ECOLOGICAL PATTERNS IN THE DEVELOPMENT, SETTLEMENT AND RECRUITMENT OF ARCHAEOGASTROPODS FROM THE OREGON COAST

by

MATTHEW CURRY KAY

A THESIS

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Dr. Richard B. Emlet, Chair of the Examining Committee

Date

Committee in charge:	Dr. Richard B. Emlet Dr. Jan Hodder Dr. Alan L. Shanks Dr. Stephen S. Rumrill

Accepted by:

Vice Provost and Dean of the Graduate School

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Approved: ____
Dr. Richard B. Emlet

Larvae of the limpets *Lottia digitalis* and *Lottia asmi*, as well as larvae of the flat abalone *Halioits walallensis*, develop into lecithotrophic veliger larvae and settle into benthic habitat after a breif planktonic stage. Larvae of *L. digitalis* settled and metamorphosed upon rocky substrata, as well as the barnacle *P. polymerus*, collected from an adult habitat high in the rocky intertidal. In contrast, substrata from mid and low intertidal zones failed to induce settlement and metamorphosis in larvae of *L. digitalis*. These results suggest that recruitment into high intertidal habitat is driven by settlement rather than post settlement processes. New recruits within an adult habitat were most abundant low within the adult range and upon north-facing slopes of rocks. Larvae of *H. walallensis* that experienced a five-day extension of their competence period exhibited accelerated rates of metamorphosis, as well as accelerated juvenile growth rates, relative to larvae that were presented with settlement surfaces at initial competence.

CURRICULUM VITA

NAME OF AUTHOR: Matthew Curry Kay

PLACE OF BIRTH: Santa Barbara, California

DATE OF BIRTH: January 29, 1973

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon
Universidad San Francisco de Quito (Quito, Ecuador)

DEGREES AWARDED:

Master of Science in Biology, 2001, University of Oregon
Bachelor of Science in Biology, 1996, University of Oregon

AREAS OF SPECIAL INTEREST:

Natural History of Coastal Marine Invertebrates
Reproductive Biology and Ecology of Marine Gastropods

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, Oregon Institute of Marine Biology (University of Oregon), Charleston, 1998-2000

Undergraduate Teaching Assistant, Department of Biology, University of Oregon, Eugene: Fall 1993, Fall 1995, Winter 1995

AWARDS AND HONORS:

Neil Richmond Memorial Fellow, 2000
Conchologists of America Student Research Award, 1999

GRANTS:

Western Society of Malacologists Student Research Grant, 1999
PADI: Project AWARE Research Grant, 1999

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DEDICATION

This thesis is dedicated, however inadequately, to the memory of the late Neil Richmond. Neil's premature death paradoxically served as a reiteration of life's most fundamental lesson: how to live. I am reminded of Neil, and his love for life, when I read the following passage from John Steinbeck's "The Log From the Sea of Cortez". The following reflections were inspired as the Crew of the *Western Flyer* steamed south through rolling seas along the California coastline – a scene in which, no doubt, Neil would have glowed.

"We sat on a crate of oranges and thought what good men most biologists are, the tenors of the scientific world – temperamental…loud-laughing, and healthy. Once in a while one comes across the other kind – what used in the university to be called a "dry-ball" – but such men are not really biologists. They are the embalmers of the field, the picklers who see only the preserved form of life without any of its principle. The true biologist deals with life, with teeming boisterous life, and learns something from it, learns that the first rule of life is living… Your true biologist will sing you a song as loud and off-key as will a blacksmith…and meanwhile he is very good company…"

For Neil: September 24, 1954 – June 8, 1999

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CHAPTER I

GENERAL INTRODUCTION

Benthic marine invertebrates with planktonic larval stages impose an apparently critical challenge on their progeny. Not only must offspring survive various perils of the plankton, they must also return to suitable adult habitat if they are to recruit into reproductive populations. This thesis describes experiments and observations that were conducted in an effort to better understand how marine gastropod larvae achieve the transition from plankton to benthos. The organisms that I employed in my studies were the ribbed limpet, *Lottia digitalis* (Patellogastropoda: Lottiidae) (Rathke 1833) and the flat abalone, *Halitois walallensis* (Vetigastropoda: Haliotidae) (Stearns).

Knowledge of reproduction and larval development in a given organism is fundamental to an understanding of its settlement and recruitment biology. Appropriately, I provide a thorough description of the reproduction and larval development for both of the organisms under study in this thesis. Chapter II is focused entirely on the spawning and development of *L. digitalis*, as well as its congener *Lottia asmi*, whereas larval development of *H. walallensis* is described in Chapter V. These descriptions are of value not only in the context of this thesis, but have broader application since there is no published, complete description of larval development for *L. digitalis, L. asmi* or *H. walallensis*.

Chapter III of this thesis describes settlement experiments in which larvae of *L. digitalis* were exposed to various substrata from an adult habitat. The purpose of these experiments was to identify likely natural settlement surfaces for this species. *L. digitalis* is found high in the intertidal where individuals occupy one of two habitat types: rocky substrata and the stalked barnacle *Pollicipes polymerus* (Morris et al. 1980). Whereas the recruitment of *L. digitalis* onto *P. polymerus* has not been investigated, distributional studies based on length-frequency data suggest that *L. digitalis* settles and recruits directly into rocky, high-intertidal adult habitat (Frank 1965; Breen 1972). Two factors, however, suggest that *L. digitalis* larvae might settle lower in the rocky intertidal, and then undertake an upward postlarval migration. First, the youngest individuals observed by Frank (1965) and Breen (1972) were consistently seen at lower elevations within the adult habitat range, but were many months old by the time they were visible. Second, a similar indirect recruitment pattern has been hypothesized for other species of limpets that live high in the rocky intertidal (Corpuz 1981; Delany et al. 1998). Previous descriptions of *L. digitalis* recruitment do not address the possibility of indirect recruitment in this species. Information regarding settlement preferences presented in this report, when considered in tandem with size distributions previously observed by Frank (1965) and Breen (1972), addresses the relative importance of initial settlement versus post-settlement processes during the recruitment of *L. digitalis* into rocky high intertidal habitat. Additionally, I exposed larvae of *L. digitalis* to live specimens of *P. polymerus* to determine if the former settles (and possibly recruits) directly onto this host.

Chapter IV of this thesis reports on observations of the abundance and distribution of *L. digitalis* recruits (0-1 year-olds) in an high intertidal, boulder field habitat. This habitat allowed me to observe recruit abundance relative to a) height on the shore, and b) the directional orientation (i.e. north vs. south facing) of substrata in the habitat. Chapters III and IV provide complementary perspectives of recruitment of *L. digitalis* into high intertidal habitat.

Chapter V of this thesis, in addition to describing larval development of *H. walallensis,* reports on the effect of a five-day extension of metamorphic *competence* (the stage at which larvae are morphologically and metabolically able to metamorphose and inhabit benthic habitat) in larvae of *H. walallensis*. The significance of this research, and relevance to this thesis, is as follows. Since larvae of benthic marine invertebrates are weak swimmers, and readily become entrained in currents, they have limited spatial control over their return trip to adult habitat (Shanks 1995). However, they might exert some temporal control by extending their competence periods, thereby increasing the probability that they will encounter suitable settlement surfaces (Morgan 1995). The ecological benefit of an extended competence period, however, could be negated if the extension were to impose energetic or physiological stress on a larva (Wendt 1998). This is especially true for non-feeding larvae with a finite supply of endogenous nutrition (Pechenik et al. 1998). The ways in which an extended competency period can compromise postlarval performance are manifold. The most severe repercussion is the inability of a larva to successfully undergo metamorphosis once an appropriate surface is encountered (Woollacott et al. 1989; Pechenik and Cerulli 1991). When larvae that have experienced prolonged larval periods do successfully undergo metamorphosis, they often experience suppressed juvenile growth rates (Pechenik et al. 1993; Woollacott et al. 1989). Retarded growth, no matter what the cause, has ostensibly grave implications for organisms that must secure resources (i.e. space) in highly competitive environments. A more subtle physiological consequence of extended larval period, which has yet to be thoroughly researched, is the ability of newly-settled juveniles to endure physical and biological stresses (Pechenik et al. 1998). The extension of competence periods in marine invertebrate larvae, although ecologically adaptive, is not always an inconsequential event. Chapter V explores the postlarval affects of a five-day extension of the metamorphic competence period in larvae of *H. walallensis*.

In essence, this thesis explores the patterns in marine gastropod larval ecology that facilitate successful recruitment into adult habitats. As evidenced by the results, these patterns manifest on both spatial and temporal scales. The mere existence of these patterns suggests that settlement and recruitment is not a random process in the taxa I studied. Furthermore, as the field of larval ecology develops and grows, the insights of its practitioners must be integrated into knowledge of adult biology and ecology in order to achieve a more complete understanding of the natural history of marine invertebrates.

