The Effects of Natural Foods on the Growth and Development Of Queen Conch Larvae (*Strombus gigas*)

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ABSTRACT

Phytoplankton biomass and quality caused variation in the larval life span of queen conch, *Strombus gigas*. Conch larvae were grown in the laboratory on two sources of natural seawater, Great Bahama Bank and Exuma Sound, and on cultured algae, *Caicos Isochrysis* and *Chaetoceros gracilis*. Larvae were also fed a continuous supply of natural phytoplankton from Bahamian waters in a field-enclosed mesocosm system. Metamorphic competence was achieved in the shortest time (day 16) in the mesocosm system. In the laboratory larvae were competent by day 26 in the Bank water and day 20 in the cultured algal treatments. Veligers fed Sound waters did not achieve metamorphic competence. Food source was best characterized by cell counts and composition, than Chl *a* concentrations. Bank and Sound waters had similar Chl *a* levels, however, Bank water had larger cells, more taxa and higher cell count. Natural foods can be used as an alternative food source for culturing *S. gigas* larvae. Results from feeding studies using natural foods can also be used to predict dispersal potential in the field.

KEY WORDS: Growth, larvae, mesocosm, natural foods, phytoplankton, *Strombus gigas*

INTRODUCTION

The large gastropod *Strombus gigas* (queen conch) inhabits the shallow seagrass beds of Florida and the Caribbean region (Randall 1964). Since the 1970's the queen conch has been an important fisheries species in this region. The recent landings for conch are estimated at 6,000 MT with a value of 60 million dollars US (Chakalall and Cochrane 1997). Overfishing of this species has necessitated the need for management regulations in many countries (Appeldoorn 1994, Chakalall and Cochrane 1997). As of 1992 queen conch was added to Appendix II under CITES (Convention for the International Trade of Endangered Species). Now countries that export conch need permit approval from CITES management to ensure that the species is harvested at a level consistent with its fisheries population. However, management practices may not be enough to bring back overfished stocks or maintain fisheries populations over the long term.

Methodologies to culture queen conch larvae to the juvenile stage for stock

enhancement and for growout markets were established by several organizations between the 1970s and 1990s (Creswell 1994, Davis 2000). Typical culture methods start with collection of egg masses from the field. Larvae (veligers) are cultured for approximately 21 days and fed cultured phytoplankton. Juvenile conch are fed an artificial pellet diet and are grown in sand trays and shallow ponds. Final growout of conch is accomplished in field enclosures.

In this field and laboratory study veligers of *Strombus gigas* were fed natural foods from two adjacent tropical oligotrophic waters in the Bahamas (Great Bahama Bank and Exuma Sound) and cultured phytoplankton to determine how differences in food quantity and quality affect larval growth and survival to metamorphic competence. The results from this investigation present alternative larval food sources and systems for culturing veligers of *S. gigas*. The data can also be integrated with oceanographic processes to estimate dispersal potential and supply of larvae to settlement sites.

MATERIALS AND METHODS

Field and laboratory studies were conducted at the Caribbean Marine Research Center (CMRC) field station on Lee Stocking Island (LSI), Exuma Cays, Bahamas from July to August 1994 and from June to July 1995. One egg mass was collected each experimental year from an offshore reproductive site located at a depth of 18 m on the island shelf approximately 1 km east of LSI, in the Exuma Sound. The newly laid egg mass was incubated in the laboratory for 4 days in a flow-through system (Davis 1994a). On the day of hatching several strands of the egg mass were placed in individual 8 L plexiglass vessels. The following morning (day = 0) the newly hatched veligers were used for the field and laboratory studies to test for the effects of different food types on growth of conch veligers.

