Investigation of a Deep Sea Naticid Gastropod, Including Morphological Characteristics of the Egg Collar, Veliger Clearance Rates of the Cyanobacterium, Synechococcus, and Sinking Rates of Veligers

Katie Bennett

Introduction

The deep sea presents scientists with the opportunity to study an environment about which there is still relatively little known. With the information garnered from early, deep-water trawls, combined with that obtained using current-day manned submersibles and ROVs, scientists have tried to tell the story of the deep. To begin to tell that story, one must first address some of the fundamental questions of life in the deep-sea. How do deepwater organisms deal with parameters that are so different from their shallow-water counterparts? How does the increase of pressure, the decrease of temperature and the virtual absence of light drive reproduction and life histories? What, in the deep sea, takes the place of photosynthetic algae as the primary food source of larval invertebrates?

Numerous studies have shown that there is a range of developmental modes among deep-sea invertebrates (e.g. Tyler and Gage 1984, Young et al. 1989, Young et al. 1997). What do these microscopic larvae eat? Is it possible that they swim to surface waters to feed, before returning to depth to settle?

We were fortunate, on a recent research trip to Tongue of the Ocean, Bahamas, to find egg collars containing multiple embryonic stages of an unknown Naticid gastropod. In this paper, we test the ability of swimming veligers to ingest the cyanobacterium, synnechococcus, which is found as deep 4000m. We also test the sinking rates of veligers as a tool to infer possible vertical distribution and describe morphological characteristics of the egg collar as an aid in taxonomic classification.

Materials and Methods

Multiple egg collars were collected on May 16, 2008, using the Johnson Sea-Link II submersible (Harbor Branch Oceanographic Institute, Fort Pierce FL, U.S.A.), at a depth of 410 m and an in-situ temperature of 17-18°C. Specimens were brought to the surface in a container of seawater then immediately placed in fresh, filtered seawater in a cold-room that was maintained for the duration of the cruise (11 days) at an ambient temperature of 13°C. Portions of some egg collars containing developing embryos were shipped to Oregon following the cruise, where they are maintained at a temperature of 11°C and frequently monitored for development.

Egg collar morphology

To quantify egg density collar^{-1}, embryonic capsules were removed from a section of the egg collar using forceps. Embryos were counted and fixed for later SEM (for a detailed report of embryonic development see Cziko unpublished). The area of the egg collar where embryos had been removed was measured using Image-J Software. Average density collar^{-1}

was then calculated using the total area of collar (excluding the basal and apical margins where no embryos are present).

Feeding: Clearance rate experiment

Only macroscopically visible, swimming veligers were used for this experiment. Veligers were transferred by Pasteur pipette into a rinse of clean filtered seawater then, in a volume of seawater sufficient only for the prevention of dehydration, to glass scintillation vials for the experiment.

We diluted a personal isolate of marine synechococcus to a concentration of 35,000-42,000/mL; about three times the typical synechococcus concentration at deep chlorophyll maximum in subtropical waters. 10.1 mL of the synechococcus dilution was added to vials containing 0, 10 or 20 veligers.

At time point zero, 1 mL of solution was removed from each vial and quantified using direct cell counts on filter membranes following the procedure described by Sherr et al. (2001). Between time points, the experimental vials were kept in near-darkness is a closed cardboard box at 13°C. After a 24 hr time interval, two 1 mL aliquots were taken from each vial and enumerated separately. A third count with two replicates was taken 24 hrs later. After completion of the feeding experiment, the veligers were examined using blue light excitation epi-illumination to see if marine synechococcus was visible in the gut.

Sinking rate

To determine sinking rate, veligers were placed in a 200 mL graduated cylinder using a Pasteur pipette and timed as they sank a distance of 16 cm. Because the veligers often retracted their vela when handled, we anesthetized half of the veligers in 7.5 % $MgCl_2$ prior to the experiment in the hope that their vela would be extended. To ensure a consistent density of the seawater medium, temperature was maintained at 19-22°C throughout the experiment.

Results

Egg collar morphology

The deep-sea naticid egg collars collected from Green Cay are rigid, with apical and basal acapsular borders and a wide, plicated basal margin (Fig. 1a,b). They are sand-encrusted, and the grain-size and variable in coarseness reflect the grain size of sediment at place of collection (Fig. 1e). Embryos are intracapsular, and are held together by a sac of capsular jelly, which is easily perforated with forceps. The number of embryos per capsule is variable within a single egg collar, and a single capsule may contain 3,4 or 5 eggs (Table 1, Fig. 1c,d) A vertical slice of the collar shows that embryos are arranged in a monolayer (Fig. 1d). Developmental stages were synchronous within an individual egg collar, but were asynchronous between collars. On average, there were 4.33 embryo mm^{-2}, and ~10,000 embryos in a single egg collar. Based on embryological developmental rates during the course of this study, we estimate about 25 days time between the laying of the egg collar and hatching if embryos were kept at a constant seawater temperature of 17-18°C, the temperature at which they were collected (Table 1).

