ABSTRACT

SMITH, MELANIE. Turbulence and Seagrass Epiphytes as Settlement Cues for Conch (Strombus alatus) Larvae. (Under the direction of Thomas G. Wolcott.)

Knowledge of the mechanisms underlying larval settlement and recruitment of marine organisms is needed to manage fishery stocks, conserve threatened species, manage vital habitats and predict responses of populations, communities, and ecosystems to global change. Recruitment in benthic invertebrate species with sedentary adults often depends in part on settlement of larvae within suitable habitats. Most larvae are not just passive particles, and the interactions of larval behavior and the local current regime play an important role in settlement patterns. Conch larvae respond to contact with benthic trophic cues in nursery habitats by metamorphosing. I hypothesized that while planktonic, they also respond to water-borne cues and increased turbulence (symptomatic of shallower water) as indicators of potential nursery habitats. By swimming down or sinking, they would increase their chances to explore the substrate and come in contact with metamorphic cues. When exposed to turbulence typical of shallow tidal flows, laboratory-reared larvae competent to metamorphose, generally already low in the water column, showed no response. Pre-competent larvae, on the other hand, withdrew their velar lobes and sank. This would favor transport along or near the bottom and increase the probability of encountering suitable nursery habitats and settling there. Competent larvae responded to water-borne cues from epiphyte communities common on Thalassia testudinum blades by swimming faster in all directions and exploring the cue source, which would increase the frequency of contacts with substrates within a habitat matrix. They also metamorphosed in response to the epiphyte cue. Both phenomena would increase the probability of settling in suitable nursery habitats.
Turbulence and Seagrass Epiphytes as Settlement Cues for Conch (Strombus alatus) Larvae

by
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DEDICATION

To my son, Oliver Jack, who was taking shape in my womb as this thesis took shape in my mind. His conception and development reminded me of the wonders of the natural world that triggered my passion for and pursuit of the biological sciences. I look forward to sharing in his joy of discovery as can only be experienced by an innocent soul with an open mind.
Melanie Smith was born and raised in the small village of Fredonia in western New York. Ample time spent outdoors in this rural environment nurtured a strong appreciation for the natural world, and cultivated her curiosity about biological processes. Melanie was employed as a quality assurance technician in a condiment factory in her hometown for several years before deciding to pursue a higher education. Driven in part by her realization that human activities, including the industrial processes that became so familiar to her during her employment, were having devastating effects on our living planet, Melanie enrolled in the Biology program at Jamestown Community College. Having earned an Associate’s degree in Biology in 2003, she transferred into the Environmental Science program at the State University of New York in Fredonia where she earned her Bachelor’s degree and graduated Summa Cum Laude in May 2006. Compelled by the knowledge and experiences gained at her undergraduate institutions, Melanie finally departed from her lifelong home state to seek a Masters degree in biological oceanography at North Carolina State University. Life at NC State and in the city of Raleigh exposed her to an astounding array of academic and cultural diversity, as did a summer 2008 internship at Harbor Branch Oceanographic Institute where she performed experiments in aquaculture for her thesis research. As she was interpreting and writing up the results of her investigations in marine biology, Melanie learned that she had become engaged in another biological venture: pregnancy. As of this writing she is enjoying a more profound reverence for the marvels of life, and an even deeper cognizance of the need to preserve Earth’s ecological integrity.
ACKNOWLEDGMENTS

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Introduction

It seems reasonable to assume that many organisms possess the capacity to detect environmental attributes that denote suitable habitat for their species. This would be especially important for those species that undergo ontogenetic habitat shifts during their life cycles and those for which suitable habitat is patchy. In light of recent rates of habitat loss and of extinction (Parnell et al 2006, Hodges & Elder 2008), it is particularly important that scientists and fisheries managers identify and conserve critical habitats. Before this can be achieved, however, we must identify the criteria for optimal habitat from the perspective of the species in question. What are the indicators of advantageous characteristics to an organism seeking settlement habitat?

What constitutes suitable habitat often changes during an organism’s ontogeny. Numerous marine benthic invertebrates exhibit complex life histories wherein a planktonic larval stage precedes the benthic juvenile and adult stages. The larvae must find suitable settlement habitats, and this involves a) descending from the water column to explore the benthos, b) sensing appropriate metamorphic cues there, and c) settling and metamorphosing successfully. Considerable attention has been given to b) and c), but literature on a) is sparse. I therefore decided to investigate potential environmental stimuli that could be used by marine invertebrate larvae (those of a commercially important gastropod species) to initiate the transition between a pelagic and a benthic existence.

During their residence in pelagic habitats, larvae have high potential for widespread dispersal. This is the primary mechanism by which subpopulations in geographically isolated
habitat patches are connected, and the spatiotemporal scales and patterns of dispersal influence the geographic distribution and population dynamics of a species (Kinlan and Gaines 2003). Dispersal is also an important mechanism by which species colonize new habitats, reduce intraspecific competition, promote gene flow, and avoid meta-population extinction due to local perturbations. Dispersal plays an important role in shaping the structure of populations, communities and ecosystems.

In order for a population to persist or grow, the following sequence of events needs to occur. First, a sufficient number of larvae must be transported from where adults spawn to suitable nursery habitats. Larval supply to nurseries is variable, and this can be a bottleneck for many species (Rodriguez et al. 1993). Second, once larvae have arrived in suitable habitat, a sufficient number of new individuals must successfully settle, defined here as making the permanent physical transition from the pelagic to the benthic environment. Recruitment of individuals to the adult population begins with the completion of these events.

If the interactions of hydrographic and behavioral factors serve to deliver larvae to favorable locations, thus setting up an opportunity for settlement, larvae also need a mechanism to bring them to (or very near) the substratum and keep them there long enough to metamorphose into the juvenile stage (Hadfield 2004). An ability to detect and respond to cues indicative of sites that are appropriate for metamorphosis and growth would provide a selective advantage.

Larval settlement is essentially a two-stage process: larvae 1) descend from the water
column to the benthos and 2) explore the substrate and metamorphose in response to contact with appropriate benthic cues. Considerable attention has been given to the second stage of settlement (e.g., Mullineaux & Butman 1991, Abelson 1997, and Walters et al. 1999), and many metamorphic cues have been identified, both physical (substrate contour, texture, thermal capacity) and chemical (microbial films, “odors” of conspecifics, heterospecifics, or food sources) that work alone or in combination (Pawlik 1992, Davis & Stoner 1994, Hadfield and Koehl 2004, Tamburri et al. 2008). Species differ in their dependence upon, and specificity for, cues (Morse 1990). The presence or absence of appropriate stimuli can influence settlement success, recruitment patterns and post-metamorphic processes including growth and survival (Rodriguez et al. 1993).