CHAPTER II

SPAWNING, LARVAL DEVELOPMENT AND METAMORPHOSIS OF THE LIMPETS *Lottia digitalis* AND *Lottia asmi* (PATELLOGASTROPODA: LOTTIDAE)

Introduction

The patellogastropod limpets *Lottia digitalis* (Rathke, 1833) and *Lottia asmi* (Middendorff, 1847), are members of the Lottiidae Gray 1840 (superfamily Acmaeoidea), which is one of three families formed from the recently fragmented Acmaeidae (then superfamily Patellacea) (Lindberg, 1986). The 25 lottiid species that inhabit the northeastern Pacific (Lindberg, 1981) serve as an impressive example of ecological radiation, as some species have formed strict associations with particular algal species (Test, 1945; Yonge, 1962; Proctor, 1968), gastropods (Test, 1945; Eickenberry & Wickizer, 1964), and vertical habitat zones (Rickets & Calvin, 1968; Underwood, 1979; Morris et al., 1980).

Lottia digitalis and *L. asmi* occupy very different microhabitats in the intertidal zone. *L. digitalis* is common in high intertidal rocky habitats from the Aleutian Islands (Alaska) to southern Mexico (Morris et al., 1980). In contrast, *L. asmi* is restricted almost exclusively to the shells of its gastropod hosts, *Tegula funebralis* and *Tegula gallina*, throughout its range from British Columbia to Isla Socorro, Mexico (Morris et al., 1980). The adult biology and ecology of *L. digitalis* have been studied extensively (Castenholz, 1961; Frank, 1965; Hardin, 1968; Breen 1971; Wootton, 1992, 1993; Hobday 1995), and the same is true for *L. asmi* (Test, 1945; Eickenberry & Wickizer, 1964; Alleman, 1968; Evans, 1992; Lindberg, 1990; Lindberg & Pearse, 1990).

Despite the scrutiny placed upon the adult biology of *L. digitalis* and *L. asmi*, larval development for *L. digitalis* has been described only to the veliger stage (Koppen et al., 1996; Holyoak et al., 1999), and there is no published description of development for *L. asmi.* Aside from work with *L. digitalis*, and Lindberg's (1983) description of reproductive anatomy in the brooders *Erginus* spp., detailed developmental study of the 25 northeastern Pacific lottiids has been restricted to *Tectura scutum* (Karp, 1970) and *Acmaea incessa* (Proctor, 1968). Developmental studies are important because developmental mode influences the dispersal ability, genetic structure, ecological resilience, and evolutionary persistence of marine invertebrate taxa (Strathmann, 1985; Pechenik, 1999). Accounts of species development also contribute to our understanding of life history evolution (Grahame & Branch, 1985; Havenhand, 1993; Goddard, 1996) and phylogenetics (Emlet, 1988; Hickman, 1992; Ponder & Lindberg, 1996).

This study describes and compares the laboratory spawning, larval development, and metamorphosis of *L. digitalis* and *L. asmi*. My observations reveal that these ecologically unique species share nearly identical patterns of early development. Both species freely spawn their gametes, which develop into lecithotrophic veliger larvae that reach competence at 5.25-5.5 days after fertilization (13^{O}C). *L. asmi* shed smaller eggs than *L .digitalis*. Small egg size may be an adaptation to increase fecundity in the small-bodied *L. asmi*, but this conclusion is hampered by a lottiid developmental literature that: a) represents relatively few species, and b) suggests that intraspecific variation in egg size may not be uncommon in the family. Because free spawning lottiids do not need to secure egg masses in safe habitats for development, this reproductive mode may have been conducive to their ecological radiation into novel benthic habitats.

Materials and Methods

Spawning and Gamete Collection

Adult *Lottia digitalis* were collected from South Cove of Cape Arago, OR. (43^{O} 18' N; 124^{O} 24' W), during winter and spring of 2000 (Table1). During the same time period, adult *Lottia asmi* (on their hosts, *Tegula funebralis*) were collected from the Cape Arago site, and also from Sunset Bay State Park, OR. (43^{O} 20' N; 124^{O} 22' W). The limpets were held in a 13^{O}C incubator at the Oregon Institute of Marine Biology (OIMB), Charleston, OR., where they were spawned within one day of collection.

To induce spawning, groups of 6-8 adult *L. digitalis* and *L. asmi* (removed from their hosts) were separately placed in seawater in 2L glass finger bowls (18cm diameter, 7cm tall) and exposed to a weak solution of hydrogen peroxide (H_2O_2), thermal shocking, and/or vigorous bubbling. Hydrogen peroxide treatments were prepared according to Hahn (1989). Specifically, each liter of seawater was buffered with 6.6ml of 2M Tris(hydroxymethyl)aminomethane (Tris-base), allowed to equilibrate for 15 minutes, then spiked with 3ml of a freshly prepared 6% H_2O_2 solution. Adult limpets were soaked in this solution for two hours. In the thermal shocking treatments, limpets were held in water while the temperature was increased from $13^{O}C$ to $16\text{-}18^{O}C$ over a two-hour period. Seawater in the vigorously bubbled treatments was agitated for two hours using standard aquarium airstones. All three spawning treatment stimuli were terminated by the addition of 0.45μm filtered seawater (FSW) at $13^{O}C$, which thoroughly rinsed and refilled each vessel. Each vessel of treated adults was then placed in an incubator at $13^{O}C$ with a photoperiod representative of field conditions, and monitored continuously for gamete release.

Immediately upon release, eggs were reverse-siphoned through a 300μm nitex mesh sieve and pipetted into 500ml beakers of isothermal 0.45μm FSW. After the eggs had settled to the bottom of these beakers (~10 minutes), the vessels were decanted and refilled twice for a total of three rinsings. Sperm were collected from spawning males when possible, but in some cases they were stripped with a fine bore hypodermic syringe and activated in seawater. A stock solution of sperm in seawater, typically ~ 5×10^{6} sperm per ml FSW, was prepared using a hemocytometer to calculate cell density.

Larval Culture

Larval culture took place in 0.45μm FSW at $13^{O}C$ under a photoperiod representative of field conditions. Rinsed, unfertilized eggs were settled into uncrowded monolayers (~ 500 eggs/cm^{2}) upon the bottom of 1L glass beakers. Sperm were then added from the stock solution to yield a final concentration of 200,000 sperm per ml of 0.45μm FSW. This concentration was maintained for five minutes, after which time the cultures were decanted and refilled with fresh 0.45μm FSW. Control batches of eggs (which were not exposed to sperm solution) were maintained along with the fertilization vessels to ensure that fertilization occurred at a known time. Cultures were periodically agitated during early development.

Soon after trochophores began to swim, the upper half of each culture was decanted into a 2.5L culture vessel and diluted to a density of 1 larva per 2-4 ml FSW. In this manner, waste and debris accumulated in the fertilization vessels were eliminated and healthy, actively swimming trochophores were isolated for final culture. From this point in time until completion of the protoconch, cultures were unrinsed and periodically agitated.

When larvae first displayed the ability to withdraw soft tissues into their shells (i.e. protoconch and operculum fully formed), they were rinsed in a partially submerged 100 μm nitex sieve. From this point in time until settlement, larvae were rinsed and transferred to fresh 0.45μm FSW once daily, and the cultures were agitated with paddles that swung at 12 beats min.^{-1} (described in Strathmann, 1971). To observe metamorphosis, larvae of both species were settled onto fragments of rock that were collected from the adult habitat of each species. To measure the effect of temperature on development of *L. digitalis*, the above protocol was followed at 8.5^{O}C (in addition to 13^{O}C) for adults collected on February 12, 2000.

Light and Scanning Electron Microscopy

Larval development was monitored with a compound microscope, and major stages were recorded with photomicrographs. Ciliated blastulae, trochophores, and early stage veligers were fixed for photography in a buffered solution of 4% formaldahyde (1% sodium borate). Late-stage veligers were exposed to low levels of $MgCl_2$ (2-4 drops of 7.5% $MgCl_2$ per ~20ml of FSW) for 2-12 hours in order to prevent velar retraction. Developmental features were measured to the nearest 4.4 μm with an ocular micrometer. Measurements of larval shells were taken: a) across the shell opening, perpendicular to the velum's bilateral symmetry, and b) from the tip of the shell opening to posterior end of the protoconch. These two measures were termed length and width, respectively, and are diagrammed by Hadfield & Strathmann (1990).

The veliger shells were also observed with scanning electron microscopy (SEM). To clear tissues from larval and postlarval shells, anaesthetized individuals were soaked for six hours in a 3:10 solution of bleach and distilled water. To dissolve residual lipids, shells were held in distilled water and gradually introduced to 100% methanol, exposed to a 1:1 mixture of methanol and chloroform (1 hour), then rehydrated through the methanol series to distilled water (Pederson & Page, 2000). Once the shells were cleaned, they were placed in absolute acetone and stored until examination. Specimens were subsequently air-dried, stub mounted, platinum coated, and examined with an AMR 1000A scanning electron microscope. All larval shell SEM work employed shells from competent larvae.

Results

Spawning and Gamete Collection

Lottia digitalis

Of the animals collected on February 12, 2 of 30 spawned after immersion in H_2O_2, whereas 7 of 30 adults spawned after exposure to vigorous bubbling (Table 1). Similarly, of the animals collected on March 2, 3 of 24 spawned after immersion in H_2O_2, whereas 8 of 24 adults spawned after exposure to vigorous bubbling. In light of these results, vigorous bubbling was employed throughout the season to induce gamete release in *L. digitalis*. (In some instances, thermal shocking and/or H_2O_2 were also applied, but never in the absence of bubbling.)