Clearance rate

Over a time period of 48 hours, there was a decrease of synechococcus concentration in the vials containing veligers and an increase in the synechococcus concentration in the control (Fig. 2). This suggests that the veligers were, in fact, clearing synechococcus from the seawater. On average, the 10-veliger treatment had a slightly higher clearance rate than the 20-veliger treatment (Table 2). A post-experimental examination of the veligers under an epi-fluorescent scope revealed no obvious synechococcus in the gut or on the external tissue (Fig. 3).

Sinking rates

Veligers that were not relaxed with $MgCl_2$ sank faster than those that were, which confirms the intuitive notion that the protraction and retraction of the velum changes sinking rate (Table 3). Based on our sinking-rate estimates, a veliger with a partially open velum will sink at a rate of 316 m day^{-1} (in the absence of a flow regime).

Discussion

The morphological characteristics detailed in this paper may allow us to determine, at a later date, the taxonomic identity of this gastropod. The gelatinous egg ribbon embedded with sand particles is representative of the caenogastropod family, Naticidae, to which we have tentatively assigned this gastropod. Using existing taxonomic keys, we were unable to ascribe this organism to any particular genera, although the rigid structure and plicated basal margin of the egg collar do allow us to rule out a number of possible identities (Giglioli 1955). We have isolated DNA, and hope in the future to do a genetic analysis of this organism. Input from molluscan experts regarding the Scanning Electron Micrographs of the protoconch and of the different stages of development (Cziko unpublished) may also provide further clues as to this organism's identity.

It is common practice to use the size of the protoconch to infer developmental mode. In general, small protoconchs (of less than 175 μm) signify planktotrophic development, and protoconchs larger than 325 μm indicate a non free-swimming larva (Strathmann 1987). Using these guidelines, this egg mass would give rise to crawl-away juveniles. However, we observed in the lab that veligers hatched as swimming larvae and retained that ability to swim for at least seven days.

The feeding experiment provides an interesting conundrum. The decreasing concentration of synechococcus in the veliger trials suggests some kind of uptake. If cell concentration were decreasing as a result of adherence to mucous on the vela of the veligers, as has been suggested by our colleagues, then we would expect to see it fluoresce on the exterior of the veligers examined under epi-fluorescence after the experiment (Fig. 3). It is possible that the veligers are drinking the seawater, and thus entraining the cyanobacteria. If this is the case, then the fate of cyanobacterium after ingestion in this way is unknown. It is also possible that the synechococcus no longer fluoresces after digestion.

The sinking rate experiment provides some clues to the dispersal potential of this species. With their relatively fast sinking rate and large shells, it is unlikely that this species would be capable of a vertical migration into the euphotic zone. Due to the vela being only partially protracted, and due also to the continued beating of the velar cilia, it is hard to assess the impact of velum extension on sinking rate. Perhaps the addition of tobacco to the $MgCl_2$ would better stop the ciliary action of the vela and produce more definitive results. Further information regarding the dispersal potential of this organism might be gleaned from

experiments on the physiological constraints of veligers. In their 1998 study, Young et. al tested the thermal tolerances of embryos and larvae of deep-sea echinoderms and used those data to infer at what depths within the water column, and during which life stages, those organisms would be capable of normal development.

By using the morphological description of the egg collar, the description of the protoconch and the embryological development timetable presented in Cziko (unpublished), as well as further analysis using DNA sequence data, we hope to soon have an identity for this species. The feeding and sinking experiments provide valuable insight into early life stage feeding and swimming behavior of a deep-sea larva.

Acknowledgements

Best trip ever!! Thanks very much to Dr. Young, Dr. Tyler, Dr. Emlet, Dr. Jaekle and Dr. Wood for allowing me this incredible opportunity. Thanks also for your guidance and patience throughout the trip. Thanks to Paul Cziko, the co-author of this work, for never sleeping during this project, and for teaching me so much.

Literature Cited

Giglioli, M.E.C. 1955. The Egg Masses of the Naticidae (Gastropoda). *J. Fish. Res. Bd. Canada* **12:** 287-327.

Sherr, B., E. Sherr, P. del Georgio. Enumeration of Total and Highly Active Bacteria. *Methods in Microbiolgy* Vol. 30, Academic Press Ltd.