Less progress has been made in understanding the mechanisms by which larvae are transported through the water column to benthic habitats (Fuchs et al. 2004). Because so many invertebrate larvae tend to remain near the surface, transport toward the bottom is a crucial step in achieving contact with metamorphic cues in appropriate benthic habitats.

For the larvae of some invertebrate species, descent in the water column is a passive process. Water flow can exert hydrodynamic forces that affect the larva’s encounter with the substratum, subsequently influencing settlement. Hydrodynamic mechanisms by which particles are passively delivered to the substratum include direct interception (neutrally buoyant particles are carried to within one particle radius of substratum on laminar streamlines), inertial impaction (particles denser than fluid continue moving toward the substratum as laminar streamlines are deflected by the bottom), gravitational deposition
(particles deviate from laminar streamlines due to sinking), diffusional deposition (tiny particles exhibit random, Brownian motion that brings them into contact with the substratum), or turbulent “sweeps” (high-speed fluid jets penetrate the viscous sublayer, carrying propagules to the substratum) (Abelson and Denny 1997). Substratum topography and structure have been found to exert a strong influence on passive larval settlement patterns (Eckman 1990, Harvey and Bourget 1995, Koehl 2007).

In contrast to these passive mechanisms, there are some instances where marine invertebrate larvae have been shown to descend toward the benthos via behavioral responses to environmental stimuli in the water column (e.g., Chia & Koss 1988, Brown & Zimmer 2001, Fuchs et al. 2004, and Hadfield & Koehl 2004). Beyond these few examples, it is not clear how commonly larvae initiate settlement via behavioral responses to conditions in the water column. Settlement and recruitment patterns exert a strong influence on the structure of benthic communities, indicating the need for further identification of potential water-borne signals and their role (Pawlik 1992).

Many invertebrate larvae demonstrate the capacity to influence horizontal transport by modifying their vertical position in the water column in response to environmental stimuli (e.g., Smith & Stoner 1993, Olmi 1994, and Bradbury & Snelgrove 2001). Given this potential for depth regulation, it seems a reasonable assumption that some species descend through the water column in response to water borne cues. A logical first step in determining possible settlement cues for a particular species would be to consider the types of stimuli available in the vicinity of their juvenile habitats.
The planktonic larvae of numerous marine invertebrate species develop in offshore shelf waters prior to recruiting to inshore benthic habitats (Stoner and Davis 1997a, Garland et al. 2002, Rilov et al. 2008). Physical and chemical characteristics in the water column certainly differ between these environments, and individual species are likely to possess the ability to detect specific contrasts that could serve as settlement cues. The objective of this research was to identify potential water-borne settlement cues for the larvae of a commercially important invertebrate species, and to test for and interpret behavioral responses to the selected stimuli. The ultimate goal was to build a more complete understanding of the factors that determine settlement patterns in the selected species, thus contributing to the identification of key habitats and effective management strategies.

**Study Species**

The “ideal” species for this study would be the queen conch (*Strombus gigas*) because of its economic value and commercially endangered status, but the Florida fighting conch (*Strombus alatus*) was selected as an appropriate surrogate for practical reasons. Both are Strombid gastropods, which have long been harvested for food and for shell to be used as building material and decoration (Shawl et al. 2003). Of the seven *Strombus* species found in the Caribbean and Florida region, the queen conch (*S. gigas*) holds the highest commercial value: approximately $40 million US annually (Appeldoorn, 1994). In recent decades, the combined effects of increased fishing pressure and habitat loss have elicited a precipitous decline of queen conch populations, which have been slow to recover despite fishery closures and stock restoration efforts (Glazer and Delgado 2003). Consequently, the species has been
listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) for nearly twenty years.

Because fishing pressure has outpaced population recovery, captive breeding and larval rearing techniques have been developed to supply juvenile *S. gigas* for restocking efforts and for the market (Davis 2000). The success of captive breeding efforts has been limited by the failure of *S. gigas* to spawn in captivity, and the subsequent need to rely upon egg masses collected from the wild (Shawl *et al.* 2003). The results of stock restoration attempts have been similarly troublesome, demonstrating that numerous complicating variables including season, lunar phase, size at release, water quality, predator density, behavioral deficits, morphological deficits, previous predator exposure, and cost of outplanting captive-bred stock must be carefully considered (Glazer 2001).

While captive breeding of *S. gigas* to supplement stocks and markets has proven problematic, the closely related Florida fighting conch, *S. alatus*, has demonstrated the potential to serve as an alternative food species. Fighting conch are similar to the queen conch in terms of the appearance and taste of their meat, but they breed more readily in captivity, grow at a faster rate under comparable conditions, are more tolerant of fluctuations in water quality, have hardier larvae, and are not a CITES regulated species (Davis and Shawl 2005).

As aquaculture facilities progress toward commercial production of *Strombus* species, market demand for their meat will be met more readily and fishing pressure on wild *S. gigas* populations may ease. However, development of effective long-term strategies for the
recovery and management of those wild populations requires a thorough understanding of early life history, the recruitment process, and habitat requirements (Appeldoorn 1994, Stoner 1994, Stoner 2003).

The life histories of juvenile and adult *S. gigas* and *S. alatus* are relatively well known. Both species inhabit seagrass beds, shallow reefs, and sand and rubble seafloor that support the growth of diatoms and other epiphytic algal food sources (Davis 2005, Davis and Shawl 2005). The historical geographic range of *S. gigas* extends from Bermuda to southern Florida and from Mexico to Venezuela, encompassing much of the Caribbean region (Stoner 2003). The range of *S. alatus* overlaps with that of *S. gigas* in southern Florida, but also extends throughout the Gulf of Mexico and as far north as coastal North Carolina (Davis and Shawl 2005). While the species differ in size and age at maturity, both are sexually dimorphic, reproduce by internal fertilization, spawn during the warm summer months, and lay crescent-shaped egg masses from which free-swimming planktonic veligers hatch after 3 days (Davis 2005, Davis and Shawl, 2005).