Physical contact between/among spawning males and females was not typical. Eggs and sperm were extruded from beneath the right anterior portion of the adult shells. Eggs were released singly, while sperm were extruded as a viscous milk-like fluid. Male and female gametes were negatively buoyant. The eggs were "rusty" brown in color upon release, but turned a pale green color after ~5 minutes.

The spherical eggs released by 5 different females had mean diameters of 150.9 μm (SD = 0.69, n = 25), 151.0 μm (SD = 1.5, n= 25), 151.1 μm (SD = 1.5, n = 25), 152.2 μm (SD = 4.1, n = 25), and 167.4 μm (SD = 3.1, n = 25). Each egg was surrounded by a transparent jelly coat, (262.3 μm in diameter, SD = 4.6, n = 25 eggs from a single female), which was visible when eggs were suspended in "Sumi" ink particles

(Strathmann, 1987). The smallest female to release eggs was 16mm long, and the largest number of eggs released was 42,000 (Fig. 1). As evident in Figure 1, there was a trend of increased fecundity with increased adult size in *L. digitalis*. The lanceolate sperm from one male had mean head and tail lengths of 8.0 μm (SD = 0.8) and 70.0 μm (SD = 3.7), respectively (n = 16).

Lottia asmi

Ten of twenty adults that were collected on April 19 spontaneously released gametes on April 20 (Table 1). Of the animals collected on April 25, 8 of 20 spawned after immersion in H_2O_2, while 3 of 20 spawned after exposure to rigorous bubbling. Efforts to induce spawning after this date were met with limited success. Eggs and sperm were extruded in the same manner as in *L. digitalis*.

The spherical eggs released by 5 different females had mean diameters of 135.9 μm (SD = 2.8, n = 25), 130.5 μm (SD = 4.4, n = 25), 135.0 μm (SD = 3.1, n = 25), 134.6 μm (SD = 2.8, n = 25), and133.2 μm (SD = 3.4, n = 25). Eggs were rusty brown in color upon release, but turned pale green within 5 minutes. The smallest female to release eggs measured 7 mm, and the largest number of eggs released was 13,000. The lanceolate sperm from one male had a mean head and tail length of 7.0 μm (SD = 1.6) and 63.5 μm (SD = 2.4), respectively.

Larval Development

The sequence and timing of developmental stages were nearly identical in *L. asmi* and *L. digitalis*. Due to this similarity, some early stage photographs for *L. asmi* and *L. digitalis* are presented in alternating order in Figures 2 and 3. For example, Figure 2B illustrates the two-cell stage for *L. asmi*, whereas Figure 3B illustrates an 8-cell embryo of *L. digitalis*. The timing of developmental events at 13.0^{O}C was synchronous within each species, but differed slightly between the two (Table 2). *L. digitalis* larvae reared at 8.5^{O}C took nearly twice as long to reach metamorphic competence as larvae reared at 13^{O}C (see Table 2 for the specific timing of developmental events).

In both species, the first reliable evidence of fertilization was the formation of a polar body (Figs. 2A, 3A). In contrast, separation of the egg membrane from the egg was imperceptible or extremely slight. The first two cleavages were complete, equal, meridional and always initiated near the polar body (Fig. 2B). The third cleavage was unequal, equatorial, and dexiotropic, such that the resulting 8-celled embryos were comprised of four macromeres and four micromeres surmounted by the polar body (Fig. 3B). The fourth and fifth divisions were synchronous, but the 6th division was asynchronous.

At approximately 9 hrs (13^{O}C) post fertilization, the gastrulae bore three tiers of cilia which beat feebly and rotated the embryos at rates no faster than 1 revolution 20 seconds^{-1} (Fig. 2C). At this stage, the polar body persisted atop the animal pole. At approximately 10 hours, however, the polar body was resorbed and apical cilia appeared in its place. Unfortunately, I was unable to determine the process by which gastrulation occurred.

Trochophores were well formed and swam actively 14-16 hours after fertilization. Soon after each species began active swimming, the telotroch (anal tuft) and refractive bodies were formed (Fig. 2D). In early trochophores of both species, steady swimming was often punctuated with abrupt bursts of speed. During these short bursts, which lasted less than 0.25 sec., larvae swam distances of >10 body lengths. This sprint behavior was consistently induced when larvae were touched with an eyelash probe, and they often responded in this manner when a probe passed them in close proximity.

At 16 hours, the stomodaeum was visible immediately beneath the prototrochal girdle (Fig. 3C). The prototrochal girdles of each species were comprised of 21 individual cells. Girdle cells in *L. digitalis* and *L. asmi* were approximately 35-40 and 30-35 μm long, respectively. *L. digitalis* trochophores were 177 μm long (SD = 8.5, n= 30) and 164 μm wide at the prototrochal girdle (SD = 7.6, n = same 30), while *L. asmi* trochophores were 155 μm long (SD = 4.4, n= 30) with a 145 μm girdle width (SD = 8.4, n = same 30).

During protoconch formation, the telotroch migrated anteriorly along the ventral surface of the larva, until it was located immediately posterior to the foot rudiment (Figs. 2E, 3E). Torsion was not a punctuated event in either species, but rather, it took place over a 2-4 hour period. Soon after torsion was completed, the operculum, the first epipodial tentacle, and both eyespots were formed. At this stage, larval behavior changed markedly. Rather than resorting to flight as a defensive strategy (as in trochophores), veligers withdrew into their shells at the slightest provocation. Both species swam upwards, as trochophores and veligers, but the roles of photo- and geotaxis were not determined.

The veliger larvae of *L. digitalis* and *L. asmi* (reared at 13.0^{O}C) reached metamorphic competence at 5.5 and 5.25 days, respectively. Larvae were deemed competent when they crawled on/adhered to glassware, and when they bore 3^+ tubercles on their cephalic tentacles (as described in Seki & Kan-no, 1981). Upon settling, larvae reared forward on their shells and swept laterally across the substratum with their cephalic region. Velar loss, the first evidence of metamorphosis, and the presence of food (plant matter) in the gut were detectable 1 day after settlement in both species. It was not determined whether velar cells were shed, resorbed, or ingested by the newly formed juvenile.

Development of the radular complex was rapid in postlarvae of both species, and was particularly visible in *L. digitalis* (Figs. 2H and 3H). By 2 days after settlement, the ribbon-like radulae of postlarvae were clearly visible. By 10 days after settlement, *L. digitalis* postlarvae developed well-vascularized radular muscles that were red in color when oxygenated, but quickly turned blue-purple when postlarvae were placed between a microscope slide and cover slip for observation (and ostensibly deprived of oxygen). In contrast, *L. asmi* postlarvae did not develop obvious vascularisation of their radular muscles until 20-25 days after settlement, and even then the muscles were not as intensely red as in *L. digitalis*. Postlarvae of both species actively rasped the substratum with their radulae, but they were sedentary and typically remained in small (9-16mm^2)

areas for consecutive days. In many instances, larvae settled in clusters of 4-10 individuals. It is unclear, however, whether this was a display of gregarious settlement or a pattern caused by patchiness of substratum quality. At 13^{O}C, *L. digitalis* grew to a size of 1mm in 35-40 days, whereas *L. asmi* took 45-50 days to reach that size (Table 2).

Larval shells of *L. asmi* averaged 206.2 μm wide and 144.5 μm long (SD's = 2.5 and 1.6, respectively; n = 10 individuals from one female with average egg diameter = 133.2 μm). See Hatfield and Strathmann (1990) for a description of dimensions. Larval shells of *L. digitalis* averaged 224.2 μm wide and 154.9 μm long (SD's = 2.33 and 3.2, respectively; n = 10 individuals from a female with average egg diameter = 151.0 μm). There was no difference between *L. asmi* and *L. digitalis* with regard to protoconch sculpturing (Fig. 4). Both species initiated adult shell growth 2-4 days after settlement. The adult shell was initially secreted symmetrically, as illustrated in Figs. 4 C&F. In slightly older postlarvae, however, the protoconch appears as to be displaced clockwise relative to the adult shell's long axis (Fig. 5).

Discussion

Spawning

Broadcast spawning is a common reproductive mode among littoral archaeogastropods of the northeastern Pacific (Karp, 1970; Strathmann, 1987; Holyoak, 1988; Hickman, 1992; Moran, 1997). One potential benefit of broadcast spawning is that, unlike eggs tethered in benthic egg masses, free-spawned gametes are delivered from potential stresses encountered in the adult habitat (Underwood, 1979; reviewed by Pechenik, 1999). This advantage has been linked to the prevalence of broadcast spawning among high-intertidal littorinids (Mileikovsky, 1975), although Reid (1990) has proposed an alternative phylogenetic hypothesis for this trend. Among northeastern Pacific lottiids, almost all of which free spawn their gametes, it is unclear if broadcast spawning has been selected for ecologically or is constrained phylogenetically. The occurrence of nonpelagic development in the northeastern Pacific lottiids *Erginus apicina*, *Erginus moskalevi*, and *Erginus sybaritica* (Lindberg, 1983), however, suggests that developmental mode is not under strict phylogenetic constraint in the Lottiidae. Since free-spawning adults are not obligated to locate safe microhabitats for benthic egg development, this reproductive mode may have been permissive in the ecological radiation of the Lottiidae.

