Spring 2007

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Recommended Citation
Goldstein, Jason S.. "Behavioral Enhancement of Onshore Transport by Postlarval Caribbean Spiny Lobster (*Panulirus Argus*)" (2007). Master of Science (MS), Thesis, Biological Sciences, Old Dominion University, DOI: 10.25777/t8mn-yf04
https://digitalcommons.odu.edu/biology_etds/202

BEHAVIORAL ENHANCEMENT OF ONSHORE TRANSPORT BY POSTLARVAL CARIBBEAN SPINY LOBSTER (*PANULIRUS ARGUS*)

by

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A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

MASTER OF SCIENCE

BIOLOGY

OLD DOMINION UNIVERSITY
May 2007

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ABSTRACT

BEHAVIORAL ENAHNCEMENT OF ONSHORE TRANSPORT BY POSTLARVAL CARIBBEAN SPINY LOBSTER (*PANULIRUS ARGUS*)

Jason Seth Goldstein
Old Dominion University, 2007
Director: Dr. Mark J. Butler IV

The onshore transport of meroplanktonic marine larvae or postlarvae is often complex, involving both active (i.e., behavior, swimming) and passive (i.e., oceanographic elements) transport mechanisms. Behaviors that modify passive transport have presumably evolved to situate larvae in settlement habitats where survival is enhanced. Active transport mechanisms have not been described for the puerulus postlarvae of any species of spiny lobster, despite their extraordinary mobility and known preference for specific settlement habitats. In the Florida Keys, for example, Caribbean spiny lobster (*Panulirus argus*) pueruli travel from oceanic waters into coastal areas where they settle within bushy, red macroalgae. I conducted a series of laboratory experiments to examine puerulus orientation, settlement, and metamorphosis in response to cues characteristic of the nearshore nursery environment. Results from ≈ 270 trials testing puerulus choice of water sources in a custom acrylic choice tube indicate that pueruli are attracted to coastal water and the metabolites of red macroalgae (*Laurencia* spp.) compared to oceanic water and artificial seawater treatments. Postlarvae are not attracted to waterborne cues from seagrass, and are repelled by hyper- or hypo-saline water. Postlarvae incubated in bay water or artificial seawater containing macroalgal metabolites metamorphosed about 2 days earlier (20 – 30% faster) than those exposed to artificial seawater. I used a pressurized test chamber to determine that pueruli settled under pressures simulating shallow depths (< 5m) indicative of nursery areas, particularly in the presence of the red macroalgae *Laurencia* spp. Pueruli swam up into the water column when subjected to higher pressures that simulated depths of ≈ 10 – 15 m. Combined,

these results highlight the role that chemical and pressure cues play in altering the behavior and metamorphosis of postlarval spiny lobsters during onshore transport and settlement.

About 10 years ago I filled out an application and was accepted as a volunteer working at the New England Aquarium's Edgerton Research Lab. Located up on the fifth floor far away from the sea lions, coral reefs, dolphins and the hustle and bustle of the public exhibit galleries, was the almost windowless domain known simply as the ERL. What I became acquainted with not to shortly after was a damp, cold, and salty wetlab and my new but aggressive friends with claws and spines, the lobsters. My time spent in the lobster research hatchery started a unique journey for me that has stuck with me ever since. Although my initial duties were rudimentary and my knowledge slim, I took to the role in a very passionate and serious way. As a former staff and researcher of almost seven years since, I was always given the encouragement and resources to expand my interests of conducting basic and applied research at ERL and abroad. I believe that this experience helped me to become the person and scientist I am today.

Additionally my parents with their constant unyielding enthusiasm and support provided me with an atmosphere of opportunity to pursue my interests. I therefore dedicate my thesis to my parents for giving me the encouragement and inspiration to follow my heart and to the New England Aquarium for its support of my work and cultivation and growth of my skills and scientific creativity.

ACKNOWLEDGMENTS

There are many individuals, groups, and institutions who all played significant and vital roles in the fruition and success of my research. Their support, encouragement, cooperation, and 'team spirit' attitude have given me a positive experience of what doing marine research should be and how enjoyable and fulfilling it can be. I would like to acknowledge and thank my fellow ODU graduate students and colleagues in the Butler Lab whose time and efforts were greatly appreciated in both the lab and the field. In particular, D. Behringer Jr. and S. Donahue for their initial insight and advice in how to conduct research in the sometimes challenging Florida Keys environment. Also, J. Lear, T. Dolan, R. Carter, and K. Kauffman for both their on-campus support and field assistance. A special thanks to B. Powell of the ODU machine shop for his construction of acrylic tube tanks. A very sincere thanks and appreciation goes to P.E. Bouwma of Florida State University whose tremendous physical stature and 'good naturedness' allowed me to collect copious numbers of animals from bridge tows. 'Big Pete' was always around to lend me a hand. I also would like to acknowledge Bill 'Doc' Herrnkind for his insight, advice, and more than 30 years experience of doing lobster research in the Keys. Tremendous thanks goes to the hard-working and congenial staff at the Keys Marine Laboratory on Long Key for their continued generosity and use of facility resources. Thanks also to Capts. J. Rothschild of the R/V Coral Reef II in Miami and J.C. Humphrey of the Florida Institute of Oceanography for their services in providing oceanic seawater from offshore. The researchers and staff located at the Florida Wildlife Research Institute (FWRI) in Marathon were certainly an enormous resource and great help to me. In addition to their professionalism and continued advice as colleagues, FWRI helped to supply me with lab space, field materials for collectors, animals, and boats. Therefore, I extend a gracious thanks to the director J.H. Hunt and the lobster research team there:

T. Matthews, R. Bertelsen, and C. Cox. A special thanks from that group goes to W.C. Sharp whose analytical and editorial skills as well as his patience and encouragement were indispensable to me both in bridge tows and in front of the computer crunching data. Also from FWRI thanks to B. Glazer and G. Delgado for use of their dissection scope and associated equipment. I would also like to recognize and thank Dr. M. Tlusty of the NEAQ for his thoughtful insight and assistance with some of my data.

Finally, and certainly not least, I owe my gratitude and appreciation to my advisor and mentor, Prof. Mark J. Butler IV, whose passion and extensive knowledge in ecological research and the Florida Keys provided me with the initiative and confidence to design and carry out a successful research project. In addition to supplying me with the resources of boats, nets, lab space and supplies, under Mark's guidance, I have gained a genuine and new appreciation and respect for conducting well-designed and thought out research.

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INTRODUCTION

The initial release and subsequent passive distribution of marine meroplanktonic larvae within the sea is subject to the intricacies of large-scale physical forces (e.g., surface winds, advection, currents, gyres) that operate over variable temporal and spatial scales. However, at smaller scales larvae engage in behaviors (e.g., directional swimming, vertical migration, taxes, kineses) and are directed by mesoscale hydrodynamics (e.g., tides, fronts, wind-driven transport, internal waves, etc.) that often situate them in favorable habitats; thus the processes in play during the larval stage help to shape the subsequent population dynamics and community structure of benthic marine species (Thorson, 1950; Gaines and Roughgarden, 1985; Roughgarden et al., 1985 and 1988; Scheltema, 1986; Cowen et al., 2000; Sale and Kritzer, 2003; Cowen et al., 2006; Incze et al., 2006).

Historically, more emphasis was placed on the importance of passive larval dispersal, the assumption being that weakly swimming larvae are generally advected as particles irrespective of their size, shape, or density (Hannan, 1984; Butman, 1987; Zimmer and Butman, 2000). However, more recent evidence (especially for larval fishes) indicates that the patterns of larval dispersal and retention, hence population connectivity, are not necessarily at the mercy of advective processes.

The model for this thesis is the Journal of Experimental Marine Biology and Ecology.

This is especially true if larvae possess well-developed sensory capabilities (i.e., vision, sound, smell) and are competent swimmers (Wolanski et al., 1997; Cowen et al., 2000; Fisher et al., 2000; Kingsford et al., 2002; Leis and McCormick, 2002; Mora and Sale, 2002; Sale and Kritzer, 2003; Cowen et al., 2006). Although species with long pelagic larval durations (e.g., many crustacean and fish larvae) are presumably able to disperse more widely than those that spend less time in the plankton (e.g., coral and sponge larvae), both experimental studies (Swearer et al., 1999; Jones et al., 2005) and biophysical modeling (Wolanski et al., 1997; Jones et al., 2002; Carr et al., 2005; Cowen et al., 2006) suggest that local retention of behaviorally adroit larvae can be significant.

Whether larvae disperse widely or remain just offshore of their natal source, at the conclusion of their larval period they somehow must still transit from the pelagic realm to the coastal benthic nursery where they settle and metamorphose to begin a demersal existence. This involves two steps. First, orientation and transport from offshore to inshore, then the selection of a specific settlement habitat. A host of processes (both physical and behavioral) are implicated in the directed movement by larvae from offshore to coastal areas. Physical factors such as tidal fronts, internal wave slicks, turbulence, and Ekman transport (see Shanks, 1995 for review) work in tandem with larval behaviors (e.g., selective tidal stream transport, attraction to surface, depth regulation) to situate larvae in the "right" place (i.e., in surface slick, near the surface, rising tide, etc.) (see Young, 1995 for review).

Upon arrival to the nursery, the process of settlement consists of two distinct phases (Rodriguez et al., 1993). The first is an exploratory phase in which larvae search for appropriate settlement habitats, and the second is the resident or attachment phase typically involving metamorphosis into a benthic dwelling form. In most cases, the settlement of benthic marine invertebrates is initiated through a behavioral cue associated with a particular habitat (Scheltema, 1986; Butman, 1987; Pechenik, 1990; Rodriguez et al., 1993). The same cues that attract settlers may also trigger a series of neuronal and hormonal events that culminate in the morphological and physiological changes associated with metamorphosis (Burke, 1983; Trapido-Rosenthal and Morse, 1986). Settlement cues include a variety of exogenous factors including changes in current speeds and substratum contours (Sebens, 1983; Pawlik, 1991), heterogeneity of substratum (Herrnkind and Butler, 1986; Butler and Herrnkind, 1991; Kingsford, 1995), luminosity (Crisp and Ritz, 1973), chemical cues (Zimmer-Faust, 1989; Zimmer and Butman, 2000; Morse, 1991; Pawlik, 1992; Boudreau et al., 1993; Finelli, 1999, Hadfield and Paul, 2001), and hydrostatic pressure (Knight-Jones and Morgan, 1966; Sulkin, 1984; Forward, 1990) among others. Endogenous bio-physiological changes in larval condition, such as lipid mobilization and reallocation (Jeffs et al., 1999 and 2001), also play a role in the timing and location of settlement. Despite this long, yet non-exhaustive list of examples, the factors that enhance settlement by attracting larvae or influencing their choice of settlement substrates remains poorly understood (Butman, 1987; Pawlik, 1992; Zimmer and Butman, 2000; Hadfield and Paul al., 2001). Despite a burgeoning literature on larval behavior and its importance in determining patterns of dispersal and settlement, few studies have focused on taxa, such as lobsters, whose larval durations are among the longest for marine animals (Kittaka, 1994).

Evidence of orientation and settlement cues in marine larval decapod crustaceans varies widely across taxa (see Young, 1995 for review). For example, megalopae of the blue crab (*Callinectes sapidus*) use a suite of behavioral responses to scalar cues (pressure, salinity, temperature, chemical odors) and vector cues (gravity, light, currents) (Tankersley et al., 1995; reviewed in Forward et al., 2003b) in addition to endogenous swimming rhythms (Forward et al., 1997) to orient shoreward to estuarine nurseries (Welch el al., 1997; Diaz et al., 1999; Forward et al., 2003b; Forward, 1988 and 1989; Forward and Buswell, 1989). Similarly, postlarval clawed lobsters (*Homarus americanus*) use a variety of cues to orient to coastal nurseries and select settlement substrates, including thermal gradients (Boudreau et al., 1992; Annis, 2005) and chemical odors from predators, water masses (e.g., rocky versus sandy substratum), conspecifics, and macroalgae (Boudreau et al., 1993).

In palinurid (spiny) lobsters, the offshore distribution of the specialized and highly-mobile postlarval (puerulus) stage suggests that directed onshore movement as opposed to simple random dispersal is necessary for successful settlement in coastal nurseries (Ritz, 1972; Chiswell and Booth, 1999). Based on calculations of the shoreward movement of pueruli of the New Zealand rock lobster (*Jasus edwardsii*; Chiswell and Booth, 1999), laboratory experiments on metabolic requirements for swimming, and field observations of metabolic reserves (Jeffs et al., 1999), it appears that pueruli are capable of swimming from as far as 200 km offshore. Although capable swimmers, how the pueruli of *J. edwardsii* or any other palinurid orient toward shore is unknown. A number of cues used by fishes and adult lobsters (e.g., underwater sound, magnetic and electric fields, celestial cues) might be used by pueruli for shoreward navigation, but none have been verified (see Jeffs et al., 2005 for review). For instance, sound has been implicated in the orientation of larval and postlarval marine crabs and fishes (Jeffs et al., 2003; Leis et al., 2003; Simpson et al., 2004; Tolimieri et al., 2004). Chemical cues, however, represent perhaps the strongest candidates for puerulus orientation given their pervasive influence during the rest of the life history for most palinurids (see Butler and Herrnkind, 2000 for overview).

Pueruli enter the nearshore waters in monthy pulses (Acosta et al., 1997), and in the Florida Keys they preferentially settle into foliose macroalgae (Marx and Herrnkind, 1985a; Butler et al., 1997). Of particular importance are the assemblages of red macroalgae (*Laurencia spp.*) that can cover > 90% of the bottom in some hard-bottom areas (Chiappone and Sullivan, 1994; Butler et al., 1997; Herrnkind et al., 1997; Behringer and Butler, 2006). Secondary plant metabolites associated with marine algae, particularly rhodophytes, are important cues for settlement and metamorphosis of a variety of larval invertebrates including: echinoids, asteroids, polychaetes, gastropods, corals, and hydroids (Morse, 1992; Boettcher, 1996; Hadfield and Koehl, 2004; Swanson et al., 2004).

Along with the induction of settlement, these compounds also retard herbivory and thus reduce the risk of predation for new settlers (Hay and Fenical, 1988; Hay et al., 1990). Given the preference of *P. argus* pueruli for red macroalgae as a settlement habitat, where their survival and growth is high (Marx and Herrnkind 1985a,b; Herrnkind and Butler 1986; Butler et al. 1997), it is likely that they too are attracted to it by chemical signals. Chemical signals from settlement substrates also hasten the metamorphosis process in a number of fish and invertebrates (Pechenik, 1990 and 1999), which would significantly reduce the probability of mortality for the vulnerable puerulus of *P. argus* (Acosta and Butler, 1997; Butler et al., 1997).

Marine meroplanktonic larvae are also sensitive to changes in pressure, and as such, barokinesis remains a reliable metric for quantifying orientation and settlement cues (see review by Knight-Jones and Morgan, 1966; Sulkin, 1984; Forward, 1989 and 1990). The ability of larvae to control their position in the water column has significant ecological advantages (e.g., avoiding predation, optimizing feedings) that play a significant role in shaping settlement behavior (Sulkin, 1984; Acosta and Butler, 1997; Mundy and Babcock, 1998; Stake and Sammarco, 2003). If settlement of spiny lobster pueruli is depth limited, then much of the potential nursery habitat across the Caribbean would be unsuitable and may explain the absence of recently recruited lobsters in deeper waters (> 5 m) and in areas with deep, narrow coastal shelves compared to regions with shallower shelf systems (e.g., Florida Keys, Gulf of Batabanó, SW Cuba) (Cruz and Adriano, 2001). In the Florida Keys, the occurrence of recently settled and metamorphosed early-benthic-phase juvenile *P. argus* is higher in the shallow (< 3m) bay- and gulf-side of the islands than in the deeper ocean-side lagoon behind the reef (Butler and Herrnkind, 1997; Acosta and Butler, 1999). Differences in predation-driven post-settlement survival between bay – and ocean-side habitats is one possible explanation for this phenomenon (Acosta and Butler, 1999), and differences in puerulus settlement in response to depth is another, yet untested possibility.

