A New Approach to Comparative Studies of *Strombus gigas* Larvae at the Developmental and Nutritional Levels

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ABSTRACT

In the past, *Strombus gigas* L. larval studies were conducted with the immediate purpose of hatchery-reared production of juvenile queen conch. 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. *S. gigas*, as a species, needs to be studied in relation to its natural environment. The ultimate goal of the larval ecology perspective is to investigate ecological mechanisms at work during the larval phase of *S. gigas* life history. Comparative studies were conducted to assess growth, nutritional requirements and metamorphosis of queen conch larvae in natural conditions. This paper presents new methods used in these studies along with initial results obtained during the 1988 reproductive season.

INTRODUCTION

Although we have considerable knowledge of the general life history of *Strombus gigas* Linne (Randall, 1964; Hesse, 1976) and have learned about

quantitative context of its natural environment. As part of the Benthic Ecology Program at Lee Stocking Island (Caribbean Marine Research Center), a new approach to the study of *Strombus gigas* larvae was initiated in April 1988. The ultimate purpose of the larval ecology component of the Benthic Ecology program at CMRC is to elucidate mechanisms and strategies crucial to the natural distribution of *Strombus gigas* populations around Lee Stocking Island, Exumas. 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 distinctly lacking.

"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 need 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 longer 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, a longer planktonic period may enhance larval dispersal (Strathmann, 1980), and settlement habitat selection (Doyle, 1975; Obrebski, 1979). Strathmann (1980) speculates that if a larva has a long precompetent period it may require a similarly long competent period to be transported to a suitable locality for settlement (*e.g.* Pechenik, 1980; Jackson and Strathmann, 1981). Yamaguchi (1975) hypothesized that size at metamorphosis, which in turn depends on larval growth, has an effect on post-settlement 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 separate 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 strains). Algal cells were centrifuged and resuspended in filtered sea water to remove culture medium before feeding.

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

Egg mass

Egg masses were collected under a spawning female at a site located offshore Lee Stocking Island at 20 m depth on a coarse sandy bottom. Back in the laboratory, they were suspended on a Nitex mesh (500 μm) in filtered sea water changed daily until they hatched.

Larval cultures

Twenty-four hours after hatching, veligers were transferred into:

1. 8.0 liter Nalgene culture jars with 6.0 liters of filtered sea water,
2. 1.0 liter glass breakers, or
3. small Nalgene dishes with 200 milliliters 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 state. Temperature was kept at 27°C. Average temperature in the field varies from 25°C (April) to 29.5°C (August–September) during the *Strombus* reproductive season. When changing culture containers, water was siphoned out of the jars 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. If only water needed to be changed, water was siphoned in the same way and new water was added slowly to the same container.

Larval Development and 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 examined following these criteria: velum development (size and stage), siphon development, stomach color, shell development and foot pigmentation. At regular intervals, ten larvae were removed from each culture and shell length was measured.

Strombus gigas larvae from the same egg mass were reared in the laboratory on Lee Stocking Island using two treatments. One set of larvae (25/liter) was fed *Isochrysis* with filtered sea water; the other set was fed the natural assemblage of phytoplankton, bacteria and dissolved organic matter found in natural sea water.

Another experiment tested the effect of different feeding initiation times on veliger development. Veligers were fed *Isochrysis* at 24, 48, 72 and 96 hours after hatching.

Competence and 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:

1. in plastic petri dishes covered with a natural algal/bacterial film (as a positive test), and
2. in clean petri dishes (as a negative test).

Other treatments such as *Acantophora*, *Acantophora* extract, and clean petri dish with suspended food in the water (*Isochrysis*) were tested in the same manner.

RESULTS AND DISCUSSION

Hatching Time

Since only 1/3 of the total mass was collected as it was being laid, the majority of the eggs were at a similar development stage, usually the single cell stage. Veligers started to hatch 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.

