Metamorphic Response of Queen Conch (*Strombus gigas*) Larvae Exposed to Sediment and Water from Nearshore and Offshore Sites in the Florida Keys

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ABSTRACT

Queen conch, *Strombus gigas,* is an important fisheries species that has been over-harvested in many locations throughout the Caribbean including Florida. The conch population in the Florida Keys has been slow to recover due, in part, to diminished recruitment and declining environmental conditions. Therefore, it is crucial to evaluate the efficacy of management strategies aimed at conserving and restoring queen conch populations. As such, this study examined the effects of juvenile conch habitat quality on metamorphosis. Competent conch larvae were exposed to sediment and water collected from two nearshore sites adjacent to the land and two offshore sites along the reef tract in the Florida Keys. Juvenile conch aggregations were present at all sites. Metamorphic response to nearshore and offshore treatments were similar ($p < 0.05$), and the average number of larvae that metamorphosed ranged from 62% to 85%. In addition, there was no significant difference in metamorphic response for larvae exposed to site sediment with site water or to those larvae exposed to site water only ($p < 0.05$). However, larvae that metamorphosed when exposed to nearshore treatments were not as robust (defined as crawling on the substrate and searching for food with proboscis) as those exposed to offshore treatments. These findings indicate that both nearshore and offshore habitats are favorable settlement locations for competent larvae; however, nearshore sites may not have the same quality as offshore sites. Resource managers can apply these results to assist in defining critical juvenile nursery grounds for conservation and stock enhancement.

KEY WORDS: Queen conch, metamorphosis, *Strombus gigas*

La Respuesta Metamórfica de las Larvas del Caracol (*Strombus gigas*) Expusieron a Sedimento y Agua de Sitios Cercanos a la Costa y Arrecifales de los Cayos de la Florida

El caracol, *Strombus gigas*, es una especie importante de pesquerías que ha sido sobre-explotado a través del Caribe inclusive en la Florida. La población del caracol en los Cayos de la Florida se ha recuperado lentamente debido, en parte, a un reclutamiento mínimo y ha condiciones ambientales en deterioro. Es importante evaluar la eficacia de estrategias de manejo que proponen a conservar y restaurar poblaciones del caracol. Por lo tanto, este estudio examinó los efectos de la calidad del habitat juvenil sobre la metamorfosis de las larvas del caracol. Larvas competentes fueron expuestas a sedimento y agua colectado de dos sitios cerca de la costa y dos sitios arrecifales en los Cayos de la Florida. Agregaciones de juveniles existen en todos sitios. La respuesta metamórfica en los tratamientos cercanos a la costa y arrecifales fueron similares ($p < 0.05$), y el porcentaje de larvas que metamorfosearon fue de 62% a 85%. Además, no había diferencia significativa en la respuesta metamórfica de larvas expuestas al sedimento del sitio con agua del sitio ni a esas larvas expuestas al agua del sitio sólo ($p < 0.05$). Sin embargo, las larvas que metamorfosearon cuando expuesto a tratamientos cerca de la costa no fueron tan robustas (definido como arrastrándose en el sustrato y buscando alimento) como esos expuesto a tratamientos arrecifales. Estos resultados indican que los habitates cercanos a la costa y arreciflaes son areas favorables de reclutamiento para larvas competentes; sin embargo, parece que los sitios cerca de la costa no tienen la misma calidad que los sitios arrecifales. Estos resultados se pueden aplicar para definir el habitat crítica de los juveniles.

