells with a microcapillary using an inverted optical microscope [19]. Stock cultures were cultured using modified Jørgensen's medium [20] supplemented with 0.05 µg/L Vitamin B_{12} and maintained under a light intensity of 150 µE/m^2/sec at 12:12 LD cycle in an air-conditioned room.

Five isolated diatom strains (*Navicula mollis, Stauroneis* sp., *N. ramosissima, Pleurosigma* sp., and *Cocconeis* sp.) were used for settlement and early feeding experiments. The diatom cultures were not axenic.

2.2 Preservation and Identification of Diatoms

Light microcopy and scanning electron microscopy were used for examination of whole cells to identify the diatom species [21]. The diatoms were scraped, washed, centrifuged, acid-cleaned [22], fixed with 3% glutaraldehyde, washed with 0.1 M Phosphate buffer, post-fixed with 1% osmium tetroxide, buffer washed again, dehydrated in ascending ethanol series, stub mounted and coated with gold, and viewed up to 2,000 X using a scanning electron microscope (SEM Hitachi S-510).

2.3 Abalone larvae

Abalone larvae were obtained from eggs and sperm collected from natural spawning at the hatchery. About 38-39 h after fertilization, larvae with creeping ability were used for the experiments. Only larvae from the same batch were used in each experiment. Two separate batches were used for the 2 settlement experiments.

2.4 Settlement Experiments

Five monocultured species of benthic diatoms were used for settlement experiments (Table 1). The diatoms were cultured in 12-well polystyrene tissue culture plates (Corning Cat. No. 3512). The cell dimensions, density, and growth forms of each diatom species on the bottom surface of the wells at the beginning of the experiments are shown in Table 1. The diatoms were inoculated in the wells about 1-3 days before the start of the settlement experiments. Two separate settlement experiments were conducted.

In both experiments, five larvae (219.53±5.54 µm SL)) per well were reared on each species of diatom at 120-150 µE/m^2/sec with a 12h L:D photoperiod. Both experiments were conducted with 5 replicates each. Each well contained 3.5 ml filtered seawater (FSW). The culture medium for the diatoms in the wells was changed with 0.2 µm (25 mm Acrodisc Syringe Filter, Gelman) FSW prior to the start of each experiment. Observation periods of attachment and metamorphosis were done after 6 h, 1 d, 2 d, 3 d, 4 d, 5 d, and 7 d using an inverted microscope at 5X magnification.

The seawater in the wells was changed daily by removing 2 ml using a micropipette and replacing it with FSW, being careful not to remove animals. No antibiotics were used during the settlement experiments.

Attachment, metamorphosis, and shell growth was quantified by inspecting the bottom and sides of the dishes with the inverted optical microscope.

Table 1: Mean cell size (length and width), initial density, and growth form of 5 benthic diatom species used in settlement experiments

Diatom Species	Cell Length (µm, Mean±SE)	Cell Width (µm, Mean±SE)	Initial Density (cells/cm^2, Mean±SE)	
			Trial 1	Trial 2
Navicula mollis	26.54±0.45	7.36±0.22	(1.09±0.34) X10^5	(4.98±0.86) X10^4
Stauroneis sp.	19.18±2.18	5.64±0.11	(2.76±0.29) X10^5	(5.31±0.22) X10^4
Navicula ramosissima	16.82±0.48	7.54±0.18	(2.57±0.11) X10^5	(3.83±0.35) X10^4
Pleurosigma sp.	73.72±1.92	15.18±0.64	(1.30±0.17) X10^4	(3.43±0.11) X10^3
Cocconeis sp.	13.64±0.29	6.09±0.11	(3.45±0.24) X10^5	(1.79±0.41) X10^5

Growth forms based on classification by Kawamura and Hirano (1992). They are classified into 7 types (A-G), based on mode of attachment, whether solitary or colonial, and by their motility and adhesive strengths. All diatoms used were Type A species except *Cocconeis* which was a Type B species.

2.5 Data Analysis

Frequency distributions, percentages and arithmetic means were used to present and analyze data. The differences between percentage attachment, metamorphosis, and shell growth among the different treatments were tested using single factor analysis of variance (ANOVA).

3. Results

3.1 Effects of Diatom Films on Attachment and Metamorphosis

Larvae of *H. asinina* at the outdoor hatchery began to display attachment behavior at about 32 h after fertilization as they began to sink and orient toward the substratum.