Marine species whose larvae settle in habitats close to shore are also exposed to habitats where salinity varies spatially and temporally. Although species that recruit into estuaries are adapted to such conditions (e.g., horseshoe crab larvae; Ehlinger and Tankersley, 2004), truly marine taxa are not. The detection of inappropriate salinities is advantageous to a widely dispersing stenohaline marine organism whose larvae/postlarvae cannot tolerate non-marine salinities, yet which settle in shallow coastal nurseries where freshwater runoff and riverine input are likely (Richmond and Woodin, 1996). For example, the re-engineering of freshwater input into an already variable salinity environment such as Florida Bay (Field and Butler, 1994) will invariably pose challenges to the recruitment dynamics of a variety of marine animals and plants in the ongoing Everglades restoration (CERP, 2006). It is anticipated that equally massive changes in the marine community structure in the region will occur in response, although the precise impact and their geographic extent are unknown.

The environmental cues that govern the coastal orientation and settlement of postlarvae of any species of palinurid lobster have not been determined. Therefore, in this study I carried out a series of laboratory studies to investigate cues characteristic of the nearshore nursery environment that may be used by pueruli during transport and settlement, specifically: (a) chemical cues (odors), (b) salinity, and (c) hydrostatic pressure.

MATERIALS AND METHODS

Collection of Postlarvae

For all experiments, transparent pueruli (mean = 6.25 ± 0.36 mm carapace length (CL), n ≈ 280) were collected at night on rising tides using plankton nets (1 m diameter, 2.5 m long, 750 µm mesh size, Sea Gear Corp., Miami, FL USA) suspended from Tom's Harbor Channel Bridge (between Grassy Key and Duck Key, 80^0 55.25'N, 24^0 46.75'W, Florida, USA) during new and first quarter lunar periods between June 2002 and April 2004 (see Appendix B). Upon capture, pre-competent pueruli characterized as fully transparent with the exception of eyes and thorax (confirmed using staging criteria from Lewis et al., 1952) were acclimated in a 4 l cooler to ≈ 1:1 mixture of artificial seawater (Instant Ocean®, Aquarium Systems, Ohio, USA: salinity = 32 - 35, pH_{range}= 7.8 - 8.0) and ambient seawater before transport to the Keys Marine Laboratory (KML), Long Key, Florida. Once at KML, lobsters were held in featureless 4 l static tank systems filled with artificial seawater (denoted hereon as AS) at ambient temperatures. To help supplement the often low numbers of pueruli collected from bridge tows, additional pueruli were collected from modified Witham floating surface collectors (i.e., n = 5 - 6 collectors) (see Witham et al., 1968 and Cox et al., 1997 for design details) within the same lunar period as bridge-caught animals. Pursuant to using collector-caught animals, I conducted a preliminary study to investigate if habitat selection by collector-caught animals was similar to that of those obtained from the plankton (see Appendix B for full study description). I found no significant difference in the settlement patterns and rates of emigration from inappropriate habitats between collector- and plankton-caught pueruli, and therefore used pueruli from both sources in subsequent experiments.

Chemotactic Response of Postlarvae

To determine if *P. argus* pueruli respond to chemical cues associated with nearshore settlement habitats, I conducted a series of laboratory experiments testing pueruli preference for several different water masses including: (a) inshore vs. offshore water sources, (b) macroalgal vs. seagrass settlement habitats, and (c) seawater of different salinity. In brief, I tested the "choice" of pueruli for different water sources at night in four custom-made acrylic choice chambers (0.95 cm long x 20 cm dia; Fig. 1) in which pueruli were given the opportunity to swim upcurrent toward one of two cues, or remain in the central chamber where the waterborne cues mixed. Experimental test conditions were: (1) AS vs AS; (2) oceanic water vs. AS; (3) oceanic water vs. bay water; (4) macroalgae vs. AS; (5) seagrass mix vs. AS, and a series of salinity trials (25, 30, 45, 50) each vs. AS at 35 (see Table 1 for preparation details). All test solutions were replenished daily prior to new trials and were all allowed the same incubation times.

Test Solutions

Artificial seawater served as the control water treatment in all of the experiments and served as a stock solution in which other factors of interest could be mixed and tested independently. A stock of artificial seawater was made up by subjecting two freshwater reservoirs (208 l plastic barrels) to 12 hours of heavy aeration followed by dechlorination with a water conditioner (NovAqua, Kordel, Inc.) and passive filtration with activated carbon (Proline, Aquatic Ecosystems (AES), Apopka, FL USA). I then added Instant Ocean® Seasalt (≈ 1 kg / 37.5 l) to obtain a salinity of 33 - 35 (pH = 7.8 - 8.0). For plant treatments, live material (algae or seagrass) (≈ 500 – 700 g / 50 l) was added to a 6 l perforated bucket with a lid and allowed to seep into a 50 l treatment tank filled with AS for 12 – 15 hrs. Fresh samples of all marine plants were collected by snorkeling or SCUBA in Florida Bay (Long Key area) 1 - 2 days prior to the start of each month's experiments.

The macroalgae treatment contained predominantly *Laurencia intricata* and *Laurencia poiteaui* as confirmed by botantical descriptions provided in Littler and Littler (2000). The seagrass treatment was a mix of the three dominant seagrasses in southern Florida Bay: *Thalassia testudinum, Syringodium filiforme, and Halodule beaudettei.* Plant samples were washed and cleaned with AS to rinse them of epiphytes, debris, and clinging invertebrates prior to use. Blades of seagrass were washed lightly and the obvious, loosely attached epiphytes removed.

Oceanic water was obtained offshore beyond the reef tract (≈ 10 - 12 km offshore) on flood tides 1-2 days before the monthly experimental trials were to begin. Seawater was pumped from just below the surface and filtered through a 0.5 µm polypropylene-wound inline cartridge filter (AES) into large storage barrels on board a boat then transported to the laboratory. Bay water was obtained by boat in Florida Bay on ebb tides and processed similarly to oceanic water. All treatment stocks were aerated and small powerheads (Aquaclear model A301, AES) (recirculation rate = 644 l / hr) provided continuous mixing of the seawater.

Table 1
Treatment descriptions and preparations used in the test of pueruli response to chemical cues

Test Solution	Treatment Preparation
Control	Artificial seawater (AS) (Instant Ocean® seasalt) added to dechlorinated freshwater (dechlorinated using aeration, water conditioner, and filtration through activated carbon) and mixed to achieve a salinity of 33 - 35 and stored in aerated tanks.
Offshore (oceanic)	Seawater obtained ≈ 10 - 12 km beyond the reef tract (near Tennessee Reef) just below surface on flood tides 1 - 2 days prior to trials; water filtered through 0.5 µm polypropylene cartridge filter and stored in aerated tanks.
Inshore (bay)	Seawater collected on bayside of Long Key < 1 km from shore just below surface on ebb tides 1-2 days prior to trials; water filtered through 0.5 µm polypropylene cartridge filter and stored in aerated tanks .
Macroalgae (metabolites)	*Laurencia intricata* and *L. poiteaui,* collected by SCUBA from sites bayside of Long Key 1-2 days prior to trials; ≈ 500 – 700 g / 50 l (10 - 14 % vol) cleaned and washed of major epiphytes and debris, placed into 6 l perforated buckets within 50 l barrels to seep in AS for 12 - 15 hours.
Seagrass (metabolites)	*Thalassia testudium, Syringodium filiforme,* and *Halodule beaudettei.* Obvious epiphytes removed. Collected and processed similarly to macroalgae.
Salinity Series	Hypersaline (45), Hyposaline (25), and two variable salinities (30 and 50) all mixed from AS stock solutions with the addition of Instant Ocean® and/or dechlorinated freshwater.

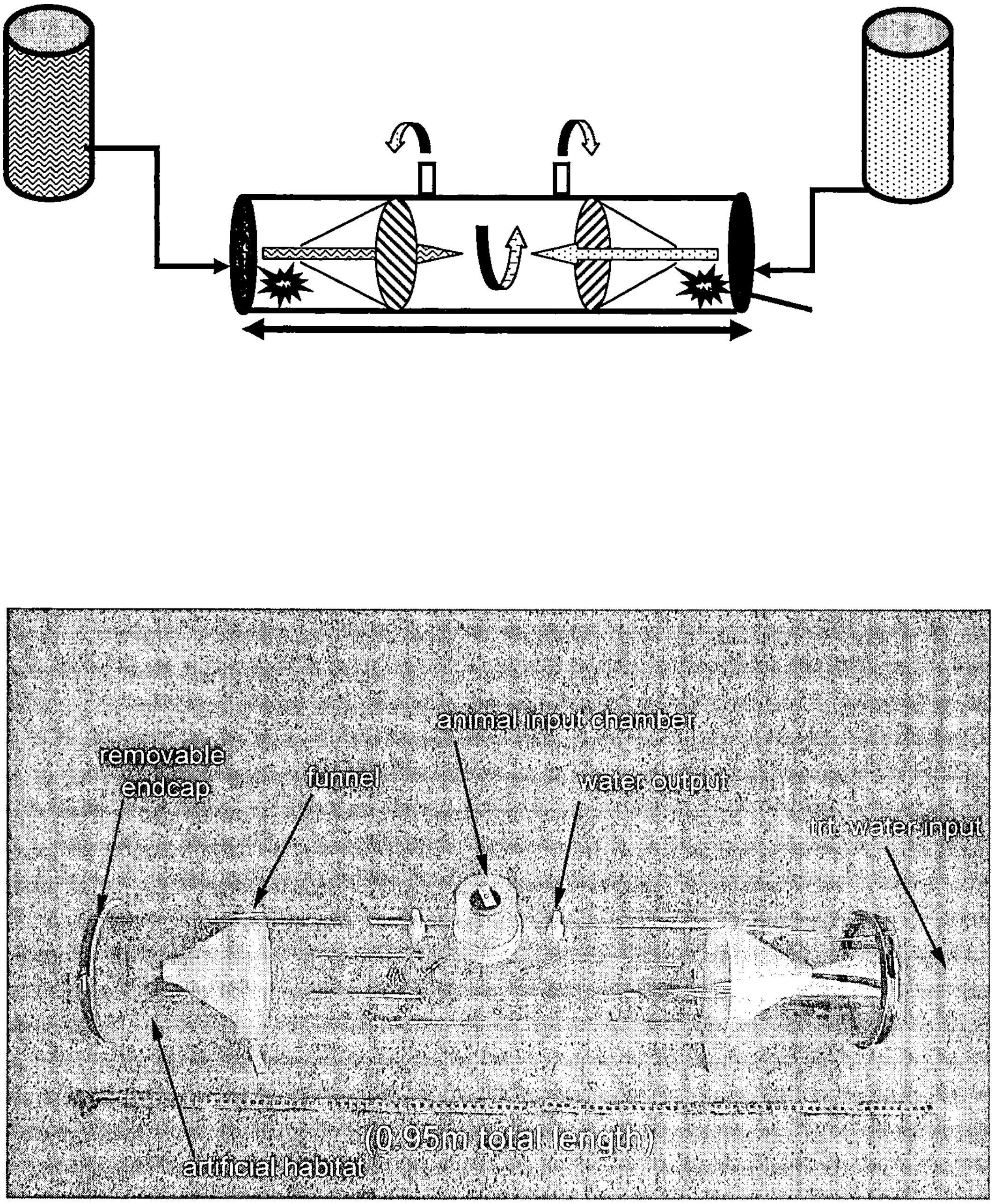

Fig. 1. Top: Schematic drawing of choice tube chamber apparatus. Arrows indicate directional flow of water. Bottom: Picture of acrylic choice tube chamber apparatus without the two headtanks that supply water to the apparatus.

Experimental Trials

Chemotactic and directional responses of lobster pueruli were tested in the lab at night between the new and first quarter lunar periods when pueruli are normally moving onshore each month (Little, 1977; Little and Milano, 1980; Marx, 1986; Ward, 1989; Bannerot et al., 1992; Herrnkind and Butler, 1994; Briones-Fourzán, 1994; Eggleston et al., 1998; Quinn et al., 1998 Acosta and Butler, 1999; Gordon and Vasquez, 2006). All preparation work and initial observations were made under filtered red light (600 - 700 nm, Roscolux light gel # 26, 12% transmittance, Hollywood, CA USA) after which lights were turned off for the duration of the trials. Prior to and between trials, choice chambers and head tanks were washed thoroughly with a non-toxic soap and allowed to air dry before use. To initiate a trial, I filled each choice chamber with 7.5 l AS and then filled the two 11 l head tanks that supplied each chamber with the appropriate test solution. Seawater test solutions flowed into the opposite ends of each choice chamber through a 50 cm segment of 6 mm diameter airline tubing from the two head tanks. After entering the ends of the chamber, the water passed through the funnel barriers and into the central section of the chamber where it mixed then flowed out of the chamber through valves at the chamber top. Once placed in the central portion of the choice chamber, pueruli could remain in that part of the chamber or swim into the current toward a chemical cue through funnels leading into either of the adjoining end sections of the chamber. However, once in an end section, it is unlikely that pueruli could swim back out through the inverted funnel (Fig. 1). It is within the two end chambers that pueruli then remained until daybreak when their position was recorded and each animal removed. The dynamics of this chamber design allow for two chemically distinct water gradients within each chamber. I conducted and videotaped chemical dye tests prior to some trials to confirm this flow regime. Flow in all trials flow was set at ≈ 0.15 – 0.20 l / min.

Into the center of each tube chamber I placed a clear segment of 2.5 cm diameter x 30 cm long PVC into which I added a single puerulus. After five minutes, the PVC tube was slowly lifted out of the tube and the pueruli freed. The acclimation period was vital in avoiding an initial escape response (i.e., tailflipping) by the pueruli. Water flowed into each choice chamber for $\approx 2.0 \pm 0.5$ hours after the start of the trial. The next morning, water was drained from each chamber and the position of the postlarvae noted. Similarly to procedures given in Boudreau et al. (1993), I tested some pueruli for behavioral consistency. That is, I retested a random subset of the unpigmented pueruli under the same treatment conditions the next night. Water temperature was measured prior to each trial ($\text{temp}_{\text{avg}} = 26\ {}^0\text{C}$; range = $20\ {}^0\text{C} - 28\ {}^0\text{C}$). A total of four choice chambers (replicates) were run each night simultaneously, one puerulus per tube.

A three-way log-linear contingency table analysis was used (SPSS v.14.0, Chicago, Illinois) to assess whether significant differences existed among the three factors tested: postlarval source (2 levels; collector- or plankton-caught postlarvae), water treatment type (7 levels), and choice (2 levels; choice of AS control or treatment seawater). The test for a 3-way effect (source x treatment x choice) was non-significant, as was the 2-way test of the source effect (collector- or plankton-caught) on choice (Table 2). This result was corroborated in a preliminary study of the effect of puerulus source on settlement and emigration behavior (see Appendix B). Consequently, I pooled the data for pueruli from both collection methods (Zar, 2006) and ran a 2-way loglinear contingency table analysis (G test with Williams adjustment; Williams, 1976) to examine the effect of each treatment on puerulus choice of water sources (treatment or control).