PALABRAS CLAVES: Caracol, metamorfosis, *Strombus gigas*

INTRODUCTION

Queen conch (*Strombus gigas*), a marine gastropod, is found throughout the Caribbean and southern Florida (Randall 1964). The conch industry is a valuable commercial fishery for the Caribbean exceeded only by the harvest of spiny lobster (Appeldoorn 1994). Due to intense pressure from fishing, queen conch was listed in the Convention on International Trade of Endangered Species (CITES) in the 1980s and in 1992 was listed in Appendix II of CITES. In Florida, the decline of the queen conch population resulted in the government enacting legislation (Florida Administrative Code 1985) that made the collection of queen conch illegal (Glazer and Berg 1994). Though pressure from fishing has decreased, recovery of the queen conch population in the Florida Keys has been slow (Glazer and Berg 1994). In 1992, belt-transects estimated that 5,800 adult conch were present in the Florida Keys. In 2003, approximately 37,000 adult were present in the Keys with estimates of another 25,000 juveniles (Delgado Pers. comm.). Even with the increase in abundance, the current population would not be sustained if fishing were allowed again.

In the Florida Keys, two distinct conch populations exist in nearshore and offshore waters (Glazer and Quintero 1998). The two populations do not exchange genetic information, which has been attributed to the Hawk Channel (Delgado et al. 2004). The channel acts as a physical barrier due to its composition of soft sediment, which the conch do not inhabit (Glazer and Berg 1994). The slow recovery of the conch has been attributed to smaller spawning aggregations and decreased recruitment between populations as a result of the channel (Delgado et al. 2004). Adding to problems of low abundance, nearshore aggregations have not reproduced since the early 1990s (Glazer and Berg 1994). Nearshore aggregations lack gonadal development required for reproduction, while offshore aggregations have normal gonadal development (Delgado et al. 2004). Translocation has been an effective way to improve reproductive success.

Possible cues that induce larvae to settle include the presence of adults, a food source, or an appropriate habitat (Walters et al. 1996). The quality of water at habitats may also affect the development of larvae (Glazer and Quintero 1998). It is known that larvae will go through metamorphosis when exposed to the red algae *Laurencia*, but only in high concentrations (Davis 1994, Boettcher and Targett 1996). When exposed to different substratum and sediment samples found in conch nurseries, larvae showed a high rate of metamorphosis (Stoner et al. 1996b). To better understand the quality of the two habitats, larvae were exposed to sediment from nearshore and offshore sites where conch were present. Sediment was also taken from directly beneath juveniles at an offshore site to determine if there was a response to conspecifics.

MATERIALS AND METHODS

Culture

Queen conch egg masses were collected from the Florida Keys on June 16, 2004 and cultured at Harbor Branch Oceanographic Institution from June 17-July 7, 2004 following procedures described by Davis (1994). The egg masses were incubated until they were ready for hatch. Larvae were raised in 700 L larval tanks under static conditions. A complete water change occurred every 48 hours, and larvae were fed daily with Tahitian *Isochrysis*. Larvae were raised until competent for metamorphosis, which was at twenty-one days, when pigment on the foot changed from orange to dark green (Davis 1994).

A test set was performed prior to the experiment to determine if larvae were competent for metamorphosis and to determine which concentration of *Laurencia* to be used during the experiment. The *Laurencia* treatment was prepared as described in Davis and Shawl (In press). Twelve larvae were exposed to *Laurencia* extract at a concentration of 7ml/L of seawater. Ten larvae were placed in 100 ml glass dishes and exposed to Laurencia concentrations of 10, and 15ml/L of seawater. Larvae were exposed to the *Laurencia* treatments for 4 hours and then percent metamorphosis was determined. The larvae had complete metamorphosis when their velar lobes were lost, they were crawling around with the propodium, and they were searching for food with the proboscis (Davis 1994). Dosage was determined to be effective when 60% or greater of the larvae have gone through metamorphosis. It was found that 15ml/L had the best rate of metamorphosis.

Experiment

On July 7, 2004, competent larvae were transported in gallon plastic bags placed in coolers. Approximately 160 conch were placed in each bag, with a total of twelve bags. Temperature within the bags was 28.6ºC. Larvae were in the bags for five and a half hours prior to their arrival in the Keys.