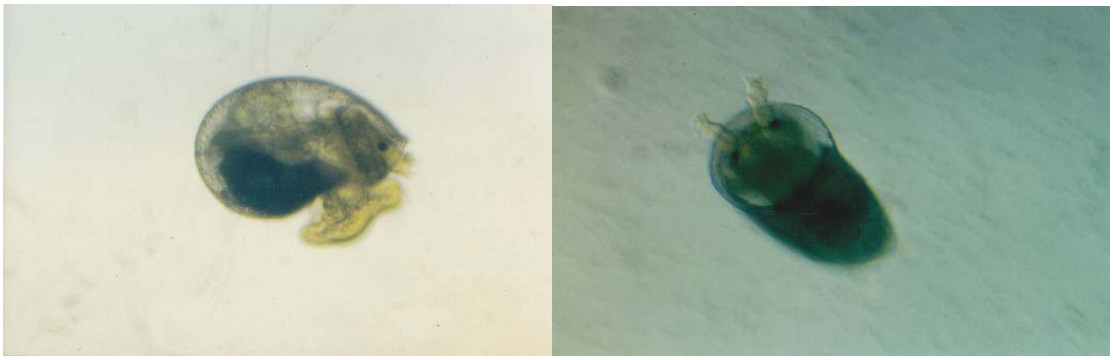

Fig 1: a, Larva at the start of the experiment; b, Larva began to attach and crawl on the bottom and sides, including control dishes 6 h after start of experiment.

At 6 h after the experiment began, almost all (>80%) of these 45 h old larvae began to attach and crawl on the bottom and sides (Figure 1), including control dishes with no significant differences among treatments (P>0.05). However, only a low percentage of these attached larvae survived and went through metamorphosis. Initiation of metamorphosis characterized by the shedding of velum was observed on *Cocconeis* sp., *N. mollis, N. ramosissima, Pleurosigma* sp., and the control treatment after 72 h of the experiment (Figure 2). Shell growth was observed only on the diatoms *N. mollis, Pleurosigma* sp., and *Cocconeis* sp. Only 2 post-larvae (8%) in the control dishes displayed little peristomal shell growth in Trial 1. The highest percentage metamorphosis and shell growth were observed consistently on *Cocconeis* sp. (52.00±10.20% and 60.00±10.95% metamorphosis after 3 d and 12.00±4.90% and 16.00±7.48% shell growth after 5 d in Trials 1 and 2, respectively) in both settlement experiments (Figure 3). These values for *Cocconeis* were significantly different from the other treatments (P<0.05).

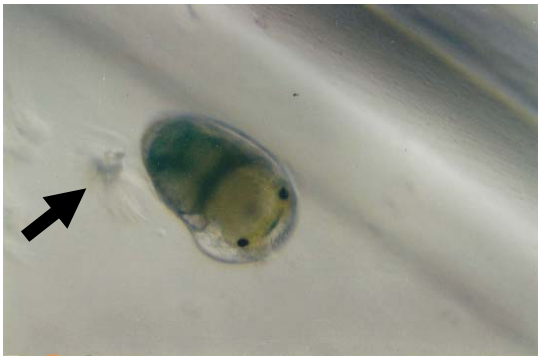

Fig 2: Initiation of metamorphosis characterized by the shedding of swimming cilia (arrow).

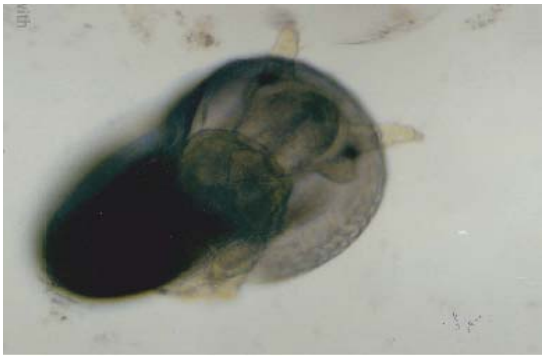

Fig 3: Peristomal shell growth indicating completion of metamorphosis

3.2 Early Post-Larval Feeding

The few surviving ones with obvious peristomal shell growth reared on *Cocconeis* sp. attained an average shell length of 280.6 (n=5) for a daily growth rate of 8.73 µm/d after 7 d (Trial 2). Slightly faster growth rates were attained by the few surviving ones (n=3, Trial 2) with shell growth reared on *N. mollis*, a species with higher amounts of extracellular polysaccharides at 15.64 µm/d after 5 d. On the other hand, only 1 individual (Trial 2) survived in the control, but with no shell growth. Two survivors on *Cocconeis* sp. survived up to 15 d and attained a shell length of 272.70 and 420.41 µm.