Conch larvae spend 18 to 26 days adrift in the water column as plankton before metamorphosing into juveniles (Davis 1994). The ecology of larvae is difficult to study in the field, compared with that of life stages that follow recruitment to the benthos, and virtually nothing is known concerning survival in the plankton. Because there is a strong positive correlation between *S. gigas* larval supply and size of the subsequent juvenile population (1 year later) in both the Exuma Cays, Bahamas and the Florida Keys (Stoner et al. 1996), knowledge of larval ecology clearly is vital to management efforts.
It is imperative that conch larvae encounter and settle within suitable benthic habitat as they approach competency, and (in the case of S. gigas in the Caribbean,) this involves transport from island shelf habitats into shallow tidal channels and on to shallow seagrass beds (Stoner and Smith 1998). In the Exuma Cays, the larvae arrive on flood tides, as water rapidly flows from Exuma sound through shallow (2-6 m deep) inlets between cays, and subsequently slows as it mixes with shallower waters of the 1-3 m deep Great Bahama Bank (Stoner and Davis 1997a). Shallow seagrass beds are the generic nursery habitat for newly-settled and juvenile conch, but optimum habitat is defined by a specific combination of characteristics that include medium seagrass shoot density, regular tidal flushing, and high production of macroalgal food sources (Stoner 2003). The importance of settling within these habitats is heightened by the fact that larvae settling even slightly outside optimal areas are subject to higher rates of predation (Ray and Stoner 1995).

Previous behavioral experiments in the laboratory and in field mesocosms (Barile et al., 1994) and intensive depth-stratified sampling in conch habitats in the Bahamas (Stoner and Davis 1997b) indicate that conch larvae are positively phototactic and tend to be concentrated near the surface. However, Barile et al. (1994) also observed that negative phototaxis becomes important at high light intensities, and that larvae sink when physically disturbed. Furthermore, Stoner and Davis (1997b) observed that surface conditions (i.e. turbulence) appear to affect vertical distributions to an even greater extent than does time of day, demonstrating that conch larvae modify their behavior in response to environmental stimuli.
Do conch larvae exhibit behaviors that facilitate settlement within suitable nursery grounds as we would expect from the apparent selective advantage they would provide? The first step toward answering this question is to identify potential cues that would alert *Strombus* veligers to the presence of optimal habitat.

Fuchs *et al.* 2004 found that mud snail (*Ilyanassa obsoleta*) larvae sink when exposed to elevated turbulence such as they would experience when currents transport them from open water into a tidal channel, thus increasing their potential for contact with benthic metamorphic cues. The observations that conch larvae sink when disturbed, and that they tend to be lower in the water column when surface waters are rough, suggest that turbulence could likewise serve as an initial settlement cue for *Strombus* species.

Trophic cues also can induce behavioral responses that facilitate settlement of invertebrate larvae on suitable substrates, as demonstrated by the sinking response of nudibranch (*Phestilla sibogae*) larvae following exposure to a water-borne chemical from their coral prey (Hadfield and Koehl 2004). The horizontal distribution of *S. gigas* veligers relative to historically important juvenile habitats in the central Bahamas suggests that they also respond behaviorally to environmental cues indicative of “good” settlement sites. Higher densities were found in the water column over an established tidal channel nursery site than either up-current at the tidal inlet, or down-current beyond the nursery habitat (Stoner and Davis (1997a). Furthermore, downstream from nursery sites, early-stage larvae were collected, but never mid or late-stage larvae. It appears that all but early-stage larvae disappear from a water mass as it moves over an established juvenile habitat. These observations cannot be
accounted for by hydrodynamics alone. Although currents have certainly played a role in establishing the locations of these habitats, because all stable nurseries occur along primary flow axes in tidal channels, the concentration of larvae in a water mass cannot change unless the larvae move relative to the water, i.e., into or out of that water mass. This would require larvae to actively deviate from the direction of water flow, i.e., to “behave.”

The aggregation of larvae near nursery grounds led me to explore responses to water-borne cues, beyond those to contact cues that have been previously demonstrated. Queen conch larvae exhibited a stronger metamorphic response to contact with natural substrata collected from within established nursery sites than to those collected elsewhere (Davis and Stoner 1994). When the components of these substrata (sediments, seagrass (*Thalassia testudinum*) blades, and detritus with epiphytes, without epiphytes and sterilized) were isolated and tested individually, live epiphytes were found to be an important metamorphic cue (Davis and Stoner 1994). Stoner *et al.* (1996) likewise found that nursery ground substrata with complex biotic structures and algal matrices elicited both high metamorphic responses and high postlarval growth rates. It therefore seems reasonable to assume that these nursery sites also possess water-borne cues that could induce behavioral responses (e.g., sinking, swimming down) that favor contact with metamorphic cues.

Turbulence and chemicals from seagrass epiphytes were selected as the most likely water-borne settlement cues with which to test the behavioral responses of *Strombus* spp. larvae, given the situations in which elevated concentrations of larvae were observed. The first hypothesis was that late-stage conch larvae would exhibit a down-swimming and/or sinking
response when exposed to elevated turbulence as would be experienced during transport from open water into a shallow coastal environment. This behavior would enable substrate sampling and heighten the probability of successful settlement. The second hypothesis was that late-stage conch larvae in the water column would exhibit a similar down-swimming and/or sinking response when exposed to a filtrate from seagrass epiphytes. This reaction would facilitate contact chemoreception of the substratum and subsequent attachment.

**Materials and Methods**

**Larval Culture**

Larvae of Florida Fighting Conch, *S. alatus*, were used as surrogates for those of *S. gigas* because they are readily produced in culture, while egg masses from wild *S. gigas* were not available during the study period. This is a reasonable substitution because the two species share many characteristics and both are economically significant. Inferences from experiments with *S. alatus* larvae may reasonably be extrapolated to those of *S. gigas*.

*S. alatus* larvae used in these laboratory studies were cultured at Harbor Branch Oceanographic Institution in Ft Pierce, Florida. A small broodstock maintained in the Aquaculture Division provided the 5 egg masses from which 5 separate batches of larvae were reared during the months of June and July, 2008 (See Table 1 for relationship between batches and experiments). Veligers were reared in 1-, 3- and 6 liter static culture vessels containing seawater that was pumped from shallow wells, passed through 10 µm filters, and UV treated. Complete water exchanges were performed every 48 h beginning the day after hatching. Cultures were kept in incubators at 28 º C with a 12:12 light-dark cycle. Initial
larval density was approximately 200 per liter, and this was gradually reduced during water exchanges to a final density of approximately 12 – 40 per liter. Larvae were fed cultured diatoms (*Isochrysis galbana*) daily. Details of the techniques used for conch larviculture are presented in Davis (1994). Those larvae reared in 1- and 3-liter containers were utilized for preliminary trials, while those cultured in 6-liter vessels were utilized for the primary experiments.