On arrival, the larvae were exposed to a 16 different treatments to induce metamorphosis: a positive control of Laurencia extract (15ml/L seawater), a negative control of Harbor Branch seawater (27.5 ºC), which was filtered and UV treated were used. Queen conch larvae were exposed to sediment samples from two offshore sites and two nearshore sites (Table 1). All sediment samples were taken from areas where conch were present (Figure 1). Each treatment used 75 ml of water, site water or HBOI water. There was a slight variation in water temperature between nearshore and offshore sites (Table 1). Each treatment and control had four replicates with 10 larvae per replicate (Table 2). Each treatment sat overnight in dark conditions (19 hours), in a water bath of 28 ºC. Ending water temperature for the treatments was 26 ºC. Larvae from the *Laurencia* treatment were removed after four hours and placed in negative control water and fed. They were analyzed when the other treatments were viewed.

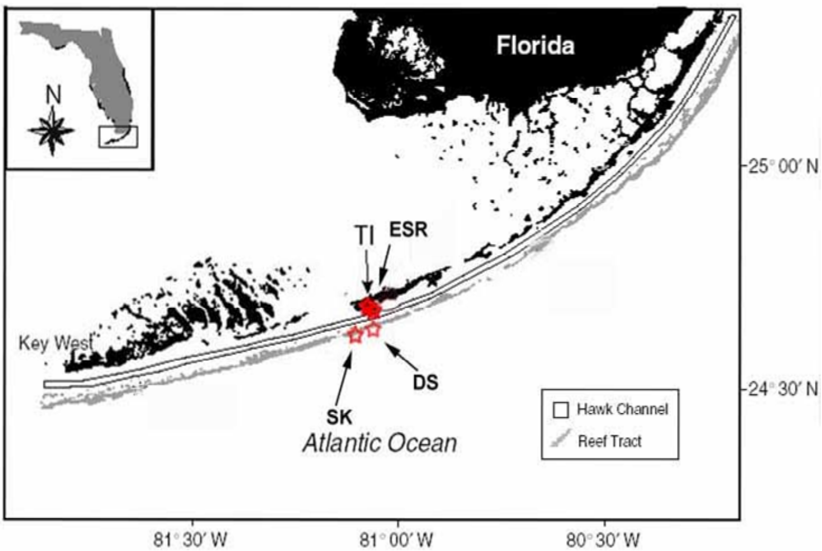

Figure 1. Location of sediment sampling sites in the Florida Keys. Arrows point to the general location of sampling sites. (TI) Tingler Island, (ESR) East Sister Rock, (SK) Sombrero Key, (DS) Delta Shoal

Table 1. Description of sediment samples from nearshore and offshore sites in the Florida Keys, July 8, 2004

Site	Location	Depth (ft)	Tide	Water Temp (ºC)	Vegetation	Sediment Description
Tingler Island (TI)	Nearshore	6	Slack low	30.7	Calcareous red algae, possible *Dictyota* sp.	Hard bottom, mix of rubble and coarse sand, has pieces of algae (*Halimeda*, calc. red algae), snail shells, color: dark tan and brown
East Sister's Rock (ESR)	Nearshore	6	Slack low	30.7	A little *Bataphora*, many sponges, Bivalve pieces encrusted with red algae	Little rubble, mostly fine sand, color: dark tan
Sombrero Key (SK)	Offshore	10	Ebb	30.1	*Thalassia* green and detrital blades, *Halimeda* live and dead, possible *Hypnea*	Few rubble pieces (*Halimeda*), majority of course sand with some fine sand, color: light tan
Delta Shoal (DS)	Offshore	6	Ebb	30.1	*Halimeda incrussata*, *Thalassia*, green and detrital blades, *Halimeda opuntia*	Few rubble pieces (*Halimeda*), mostly course sand, with some fine sand, color: light tan
Delta Shoal (DS)	Offshore under conch	6	Ebb	30.1	*Thalassia,* Seagrass live and detrital	Mostly fine sand with some coarse sand, pieces of coral rubble, live *Halimeda*, color: light tan