Table 2
Hierarchical log linear analysis results for k-way effects of puerulus source (plankton or collector-caught), treatment water (see Table 1), and choice (left or right in choice tube) for chemotactic response experiments

Effect Name	df	G	P
Source*trt*choice	5	3.24	0.667
Source*choice	1	1.98	0.159
Trt*choice	5	40.90	0.001

Effect of Odor on Postlarval Time-to-Metamorphosis

In this experiment, I individually exposed transparent pre-competent pueruli to one of three water treatments (AS, AS conditioned with *Laurencia*, bay water) to determine whether time-to-metamorphsis is fixed or is a plastic response to local environmental conditions. I placed 45 400 ml plastic nalgene beakers (n = 15 for each of three water treatments, AS, bay, and AS + *Laurencia* spp.) within two large plastic bins (dimensions: 60 cm x 60cm x 30 cm) containing 2 - 3 cm of seawater that kept all of the containers thermally equalized. Pueruli were then randomly assigned to containers (1 puerulus / beaker), each containing the assigned seawater treatment and a small 9 cm^2 clump of window mesh screening to serve as a refugia. Treatment water was exchanged in each container and replenished with fresh treatment water daily. All test solutions were maintained at salinities 32 - 33. Each day, pueruli were checked for qualitative signs of pigmentation (i.e., the start of metamorphosis). The primary discerning trait in most instances was the pinkish/purple color that could be seen at the base of the third basal segment of the antennae (Lewis et al., 1952). Pueruli were removed from the assay upon the start of metamorphosis. Assays were replicated over the course of five separate months (Aug, Nov, and Dec 2002 and Jan-Feb 2003). Pueruli used for these trials were all collected from the plankton on the same night and assumed therefore to be of the same cohort. These data were analyzed using a

1-factor model I randomized block ANOVA (SPSS v.14.0, Chicago, Illinois) where the factor of interest was water treatment (3 levels), the block was month (to control for the effect of temperature differences on metamorphosis), and the response variable measured as the mean number of days to pigmentation (SE). The raw data were ln+1 transformed to meet parametric assumptions.

Effect of Hydrostatic Pressure on Puerulus Settlement

I tested the hypothesis that *P. argus* pueruli respond to changes in hydrostatic pressure and habitat structure in ways that would facilitate their selection of shallow, structurally complex nursery habitats. To assess this, I conducted a series of laboratory experiments testing pueruli swimming behavior and position relative to the benthos when exposed to different hydrostatic pressures indicative of depth changes of approximately 0 – 14 m, and three habitat structure types: (a) no structure, (b) artificial structure, and (c) macroalgal structure.

I monitored the depth of pueruli in the water column in relation to the three structure types at night using three 4.5 l commercially manufactured clear, cylindrical mechanical filter canisters (dimensions: OD = 24 cm, ID = 23 cm, height = 21cm height, Red Sea Pharm Co., USA) (Fig. 2) designed with a single threaded screw-on lid. Incremental pressures of 0, 35, 69, 103, and 137 kPa corresponding to 0, 3.4, 6.8, 10.2, and 13.5 vertical depths (m) were used to reflect a reasonably realistic stratified gradient from inshore Florida Keys waters, through Hawk's channel (lagoon) and out to the fore reef. Treatments were further constrained within the chamber's technical specifications. An external oil-filled pressure gauge (Dixon ABG 15, AES) was threaded into one side of the canister and provided pressure readings in increments of 1 psi. (0.145 kPa). Pressure was applied in regular increments to the chamber by injecting air into the chamber via a foot pump connected to a stem valve that was mounted into the in-port of the chamber (Fig. 2). Once at the desired pressure(s), the chamber was de-pressurized incrementally by depressing the stem valve release. A measuring stick (marked in 1 cm increments, up to 21 cm) was mounted to the outside chamber to quantify the vertical position of a puerulus in the water column at any time.

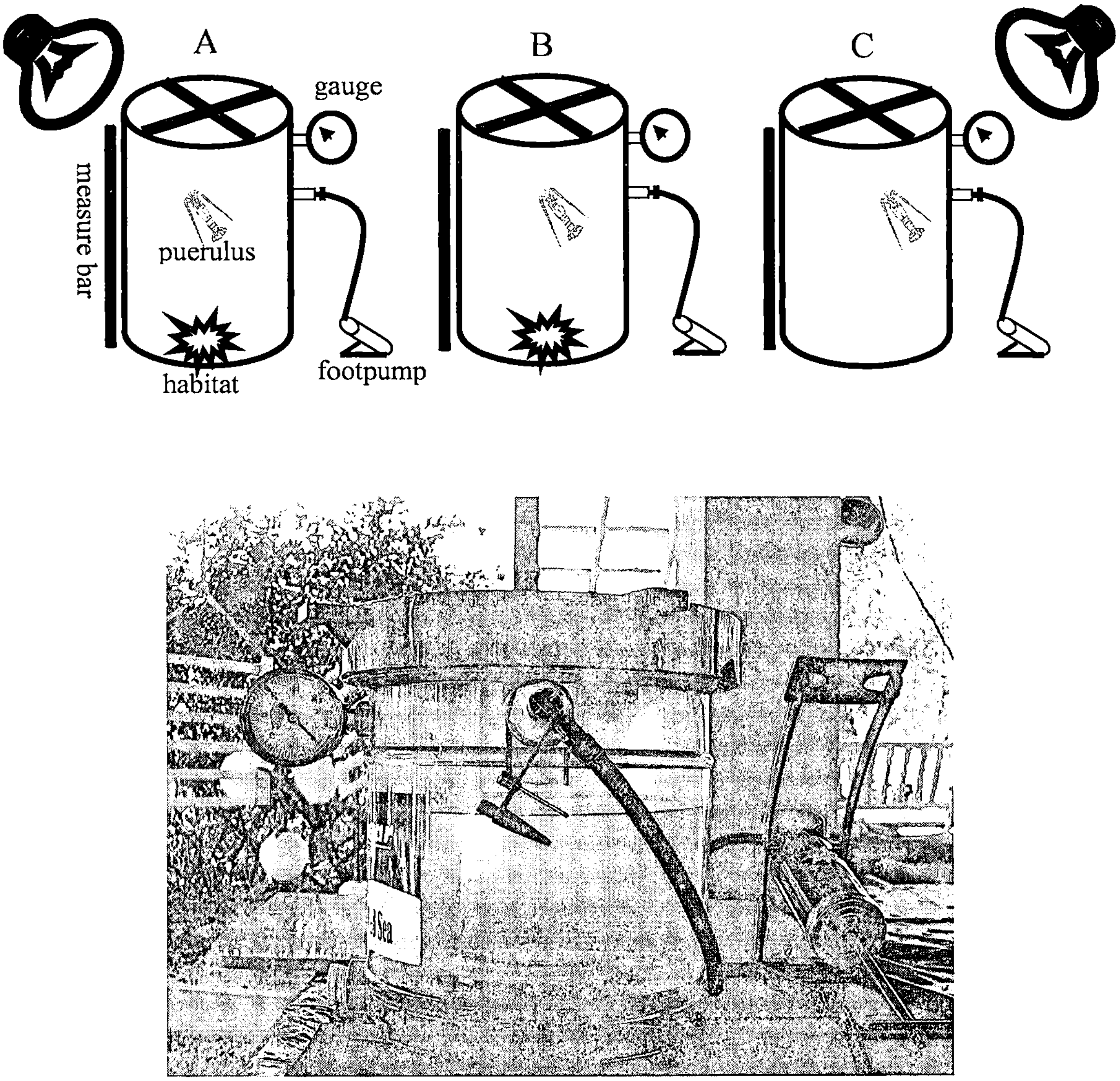

Fig. 2. Top: Schematic drawing of pressure chamber apparatus. Treatments are denoted as follows: (A) *Laurencia* spp. habitat, (B) artificial habitat and (C) no habitat. Bottom: Picture of pressure chamber apparatus (see text for details).

Prior to a set of trials, each chamber array was filled to the top with ≈ 4.5 l AS and fitted with the appropriate habitat treatment: either one small ≈ 9 cm^2 tuft of *Laurencia* spp., window screening (artificial habitat), or featureless (no habitat). Chambers were allowed to sit for ≈ 20 minutes. A single puerulus was introduced into each chamber using a segment of pvc pipe (methods similar to choice chambers) and allowed to acclimate for roughly 5 minutes on the bottom. Once the pipe segment was removed, the lid was screwed on tightly and each puerulus was free to swim and move around the chamber. All trials were conducted under red light as described in choice chamber experiments. For each incremental pressure treatment, an interval of one minute was allotted before pressure was either increased or decreased to the next value for a total time budget (including acclimation) of 15 minutes per trial / puerulus. Additionally, I observed the behavior of pueruli during each trial using the behavioral categories defined by Calinski and Lyons (1983), including: forward swimming, sinking, approach, and retreat to and from bottom habitat structure.

This series of experiments was analyzed using a 2-factor repeated measures ANOVA (Zar, 2006) (SPSS v.14.0 Chicago, Illinois). Habitat (3 levels) was treated as a model I factor and pressure treatments (10 levels total, 5 increasing and 5 decreasing) treated as separate time elements in a repeated measures factor. The position of each animal at the end of each two-minute period and how fast or slow that change occurred (i.e., rate of change) was calculated and pooled for all pueruli for each treatment.

RESULTS

Chemotactic Response of Postlarvae

Panulirus argus pueruli were attracted to moderate salinity seawater and chemical cues from coastal waters, specifically red macroalgae (Figs. 3 and 4; summarized in Table 3). Pueruli showed no preference for two identical AS controls (53% vs 47%), and could not distinguish between oceanic water (57%) and the AS control (43 %). Pueruli also showed no preference for AS containing seagrass odors over AS alone (47% vs. 53%). Conversely, pueruli were significantly attracted to odors from *Laurencia* spp. (62% vs. 38% for oceanic water) and to bay water (70% vs. 30% for oceanic water), presumably in response to the secondary metabolites found in each.

In only 8 cases out of 272 trials did pueruli remain in the central portion of the chamber and thus did not choose a water source. Furthermore, 17 of the 21 pueruli (81%) that were re-tested made the same choice of water mass in the subsequent trial. These findings lend credence to the precision of the methodology and the robustness of the results.

Effect of Odor on Postlarval Time-to-Metamorphosis

Pueruli incubated in both bay and *Laurencia* spp. water treatments pigmented significantly faster compared to those in AS ($F = 26.86$; $df = 2,180$; $p < 0.001$; REGWF post-hoc comparisons), but did not differ between each other ($p > 0.05$; REGWF post-hoc test) (Fig. 5 and Table 4). As expected, time (days shifted) to pigmentation (TTP) varied among months because of differences in seawater temperatures (block effect in analysis; $F = 37.54$; $df = 4$; $p < 0.001$). TTP for all treatments in August averaged 5.6 days compared with 11.6 days in January; monthly temperature averages were: 26.5 ^{0}C (Aug), 25.3 ^{0}C (Nov), 24.1 ^{0}C (Dec), 21.3 ^{0}C (Jan), and 23.8 ^{0}C (Feb). Despite these temperature differences, bay and *Laurencia* spp. water treatments consistently lowered TTP from 1.9 - 2.9 days (avg = 2.4 days) compared with AS (Fig. 6).

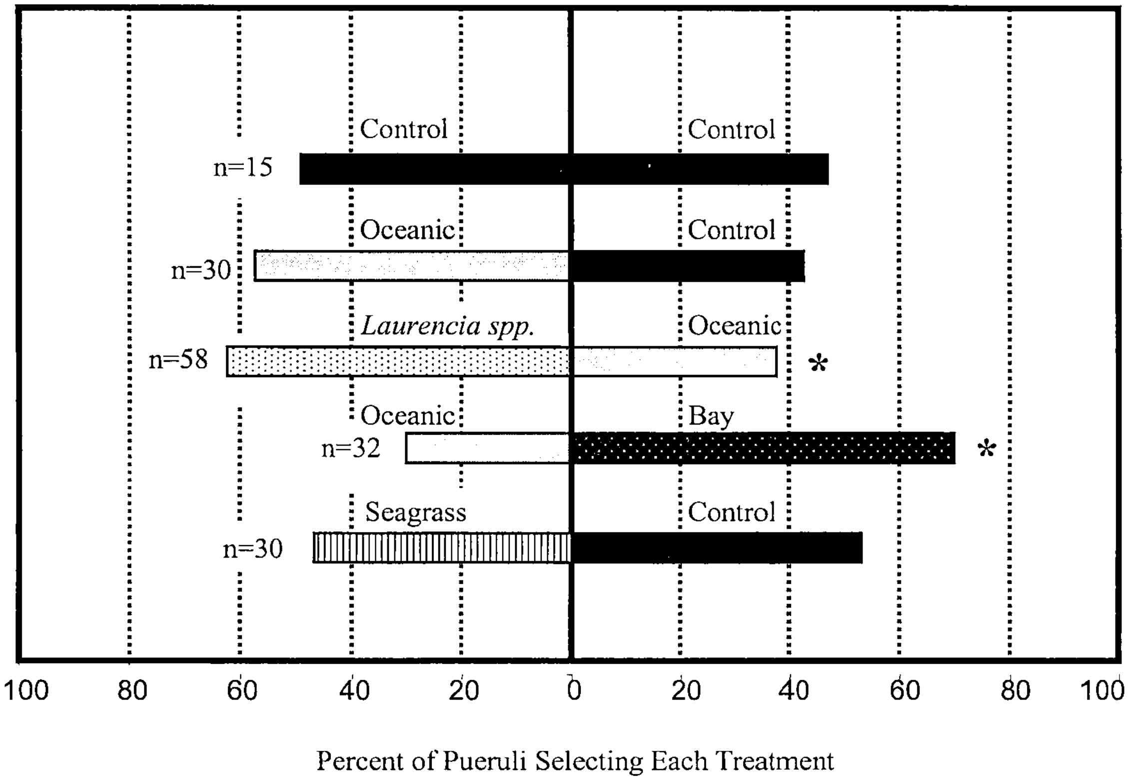

Fig. 3. Choice of *P. argus* pueruli for seawater sources and odor cues in laboratory experiments. An '*' indicates treatment comparisons that were significantly different ($p < 0.05$) (G test with Williams adjustment) at the $\alpha = 0.05$ level. Histograms of the same color or fill pattern indicate the same treatment.