Both metamorphically competent and nearly competent larvae were used for these experiments. Metamorphic competence was indicated by morphological characteristics (as identified by Davis, 1994) including the presence of gills, dark pigment spots on the larval operculum, shrinking of the velar lobes, and swim-crawl behavior. Larvae cultured for these experiments became competent between 18 and 23 days post-hatch, when their average shell lengths were just over a millimeter.

**Turbulence Tank**

To test the effects of turbulence on conch larval behavior, a variable-speed grid-stirred turbulence tank comparable to that described in Fuchs, *et al.* (2004) was constructed (Figure 1). The dimensions of the tank are 25.4 cm by 25.4 cm by 50.8 cm with a total volume of 32.8 liters. The grid consisted of a 0.9 cm thick plastic plate into which an array of 3.86 cm square holes was milled. The bars between holes were 0.9 cm wide, yielding 34% open area. The turbulence generated in the tank, reported as turbulent dissipation rate ($\varepsilon$), was calculated using a formula from Peters and Marrase, 2000:
\[
\bar{\varepsilon} = \frac{1}{(T/4)} \int_{0}^{T/4} \frac{C_d A}{V} [u(t)]^3 dt
\]  

(1)

in which

\(T\) = period of oscillation \((1/f)\) in seconds.

\(C_d\) = drag coefficient (constant of 0.7 used)

\(V\) = volume of fluid in container in \(cm^3\).

\(A\) = grid solid area (percentage of tank open area), 34%.

\(u(t)^3\) = vertical velocity of grid (from oscillation frequency and stroke length) in cm/sec.

After integration and minor simplification (Dr. P.T. Shaw, NC State University, Raleigh N.C., pers. comm.), this was reduced to:

\[
\varepsilon = 100 \left( \frac{L^3}{T^3} \right) (C_d* A/V) \text{ in } cm^2s^{-3}
\]  

(2)

in which

\(L\) = \(1/2\) stroke length

The intensity of the turbulence generated in the tank is controlled by the voltage to the drive motor, which varies the oscillation frequency, and by the eccentricity of the flywheel crank pin, which varies stroke length and oscillation amplitude. Treatment levels were chosen to generate turbulent dissipation rates ranging from those representative of open water up to those found in tidal channels (Table 2.)
Flow velocity measurements

While equation 2 provided a theoretical estimation of the turbulence generated in the tank, minor variations in the grid and tank geometries could result in deviations from this empirical formula (McKenna 2000). To “ground truth” these theoretical calculations, I quantified flow in the tank using a SonTek 10MHz acoustic Doppler velocimeter (ADV). This instrument makes remotely-sensed, 3D measurements of water velocity within a very small sampling volume (Fig. 2).

The velocity range and sampling rates of the ADV (ranging from ±3 to ±250 cm/sec and 0.1 to 25 Hz respectively) are programmable, and measurement error is specified by the manufacturer as ±1% of the measured velocity range. A velocity range of ±30 cm/sec and a sampling rate of 25 Hz were used for my experiments as suggested by the instrument manual and as used by McKenna (2000).

Two additional indicators of data quality are measured during ADV operation; the signal to noise ratio (SNR) and the correlation coefficient. The SNR, reported in dB, is a measure of the intensity of the reflected acoustic signal. The correlation coefficient is expressed as a percentage where 100% indicates a reliable, low noise velocity measurement and 0% indicates a signal dominated by noise. A correlation coefficient is computed for each of the three acoustic receivers with each velocity sample. In my experiments SNR was above 15 dB and correlation coefficients were between 70 and 100%, well above the manufacturer’s recommended minima.

Measurements of flow velocity in the turbulence tank were replicated twice. The probe
head was positioned so that its sampling volume was 10 cm below the water surface, above
the grid, and somewhat off-center of the tank’s vertical axis (Figure 3.) For each of the three
turbulence treatments, a 15 minute warm-up period was followed by 15 minutes of
continuous data collection. Fifteen minutes is ample time for the flow to reach steady state,
and 15 min. also gives a long enough time series for meaningful statistical analysis
(McKenna 2000). Following each treatment, water movements were allowed to dissipate in
the unstirred tank for a minimum of 15 minutes before the next treatment was begun.

The ADV software reports 3D water velocity in cm/sec. In order to compare the
theoretically determined turbulence intensities to the flow measured in the tank, the ADV
data had to be converted to turbulent dissipation rate ($\varepsilon$) in cm$^2$s$^{-3}$. Dr. S. Pal Arya (professor
of fluid mechanics, NC State University, Raleigh, NC), recommended the use of two
equations to arrive at an acceptable approximation (within an order of magnitude) of this
value. First, the turbulent kinetic energy ($E$) was estimated using:

$$E = \frac{1}{2} \left( \sigma_{\mu}^2 + \sigma_{\nu}^2 + \sigma_{w}^2 \right) \quad (3)$$

Where:

$\sigma_{\mu}^2$, $\sigma_{\nu}^2$ and $\sigma_{w}^2$ = variance of velocity in x, y and z dimensions respectively.

Next, $\varepsilon$ was approximated from $E$ using:

$$\varepsilon = C_{\varepsilon} E^{3/2} / \ell_{\varepsilon} \quad (4)$$

Where:

$C_{\varepsilon}$ = empirical constant, $\approx 0.1$

$E$ = turbulent kinetic energy

$\ell_{\varepsilon}$ = largest eddy lengthscale (grid L) = 4.76 cm
Turbulence Experiments

To determine whether competent *S. alatus* larvae would exhibit a behavioral response to turbulence, fifty actively swimming (18 day old) larvae were gently pipetted into the tank filled with filtered seawater and allowed to acclimate for twenty minutes. They then were subjected to successive treatments that included 20 minute periods of low (mixed layer) and high (tidal channel) turbulence followed by 20 minute still recovery periods.

Once the results had indicated that competent larvae do not respond to turbulence, turbulence experiments were replicated four times with nearly competent *S. alatus* larvae. Those utilized for these experiments were derived from two separate batches, each comprising four 6 liter culture vessels (approximately 200 larvae reared in each vessel). On each of two days, two replicates were preformed utilizing larvae from one batch. Although all larvae were the same age (19 days) when employed for an experiment, they varied slightly in size and developmental stage. Therefore, on the day that larvae from a batch were used, ten individuals from each of the four culture vessels were examined and measured with an ocular micrometer under a dissecting microscope (40×) to obtain the current larval size distribution in that batch.