Table 2. List of treatments in metamorphosis experiments on *Strombus gigas* conducted at Florida Fish and Wildlife Center in the Florida Keys. HBOI: Harbor Branch Oceanographic Institution; TI: Tingler Island; ESR: East Sister Rock; SK: Sombrero Key; DS: Delta Shoal

Treatment	Source	Quantity Sediment	Quantity water
Control			
Positive: Laurencia	*Laurencia*		75mL
Negative: HBOI seawater	HBOI seawater		75mL
Nearshore			
TI sediment w/ TI water	Tingler Island	2mL	75mL
TI sediment with HBOI water	Tingler Island	2mL	75mL
TI water only	Tingler Island		75mL
ESR sediment w/ ESR water	East Sister's Rock	2mL	75mL
ESR sediment with HBOI water	East Sister's Rock	2mL	75mL
ESR water only	East Sister's Rock		75mL
Offshore			
SK sediment w/ SK water	Sombrero	2mL	75mL
SK sediment with HBOI water	Sombrero	2mL	75mL
SK water only	Sombrero		75mL
DS sediment w/ DS water	Delta	2mL	75mL
DS sediment with HBOI water	Delta	2mL	75mL
DS water only	Delta		75mL
DS sediment under juvenile with DS water	Delta	2mL	75mL
DS sediment under juvenile with HBOI water	Delta	2mL	75mL

Percent metamorphosis was determined after larvae sat overnight (19 hours) in the treatments. Larvae were visually analyzed using dissecting microscopes and placed in clean seawater. Three classifications were used to describe the larvae: lobes remaining, metamorphosed, or dead. Larvae that had undergone metamorphosis were described as lethargic, weak, or robust: lethargic larvae had undergone metamorphosis, but were not moving around; weak larvae were moving, but not actively searching for food; and larvae that were moving around and actively searching for food were classified as robust.

Statistical Analysis

The Shapiro-Wilk test was used to test the data for normality. Data conformed to a normal distribution. Bartlett's Test was used to determine homogeneity of variance. Mean percent metamorphosis among treatments was compared using a two-way ANOVA and Tukey's Method for mean separation.

RESULTS

Larvae metamorphosed when they were exposed to all treatments including HBOI seawater negative control. There was no observed difference between nearshore and offshore treatments. A significant difference was found between the HBOI seawater control and most of the treatments (Figure 2). The control was not significantly different from Tingler Island with water from HBOI, East Sister Rock sediment with HBOI water, Delta Shoal sediment with HBOI water and Sombrero Key water only. The lowest metamorphic response (50%) was to sediment from nearshore site Tingler Island with water from HBOI. The highest response (82.5%) was from larvae exposed to sediment directly beneath a juvenile from offshore site Delta Shoal with water from Delta Shoal.

Larvae appeared to have a higher metamorphic response when exposed to site sediment and site water (Figure 3). When exposed to site sediment and Harbor Branch water, a decrease in metamorphic response was found. Larvae did metamorphose when exposed only to site water. Site water was found to have a significant effect on metamorphic response ($p < 0.05$), while sediment did not have a significant effect. Sediment and site water, and site water only were found to have a significantly better response than the HBOI control ($p < 0.05$). Mortality occurred in fifty percent of the treatments, with the highest mortality (22.5%) occurring with larvae exposed to the offshore site, Sombrero Key, water only (Figure 4). There were no consistent trends between treatments that did have mortalities.

Of the larvae that had a metamorphic response, the majority were either robust or lethargic (Figure 5). All larvae exposed to the seawater control were lethargic. The offshore site Sombrero Key had the greatest percentage of larvae that were robust, while the nearshore site East Sister's Rock had the least robust larvae. The only treatments without lethargic larvae were the *Laurencia* control and larvae that had been exposed to sediment from directly beneath a juvenile at the offshore site, Delta Shoals. The larvae exposed to the *Laurencia* control had been fed after their exposure time, which would account for why they were robust.

