ECOLOGY AND EARLY LIFE HISTORY OF *BATHYNERITA NATICOIDEA:* EVIDENCE FOR LONG-DISTANCE LARVAL DISPERSAL OF A COLD SEEP GASTROPOD.

by

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A THESIS

Presented to the Department of Biology
and the Graduate School of the University of Oregon
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"Ecology and early life history of *Bathynerita naticoidea:* evidence for long-distance larval dispersal of a cold seep gastropod," a thesis prepared by Ahna Lea Van Gaest in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

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Dean of the Graduate School

An Abstract of the Thesis of

Ahna Lea Van Gaest for the degree of Master of Science

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Title: ECOLOGY AND EARLY LIFE HISTORY OF *BATHYNERITA NATICOIDEA:* EVIDENCE FOR LONG-DISTANCE LARVAL DISPERSAL OF A COLD SEEP GASTROPOD.

Approved:

Dr. Craig M. Young

Bathynerita naticoidea is a bathyal gastropod endemic to hydrocarbon seeps in the Gulf of Mexico and Caribbean at depths from 400-1700m. At a brine pool seep in the Gulf of Mexico, *B. naticoidea* were less abundant near the pool than several meters away. Snails suffered 100% mortality in salinity of 15, 32.5% mortality in salinity of 60, and, when given the choice, did not enter salinities greater than 60. Egg capsules are deposited seasonally, with peak oviposition between December and February. Embryos hatch as swimming veligers in the spring. Larvae were collected in plankton tows from the upper 100m of the water column in February, suggesting that they disperse for at least 8 months. Larvae tolerated temperatures up to 32°C and salinities from 15 to 60. The early life-history characteristics of *B. naticoidea* suggest the potential for long distance dispersal and provide strong evidence for ontogenetic vertical migration.

This thesis includes my co-authored materials.

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CHAPTER I

GENERAL INTRODUCTION

Chemosynthetic bacterial communities flourish year-round at cold seeps where continual seepage of oil and methane and production of hydrogen sulfide provide a constant source of energy (MacAvoy *et al.,* 2002). Primary production by chemosythetic bacteria supports the maintenance of a dense benthic assemblage in areas otherwise characterized by low diversity and biomass. Communities of chemosynthetic tubeworms, clams, and mussels were first discovered at cold seeps along the northern Gulf of Mexico continental slope in the mid-1980's (Kennicutt *et al.* 1985). Dense mussel beds and tubeworm bushes provide food and habitat for a diverse community of consumers including gastropods, orbinid worms, alvinocarid shrimp, and galatheid crabs (MacAvoy *et al.* 2002; Bergquist *et al.* 2005).

Early life history processes, such as the initiation of gametogenesis, spawning, larval duration, and release of larvae, are critical to the formation and maintenance of benthic communities, however, few studies have investigated the reproductive processes in cold seep assemblages (but see Young *et al.,* 1996c; Eckelbarger *et al.,* 2001).

When a population undergoes synchronous gametogenesis or spawning events, an exogenous factor such as food availability, temperature, day length, or phase of the moon is often responsible for controlling gamete production (Giese and Pearse, 1974). For example, phytoplankton blooms have been reported to induce spawning events in some shallow-water invertebrate species (Starr *et al.,* 1990), and gonad development has been correlated to the timing of phytodetrital food pulses in deep-sea communities that are influenced by photosynthetic primary production (Tyler and Gage, 1983, 1984; Tyler, 1988).

The extent and direction of larval dispersal of benthic invertebrates is dependent upon the length of larval life, swimming behavior of the larva, and the hydrographic regime the larva encounters in the water column (Thorson, 1961; Scheltema, 1966, 1971). Planktotrophic larvae generally have longer planktonic lives and a potential for wider dispersal than lecithotrophic larvae. In addition, long-lived planktotrophic larvae may have the potential for trans-oceanic dispersal (Thorson, 1961; Scheltema 1966, 1971; Scheltema and Rice, 1990). However, the potential of deep-sea larvae to undergo long-distance dispersal probably depends upon their ability to vertically migrate to the productive surface waters where high currents are prevalent.

Moseley first suggested that the larvae of deep-sea animals could have surface-dwelling larvae in 1880, but this idea was overshadowed by Thorson's (1950) view that the vertical migration of abyssal larvae was energetically impossible. A number of recent papers have focused on disproving "Thorson's Rule" as a common phenomenon in the deep-sea (reviewed by Pearse, 1994; Young, 1994, 2003). Larvae belonging to several taxa, including holothurians, gastropods, and brachiopods, have been found in surface plankton tows, providing direct evidence for ontogenetic vertical migration (Ashworth, 1914; Pawson *et al.*, 2003; reviewed by Bouchet and Warén, 1994). Protoconch analyses in deep-water mollusks suggest that vertical migration of planktotrophic larvae to the euphotic zone may not be uncommon (Rex and Warén, 1982; Bouchet and Warén, 1994). In addition, energetic models of bathyal echinoids with planktotrophic larvae suggest that energy stores may not limit the ability of many deep-sea species to vertically migrate (Young *et al.*, 1996a). Larval physiological tolerances to the physical conditions of the euphotic zone may be more useful indicators of the potential for ontogenetic migration than energetic models (Young and Tyler, 1993; Young *et al.*, 1996a; Young *et al.*, 1996b).

Deep-sea hydrothermal vents and seeps are patchy habitats that can be tens to hundreds of kilometers apart. Given these distances, one would expect to find a high proportion of endemic species with long-lived planktotrophic larvae capable of

dispersing long distances in order to successfully colonize these isolated habitats. However, there seems to be a dominance of lecithotrophic development within the hydrothermal vent fauna (Lutz *et al.,* 1984; Lutz, 1986, 1988; Tyler and Young, 1992; Gustafson and Lutz, 1994; Mullineaux and France, 1995). Less is known about the reproductive processes of cold-seep assemblages, but the few studies that have examined the developmental mode suggests a similar trend to the hydrothermal vent fauna so far examined (Gustafson and Lutz, 1994; Young *et al.,* 1996c; Eckelbarger *et al.,* 2001).

Bathynerita naticoidea Clarke 1989 (Gastropoda: Neritacea) is a bathyal gastropod endemic to hydrocarbon seeps in the Gulf of Mexico and the southern Barbados Prism at depths from 400-1700 m (Carney 1994; Olu *et al.* 1996; Zande and Carney 2001). *B. naticoidea* is often the dominant heterotroph in cold-seep mussel beds at sites in the northern Gulf of Mexico continental slope (Bergquist *et al.* 2005). *B. naticoidea* grazes on methanotrophic bacteria and detritus from hard substrata, primarily the shells of the mussel *Bathymodiolus childressi* (Zande and Carney 2001). A nerite similar to *B. naticoidea* has been found in Miocene deposits in Italy and also at middle Eocene fossil seeps in western Washington, USA, suggesting a long history of association with cold seeps (Taviani 1994; Squires and Goedert, 1996).

The complex geology associated with the extensive salt diapers beneath the northern Gulf of Mexico can create pockmarks filled with methane-enriched brine on the seafloor. One such pockmark, Brine Pool NR1 ("the Brine Pool", 27º43.4157N, 91° 16.756W, ~650m depth) is surrounded by a dense bed of *Bathymodiolus childressi*, a hydrocarbon seep mussel with methanotrophic endosymbionts (Childress *et al.* 1986; MacDonald *et al.* 1990). The mussel community extends from 3 to 7m in width (~540 m^2) and can be divided into two distinct zones, the inner and outer zones, which vary in their water chemistry and biological community. The inner zone is characterized by high densities of small mussels, while the outer zone is dominated by lower densities of large mussels and disarticulated shells (Smith *et al.* 2000; Arellano, unpublished data). The transitional area between the inner and outer zones (the "middle zone") has

characteristics of both the inner and outer zones and is heterogeneous in both water chemistry and biological patterns (MacDonald *et al.* 1990; Smith *et al.* 2000; Bergquist *et al.* 2005).

Physical and chemical factors that may influence distribution patterns in the Brine-Pool mussel bed include high hydrogen sulfide concentrations and lower oxygen concentrations at the outer zone (Smith *et al.* 2000; Bergquist *et al.* 2005). However, on a fine scale, the water chemistry across the Brine Pool mussel bed is quite heterogeneous (Smith *et al.* 2000; Bergquist *et al.* 2005). Lateral seepage of brine from the pool across the mussel bed is apparent, although the extent of seepage is unclear (Smith *et al.* 2000).

Although a previous study of the development of *Bathynerita naticoidea* concluded that this species undergoes direct development and hatches out of capsules as crawl-away juveniles (Zande, 1994), the large number and relatively small size (135-145 μm) of oocytes in egg capsules laid by *B. naticoidea* suggest planktotrophic development (Warén and Bouchet, 2001). In addition, the limited dispersal potential inherent in direct development provides no obvious mechanism for the colonization of new seeps, nor does it explain the wide distribution of this species at isolated cold seeps. The success of *B. naticoidea* at colonizing patchy cold-seep sites across a wide geographic range probably results from dispersal of a free-swimming larval stage. This study aims to re-investigate the developmental mode and early life history processes of this species. Do the larvae of *B. naticoidea* undergo ontogenetic vertical migration in order to feed on plankton in the euphotic zone, therefore encountering high currents and potentially dispersing long distances?

CHAPTER II

SEASONAL BREEDING, OVIPOSITION, AND DEVELOPMENT IN THE COLD-SEEP GASTROPOD *BATHYNERITA NATICOIDEA*

Introduction

Cold seeps in the marine environment are characterized by continual seepage of hydrocarbons and production of hydrogen sulfide, allowing chemosynthetic bacterial communities to flourish year-round (MacAvoy *et al.*, 2002). Primary production by bacteria, in turn, supports formation and maintenance of a diverse heterotrophic community. Orton (1920) first postulated that organisms living in the deep-sea would not be subject to seasonal fluctuations in food availability or environmental factors (i.e., temperature, day length) and should therefore reproduce continuously. However, a growing body of evidence indicates that many deep-sea invertebrates reproduce seasonally. Reproductive seasonality has been documented in a variety of deep-sea taxa, including isopods, brachiopods, bivalves, and echinoderms (Harrison, 1988; Tyler, 1988; Bishop and Shalla, 1994). Energy allocated to reproduction in the deep sea can be obtained from either surface primary production (in the form of phytodetritus) or chemosynthetic primary production originating at cold seeps or hydrothermal vents (Childress and Fisher, 1992; Van Dover, 2000).

Continuous breeding in the deep sea can often be recognized by gametogenic asynchrony among individuals in a population. Asynchrony can occur either when individual gametogenic cycles are staggered, causing the population to spawn year-round even if individuals spawn only once, or when individuals undergo continuous gametogenesis and spawn frequently (Rokop, 1974; Eckelbarger and Watling, 1995). Seasonal reproduction is indicated by synchronous gametogenic cycles between individuals in a population, with a periodic signal in mature vitellogenic oocytes (Grant

and Tyler, 1983). Oocyte size-frequency analysis is a more powerful means of detecting seasonal gametogenic cycles than gonad or maturity indices (Grant and Tyler, 1983).

The reproductive cycle of marine invertebrates entails several discrete processes, including initiation of gametogenesis, spawning or oviposition, larval duration, and, in the case of snails that lay benthic egg capsules, release of larvae (Tyler *et al.*, 1982; Eckelbarger and Watling, 1995). When a population undergoes synchronous gametogenesis or spawning events, an exogenous factor such as food availability, temperature, day length, or phase of the moon is often responsible for controlling gonad development (Giese and Pearse, 1974). For example, phytoplankton blooms induce spawning events in some shallow-water invertebrate species (Starr *et al.*, 1990), and gamete production frequently is correlated with the timing of phytodetrital food pulses in deep-sea communities that depend entirely on photosynthetic primary production (Tyler and Gage, 1984; Tyler, 1988).

Larvae of marine invertebrates may generally be categorized as planktotrophic or lecithotrophic (Thorson, 1950; Mileikovsky, 1971), but some larvae exhibit facultative planktotrophy, an intermediate pattern in which larvae are given enough yolk reserves to reach metamorphic competence without feeding, but have structures (i.e., mouths, ciliated bands) that enable supplemental feeding during the planktonic lifetime (Mileikovsky, 1971, Hadfield, 1972, Perron, 1981, Kempf and Hadfield, 1985). The extent of feeding during the planktonic stage can result in differences in larval body size at metamorphosis, juvenile growth, and juvenile mortality (Emlet, 1986).

Bathynerita naticoidea Clarke 1989 is a bathyal detritivorous snail endemic to hydrocarbon seeps in the Gulf of Mexico and the southern Barbados accretionary prism at depths from 400-2100m, where it is the most abundant gastropod in mussel bed communities (Carney 1994; Olu *et al.* 1996; Zande and Carney 2001; Bergquist *et al.* 2005). Although a previous study of the life history of *B. naticoidea* provided evidence that this species undergoes direct development and hatches out of capsules as

crawl-away juveniles (Zande, 1994), the large quantity and relatively small size (135-145 µm) of oocytes in egg capsules laid by *B. naticoidea* suggest planktotrophic development (Warén and Bouchet, 2001). Direct development is also problematic because limited dispersal provides no obvious mechanism for the colonization of new seeps, nor does it explain the wide distribution of this species at isolated cold seeps.

This study investigates the timing of reproduction in *Bathynerita naticoidea* using histological methods and observations of oviposition and egg capsule contents both in the laboratory and *in situ*. In addition, the embryology of *B. naticoidea* is described and the developmental mode explored.

Materials and Methods

Study Site and Sample Collections

Adult *Bathynerita naticoidea* were collected by the Johnson-Sea-Link I & II submersibles (Harbor Branch Oceanographic Institution) on cruises between 2002 and 2005 to Brine Pool NR1 ("the Brine Pool") and several other hydrocarbon seeps along the Louisiana continental slope of the Gulf of Mexico (Table 2.1). No attempt was made to keep snails from different sites separate. The Brine Pool, located approximately 180 km south of New Orleans, LA, in the Gulf of Mexico, is a methane-enriched "lake" of brine (salinity 120) surrounded by a dense bed of *Bathymodiolus childressi*, a hydrocarbon seep mussel with methanotrophic endosymbionts (Childress *et al.* 1986; MacDonald *et al.* 1990). The mussel bed provides habitat for an assemblage of endemic heterotrophic fauna including gastropods, galatheid crabs, alvinocarid shrimp and orbinid worms (MacAvoy *et al.*, 2002).

Table 2.1. *Bathynerita naticoidea* collections from several cold seeps on the northern Gulf of Mexico continental slope. Gametogenic samples collected and processed for histological analysis of oocyte size frequency; N refers to the number of females processed and n refers to the number of oocytes measured. Live adult samples were used in embryological and development mode studies. Distribution samples of egg capsules were collected haphazardly from the inner, middle, and outer zones of the Brine Pool mussel bed by the Johnson Sea Link submersibles (HBOI).