In each replicate, approximately 200 larvae were introduced into the tank and allowed to acclimate for 20 minutes. Treatment levels included 20 minute exposures to low (mixed layer), then medium (intermediate) and finally high (tidal channel) turbulence, with a 20 min recovery period without agitation between each intensity. Thus, the total duration of each replicate was 2 hours and 40 minutes. All experiments were conducted under ambient fluorescent light. The order of increasing turbulence exposure in the experimental design
might have had a cumulative effect on the larval behavior in each replicate. Even though the 20 minute still water recovery period between treatments should have helped to offset such effects, a Repeated Measures ANOVA was used for statistical analysis of the data since the treatments could not technically be considered independent.

The second ten minutes of the acclimation period, each treatment intensity, and each recovery period were recorded with a miniature video camera positioned at the vertical midline of one side of the tank, halfway between the upper grid position and the water surface (Figure 4).

**Quantification of larval trajectories**

The Expert Vision Motion Analysis System was utilized to analyze video records from the turbulence experiments. The distance scale was determined by placing a metric ruler in the focal plane and calculating the pixel to micrometer ratio. The paths of all larvae passing through the video frame were digitized in 30-second segments at a rate of 10 frames per second. From these digitized paths, the speed and vertical velocity of larval movements were quantified. For each of the four replicates, ten paths from each still water acclimation period and twenty paths from each of the three turbulence treatments were randomly selected for statistical analysis and comparison between treatments. Preliminary analyses indicated that these numbers of paths were sufficient to encompass the variability in speed and vertical velocity.

**Description of trophic cues**

Live seagrass epiphytes are an important contact metamorphic cue for *S. gigas* larvae (Davis and Stoner 1994), so they were selected as the source for potential water-borne
settlement cue for these experiments. Blades of the seagrass *Thalassia testudinum* were collected from the Indian River Lagoon (a location within the known geographic range of *S. alatus*.) for quantification of epiphytes and debris per blade area, and for use in trophic cue experiments. Samples consisted of four live blades (approximately 20.0 cm long x 0.60 cm wide) placed in vials with 50 ml seawater. Each blade was scraped free of epiphytes and debris under a dissecting microscope using a scalpel, and the scrapings were placed in vials with seawater (15 ml total volume). Blades were collected 1 to 2 days before, and prepared 1 day prior to, measurements and experiments.

Samples utilized for quantification of epiphytes and debris per unit area were allowed to settle in 15 ml centrifuge tubes for 24 hours. 8 replicates were performed to determine the mean wet volume per 4 blades. On two collection days, several additional *T. testudinum* blades were collected for qualitative analysis of the dominant epiphytes in assemblages. The epiphytes scraped from these blades were observed and photographed under a compound microscope (40×).

**Dispersal of trophic cues**

To visualize the spatial and temporal dispersal of the trophic cue in the experimental glass columns (10.16 cm square by 50.80 cm tall, filled with 4.5 l filtered seawater), a series of trials were conducted using fluorescein dye (Sargent-Welch, product # WLC94545-02). Powdered dye was mixed with seawater to a concentration (0.1 mg/ml) that remained visible for an hour when 5 ml of the solution were introduced at the bottom of the experimental columns. Samples of the epiphyte cue mixed with 5 ml fluorescein solution were subsequently injected into the columns, and the dispersal of the mixture was observed for one
hour. For comparison, 5 ml fluorescein solution was mixed with 5 ml seawater and the dispersal of this mixture was observed using the same procedure.

**Tests of fluorescein toxicity**

To ensure that the fluorescein dye solution would not result in larval mortality, a toxicity test was performed. This test also served to establish whether the dye solution had any obvious effect on larval behavior. Twelve competent veligers were exposed for 48 hours to 1 ml fluorescein solution in 1000 ml filtered seawater, a concentration similar to that to be used in the trophic cue experiments. Immediately following introduction into the fluorescein solution, larvae were observed several minutes for any noticeable changes in behavior. They were also observed after 24 and 48 hours of exposure for apparent changes in behavior and for mortality.

**Trophic cue experiments**

Trophic cue experiments were conducted under ambient fluorescent light in the experimental glass columns containing 4.5 l filtered seawater. A 20 µm filter bag was inserted to rest on the bottom of each column; from the bag a length of plastic tubing led to above the water surface, allowing remote introduction of cue fluids. The filter bag allowed only water-borne cues (soluble or very fine particulate matter) associated with seagrass epiphytes to enter the water, but not debris or large cells that could serve as contact cues. In all experiments, 15 or 24 actively swimming *S.alatus* veligers were gently pipetted into each column and allowed to acclimate for twenty minutes. Immediately following acclimation, the larval distribution in each column (number observed in top, middle and bottom thirds) was recorded. Following introduction of the cue (as described below), larvae were allowed to
swim in the columns for one hour without disturbance, and their vertical distributions were recorded every 15 minutes. After completion of each experiment, from each treatment one liter of water and the included larvae were transferred into beakers. Those larvae were observed under a dissecting microscope 24 and 48 hours after experiments, and the proportion of them that had metamorphosed was recorded.

The first trophic cue experiment comprised two treatments: a seagrass epiphyte cue and a seawater control. Each treatment was replicated four times with groups of 24 competent larvae. For the cue treatment, epiphytes from 4 seagrass blades in seawater (total volume of 10 ml) were slowly (approx. 1 ml/sec) injected into the filter bag via the plastic tubing, using a pipette. Ten ml seawater was injected immediately thereafter to flush any remaining cue from the tube into the bag. For the control, 20 ml seawater was injected into the filter bag.

A trial experiment was conducted to determine whether pre-competent *S. alatus* larvae would exhibit a behavioral response to the seagrass epiphyte trophic cue. Two treatments, the epiphyte cue labeled with fluorescein and a fluorescein control, were each administered to 24 pre-competent (15 day old) veligers.

The second trophic cue experiment consisted of four treatments: a seawater control, raw epiphyte cue, a fluorescein control, and cue labeled with fluorescein. These were administered to 15 competent (19-20 day old) larvae in each of four replicates.

**Results**

**Flow velocities**

The empirically determined turbulent dissipation rates in the tank were within an order of magnitude of the theoretical (calculated) values (Table 2). This level of correspondence was
deemed acceptable because, in all cases, both values fell within the range of turbulence intended for each treatment.