Strathmann, M.F. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast.* Pp.222. University of Washington Press.

Tyler, P.A., J.D. Gage 1984. The Reproductive Biology of Echinothuriid and Cidarid Sea Urchins From the Deep Sea (Rockall Trough, North-East Atlantic Ocean). *Mar. Bio.* **80:** 63-74.

Young, C.M., S.U.K. Ekaratne, J.L. Cameron 1998. Thermal Tolerances of Embryos and Planktotrophic Larvae of *Archaeopneustes hystrix* (A. Agassiz) (Spatangoidea) and *Stylocidaris Lineata* (Mortensen) (Cidaroidea), Bathyal Echinoids from the Bahamian Slope. *J.of Exp. Mar. Bio. And Ecol.* **223:** 65-76.

Young, C.M., M.A. Sewell, P.A. Tyler, A Metaxas 1997. Biogeographic and bathymetric Ranges of Atlantic Deep-Sea Echinoderms nad Ascidians: the Role of Larval Dispersal. *Biodiversity and Conservation* **6:** 1507-1522.

Young, C.M. J.L. Cameron, K.J. Eckelbarger 1989. Extended pre-feeding Period in Planktotrophic Larvae of the Bathyal Echinoid *Aspidodiadema jacobyi. J. Mar. Biol. Ass. U.K.* **69:** 695-702.

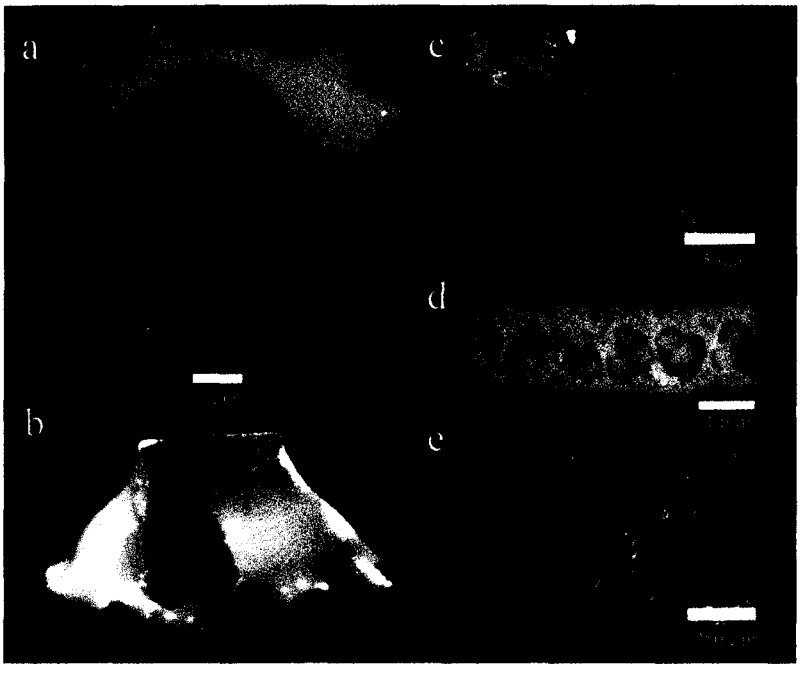

Figure 1. Egg collar. Top view (a) and lateral view (b), tangential section (c) with surface sand grains removed with forceps, cross section (d), and SEM micrograph of sediment on surface of collar (e). Note plicate basal margin and transmitted light through lateral view of collar showing individual capsules (light spots), and apical and basal acapsular membrane regions.

Table 1. Measurements of egg collar and embryos and other data

Measurement or calculation	**Value ± SE**
Number of embryos per capsule	3, 4, or 5[#]
Number of capsule layers in cross section	1
Length of collar apical margin	7.0 cm
Length of collar basal margin	20.7 cm
Internal diameter of erect egg collar	Approx. 2.5 cm
Outside diameter of erect egg collar	Approx. 6.5 cm
Max width of collar (min - max)	30-38 mm
Mean embryos per area of collar* (Min, Max)	4.33 ± 0.43 (4.33, 5.48) mm^{-2}
Width of egg capsule layer in collar (min – max) *	19-30 mm
Thickness of collar at middle	1.56 ± 0.07 mm[$]
Vitelline membrane diameter (30-80-cell stage)	497 ± 16 µm[$]
Estimated development time (fertilization to hatching)	25 days[&]
Depth of collection	410 m
Water temperature at collection site	17-18°C

* Area of collar does not include the basal or apical acapsular membranes.
[#] Number of embryos per capsule often variable within a single egg collar
[$] Value ± standard deviation
[&] Inferred from development at 10-12°C of asynchronous cultures. See Cziko and Bennett, unpublished.