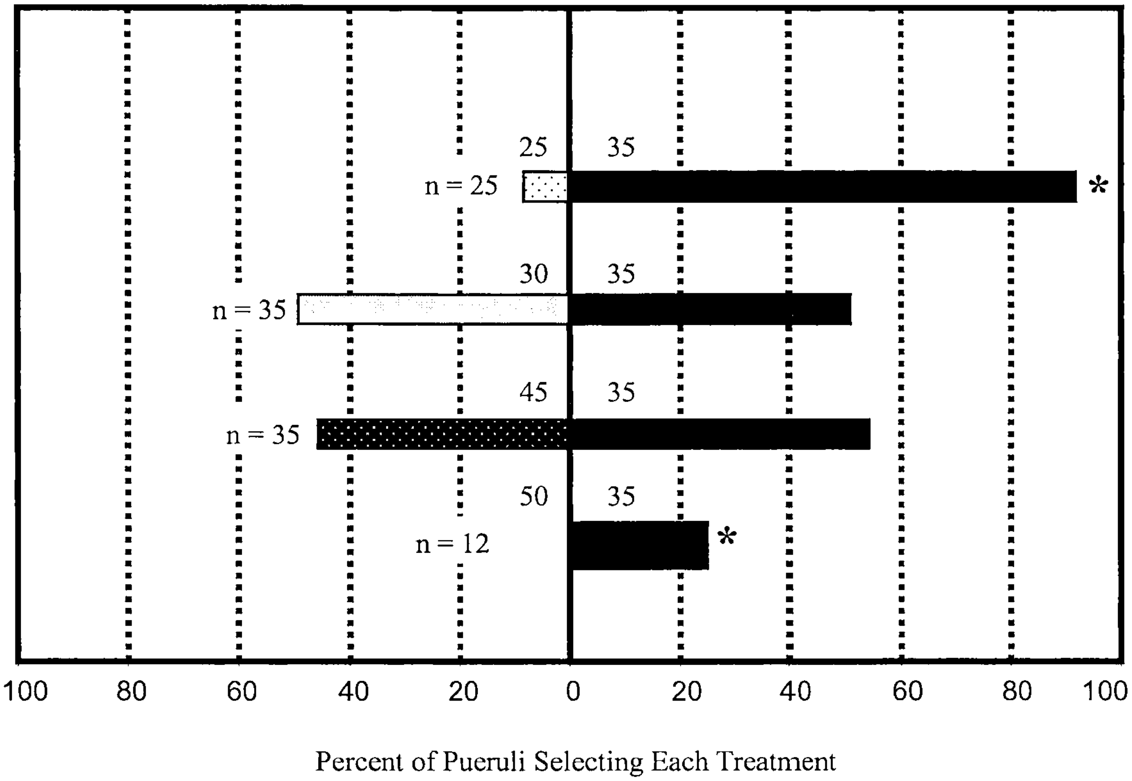

Fig. 4. Choice of *P. argus* pueruli for salinity cues in laboratory experiments. An '*' indicates treatment comparisons that were significantly different ($p < 0.05$) (G test with Williams adjustment) at the $\alpha = 0.05$. Histograms of the same color or fill pattern represent the same treatment.

Table 3
Summary of laboratory experiments testing *P. argus* puerulus choice of water sources and salinities. Results are pooled for pueruli obtained from the plankton and from collectors, but the sample sizes of each are shown in the columns: $N_{plankton}$ and $N_{collectors}$, respectively. Degrees of freedom = 1 for each 2-way G-test

Treatment	$N_{Plankton}$	$N_{Collectors}$	N_{Total}	% Choice	G_{adj}	P
control vs. control	10	5	15	53 : 47	0.25	ns
oceanic vs. control	19	11	30	57 : 43	0.12	ns
Laurencia spp. vs. oceanic	44	14	58	62 : 38	7.12	0.010
oceanic vs. bay	24	8	32	30 : 70	5.53	0.017
seagrass vs. control	18	12	30	47 : 53	0.20	ns
Salinities						
25 vs. 35	11	14	25	8 : 92	27.53	0.001
45 vs. 35	23	12	35	46 : 54	0.44	ns
30 vs. 35	17	18	35	49 : 51	0.03	ns
50 vs. 35	0	12	12	0 : 25		
Totals	166 (61 %)	106 (39 %)	272			

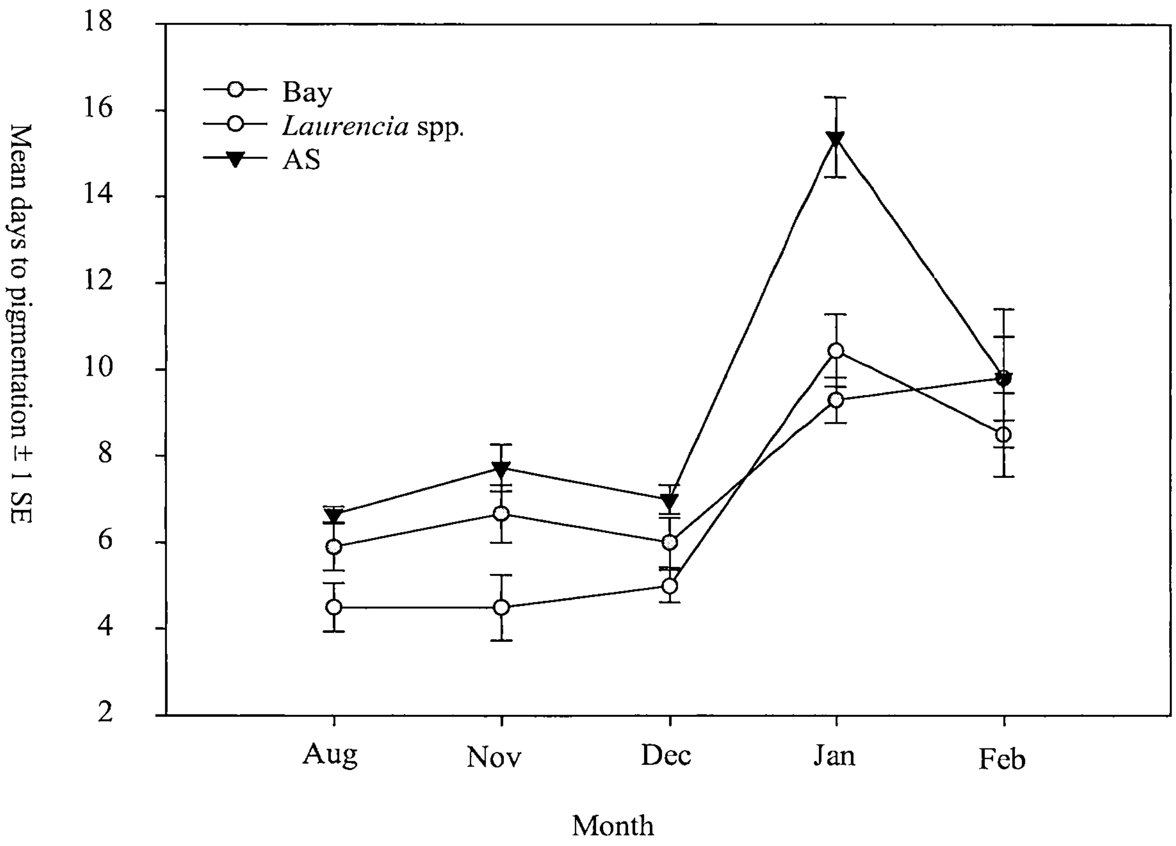

Fig. 5. Time-to-pigmentation (TTP; mean days to pigmentation ±1 SE), an indication of the onset of metamorphosis, for *P. argus* pueruli by month (n = 10-15 animals / trt. / month).

Table 4
1-factor model I randomized block ANOVA results testing the effect of seawater treatment and month (block) on time-to-pigmentation (metamorphosis) of *P. argus* pueruli

Source	df	SS (Type III)	MS	F	P
Block (month)	4	8.4	2.1	37.62	0.001
Treatment	2	1.42	0.71	26.86	0.001
Error	150	0.795	0.0053		
Totals	180				
		Post-hoc REGWF Results ($\alpha = 0.05$)			
Treatment Group:	AS^a		Bay^b		Laurencia^b

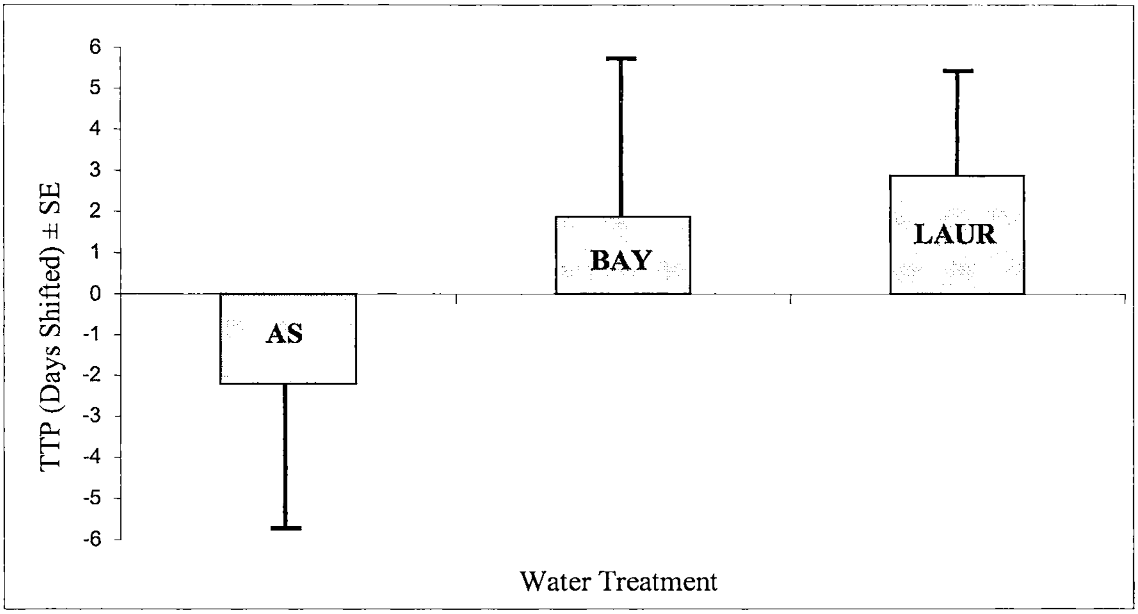

Fig. 6. Average deviation (± SE) from the mean (in days) that each water treatment condition shifted time-to-pigmentation (TTP) of *P. argus* pueruli (n ≈ 45 / trt). Data from all monthly trials combined. Both bay and *Laurencia* spp.(LAUR) treatments accelerated TTP by 1.9 - 2.9 days (avg = 2.4) compared to TTP in artificial seawater (AS).

Effect of Hydrostatic Pressure on Puerulus Settlement

The raw data from this experiment were ln +1 transformed to meet parametric assumptions of normality, heterogeneity of variance or independence of errors. Pueruli responded to changes in pressure by swimming upward into the water column at higher pressures and down to the bottom where they ceased swimming when pressure was lower (2-factor repeated measures ANOVA; $F = 38.92$; df = 2,90; $p < 0.001$) (Fig. 7 and Table 5). The response of pueruli differed even more so in the treatment containing *Laurencia* spp. (L) compared to the other two treatments containing artificial (A) structure or no structure at all (control, C) ($F = 21.73$; df = 2,9; $p = 0.023$; REGWF post-hoc test) (Table 5).

As pressure increased, pueruli in the no structure control swam rapidly and higher (4.6 ± 1.04 cm compared with mean heights of 0.43 ± 0.31 cm and 0.67 ± 0.52 cm for L and A treatments, respectively. Pueruli swam off of the tank bottom in the no structure control when pressures were as low as 0-1 kPa. In contrast, pueruli in the *Laurencia* and artificial structure treatments remained within structure on the bottom until pressures increased to ≈ 69 kPa and then swam to heights comparable with animals in the control treatment (9.5 – 13.7 cm over all three treatments; Fig. 7). Under conditions of decreasing pressure, pueruli in the *Laurencia* and artificial structure treatments responded similarly by swimming downward from their maximum mean heights of 14.2 cm (L) and 16.6 cm (A) at 137 kPa, whereas pueruli in the control treatment remained within the water column significantly longer over a range of decreasing pressures.

Once pueruli made contact with structure on the bottom of the tank, either *Laurencia* or artificial structure, they spent more time on the bottom and thus less time in the water column (Fig. 7). When pressure was increased, pueruli in the *Laurencia* treatment remained in structure on the bottom longer and were often the first to leave the water column and settle to the bottom under conditions of decreasing pressure. Regardless of the pressure, pueruli remained (on average) closer to the bottom (6.32 cm) when in the presence of *Laurencia* compared to those in the artificial structure treatment (8.73 cm) and no structure control (11.59 cm).

Pueruli maintained in featureless chambers (control) often swam erratically. Pueruli in the control treatment made more excursions to the bottom, but they were shorter in duration and the pueruli did not explore the benthos. In contrast, animals in both the *Laurencia* and artificial structure treatment chambers often swam toward the tufts of *Laurencia* or artificial structure and conducted short (1 - 2 s) tactile interactions (‘touch-downs’ and ‘lift-offs’) with the structures.

Table 5

2-factor repeated measures ANOVA results testing the effect of benthic structure (3 levels, fixed) on vertical position and a series of increasing and decreasing pressure treatments (10 levels) of *P. argus* pueruli (n = 30 / trt)

Source	df	SS (Type III)	MS	F	P
Height	2	83.21	41.605	38.92	0.001
Pressure (repeated)	9	510.228	56.692	52.303	0.001
Height*pressure	18	153.01	8.500	21.73	0.023
Error	87		1.084		
Total	116				

	Post-hoc REGWF Results ($\alpha = 0.05$)		
Treatment Group:	Control^{a}	Artificial^{b}	Laurencia^{b}

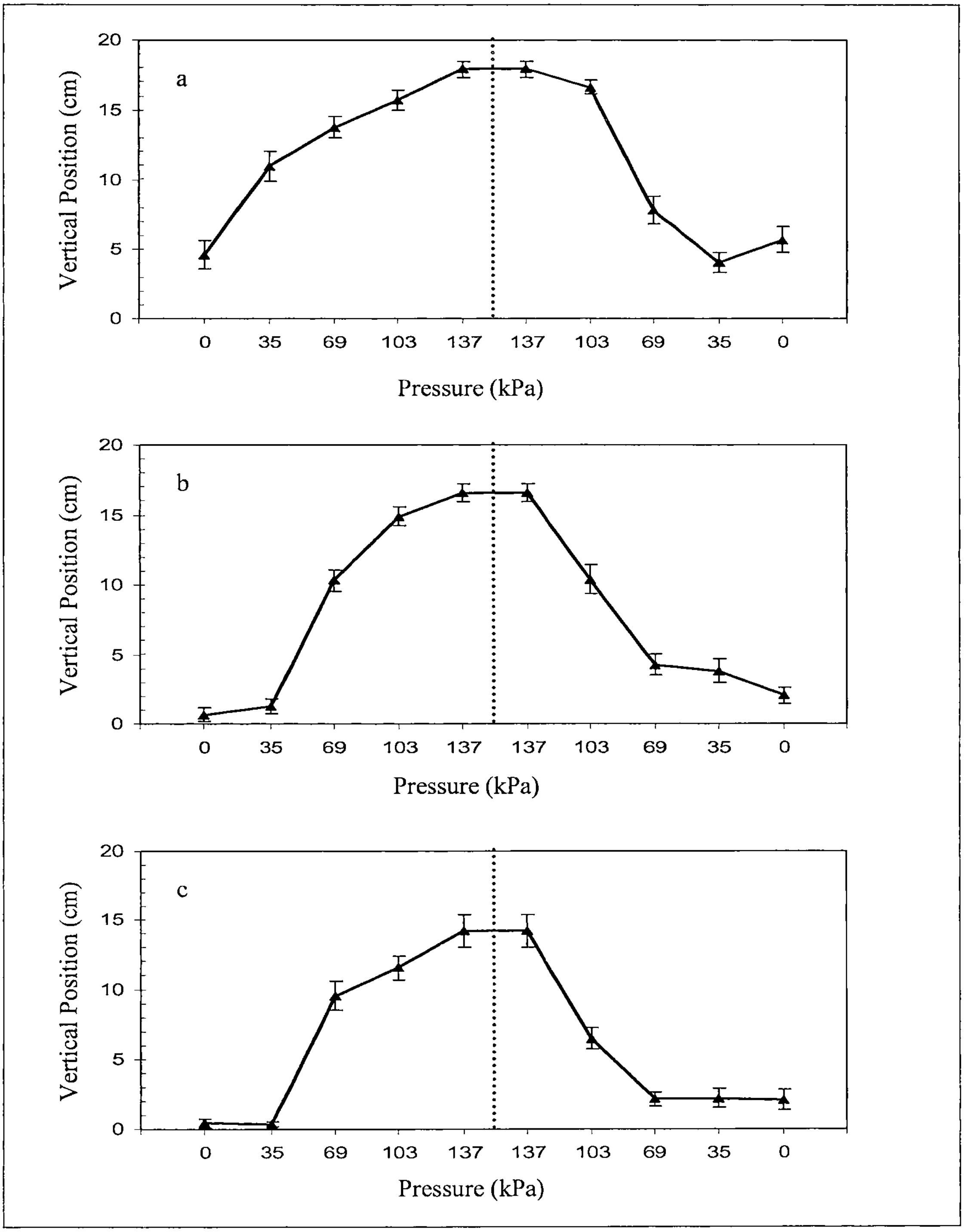

Fig. 7. Summary results testing the swimming response of *P. argus* pueruli to different pressures indicative of potential settlement depths. Puerulus response (n = 30 / treatment) was also tested in chambers containing either (a) no structure (control), (b) artificial structure, or (c) the macroalgae *Laurencia*, in which they prefer to settle. Shown is the mean (± 1 SE) vertical position in the water column for each treatment under increasing and then decreasing pressures.