**Turbulence**

During the trial turbulence experiment, competent *S. alatus* larvae did not exhibit the hypothesized sinking or swimming down behavior when exposed to turbulence representative of shallow water habitats. The few veligers that were struck by grid bars rather than simply passing through the holes withdrew their velar lobes briefly, but quickly re-emerged. Swimming behavior in low or high turbulence was not obviously different from that in still water. Most competent larvae hovered near the bottom of the tank except when stirred into the water column during the high turbulence treatment.

Pre-competent *S.alatus* veligers did not appear to swim down or sink during low or intermediate intensity turbulence treatments. Like competent larvae, they did withdraw their velar lobes when struck by the grid, but quickly re-emerged and resumed swimming. In contrast, nearly all veligers withdrew into their shells and began sinking immediately following the onset of the high intensity turbulence treatment. Most of them remained completely or partially withdrawn for the duration of this treatment. This behavior was expressed consistently in all experimental replicates.

**Quantification of Larval trajectories**

The mean speed and vertical velocity of larval movements were compared by image analysis of video records from the turbulence experiments (Figures 5 and 6, respectively.) It was not possible to partition larval movement by locomotion from that due to passive transport by eddies. However, while larval speed and velocity are expected to increase in
increasing turbulence, I reasoned that because turbulent eddies are random, a net velocity in any direction should be reflective of either swimming or sinking behavior. The mean vertical velocities were statistically different between treatments ($F = 39.91$, $df = 3$, $p < 0.001$). The negative (downward) vertical velocity of the pre-competent larvae in the high (tidal channel) turbulence treatment was significantly greater than that in either of the other turbulence treatments or the still water control, and that in the medium (transitional) turbulence treatment was significantly greater than that in the still control (Table 3).

Exploratory ADV measurements indicated slight net vertical velocities in the turbulence tank (both positive and negative) that varied with the probe location. These observations were most likely a consequence of weak circulation generated by the grid motion (McKenna 2000, Fuchs 2004). While grid-generated turbulence in this type of tank is thought to be nearly isotropic given proper tank design and operation, experiments have shown that exceptions occur (e.g., immediately behind the grid where turbulence is more intense due to shear and where tank side walls form planes of symmetry with grid ends) that yield minor flow variations and secondary motions (DeSilva and Fernando 1994, McKenna 2000). Because there was no obvious pattern to these velocities, and they were smaller (by at least an order of magnitude) than the larval velocities, they were disregarded during subsequent data analysis.

**Trophic cues**

The mean settled wet volume of epiphytes from 4 seagrass blades was 2.8 ml +/- 0.6 ml (s.d.), or 0.12 ml/ cm$^2$ of grass blade area. The common epiphytes associated with the sampled *Thalassia testudinum* blades were diatoms from the genera *Pleurosigma*,
Licmophora, Nitzschia, Thalassionema, Synedra, Rhopalodia, Mastogloia, Navicula and Amphora (Fig 7). While S. alatus have not been studied extensively in the field, and we cannot state whether the Indian River Lagoon T. testudinum collection site serves as a nursery for the species, the epiphyte communities identified in my samples were qualitatively similar to those described by Davis and Stoner (1994). A negligible number of epifauna were observed in the T. testudinum epiphyte samples.

**Dispersal of trophic cues**

The epiphyte cue mixed with fluorescein dye slowly crept across the bottom of the experimental columns, while the dye alone dispersed more readily as though it were less dense (Fig 8). The fluorescein dye quickly ascended in the experimental columns (within 3 minutes of introduction), and after one hour, the dye alone had mixed more homogenously throughout the column than had the dye mixed with cue.

**Fluorescein toxicity**

Fluorescein dye did not appear to be toxic to S. alatus larvae at the concentration utilized for these experiments. After 48 hours of exposure to 1 ml fluorescein solution (0.1 mg/ml) in 1000 ml seawater, all 12 competent larvae tested were alive and active. Likewise, the dye did not have any noticeable effects on larval behavior at any point during the 48 hour exposure.

**Trophic cue experiments**

Larvae did not exhibit the expected sinking or down-swimming behavior when they were exposed to the epiphyte cue. Likewise, there were no obvious changes in their vertical distributions in the water column, compared to controls, following introduction of the trophic cue (e.g., Fig 9).
During the trial trophic cue experiment with pre-competent larvae, there was no metamorphic response to the cue treatment versus the seawater control. No veligers had undergone metamorphosis after 24 or 48 hours.

During the first trophic cue experiment with competent larvae, the presence of cue significantly increased the proportion of larvae metamorphosing relative to the seawater control \((F = 11.45, \text{df} = 3, p < 0.001)\). The difference in metamorphic response in each treatment between 24 and 48 hours was not significant (Fig 10).

Following the second trophic cue experiment, significantly more larvae in cue treatments metamorphosed than did in the controls \((F = 4.74, \text{df} = 7, p = 0.0019)\). The metamorphic responses between the seawater and fluorescein controls and between the cue and cue mixed with fluorescein treatments were not significantly different, but the data suggest that fluorescein dye might have inhibited metamorphosis slightly (Fig 11). As in the first trophic cue experiment, the differences in metamorphic responses in each treatment between 24 and 48 hours were not significant.

**Discussion**

Conch larvae exhibited behavioral responses to cues in the water column that could indicate their proximity to favorable juvenile habitat. The observed behaviors were not always what I expected, but they do appear adaptive in that they would increase the probability of larvae encountering favorable benthic habitats.

Pre-competent *S. alatus* veligers withdrew their velar lobes and sank when exposed to turbulence such as would be experienced during transport from open water into shallow nursery habitats. This suggests that conch larvae, like those of mud snails (*Ilyanassa*)
obsolete), may indeed utilize turbulence intensity as an initial settlement cue when they are approaching competency. Early to mid-stage conch larvae tend to spend more time higher in the water column than do late-stage veligers. Because they possess heavy shells as ballast, they sink most rapidly when they withdraw completely into their shells (Noyes 1996). Pulling in their velar lobes when they encounter threshold levels of turbulence is thus an effective mechanism for arriving at the benthos. If the appropriate turbulence stimulus is persistent, a sinking response favors transport along or near the bottom, increasing the probability of encountering suitable substrates and having opportunities to settle there when competent.