Field Studies

The *in-situ* flow-through mesocosm system was moored in 3-m deep water near Lee Stocking Island. The system was comprised of a 3.6 m diameter hexagon shaped platform that supported 6 mesocosms, solar array, batteries and pumping system (Davis et al. 1996). Each mesocosm was a 1.8 m long x 0.5 m diameter transparent fiberglass cylinder with a 45° conical bottom. The 200 L mesocosm was submerged except for the top 0.5 m to prevent sample loss and wash-over by waves. To avoid contamination, each mesocosm was covered with a transparent fiberglass top. A solar charged battery powered a 12-volt bilge pump, which provided a continuous flow of ambient water (1 L/min, 8 exchanges of water per day), to each mesocosm. The water was pumped from 1 m below the system. To retain the larvae, each cylinder was equipped with a porous standpipe. Seawater was filtered through 50 μm bags, which allowed

phytoplankton to enter the mesocosms, but blocked potential predators and competitors. These bag filters were changed daily.

Newly hatched larvae were stocked into 2 mesocosms at a concentration of 20 veligers/L. Every four days density was reduced by counting veligers out of one mesocosm into a newly-filled mesocosm. Final density at the end of the experiment was 0.7 veligers/L. Larvae were grown until <50% of the veligers showed signs of metamorphic competence (Davis 1994b). These signs include dark green pigmentation on the propodium. The temperature fluctuated during the day according to tide and ranged from 28 to 30 °C. To determine growth and development rates of veligers, shell lengths of 15 veligers were made every other day using a dissecting microscope (40X) equipped with an ocular micrometer. Replicate water samples (800 ml) from the mesocosm were filtered every other day to determine Chl *a* concentration (ng/L). Extraction of Chl *a* and fluorometric readings were performed according to standard methods (Strickland and Parsons 1972).

Laboratory Studies

In the 1994 laboratory study, veligers were fed phytoplankton assemblages from three different sources:

i) natural foods from Great Bahama Bank water (Bank),
ii) natural foods from Exuma Sound water (Sound), and
iii) an algal mixture of cultured Caicos *Isochrysis* and *Chaetoceros gracilis*.

In 1995, veligers were only fed natural foods from Bank and Sound water. Shallow Bank water was collected on ebb tide and Sound water was collected from the shelf on the flood tide (Figure 1). The temperature for the Sound water was 27.5 ± 0.8 °C and for Bank water 29.0 ± 1.3 °C. The seawater was filtered through a 250 μm mesh sieve to exclude most of the potential predators, while conserving food cells in the culture filtrate.

The cultured algae, Caicos *Isochrysis* and *Chaetoceros gracilis*, fed to larvae in this study are typical foods used in conch larviculture (Davis 1994a). The algae were grown in 250 ml flasks using Guillard's (1975) methodology and fed to achieve a final density of 5000 to 10,000 cells/ml. To avoid introducing natural phytoplankton into cultured algal treatments, all water was 0.45 μm filtered prior to adding algae. When veligers were cultured in only filtered seawater they did not survive past day 8.

The newly hatched veligers were stocked at 12 veligers/L in 800 ml transparent polypropylene containers. On day 10 the concentration was reduced to 5 veligers/L. There were five replicates per treatment for the 1994 study and four replicates per treatment for the 1995 study. To maintain densities additional veligers were cultured under the same treatment conditions to replace animals that died. To keep track of mortality, the number of veligers that died was

recorded on each measuring date. When all the veligers died, the replicate was discarded. The treatments were run until veligers showed signs of metamorphic competence, halted development, or died.