DISCUSSION

Understanding how pelagic marine larvae detect suitable coastal settlement habitat is crucial for predicting patterns of recruitment given local environmental conditions, which can be dynamic and altered by human activities. The notion that meroplanktonic larvae are passive particles whose transport is driven solely by ocean currents has largely been discounted as proof of their remarkable behaviors grow (Katz et al., 1994; Wolanski et al., 1997; Cowen et al., 2000; Sponaugle et al., 2002; Bradury et al., 2003; Epifanio, 2003; Sale and Kritzer, 2003; Cowen et al., 2006; Incze et al., 2006). My results demonstrate for the first time that palinurid lobster postlarvae are among those taxa that respond to coastal cues. Specifically, I have shown that *P. argus* pueruli are: (1) repulsed by salinities much different than 35, (2) attracted to coastal seawater, particularly chemical cues derived from the macroalgae in which they settle, and (3) alter their swimming behavior in response to pressure cues that signal their arrival in shallow coastal nurseries. I also show that macroalgal odors hasten the metamorphosis of postlarvae to the benthic juvenile stage, a transition that significantly reduces their risk of predation (Acosta and Butler, 1999).

The metamorphosis of the teleplanic and highly-branched phyllosoma to the compact and fusiform-shaped puerulus was described by Gurney (1942) as "the most profound transformation at a single moult known in the Decapoda". Although great strides have been made to understand and interprept various aspects of the biology of this important transitional phase, our knowledge of puerulus behavior and how it influences their onshore transport and selection of settlement habitat is still rudimentary (Butler and Herrnkind, 2000; Jeffs et al., 2005). Yet, there is convincing evidence that behavioral and physiological adaptations play a pivotal role in the onshore transport of pueruli to nearshore habitats (Phillips and McWilliam, 1986; Acosta and Butler, 1999; Jeffs et al., 2005).

Evidence that physical forcing alone can describe the onshore arrival of palinurid postlarvae is generally weak, and comes from studies associating indices of postlarval supply with measurements of potentially important advective processes (Booth, 1986; Yeung and McGowan, 1991; Caputi and Brown, 1993; Booth, 1994; Caputi et al., 1995; Polovina and Moffitt, 1995; Acosta et al., 1997; Eggleston et al., 1998; Yoshimura et al., 1999; Sekiguchi and Inoue, 2002; Yeung and Lee, 2002). For example, Acosta et al. (1997) examined monthly settlement of *P. argus* pueruli on artificial collectors over an eight year period and found only a weak correlation between onshore wind-forcing and settlement. Similar results were reported by Eggleston et al. (1998) in the Bahamas (6 year data set) and Yeung et al., (2001) in the Florida Keys (2 year study). Mesoscale currents operating over various spatial and temporal scales improve this relationship (Acosta et al., 1997; Booth et al., 2000; Yeung et al., 2001), but do not explain the majority of the variance in postlarval abundance, with the notable exception being the evidence that seasonal changes in the strength of the Leeuwin Current off Western Australia may block the onshore arrival of *P. cygnus* postlarvae (Caputi et al., 1995). My results and those of others (Calinski and Lyons, 1983; Herrnkind and Butler, 1986; Acosta and Butler, 1999) indicate that the onshore arrival of *P. argus* pueruli is enhanced by behaviors (i.e., attraction to macroalgal chemical cues, nighttime surface transport during new moon, and affinity for shallow, structurally complex habitat) coupled with morphological and physiological characteristics (e.g., transparency, stored energy reserves that sustain their strong swimming) (Jeffs et al., 1999; Phillips et al., 2005) that make them specialized agents for transport and settlement.

Palinurid pueruli are indeed strong swimmers with average sustained swimming speeds of 15 cm/s and burst speeds of 30 cm/s or more (e.g., *P. cygnus*: Phillips and Olsen, 1975; *P.*

interruptus: Serfling and Ford, 1975; *P. argus*: Calinski and Lyons, 1983; *Jasus edwardsii*: Jeffs and Holland, 2000), which rival or surpass the speeds achieved by marine fish larvae (see Fisher et al., 2000 and 2005). Yet, pueruli are incapable of feeding (Wolf and Felgenhauer, 1991; Lemmens and Knott, 1994; Takahashi et al., 1994; Nishida et al., 1995) and maintain these metabolically costly swimming speeds for weeks through catabolism of phosolipid reserves (Lemmens, 1994a,b, 1995; Jeffs et al., 1999; Jeffs and Holland, 2000; see Appendix C). Numerous studies of the spatial distribution of planktonic palinurid larvae (Ritz, 1972; Yeung and McGowen, 1991; Sekiguchi, 1997; Chiswell and Booth, 1999; Jeffs et al., 2001; Sekiguchi and Inoue, 2002) have shown that late-stage phyllosomata and pueruli are routinely caught near or beyond the continental shelf break where metamorphosis to the puerulus stage presumably occurs. Jeffs et al. (1999) estimate that in New Zealand *J. edwardsii* pueruli swim as far as 200 km to shore (mean = 92km) and up to 17% arrive onshore metabolically exhausted. Thus, mechanisms that promote the arrival and early metamorphosis of pueruli in coastal nurseries, such as those demonstrated in this study, would be of obvious adaptive benefit in reducing larval wastage and maximizing the metabolic reserves available at the energetically costly metamorphosis to the benthic juvenile stage.

Chemotaxis and Metamorphosis

Chemotaxis is the most common cue used by decapod crustaceans for orientation (Childress and Herrnkind, 1997; Nevitt et al, 2000; Forward et al., 2003a; Jeffs et al., 2003; Keller et al., 2003). Yet until this study, there was no published evidence that palinurid pueruli use chemotaxis for onshore transport, although comparable studies exist for some marine larval fishes (Sweatman, 1988; reviewed in Montgomery et al., 2001). For example, Atema et al. (2002) documented that settling apogonids preferentially chose lagoon water over ocean water in laboratory choice tests. Here I report that *P. argus* pueruli are also attracted to seawater collected within 1 km of shore bayside of the Florida Keys within an area of mixed seagrass and hard-bottom, typical of lobster nursery habitat in the region. Moreover, I show that pueruli are attracted to chemical substances associated with red macroalgae (*Laurencia* spp.), but not seagrasses, and that odors from *Laurencia* speed up development (i.e., time-to-metamorphosis).

The bushy red algae *Laurencia* spp. is a ubiquitous component of shallow hard-bottom habitat in the Florida Keys (Chiappone, 1996; Robles et al., 2005), although this type of habitat is not as abundant throughout the Caribbean as it is in Florida more than 5,000 species of Rhodophyta exist (Littler and Littler, 2000). *Laurencia* is the preferred settlement substrate for *P. argus* pueruli (Marx and Herrnkind, 1985a; Herrnkind and Butler, 1986), and pueruli may be attracted to its chemical metabolites (this study), that are characterized as water soluble, of low molecular size (< 1 kDa), stable over time (≈ 12 months), and unaffected by boiling for 10 minutes (Boettcher et al., 1996). Not only does *Laurencia* serve as an architecturally complex refuge for the vulnerable puerulus and early benthic juvenile stages of *P. argus*, it also induces metamorphosis of pueruli and other marine invertebrate larvae. Chemical cues associated with *Laurencia* enhance metamorphosis in mollusks (*Strombus gigas*, *Haliotis* spp.*)*, echinoderms *(Strongylocentrotus droebachiensis),* polychaetes (*Spirobis rupestris),* and now decapods (Gee, 1965; Morse and Morse, 1984 and 1990; Pearce and Scheibling, 1990; this study). Application of naturally occurring chemical cues are widely used in shellfish aquaculture to induce larval settlement and metamorphosis in oysters (Coon et al., 1990; Doroudi and Southgate, 2002) and other commercial molluscs (Tetrault and Rice, 1994) including queen conch (*Strombus gigas*) (Davis et al., 1990; Davis and Stoner, 1994; Boettcher and Targett, 1996; Boettcher et al.,1997).

Equally important as the response of pueruli to red macroalgae was their lack of response to artificial seawater containing a mix of locally abundant seagrasses. In contrast, some decapod megalopae, such as those of the blue crab (*Callinectes sapidus*) detect and orient toward water masses laden with seagrass (*Zostera marina*), a documented nursery habitat for blue crabs (Forward et al., 2003a). Despite the abundance of seagrass in the shallow waters of the Florida Keys and the Caribbean, there is no evidence that *P. argus* pueruli preferentially settle in seagrass, where mortality rates are higher than in algae (Herrnkind and Butler, 1986). Some undoubtedly settle in seagrass and in other architecturally complex substrates (e.g., mangrove roots), but these are not preferred habitats (Acosta and Butler, 1997). The use of ecologically relevant concentrations of odors in studies of chemically mediated behavior (see Weissburg, 2000 for review) is of potential importance in studies such as this and may explain the lack of response by pueruli to seagrass odors. However, the seagrass and macroalgae treatment cues used in this study were "natural" odors, not processed metabolites, and represented equivalent, ecologically relevant, but unknown concentrations of *Laurencia* and seagrass metabolites. I did not investigate which compounds in the *Laurencia* metabolites are attractive to pueruli. However, a number of compounds are known to induce metamorphosis in marine larvae including free amino acids (e.g., isoleucine, valine, histamine), potassium, hydrogen peroxide, and the neurotransmitters DOPA and GABA, which mimic the effects of natural compounds such as those found in red algae (Boettcher and Targett, 1998; Swanson et al., 2004). Now that I have shown that pueruli respond strongly to macroalgal cues, tests of their response to a gradient of declining metabolite concentration indicative of onshore-offshore dilution are a logical next step.

Salinity Detection

In addition to discerning among chemically distinct water masses, pueruli were also adept at discriminating salinity and avoided hyper – and hypo-saline conditions (e.g., 50 and 25, respectively) that are lethal. These findings corroborate work conducted by Field and Butler (1994) who reported that combinations of extreme salinity and temperatures (particularly high temperatures and salinity) reduced survivorship and recruitment in *P. argus* pueruli in both laboratory and field studies. Similarly, the settlement of clawed lobsters (*Homarus americanus*) is influenced by gradients in temperature and salinity, as has been documented in the field (Annis, 2005) and the laboratory (Boudreau et al., 1992). Salinity stress has been shown to affect a cadre of biotic metrics in marine larvae (e.g., growth, feeding efficiency, metamorphic competence) and coupled with the effects of sub-optimal temperatures has also been implicated in retarding growth, metamorphic delay, and decreased survivorship (Field and Butler, 1994; Richmond and Woodin, 1996; Pechenik et al., 1998; Tindle et al., 2004).

Response to Hydrostatic Pressure

My results also indicate that pueruli swim upward when exposed to increases in pressure. Conversely, decreases in pressure triggered pueruli to swim down to the bottom albeit at different rates contingent on the type of settlement structure available. In all cases, pueruli remained closer to the bottom when *Laurencia* spp. was present, as compared to tests with either artificial structure or no structure. Negative barokinesis has been demonstrated in several marine larvae (Forward, 1990), but this is the first report of barokinesis in the postlarvae of any species of spiny lobster. Although there are anecdotal reports of newly settled pueruli and juveniles found (often on artificial structures) at depths up to 30 m, these observations are generally thought to be instances of 'temporary settlement' where emigration from these locations is imminent or mortality likely (Jeffs et al., 2005). The extended cross-shelf journey traversed by pueruli coupled with the paucity of pueruli in daytime plankton tows suggests that pueruli are involved in diurnal periods of sinking (daytime) and swimming (nighttime), which might also place them at midwater depths or in temporary bottom refugia as they move shoreward. Yet, with a few exceptions (e.g., *Palinurus elephas* in the Mediterranean), most palinurid pueruli settle in shallow water (< 5 m; Butler et al., 2006). However, pressure alone is not the sole trigger for settlement of *P. argus*. My results and those of earlier investigators (Herrnkind and Butler, 1986) suggest that at least three mechanisms promote settlement of *P.argus* pueruli in shallow coastal areas: chemotaxis, barotaxis, and thigmotaxis. *Panulirus argus* pueruli are drawn to coastal habitats by the odors produced by red macroalgae. Upon arrival on the shelf, pueruli respond to incremental changes in pressure and move toward the bottom at pressures that correspond to depths of < 5 m. Finally, the presence of a physically (and perhaps chemically) appropriate substrate promotes bottom seeking, exploratory behavior and presumably settlement.

Sensory Structures and Mechanisms of Detection

A host of sensory structures, mostly on the antennae, have been implicated as important receptors of chemical and vibrational stimuli in the pueruli of several species of palinurid lobsters (Phillips and Penrose, 1985; Phillips and Macmillan, 1987; Macmillan et al., 1992; Jeffs et al., 1997). Several studies have detailed the olfactory system of *P. argus* juveniles and adults, which is centered in the lateral antennules that are furrowed with rows of aesthetascs (chemo-sensing hairs). These structures are capable of detecting a variety of complex chemical odors (Livermore et al., 1997), including those produced by prey, predators, and conspecifics (Childress and Herrnkind, 1997; Ratchford and Eggleston, 1998; Butler et al., 1999; Nevitt et al., 2000; Horner et al., 2006) – even diseased conspecifics (Behringer et al., 2006). It is also postulated that the arrangement of pinnate sensory setae on the antennae of pueruli play a role in vibrational detection (MacMillan et al., 1992; Jeffs et al., 1997) although they may also function a dual role as chemosensory detection organs. In contrast, it is not clear which structures in pueruli operate in salinity discrimination, although *P. argus* larvae (Scarratt and Raine, 1967) and postlarvae (Field and Butler 1994, this study) clearly avoid salinities much different from 35. The antennae of the spiny lobster *Panulirus japonicus* as reported by Tazaki (1975) contain mechanoreceptors that function as osmoreceptors. Davenport and Wankowski (1973) found that the walking legs of the porcelain crab (*Porcellana platycheles*) were used in detection of changes in salinity, whereas

Dufort et al. (2001) reported that primary receptors within or near the branchial chambers of adult North American lobster (*Homarus americanus*) could delineate salinity reductions to within ± 1. Although these examples provide evidence that the ability to detect different salinities is widespread among lobsters and crabs and probably operates via receptors on the legs and antennae, the mechanistic details are poorly understood.