Date	Location	Coordinates	Zone	Collection type	N	
March 9, 2002	Brine Pool	27°43.4157N, 91°16.756W		Gametogenesis	4	400
October 2002	MC 929	28°01.471N, 89°43.634W		Live Adults		
October 2002	Brine Pool			Live Adults		
February 2003	GC 234	27°44.7318N, 91°13.4355W		Live Adults		
February 2003	Brine Pool			Live Adults		
February 2003	Brine Pool		Inner	Distribution	1	
February 2003	Brine Pool		Middle	Distribution	1	
February 2003	Brine Pool		Outer	Distribution	1	
February 13, 2003	Brine Pool			Gametogenesis	10	1000
November 2003	Bush Hill	27°46.9478N 91°30.5266W		Live Adults		
November 2003	GC 234			Live Adults		
November 2003	Brine Pool			Live Adults		
November 2003	Brine Pool		Inner	Distribution	3	
November 2003	Brine Pool		Middle	Distribution	3	
November 2003	Brine Pool		Outer	Distribution	3	
December 16, 2003	Brine Pool			Gametogenesis	10	1000
February 2004	Brine Pool			Live Adults		
February 24, 2004	Brine Pool			Gametogenesis	7	700
July 2004	Brine Pool			Live Adults		
July 2004	Brine Pool		Inner	Distribution	3	
July 2004	Brine Pool		Middle	Distribution	3	
July 2004	Brine Pool		Outer	Distribution	6	
July 7, 2004	Brine Pool			Gametogenesis	11	1100
September 2005	GC 234			Live Adults		
September 19, 2005	GC 234			Gametogenesis	11	1100

Oogenesis

Bathynerita naticoidea were collected in February, March, July, September, and December between 2002 and 2005 (Table 2.1). Gonad samples span the course of several years and are grouped into a composite "year" to investigate maturation patterns of oogenesis. Samples are designated in the text by their month only, except in the case of February, for which two samples from different years were processed.

Freshly collected snails were preserved immediately in 4% formalin for 24 hours then transferred into 70% ethanol for storage. Shells and opercula were removed manually with forceps before dehydration in an ethanol series. Specimens were transferred into toluene for 24 hours then embedded in paraffin (mp = 52°C) blocks by the step-wise addition of liquid paraffin. Seven-µm sections were cut, mounted on glass slides, stained with Mayer's hematoxylin and counterstained with 0.5% Eosin. Finally, glass coverslip were permanently fixed to the slides using Permount adhesive.

Photographs of at least three slides from each animal were taken with an Optronics Microfire model S99808 camera system mounted on an Olympus BX50 compound microscope. The areas of one hundred oocytes from each individual were measured using UTHSCSA Image Tool analysis software. To avoid resampling oocytes, only sections with a visible nucleolus were measured. Since oocytes are packed tightly into the ovary they tend to be irregular in shape, therefore oocyte feret diameters were calculated [Feret diameter = $((4 \times \text{area}) / \pi)^{1/2}$] to standardize oocyte size and used in all analyses. Mean oocyte sizes were compared within and between months for reproductive synchrony by a random effects nested design ANOVA.

Egg Capsule Distribution

To determine the spatial distribution of egg capsules laid by *Bathynerita naticoidea,* samples were collected at the inner (next to the brine), middle, and outer (bordered by sediment) zones of the Brine Pool mussel bed in February 2003, November 2003, and July 2004 (Table 2.1). Each sample was collected haphazardly by taking several adjacent scoops with the Johnson-Sea-Link submersible arm within a

discreet area of the mussel bed. Only one sample was taken in each of three zones in February 2003. Three replicate samples in each zone were taken in November 2003 and July 2004 except for the outer zone in November 2003, where six replicate samples were obtained. Because the areas sampled were not quantitative, we expressed egg capsule abundance within each sample in terms of capsules per square meter of mussel surface area. The total mussel surface area of each replicate sample was calculated by measuring the length of each mussel (L) within a sample and converting it to surface area (SA) using the equation $SA = 0.5794^{L2.06}$ (Bergquist, personal communication). Because no egg capsules were found in July and replicate samples were not taken in February 2003, egg capsule density between zones of the Brine pool was analyzed statistically for November 2003 only. Log-transformed density was analyzed by one-way ANOVA followed by Tukey's HSD post hoc multiple comparisons.

Culture Methods

Bathynerita naticoidea began to lay egg capsules on *Bathmodiolus childressi* mussel shells in the laboratory during the winter 2003. Observations spanned three reproductive periods between 2003 and 2005. All adults and egg capsules were housed in a walk-in cold room at 7-9 °C throughout the reproductive season. Egg capsules were separated from each other immediately after deposition and placed in 2-ml wells filled with cold (8 °C) 0.45µm-filtered seawater (CFSW) until hatching. The water was changed in these wells once a week. Veligers hatched out of capsules from May to July. Once hatched, all veligers from each capsule were placed into either 175-ml glass dishes with CFSW or combined with many capsules that hatched on the same day into 2-L glass jars. Several methods of larval culture were used in 2003 in the attempt to keep larvae alive for extended periods of time. The use of antibiotics was necessary to maintain cultures longer than two weeks. Thus, all larval cultures and experiments in 2004 and 2005 were maintained in CFSW with 10 µg/L chloramphenicol. Water was changed using a reverse filtering method every 2 to 3 days with a 100 µm nitex-mesh filter. Veligers were fed a mix of *Thalassiosira pseudonana* and *Isochrysis galbana* at

concentrations of 5,000 to 10,000 cells/ml every other day (Strathmann, 1987). These methods apply to all larval experiments and observations unless otherwise indicated.

Several hundred egg capsules were pried open with forceps to observe developmental stages between January and May 2004 and 2005. Embryos within 175 egg capsules were allowed to develop completely within capsules to re-investigate development mode.

Developmental Mode

To examine feeding capabilities at the ambient temperature at the Brine Pool and at temperatures found throughout the water column in the Gulf of Mexico, twenty recently hatched larvae were placed at 8, 10, 12, and 15 °C within 20-ml glass scintillation vials. Only one sample was examined at each temperature. Two water baths (5 and 40 °C) circulated water through open cavities at each end of a solid aluminum block, cooling and heating the respective sides to maintain a stable temperature gradient across the block. The block was designed to hold 20-ml glass vials in 10 rows of 4, spaced at equal distances along the block. Temperature was monitored in one well in each row throughout the experimental period. Larvae were placed in 20 ml of CFSW then acclimated to the desired temperature. Larvae were starved for three days and then fed a mix of *Rhodomonas lens* and *Isochrysis galbana* at concentrations of 5,000 to 10,000 cells/ml. Within twenty minutes, gut fluorescence was visualized in five larvae from each treatment using an Olympus BH-2 compound epifluorescence microscope with a 100W mercury vapor lamp and a blue excitation filter (488 nm). A control treatment placed at 8 °C was starved and checked to ensure the larval gut did not autofluoresce.

Results

Oogenesis

Oocytes examined in this study ranged from 5 to 180 µm in feret diameter. Vitellogenesis in this species begins at oocyte diameters of 25-30 µm. Previtellogenic oocytes are defined in this study as those less than 30 µm in diameter, vitellogenic oocytes between the diameters of 31 and 80 µm, and late vitellogenic oocytes, characterized by the abundance of lipid droplets within the oocyte, are defined as those greater than 81µm in diameter. Ova are 135-145 µm in diameter when deposited in egg capsules.

Synchronicity of Oocyte Development

Comparisons of oocyte size-frequency distributions among individuals in each sample indicate synchronous gametogenesis. Cohorts of vitellogenic oocytes began to develop in July in 91 % of the individuals sampled (Figure 2.1). These cohorts of vitellogenic eggs appear to increase in size in all individuals in the September sample relative to the July sample. The variation in oocyte size-frequencies between individuals seen in December and February are consistent with a prolonged spawning period during which *Bathynerita naticoidea* presumably lays numerous egg capsules.

Reproductive Seasonality

A clear seasonal pattern in mean oocyte size-frequency across months was detected by a random effects nested design analysis of variance ($F = 7.29$, $p < 0.001$). Mature oocytes are deposited in egg capsules in the late winter (December and February samples) and gonads appear spent by March as indicated by the decrease in proportion of vitellogenic and late vitellogenic oocytes (Figure 2.2). Vitellogenesis is initiated in the summer, as indicated by the increase in the proportion of vitellogenic oocytes (~50%) in the July sample relative to March and February 2003. However, no

difference was detected between the July and February 2004 oocyte size frequencies (Tukey post hoc, $p = 0.1$).

Large oocytes were present in individuals from all months, indicating incomplete spawning of oocytes, however the relative proportion of vitellogenic and late-vitellogenic oocytes in each sample showed a seasonal pattern. A high ratio of previtellogenic: vitellogenic oocytes (26% to 65%) was found in all individuals in all months, resulting in a bimodal distribution in the July, September, December and February 2004 samples (Figure 2.3). Previtellogenic oocytes dominated the size-frequency distributions in December, February 2003, and March samples (> 50%), resulting in a left-skewed, unimodal distribution in the February 2003 and March samples. The December and February 2004 sample showed increased variability among individuals in oocyte size frequency, with some spent snails with decreased proportions of large oocytes relative to the September sample, which is consistent with the onset of spawning in the population in December. Vitellogenic and late vitellogenic oocytes dominated the February 2004, July, and September samples. The September sample was in an advanced state of vitellogenesis as seen by the high proportion of vitellogenic (49%) and late vitellogenic oocytes (26%). The oocyte size-frequency from populations collected in February 2003 and 2004 were significantly different ($p < 0.001$, Tukey's HSD post hoc).

Egg capsule distribution

Egg capsules were relatively abundant in February and November, but no intact egg capsules were collected from the July samples, suggesting periodicity in oviposition of *Bathynerita naticoidea.*

Egg capsule density was significantly less in the inner zone than the middle zone of the Brine Pool mussel bed in November 2003 ($F = 8.5$, $p = 0.018$; Figure 2.4). Density was not significantly different between the inner and outer zone, or the middle and outer zone (Tukey's HSD post hoc multiple comparison, $p = 0.051, 0.671$, respectively).

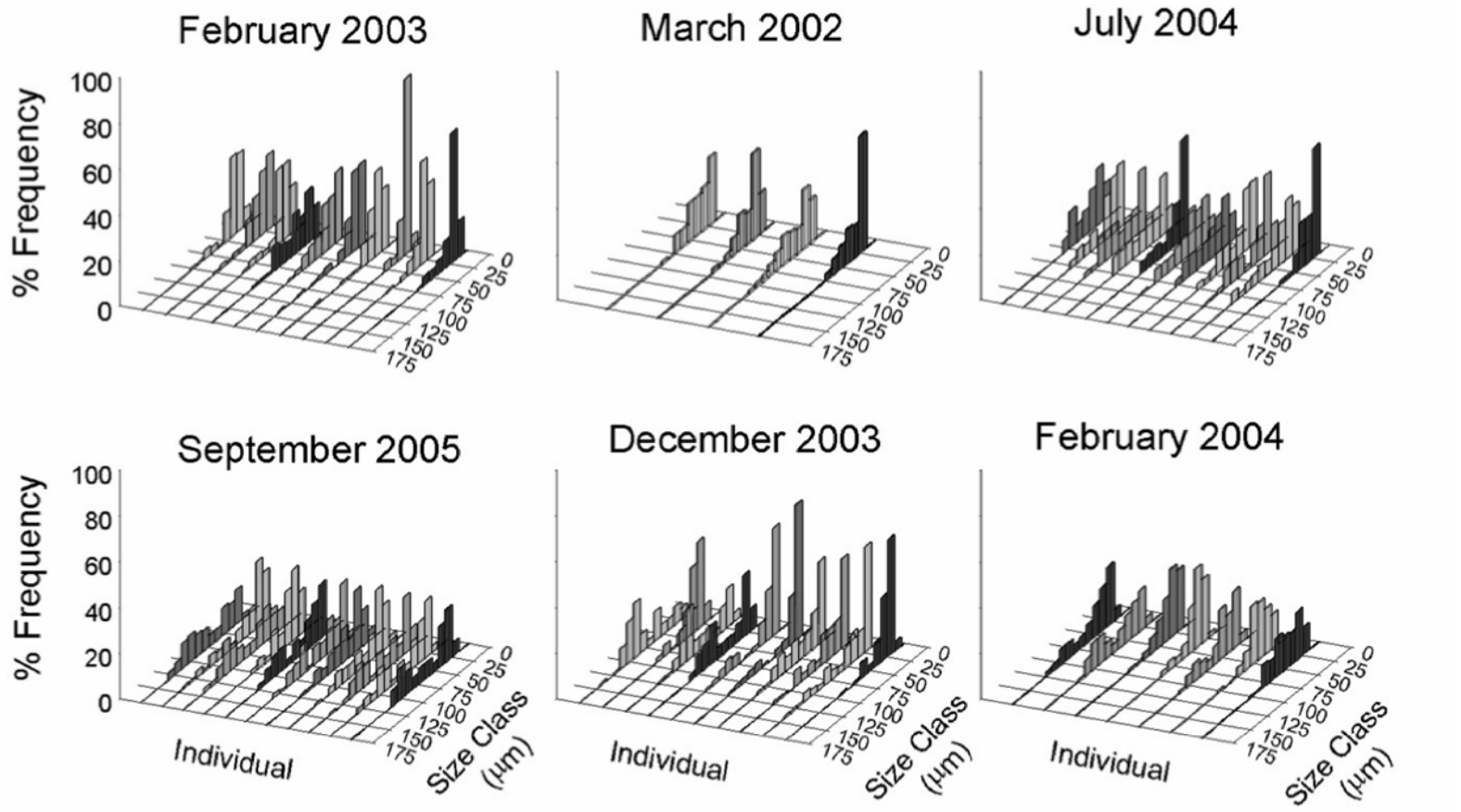

Figure 2.1. Size-frequency distributions of 100 oocytes each taken from individual *Bathynerita naticoidea* in December 2003, February 2003 and 2004, March 2002, July 2004, and September 2005. Note synchronous gametogenesis in July and September samples and increased variability among individuals in samples collected in December and February 2003 and 2004, indicating spawning.