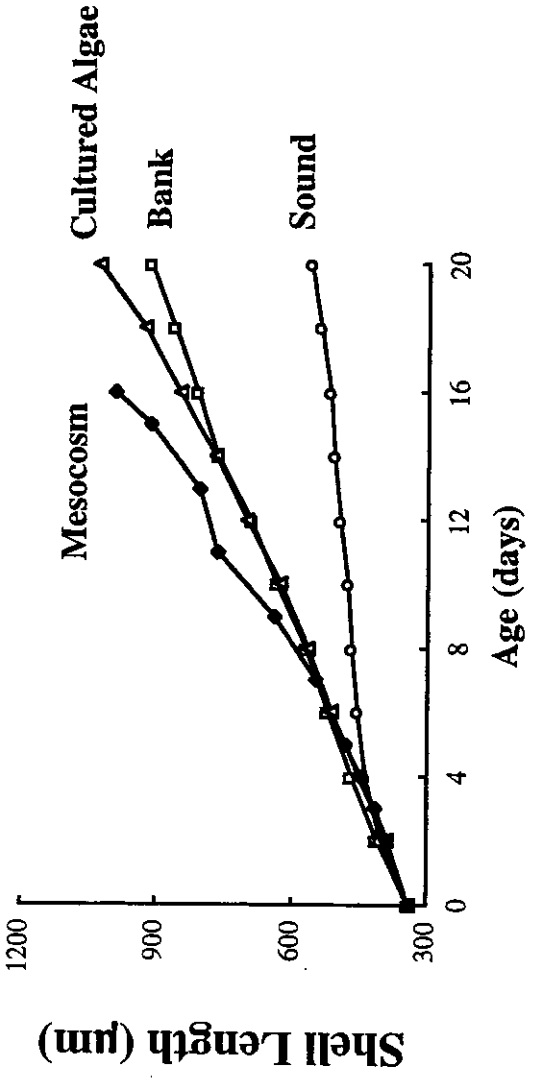

Figure 1. Growth curves for veligers of *S. gigas* fed natural foods (Bank, Sound, and mesocosm waters) and cultured algae (*Isochrysis* and *Chaetoceros*).

Veligers were cultured in an environmentally controlled incubator (28-29 °C, 38 ‰, 12 hr light: 12 hr dark). Water in the culture vessels was static and changed daily by pouring the veligers and water through a submerged sieve (180 μm mesh). A wash bottle with 0.45 μm filtered seawater was used to move the veligers from the sieve into the treatment water and culture vessel.

To determine growth rates of veligers in each treatment, shell length of all veligers in each replicate was measured from the apex to the siphonal canal every other day using a dissecting microscope (40X) equipped with an ocular micrometer.

Replicate water samples (800 ml) from each treatment were collected every other day to determine Chl *a* levels. To identify and count natural food cells, 15 L from each water mass (Bank and Sound) was concentrated to 1 L using the tangential filtration system (Davis 1998). Water was collected four times from the Bank and three times from the Sound over a one week period (20-26 June 1995) during the experiment. Five samples were taken from the concentrate and cells were counted and observed using a 4 chamber hemacytometer and a compound microscope (400 - 1000 X).

RESULTS

In 1994, when veligers were fed Bank water or high concentrations of cultured algae (7,939 cells/ml $\pm$ 1,211) they grew at similar rates from day 6-18, but on day 20 shell length (SL) was statistically smaller for veligers fed Bank water ($p < 0.05$) (Figure 1). On day 20, 35% of the veligers fed Bank water were metamorphically competent and shell length (SL) was 915 $\pm$ 62 μm. For veligers fed cultured algae, 80% were competent and SL was 1030 $\pm$ 28 μm. None of the veligers fed Sound water became competent by day 20. Development was arrested and they only attained 560 $\pm$ 21 μm SL (Figure 1). Veligers in the mesocosm system grew faster than their laboratory counterparts. Metamorphosis for veligers fed 50 μm filtered phytoplankton was first seen on day 13. By day 16, 95% of the veligers were competent or had completed metamorphosis with an average size of 933 $\pm$ 51 μm SL.

Although veligers fed cultured algae grew at rates (35 μm/day) equal to or slightly faster than veligers fed Bank water (29 μm/day), the Chl *a* concentrations were remarkably different. Average concentration was 4,700 ng/L for cultured algae and 176 ng/L for the Bank water, a 25-fold difference. The Chl *a* concentration in the mesocosm was 160 ng/L that was not statistically different than Bank water ($p > 0.05$). However, growth rates were faster for veligers grown in the mesocosm (41 μm/day) than on Bank water (29 μm/day). Chl *a* concentrations were statistically higher for Bank water than Sound water (107 ng/L) ($p < 0.05$). However, this Chl *a* difference (69 ng/L) was not large, and in 1995 studies ambient Chl *a* concentrations for Bank (147 ng/L) and Sound

(109 ng/L) water were not significantly different ($p > 0.05$). Even though Chl *a* concentrations were similar or slightly higher for Bank water, the average growth rates for veligers were considerably faster in Bank water than Sound water in both 1994 (29 μm/day vs 11 μm/day) and 1995 (26 $\mu m^{/day}$ vs 7 μm/day) (Figure 2).