Structures that might aid in barokinesis of *P. argus* pueruli have not been described. However, statocysts in other taxa appear to operate in the detection of hydrostatic pressure (Fraser, 2001) and have been described in pueruli of *Jasus edwardsii* (Sekiguchi and Terazawa, 1997). The lipid stores that pueruli rely on to fuel their swimming may also serve as rudimentary buoyancy devices (i.e., lipid vacuoles) that may aid in their depth regulation and detection. It is possible that lipid vacuoles mechanically compress and expand in response to differential pressures and as such may elicit sufficient cues for barotaxis, as has been suggested for the planulae larvae of the coral *Porites astreoides* (Stake and Sammarco, 2003). If so, then as pueruli metabolize and deplete lipid reserves as they move into shallower waters, their depth regulation would become less sensitive as the time to settlement looms. Thus, chemosensory and tactile cues become more significant at shallower depths where nursery habitat is present and pressure cues less reliable.

Enhancement of Onshore Transport

Lagoons and bays produce chemical fingerprints characteristic of their ecological constituents (Montgomery et al., 2001; Atema et al., 2002; Kingsford et al., 2002). Thus, the presence and influence of a large, heavily vegetated coastal shelf off the southern tip of the Florida (including Florida Bay and the Florida Keys) creates a scenario that presumably provides a potent chemical plume offshore by which pueruli can orient. Studies of coastal hydrodynamics in the Florida Keys indicates that the net tidal flow of water moves north to south from the Gulf of Mexico through the bays and lagoons surrounding the Florida Keys and out over the reef tract to the Straits of Florida (Smith, 1998 and 2002). Although the oscillation of tides, incursions of oceanic water, coastal countercurrents, local gyres and eddies, and significant wind-driven currents complicate this flow, and presumably the coastal odor plumes it contains, the net flow is offshore (Pitts, 1994; Lee and Smith, 2002; Smith, 1994, 1998 and 2002) and no doubt sets up a large-scale "coastal odor" gradient through which fast swimming larvae and postlarvae can navigate. "Chemo-sensing" by marine animals at small scales may be enhanced by eddychemotaxis (Atema, 1996) or via sampling of fine-scale eddy structure in coastal waters. Indeed, preliminary results of laboratory studies testing puerulus choice of seawater from varying distances offshore of the Florida Keys suggest that pueruli are attracted to coastal cues out to distances of at least 20 km (M. Butler, Old Dominion University, unpublilshed data). There is no reason to suspect that the oceanographic conditions or coastal odors present in the Florida Keys differ substantially from those elsewhere in the Caribbean where a multitude of red macroalgae (5,000+ species) persist species of red algae are prevelant, a region throughout which pueruli settle. Therefore, the physical conditions appear right and the available data supportive of the hypothesis that the transport of pueruli to coastal areas is enhanced by their detection and directed swimming in response to coastal chemical cues.

Implications of Coastal Degradation on Postlarval Settlement

Determining how natural chemical cues influence the settlement of marine species with planktonic larvae is important for understanding the recruitment process and its limits. Given the current state of the world's seas, it is also important to examine whether anthropogenic changes in coastal water quality, nursery habitat structure, and density of conspecifics adversely affect recruitment processes (Worm, et al., 2006). The Florida Keys are a crucible for a range of planned and unplanned ecological perturbations that may negatively affect the settlement dynamics of spiny lobster, which support the most valuable fishery in the state (Muller et al., 1997; Hunt, 2000). For example the impacts of dissolved organics, pesticides, and herbicides have not fully been investigated for the nearshore waters of the Florida Keys (Glazer and Quintero, 1998; Boyer et al., 1999), nor have the potential impacts of sewage discharge (both residential and commercial) all of which may adversely effect the early-life stages of aquatic invertebrates (Szmant and Forrester, 1996). Anecdotal evidence suggests that pueruli are very sensitive to pesticides, but their response to these or any other pollutants have not been quantified despite their growing threat in Caribbean coastal nurseries where tourism and coastal development are growing. Wastewater and agricultural runoff from land-based sources constitute a major source of pollution (e.g., organic nitrogen, ammonia, nitrate, nitrite, phosphorus) into Florida Bay (Lapointe and Clark, 1992) and have been implicated in massive seagrass die-offs, chronic algal blooms, and wide-spread sponge mortality events (Butler et al., 1995). Poor and degrading nearshore water quality is suspected to have led to decreased abundance of conch larvae, which are an order of magnitude lower compared with offshore populations (Stoner et al., 1996), although local variability in current patterns and post-settlement predation may also play a role (Glazer and Quintero, 1998). Herrnkind et al. (1988) documented clear differences in *P. argus* pueruli settlement patterns in heavily silted versus unsilted patches of *Laurrencia* spp. More recently, Peachey (2005) reports a positive correlation between larval mortality and the synergistic effects of polycyclic aromatic hydrocarbon pollutants (PAHs) and UV radiation on four species of larval marine crabs (spider, stone, mud, and blue crabs) found in estuaries and coastal waters. What these and other studies suggest is that coastal invertebrates with complex larval life-histories are vulnerable to a host of anthropogenically-influenced factors that have not been fully realized.

Looming in the next few years is perhaps one of the largest ecological perturbations yet to impact Florida Bay and portions of the Florida Keys, that being the "downstream" consequences of the massive effort to restore the historical flow of freshwater in the Everglades. It is projected that vast quantities of freshwater that had historically been diverted away from Florida Bay for storm water management and agricultural and urban uses, will soon be diverted back into Florida Bay resulting in potentially massive changes in salinity and water quality. Estimates of the impact on spiny lobster recruitment, derived from preliminary modeling studies, suggest that local decimation of spiny lobster nursery habitat and lobsters is likely with a projection of a 5 – 10% loss of recruitment potential for the entire south Florida region (Butler, 2003 and 2005). Much of the projected direct effect of salinity change on *P. argus* is via mortality of pueruli and juveniles, because adults and large juveniles emigrate to avoid low salinity, which does not result in their immediate mortality. These simulations do not consider avoidance by pueruli of low salinity areas, as my results suggest is possible.

CONCLUSIONS

In conclusion, the results of this thesis provide the first demonstration of chemically- and physically-mediated orientation in spiny lobster postlarvae. First, I demonstrated that the pueruli of *P. argus* discern and are attracted to coastal water masses, particularly odors associated with the red macroalgae *Laurencia* spp., a preferred settlement habitat. Chemicals released from *Laurencia* also accelerate the time-to-metamorphosis of pueruli to the early benthic juvenile stage. I also showed that pueruli avoid seawater whose salinity differs markedly (low or high) from oceanic salinities near 35, confirming the results of earlier studies indicating the lethality of such salinities. Finally, I discovered that pueruli respond to increases and decreases in hydrostatic pressure and habitat structure in ways that would facilitate their selection of shallow, structurally complex nursery habitats.

These chemosensory cues, perhaps in conjunction with others, represent a suite of stimuli that influence the orientation and selection of nursery habitat by postlarval *P. argus*. In a broader sense, these results confirm the significance of behavior in the recruitment of larval and postlarval spiny lobsters and, when coupled with physical parameters such as water quality and oceanographic hydrodynamics, will be integral in determining population connectivity and the local impediments to successful recruitment of this species throughout the Caribbean.

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APPENDIX A

VITAL STAINING SURVIVORSHIP STUDY

Rationale

Natural biological dyes (i.e, vital stains) are complex organic compounds commonly used for safely staining living biological components and are capable of penetrating cell membranes of living cells or organisms due to their lipophilic properties (Takahashi et al., 1994; Kiernan, 2002;). The objective of this preliminary study was to evaluate the efficacy of vital staining of pueruli for purposes of night-time observations under infrared light, as well as for subsequent experiments in keeping sources of animals separate.

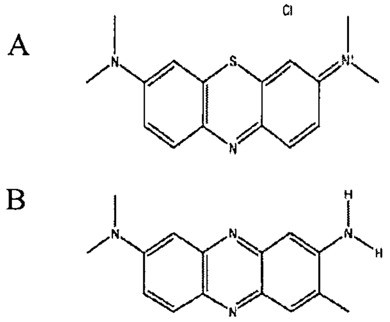

Fig. 8. Molecular composition of biological dyes used for puerulus vital stain survivorship study. (A) methylene blue (B) neutral red (from Kiernan, 2002 with permission).

More specifically, I wanted to confirm that vital staining had no deleterious effects on the pigmentation and metamorphosis process from puerulus to early benthic juvenile as compared to unstained animals.

Study Design

For this study, two commercial and biologically certified stains, methylene blue ($C_{16}H_{18}ClN_3S$, M291-25, Fisher Scientific, USA) and neutral red ($C_{15}H_{17}ClN_4$, N128-25, Fisher Scientific, USA) were chosen based on their history as traditional and reliable crustacean stains (Kiernan, 2002) (Fig. 8). A total of 40 transparent pueruli collected at night on rising tides from plankton net tows (1 m diameter, 2.5 m long, 750 m mesh size) at Tom's Harbor Channel Bridge (between Grassy Key and Duck Key, Florida) were immediately transported via a cooler to the Keys Marine Laboratory (Long Key, Florida) and retained overnight in the same water from which they were collected. The following day, pueruli were randomly assigned to 400 ml plastic beakers (1 puerulus / beaker) and maintained in artificial seawater (Instant Ocean®, Aquarium Systems, Ohio, USA: salinity = 35, pH = 7.5-8.0) using dechlorinated (NovAqua, Kordon, Inc.) freshwater as a base solution. Each beaker also received a 9 cm^2 knotted ball of window screening material to mimic vegetative settlement habitat and refuge. All beakers were held in a temperature-controlled water bath using a drop-in titanium coil chiller (model AE4D, Aquatic Ecosystems, Apopka, Florida, USA) maintained at $25 \pm 1^{0}C$ and subjected to ambient summertime lighting conditions. Approximately 1 g of each dye powder was added to a clean, 1 l glass beaker and homogenized thoroughly with ≈ 850 ml of artificial seawater.

Twenty pueruli were then added to each dye solution for a total of 10 minutes after which, the solution was diluted by 50% and animals were retained for an additional 10 minutes. Pueruli were returned to their individual beakers and their survivorship through to metamorphosis was monitored daily over the course of 10-12 days. Complete water changes on each beaker were conducted daily with a stock solution of artificial seawater. Qualitative observations were also made for color retention by each dye and its magnitude.

Results and Conclusions

Statistical analyses were not conducted due to the clear patterns of survival to metamorphosis of all animals from both treatments within the 10-12 day period at 24-25^{0}C.Animals in the methylene blue treatment all lost their artificial iridescent blue pigmentation after just three days, while neutral red was retained in all pueruli through metamorphosis. Neutral red remained concentrated in the dorsal section of the animal, presumably staining the highly lipid-rich hepatopancreas, (Jeffs et al., 1999; Takahashi et al., 1994; Nishida et al., 1995). Thus, I chose to use neutral red as a marker for individuals used in two studies (i.e., puerulus source study below and response to pressure studies described in thesis text) because of its efficacy in providing a non-invasive but effective method for puerulus tagging.

APPENDIX B

PUERULI SOURCE STUDY

Rationale

Spiny lobster pueruli in the Florida Keys can ordinarily be obtained from two sources: (1) plankton nets suspended from tidal channel bridges on or around new moon periods (Acosta and Butler, 1999) (Table 6) and (2) from floating artificial collectors (Witham et al., 1968; Butler and Herrnkind, 1992; Cox et. al., 1997) set around coastal waters and checked by boat on or around the new moon lunar period. It is possible, however, that animals obtained from collectors are not behaviorally equivalent to bridge-caught (planktonic) pueruli with respect to their propensity for settlement. (i.e., larval competency level may differ between these two groups) (Pechenik, 1990; Rodriguez et al., 1993; Moksnes and Wennhage, 2001). Additionally, as 'temporary settlers', collector- caught pueruli may undergo subsequent biochemical changes (e.g., lipid allocation and catabolism) (Jeffs et al., 1999; Phillips et al., 2006) associated with the attachment to a substrate versus a wholely planktonic, bridge-caught puerulus that would not necessarily have been in physical contact with settlement structure.

Table 6

Bridge-tow data (raw) from pueruli collected at Tom’s Harbor Bridge (80^0 55.25’ N; 24^0 46.75’ W) between 2002-2003 in the Florida Keys, USA. At each trial 1-2 nets (1 m diameter, 2.5 m long, 750 μm mesh size) were suspended from the bridge and allowed a soak time of ≈ 20 min. All data based on an average of ≈ 6 tows per night for 3 - 5 nights per lunar month (for standardizing CPUE). Highlighted dates in bold indicate new moon date

* Tow # 6 represents the traditional 2 hour sampling time cut-off (from Butler and Herrnkind, pers. comm.)

** Assuming a mean volume of water sampled @ Tom's Harbour = 377m^3 (calculated from Butler and Hunt, unpub. data)

MOON DATE	TOW NUMBER								PUERULUS DATA			
	1	2	3	4	5	6*	7	8	PLs Caught	# Nets Fished	Mean # PLs / Tow	Mean # PLs / m^3 Water**
7/09/02	0	1	1	2	0	0			4	1	0.67	0.0018
7/10/02	3	7	12	6	14	8			50	2	8.33	0.0221
7/11/02	0	3	2	11	4	0			20	2	4.00	0.0106
8/08/02	0	1	2	3	0	2			8	2	1.33	0.0035
8/09/02	11	14	17	14	13	9			78	2	13.00	0.0345
8/10/02	5	14	11	21	9	10			70	2	11.67	0.0309
9/10/02	0	6	15	0	4	3			28	1	4.67	0.0124
9/12/02	0	0	2	0	0				2	2	0.40	0.0011
9/13/02	4	0	2	6	0	0			12	2	2.00	0.0053
10/06/02	0	0	0	0	0	0			0	2	0.00	0.0000
10/07/02	0	3	2	2	0	0			7	1	1.17	0.0031
10/08/02	0	0	1	0	0	0			1	2	0.20	0.0005
10/09/02	0	0	3	1	0	0			4	2	0.80	0.0021
10/10/02	0	1	0	0	0	0			1	2	0.17	0.0004
10/11/02	0	0	4	5	3	3			15	2	2.50	0.0066
11/04/02	18	13	8	5					44	2	11.00	0.0292
11/06/02	0	0	0	0					0	2	0.00	0.0000
11/07/02	7	13	11	1	0				32	1	1.00	0.0027
12/04/02	5	3	1	2	2	0			14	2	2.00	0.0053
12/05/02	0	0	2	10	14	7			33	2	3.00	0.0080
12/06/02	6	20	11	2	0				39	2	4.00	0.0106
01/02/03	1	4	0	0	1	0			6	2	5.00	0.0133
01/03/03	5	16	13	8	12				50	1	6.00	0.0159
01/04/03	5	6	4	3	0				18	2	7.00	0.0186
02/01/03	0	0	0	1	1	0			2	1	0.40	0.0011
02/02/03	0	3	5	2	7	9	6		32	2	4.57	0.0121
02/03/03	0	1	3	2	2	0			8	2	1.33	0.0035
03/03/03	0	0	0	1	2	1	1		5	2	0.71	0.0019
03/04/03	0	0	0	0	1	0			1	2	0.17	0.0004
03/05/03	0	0	1	1	1	2			5	2	0.83	0.0022
03/06/03	0	0	1	2	1	1			5	2	0.83	0.0022
03/07/03	1	1	1	0	2	1	1		7	2	1.00	0.0027
04/01/03	4	12	6	42	7				71	2	14.20	0.0377
05/01/03	1	1	1	1	17	2	20	2	45	2	6.14	0.0163
05/02/03	3	3	29	0					35	2	8.75	0.0232
05/31/03	0	0	0	0	0	3	4		7	2	1.00	0.0027
									569 **TOTAL**		**3.61** **AVG**	**0.0096** **AVG**

Thus, the objective of this preliminary study was to determine if spiny lobster pueruli collected from two different sources (bridge-caught vs. collector-caught) exhibited similar responses to 'good' vs. 'poor' habitat and make similar behavioral choices in vacating an inappropriate habitat. Specifically, my hypothesis tested the following:

H_0: artificial collector-caught lobster pueruli are not behaviorally different (significantly) from bridge-caught pueruli with respect to vacating adverse environmental conditions.

