A NEW APPROACH TO COMPARATIVE STUDIES OF *Strombus gigas* LARVAE AT THE DEVELOPMENTAL AND NUTRITIONAL LEVELS

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ABSTRACT

A new approach to the study of queen conch larvae was initiated in April 1988 at the Caribbean Marine Research Center, Lee Stocking Island, Exumas. This work investigates ecological mechanisms influencing *S.gigas* larval stages. During the 1988 reproductive season, studies were conducted to assess growth, nutritional requirements and metamorphosis of queen conch larvae reared in sea water and compared to artificial conditions in the laboratory.

INTRODUCTION

As part of the Benthic Ecology program at the Caribbean Marine Research Center, a new approach to the study of *Strombus gigas* larvae was initiated in April 1988. Although we have considerable knowledge on the life history of *Strombus gigas* Linne (Randall, 1964; Hesse, 1976) and larval culture in the laboratory (D'Asaro, 1965; Iversen, 1983; Laughlin and Weil, 1983; Davis *et al.*, 1985), the larval phase of this species has rarely been studied in quantitative context of its natural environment. In the past, larval studies were conducted with the immediate purpose of hatchery-rearing feasibility and mass production of juveniles. Information on larval dispersal, nutrition, predation, settlement and recruitment of *Strombus gigas* is still lacking. With this approach, we propose to elucidate mechanisms and strategies crucial to recruitment and natural distribution of *Strombus gigas* populations in the Exumas.

"Larva is a developmental stage, occupying the period from post-embryonic stage to metamorphosis, and it differs from the adult in morphology, nutrition and habitat" (Chia, 1974). As Roughgarden *et al.* (1988) imply, ecological studies of marine organisms with a complex life cycle needs to involve both habitats (larval & adult).

Length of the pelagic life is an important factor both in recruitment of the adult populations and geographic distribution of the species (Thorson, 1961; Mileikovsky, 1971; Scheltema, 1977). On one hand, a long larval period due to colder temperatures or poor nutrition could result in greater risk of starvation and predation or of transport away from habitat favorable for settlement (Thorson, 1950). On the other hand, longer planktonic period may enhance larval dispersal (Strathmann, 1980), and settlement habitat selection (Doyle, 1975; Obrebski, 1979). Yamaguchi (1975) hypothesized that size at metamorphosis, which in turn depends on larval growth, has an effect on post-settling growth and mortality rates. Therefore, growth during larval planktotrophic life is critical for survival of the newly metamorphosed Strombus gigas juveniles.

Our approach stems from research interests in larval invertebrate development and factors that may or may not alter its course in the natural environment. The **proximal objectives** of the study in progress are to determine the role of temperature and food supply on developmental rates, developmental timing, lengths of precompetent and competent periods, induction of metamorphosis, and post-settlement growth and survival.

The results reported here represent our specific approach to the study of Strombus gigas larvae. The detailed results of the experiments conducted in 1988 will be presented in seperate papers.

MATERIALS AND METHODS

Food supply

Cultured algae were grown in pure culture in Guillard's modified medium (Guillard and Ryther, 1962). Algal species were *Isochrysis* (**caicos & tahitian strain**). Algal cells were centrifuged and resuspended in filtered sea water to remove culture medium before feeding.

Filtered sea water (5 & 0.4 um) was used for both egg masses and larvae. Natural sea water, changed daily, was collected at the surface (< 1m) in Adderly Cut, just north of Lee Stocking Island and on the bank west of Lee Stocking. It was filtered through 500 um mesh before transferring into culture jars. Each food treatment consisted of two replicate containers.

Egg mass

Egg masses, collected in 20 m depth on the eastern edge of the Exuma sound, were suspended on a Nitex mesh (500 um) in filtered sea water changed daily until they hatched.

Larval cultures

Twenty-four hours after hatching, veligers (25/liter) were transferred into 1) 8 L Nalgene culture jars with 6 L of filtered sea water, 2) 1 L glass beakers, or 3) small Nalgene dishes with 200 ml of filtered sea water depending on the specific experiment. In some experiments water was kept in motion by a revolving paddle. Culture water was changed daily when using natural sea water; otherwise, water changes occurred every two to four days depending on the veligers developmental stage. Temperature was maintained at 27°C. Average temperature in the field varied from 25°C (April) to 29.5°C (August-September) during the 1988 *Strombus gigas* reproductive season. When changing culture container, water was siphoned by reverse filtration, i.e. larvae stayed in the jar as the culture water was lowered. Larvae concentrated in a smaller volume (100 ml) were then transferred into a clean jar filled with sea water.

Larval development & growth

Developmental sequence and characteristics were recorded relative to the age of the larvae. At each container change, larvae from each culture were observed with a dissecting microscope. Development was determined by the following: 1)velum development (size and stage), 2)siphon development, 3)stomach color, 4)shell development and 5) foot pigmentation. At regular intervals, ten larvae were removed from each culture and shell length was measured.

In the laboratory, one set of larvae was fed *Isochrysis* with filtered sea water; the other set, from the same egg mass, was fed the sea water with its natural assemblage of phytoplankton, bacteria and dissolved organic matter.

To test the effect of different feeding initiation times on veliger development, veligers were fed *Isochrysis* at 24, 48, 72 and 96 hours after hatching.

Competence & Metamorphosis

Larvae were observed to determine the relationships between age/size/development at the onset of competence (shell length = 1.2 mm).