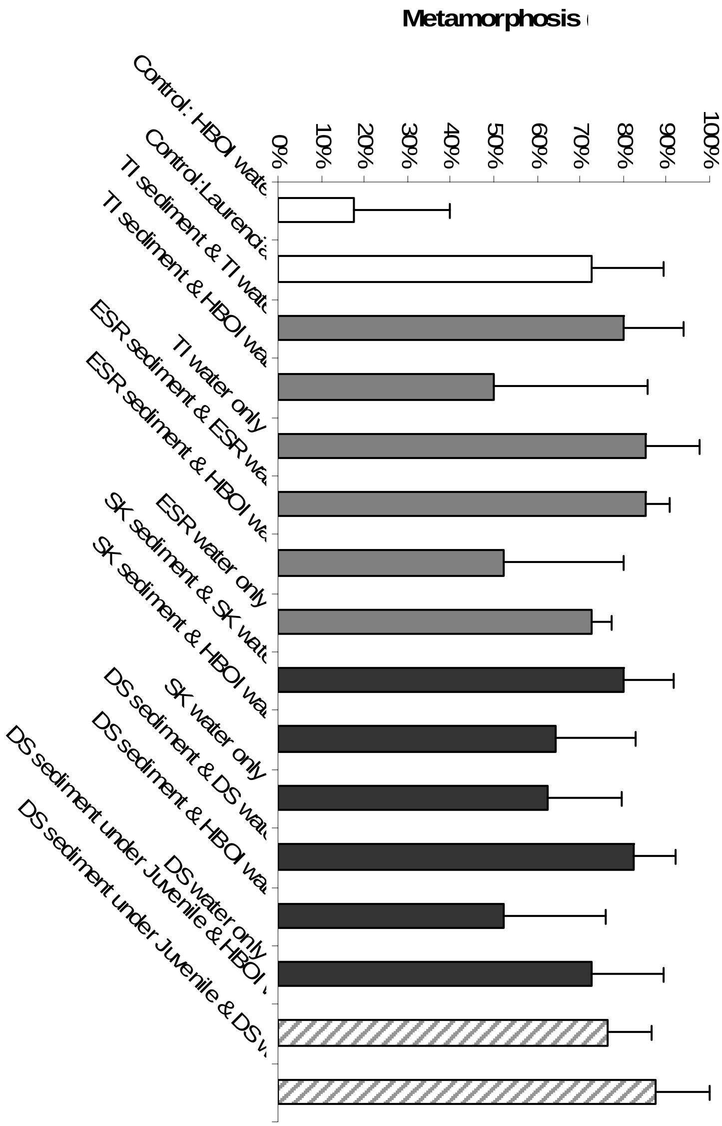

Figure 2. Percent metamorphosis treatments grouped by nearshore and offshore sites. White: controls; Light Gray: nearshore; Black: offshore; Striped: Offshore underneath Juvenile. TI: Tingler Island, ESR: East Sister Rock, SK: Sombrero Key, DS: Delta Shoal