The size of mouth after 5 d is about 23 µm. Active feeding movements were observed after 3 d but ingestion was observed 2 d after metamorphosis.

4. Discussion

4.1 Settlement Experiments

High percentage attachment (>80%) was observed in all diatom films including clean dishes at the beginning of the experiments. However, larvae can easily detach and swim actively at this stage. Similar substrate testing behavior has been reported for other abalone species [5, 23]. Similar results were observed for a closely related small abalone *H. diversicolor* that diatoms induce attachment but slow and gradual metamorphosis [24], indicating diatom as a sub-optimal cue for settlement compared to CCA [8, 12, 25]. Slow metamorphosis over several days have also been observed on diatoms in other temperate abalone species [2, 7, 11, 25]. On the other hand, the complete and rapid settlement was induced by CCA for other abalone within a few days [26]. For instance, complete metamorphosis and shell growth was observed on CCA for *H. iris* in 2 d, but over 5 d on other cues such as CCA extracts, GABA, KCl, and some diatom strains [12]. However, there are some diatoms that induce rapid settlement response compared to CCA (e.g., *Nitzschia* on *H. iris* [12] and *Navicula* on *H. laevigata* [8]). In another experiment using 5 isolated diatom strains, settlement of *H. rubra* was still low in all species ranging from 1-6% [10]. Hence, it is believed that settlement of abalone larvae in response to diatom films depends on the abalone species, the diatom species tested, and the density of diatoms and only specific diatom films can be as inductive as the natural settlement substratum [8]. At any rate, positive controls such as CCA should be included in experiments to confirm larval competence and speed of settlement response.

In the present study, high densities of diatoms were used as compared to previous studies [8]. It is likely that too high densities of diatoms have a negative effect on settlement and growth. The settlement was low on three-dimensional diatom

films [6] and heavy mortality of young post-larvae has been observed in abalone hatcheries when diatom films become too dense [11]. The high densities at the start of settlement assays could be a reason for the low settlement rates observed in the present study. There was evidence of physical interference with settlement by some diatom strains. For instance, *Stauroneis* sp. was so motile and dense that attached larvae became smothered and coated with diatoms, and subsequently died without metamorphosing. Some larvae were also observed with their shell entangled with diatom secretions preventing metamorphosis. An excessive biofilm also may cause adverse conditions for post-larvae [9]. Dense diatoms may become unstable causing parts to be lifted off the substrate.

Almost in all cases where diatoms were used compared to other treatments, the combination of a diatom film and foot mucus of a conspecific, was most effective for attachment and metamorphosis [2, 23-25, 27]. At SEAFDEC/AQD, pre-grazed diatom film is the method of choice to increase settlement rates of *H. asinina* in the hatchery (Gallardo of SEAFDEC/AQD, pers. comm.). Grazing of juveniles also increases the settlement of *H. rubra* on a prostrate green alga *Ulvella lens* [10]. In fact, mucus alone can induce *H. discus hannai* to settle and serve as initial food source for post-larvae [27]. Since abalone larvae attach readily on diatom films and even on clean substrates, metamorphosis could be possibly increased by allowing conspecific juveniles to crawl on the settlement plates for several days to coat it with mucus and reduce the density of diatom film which may be harmful to post-larvae after attachment. The chemical basis of settlement induction by trail mucus or pre-grazed diatom films has not been studied.

Another explanation of the advantage of low-density diatom substrate (e.g., pre-grazed mucus) is that it offers a stable substratum for pedal adhesion and further development during metamorphosis. Larvae exhibit characteristic behaviors such as exploration, inspection, and orientation movements prior to metamorphosis. After being cued to metamorphose, a larva orients itself to suitable microhabitat in which mucus is secreted for firm attachment prior to metamorphosis [23].

The interpretation of results should be treated carefully when comparing settlement rates of different batches of larvae because they can vary in larval fitness which can affect survival rates. Larval and post-larval abalone have considerable ability to survive without particulate food because of yolk reserves [28], so any high mortality within a few days of settlement induction is likely to be caused by factors other than starvation. Other authors observed variability in settlement success of different batches of larvae on same diatom strains [10] and during different seasons [2]. Variations can also be caused by differences in rearing conditions (Roberts, pers. comm.).