Fortuitous observations indicated possible effects of temperature on hatching time. There is no published work testing the effect of various temperatures on the length of the precompetent period. Furthermore, the effects of temperature changes on embryos, larvae and metamorphosis should be integrated within the same study (Cameron *et al.*, 1985; Boidron-Metairon, 1987). 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* & natural sea water) were comparable. The average number of days from egg collection (single cell developmental stage) to larval competence was 27 (± 2) days. At that time, larvae were tested for competence to metamorphosis, having reached a shell length between 1.1 mm and 1.3 mm.

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 successful larval development, *i.e.*, leading to metamorphosis, and the concentrations of food for optimal/minimal growth. None of these measurements can be made in the field; however, laboratory studies can be conducted on a quantitative basis for comparison with results using natural sea water. 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 not well known. An important consequence of a larval period lengthened by a food-limited environment is 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 to the larvae in near-natural conditions.

Any developmental section of most papers on *Strombus gigas* larvae refers to D'Asaro (1965). All of the published work is oriented mainly towards hatchery applications and is not concerned with developmental biology per se. The objective of this part of the study was to observe 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., a).

D'Asaro (1965) stated that *Strombus* larvae started to feed four days after hatching, whereas Siddall (1983) said larvae were feeding 6 hours after hatching. Nevertheless, the effects of feeding initiation on veliger development had not been tested previously. Later feeding decreased developmental rate initially (slower growth rate), caused offset developmental sequences and increased length of larval period (between treatments) (Boidron-Metairon and Sandt, in prep., a). These results also show that differences in developmental stages do not necessarily reflect differences in age (Boidron-Metairon, 1989).

Larval Competence

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 natural sea water metamorphosed after 29 days of total developmental time. Larval shell length prior to metamorphosis was 1.2 to 1.3 mm. Shell morphology is quite distinct, as the last whorl edge of the shell becomes round instead of its prior pointed form. Establishing relationships between age/size/development state at the onset of competence is another means of learning about larval development in natural conditions (Boidron-Metairon, in prep., b).

When larvae are competent to metamorphose, the absence of the metamorphosing cue will delay metamorphosis. Competent period (length of competence) is variable with the species. No published study has shown the maximum time that metamorphosis can be delayed by *Strombus gigas* larvae. 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) (Boidron-Metairon and Sandt, in prep., b). The developmental state of the competent larvae, which is dependent on larval nutrition, may have a role to play in the post-metamorphic growth and survival of *Strombus gigas*.

Metamorphosis

Metamorphosis of competent *Strombus gigas* larvae was induced with a natural algal/bacterial film. Metamorphic success was less than 100%. This substrate has been used for successful metamorphosis (100%) of other invertebrates larvae such as sea urchins (Cameron and Hinegardner, 1974). We still need to determine a natural metamorphosing cue (present where larvae are settling) which will yield 100% metamorphosed juveniles using competent larvae.

Two different metamorphic processes were witnessed during the course of this past reproductive season:

1. Larvae were observed to "shed" their entire 6-lobed velum within twenty minutes.
2. Larvae were observed to test the substrate and subsequently to resorb their velum over two or three days after the onset of the experiment.

Past published work usually separated the metamorphic process in two phases:

1. Settlement behavior – when the larva crawls on the bottom of tanks (with a resorbing velum, pers. observ.).
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 metamorphic mechanisms indicates that *Strombus gigas* metamorphosis is more complex than believed earlier and needs to be further observed and investigated.

CONCLUSIONS

This new approach to the study of *Strombus gigas* larvae is starting to give us partial answers to important ecological questions as presented in the introduction. We still need more time to refine and integrate the results into our experimental analysis of *Strombus gigas* life history.

Our research objectives will be pursued as stated in the introduction. The following specific questions will be addressed:

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 *Strombus gigas* larvae delay metamorphosis successfully?
4. Can the nutritional history of the larvae influence size at metamorphosis and post-settlement growth and survival?
5. What are the natural inducers of metamorphosis?

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