An additional hurdle to egg mass formation might also exist for *Lottia asmi*. It is estimated that adult *L. asmi* change hosts once every 13 to 24 hours (Test, 1945; Eickenberry and Wickizer, 1964), and that in a given night 40% of animals transfer hosts (Lindberg, 1990). Given these movement regimes, deposition of an egg mass upon a *T. funebralis* host shell would be tantamount to abandonment. Since *T. funebralis* are highly mobile, and egg masses seldom confer complete protection from abiotic environmental stresses (Pechenik 1999), a female *L. asmi* who has abandoned her egg mass would have little control over the microclimate in which it develops. If a female were to brood her egg mass on a *T. funebralis* shell, and thereby buffer the microclimate, she would be forced to fast since adult *L. asmi* consume up to 7.0% of the algae on a typical host every hour (Eikenberry & Wickizer, 1964).

Since at least the 1960's, it has been observed that certain patellaogastropods spawn coincidentally with heavy seas (Fretter and Graham, 1962). More recently, Shanks (1998) observed that field spawning in *Lottia digitalis* is apparently associated with winter storm events. The effectiveness of vigorous bubbling as a means of inducing gamete release, during the course of this study, may be related to this life history trait in *L. digitalis*. Indeed, winter spawning (when storms are most frequent) is common among eastern Pacific patellogastropods (Fritchman 1961a, b, c, 1962; Phillips 1981; Koppen et al., 1996). In light of this seasonal timing, the high sperm viscosity of *L. asmi* and *L. digitalis* may be an adaptation to resist dilution in vigorously agitated water (e.g. Thomas, 1994).

Koppen et al. (1996) and Holyoak et al. (1999) report mean egg sizes of 197.5 and 146.3 μm, respectively, for *L. digitalis*. The 150.9–167.4 μm egg sizes that I measured approach the observations of Holyoak et al., but are much smaller than those of Koppen et al. While it is challenging to account for such a wide variation in intraspecific egg size, and Holyoak et al. suggest that an erroneous micrometer calibration may be culpable, such patterns are not unprecedented in the lottiid literature. For example, Karp (1970) and Nicotri (1974) report egg sizes of 150 and 190 μm, respectively, in *Tectura scutum*. In three individual *Lottia pelta* females, collected simultaneously from the San Juan Islands, egg sizes ranged from 128-144 μm (Hadfield & Strathmann, 1996). Due to the relatively sparse literature on lottiid development, it is uncertain whether intraspecific egg size variation is the rule or the exception in this family.

Egg sizes in *L. asmi* are smaller than egg sizes described for most other eastern Pacific lottiids (Table 3). The magnitude of the differences is best appreciated when one considers the volumetric differences calculated from the measured egg diameters. For example, egg diameters of 130 µm for *L. asmi* and 150 µm for *L. digitalis* indicate that eggs of the latter are more than 1.5 times the volume of the former.

Reduced egg size, relative to other lottiids, might be a distinct result of the ecological constraints imposed by habitat choice and reproductive mode in *L. asmi.* For marine invertebrates with planktonic development, reproductive success is dependant upon high fecundity (Grahame & Branch, 1985; Emlet et al., 1987; Rumrill, 1990). Fecundity in small-bodied invertebrates, however, is acutely limited by the adults' ability to allocate energy and space for gonad development (Menge, 1975; Underwood, 1979; Gallardo & Perron, 1982; Strathmann & Strathmann, 1982) Body size in *L. asmi* is constrained by the shape and size of its host, *Tegula funebralis* (Lindberg & Pearse, 1990), but the association provides *L. asmi* with an escape from high interspecific competition among intertidal limpets (Murphy, 1976). Thus, the ecological benefits of this commensalism impose limitations on adult size and, by extension, gonadal bulk in *L. asmi.* Reduced egg size, however, permits higher fecundity in a gonad of fixed size (Strathmann, 1986). Therefore, reduced egg size in *L. asmi* might be an adaptation to increase fecundity in this small-bodied broadcast spawning limpet.

Unfortunately, the strength of this argument is handicapped by the paucity of published egg sizes and fecundities for eastern Pacific lottiids. Furthermore, intraspecific variations in egg size (as described above for *L. digitalis, L. pelta* and *T. scutum*) confound efforts to determine the adaptive significance of this life history trait within the Lottiidae. Finally, recruitment successes attributable to higher fecundity must compensate for any liabilities associated with production of smaller offspring (e.g. Emlet & Hoegh-Guldenberg, 1997).

Larval Development

Early cleavage patterns in *L. digitalis* and *L. asmi* were typical of development in lottiids and other patellogastropods (Smith, 1935; Kessel, 1964; Karp, 1969; Van Den Biggelaar & Haszprunar, 1996). The absence of a 24-cell resting stage during 5th cleavage (synchronous 5th cleavage) was observed in both species and is presumed to be ancestral among the gastropods (Van Den Biggelaar, 1993). This feature has been used to phylogenetically distinguish the patellogastropoda from members of the closely related vetigastropoda (Van Den Biggelaar & Haszprunar, 1996). No cytoplasmic markers or pigments, as have been observed in some gastropods (Collin 1997; Moran, 1997; Kay, pers. obsv.), were present or segregated during early cleavage.

The timing of development in *L. digitalis* and *L. asmi* was similar to other lottiids and patellogastropods (Smith, 1935; Proctor, 1968; Strathmann, 1987; Hadfield & Strathmann, 1996). The fact that *L. asmi* embryos developed slightly faster than those of *L. digitalis,* while egg size in the former was smaller than in the latter, is consistent with the "egg-to-juvenile" period model posited by Havenhand (1993). The effect that temperature had on developmental time in *L. digitalis* is consistant with observations of other gastropod larvae (Leighton, 1972; Hahn, 1989; Goddard, 1996)

Neither Koppen et al. (1996), nor Holyoak et al. (1999), note polar body formation in *L. digitalis*. Similarly, polar body formation is not described in *P. vulgata* (Smith, 1935; Dodd, 1957) or *A. testudinalis* (Kessel, 1964). Although the evolutionary significance of this structure might be negligible, the omission of such a conspicuous developmental feature is vexing. At the very least, polar body formation was practically useful, in the absence of egg membrane lifting, as early evidence of fertilization. I was unable to see lifting of the egg membrane in *L. digitalis*, as described by Koppen et al.

Presence of the telotroch (anal tuft) is inconsistently reported in the patellogastropod literature (but see Smith, 1935; Proctor, 1968), yet this structure might play an important sensory role in trochophores. The "sprint" behavior described above (also noted by Kessel, 1964) must be facilitated by the ability of trochophores to receive physical and/or optical stimuli. Smith (1935) proposed such a role for refractive bodies in *P. vulgata,* due to their ciliation and optically refractive properties. The anal tuft and refractive bodies bear several features in common: a) they appear on the extreme (but opposite) tips of trochophores, b) they are protrusions that bear non-motile cilia, and c) they disappear during formation of the veliger stage. Structural similarities of refractive bodies and the telotroch suggest a common function, and it is possible that both are sensory bodies in patellogastropod trochophores. This idea is bolstered by the fact that haliotid trochophores, from the closely-related pleurotomarioidea, possess neither refractive bodies nor telotrochs and do not display the same sprint behavior (Crofts, 1937; Kay, pers. obsv.). In contrast, the reflexive tendency of veligers to retract into their protoconchs is a behavior typical of this larval form (Garstang, cited in Hardy, 1958).

Although no feeding experiments were conducted with veligers of *L. digitalis* and *L. asmi*, they appear lecithotrophic. This conclusion is based upon the lack of a post-oral ciliary band and a well-defined mouth on the velum, both of which are present and conspicuous in planktotrophic gastropods (Hickman, 1992; Pederson & Page, 2000). Further evidence of their lecithotrophy is derived from the velar aspect ratio (VAR) scheme of Moran (1999). Both species had VAR's (VAR=width of velum divided by total length of larva) conservatively estimated between 0.66 and 0.85, which are well within the range of VAR values for lecithotrophic taxa surveyed by Moran. Additionally, the larvae of *L. digitalis* and *L. asmi* raised during this study were not provided food, but experienced negligible mortality during latter stages of culture.

The series of lines on larval shells from competent *L. digitalis* and *L. asmi* does not extend over the entire surface of the protoconch. This sculpture pattern contrasts with western Pacific lottiids described by Amio (1963), whose larval shells are completely sculptured from the posterior to the aperture. It is possible that these sutures were present in early stage veligers, but they were lost during subsequent shell building and/or maintenance processes. Most likely, these sculpture lines are a product of the larval shell growth pattern, but lack the defensive value of protoconch features present in gastropod larvae with longer planktonic developments (Hickman, 1999). These patterns might be of use to students of zooplankton, since protoconch morphology is reliably employed in family-level identification of eastern Pacific gastropod veligers (Goddard, in press).

In addition to any diagnostic utility, the phylogenetic value of protoconch morphology has been considered for some haliotids (Hayashi, 1983), trochaceans (Hickman, 1992), and opisthobranchs (Thompson, 1961). Although prosobranch phylogeny at the ordinal and familiar level is based primarily upon adult features (Lindberg, 1988; Bieler, 1992), protoconch morphology has potential for resolution of lower-order relationships (Hickman, 1992). Such endeavor is dependant upon a vast library of published images for any given taxon, but unfortunately no such images exist (to my knowledge) for the patellogastropoda.