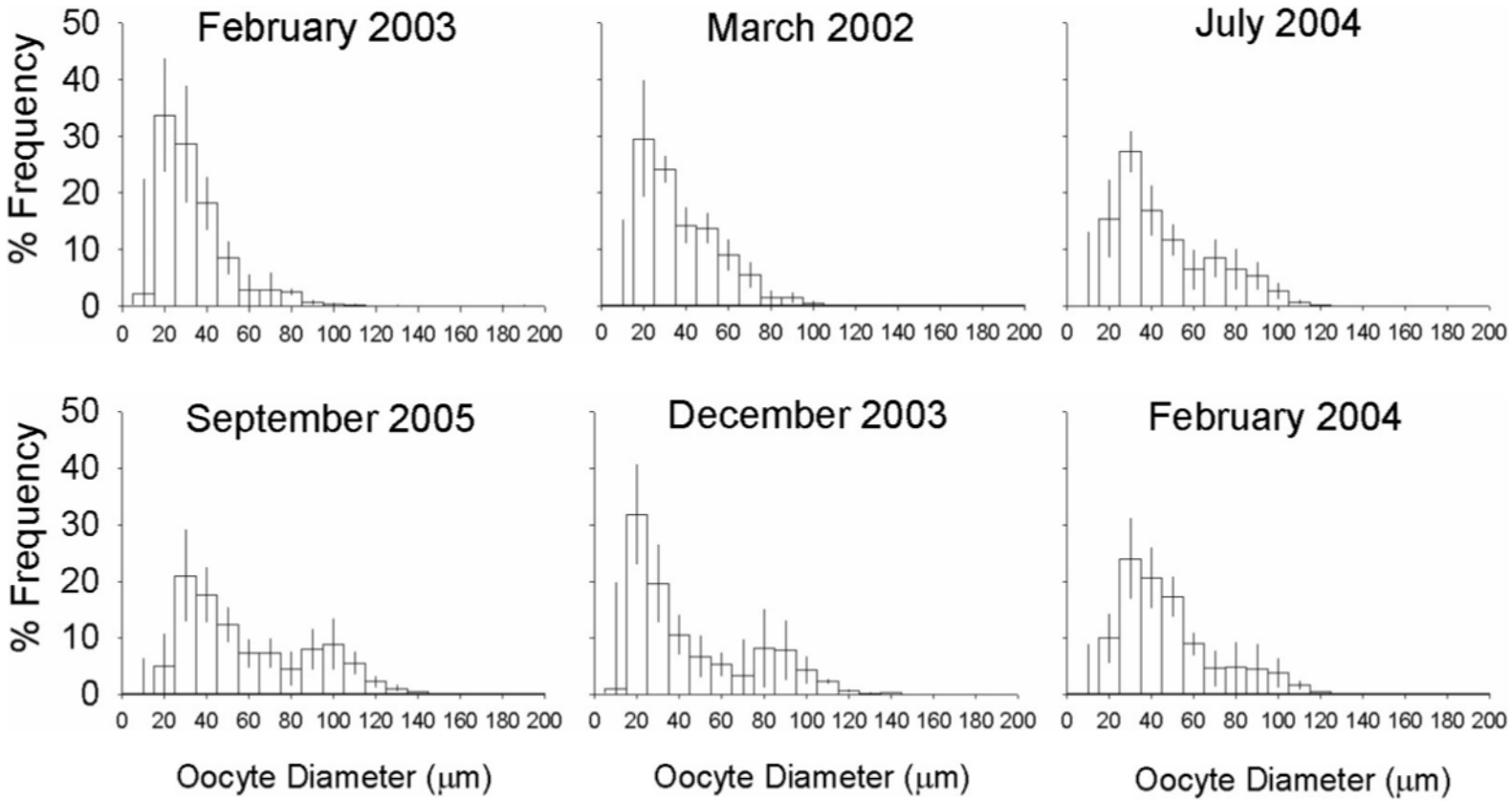

Figure 2.2. Mean size frequency of oocytes of *Bathynerita naticoidea*. A cohort of vitellogenic oocytes appear in July which show an increase in maturity and proportion in September. December shows a slight decrease in the proportion of late vitellogenic oocytes suggesting onset of oviposition. Error bars are ± 1SD.

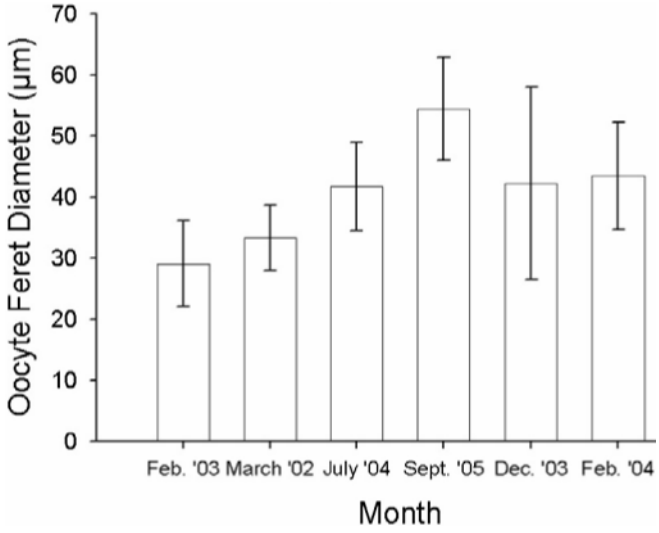

Figure 2.3. Composite monthly mean oocyte diameters of *Bathynerita naticoidea* indicate a seasonal pattern of gametogenesis. Error bars refer to ± 1 SD.

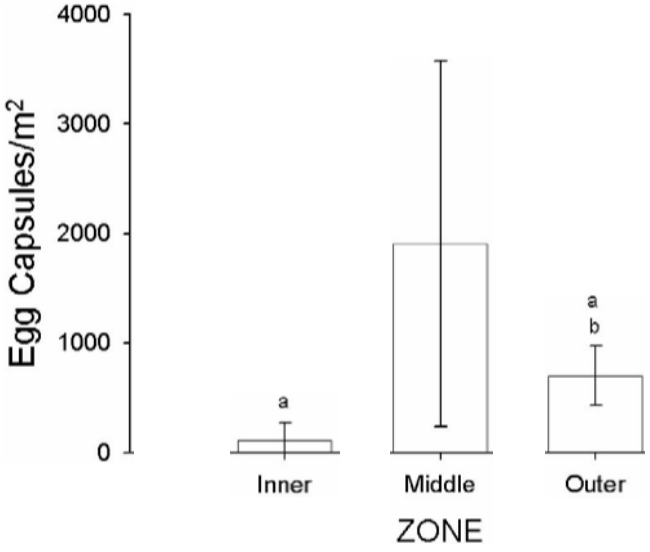

Figure 2.4. Mean ± 1 SD egg capsule distribution across zones of the Brine Pool mussel bed.

Descriptive Embryology

Egg capsules from *Bathynerita naticoidea* were found on various hard substrata in the field and laboratory including *Bathymodiolus childressi* mussels, disarticulated mussel shells, the shells of conspecifics and other snails, tubeworm tubes, and glass aquaria. Egg capsules ranged in size from 1.2 x 0.9 mm to 2.9 x 2.15 mm, and contain between 25 and 180 embryos (Waren & Bouchet, 2001; Zande, 1994). Although the top of the egg capsule is reinforced with mineral particles (Andrews, 1935; Zande, 1994), it is sufficiently thin that the general developmental state of embryos within capsules can be predicted by the color of the capsule. Capsules containing oocytes, early embryos, or pre-veligers maintain a creamy ivory color. Late-stage veligers can be detected by the dark purple color of the capsule due to the pigmentation of the larval eyespots, esophagus, gut, and intestine of the veligers within.

Live encapsulated oocytes were 143.7 ± 1.6 μm (1 SD, n=17) in diameter and lacked an egg envelope. Bouchet and Waren (2001) report egg diameters of 90-100 microns, however, the oocytes examined were probably shrunken by fixation. Eckelbarger and Young (1997) report the largest egg sizes seen in gonadal tissue samples fixed for TEM were 135-145 μm, and this value corresponds with the maximum egg size seen in histological sections of the ovary, and live oocytes measured in this study. We did not observe nurse eggs within the egg capsules. However, a viscous liquid was observed in capsules containing oocytes, but not in capsules containing later stage veligers.

Large germinal vesicles are present in oocytes immediately after encapsulation, which demonstrates that, like many prosobranch species that deposit egg capsules, oocytes are deposited immediately after fertilization before the germinal vesicle has time to break down (Webber, 1977). This is consistent with other neritids, which store sperm in the pallial oviduct, and fertilization occurs as the oocytes pass by this structure (Webber, 1977). Embryonic development follows the holoblastic spiral cleavage pattern typical of gastropods (Figure 2.5). First cleavage is meridional and

occurs approximately 60 hours after capsule deposition. Successive cleavages take place approximately every 24 hours. The first two cleavages are equal and a polar lobe is not present. The third cleavage is unequal and typically spiral with a clear segregation of yolk to the large macromere quartet. A “molluscan cross” typical of the phylum appears in 32-cell embryos. An elongate ciliated trochophore stage is passed within the capsule. The earliest veligers observed in capsules were not pigmented and possessed both a ciliated larval foot and velum. Oocytes and embryos were negatively buoyant.

Encapsulated development occurs for approximately four months at ambient temperature (8 °C) in the laboratory. *Bathynerita naticoidea* laid egg capsules in the laboratory from October to March, with the majority of capsules laid between late December and February. Between May and early July, swimming veligers hatched out of the capsules that were laid between late December and February. Development of embryos in the capsules laid prior to December was not followed. If we assume that all embryos require approximately four months to develop, hatching would take place between February and July, with the majority of capsules hatching between May and early July. Hatched veligers measured 170.6 µm ± 4.9 SD (size range: 120 to 278 µm, n = 28) along the longest axis at hatching and possess a ciliated foot, pigmented eyespots, gut, and digestive tract.

Developmental Mode

Algal fluorescence within the larval gut of recently hatched veligers was observed but not quantified in all four temperature treatments (8, 10, 12, and 15 °C), with no observable difference between treatments (Figure 2.6). The gut of the unfed control larvae did not autofluoresce.

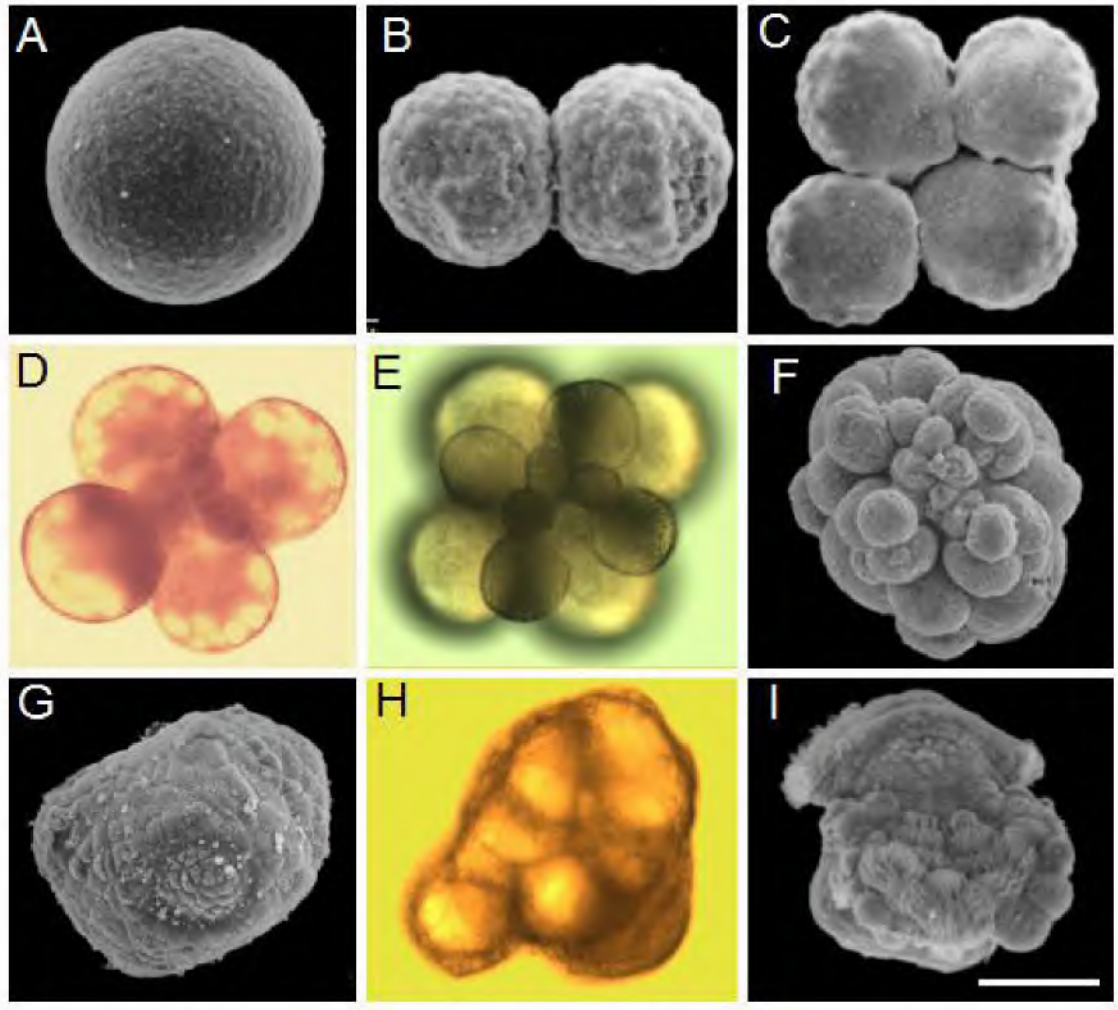

Figure 2.5. Intracapsular development of *Bathynerita naticoidea.* (a) oocyte, (b) 2-cell, (c) four-cell, (d) 8-cell, (e) 12-cell embryo illustrates staggered cleavage of the micro- and macromeres, (f) 32-cell exhibits the “molluscan cross” typical of the phylum, (g) trochophore, note the developing shell gland, (h) pre-veliger, (i) pre-veliger with a ciliated velum and foot. Scale bar represents 50 µm and applies to all photographs.