Diatom films also contain many bacteria and other microbes, as well as a variable amount of extracellular secretions and various other organic substances [29]. All of these do change physically and chemically as the biofilm ages. Hence, it is not surprising that the knowledge of the characteristics of a biofilm responsible for settlement is poorly understood [1].

The present study showed that *Cocconeis* sp. can be used as settlement substrate but a low density or pre-grazing by larger juveniles (not studied here) has the potential to increase rates of metamorphosis.

4.2 Early Post-Larval Feeding

Slow growth rates were observed in the present study compared to other abalone growing at about 20-30 µm/d in the first 10 d after settlement [30]. In the present study, those reared on *Cocconeis* sp. had slower growth rates than the other diatoms probably because of the small amount of extracellular substances of this low-volume diatom and they are probably not ingested and digested efficiently [30]. For smaller post-larva <800 µm SL, the extracellular secretions of diatoms, associated bacterial flora, and pedal mucus of juveniles are considered important food sources. They do not seem to require diatom contents as they cannot digest its contents in this size range [30-31]. It appears that small post-larvae lack the digestive capabilities to fully utilize diatom cell contents even if cell walls were ruptured. Digestive enzyme activity was detected in *H. discus hannai* at 1 mm SL, but not at 0.5 mm SL [32].

Several progressive changes were observed in the post-larval radula as they grow [33]. Post-larvae <1 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. On the other hand, larger post-larvae had higher clearance angles, which enable it to become suitable for collecting large particles and effectively scrape the substrate.

The ability to maintain a suitable quantity of food is also important in abalone hatcheries. Ingestion rates increase exponentially as they grow [34, 35], so rapid clearing of diatom films is common. It is interesting to note that post-larvae are relatively tolerant of starvation, but should not be starved for a period longer than a week [28].

It is very important to provide a 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 and the 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 should improve the juvenile production.

More settlement experiments using low density diatoms must be done in order to provide a more conclusive statement and that a positive control such as various species of crustose coralline algae (CCA), the known natural and preferred substrate, should be included in experiments to confirm larval competence and speed of settlement response.