In postlarval *L. asmi* and *L. digitalis*, the protoconch appears to be displaced clockwise relative to the adult shell's long axis (Fig. 5). This offset has been described in other patellogastropods (Morse, 1910; Thompson, 1912; Smith 1935) and in a fissurellid keyhole limpet (Pernet, 1997). Because this alignment is not established during initial shell growth (Figs. 4C&F), and because the protoconch is soon lost to abrasion (pers. obsv; also noted by Morse, 1910; Pernet, 1997), any functional value of this alignment should be dismissed. The most logical interpretation of this phenomenon, in the absence of function, is as a developmental vestige of coiled ancestry. Indeed, Lindberg (1988) has cited this feature in his assertion that the patellogastropod ancestor bore a coiled teloconch. Alternatively, Haszprunar (1988) argues compellingly that the ancestor resembled extant limpets and had a bilaterally symmetrical adult shell.

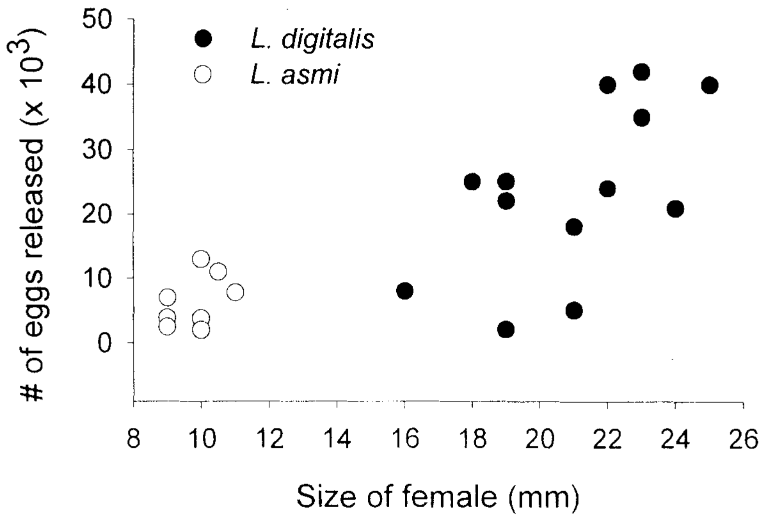

FIGURE 1. The total number of eggs released as a function of shell length for individual females induced to spawn in the laboratory.

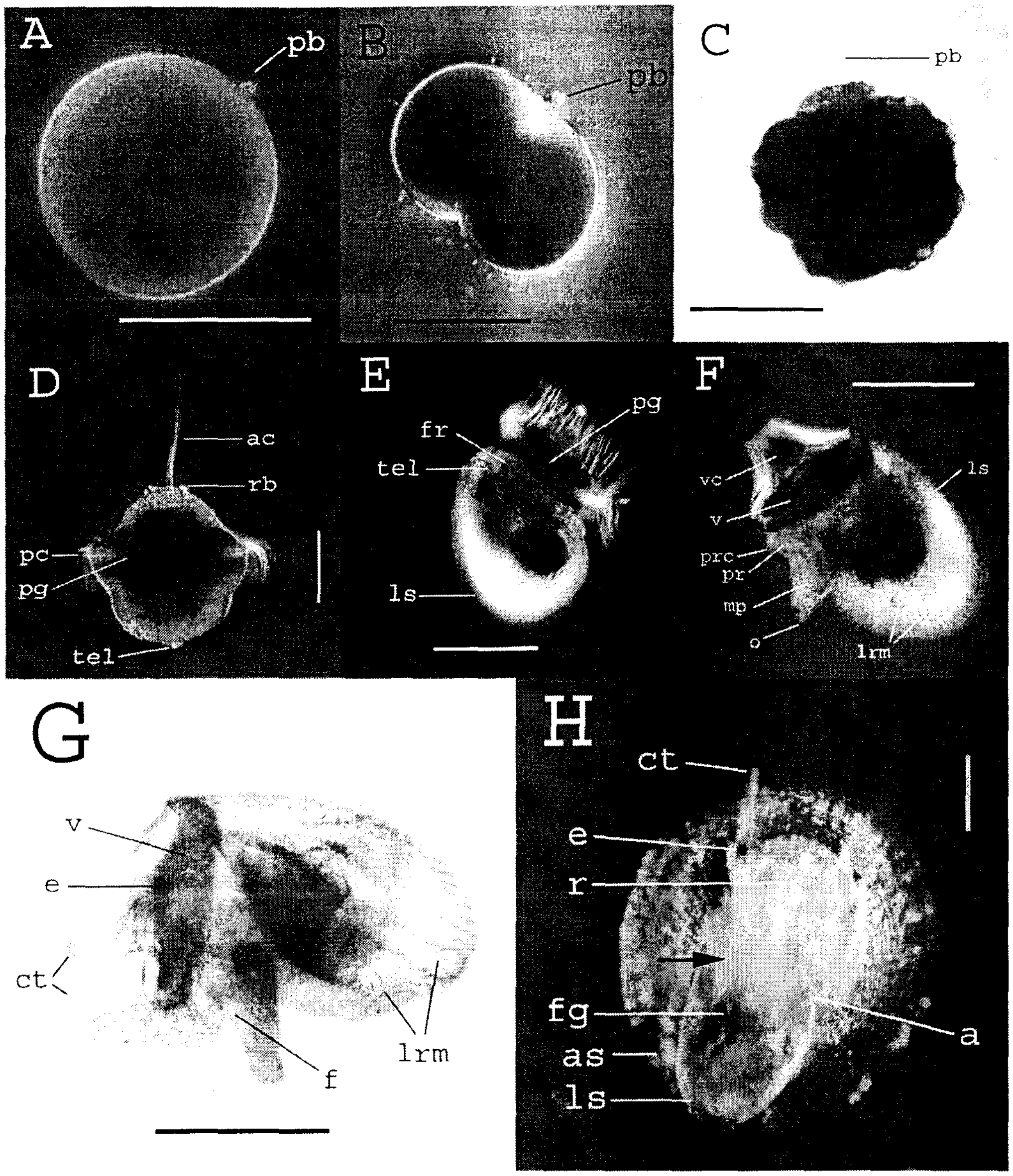

FIGURE 2. Light micrographs of developmental stages in *Lottia asmi*, reared at 13°C. (A) fertilized ovum displaying polar body (pb) (0.5 hr.). (B) first cleavage (1 hr.). (C) Gastrula surmounted by persistent polar body (9hrs.). (D) Completely formed trochophore (20 hrs.). ac, apical acilia; pc, prototrochal cilia; pg, prototrochal girdle; rb, refractive bodies; tel, telotroch. (E) Early pretorsional veliger (35 hrs.) with incomplete larval shell (ls) and telotroch still visible beneath the foot rudiment (fr). (F) Post-torsional veliger (62 hrs.) with larval retractor muscles (lrm) and operculum (o) completely formed. Larvae withdraw into protoconch at this stage. mp, metapodium; pr, propodium; prc, propodial cilia; v, velum; vc, velar cilia. (G) Competent veliger (5 days). Velar cilia are present, but not visible in this photograph. ct, cephalic tentacle; e, eye; f, foot. (H) Postlarval individual, arrow marks the break between larval and adult shells. a, anus; as, adult shell; fg, foregut; r, radula. All scale bars are 100 μm.