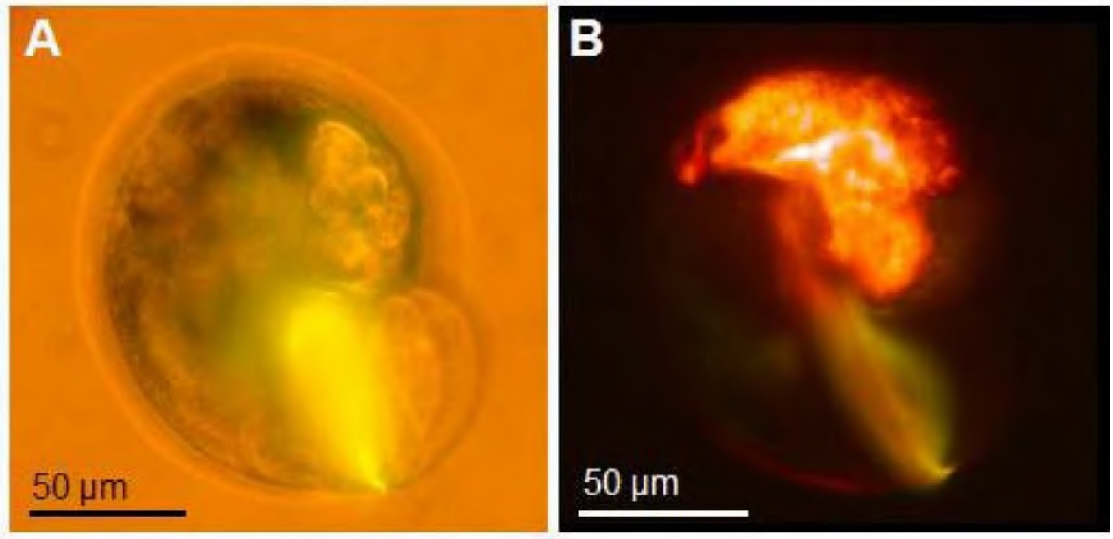

Figure 2.6. Recently hatched *Bathynerita naticoidea.* (a) light micrograph of an unfed larva and (b) epifluorescence micrograph of a larva fed a mix of *Thalassiosira pseudonana* and *Isochrysis galbana.* Microalgae fluoresces bright red in the larval gut, indicating ingestion of microalgae.

Discussion

Gametogenesis appears to be synchronous and seasonal in *Bathynerita naticoidea* at cold seeps on the Northern Gulf of Mexico continental slope. Variation in oocyte size frequencies was greater between months than between individuals. Cohorts of vitellogenic oocytes begin to develop in the summer and increase in size and proportion through the fall. The variation in oocyte size-frequencies among individuals seen in December and February is consistent with a prolonged period of oviposition in the winter months.

Cohorts of pre-vitellogenic oocytes were found in all individuals examined. Ultrastructural evidence indicates that *B. naticoidea* uses both heterosynthetic and autosynthetic pathways of vitellogenesis consistent with “fast” egg production

(Eckelbarger and Watling, 1995; Eckelbarger and Young, 1997). It is common for prosobranch gastropods to have all stages of gametogenesis regardless of seasonality (Webber, 1977). It appears that *B. naticoidea* produce pre-vitellogenic oocytes throughout the year and store mature oocytes until oviposition.

Egg capsules were abundant in samples collected from the Brine Pool in February and November. While the limited sampling frequency is not ideal for seasonal studies, the fact that nearly 2000 egg capsules were collected in November and no egg capsules were collected in July by the same sampling effort provides strong evidence that oviposition is periodic at the Brine Pool.

Egg capsule abundance was significantly lower in the inner zone of the Brine Pool than the middle zone in November 2003. Egg capsule density was also less in the inner zone than the outer zone, but not significantly so ($p = 0.051$). The pattern of abundance in the middle zone was highly variable between collection dates, a fact that reflects the patchy distribution of egg capsules and supports the conclusion made by Smith *et al.* (2000) that the middle zone is not a distinct zone, but a transitional area between the inner and outer zone.

Within the Neritidae, parental investment in nutrition of encapsulated eggs involves the inclusion of varying amounts of albumin or nurse eggs (Andrews, 1935; Webber, 1977). Typically, nurse eggs are not fertilized or abort at an early stage (Webber, 1977). Although nurse eggs are not provided by *B. naticoidea,* several lines of evidence indicate that veligers may be feeding while encapsulated. First, encapsulated early veligers continually beat their vela, presumably to feed off nutrients contained within the capsule. When the inner membrane that encloses oocytes is broken open, a viscous liquid seeps out, but when this membrane is broken in capsules with late-stage veligers, the liquid was no longer viscous. While this study did not investigate the nutritive contents of the egg capsule, we suspect that the viscous liquid initially enclosed in the inner capsule membrane is albumin. In addition, one live veliger from a mechanically opened capsule in which dead embryos and a copious amount of lipid was contained measured 278 μm in length, nearly 100 μm larger than

the average, indicating that encapsulated veligers are capable of nutritional uptake and may eat dead sibling embryos when available.

Embryos develop within capsules for approximately four months. Egg capsules were deposited in the laboratory between October and March, with peak oviposition between December and February. Veligers were observed to hatch out of capsules between May and early July, although the development of embryos from capsules deposited before December was not followed. Assuming that all embryos require four months to develop before hatching as swimming veligers, hatching in the laboratory would be expected between February and July, with peak hatching between May and early June.

The relatively large number and small size of embryos within egg capsules laid by *B. naticoidea* indicate planktotrophic development (Thorson, 1950; Bouchet and Waren, 2001). This study provided direct evidence of planktotrophy in the larvae of *B. naticoidea.* Larvae were shown to feed on microalgae immediately after hatching, however, we were not able to demonstrate whether they are obligate of facultative planktotrophs. Nevertheless, the long larval lifespan (> 90 days) and large size of larvae at settlement (600 – 700 μm) suggests that *B. naticoidea* are probably obligate planktotrophs (Chapter 3).

While no attempt was made to investigate a causal relationship between reproductive seasonality and environmental variables, cues to the seasonal flux of phytodetritus from the surface waters may be provided by the detritivorous feeding behavior of adult snails (Zande and Carney, 2001). Reproduction in a number of deep-water echinoderms with inferred planktotrophic development from the Rockall Trough synchronized reproduction so that release of larvae coincided with the spring phytoplankton bloom (Tyler and Gage, 1983). In contrast, echinoderms with inferred lecithotrophic development from the same collections exhibited asynchronous reproduction (Tyler and Gage, 1983). The larvae of *B. naticoidea* are released during the spring, roughly around the time of the spring phytoplankton bloom in the Gulf of Mexico, which suggests a coupling between production in surface waters and the

release of larvae (Müller-Karger *et al.*, 1991). The selective pressure for seasonal reproduction in this species probably derives from the necessity of the planktotrophic larvae to obtain an adequate amount of food in the euphotic zone during the spring phytoplankton bloom.

The seasonal reproduction of *Bathynerita naticoidea* shown by histological studies of vitellogenesis has important implications for the early life history and distribution of this species. The planktotrophic developmental mode, and timing of release of larvae of *B. naticoidea* during the spring, roughly around the time of the spring phytoplankton bloom in the Gulf of Mexico, suggests a coupling between production in surface waters and the release of larvae. The selective pressure for seasonal reproduction in this species probably derives from the necessity of the planktotrophic larvae to obtain an adequate amount of food in the euphotic zone during the spring phytoplankton bloom. The correlation between release of larvae and the spring phytoplankton bloom led to the following investigation into the potential ontogenetic migration and dispersal potential of *B. naticoidea*.

CHAPTER III

LONG-DISTANCE LARVAL DISPERSAL OF THE COLD-SEEP GASTROPOD *BATHYNERITA NATICOIDEA*

Introduction

The larval dispersal of benthic invertebrates is dependent upon the length of larval life, swimming behavior of the larva (i.e., depth distribution), and prevailing current patterns (Thorson, 1961; Scheltema, 1966, 1971). Planktotrophic larvae generally have longer planktonic lives and a potential for wider dispersal than lecithotrophic larvae. Trans-oceanic (teleplanic) dispersal may not be uncommon; several genera of shallow-water planktotrophic gastropod veligers with widespread adult distribution have been found in mid-Atlantic Ocean surface plankton tows (Thorson, 1961; Scheltema, 1966, 1971), and several forms of sipunculan larvae have also been found across the tropical Pacific, Atlantic, and Indian Oceans (Scheltema and Rice, 1990). However, the potential of deep-sea larvae to undergo trans-oceanic dispersal probably depends upon their ability to vertically migrate to the productive surface waters where high currents are prevalent.

Whether deep-sea animals produce planktonic larvae that migrate vertically to the euphotic zone has been a subject of much debate for the past century. The idea that deep-water animals could have surface-dwelling larvae was first suggested by Moseley (1880) following the Challenger expedition. This idea was then overshadowed by Thorson's (1950) view that the vertical migration of abyssal larvae was energetically impossible due to food limitation.

A number of recent papers have effectively disproven “Thorson’s Rule” as a common phenomenon in the deep-sea (reviewed by Pearse, 1994; Young, 1994; Young, 2003). Larvae of some deep-sea gastropods, brachiopods, and holothurians have been found in surface plankton tows, providing direct evidence for ontogenetic vertical migration (Ashworth, 1914; reviewed by Bouchet and Warén, 1994; Pawson *et al.*, 2003). Protoconch analyses in mollusks and the presence of larval eyes in many abyssal species whose adult forms lack eyes suggest that vertical migration of planktotrophic larvae to the euphotic zone may be widespread (Rex and Warén, 1982; Bouchet and Warén, 1994). In addition, energetic models of bathyal echinoids with planktotrophic larvae suggest that energy stores may not be limiting in many deep-sea species (Young *et al.*, 1996a). Indeed, contrary to Thorson’s hypothesis, larval physiological tolerances to the physical conditions of the euphotic zone may be more important indicators of the potential for ontogenetic migration (Young and Tyler, 1993; Young *et al.*, 1996a; Young *et al.*, 1996b).

Deep-sea hydrothermal vents and seeps are patchy habitats that can be tens to hundreds of kilometers apart. Given these distances, one would expect to find a high proportion of endemic species with long-lived planktotrophic larvae capable of dispersing long distances. However, there seems to be a dominance of lecithotrophic development within the hydrothermal vent fauna (Lutz *et al.*, 1984; Lutz, 1986, 1988; Tyler and Young, 1992; Gustafson and Lutz, 1994; Mullineaux and France, 1995). For example, out of 39 mollusk species endemic to the hydrothermal vents examined, only five have inferred planktotrophic development (Gustafson and Lutz, 1994). However, low metabolic rates and developmental arrest in some vent larvae in the cold environment (~2 °C) found between hydrothermal vents may increase the potential for larval dispersal of lecithotrophic vent species (Tyler and Young, 1999; Pradillon *et al.*, 2001; 2005).

We know even less about development and dispersal in cold seep assemblages than in hydrothermal vent fauna. Studies on the reproduction and early development of vestimentiferan tubeworms, *Lamellibranchia luymesi* and *Escarpia* sp. (Young *et al.*,

1996c), and preliminary studies on the iceworm, *Hesiocaeca methanicola* (Eckelbarger *et al.,* 2001), infer lecithotrophic development and a planktonic duration of at least three weeks for all three species. Based on prodissoconch morphology, Gustafson and Lutz (1994) concluded that the seep mussel *Bathymodiolus childressi* undergoes planktotrophic development, but no estimate for the length of planktonic life has been made.

Bathynerita naticoidea Clarke 1989 (Gastropoda: Neritacea) is a bathyal gastropod endemic to hydrocarbon seeps in the Gulf of Mexico and the southern Barbados Prism at depths from 400-1700 m, where it is the most abundant snail in mussel bed communities (Carney 1994; Olu *et al.* 1996; Zande and Carney 2001; Bergquist *et al.* 2005). A previous study of *B. naticoidea* development provided evidence that this species undergoes direct development and hatches out of capsules as crawl-away juveniles (Zande, 1994). However, the large quantity and relatively small size (150-200 μm) of encapsulated embryos (Warén & Bouchet, 2001), and recent evidence on feeding and hatching mode demonstrates that this species has a planktotrophic veliger stage (see Chapter 2). The success of *B. naticoidea* at colonizing patchy cold-seep sites across a wide geographic range probably results from dispersal of a free-swimming larval stage. Do the larvae of *B. naticoidea* undergo ontogenetic vertical migration in order to feed on plankton in the euphotic zone, therefore encountering high currents and potentially dispersing long distances? Alternatively, do they take up a demersal life, dispersing near the sea floor in relatively slow currents and cold temperatures, potentially colonizing cold seeps following the stepping-stone dispersal model?

The possibility of vertical ontogenetic migration to the euphotic zone by the larvae of *Bathynerita naticoidea* was investigated by examining larval swimming speeds and behavior, by testing the physiological tolerances of larvae to the thermal and salinity conditions of the upper water column, and by seeking larvae in plankton tows collected from the upper water column. The possibility of long-distance dispersal was addressed by investigating the duration of the planktonic stage in the laboratory,

and by assessing the size range and occurrence of larvae collected in the water column and in sediment traps on the seafloor.

Materials and Methods

Study Site and Sample Collection

Adult *Bathynerita naticoidea* and egg capsules were collected primarily from Brine Pool NR1 (27°43.4157N, 91°16.756W, ~650 m depth) located approximately 180 km south of New Orleans, LA, in the Gulf of Mexico (Figure 3.1, 3.2; Table 3.1). The Brine Pool is a methane-enriched "lake" of brine (salinity 120) surrounded by a dense bed of *Bathymodiolus childressi,* a hydrocarbon seep mussel with methanotrophic endosymbionts (Childress *et al.* 1986; MacDonald *et al.* 1990), which provide habitat for a suite of endemic heterotrophic fauna including gastropods, galatheid crabs, alvinocarid shrimp and orbinid worms as well as vagrant animals such as sea stars, hagfish and brachyuran crabs from the surrounding slope community (MacAvoy *et al.*, 2002; 2003; 2005). Larval tube traps were deployed at the Brine Pool and MOCNESS plankton samples were collected from the water column above or in the immediate vicinity of the Brine Pool. Samples were collected and equipment deployed by the Johnson-Sea-Link submersibles (HBOI).