Competent larvae did not sink or swim down when exposed to turbulence representative of shoaling environments. A possible explanation for this behavioral divergence is that these late-stage conch veligers already tend to spend most of their time hovering near the bottom (pers. obs., M. Davis, pers. comm.) There would not be an adaptive advantage to responding to elevated turbulence by moving toward the substratum if they are already there.

Neither pre-competent nor competent larvae sank or swam down when exposed to water-borne cues from epiphyte communities common on *Thalassia testudinum* blades, contrary to my expectations. However, competent larvae did appear to be stimulated by exposure to this cue, as evidenced by other changes in behavior. Shortly (15 – 30 min) after introduction of epiphyte cue into the experimental columns, larvae became visibly more active. They ascended and descended more quickly and frequently relative to those in the controls, rapidly changed direction seemingly at random, and in many cases were observed exploring the filter bag containing the seagrass epiphytes.
These responses probably are adaptive, given the spatial heterogeneity of conch nursery habitats. Seagrass meadows have complex, three-dimensional topography that interacts with ambient water flow along the substratum and affects the dispersal of dissolved materials (Hadfield and Koehl, 2004). When a larva detects a cue arising from the substrate, down-swimming or sinking would not necessarily be advantageous responses since the source of the stimuli may not be directly below the larva. However, increases in swimming speed (even in random directions) and exploratory behavior could expedite location of and attachment to an appropriate substrate within a seagrass habitat matrix, thus contributing to successful settlement and recruitment.

Seagrass epiphytes can serve as a metamorphic cue for *S. alatus*; the proportion of competent larvae metamorphosing when exposed to the cue treatments was significantly greater than that in the controls. *S. gigas* larvae have been shown to undergo metamorphosis following contact with seagrass epiphytes (Davis and Stoner 1994). My experiments now demonstrate that larvae also are stimulated to metamorphose in response to a dissolved natural cue (passing a 20 micrometer filter) without any physical contact with the substrate, contrary to the assumptions of earlier studies (Davis and Stoner 1994, Stoner *et al.* 1996). I cannot be sure whether this phenomenon occurs in the field because I have no means by which to compare my experimental cue concentrations to those encountered in nature.

*S. gigas* exhibits a stronger metamorphic response to cues collected within established nursery sites than to those collected outside nursery peripheries (Davis and Stoner 1994). Unfortunately, *S. alatus* have not been studied as extensively, and I cannot be certain whether the potency of the cues I used was comparable to those from a preferred settlement habitat.
for this species. The epiphytic diatom communities identified in my samples were qualitatively similar to those described by Stoner and Davis (1994), but stronger behavioral and metamorphic responses might have been observed had I been able to identify specific *S. alatus* nurseries and utilize cues from these habitats.

Pawlik (1992) suggested that invertebrate larvae utilize a hierarchy of cues during the settlement process: those that indicate an initial encounter with generally suitable habitat, and then those that prompt selection of a specific substrate. Likewise, Crisp (1984) conjectured that a series of behaviors allow larvae to respond to a succession of physical and biological cues during the settlement process, but to return to the previous step in the progression if a particular circumstance is deemed unacceptable. My results are consistent with such a paradigm. Pre-competent conch veligers sank in response to turbulence intensities representative of suitable juvenile habitats, but they resumed swimming following cessation of this treatment. Elevated turbulence could therefore serve as a preliminary indicator of potentially favorable habitat and place larvae near the benthos. Davis and Stoner (1994) suggested that concentrations of soluble cue sufficient to elicit behavioral responses are available only near the substratum. Assuming this is true, pre-competent larvae, which tend to be high in the water column, would not be able to detect chemical cues arising from the substrate and hence would be unlikely to have evolved a response to them. Late-stage veligers, on the other hand, tend to linger close to the benthos. They would be more likely to encounter such cues, and to derive selective advantage by developing a response to them. The observation that competent larvae exhibited a behavioral “searching” response to the water-borne epiphyte cue supports this supposition. Trophic cues seem more likely to prompt
habitat selection at smaller scales than would turbulence, as it was only after exposure to the epiphyte cue that the larvae attached to the substrate and metamorphosed.

Cue detection and specificity in larval settlement and metamorphosis influence the distribution of conch recruits in the natural environment (Davis and Stoner 1994). The behavioral responses to turbulence and water-borne epiphyte cues documented in my study may similarly help to account for the observed aggregation of *S. gigas* larvae in the vicinity of established nursery sites (Stoner and Davis 1997a). If larvae move relative to the water mass in which they arrive (i.e., sink in response to elevated turbulence on flood tides when pre-competent and actively seek substrate in response to epiphyte cues arising from juvenile habitats when competent), they have satisfied the conditions required to become concentrated in a particular locale.

These investigations have identified probable settlement cues utilized by *Strombus* species, and the likely behavioral responses associated with these stimuli. Knowledge of the cues that conch larvae utilize during settlement and the habitat characteristics that these cues represent can assist scientists in locating previously undiscovered nurseries as well as sites that might serve as optimal habitat for hatchery reared juveniles but are “undersaturated” as a consequence of poor larval supply. These critical habitats can then be preferentially conserved, contributing to more efficient management and effective recovery of wild conch populations.

While I have demonstrated that conch larvae respond both behaviorally and metamorphically to a water-borne cue from seagrass epiphyte communities common to *Thallasia testudinum* blades, further investigations are needed to refine our understanding of
this cue. Davis and Stoner (1994) reported that *Thalassia testudinum* detritus elicited metamorphosis in conch larvae, and that the associated epiphytes were the primary components responsible for this response. In particular, they found that the metamorphic response to detritus collected from within established nursery sites was statistically higher than to that collected outside the nursery peripheries, as are growth and survival (Stoner et al., 1996.) This disparity indicates that the quality of *T. testudinum* detritus varies between habitats, that conch larvae are adapted to detect these differences, and (from my observations) that they respond in ways that increase the probability of settling in high-quality habitats. This is tantalizing, but our ignorance of the exact nature of these chemical cues and of conch sensory abilities makes the task of identifying superior habitat for conservation difficult. Future investigations should concentrate on elucidating the specific characteristics that make epiphyte communities from nursery habitats especially effective inducers of behavioral and metamorphic responses. Before we can determine how the signals differ between propitious and less favorable habitats, we must also identify more precisely the stimuli that conch sense and respond to when selecting a site for settlement. Resolving these conundrums would enable us to refine our skills with regards to management and conservation of optimal conch habitats, further benefitting the species.