H_A: artificial collector-caught lobster pueruli are behaviorally different (significantly) from bridge-caught pueruli with respect to vacating adverse environmental conditions.

Study Design

For this study, four large, round polyethelene tank mesocosms (155 cm diameter x 91 cm depth, 850 l / ea.) were used (Fig. 9).and two macroalgal units (MAUs, plastic mesh cages, 20 cm x 8 cm x 8 cm / ea., filled with fresh *Laurencia* spp.) were placed in each tank (2 cages / tank) and exposed to a different suite of physical factors. Within each tank (replicate) one MAU (experimental unit) was left as is (i.e. favorable or good settlement conditions) while the other MAU was subjected to a freshwater plume and added siltation (poor settlement conditions), both adversive factors to pueruli settlement and retention (Herrnkind et. al., 1988; Field and Butler, 1994).

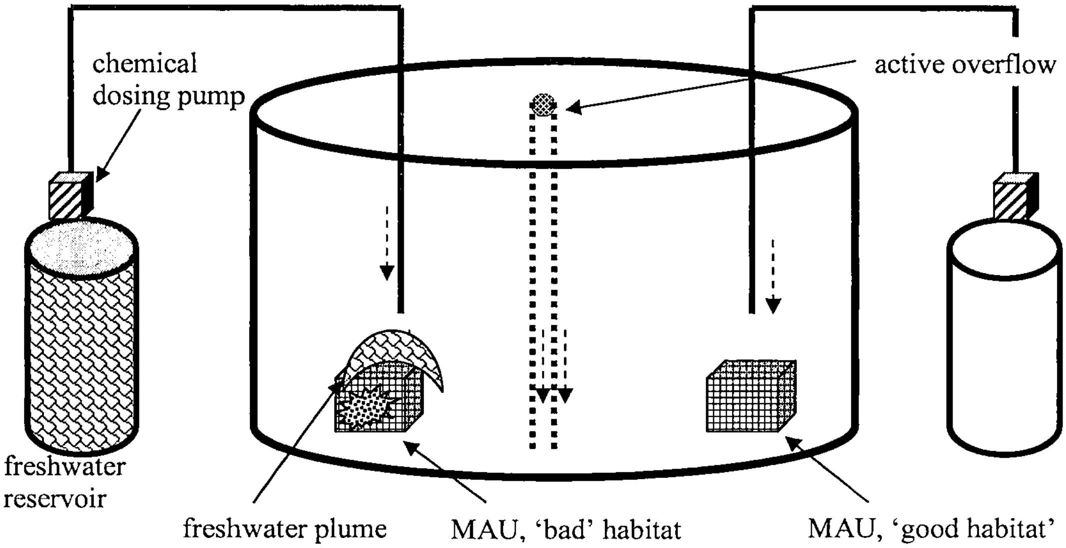

Fig. 9. Tank mesocosm setup for pueruli source experiment. Pueruli placed in 'bad' macroalgal units (MAUs) were subjected to a plume of freshwater via a dosing pump as well as silt-ladden *Laurencia* spp. tufts; 'Good' MAU cages contained fresh *Laurencia* spp. and a pulsing of ambient seawater as a control.

Control trials consisting of two unadulterated MAUs in each tank were also tested. *Laurencia* spp. was collected as needed by snorkeling or SCUBA in Florida Bay (Long Key area). Two chemical dosing pumps (model MP61, Aquatic Ecosystems, Apopka, Florida, USA) delivered timed pulses of either dechlorinated (NovAqua, Kordel, Inc.) freshwater (26-28^{0}C) or ambient tank seawater (salinity = 32-35, 26-28^{0}C) into each MAU at a rate of $\approx$ 7.5 l / hr from an external source tank (Fig. 9). Dosing pumps were used for both MAU treatments to guard against artifacts associated with the pumping and dosing action into each cage. In addition to freshwater pulses, a combination of fine silt and course gravel were sprinkled on the poor habitat MAUs as an additional source for poor habitat quality (Herrnkind et al., 1988). Bridge (n = 16, from Tom's Harbor Bridge Channel, see materials and methods section for full details) and collector-caught (n = 16, from floating surface collectors off of Long Key, Florida, see Witham et al., 1968 and Cox et. al., 1997 for design details) pueruli were added together for each tank trial equally among good and poor habitats. Identification of each respected lobster source was accomplished by applying neutral red biological staining agent (see vital stain survivorship study above) to one group at random (collector or bridge) and then alternating groups throughout trials.

Pueruli (two from each source / basket x two baskets / tank, n_{total}= 4) were introduced into a MAU via a clear segment of 2.5 cm diameter x 15 cm long PVC tubing inserted into the cage so that animals could acclimate and not tailflip away. After five minutes, the tube was slowly lifted out of the cage and pueruli were unrestrained with respect to their ability to emigrate or remain within each cage. Each trial was conducted over a 12 hr period at dusk and during new lunar periods (the active movement and swimming phase for these animals) (Acosta and Butler, 1999) and repeated over four non-consecutive nights for a total of 32 pueruli tested. Tanks were maintained minimally as flow-thru and excess water was allowed to exit out of the tank through a fine mesh overflow tube as to not significantly alter water quality or salinity with respect to treatments being applied. At the end of each trial, each MAU was carefully surrounded with a fine mesh bag and pulled out of its tank. MAUs were thoroughly examined and scored for the number of lobsters that were present. Mesocosm tanks were then examined visually for animals not found in either MAU.

This study was designed using a 2-factor split-plot ANOVA (SPSS v.14.0, Chicago, Illinois) with the following parameters: factor a = puerulus type (2 levels: bridge-caught and collector-caught), factor b = habitat type (2 levels: good and poor), whole-plot factor = bridge-caught, subplot factor = habitat type, dependent variable = movement out of or residency in each cage treatment, treated as a proportion. As a consequence, raw data were arcsin transformed to adequately meet parametric statistical assumptions (Zar, 2006).

Results and Conclusions

There were no significant difference in the propensity of pueruli from either source (collector or bridge-caught) to vacate good habitat or remain in poor ones (split plot ANOVA, $F = 14.73$; $df = 1,30$; $MS_E = 41.60$; $p = 0.738$; $\alpha = 0.05$; $1-\beta = 0.57$). In only two cases did pueruli remain in poor MAUs, and those were found dead. In several other runs however, some pueruli were in neither MAU and instead on the bare tank bottom or sometimes wedged under the lip of the tank's standpipe. It is not known in these cases if animals were attempting to vacate MAUs and were in an exploratory phase or if pueruli actually were emigrating from this habitat as has been documented in at least three other larval decapod species (shore crab, *Carcinus maenas*; brown shrimp, *Crangon crangon*; grass shrimps, *Paleamon* spp.) (Moksnes and Wennhage, 2001; Moksnes et al., 2003).

Based on these results, *P. argus* pueruli from either artificial floating collectors or bridge-caught (wholely planktonic) nets are equivalent with respect to their use in all subsequent studies described herein. As a caveat it should be stated that using collector-caught pueruli that can be discerned qualitatively as completely transparent is also essential (Stage 1A). This has been the current field sampling technique used for over a decade by research biologists in the Florida Keys during their monthly pueruli assessments of surface collectors whereby 'clear' or fully transparent animals are categorized separately from 'cloudy' (i.e., visible lipid concentrations in hepatopancreas) (see Fig. 1 in Jeffs et al., 1999) or pigmented (Phillips et al., 2005). Lewis et al. (1952) (for *P. argus)* and Matsuda et al. (2001) (for a congener, *P. japonicus)* document such descriptors more formally noting distinct stages within the puerulus phase are characterized (among other things) by changes in eye color, antennual markings, and overall patterns in pigmentation. Two other studies carried out in this thesis (choice chamber and hydrostatic pressure studies) statistically considered differences among these two puerulus sources; neither study found any significant differences (see methods section). Only in the time-to-pigmentation (metamorphosis) study were bridge-caught pueruli solely used where cohorts of animals of known age were pivotal to the hypothesis addressed.

APPENDIX C

PUERULUS SIZE AND CONDITION FACTOR STUDY

Rationale

The pelagic and nektonic phase (puerulus) of spiny lobster (Palinuridae) development is characterized as a clear, non-feeding, fusiform-shaped, strong swimming lobster that, at an undetermined distance from shore is capable of seeking appropriate settlement habitat, primarily in shallow nearshore environments (Marx and Herrnkind, 1985a; Phillips and McWilliam, 1986; Acosta and Butler 1999; Jeffs et al., 1999 and 2005). The onshore arrival of these animals, using combinations of both active swimming and passive transport via cross-shelf mechanisms and other oceanographic elements, may originate from the continental shelf or beyond although no conclusive indication of this has been shown to date (Phillips and McWilliam, 1986; Jeffs et al., 2005). Convincingly strong morphological and biochemical evidence suggests that this life-history phase does not feed (Gilchrist, 1916; Lewis et al., 1952; Nishida et al., 1990; Lemmens, 1991 and 1994a; Jeffs et al., 1999) rather, pueruli are 'fueled' by lipid reserves that have been acquired and built up over the protracted larval (i.e. phyllosomal) period (Jeffs et al., 1999 and 2001). Extensive work by Jeffs et al. (2001) for example with the New Zealand rock lobster (*Jasus edwardsii*) showed that phospholipids comprised the majority of stored lipid stock (86-96 %) and were found at lower levels in pueruli arriving onshore (< 1 km) ($n_{total} = 25$) compared to animals taken 20 km ($n_{total} = 35$) at the edge of the continental shelf.

A similar pattern is conjectured to take place in *P. argus* pueruli in the Florida Keys although oceanographic elements of onshore passive transport are notably different and unique to the region (Yeung et al., 2001; Acosta et al., 1997; Smith, 2002; Acosta and Butler, 1999). Aside from visually assessing pueruli according to varying degrees of larval competency (i.e. transparent, semi-pigmented, fully pigmented) (Lewis et al., 1952; Phillips et al., 2005), lab techniques have been developed and substantiated for quantifying changes in lipid class composition or RNA:DNA and C:N:H ratios for comparing biochemical synthesis (i.e. nutritional condition or condition factor, CF) over spatial and temporal scales (Dawirs, 1980; Anger et al., 1989; Fraser, 1989; Lemmens, 1994b; Nishida et al., 1995; Jeffs et al., 1999; Robertson et al., 2000; Gwak et al., 2002) for a variety of larval marine fishes and crustaceans. Although these condition indicies are reliable in their ability to discern nutritional differences, the methods can be cumbersome and expensive.

In at least two other studies, lobster (*Homarus americanus*) (Tlusty et. al., 2005), and seahorses (*Hippocampus abdominalis*) (Woods, 2003) researchers adopted CFs to reliably assess nutritional condition (among other parameters) using more rudimentary techniques (e.g. larval dry weights and body metrics). CFs can provide an accurate and comparable proxy eliminating many of the methodological complexities that are involved in full-scale biochemical analyses. The Florida Marine Research Institute has been monitoring pueruli settlement indicies from floating collectors for > 15 years (J. Hunt, pers. comm.) comprising one of the largest continuous data sets of this kind with the exception of the Western rock lobster (*Panulirus cygnus)* (Phillips, 1995). In this small-scale but potentially valuable study, I sought to provide an additional method for assessing differences in puerulus condition from monthly sampling techniques in the Florida Keys using the dried weights and carapace lengths of subsamples of pueruli to calculate CFs. I also tested CFs in their ability to discriminate pueruli from pre-competent and competent stages.

Study Design

Pueruli were collected and processed in the Florida Keys from floating modified Witham surface collectors (Witham et al., 1968; Cox et al., 1997) located nearshore on the ocean side of Long Key (upper Keys, N 24^{0} 37.08 W 81^{0}23.66') and Big Pine Key (lower Keys, N 25^{0} 48.31'W 81^{0} 50.37') ($n = 5$-6 collectors per site) at 7 ± 1 days post new moon (i.e. first lunar quarter) each month over a two year sampling (2002-2004) period. A combination of fully transparent and initially pigmented (base of third antennae) animals were staged and sorted qualitatively for this study as described in Lewis et al. (1952). Upon capture, all pueruli were immediately preserved in histological tissue cassettes, soaked in 5% formalin and filtered seawater and archived-preserved 24 hours later in 70% ethanol. Significant effects of this chemical preservation method for zooplankton weights and measurements are well documented and do not seem to have deleterious effects (Giguere, 1989; Ouellet and Plante, 2004).

In the laboratory carapace lengths (CLs) were measured to the nearest 0.1 mm along the middorsal line from the anterior end between the postorbital spines and the posterior edge using a WILD M3Z (Heerbrugg, Switzerland) stereo dissecting scope at 16 x (Fig. 10). A supplemental group of pueruli that had been previously frozen (-20^{0}C) were also incorporated, and there were no significant discrepancies in measurements between larvae subjected to the two preservation methods ($F= 3.36$; $df = 1,24$; $p < 0.001$; $\alpha = 0.05$). Animals that had been measured were next dried in an oven (Fisher Isotemp 500 Series) at 35^{0}C for 24 hr, after which dry-weights (DWs) were obtained to the nearest 0.0001 mg. on a Mettler Toledo analytical balance (model AG204). Because many animals were prone to antennule breaks from field handling methods and preservation, both antennae were removed with a pair of fine dissecting scissors at the third basal segment prior to any drying or weighing. A relative condition factor (CF) was then calculated for each puerulus using a modified von Bertalanffy growth equation ($q = DW * CL^3$) similarly to

Tlusty et al., (2005) where q is the CF for any individual animal proportional to it's DW (mg) divided by the cube of its CL (mm).

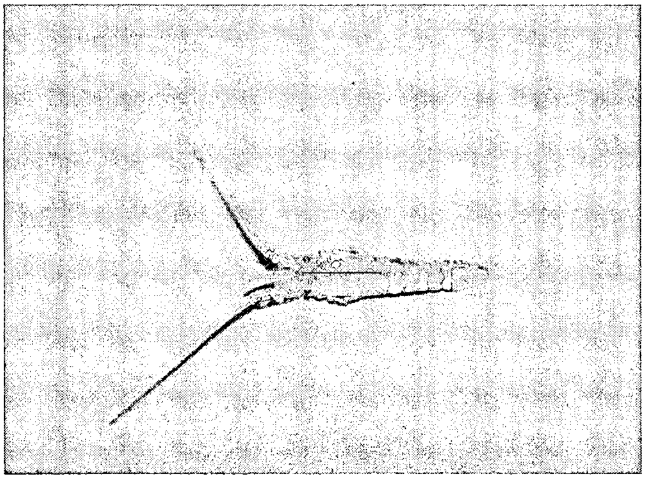

Fig. 10. Picture of carapace length (CL) (dark line) measurements for pueruli of *P. argus*. Also note the completely transparent body and lack of pigmentation except for black eyes confirmed as a Stage 1A puerulus according to staging methods by Lewis et al. (1952).