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6. References

1. Roberts RD. A review of settlement cues for larval abalone (*Haliotis* spp.). J Shellfish Res 2001; 20:571-586.

2. Slattery M. Larval settlement and juvenile survival in the red abalone (*Haliotis rufescens)*: An examination of inductive cues and substrate selection. Aquaculture 1992; 102:143-153.
3. Capinpin EC Jr, Hosoya M. Spawning and larval development of a tropical abalone *Haliotis asinina* (Linné). Philipp J Sci 1995; 124:215-232.
4. Singhagraiwan T, Sasaki M. Breeding and early development of the Donkey's ear abalone, *Haliotis asinina* Linné. Thai Mar Fish Res Bull 1991; 2: 83-94.
5. Hahn KO. Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, Florida, 1989.
6. Kawamura T, Kikuchi S. Effects of benthic diatoms on settlement and metamorphosis of abalone larvae. Suisanzoshoku 1992; 40: 403-409.
7. Moss GA, Tong LJ. Techniques for enhancing larval settlement of the abalone, *Haliotis iris*, on artificial surfaces. NZJ Mar Freshw Res 1992; 26:75-79.
8. Daume S, Brand-Gardner S, Woelkerling WMJ. Preferential settlement of abalone larvae: Diatom films vs. non-geniculate coralline red algae. Aquaculture 1999; 174:243-254.
9. Searcy-Bernal R. Boundary layers and abalone post-larval culture: Preliminary studies. Aquaculture 1996; 140:129-137.
10. Daume S, Krsinich A, Farrell S, Gervis M. Settlement, early growth and survival of *Haliotis rubra* in response to different algal species. J Appl Phycol 2000; 12:479-488.
11. Searcy-Bernal R, Salas-Garza AE, Flores-Aguilar RA, Hinojosa-Rivera PR. Simultaneous comparison of methods for settlement and metamorphosis induction in the red abalone (*Haliotis rufescens*). Aquaculture 1992; 105:241-250.
12. Roberts RD, Nicholson CM. Variable response from abalone larvae (*Haliotis iris, H. virginea*) to a range of settlement cues. Molluscan Res 1997; 18:131-141.
13. Capinpin EC Jr, Corre KG. Growth rate of the Philippine abalone, *Haliotis asinina* fed an artificial diet and macroalgae. Aquaculture 1996; 144:81-89.
14. Capinpin EC Jr, Toledo JD, Encena VC II, Doi M. Density dependent growth of the tropical abalone *Haliotis asinina* in cage culture. Aquaculture 1999; 171:227-235.
15. Capinpin EC Jr. Feeding, growth and survival of post-larval abalone *Haliotis asinina* post-larvae on different benthic diatoms. Science Diliman 2007; 19:49-59.
16. Norman-Boudreau K, Burns D, Cooke, CA, Austin A. A simple technique for detection of feeding in newly metamorphosed abalone. Aquaculture 1986; 51:313-317.
17. Matthews I, Cook PA. Diatom diet of abalone post-larvae (*Haliotis midae*) and the effects of pre-grazing the diatom overstorey. Mar Freshw Res 1995; 46:545-548.
18. Siqueiros-Beltrones DA, Voltolina D. Grazing selectivity of red abalone *Haliotis rufescens* post-larvae on benthic diatom films under culture conditions. J World Aquac Soc 2000; 31:239-246.
19. Brand LE. The isolation and culture of microalgae for biotechnological applications. In: Isolation of Biotechnological Organisms from Nature. Labeda DP (ed), McGraw-Hill Publishing Company, 1990, 81-115.
20. Jørgensen EG. Antibiotic substances from cells and solution of unicellular algae with special reference to some chlorophyll derivatives. Physiol Plant 1962; 15:530-545.
21. Round FE, Crawford RM, Mann DG. The Diatoms: Biology and morphology of the genera. Cambridge University Press, Cambridge, 1990.
22. Hasle GR. Some specific preparations: Diatoms. In: Phytoplankton Manual. Sournia A (ed), UNESCO, 1978, 136-142.
23. Seki T, Taniguchi K. Factors critical to the survival of herbivorous animals during settlement and metamorphosis. In: Survival Strategies in Early Life Stages of Marine Resources. Watanabe Y, Yamashita Y, Oozeki Y (eds), AA Balkema, 1996, 341-354.
24. Bryan PJ, Qian PY. Induction of larval attachment and metamorphosis in the abalone *Haliotis diversicolor* (Reeve). J Exp Mar Biol Ecol 1998; 223: 39-51.
25. Moss GA. Factors affecting settlement and early post-settlement survival of the New Zealand abalone *Haliotis australis*. N Z J Mar Freshw Res 1999; 33: 271-278.
26. Roberts RD, Kaspar HF, Barker RJ. Settlement of abalone (*Haliotis iris*) larvae in response to five species of coralline algae. J Shellfish Res 2004; 23:975-987.
27. Takami H, Kawamura T, Yamashita Y. Survival and growth rates of post-larval abalone *Haliotis discus hannai* fed conspecific trail mucus and/or benthic diatom *Cocconeis scutellum* var. *parva*. Aquaculture 1997; 152:129-138.
28. Roberts RD, Lapworth C, Barker RJ. Effects of starvation on the growth and survival of post-larval abalone (*Haliotis iris*). Aquaculture 2000; 200:323-338.
29. Hoagland KD, Rosowski, JR, Gretz, MR, Roemer, SC. Diatom extracellular polymeric substances: Function, fine structure, chemistry, and physiology. J Phycol 1993; 29:537-566.
30. Kawamura T, Roberts RD, Takami H. A review of the feeding and growth of post-larval abalone. J Shellfish Res 1998; 17: 615-625.
31. Kawamura T, Saido T, Takami H, Yamashita Y. Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. J Exp Mar Biol Ecol 1995; 194: 189-199.
32. Takami H, Kawamura T, Yamashita Y. Development of polysaccharide degradation activity in post-larval abalone *Haliotis discus hannai*. J Shellfish Res 1998; 17:723-727.
33. Roberts RD, Kawamura T, Takami H. Morphological changes in the radula of abalone (*Haliotis iris*) during post-larval development. J Shellfish Res 1999; 18:637-644.
34. Martínez-Ponce DR, Searcy-Bernal R. Grazing rates of red abalone (*Haliotis rufescens*) post-larvae feeding on the benthic diatom *Navicula incerta*. J Shellfish Res 1998; 17:627-630.
35. Roberts RD, Kawamura T, Nicholson CM. Growth and survival of post-larval abalone (*Haliotis iris*) in relation to development and diatom diet. J Shellfish Res 1999; 18:243-250.