The digestive glands of veligers fed Sound water contained a minimal amount of food and were clear to pale in color. These veligers developed 6 velar lobes; however, these lobes were stunted, deformed and wider than lobes of veligers fed Bank water. Lowest survival (62%) occurred for veligers fed ambient Sound water. Veligers cultured with Bank water, cultured algae or mesocosm water had golden brown guts full of algae, were very active, and developed 6 elongated lobes. Overall survival for these cultures was 90%, ~100%, and 73%, respectively.

Cell counts and composition of natural foods may partially explain growth differences for veligers fed natural foods from Bank and Sound water. The average total cell count for Bank water was 725 cells/ml and was statistically higher (3 times) than Sound water ($p < 0.05$), which was 234 cells/ml. The Bank and Sound water had similar dominant species and overall composition. However, the higher cell count in Bank water was attributed to the cell count in the class Bacillariophyceae and presence of cells in the class Euglenophyceae. In both water sources the dominant class was Cyanophyceae, followed by Haptophyceae or green flagellates, then colorless microflagellates. Even though the dominant classes were similar in the two water sources, the size and number of taxa differed. The Bank water contained four size categories of cells: microplankton (50 - 500 μm), nannoplankton (5 - 50 μm), ultraplankton (2 - 5 μm) and picoplankton (< 2 μm), whereas the Sound water had only the 3 smaller size categories. The Bank water also had 3.3 times more taxa and more cells in most size categories than Sound water.

During the week of 20-26 June 1995, there were fluctuations in variety and number of cells found in the water masses, especially in Bank water. Cell counts in Sound water ranged from 117 - 359 cells/ml per day and Bank water ranged from 467 - 1107 cell/ml per day. Even though fluctuations in cell count occurred on a daily basis, the growth curves for veligers fed natural foods from Bank and Sound water were similar during two years (Figure 2).