Larval competence (i.e. ability to metamorphose) was tested by using various substrates. Larvae were placed in 1) plastic petri dishes covered with a natural algal/bacterial film (as a positive test) and 2) clean petri dishes (as a negative test). Other treatments such as *Acanthophora* sp., *Acanthophora* sp. extract, and clean petri dish with *Isochrysis* in the water were tested in the same manner.

RESULTS AND DISCUSSION

Hatching time

The majority of the eggs collected were at a similar developmental stage, usually the single cell stage. Veligers began hatching after five to six days at no particular time of the day. They continued to hatch over a two day period after which we discarded the egg mass.

Our observations indicated possible effects of temperature on hatching time. The effects of temperature changes on embryos, larvae and metamorphosis need to be integrated within the same study. This approach gives the best opportunity to understand the role of the larval phase within *Strombus gigas* life history.

Larval development

Precompetent periods for larvae submitted to the two treatments (fed *Isochrysis* & available nutrients in sea water) were comparable. The average number of days from egg collection (single cell developmental stage) to larval competence (morphological/developmental milestone) was 27 days. At that time, larvae were tested for their ability to metamorphose (behavioral/developmental change), having reached a shell length between 1.1 mm and 1.3 mm.

The length of the precompetent period was dependent on larval developmental rate, which in turn was dependent upon nutrition. There is uncertainty on what type of food is suitable for larval development leading to metamorphosis, and the concentrations of food required for optimal/minimal growth. The optimal and minimal nutrition requirements still need to be determined to ascertain the length of time a larva could spend in the plankton prior to metamorphosis.

The degree to which natural quality and quantity of food limits rates of larval growth and development is poorly known. An important consequence of a food limited environment is lengthening of the larval period with a greater risk of being eaten or transported away from favorable habitats. Thus, food limitation could contribute to variation in recruitment to benthic populations. Water quality needs to be regularly assessed throughout the *Strombus gigas* reproductive season for its ability to provide adequate feeding, i.e. normal growth, to the larvae in near-natural conditions.

Most of the published work on *S. gigas* larvae is oriented towards hatchery applications and is not concerned with developmental biology *per se*. Our study, on the other hand, concerned the developmental characteristics as they follow a defined sequence for larvae reared under different feeding regimes, i.e. natural sea water versus filtered sea water with added *Isochrysis*. Comparative studies of larval development conducted in the laboratory under controlled conditions will bring us closer to the larval development followed in the field (Boidron-Metairon, in prep.).

D'Asaro (1965) stated that *Strombus* larvae started to feed four days after hatching, whereas Siddall (1983) said larvae fed 6 hours after hatching. Nevertheless, the effects of feeding initiation on veliger development had not been tested previously. In our study, delayed feeding initially decreased growth rate, caused offset developmental sequences and increased length of larval period (between treatments) (Boidron-Metairon and Sandt, in prep.). These results also show that differences in developmental stages do not necessarily reflect differences in age (Boidron-Metairon, in press).

Larval competence

The dispersal potential of a larva is determined not only by its developmental rate to competence but also by its capacity of delaying metamorphosis successfully (larvae still able to metamorphose at the end of the delay period). Competent larvae seemed to test the substrate by inverting their velum towards the dish bottom with their shell upside down from the swimming position. Larvae fed with only the nutrients contained in natural sea water metamorphosed after 29 days of total developmental time. Larval shell length prior to metamorphosis was 1.2 to 1.3 mm.

When larvae are competent to metamorphose, the absence of cues will delay metamorphosis. Competent period is variable with the species. The maximum time that metamorphosis can be delayed by *Strombus gigas* larvae has not been determined. The developmental state of the competent larvae, which is dependent on larval nutrition, may have a role to play in post-metamorphic growth and survival.

Metamorphosis

Metamorphosis of competent *Strombus gigas* larvae was induced with a natural algal/bacterial film. Metamorphic success was 78% at 30°C. We still need to determine a natural metamorphosing cue (present where larvae are settling) which will yield consistent high percentage (>50%) of juveniles using competent larvae.

We observed two different metamorphosis processes during the course of this past reproductive season: 1) "shedding" the entire 6-lobed velum within twenty minutes, and 2) testing the substrate and resorption of the velum over two or three days after the onset of the experiment.

In other studies, the metamorphic process is usually seperated into two phases: 1) settlement behavior - when the larva crawls on the bottom of tanks (with a resorbing velum, pers. observ.) and 2) complete metamorphosis (*sensu* Brownell, 1977) that takes approximately ten days (complete resorption of the velum, outward migration of the eyes, disappearance of the velum) during which the juvenile conch adapts to a benthic existence.

The observation of two different metamorphosis mechanisms indicates that *Strombus gigas* metamorphosis is more complex than believed earlier and needs to be further investigated.

CONCLUSIONS

This approach to the study of *Strombus gigas* larvae gives us answers to important ecological questions as presented in the introduction. Our study will continue in the same direction as we pursue the experimental analysis of *Strombus gigas* life history with the following research recommendations:

1) How do temperature changes occurring in the field affect embryos, larvae and induction of metamorphosis? What is the influence of various temperatures on embryonic and larval development?

2) Are *Strombus gigas* larvae food limited in the field during the reproductive season?

3) How long can <u>Strombus gigas</u> larvae delay metamorphosis successfully?

4) Can the nutritional history of the larvae influence size at metamorphosis and post-settlement growth and survival?

5) What induces metamorphosis in nature?

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