Culture methods

Bathynerita naticoidea began to lay egg capsules on *Bathymodiolus childressi* mussel shells in the laboratory during the winter 2003. Larval experiments and observations spanned three reproductive periods between 2003 and 2005. All adults and egg capsules were housed in a walk-in cold room at 7-9 °C throughout the reproductive season. Egg capsules were separated from each other immediately after deposition and placed in 2-ml wells filled with cold (8 °C) 0.45μm-filtered seawater (CFSW) until hatching. The water was changed in these wells once a week. Veligers hatched out of capsules from May to July. Once hatched, all veligers from each

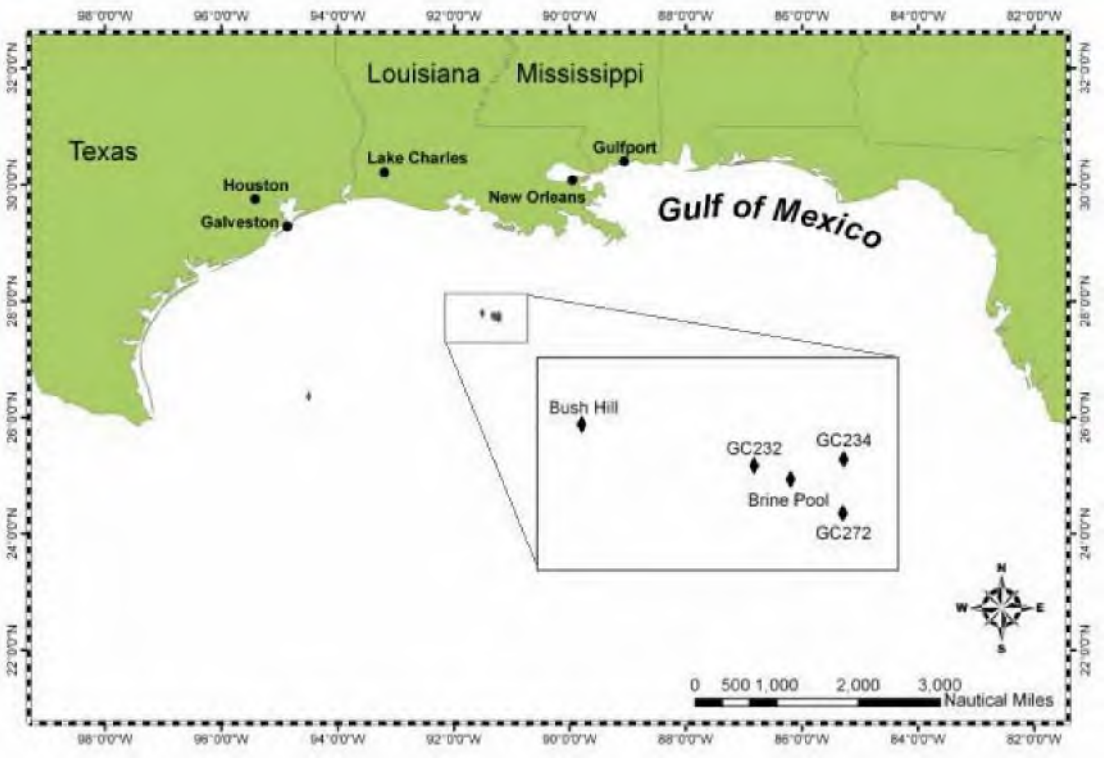

Figure 3. 1. Location of cold seep sites on the northern Gulf of Mexico continental slope.

Table 3. 1. *Bathynerita naticoidea* field collections from the Gulf of Mexico.

Date		Location	Coordinates
October	2002	MC 929	
October	2003	Brine Pool	27°43.4157N, 91°16.756W
February	2003	GC 234	27°44.7318N, 91°13.4355W
February	2003	Brine Pool	
November	2003	Bush Hill	27°46.9478N 91°30.5266W
November	2003	GC 234	
November	2003	Brine Pool	
February	2004	Brine Pool	
July	2004	Brine Pool	
September	2005	GC 234	

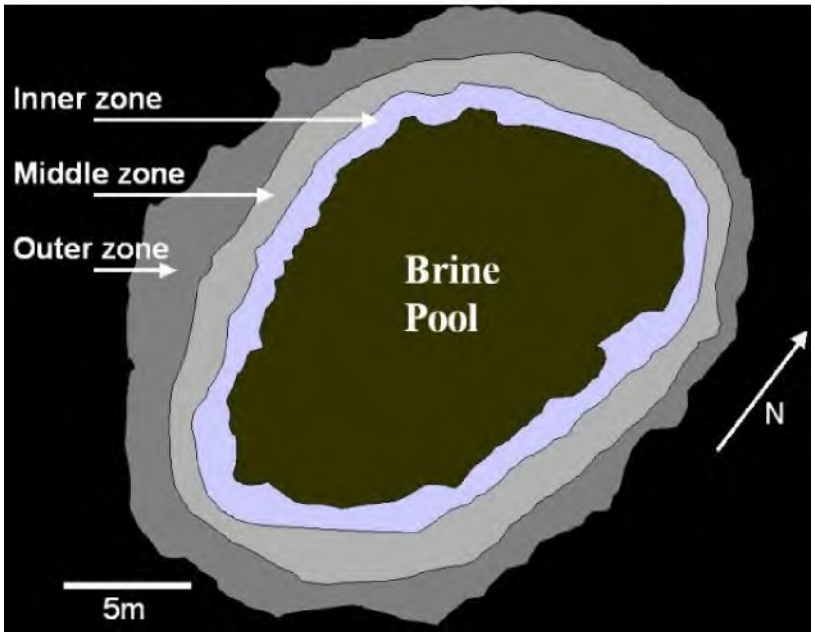

Figure 3.2. Diagram illustrating the mussel-bed zonation at the Brine Pool.

capsule were placed into either 175-ml glass dishes with CFSW or combined with many capsules that hatched on the same day into 2-L glass jars. Several methods of larval culture were used in 2003 in an attempt to keep larvae alive for extended periods of time. The use of antibiotics proved necessary to maintain cultures longer than two weeks. Thus, all larval cultures and experiments in 2004 and 2005 were maintained in CF SW with 10 µg/L chloramphenicol. Water was changed using a reverse filtering method every 2 to 3 days with a 100-µm nitex mesh filter. Veligers were fed a mixture of *Thalassiosira pseudonana* and *Isochrysis galbana* at concentrations of 5,000 to 10,000 cells/ml every other day (Strathmann, 1987). These methods apply to all larval experiments and observations unless otherwise indicated.

Larval Swimming

The initial swimming behavior was examined in recently hatched larvae of *Bathynerita naticoidea.* Two days after hatching, three subsamples of ten actively swimming larvae were taken from each of three egg capsules and placed into 40-ml tissue culture vials (8 x 4 x 2cm), for a total of nine vials. Position (swimming or resting at the bottom) of each larva within the tissue culture jars was scored daily around 2 pm. The experiment continued until fewer than 10% of the larvae were still swimming (17 days). Percent swimming was analyzed by ANOVAR using Day as a continuous fixed factor and Position as the response variable.

The vertical swimming speed of larvae of *Bathynerita naticoidea* within hours of hatching was investigated at 8, 15, 25, 30, and 35°C. Tissue culture vials (40 ml) filled with FSW were placed into a water bath at the appropriate temperature and brought up to the treatment temperature over one hour. Larvae from compiled hatched capsules were kept in an 8 °C cold room then placed directly into the desired temperature treatment. Larvae were introduced to the bottom of the vial with a Pasteur pipet. The swimming time of individual larvae was measured with a stopwatch beginning when a larva passed 1 cm above the bottom of the vial and ending at the surface, 4.6 cm above the bottom. To avoid resampling, each larva was removed with a pipet when it reached the top of the vial. Twenty larvae were placed into each of the five temperature treatments. Owing to the habit of gastropod larvae to withdraw their vela and cease swimming when disturbed, a two-hour limit was placed on each replicate so that only the first 11 to 16 larvae swimming were analyzed in each. Four additional replicates were run at 8 °C in which the first 12 to 14 larvae were timed. The top three recorded swimming speeds were extracted from each 8 °C replicate, then averaged across replicates as a measure of the maximum burst swimming speed of recently hatched *B. naticoidea* at ambient bottom temperature (8 °C).

Physiological Tolerances of Larvae

The thermal tolerance of larvae of *Bathynerita naticoidea* to temperatures found in the upper water column was investigated. A solid aluminum block designed to hold 20-ml glass vials in 10 rows of 4, spaced at equal distances along the block, was used to maintain a stable temperature gradient. Two water baths (5 and 40 °C) circulated water through open cavities at each end of the solid block, cooling and heating the respective sides. Three replicates of 20 day-old larvae from several compiled capsules were placed into five temperature treatments: 15, 25, 29, 32, and 35 °C for 72 hours and then scored for survival. Larvae were placed in glass vials filled with 20 ml of CFSW, and then all treatments were brought to their respective temperatures within ten minutes. Arcsine transformed percent survival was analyzed by one-way ANOVA with Temperature (15, 25, 29, 32, and 35 °C) as a fixed factor.

Salinity tolerance of larvae was examined in four replicates of 20-week-old larvae from several hatched capsules. Larvae were placed directly into four salinity treatments (15, 30, 45, and 60 ppt) made with Instant Ocean® sea salts. Salinity solutions were checked with a refractometer and salinity (ppt) is reported according to Re Practical Salinity Scale (PSU, 1978). Each replicate was contained in a 175-ml glass dish and kept in a dark refrigerator at 8 °C. Larval survival was scored after five days. Percent survival (arcsine transformed) was analyzed by one-way ANOVA with Salinity as a fixed factor.

Larval collections

Nine larval tube traps filled with 10% seawater-buffered formalin were placed on the mussel bed surrounding the Brine Pool from February 2003 to November 2003 (271 days) and again between November 2003 and July 2004 (247 days) for a total of 18 tube traps. Each larval tube trap was constructed of plastic PVC pipe 30 cm in length and 5 cm in diameter (6:1 aspect ratio) with a five-pound weight attached as a

base (Yund *et al.,* 1991). The contents of all tube traps were placed in 70% ethanol upon recovery for later sorting and identification.

Larvae were sampled in the water column using the Multiple Opening/Closing Net and Environmental Sampling System (MOCNESS), a sophisticated computer-controlled plankton sampling system capable of taking discreet plankton samples throughout the water column. MOCNESS tows (150 µm mesh) were taken on two cruises in the Gulf of Mexico above the Brine Pool (depth ~700 m) in February 2003 and November 2003 at either 50-m or 100-m intervals. Samples were sieved through a 1 mm-mesh and the small and large portions fixed in 10% formalin for 24 hours, then transferred into 70% ethanol for storage. The small portions of each tow were sorted for gastropod shells.

Juvenile *Bathynerita naticoidea* with intact protoconchs were collected in samples from November 2003 and intact protoconchs were used as a comparison for the larval shells from both the MOCNESS plankton tows and the sediment traps.

Results

Larval Swimming

Larvae generally swam upward immediately after hatching until they encountered the surface, at which point they retracted their vela and dropped through the water. In the first few days, most larvae dropped a few millimeters before beginning to swim again, however after several days, the larvae tended to drop all the way to the bottom before beginning to swim again. The ratio of swimming larvae dropped daily. ANOVAR results reveal a significant interaction between the number of days swimming and capsule. The larvae taken from capsule C (n=3) swam significantly longer than those of either capsule A or B ($p < 0.001$). Approximately one half of the larvae continued to swim eleven days after hatching, while the other half were rotating in circles on the bottom of the vials (Figure 3.3). Seventeen days after hatching, only 12% continued swimming.

Temperature significantly affected the vertical swimming speed of the larvae of *Bathynerita naticoidea* (ANOVA: $F = 11.8$, $p < 0.001$). Swimming speed at 8 °C was significantly slower than at 15 °C and 30 °C (Bonferroni post hoc, $p < 0.001$, $p = 0.001$, respectively), however there was no significant difference between swimming speed at 8 °C and 25 °C ($p = 0.49$) (Figure 3.4). Swimming speed was significantly slower at 25 °C than 15 °C ($p = 0.017$). Mean vertical swimming speed ± 1 SD at 8, 15, 25, and 30 °C was 0.098cm/s ± 0.033, 0.161cm/s ± 0.070, 0.115cm/s ± 0.028, and 0.151cm/s ± 0.058, respectively. The mean maximum vertical burst swimming speed± 1 SD of *B. naticoidea* at 8 °C was 0.137 cm/s ± 0.013.

Physiological Tolerances of Larvae

Survival of larval *Bathynerita naticoidea* decreased significantly with high temperatures (one way analysis of variance, $F = 207$, $p < 0.001$). *Post-hoc* Bonferroni tests revealed that the highest temperatures (32 °C and 35 °C) greatly decreased larval survival, while there was no difference in survival between temperatures below 29 ºC. All larvae in the 15, 25, and 29 °C treatments survived. Mean ± 1 SD percent larval survival was 85% ± 1.5 in the 32 °C treatment, and larvae in the 35 °C treatment suffered 100% mortality (Figure 3.5).

Between 90 and 100% of larvae survived each salinity treatment (15, 30, 45 and 60 ppt) and no significant difference in survival was found among treatments by analysis of variance ($F = 1.0$, $p = 0.42$; Figure 3.6).

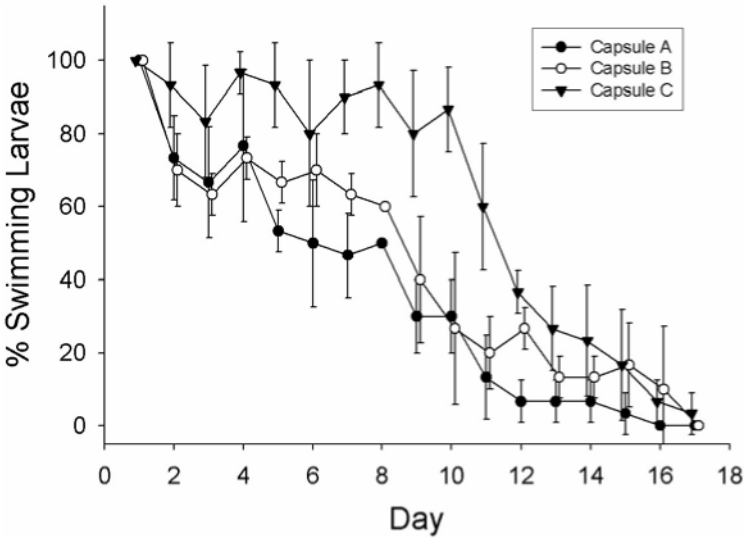

Figure 3.3. Percent of larvae of *Bathynerita naticoidea* from hatching to 18 days old that were swimming. Each line represents the mean ± 1 SD of three replicates from one capsule.

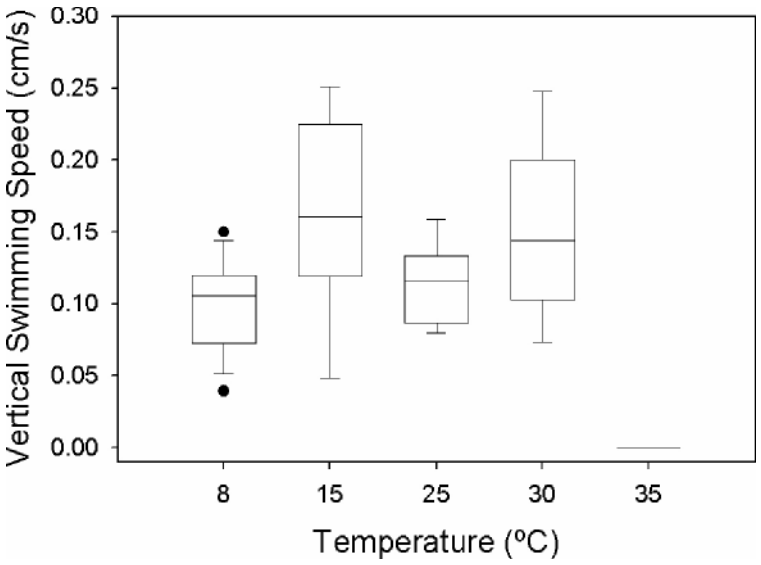

Figure 3.4. Larval swimming speeds of *Bathynerita naticoidea* at several temperatures. Each box shows the median, 25%, and 75% confidence interval. Whiskers represent the 5% and 95% confidence intervals and dots represent outliers. Note that larvae did not swim at 35 °C.