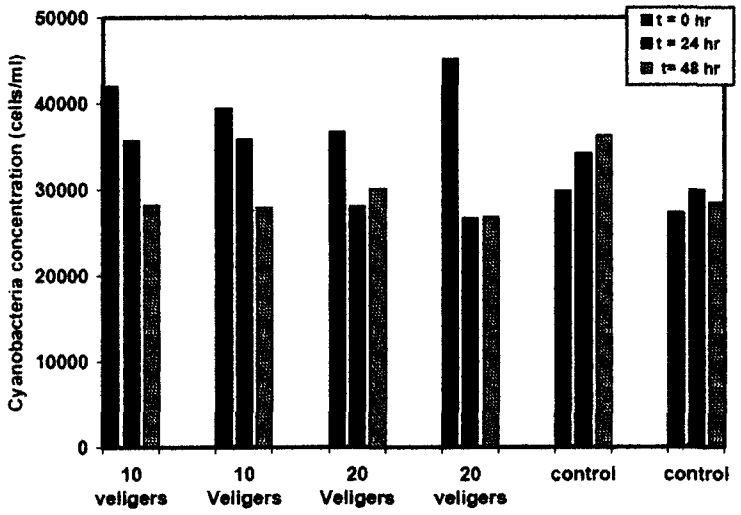

Figure 2. Results of clearance experiment. Swimming veligers were introduced into cultures containing marine synechococcus, with two replicates of each: 10 veligers (1/mL), and 20 veligers (2/mL). Both experimental trials showed a decrease in synechococcus cells suggesting clearance by veligers over a 48-hour period.

Table 2. Calculated clearance rates of synechococcus by veligers.

	Replicate 1 (cells individual^{-1} hr^{-1})*			Replicate 2 (cells individual^{-1} hr^{-1})*		
	0-24 hours	**24-48 hours**	**mean**	**0-24 hours**	**24-48 hours**	**mean**
10 veligers (~1/ml)	271	222	247	149	-28	61
20 veligers (~2/ml)	187	235	211	401	0	200

*Calculated rates do no account for the possibility of synechococcus growth and replication throughout the sampling period, thus clearance rates presented here are likely to be underestimated

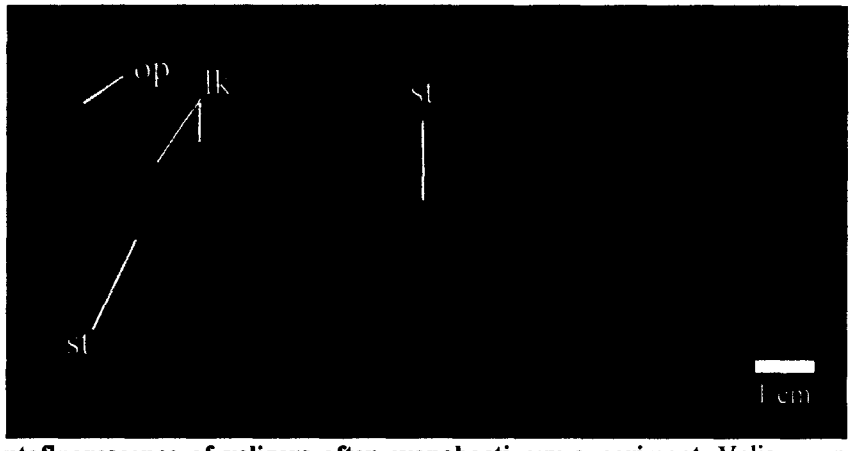

Figure 3. Autofluorescence of veligers after cyanobactivory experiment. Veligers under blue light excitation epi-illumination after completion of feeding experiment. Marine synechococcus fluoresces pale yellow under this excitation spectrum. Note autofluorescence of veliger operculum (op) and larval kidney (lk). Stomach area (likely site of cyanobacteria) is indicated (st). Fluorescence due to synechococcus was not observed.

Table 3. Sinking rates of hatched veligers.

Treatment	Sinking rate through 19-22°C still seawater column (mean ± SE)		
	cm/sec	m/h	m/day
Untreated, operculum closed, velum fully retracted	0.50 ± 0.04	18 ± 1.6	432 ± 38
Anesthetized in $MgCl_2$, velum partially protracted*	0.36 ± 0.05	13 ± 1.6	316 ± 39

*Cilia of ciliary bands on velum remain active following $McCl_2$ anesthetization.