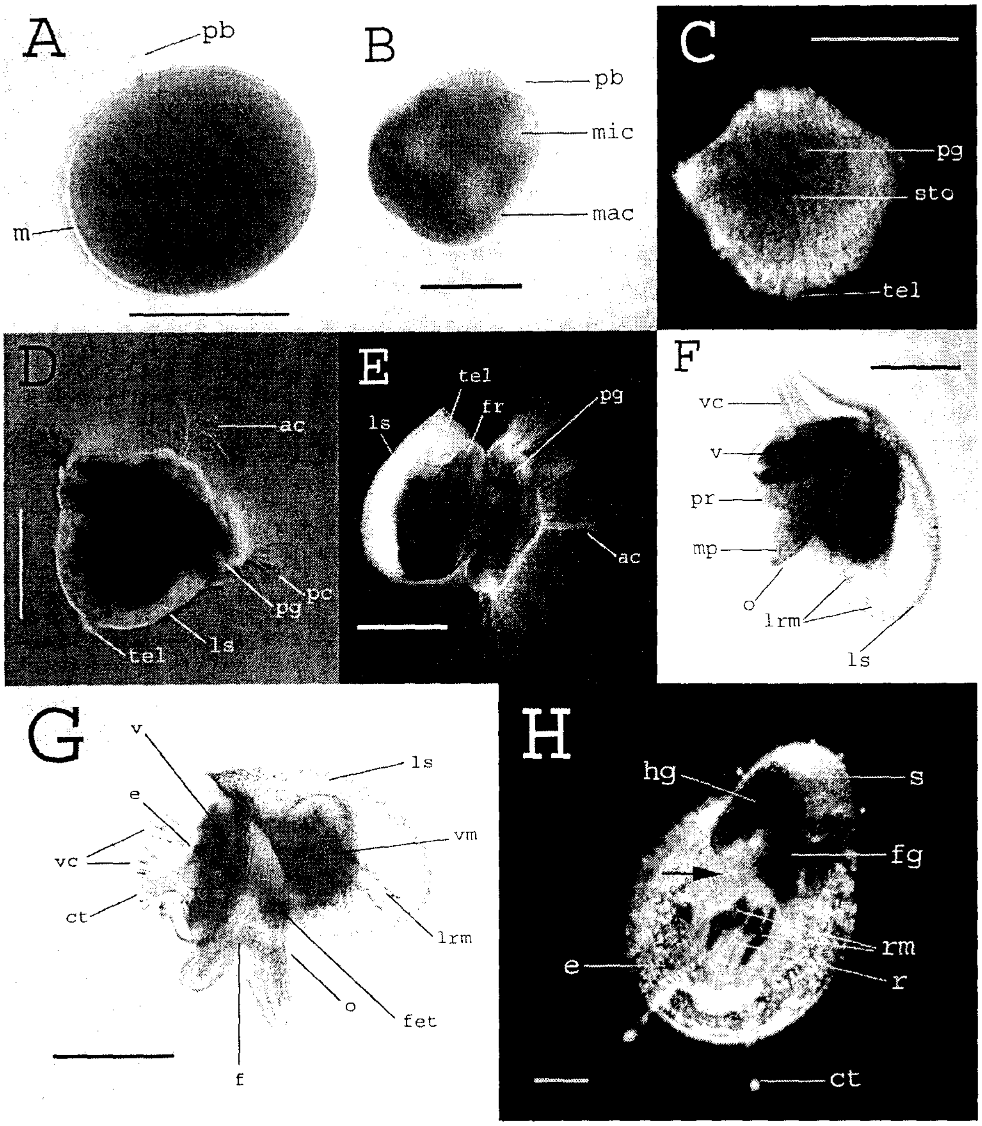

FIGURE 3. Light micrographs of developmental stages in *Lottia digitalis*, reared at 13^{O}C. (A) Fertilized ovum displaying polar body (pb) (0.5 hr.). m, egg membrane. (B) Eight cell stage (2.5 hrs.). mic, micromere; mac, macromere. (C) Newly formed trochophore with stomodaeum (sto) below prototrochal girdle (pg). tel, telotroch. (24.5 hrs. at 8.5^{O}C). (D) Late stage trochophore (35 hrs.) with larval shell formation underway. ac, apical cilia; fr, foot rudiment; pg, prototrochal girdle; tel, telotroch. (E) Pretorsional veliger with telotroch visible posterior to the foot rudiment (fr) (45 hrs.). ls, larval shell. (F) Post-torsional veliger (62 hrs.) with larval retractor muscles (lrm) and operculum (o) completely formed. Larvae withdraw into protoconch at this stage. mp, metapodium; pr, propodium; v, velum; vc, velar cilia. (G) Competent veliger (5.5 days). e, eye; ct, cephalic tentacle; f, foot; fet, first epipodial tentacle; vm, visceral mass. (H) Postlarval individual (15 days) with well developed and vascularized radular muscles (rm). fg, foregut; hg, hindgut; r, radula; s, stomach. Arrow marks the break between larval and adult shells. All scale bars are 100 μm.

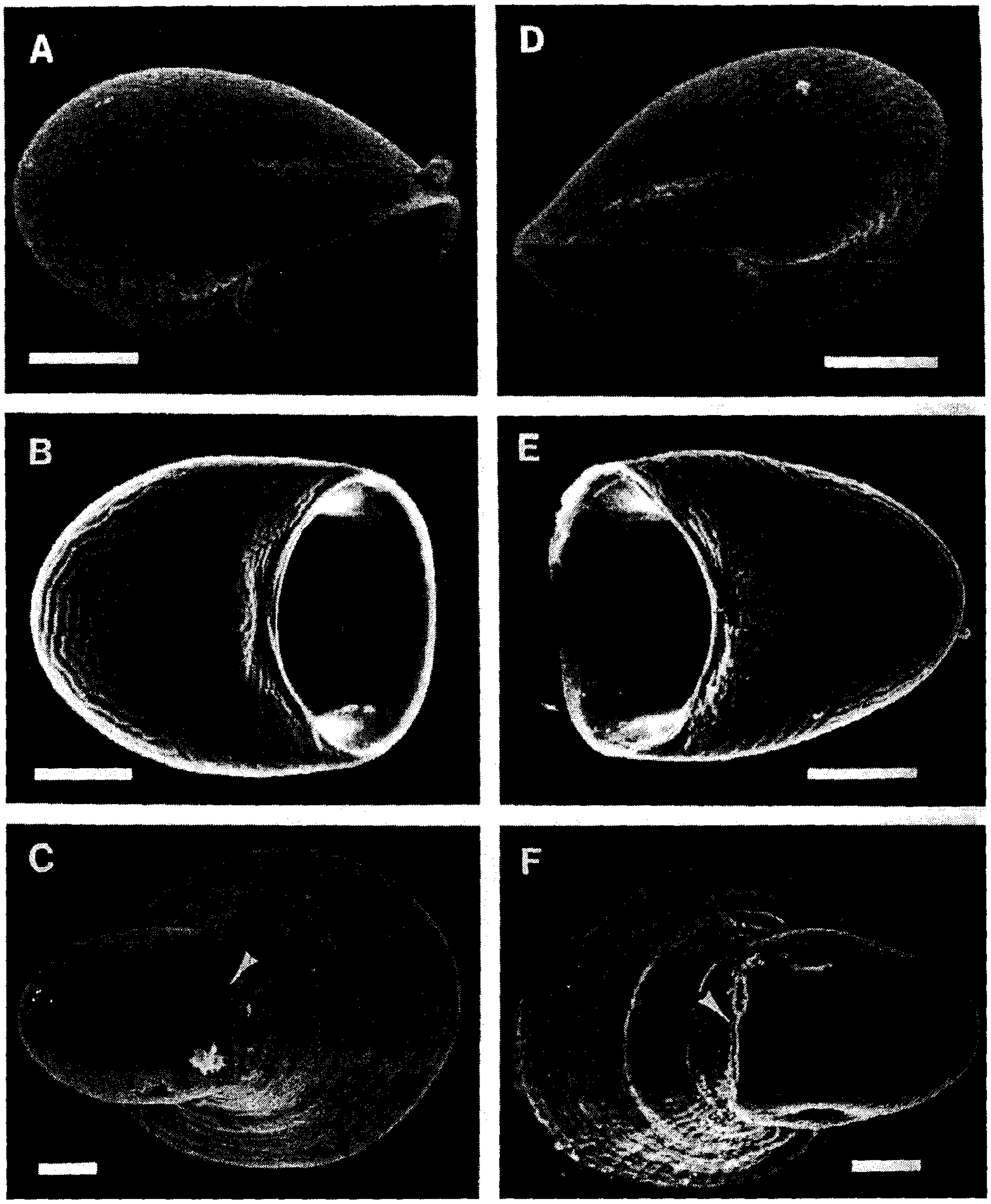

FIGURE 4. Scanning electron micrographs of: (A) *Lottia digitalis* larval shell in lateral view, rotated slightly to expose the shell aperature. (B) Bottom view of *L. digitalis* larval shell. (C) *L. digitalis* postlarva, arrow marks break between adult and larval shells. (D) *Lottia asmi* larval shell in lateral view, rotated slightly to expose the shell aperature. (E) Bottom view of *L. asmi* larval shell. (F) *L asmi* postlarva, arrow marks break between adult and larval shells. Scale bars are 50 μm.

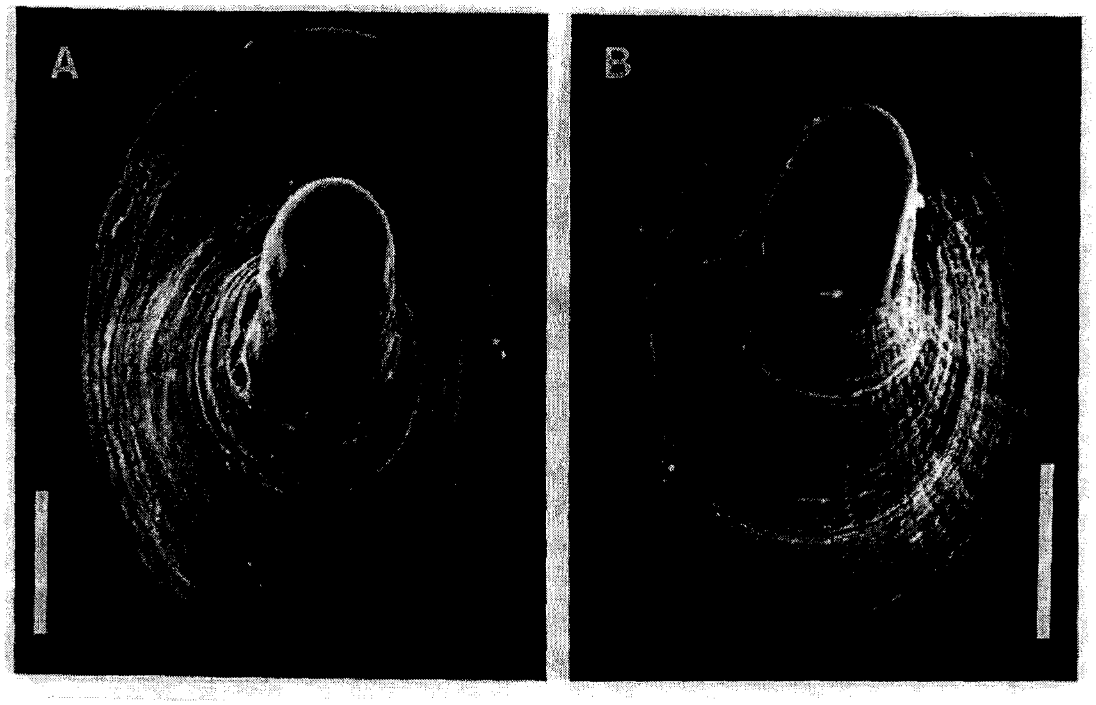

FIGURE 5. Scanning electron micrographs of postlarval (A) *Lottia digitalis* and (B) *Lottia asmi*. Note the slight clockwise offset of larval shell, relative to the long axis of the adult shell. Scale bars are 200 µm.