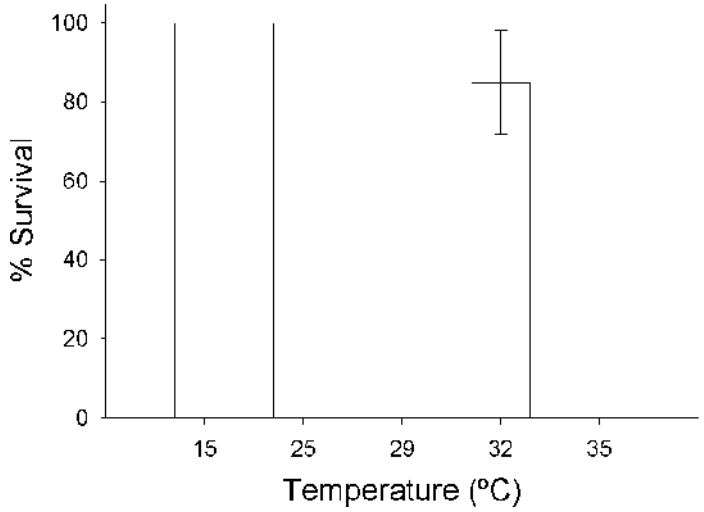

Figure 3.5. Mean thermal tolerance of recently hatched larvae of *Bathynerita naticoidea* subjected to five temperature treatments for 3 days. Error bars represent ± 1 SD. All larvae survived temperatures of 15, 25, and 29 °C and all larvae died at 35 °C.

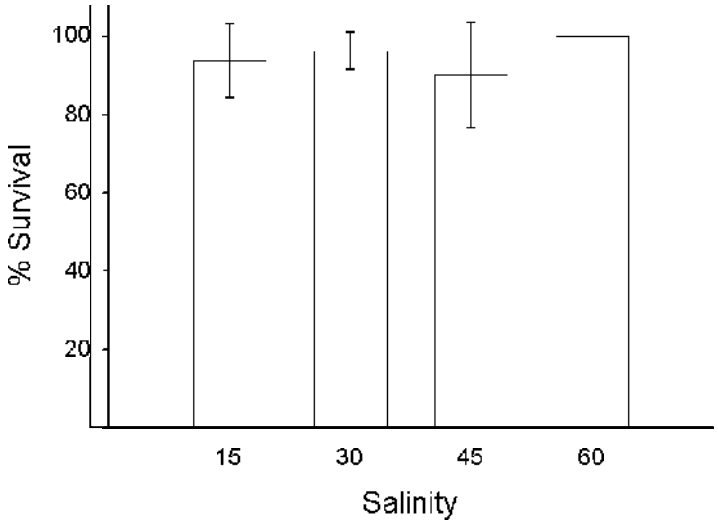

Figure 3.6. Mean percent survival of recently hatched larvae of *Bathynerita naticoidea* placed in four salinity treatments. Error bars represent ± 1 SD.

Larval collections

The protoconchs of most adult snails are eroded away, but two juvenile *Bathynerita naticoidea* with intact protoconchs were collected from the Brine Pool in November 2003. The juveniles were 1075 µm and 1400 µm in length with protoconchs 630 µm and 615 µm in length, respectively (Figure 3.5), suggesting that larvae settle at this approximate size. Morphological characteristics of these shells (growth lines, shape of the opercular opening, and size of protoconch I) were used to determine the identity of larvae collected in tube traps and plankton tows (Figure 3.5).

A total of fourteen *Bathynerita naticoidea* larvae were identified in the larval tube traps placed at the Brine Pool. The length of the larval shells ranged from 585 to 702 µm, with a mean of 667µm ± 44.0SD.

Thirteen *Bathynerita naticoidea* larvae were collected in the top 100 m of the water column above the Brine Pool in February 2003. They ranged from 389 to 668 µm in length. One larva was found in February between the depths of 300 and 400 m (length: 418 µm). Two larvae were collected in November 2003, one between the depths of 500 to 550 m (length: 403 µm) and one between 650 and 700 m (length: 677 µm) (Table 3.2). The size range of larvae found in the plankton was similar to that of the larvae collected in the tube traps, which enabled direct comparison between larval shells.

Discussion

The vertical swimming behavior immediately after hatching indicates that the majority of larvae of *Bathynerita naticoidea* swim upward for at least eleven days and may continue for seventeen days. The differences among capsules in time spent swimming upward may be attributed to genetics or the quality of eggs within each capsule (Emlet *et al.*, 1987; George, 1996). Unpublished observations in the laboratory indicate that unfed larvae swim for as long as 35 days, suggesting that access to food may affect the swimming behavior of *B. naticoidea.*

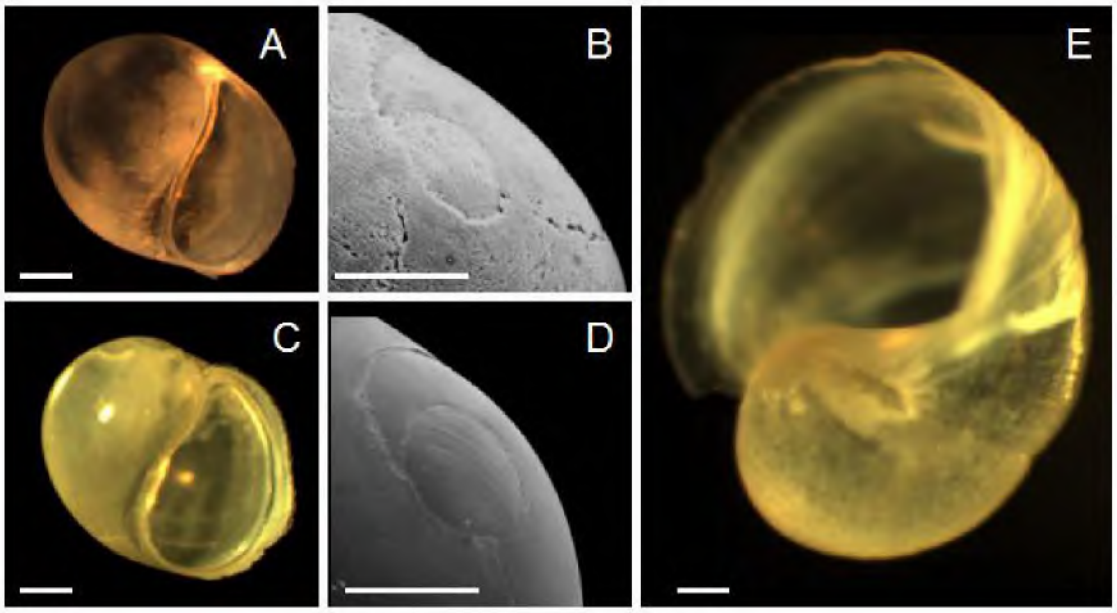

Figure 3.7. *Bathynerita naticoidea.* (a) Larval shell and (b) protoconch collected in upper 100 m of the water column, Gulf of Mexico. (c) Larval shell and (d) protoconch collected in tube traps placed at the brine pool. (e) Juvenile shell with the intact protoconch 615 µm in length. Scale bars represent 100 µm.

Table 3.2. Larval shells of *Bathynerita naticoidea* collected in MOCNESS plankton tows.

Depth (m)	Month	No. *B. naticoidea*	Size (µm)
0-100	February 2003	13	389-668
300-400	February 2003	1	418
500-550	November 2003	1	403
650-700	November 2003	1	677

If *Bathynerita naticoidea* were to swim at their maximum burst swimming speed of 0.137 cm/s, it would take between 4 and 5 days to swim from the Brine Pool (depth ~650 m) to the euphotic zone (chlorophyll maximum: 100 to 200m depth). However, the larvae probably would not sustain burst speeds for the duration of a 450-550 m vertical migration. If the larvae sustained the mean vertical swimming speed observed in the laboratory at 8 °C (mean ± 1 SD = 0.11 cm/s ± 0.03), they would arrive at the euphotic zone within 8 days. This figure is probably an underestimate, as the larvae would tend to swim faster in the warmer waters above the thermocline. The larvae would probably spend some time swimming and some time sinking, but modeling this type of complex behavior is outside the scope of this paper. However, this estimate is well within the time range that larvae will swim vertically in the lab. The observed vertical swimming behavior immediately after hatching coupled with swimming speeds suggest that the larvae of *B. naticoidea* swim to the euphotic zone soon after hatching and can probably reach the photic zone before energy reserves are depleted.

Recently hatched larvae of *Bathynerita naticoidea* are able to survive thermal conditions up to 32 °C for at least three days, suggesting that the larvae of *B. naticoidea* are tolerant of the high temperatures found in the upper water column in the Gulf of Mexico throughout the year. In addition, larvae continue to swim upward in temperatures up to 30 °C, suggesting that the larvae will continue to ascend through the upper layers of the water column despite high temperatures.

The larvae of *Bathynerita naticoidea* are able to survive salinities from 15 to 60 ppt. *B. naticoidea* often live at brine-dominated methane seeps where salinity at or just below the sediment can reach 120 ppt (Macdonald et al., 1990). Tolerance to high salinities in older larvae would help this species successfully recruit to these types of sites. High salinities may also be encountered in the Gulf of Mexico in the surface waters due to evaporation during the summer. In addition, Mississippi River effluent and tropical storms can produce lenses of low-salinity water in the upper water column of the Gulf of Mexico that reach far offshore (Müller-Karger and Walsh, 1991). The

euryhaline character of the larvae of *B. naticoidea* suggests that this species can tolerate the salinity conditions and fluctuations of the upper water column as well as moderately high salinities found in the adult habitat.

Despite the fact that the protoconchs of deep-water gastropods often dissolve or erode rapidly (Bouchet and Warén, 1994), intact protoconchs from two small juveniles were collected at the Brine Pool (630 µm and 615 µm in length). These are the only protoconchs of this species reported to date and provided an excellent opportunity to compare the shells of larvae collected in both the larval tube traps and the MOCNESS plankton samples.

Within the Gastropoda, the larvae of *Bathynerita naticoidea* dominated the tube trap collections. The similarities in the shape of the opercular opening, coloration, and growth lines between the protoconchs of juvenile *B. naticoidea* and the larvae collected in the tube traps support the conclusion that the shells collected were indeed *B. naticoidea.* The larvae ranged in size from 585 to 726 µm in length, which parallels the size range of the two juvenile protoconchs and suggests that the larvae collected in tube traps were probably competent to settle. Small larvae in the size range at hatching (~200 µm) were not collected in the tube traps, which may lend support to the laboratory observation that the larvae of *B. naticoidea* swim directly upward at hatching, or it may be a result of the effectiveness of collection by cylindrical traps, which declines with decreasing particle size (Yund *et al.*, 1991).

The larvae collected from the plankton were similar in size to those collected in the tube traps, providing the opportunity for direct comparison of shells between the two samples. The size range of larvae collected in MOCNESS samples supports the planktotrophic life history and reflects the extended seasonal oviposition of *B. naticoidea.* Egg capsules are laid in the laboratory between October and March, with peak intensity between late December and February (see Chapter 2). Release of swimming *B. naticoidea* larvae (~200 µm) from capsules is roughly timed to coincide with the spring phytoplankton bloom, between March and July (see Chapter 2). Assuming that the reproductive seasonality of *Bathynerita naticoidea* is similar across

its entire geographical range and considering the length of the larvae obtained from MOCNESS tows in February 2003 (389 to 668 µm), the larvae of *B. naticoidea* are planktonic for at least 8 to 12 months and triple in size before settling at cold seeps. This study is one of few with direct field evidence supporting the ontogenetic migration of a long-lived, planktotrophic cold seep endemic invertebrate (Bouchet and Warén, 1994).

Based on the various pieces of evidence presented here, we can make some educated guesses about potential distribution of *Bathynerita naticoidea.* This species is ubiquitous at cold seeps along the Louisiana continental slope (Zande and Carney, 2001). The major shallow-water current system within the Gulf of Mexico (the Loop Current) would tend to entrap the majority of larvae within this body of water, as there is a narrow outlet of flow out of the Gulf over an 800 m sill between Florida and Cuba (Carney, 1994). Populations of *B. naticoidea* at cold seeps on the Eastern edge of the Caribbean Plate would tend to have their larvae swept to the NW, into the Gulf of Mexico or up the Atlantic coast of the U. S. Larvae could also potentially be transported from the Caribbean across the Atlantic entrained in the North Equatorial Counter Current and Undercurrent. The larval life span and thermal tolerance of *B. naticoidea* would potentially allow for a trans-Atlantic passage to colonize cold seeps along the Western African Coast. In addition, the presence of a *Bathynerita*-like neritid in association with Miocene cold seep deposits from Italy (Taviani, 1994) and similar specimens collected from a middle-Eocene deposit in western Washington, USA (Squires and Goedert, 1996), suggest a widespread transoceanic distribution of this seep endemic or its ancestors.

The extensive salt diapers beneath the northern Gulf of Mexico continental slope and the abundant methane reserves in the Gulf of Mexico lead experts to believe that brine pools are relatively common in this area. The previous chapter concluded that the length of larval life, swimming behavior, and presence of larvae in the upper water column suggests the potential for long-distance dispersal of *Bathynerita naticoidea.* The wide dispersal capability of this species suggests that it may colonize brine pools across the northern Gulf of Mexico continental slope. *B. naticoidea* is abundant at one such brine pool, Brine Pool NR1 (27°43.4157N, 91°16.756W, ~650m depth). The fine-scale distribution of *B. naticoidea* in the mussel bed at Brine Pool NR1 was examined and the tolerance and behavioral response to salinity was investigated.

CHAPTER IV

BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO SALINITY AS DETERMINANTS OF DISTRIBUTION IN THE HYDROCARBON SEEP GASTROPOD *BATHYNERITA NATICOIDEA*

Introduction

Dense assemblages of chemosynthetic organisms were first reported along the Louisiana continental slope in the mid-1980's (Kennicutt *et al.* 1985). Mussel beds and tubeworm bushes in this region provide food and habitat for a diverse community of endemic and vagrant consumers including gastropods, alvinocarid shrimp, galatheid crabs, and orbinid worms (MacAvoy *et al.* 2002; Bergquist *et al.* 2005).