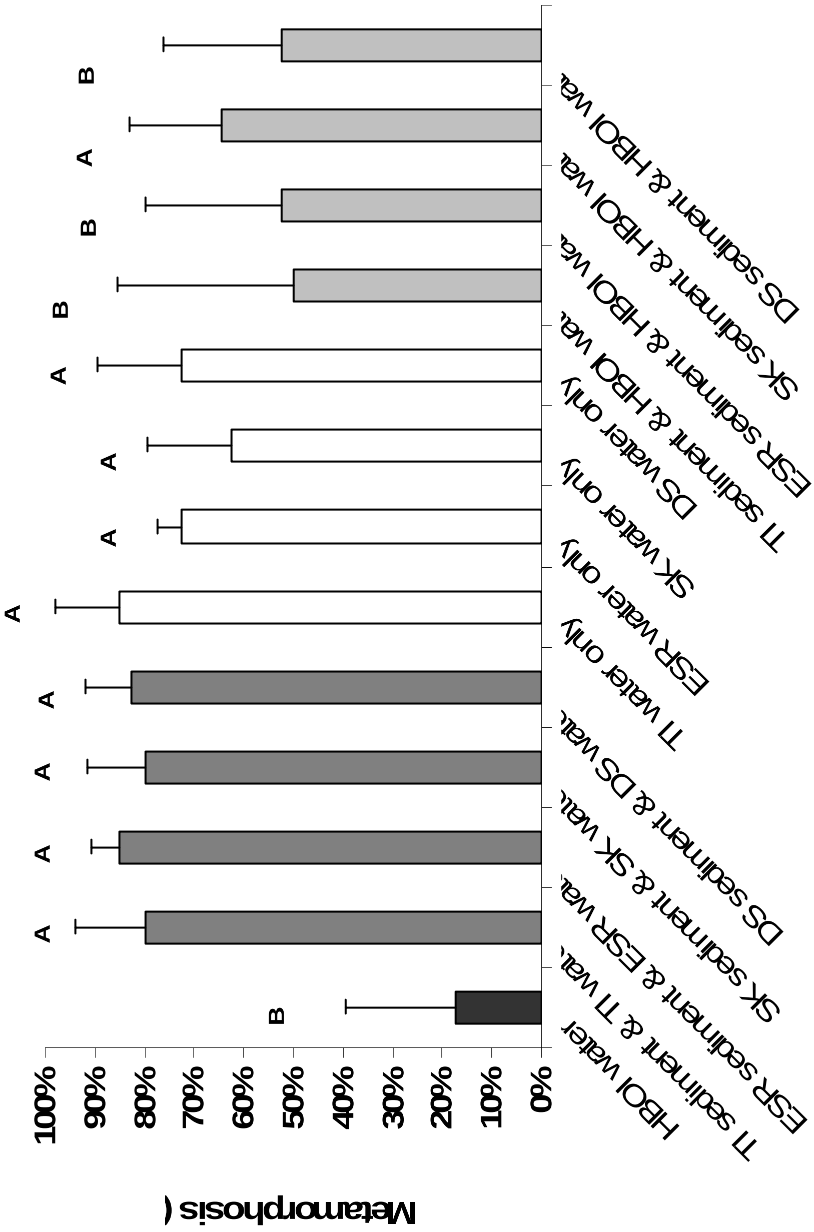

Figure 3. Percent metamorphosis grouped as site sediment to site water, site sediment to HBOI water and site water only. Black: Negative HBOI control; Dark Gray: Site sediment with site water; White: Site water only; Light Gray: Site sediment with HBOI water. TI: Tingler Island, ESR: East Sister Rock, SK: Sombrero Key, DS: Delta Shoal

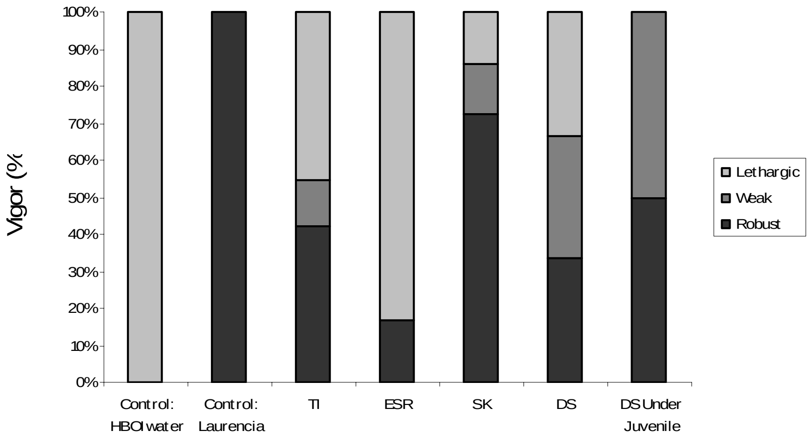

Figure 5. Condition of larvae from each treatment site. TI: Tingler Island, ESR: East Sister Rock, SK: Sombrero Key, DS: Delta Shoal