TABLE 1. Laboratory spawning summary for *Lottia digitalis* and *L. asmi*.

Date (2000)	Stimulus	#spawned/ # attempted	Lunar Phase
Lottia digitalis:			
2/12	HP	2/30	3/4
	B	7/30	
3/2	HP	3/24	New
	B	8/24	
3/6	B+TS	10/20	New
3/21	B+TS	4/45	Full
3/27	B+HP+TS	0/50	1/4
4/6	B+HP+TS	3/100	New
4/14	B+TS	11/50	3/4-Full
4/25	B+TS	7/50	1/4
Lottia asmi:			
4/14	B+TS	2/20	3/4-Full
4/20	VOL	10/20	Full
4/25	B+HP+TS	11/40	1/4
5/23	HP	0/19	1/4
5/28	HP+TS	1/20	1/4
6/6	B+HP+TS	2/50	3/4
6/16	HP	3/50	Full
	B	0/50	Full

Laboratory spawning of animals collected from the field. Spawning stimuli were hydrogen peroxide (HP), vigorous bubbling (B), and thermal shocking (TS) (see text for details). VOL = volunteer spawning event.

TABLE 2. Summary of development in *Lottia asmi* at 13.0° C, and *L. digitalis* at 8.5 & 13.0° C.

Developmental event or stage	Time since fertilization *L. asmi* (13°)	*L. digitalis* (13°)	*L. digitalis* (8.5°)
Polar body formation	0.25-0.5 hr	0.25-0.5 hr	0.25-0.5 hr
1st cleavage	1 hr	1.25 hr	1.5 hr
2nd cleavage	1.5 hr	1.75 hr	2.5-3 hr
3rd cleavage	2 hr	2.5 hr	-
4th cleavage	2.5 hr	3 hr	-
Ciliated gastrulae	9 hr	9.5 hr	16 hr
Apical cilia appear, polar body lost	10.5 hr	11 hr	17-20 hr
Swimming trochophores	15 hr	14-16 hr	24.5 hr
Telotroch (anal tuft) formed and ciliated	-	16.5 hr	-
Protoconch initiated / protoconch completed	28 hr/40-43 hr	29 hr/44-48 hr	38 hr/88 hr
Larval retractor muscles	36 hr ^{P}	-	64 hr ^{P}
Torsion started / torsion completed	41 hr/43-48 hr	48 hr/50-52 hr	88 hr/ 94 hr
Operculum fully formed	52 hr	57 hr	4.6 d
First epipodial tentacle	57-59 hr	58-60 hr	5.6 d
Eyespots, ciliation of metapodium	61-62 hr	63.5-64 hr	5.8 d
Propodium formation / cilia on propodium	64-70 hr/70 hr	64-70 hr/70 hr	-
Cephalic tentacles begin to elongate	3 d	3.5 d	5.9 d
Cephalic tentacles bearing 3+ tubercles	4.25-4.5 d	4.5 d	8.6 d
Larvae crawl (behavioral competence)	5.25 d	5.5 d	9.1 d
Vela lost	6.5 d	6.5 d	10.5 d
Food visible in gut	6.5 d	6.5 d	11 d
Adult shell growth	7-8 d	7-8 d	11.5–12 d
Animals 1mm long	~40-50 d	~35-40 d	-

Times for 13° C observations were averaged from two rearing events for both *L. asmi* and *L. digitalis*.
^{P} = visible under polarized light, but not white light.

TABLE 3. Egg sizes of free spawning northeastern Pacific Lottiids.

Species	Egg size (μm)	Source
Lottia asmi	133	(This study)
Lottia digitalis	147	(Holyoak et al., 1999)
	156	(This study)
	197	(Koppen et al., 1996)
Lottia gigantea†	130	(Daly, 1975)
Lottia pelta	128-144	(Hadfield and Strathmann, 1996)
	185	(Nicotri, 1974)
Notoacmea insesssa	150	(Proctor, 1968)
Tectura persona	200	(Strathmann, 1987)
Tectura scutum	150	(Karp, 1970)
	185	(Nicotri, 1974)
*Acmaea testudinalis**	140	(Kessel, 1964)

† Egg sizes measured from dissected gonads, not from spawned gametes.

*spawning described in a western Atlantic specimen, but this species occurs in the northeastern Pacific (Lindberg, 1979).

BRIDGE I

As demonstrated in Chapter II, adult *Lottia digitalis* from South Cove, Cape Arago, free spawn gametes that require ~5-6 days to develop and reach metamorphic competence. During this planktonic period, it is likely that tidal mixing and nearshore currents disperse larvae away from the parental habitat. Larvae must ultimately rejoin adult conspecifics, however, if they are to form part of a reproductive population and contribute their genetic material to subsequent generations.

It can be hypothesized that two general phenomena are operative in the recruitment of *L. digitalis* into adult habitat. First, larvae might settle directly into adult habitat. Second, post-settlement processes, such as migration or differential mortality, might alter patterns of random settlement, or differential settlement into habitats outside the adult habitat, and thereby determine the adult distribution pattern that is observed. Chapter III addresses the importance of settlement vs. post-settlement processes in the recruitment of *L. digitalis* into high intertidal habitat.

CHAPTER III

LABORATORY EVIDENCE THAT NATURAL RECRUITMENT IN THE LIMPET *Lottia digitalis* (PATELLOGASTROPODA: LOTTIIDAE) IS DRIVEN BY SETTLEMENT RATHER THAN POST-SETTLEMENT PROCESSES

Introduction

Many patellogastropod limpet species release gametes that ultimately develop into planktonic veliger larvae (Lebour 1937; Fretter and Graham 1962; Strathmann 1987). After a brief pelagic life-stage, these larvae settle onto appropriate surfaces where they metamorphose and commit to a benthic existence. During the benthic life stage, many limpet species form strict habitat associations with particular algal species (Yonge 1962; Proctor 1968; Choat and Black 1979; Munoz and Santelices 1989), other gastropods (Eickenberry & Wickizer 1964; Scheibling et al. 1990), and tidal zones (Haven 1972; Branch 1976; Underwood 1979). An interesting dynamic of the early life history in these limpets, in light of their habitat fidelity and planktonic larval dispersal, is the recruitment of juveniles into highly specific adult habitats. With regard to this dynamic, two general patterns emerge: 1) some limpet species are reported to settle directly into/onto the adult habitat (Graham and Fretter 1947; Proctor 1968; Scheibling et al. 1990), 2) others recruit indirectly by settling outside, and then migrating into, the adult habitat (Corpuz 1981; McGrath 1992; and Delany et al. 1998). A third possible recruitment pattern is that adjacent habitats experience equal settlement, but the habitats impose differential postlarval mortality and thereby determine juvenile/adult distributions. Although this pattern has been observed for some taxa (Raimondi 1988; Rowley 1989), it has not been reported for recruitment of intertidal limpets.

Studies of limpet recruitment are typically based on length-frequency observations, such that size-class structures are compared for adjacent habitat types (e.g. Frank 1965; Breen 1972; Quinn 1988; McGrath 1992; Delany et al. 1998). These studies are usually dependent upon observations made in the field, and they interpret the presence and/or absence of juveniles in different habitats as evidence of recruitment patterns. As such, their accuracy is limited because many months may pass between initial settlement and the time at which juveniles are large enough to be visible and identifiable in the field (Kay, Chapter IV of this thesis; Frank 1965; Parry 1982; Quinn 1988). During the interval between settlement and census, juvenile distributions across (or within) adjacent habitats can change due to differential mortality or movement (Graham and Fretter 1947; Keough and Downes 1982; Connell 1985; Rowley 1989; Rodriguez et al. 1993). Consequently, and with particular regard to mobile organisms, juvenile distributions at the time of census may not correspond to settlement locations. Recruitment studies based on length frequency observations alone, although valuable and informative, are not always unequivocal in their identification of settlement sites and early juvenile habitats.

For many benthic marine invertebrates with planktonic larvae, settlement tends to be a nonrandom process triggered by environmental cues (e.g. Burke 1983; Butman

1987; Rodriguez et al. 1993). Environmental cues are typically associated with juvenile or adult habitat, and are assumed to assist in successful recruitment. In the laboratory, where competent larvae can be manipulated under controlled conditions, settlement cues for a given taxon can be explored and identified. Indeed, laboratory studies have identified the preferred settlement substrata of numerous gastropods (Hadfield 1978; Morse and Morse 1984; Lambert and Todd 1994; Duame et al. 1999; Krug and Manzi 1999). In one such study, Proctor (1968) was able to settle larvae of the patellogastropod limpet *Acmaea incessa* onto fragments of its algal host *Egregia menziessii*, but was unable to settle larvae onto other non-host algae or bacterial films.