The bathyal neritid gastropod *Bathynerita naticoidea* Clarke 1989 is known only from hydrocarbon seeps in the Gulf of Mexico and on the Barbados accretionary prism at depths ranging from 400-2100m, where it is the most abundant gastropod in mussel-bed communities (Carney 1994; Olu *et al.* 1996; Zande & Carney 2001; Bergquist *et al.* 2005). A nerite similar to *B. naticoidea* has been found in Miocene deposits in Italy and also at middle Eocene fossil seeps in western Washington, USA, suggesting a long history of association with cold seeps (Taviani 1994; Squires & Goedert, 1996). *Bathynerita naticoidea* grazes on methanotrophic bacteria and detritus from hard substrata, primarily the shells of the mussel *Bathymodiolus childressi* (Zande & Carney 2001).

The Brine Pool (27°43.4157N, 91°16.756W, ~650m depth) is a methane-enriched lake of brine (salinity 120) surrounded by a dense bed of *Bathymodiolus childressi,* a hydrocarbon seep mussel with methanotrophic endosymbionts (Childress *et al.* 1986; MacDonald *et al.* 1990). The mussel community is from 3 to 7m in width,

covering an area of ~540 m^2 and can be divided into two distinct zones, the inner and outer zones, which vary both in their water chemistry and biological community and are separated by a transitional area (the middle "zone") (MacDonald *et al.* 1990; Smith *et al.* 2000; Bergquist *et al.* 2005). The inner zone is characterized by high densities of small mussels that rest in brine, while the outer zone is dominated by lower densities of large mussels and disarticulated shells that rest on soft sediment (Smith *et al.* 2000; Arellano, unpublished data).

Differences in the mussel bed community are apparent across zones of the Brine Pool mussel bed. For example, the orbinid polychaete *Methanoaricia dendrobranchiata* and the caridean shrimp *Alvinocaris stactophila* are more abundant in the inner zone than the outer zone (Bergquist *et al.* 2005; Copley & Young in press; Arellano, unpublished data).

Large-scale physical and chemical factors that may influence abundance patterns in the Brine-Pool community include high hydrogen sulfide concentrations and lower oxygen concentrations at the outer zone (Smith *et al.* 2000; Bergquist *et al.* 2005). However, on a fine scale, the water chemistry across the Brine Pool mussel bed is quite heterogeneous (Smith *et al.* 2000; Bergquist *et al.* 2005). Lateral seepage of brine from the pool across the mussel bed is apparent, although the extent of seepage is unclear (Smith *et al.* 2000).

In this study, we investigated the possible role of a horizontal salinity gradient in determining the abundance patterns of *B. naticoidea.* Specifically, we (1) quantify the density and horizontal distribution of *B. naticoidea* across the Brine Pool mussel bed, (2) examine the physiological tolerance to salinity and (3) examine the behavioral responses of *B. naticoidea* to several salinities they may encounter at the Brine Pool.

Materials and Methods

Field Distribution

To determine the distribution of *Bathynerita naticoidea,* three discreet samples were collected at the inner (next to the brine), middle, and outer (bordered by sediment)

zones of the Brine Pool mussel bed on two cruises in November 2003 and July 2004 for a total of 18 samples (Figure 3.1, 3.2; Table 4.1). Replicate samples were collected haphazardly by taking several adjacent scoops with the Johnson Sea Link (HBOI) submersible arm within a discreet area in three different regions of the mussel bed. Mussels and associated fauna were scooped out with the submersible arm and the remaining fauna was suctioned into a closed bucket. Because the areas sampled were not strictly quantitative, we expressed snail abundance within each sample in terms of individuals per unit of mussel surface area. The total surface area of each replicate sample was calculated by taking the length of each mussel (L) within a sample and converting it to surface area (SA) using the equation $SA = 0.5794^{L2.06}$ (Bergquist, personal communication). The density of *B. naticoidea* is therefore presented as # individuals/m^2 of mussel. Density was analyzed by 2-way ANOVA with zone as a fixed factor and month as a random factor.

Table 4.1. Collection of *Bathynerita naticoidea* at the Brine Pool. Each sample consisted of several scoops collected haphazardly with the Johnson Sea Link submersible.

Location within mussel bed	Month	No.Samples(n)
Inner	November 2003	
Inner	July 2004	
Middle	November 2003	
Middle	July 2004	
Outer	November 2003	
Outer	July 2004	

Salinity Tolerance

To test the salinity tolerance of *Bathynerita naticoidea,* ten freshly collected specimens were placed in each of four replicate 500mL plastic containers filled with cold (7 $^{\circ}$C) seawater of four salinities (15, 30, 45, 60). High- salinity water was made by freezing seawater. Low-salinity water was made by diluting seawater with distilled water. Salinity measurements were made with a refractometer and are expressed as dimensionless values (s) in accordance with Re Practical Salinity Scale PSS78. Snails were left in the salinity treatments for five days then allowed to recover in ambient seawater (salinity: 35) for 24 hours. Survival was scored by prodding the foot using a blunt probe and noting responses. If *B. naticoidea* did not respond to prodding by moving its foot or operculum then it was scored as dead. Results were analyzed by one-way ANOVA with salinity as a fixed factor.

Responses to High Salinities

Bathynerita naticoidea generally move upward in laboratory tanks, spending most of their time at the air/water interface. We exploited this behavior to investigate the behavioral responses of individual snails to high salinities. Snails were placed below inverted haloclines and allowed to crawl upward to determine if they would enter waters of three different high salinities. In each case, the higher salinity was at the top and the lower ambient salinity (always 35) was at the bottom. To make stable inverted haloclines, the density of the ambient salinity water was increased by 0.05 kg/m^3 above that of the high salinity water by adding the silica colloid Percoll ™ (Amersham Pharmacia Biotech). Percoll™ is a sterile solution composed of silica beads (15-30 nm) coated with polyvinylpyrrolidone (PVP), a non-toxic material with an osmolality of <25 mOs/kg H_2O. The osmolality of each Percoll™ solution was verified with a Vapro 5520 vapor pressure osmometer and the osmolality of each solution was corrected by the addition of high-salinity water. The three halocline treatments tested had salinities of 50, 60 and 70 as the top layer

Three isocline (control) treatments were also constructed, with dense seawater as the bottom layer and ambient seawater (salinity: 35) as the top layer, to act as a control for the addition of Percoll™. One isocline treatment was run to match each halocline treatment. In these isoclines, the densities of the bottom layers were adjusted with Percoll™ to match the densities of the bottom layers in the corresponding halocline treatments (Table 4.2).

Isocline and halocline experiments were run simultaneously. For example, the 50-halocline treatment was run at the same time as an isocline control having a bottom layer of ambient (35 salinity) seawater with Percoll™ added to match the density of the bottom layer of the 50-halocline treatment, and 35 salinity seawater without added Percoll™ as the upper layer.

Each trial consisted of one halocline and one isocline treatment with twenty replicate snails per treatment. Each trial was run separately because of time constraints, and each treatment (salinity of 50, 60, and 70) was replicated twice for a total of 6 trials. Experimental haloclines were constructed in large glass test tubes (25 x 200 mm) in a 7 $^{\circ}$C cold room and blue food coloring was used to mark the depth of all haloclines. Once the halocline stabilized, one randomly selected snail was gently dropped into each tube, taking care not to disrupt the halocline. After one hour, the position of each snail was recorded, then categorized as Top, Halocline, or Bottom. Each test tube was divided into forty units. The Top bin included the top 15 units, the Halocline bin included five units above and below the halocline (10 units total), and the Bottom bin consisted of the bottom 15 units.

A pilot experiment was conducted to investigate whether there was an effect of Percoll™ on behavior and to determine the proper time frame for the halocline experiment.

The responses of *B. naticoidea* to haloclines were analyzed as a 3-way mixed model ANOVA with treatment and salinity as fixed factors and trial as a random factor. The mixed-model 3-way ANOVA showed that trials were not significantly

different from each other, so trials were pooled for a reanalysis by 2-way ANOVA with treatment and salinity as fixed factors.

Table 4.2. Salinity and Density (units of kg/m^3) conditions in the isocline and halocline experiments. Asterisk (*) indicates that Percoll™ was added to achieve the desired density.

	Top Layer		Bottom Layer	
Treatment Name	Salinity	Density	Salinity	Density
50-Halocline	50	1.040	35	1.045*
50-Isocline	35	1.025	35	1.045*
60-Halocline	60	1.045	35	1.050*
60-Isocline	35	1.025	35	1.050*
70-Halocline	70	1.050	35	1.055*
70-Isocline	35	1.025	35	1.055*

Results

Field Distribution

A total of 393 *Bathynerita naticoidea* were collected from the Brine Pool in November 2003. When standardized by the surface area of mussel shell available in each sample, the mean density ± 1 SD in the inner, middle, and outer zone was 6.28 ± 7.4, 124.3 ± 57.7, and 462.8 ± 180.0 individuals/m^2, respectively. In July 2004, a total of 577 *B. naticoidea* were collected. Mean snail density ± 1 SD in July was 30.5 ±

44.4, 511.9 ± 461.8, and 331.6 ± 224.3 individuals/m^2 in the inner, middle, and outer zone, respectively. There was a significantly higher density of *B. naticoidea* in the outer zone than the inner zone (p = 0.03, F = 4.77; Bonferroni post hoc), with no significant effect of sampling date (Figure 4.1). No detectable difference in *B. naticoidea* density was found between the middle and either the inner or outer zone (p = 0.117, 1.0, respectively).

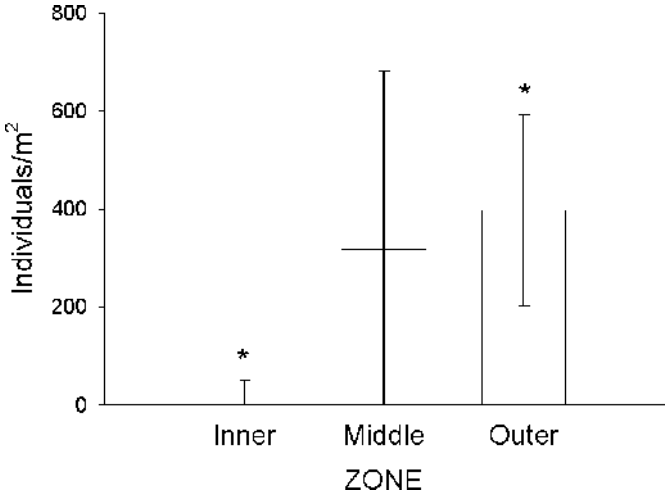

Figure 4.1. *Bathynerita naticoidea* collected in the inner, middle, and outer zone of the Brine Pool. Mean density± 1 SD standardized by available mussel shell surface area.

Salinity Tolerance

All *B. naticoidea* survived salinity treatments of 30 and 45 with no unusual behaviors noted during the experimental period or after recovery. All snails in salinities of 15 and 60 spent the entire experimental period lying upside down with the operculum open, responding slightly when prodded. After the recovery period, snails in the salinity treatment of 15 suffered 100% mortality, and those in the salinity treatment of 60 suffered 32.5+/- 0.5% mortality (p < 0.001; Figure 4.2).

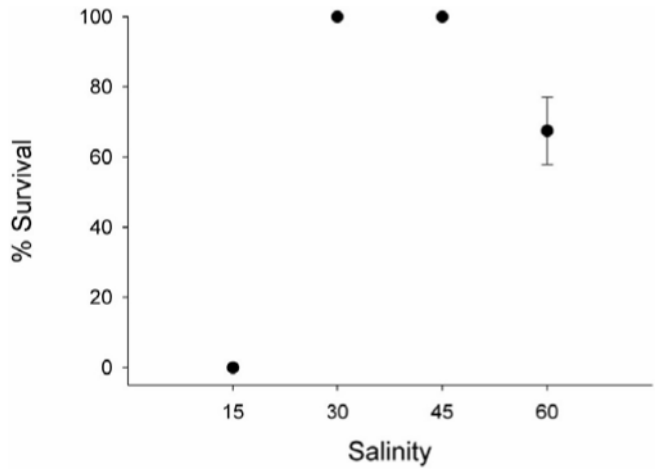

Figure 4.2. *Bathynerita naticoidea* survival after recovering for 24 hours from 5 days in four salinity treatments: 15, 30, 45, and 60. Error bars refer to ± 1 SD.

Responses to Haloclines

A significant interaction in the two-way ANOVA indicates that the behavior of *B. naticoidea* differs between the isocline and halocline treatments ($p = 0.034$, $F = 3.42$) at haloclines above 50. The 60 and 70 halocline treatments were significantly different from the 50-halocline treatment ($p = 0.001$, 0.013, respectively; Bonferroni post hoc).

Between 70 and 87.5% of the snails in the isocline treatments crawled to the surface and sat with 2-5 mm of their body sticking out of the water (Figure 4.3). In the 50-halocline treatment, 65% of the snails crawled to the top of the tube and sat at the surface. In the 60-halocline treatment, only 12.5% of the snails crawled through the high saline water to the surface, and no snails were found at the top of the 70-halocline treatment.

In the 60 and 70 halocline treatments, snails were usually found right below the halocline, with a few millimeters of foot or tentacle sticking up into the high salinity water. Interestingly, 22.5% of the snails in the 60-halocline treatment were found within five units below the halocline and another 25% were at the halocline with part of their body or shell in each layer. In the 70-halocline treatment, 35% of the snails were within five units below the halocline and 35% were at the halocline. One snail was found immediately above the halocline in the 70-halocline treatment.

Discussion

Associated fauna at the Brine Pool are generally more abundant at the inner zone than the outer zone (Bergquist *et al.,* 2005). This pattern is not mirrored by *B. naticoidea,* which is more abundant, by an order of magnitude, at the outer zone than the inner. The pattern of abundance in the middle zone was highly variable between collection dates, a fact that reflects the patchy distribution of *B. naticoidea* and supports the conclusion made by Smith *et al.* (2000) that the middle zone is not a distinct zone, but a transitional area between the inner and outer zone. The density of mussels in the inner zone tends to be higher than that of the outer zone (Smith *et al.* 2000), so standardizing density of *B. naticoidea* by surface area of mussel shell probably results in greater differences than if the data were expressed as snails per unit area of sea floor. Nevertheless, we believe the difference in *B. naticoidea* abundance across the mussel bed is real. The paucity of *B. naticoidea* collected in the inner zone is also reflected in observations of *B. naticoidea* egg capsule distribution across the mussel bed and is therefore probably not a temporal artifact but a true pattern (Van Gaest, unpublished data).