DISCUSSION

Spontaneous metamorphosis is when larvae have a metamorphic response to environments where metamorphic cues would not normally occur, such as sterile seawater. While spontaneous metamorphosis does not usually occur, Harbor Branch water did elicit a metamorphic response. Though the seawater used had been filtered and UV sterilized prior to use, nutrients or algae may have been present. It has been found that some vessels carrying seawater develop a microflora film that could induce metamorphosis (Davis and Stoner 1994).

A high response was seen when larvae were exposed to just site water. Boettcher and Targett (1996) found that certain foods that conch consume, such as Laurencia and Thalassia are water soluble. While the cues do not extend far from the substrata, the solutes may have been present in the water causing the high metamorphic response to site water only. It has been found that water soluble cues do exist less than 4mm from the substrata to in the water column for oyster larvae (Boettcher and Targett 1996). Temperature is also a known inducer of metamorphosis. When water temperatures are above culture levels, metamorphosis may be induced (Boettcher In review). Water that had been collected from the sites did have a high water temperature (> 30.1 ºC).

Queen conch larvae are known to metamorphose in response to substrata and sediment collected from juvenile conch nursery grounds (Davis and Stoner 1994, Boettcher and Targett 1996, Stoner et al. 1996b). Larvae had a high metamorphic response to sediment treatments from all sites. This may be explained by the presence of red algae at each site, many of which are known inducers of metamorphosis (Boettcher and Targett 1996). *Bataphora* sp. and *Thalassia* sp., which were present at the nearshore and offshore sites, are also known to induce metamorphosis. They are some of the most abundant macrophytes in juvenile conch habitats (Davis and Stoner 1994). Sediment is also known to contain micro-organisms, such as diatoms, and organics that have been found to be cues for conch metamorphosis. The findings agree with Davis and Stoner (1994) which indicates that metamorphosis is a trophic cue. Habitat texture may also contribute to metamorphic success. Coarse sand versus fine sand may also accumulate organics and inorganics in higher concentrations (Stoner et al. 1996b). There was some variation between sediment between nearshore and offshore sites. Nearshore sites had coarser sand with rubble pieces while offshore sites had finer sand.

Larvae that metamorphosed in nearshore treatments were not as robust as those that metamorphosed when exposed to offshore treatments. Greater percentages were lethargic after being exposed to nearshore treatments than the offshore treatments. This may be a consequence of eutrophication nearshore. Nearshore waters are subjected to a higher concentration of sewage discharge and pesticides (Glazer and Quintero 1998). Shackleton et al (2002) found that *Haliotis midae*, South African abalone, larval development was negatively affected by water with poor quality. When studying development, larvae were grown in conditions similar to offshore and nearshore conditions. Larvae raised in offshore conditions grew better than those raised in nearshore conditions (Glazer and Quintero 1998). When ready to settle and go through metamorphosis, conch appear to seek out habitats that are of good quality and appear to be areas for high survivorship (Davis and Stoner 1994). Davis (1994) hypothezied that the swim crawl stage (where lobes are still present, but they are using their foot to move around) may be an adaptation to test substrates out, but they still have the ability to swim away if the habitat is not suitable.

Larvae exhibited a metamorphic response to both nearshore and offshore habitat cues. This indicates both nearshore habitats and offshore habitats are good areas for settlement for queen conch larvae. While larvae will settle in both habitats, it does not indicate that both areas are good for post-settlement survival or reproductive activities. It is already known that nearshore habitats do not favor reproductive output due to habitat quality (Delgado et al. 2004). Resource managers can apply these results to assist in defining critical juvenile nursery grounds for conservation and stock enhancement.

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