Lottia digitalis (Rathke 1833) is a patellogastropod limpet (family Lottiidae – see Lindberg 1986) that is found high in the intertidal. *L. digitalis* occur as (at least) two different ecotypes, which are readily distinguishable based on shell morphology, that occupy rocky substrata or the stalked barnacle *Pollicipes polymerus* (See Byers 1989; Lindberg and Pearse 1990). Whereas the recruitment of *L. digitalis* onto *P. polymerus* has not been investigated, distributional studies based on length-frequency data suggest that *L. digitalis* settles and recruits directly into rocky, high-intertidal adult habitat (Frank 1965; Breen 1972). Two factors, however, suggest that *L. digitalis* larvae might settle lower in the rocky intertidal, and then undertake an upward postlarval migration. First, the youngest individuals observed by Frank (1965) and Breen (1972) were consistently seen at lower elevations within the adult habitat range, but were many months old by the time they were visible. Second, a similar indirect recruitment pattern has been hypothesized for other species of limpets that live high in the rocky intertidal (Corpuz

1981; Delany et al. 1998). Previous descriptions of *L. digitalis* recruitment do not address the possibility of indirect recruitment in this species.

This paper reports on laboratory settlement experiments conducted with larvae of *Lottia digitalis,* which were exposed to natural substrata collected from various tidal heights near and within an adult habitat. The purpose of these experiments was to identify likely settlement surfaces, and the height at which they occur on the shore, for larvae of *L. digitalis*. Information regarding settlement preferences presented in this report, when considered in tandem with size distributions previously observed by Frank (1965) and Breen (1972), addresses the relative importance of initial settlement versus post-settlement processes during the recruitment of *L. digitalis* into rocky, high-intertidal habitat.

Additionally, I exposed larvae of *L. digitalis* to live specimens of *P. polymerus* to determine if the former settles (and possibly recruits) directly onto this host. Given the results of this experiment, and the fact that the larvae I used were reared from adults taken from rocky habitat, I was able to address the possibility of a genetic basis for *L. digitalis* habitat "preference" (*P. polymerus* vs. rocky substrata) at the time of larval settlement.

Materials and Methods

Adult *Lottia digitalis* were collected from South Cove, Cape Arago, OR. (43^{O} 18' N; 124^{O} 24' W) during February and March 2000. In order to identify likely sites for natural settlement, larvae spawned from these adults were reared to metamorphic competence as described by Kay (Chapter II of this thesis), and in the laboratory they were exposed to a variety of field-collected substrata. Three of these substrata were rock fragments removed from, and representative of, dominant rocky surfaces available for intertidal settlement within and immediately below the site of adult collection. Fragments of the three rock types, termed *High*, *Mid*, and *CCA*, were removed with hammer and chisel or by hand, and were returned to the Oregon Institute of Marine Biology (OIMB) in Charleston, OR., where settlement experiments took place.

The *High* rock was collected from an adult *L. digitalis* boulder-field habitat, at a tidal height between 1 and 2 meters above mean lower low water (MLLW). Rock chips were taken from intermediate levels within the adult habitat, at about 1.5 m above MLLW. *High* rock was course sandstone, with a texture similar to 100-grit sandpaper, and was heavily colonized by diatoms and a filamentous green alga that was ubiquitous in winter throughout the adult habitat. This alga was also present on the shells of many adult *L. digitalis* at my site. The adult habitat was dominated exclusively by boulders of this rock type at the site of collection, which extended ~75 meters along the coast. Other lottiid species within this habitat zone included *Lottia paradigitalis* (moderately abundant ~1.0 – 1.4 m above MLLW), as well as *Lottia pelta* and *Tectura persona* (both were common but present in very low abundance). An individual *Macclintockia scabra* was also seen in this zone.

The *Mid* rock was collected from a tidal level 0.5 - 1.0 m above MLLW, a height at which the acmaeid limpet *Tectura scutum* was the dominant limpet species. *L. paradigitalis* and *L. pelta* also occurred in this zone but were less abundant than *T.*

scutum. The *CCA* substratum was collected from 0.0-0.5 m above MLLW as pebbles that were encrusted by a crustose corraline alga (CCA) within the family Corallinaceae (see Steneck and Paine 1986). Although I could not identify this alga, it was distinct and common at the South Cove site. At this tidal height the lottiids *Lottia asmi* (on their hosts *Tegula funebralis*) and *T. scutum*, as well as the acmaeid *Acmaea mitra,* were common but present in very low adundance.

Fragments of the three rock types were placed in 175ml (5 cm tall, 6.5 cm base) *Pyrex* custard dishes, both in the presence and absence of adult *L. digitalis* mucus, such that six settlement treatments were created (*High*, *High/Adults*, *Mid*, *Mid/Adults*, *CCA*, *CCA/Adults*). To prepare the three *Adults* treatment groups, adult *L. digitalis* were allowed to crawl on the rocks for 24 hrs. and were removed immediately prior to the experiment in order to avoid "bulldozing" of settled larvae. Each of the six treatment groups, in addition to a sterile control dish, was replicated six times (total number of dishes = 7 treatments x 6 replicates = 42 dishes). Replicate dishes were then filled with 0.45μm-filtered seawater (FSW), and 50 *L. digitalis* larvae were individually pipetted into each settlement dish. All dishes were then haphazardly arranged in a 13.0^{O}C incubator under a 12:12 Light:Dark photoperiod.

At three and six days after larvae were introduced to settlement vessels, each dish was examined under a dissecting microscope for 25 minutes. Recovered individuals were scored as: dead, unsettled, settled, vela shed, and adult shell growth (the latter two conditions are sequential events during metamorphosis). Inevitably, some individuals remained unaccounted for after the 25-minute search period. Results are presented as the percentage of larvae that fall into each of the five categories (dead, unsettled, settled, vela shed, adult shell growth). In order to calculate these percentages, the number of larvae in each category was divided by 50 (the total number of larvae introduced to each dish), rather than the total number of larvae recovered (i.e. some number < 50). In most dishes recovery success was ~70%, because larvae were often difficult to find due to their small size. Larvae that were unaccounted for probably had not metamorphosed, because those individuals with adult shell growth were relatively large and easy to see. This experiment was run twice using offspring from two pairs of parents, spawned separately on February 12 and on March 2. Larvae from the February 12 and March 2 spawning events were introduced to settlement surfaces on February 18 and March 8, respectively. Growth rates of newly settled juveniles that were spawned on March 2, and settled onto *High/Adults* and *Mid/Adults* substrata, were followed for 70 days after fertilization. Growth rates were compared for 3 dishes in both treatments that each contained 5 juveniles (number of juveniles per treatment = 3 dishes x 5 individuals/dish = 15). These juveniles were reared at 13.0°C and were immersed in 32‰ seawater throughout the 70-day period.

In addition to the *High*, *Mid* and *CCA* rocky substrata, *L. digitalis* larvae were exposed to the macroalgae *Ulva* sp., *Enteromorpha contorta*, *Alaria marginata*, and *Polysiphonia* sp., which were common in protected habitats near the site of adult *L. digitalis* collection. The design of this experiment was identical to that detailed above, with the exception that a positive control (*High* rock) accompanied the negative control (0.45μm FSW) (total dishes = 6 treatments x 6 replicates = 36 dishes). To limit algal tissue damage and/or decomposition that might have artificially deterred settlement, algae were placed in dishes as whole organisms or non-severed blades detached at a node. For *A. marginata* this was not possible, due to its large size, and so settlement surfaces from this species were pruned from the vegetative thallus. This experiment was run twice using offspring from two different parental pairs, which were spawned on March 2 and on March 6. Larvae from the March 2 and March 6 spawning events were introduced to settlement surfaces on March 8 and March 12, respectively. The larvae spawned on March 2 were siblings of those larvae exposed to the rocky substrata described above.

The results of the experiments described above (see Results, Figs. 1 and 2) indicated that specific settlement cues on the *High* rock probably included one or more of the following: 1) physical /chemical properties of the *High* rock, 2) benthic diatoms on the *High* rock's surface, 3) the filamentous green alga (FGA) observed on the *High* rock's surface, 4) the mucus or some other biochemical signature of adult *L. digitalis*. In order to distinguish among these possibilities, I conducted a settlement experiment in which larvae were exposed to each of these potential cues. Treatment groups for this experiment, which were termed *SterHigh* (*Ster*ilized *High* rock), *Diatom*, *FGA*, and *Mucus*, were prepared in the following manner: To prepare the *SterHigh* treatment, newly-collected *High* rock fragments were autoclaved in seawater for 15 minutes, scrubbed with a toothbrush, and then autoclaved a second time. To prepare the *Diatom* and *FGA* treatments, these algae were individually isolated from the *High* rock surface, and cultured in 50 ml tubes containg f/2 medium with silica (*Diatom*) and without silica (*FGA*). The diatom isolated was *Navicula* sp., and was 24.4 μm long by 12.2 μm wide (5