Figure 1. Grid-stirred turbulence tank constructed after Fuchs et al. 2004.
Figure 2. Sketch of ADV sensor probe head. (a) Probe head arrangement showing 10 MHz transmitter and three receivers. (b) Location and size of sampled volume. From McKenna 2000.
Figure 3. Position of ADV probe relative to grid during flow velocity measurements.
Figure 4. Position of miniature video camera during turbulence experiments.
Table 1. Relationship of larval batches to experiments.

<table>
<thead>
<tr>
<th>BATCH #</th>
<th>HATCH DATE</th>
<th>DURATION OF CULTURE (DAYS)</th>
<th>FATE OF LARVAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>06-10-08</td>
<td>20</td>
<td>Preliminary turbulence trial with competent larvae and first trophic cue experiment with competent larvae.</td>
</tr>
<tr>
<td>2</td>
<td>06-18-08</td>
<td>26</td>
<td>Trophic cue trial with pre-competent larvae and second trophic cue experiment with competent larvae.</td>
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<tr>
<td>3</td>
<td>06-20-08</td>
<td>24</td>
<td>Culture discarded due to bacterial and protozoan infestation.</td>
</tr>
<tr>
<td>4</td>
<td>06-28-08</td>
<td>25</td>
<td>Turbulence experiments with pre-competent larvae.</td>
</tr>
<tr>
<td>5</td>
<td>06-30-08</td>
<td>25</td>
<td>Turbulence experiments with pre-competent larvae.</td>
</tr>
</tbody>
</table>
Table 2. Intended turbulence intensities versus calculated and empirically measure values (expressed as turbulent dissipation rate, $\varepsilon$, in cm$^2$s$^{-3}$.) Empirical values are the mean (and standard error) from two replicates.

<table>
<thead>
<tr>
<th>INTENDED RANGE (FROM FUCHS ET AL. 2004)</th>
<th>THEORETICAL VALUE</th>
<th>EMPIRICAL VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LOW</strong> (open ocean)</td>
<td>10.0×10$^{-07}$ – 10.0×10$^{-02}$</td>
<td>7.80×10$^{-04}$</td>
</tr>
<tr>
<td><strong>MEDIUM</strong> (continental shelf)</td>
<td>10.0×10$^{-05}$ – 10.0×10$^{-02}$</td>
<td>1.30×10$^{-02}$</td>
</tr>
<tr>
<td><strong>HIGH</strong> (tidal channel)</td>
<td>10.0×10$^{-02}$ – 10.0×10$^{+00}$</td>
<td>3.30×10$^{-01}$</td>
</tr>
</tbody>
</table>
Figure 5. Mean speed of larval movements in turbulence experiments, from analysis of video records (Expert Vision Motion Analysis System). Values are mean ± SE, n=4 replicates per treatment.
Figure 6. Mean vertical velocities of larvae in turbulence experiments, from analysis of video records (Expert Vision Motion Analysis System). Values are mean ± SE, n=4 replicates per treatment.
Table 3. Linear contrasts (SAS 9.1.3) between mean vertical velocities of larvae in turbulence experiments, from analysis of video records (Expert Vision Motion Analysis System). DF = 1 for all contrasts.

<table>
<thead>
<tr>
<th></th>
<th>STILL</th>
<th>LOW</th>
<th>MED</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F Value</td>
<td>Pr &gt; F</td>
<td>F Value</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>STILL</td>
<td>-</td>
<td>-</td>
<td>1.64</td>
<td>0.2901</td>
</tr>
<tr>
<td>LOW</td>
<td>1.64</td>
<td>0.2901</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MED</td>
<td>263.41</td>
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<td>9.15</td>
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<tr>
<td>HIGH</td>
<td>49.67</td>
<td>0.0059</td>
<td>263.10</td>
<td>0.0005</td>
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Figure 7. Common epiphytic diatom genera found on *T. testudinum* blades collected from the Indian River Lagoon. a. *Pleurosigma*, b. *Licmophora*, c. *Navicula*, d. *Synedra*, e. *Thalassionema*. Note that all genera shown are substantially larger than 20 μm, the mesh size utilized during introduction of the epiphyte cue into experimental columns.

Photo sources:
- a) http://starcentral.mbl.edu/msr/rawdata/viewable/pleurosigma_1130609070_apw512w.jpg
- b) http://planktonnet.awi.de/repository/rawdata-PlanktonNet2/viewable/fatima_santos_lxicmophora_madeira_150606_20081229201329_small.jpg
- c) http://www.keweenawalgae.mtu.edu/ALGAL_IMAGES/bacillariophyceans/Navicula_s38_peepsock6_402.jpg
- d) http://www.keweenawalgae.mtu.edu/ALGAL_IMAGES/bacillariophyceans/Nitzschia_n43_sturgeonplant22a_40125.jpg
- e) http://content8.eol.org/content/2009/02/20/01/13105_large.jpg.
Figure 8. Dispersal of cue in experimental columns; a. = fluorescein control and b. = fluorescein mixed with cue. 1. = Prior to treatment introduction. 2. = Three minutes into treatment. 3. = Sixty minutes into treatment.
Figure 9. Vertical distribution of larvae after 20 minute acclimation (upper) and 60 minutes after onset of treatment (lower). SW = seawater control, CUE = epiphyte cue, FL = fluorescein control, FLCUE = fluorescein mixed with cue. Values are mean, n = 4 replicates per treatment with 15 larvae per replicate.
Figure 10. Metamorphic response for veligers of Florida fighting conch in a seawater control and a *T. testudinum* epiphyte cue. Values are mean ± SE, n = 4 replicates per treatment with 24 larvae per replicate. Differences in metamorphic response between the seawater control and the cue treatment were significant, but the difference in metamorphic response in each treatment between 24 and 48 hours was not (Tukey’s multiple comparison test of means).
Proportion of Larvae Metamorphosed at 24 and 48 Hours
Trophic Cue Experiments July 10-11th, 2008

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>24 hrs</th>
<th>48 hrs</th>
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<td></td>
<td></td>
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<tr>
<td>Fluorescein Control</td>
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<td>Fluorescein plus Cue</td>
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</table>

Differences in metamorphic response between the controls and the cue treatments were significant, but the difference in metamorphic response between controls, between treatments and between 24 and 48 hours was not (Tukey’s multiple comparison test of means).

Figure 11. Metamorphic response for veligers of Florida fighting conch in a seawater control Thalassia testudinum epiphyte cue, fluorescein control, and fluorescein mixed with cue. Values are mean ± SD, n = 4 replicates per treatment with 15 larvae per replicate.