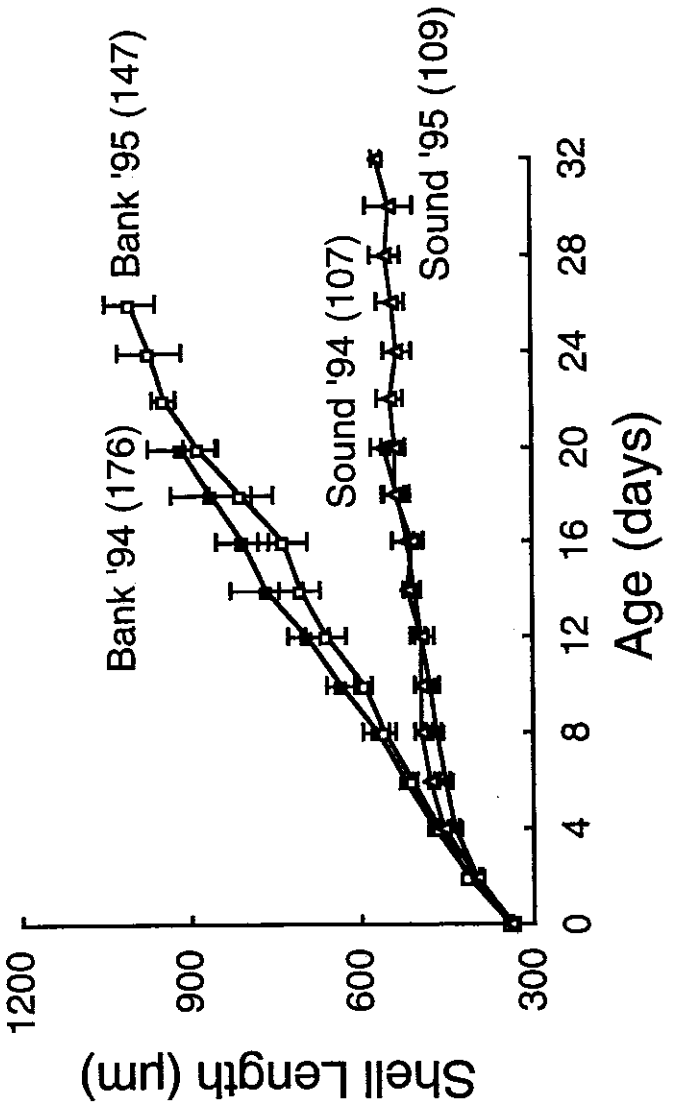

Figure 2. Comparison of growth curves for veligers of *S. gigas* fed natural foods from Bank and Sound water sources in August 1994 and June-July 1995. Data points represent mean ± sd (n = 5 replicates in 1994 and 4 replicates in 1995). Numbers in parentheses are the average Chl *a* concentrations.

DISCUSSION

Food quality and quantity are important conditions that influence length of larval life (Scheltema 1986). These are significant environmental factors from both an aquaculture and ecological viewpoint. Ideal food conditions in aquaculture promote high growth and survival rates. Traditionally, a wide range of laboratory cultured algae have been used to raise *S. gigas* larvae in research and commercial operations (Aldana-Aranda and Suarez 1998). The results from this study show that veligers of *S. gigas* can be cultured on certain types of phytoplankton assemblages collected in the waters where veligers disperse naturally. Veliger growth and time to metamorphic competence were good parameters to evaluate the nutritional quality of water.

In the laboratory, growth rates for veligers fed natural foods from Bank water were as rapid as for veligers fed cultured algae, a finding also reported by Boidron-Metairon (1992). However, to achieve this similar growth rate, veligers fed cultured algae required 11 times the number of cells and 25 times the Chl *a* level of natural foods. The mesocosm system proved to be a successful culturing unit to raise veligers to metamorphosis. Growth rates in the mesocosm were faster (16 days to metamorphosis) than in other treatments (cultured algae: 20 days, Bank: 26 days), due to continuous flow of natural foods. These results suggest that natural foods contain nutritionally superior cells compared to cells cultured in the laboratory. These findings not only suggest that natural foods can be used to raise veligers, but also underline the necessity of using natural foods for nutritional studies when field predictions will be extrapolated from the data.

Chl *a* concentrations were used as a relative index of the food availability and the quality of each water mass. However, the use of this water quality parameter proved to be misleading, corroborating the finding of Olson et al. (1987). In 1995 the ambient Chl *a* levels for Bank (147 ng/L) and Sound (109 ng/L) water were not statistically different, but growth and survival rates were considerably higher in Bank water. Veligers grown in Sound water proved to be a severely food limited and did not achieved metamorphosis. Cell composition and density may be better parameters to evaluate food quality. In this study high growth and survival rates for veligers fed Bank water may be attributed to at least three factors: larger cells, more diverse taxa (3.3 times more), and higher cell count than Sound water.

Growth rates for veligers fed natural foods from Bank water and from Sound water were similar during the peak months of two spawning seasons (June to August 1994 and 1995). The nutritional stability of these waters may be due to lack of long term or seasonal blooms which is typical of tropical, low latitude regions where phytoplankton density is low due to the lack of a constant source of nutrients (Raymont 1980, Harris 1986, Howarth 1988). It is possible that

overall growth and time to metamorphic competence maybe predictable from year to year in the peak months of the spawning season when veligers feed from the same water mass. To fully understand the relationship between growth of veligers and seasonal and short-term changes in food abundance and composition, nutritional experiments need to be conducted over the entire culturing and spawning season.

From an ecological viewpoint, food conditions in the oligotrophic waters of the Bahamas can be limiting or promote high growth and survival rates for veligers of S. gigas. Veligers suffered slow growth rates, arrested development and had low survival when fed natural foods from the Sound, whereas they grew at high rates to metamorphic competence when fed natural foods from shallow, nearshore Bank water. In the field it is likely that veligers encounter different feeding environments due to oceanographic circulation patterns and larval behavior. These differences will influence dispersal potential and settlement patterns for this important pan-Caribbean species.

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