Results and Conclusions

A mean puerulus size of 6.24 ± 0.28 mm CL (n = 528) from Long Key and 6.32 ± 0.33 mm CL (n = 397) from Big Pine was calculated among all pueruli over the course of 24 months (n_{total}= 925) (Fig. 11). In one other recently published study (Yeung et al., 2001) in the Florida Keys (July 1997-June 1999) comparative pueruli measurements were made resulting in a mean CL of 6.06 ± 0.33 (n = 597) from Long Key and 6.01 ± 0.39 (n = 740) from Whale Harbor, both upper Keys locales. Although they were no significant, differences in size between the two Keys locations

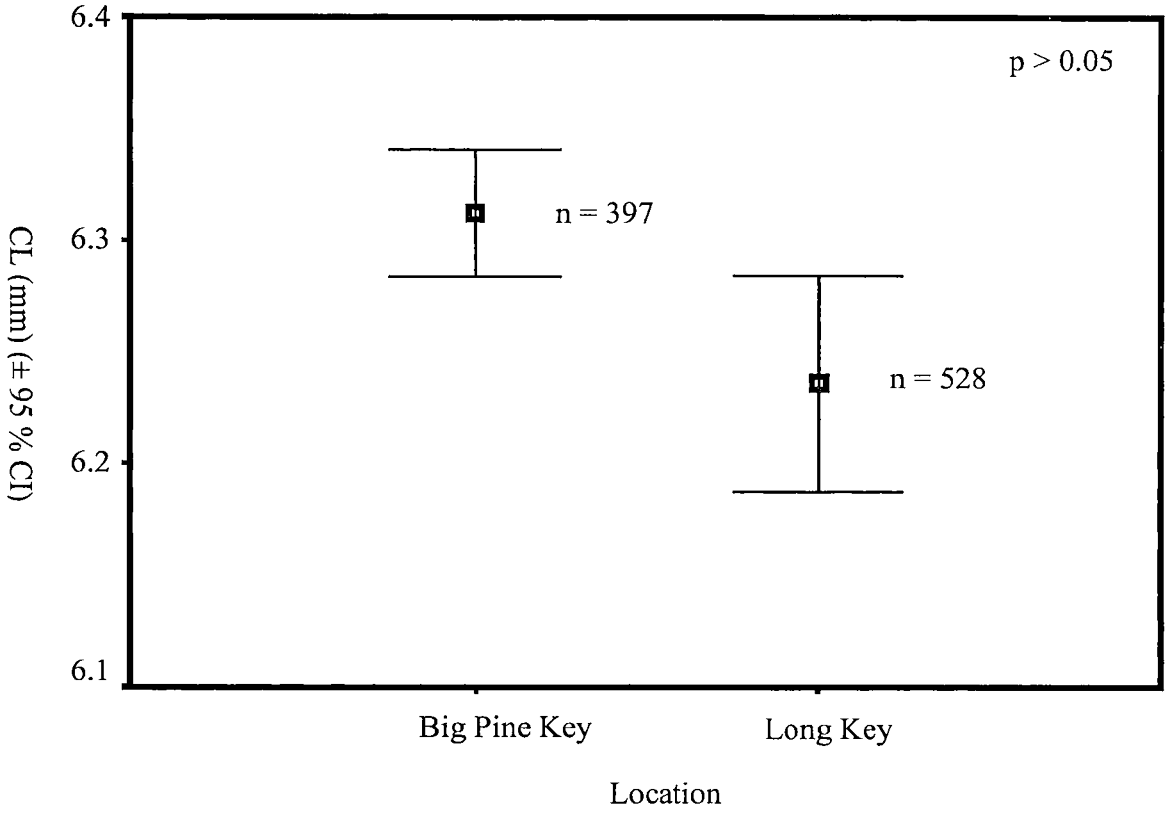

Fig. 11. Carapace lengths (CL) for *P. argus* pueruli collected at two Keys locations over 24 successive months between 2002-2004. Sites did not show significant differences in CL ($p > 0.05$, $\alpha = 0.05$).

($t = 12.06$; $df = 923$; $p > 0.05$; $\alpha = 0.05$), a trend in temporal differences was evident in CF analyses (Fig. 12). Cumulative CF measures from a total of 683 Stage 1A pueruli from 2002-2004 modeled from a Loess deterministic model showed a downward trend in CF during the summer months (range = 0.057-0.064), historically over the lowest pueruli recruitment pulses in the Keys. Conversely, an upward trend starting in September through to March yielded comparatively higher CFs (range = 0.064-0.090). A similar trend with respect to size (CL) was corroborated by Yeung et al. (2001) who reported that pueruli entering Florida Bay in winter were generally larger than those entering in summer. As a further confirmation that CFs are precise enough to discern these types of differences, CFs and DWs were compared between Stage 1A pueruli (completely transparent) and Stage 2B (initially pigmented) (Figs. 13 and 14).

Measured DWs for Stage 1A (mean = 18.68 mg, n = 600) and Stage 2B (mean = 16.12 mg, n = 325) differed significantly between these two groups (t = 8.99; df = 923; $p < 0.001$; $\alpha = 0.05$) as did CFs (0.735) and (0.678) for transparent and pigmented animals respectively (t = 16.55; df = 923; $p < 0.01$; $\alpha = 0.05$). These differences were presumably due to increased amounts of phospholipids in Stage 1A animals (Jeffs et al., 1999) that had recently arrived onshore and whose residency time on collectors was shorter. The results of this study show the efficacy in using CFs in addition to existing qualitative methods for detecting quantitative differences between pueruli of different stages and over temporal scales. It is suggested that spatial differences may also exist from disparate puerulus sources throughout the Caribbean and efforts should be coordinated more formally (Goldstein, 2006; Butler, unpublished data) to elucidate this.

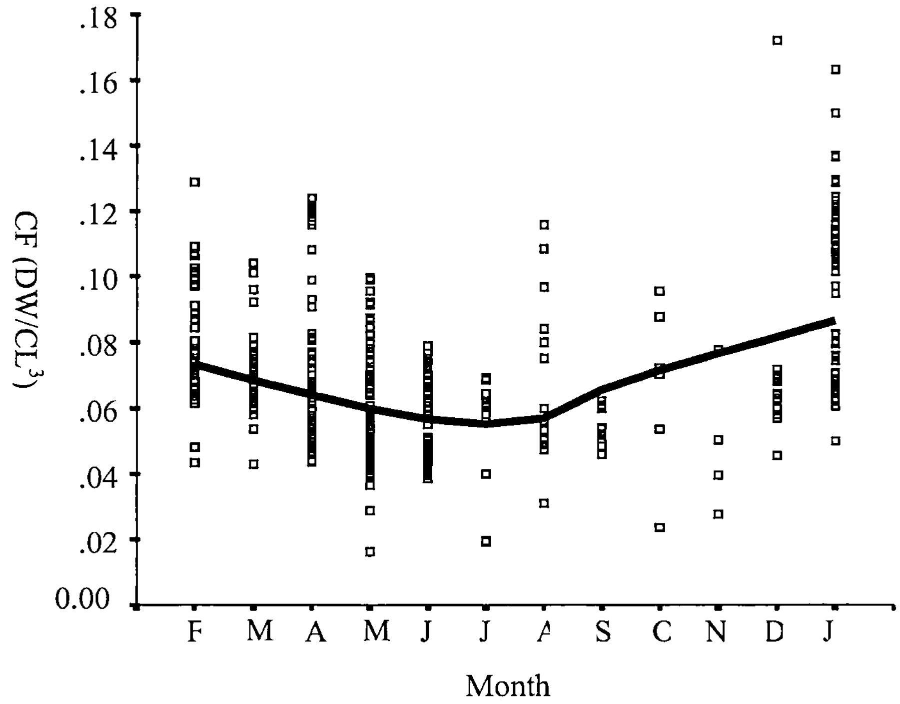

Fig. 12. Condition factor (CF) as a proxy for nutritional condition over time (2002-2004, duplicate months pooled) for pueruli (n = 683) of *P. argus* monitored and collected at two sites (Long and Big Pine Keys). Regression trend model constructed from the Loess deterministic function.

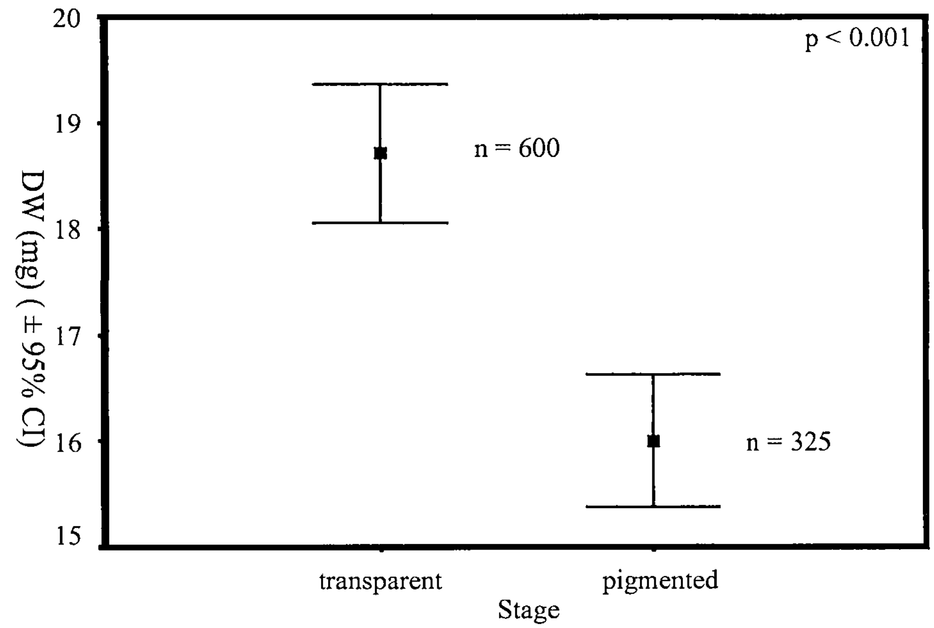

Fig. 13. A comparison of dry weights (mg) between pueruli pooled from two locations (Long and Big Pine Key) ($t = 8.99$; $df = 923$; $p < 0.001$; $\alpha = 0.05$) collected over 24 successive months between 2002-2004 from two distinct puerulus stages as described in Lewis et al. (1952).

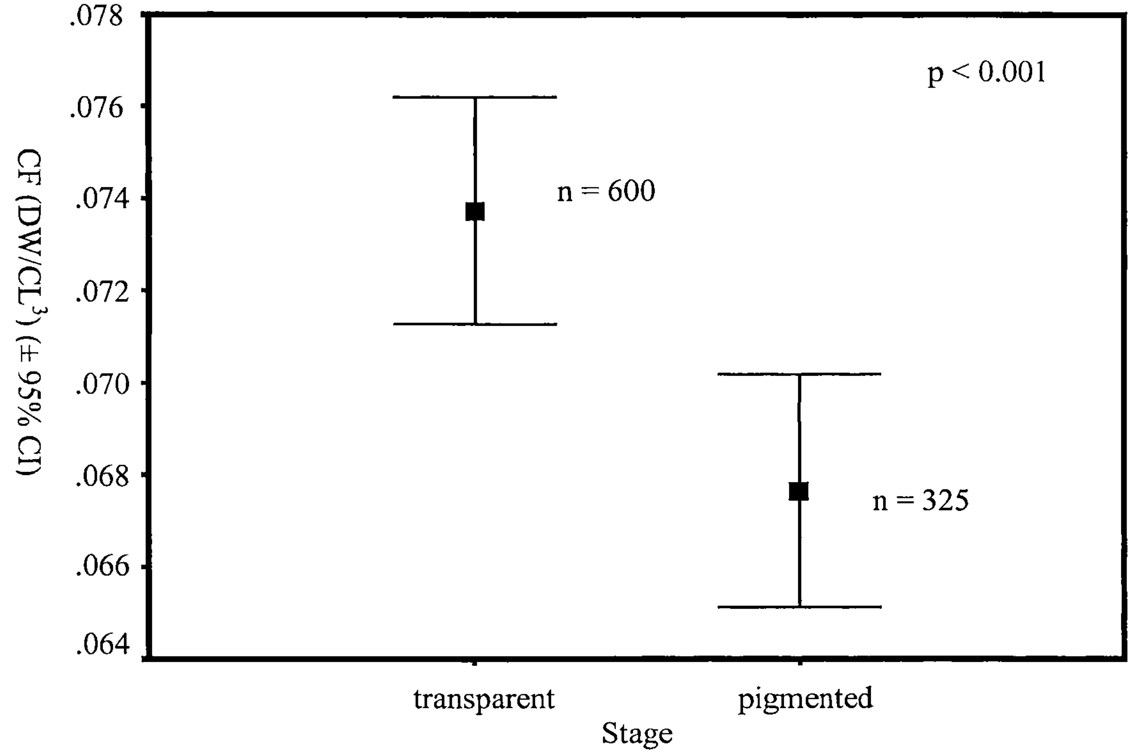

Fig. 14. A comparison of condition factor index (unitless) between pueruli pooled from two locations (Long and Big Pine Keys) (t_{STAT}= 16.55; df = 923; $p < 0.001$; $\alpha = 0.05$) collected over 24 successive months between 2002-2004 from two distinct puerulus stages as described in Lewis et al. (1952).

Oceanographic variability may influence CFs for pueruli over larger scales. It is interesting that pueruli size data differed from that of Yeung et al. (2001) and may be an artifact of changing oceanographic conditions (e.g,. changing currents or gyres) or perhaps reflective of climatic variability and oscillation. Yeung et al. (2001) and Acosta et al. (1997) both report evidence of mesoscale eddies and gyres that operate at varying distances from shore on an annual basis directing some of the onshore transport of *P. argus* pueruli into the Florida Bay system. In Western Australia, Pearce and Phillips (1988) and Caputi and Brown (1993) demonstrated positive correlations between El Nino Southern Oscillation (ENSO) and the strength of puerulus recruitment in conjunction with the Leeuwin Current for *Panulirus cygnus*. Booth (1989) documents onshore wind frequencies and the seasonal effect it has on the puerulus settlement index of *Jasus edwardsii* in New Zealand. The expansion of a more widely spread and methodologically connected Caribbean-wide study will help two-fold in understanding the mechanisms of environmental variation and its effects on both annual and seasonal puerulus settlement and recruitment strength across locations.

VITA

Jason Seth Goldstein was born in where he lived until age 12. In 1983 he moved to the Boston metropolitan area (Sharon, MA) with his family and remained there until graduating from Sharon High School in 1989. He started his undergraduate studies at the University of Massachusetts Dartmouth where he earned is B.S. in Biology in 1993. After taking an environmental internship job in Wyoming in the summer of 1993, he spent nearly seven years as a research assistant at the New England Aquarium and traveling around the world for research and holiday. During this time Jason was also awarded a graduate certificate in Museum Studies from Harvard University Extension School in 1999 having served as a curatorial intern in the marine invertebrate collections at the Harvard Museum of Comparative Zoology. Jason decided to pursue a Master's degree at Old Dominion University in 2001. He is currently enrolled as a PhD. Student at the University of New Hampshire, Department of Zoology, (Rudmam Hall, 46 College Road, Durham, NH 03824 USA) where he is continuing his studies in lobster biology and ecology.