The obvious zonation of *B. naticoidea* at the Brine Pool is reminiscent of rocky intertidal communities, in which physical gradients of wave action and tidal exposure define distinct patterns of abundance. At the Brine Pool, chemical gradients in salinity, oxygen, methane, and hydrogen sulfide might be expected to influence clines in animal

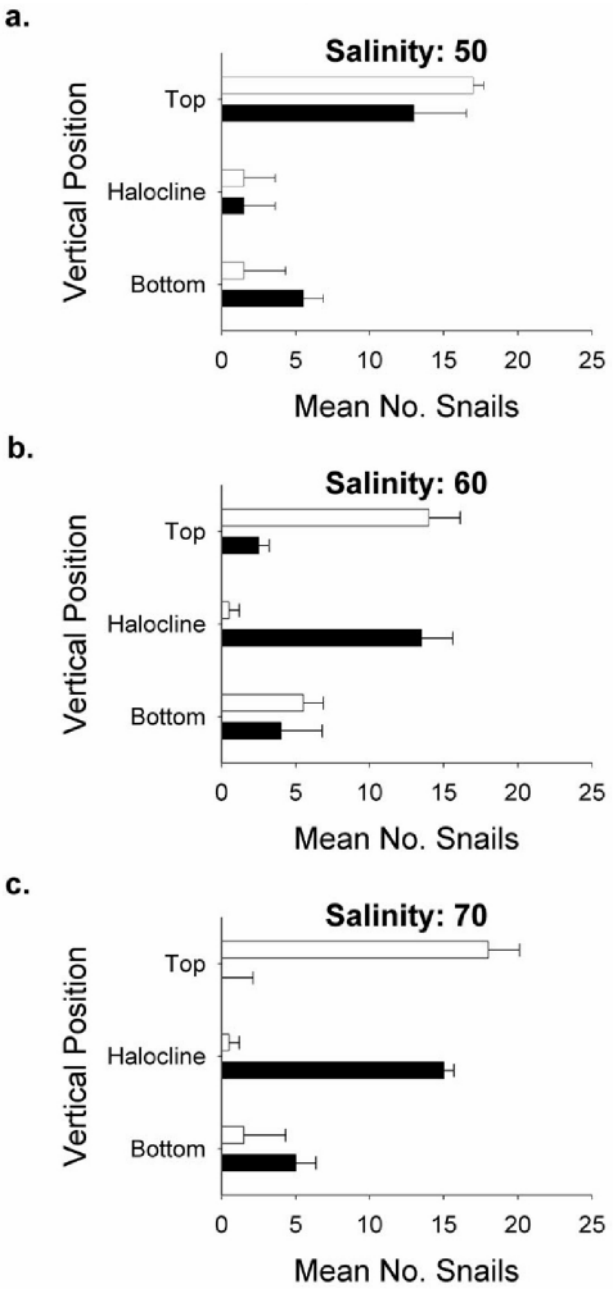

Figure 4.3. *Bathynerita naticoidea* position along a vertical test tube. Isocline treatments in white are filled with dense seawater (salinity: 35) and layered with ambient seawater (salinity: 35). Halocline treatments in black are filled with dense (salinity: 35) seawater layered with seawater of salinity (a) 50, (b) 60, or (c) 70.

distribution. However, the inner edge of the Brine Pool has more oxygen and less hydrogen sulfide than the outer edge, a fact that probably explains increased abundances of most species in this zone (Smith *et al.* 2000; Bergquist *et al.* 2005). Salinity has not been measured on a fine scale across the Brine Pool mussel bed, but is expected to show the opposite gradient, since very high salinities are present in the Brine Pool itself and the outer margin of the mussel bed rests in water of normal salinity. Thus, salinity is the one known chemical gradient that correlates well with the unexpected distribution of *B. naticoidea.*

Most neritid gastropods live in warm, tropical, shallow-water habitats and they often have life cycles that include both freshwater and saltwater stages (Fretter and Graham 1962; Clark 1989). Not surprisingly, *Bathynerita naticoidea* are euryhaline and can tolerate a wide range of salinities. *B. naticoidea* or a similar nerite has been associated with cold seeps since the Eocene, and has clearly evolved mechanisms to deal with the brine often found at cold seeps (Taviani, 1994; Squires and Goedert, 1996).

When given a choice between normal salinity and salinities of 60 or 70, *Bathynerita naticoidea* altered its behavior to avoid entering these salinities. When given a choice between normal salinity and 50, *B. naticoidea* entered 50 without a change to its behavior. Although *B. naticoidea* were not active when exposed to a salinity of 60 for a prolonged period, five individuals were found in the 60 layer and one individual was found in the 70 layer in the halocline experiment, indicating that they are tolerant and active in high salinities for short periods of time. Nevertheless, the general refusal of *B. naticoidea* to enter a layer of seawater with salinity of 70, and to a lesser extent, salinity of 60 could result in limited distribution of this species to the uppermost surfaces of the mussel bed. There is evidence of lateral seepage of brine across the mussel bed at depths of 5 and 10 cm, and in some cases 2.5 cm from the tops of the mussels (Smith et al. 2000). The behavioral response of *B. naticoidea* to high salinity would effectively limit the vertical distribution of *B. naticoidea* to the topmost 2.5, and maybe 5 cm of the mussel bed. Although there is no evidence that lateral

seepage is more common, or higher, in the inner zone of the mussel bed, detailed salinity measurements could more clearly elucidate the effect of salinity on the horizontal distribution of *B. naticoidea* at the Brine Pool.

While the behavior of *Bathynerita naticoidea* in response to high salinity may play a role in the observed distribution at the Brine Pool, biological interactions such as increased competition and predation at the inner edge may also be important factors in the observed distribution and cannot be discounted by the present study.

CHAPTER V

GENERAL DISCUSSION

Gametogenesis appears to be synchronous and seasonal in *Bathynerita naticoidea* at cold seeps on the Northern Gulf of Mexico continental slope. Variation in oocyte size-frequencies was greater among months than among individuals. Cohorts of vitellogenic oocytes began to develop in the summer and increased in size and proportion through the fall. The variation in oocyte size-frequencies among individuals seen in December and February is consistent with a prolonged period of oviposition in the winter months.

Pre-vitellogenic oocytes were found in all individuals examined, regardless of the month collected. *B. naticoidea* uses both heterosynthetic and autosynthetic pathways of vitellogenesis consistent with "fast" egg production (Eckelbarger and Watling, 1995; Eckelbarger and Young, 1997). It is common for prosobranch gastropods to have all stages of gametogenesis regardless of seasonality (Webber, 1977). It appears that *B. naticoidea* produce pre-vitellogenic oocytes throughout the year and store mature oocytes until oviposition.

Embryos develop within capsules for approximately four months. Egg capsules were deposited in the laboratory between October and March, with peak oviposition between December and February. Veligers hatched out of capsules between May and early July in the laboratory, although the development of embryos from capsules deposited before December was not followed. Assuming that all embryos require four months to develop before hatching as swimming veligers, hatching in the laboratory

would be expected between February and July, with peak hatching between May and early June.

While no attempt was made to investigate a causal relationship between reproductive seasonality and environmental variables, cues to the seasonal flux of phytodetritus from the surface waters may be provided by the detritivorous feeding behavior of the adult snails (Zande and Carney, 2001). Reproduction in a number of deep-water echinoderms with inferred planktotrophic development from the Rockall Trough synchronized reproduction so that release of larvae coincided with the spring phytoplankton bloom (Tyler and Gage, 1983). In contrast, echinoderms with inferred lecithotrophic development from the same collections exhibited asynchronous reproduction (Tyler and Gage, 1983).

The relatively large number and small size of embryos within egg capsules laid by *Bathynerita naticoidea* indicate planktotrophic development (Thorson, 1950; Waren and Bouchet, 2001). This study provided direct evidence of planktotrophy in the larvae of *B. naticoidea.* Larvae were shown to feed on microalgae immediately after hatching, however, we were not able to demonstrate whether they are obligate of facultative planktotrophs. Nevertheless, the long larval lifespan (> 90 days) and large size of larvae at settlement (600 – 700 µm) suggests that *B. naticoidea* are probably obligate planktotrophs. The larvae of *B. naticoidea* are released during the spring, roughly around the time of the spring phytoplankton bloom in the Gulf of Mexico, which suggests a coupling between production in surface waters and the release of larvae (Müller-Karger *et al.,* 1991). The selective pressure for seasonal reproduction in this species probably derives from the necessity of the planktotrophic larvae to obtain an adequate amount of food in the euphotic zone during the spring phytoplankton bloom.

The vertical swimming behavior immediately after hatching indicates that the majority of larvae of *Bathynerita naticoidea* swim upward for at least eleven days and may continue for seventeen days. If *B. naticoidea* were to swim at their mean vertical swimming speed observed in the laboratory at 8 $^{\circ}$C (0.11 cm/s), it would take

approximately 8 days to swim from the Brine Pool (depth ~650 m) to the euphotic zone (chlorophyll maximum: 100 to 200m depth). This figure is probably an underestimate, as the larvae would tend to swim faster in the warmer waters above the thermocline. However, this estimate is well within the time range that larvae will swim vertically in the lab. The observed vertical swimming behavior immediately after hatching coupled with swimming speeds suggest that the larvae of *B. naticoidea* swim to the euphotic zone soon after hatching and can probably reach the photic zone before energy reserves are depleted.

Recently hatched larvae of *Bathynerita naticoidea* are able to survive thermal conditions up to 32 $^{\circ}$C for at least three days, suggesting that the larvae of *B. naticoidea* are tolerant of the high temperatures found in the upper water column in the Gulf of Mexico throughout the year. In addition, larvae continue to swim upward in temperatures up to 30 $^{\circ}$C, suggesting that the larvae will continue to ascend through the upper layers of the water column despite high temperatures.

The larvae of *Bathynerita naticoidea* are able to survive salinities from 15 to 60 ppt. *B. naticoidea* often live at brine-dominated methane seeps where salinity at or just below the sediment can reach 120 ppt (Macdonald et al., 1990). Tolerance to high salinities in older larvae would help this species successfully recruit to these types of sites. High salinities may also be encountered in the Gulf of Mexico in the surface waters due to evaporation during the summer. In addition, Mississippi River effluent and tropical storms can produce lenses of low-salinity water in the upper water column of the Gulf of Mexico that reach far offshore (Müller-Karger and Walsh, 1991). The euryhaline character of the larvae of *B. naticoidea* suggests that this species can tolerate the salinity conditions and fluctuations of the upper water column as well as moderately high salinities found in the adult habitat.

Despite the fact that the protoconchs of deep-water gastropods often dissolve or erode rapidly (Bouchet and Warén, 1994), intact protoconchs from two small juveniles were collected at the Brine Pool (630 µm and 615 µm in length). These are the only protoconchs of this species reported to date and provided an excellent opportunity to

compare the shells of larvae collected in both the larval tube traps and the MOCNESS plankton samples.

Within the Gastropoda, the larvae of *Bathynerita naticoidea* dominated the tube trap collections. The similarities in the shape of the opercular opening, coloration, and growth lines between the protoconchs of juvenile *B. naticoidea* and the larvae collected in the tube traps support the conclusion that the shells collected were indeed *B. naticoidea.* The larvae ranged in size from 585 to 726 µm in length, which parallels the size range of the two juvenile protoconchs and suggests that the larvae collected in tube traps were probably competent to settle.

The larvae collected from the plankton were similar in size to those collected in the tube traps, providing the opportunity for direct comparison of shells between the two samples. The size range of larvae collected in MOCNESS samples supports the long-lived planktotrophic life history reported here. Assuming that the reproductive seasonality of *Bathynerita naticoidea* is similar across its entire geographical range and considering the length of the larvae obtained from MOCNESS tows in February 2003 (389 to 668 µm), the larvae of *B. naticoidea* are planktonic for at least 8 to 12 months and triple in size before settling at cold seeps. This study is one of few with direct field evidence supporting the ontogenetic migration of a long-lived, planktotrophic cold seep invertebrate (Bouchet and Warén, 1994).

Associated fauna at the Brine Pool are generally more abundant at the inner zone than the outer zone (Bergquist et al., 2005). This pattern is not mirrored by *Bathynerita naticoidea,* which is more abundant, by an order of magnitude, at the outer zone than the inner. Egg capsule abundance was also significantly lower in the inner zone of the Brine Pool than the middle zone in November 2003. Egg capsule density was also lower in the inner zone than the outer zone, but not significantly so ($p = 0.051$). The pattern of abundance of adults and egg capsules in the middle zone was highly variable between collection dates, a fact that reflects the patchy distribution of *B. naticoidea* and supports the conclusion made by Smith *et al.* (2000) that the middle zone is not a distinct zone, but a transitional area between the inner and outer zone.

At the Brine Pool, chemical gradients in salinity, oxygen, methane, and hydrogen sulfide might be expected to influence clines in animal distribution. However, the inner edge of the Brine Pool has more oxygen and less hydrogen sulfide than the outer edge, a fact that probably explains increased abundances of most species in this zone (Smith *et al.* 2000; Bergquist *et al.* 2005). Salinity has not been measured on a fine scale across the Brine Pool mussel bed, but is expected to show the opposite gradient, since very high salinities are present in the Brine Pool itself and the outer margin of the mussel bed rests in water of normal salinity. Thus, salinity is the one known chemical gradient that correlates well with the unexpected distribution of *B. naticoidea.*

Most neritid gastropods live in warm, tropical, shallow-water habitats and they often have life cycles that include both freshwater and saltwater stages (Fretter and Graham 1962; Clark 1989). Not surprisingly, *Bathynerita naticoidea* are euryhaline and can tolerate a wide range of salinities. *B. naticoidea* or a similar nerite has been associated with cold seeps since the Eocene, and has clearly evolved mechanisms to deal with the brine often found at cold seeps (Taviani, 1994; Squires and Goedert, 1996).

When given a choice between normal salinity and salinities of 60 or 70, *Bathynerita naticoidea* altered its behavior to avoid entering the higher salinities. The general refusal of *B. naticoidea* to enter a layer of seawater with salinity of 70, and to a lesser extent, salinity of 60 could result in limited distribution of this species to the uppermost surfaces of the mussel bed. There is evidence of lateral seepage of brine across the mussel bed at depths of 5 and 10 cm, and in some cases 2.5 cm from the tops of the mussels (Smith et al. 2000). The behavioral response of *B. naticoidea* to high salinity would effectively limit the vertical distribution of *B. naticoidea* to the topmost 2.5, and maybe 5 cm of the mussel bed. Although there is no evidence that lateral seepage is more common, or higher, in the inner zone of the mussel bed, detailed salinity measurements could more clearly elucidate the effect of salinity on the horizontal distribution of *B. naticoidea* at the Brine Pool.

While the behavior of *Bathynerita naticoidea* in response to high salinity may play a role in the observed distribution at the Brine Pool, biological interactions such as increased competition and predation at the inner edge may also be important factors in the observed distribution and cannot be discounted by the present study.

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