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INVERTEBRATE ZOOID POLYMORPHISM:

HYDRACTINIA POLYCLINA AND *PAGURUS LONGICARPUS*

INTERACTIONS MEDIATED THROUGH SPIRALZOOIDS

BY

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B.S. Roger Williams University, 2008

THESIS

Submitted to the University of New England
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This thesis has been examined and approved.

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DEDICATION

To my friends, family, and husband. Thank you all for your countless hours of help over the last few years.

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ABSTRACT

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Evaluating the net interaction between symbionts can be challenging when one participant is a colonial animal with polymorphic zooids, because each zooid type has unique functions. The colonial hydroid *Hydractinia polyclina* has three distinct zooid types, each of which contributes particular components to the interaction with host hermit crabs. Of these three zooid types, the function of spiralzooids is not well understood. Previously, spiralzooids have been proposed to contribute a strong negative interaction component by directly reducing their host hermit crab's reproductive output. However, this hypothesis is not supported by past or current data. I propose that spiralzooids instead function to prevent hosts from foraging on the colonies on their own shells.

I conducted a series of experiments and surveys that explored spiralzooid distribution, structure, and function. Spiralzooid distribution at the species level was quantified through an examination of the scientific literature, which documented that spiralzooids only form in colonies living on hermit crab occupied gastropod shells, regardless of crab species or geographic region. Within the local species *H. polyclina*, only colonies that were living on gastropod shells occupied by hermit crabs contained spiralzooids, regardless of collection site or colony gender.

Next, I assessed spiralzooid structure. Spiralzooids had a mean length of 1.17 ± 0.62mm and occurred at a mean density of 1.91 ± 1.03 per mm^2 along the aperture edge. Spiralzooids contain microbasic eurytele nematocysts, organized into nematocyst batteries, and battery abundance at zooid tips can be categorized into four levels. These nematocyst batteries adhered to hermit crab bodies and appendages, and adherence did not vary significantly among body parts.

Spiralzooid function was studied through several experiments. Increased hermit crab contact and the presence of a host stimulated spiralzooid formation throughout a shell, in parts of the colony that are normally devoid of spiralzooids. Although hermit crabs are active scavengers, those that are symbiotic with *H. polyclina* have never been documented consuming their own epibiont colonies. Yet, hermit crabs are commonly observed feeding on polyps in colonies on other shells. If spiralzooids prevent crabs from foraging on the colonies on their own shells, then crabs should respond to contact with spiralzooids. The act of spiralzooid lashing, as tested by probing hydroid covered shells (with a bare shell control), significantly altered hermit crab behavior. Six crab behaviors were in turn analyzed to determine their effects on initiating spiralzooid lashing. Foraging on *H. polyclina* stimulating spiralzooid lashing significantly more frequently than other hermit crab behaviors.

Lastly, hermit crab prey caloric values compiled from the literature were compared to the empirically determined caloric value for *H. polyclina* (4,011.55 ± 65.47 cal/g dry wt); hydroids ranked in the top 10% of potential hermit crab prey. In light of

these findings I suggest the data support my guiding hypothesis that spiralzooids prevent hosts from foraging on their own colonies. Under this proposed function, I suggest that gonozooids actually contribute a weak positive interaction with the host crab by potentially providing caloric value, and spiralzooids in turn contribute a weak negative interaction. Therefore, the resulting net interaction between *H. polyclina* and *Pagurus longicarpus* should be considered commensal, or weakly mutualistic.

INTRODUCTION

Hermit crab occupied gastropod shells are often utilized as attachment substrates for sessile invertebrates in marine communities, particularly in subtidal and some intertidal zones where competition for vacant hard substrate is high (McLean 1983, McDermott 2001, Reiss *et al.* 2003). Worldwide, approximately 200 species of encrusting epibionts representing four phyla live symbiotically with roughly 180 species of hermit crabs (Williams and McDermott 2004). Interactions between epibionts and hermit crabs include positive and negative components that can sum to a net parasitic, mutualistic, or commensal interaction. However, evaluating net interactions is difficult for epibionts with polymorphic zooids, because the multiple zooid types have unique morphologies and behaviors, each of which contributes a potentially different component to the interaction (Buss and Yund 1989, Harvell 1991, Taylor 1994, Dudgeon and Buss 1996, Harvell 1998, Seipp *et al.* 2007). Polymorphic colonies, such as bryozoans and several species of hydrozoans, generally have three distinct zooid types: feeding, reproductive, and defensive (Conover 1978, Harvell 1998, Damiani 2003). Each zooid type contributes unique positive and/or negative components to the interaction between the colony and the host (Dudgeon and Buss 1996, Peach and Hoegh-Guldberg 1999, Seipp *et al.* 2007).

Feeding zooids, also known as gastrozooids, consist of a mouth surrounded by tentacles in hydrozoans, or by a lophophore in bryozoans (Mills 1976, Conover 1978, Buss and Yund 1989, Harvell 1998). This zooid type is found in all epibiont colonial invertebrates and can participate in both positive and negative interactions with the host

(Conover 1978, Brooks and Mariscal 1985, Damiani 2003). Gastrozooids indirectly interact positively with their hosts by consuming other epibionts, both adults and larvae, which would ultimately add weight and can degrade gastropod shells over time (Conover 1978). However, gastrozooids are not selective feeders and consume most available larvae, potentially including those of their female hosts, therefore interacting negatively with female hermit crabs (Conover 1978, Damiani 2003). Gonozooids, also known as reproductive zooids, contain gametes and lack tentacles in hydrozoans or a lophophore in bryozoans (Mills 1976, Conover 1978, Buss and Yund 1989, Harvell 1998). Gonozooids are distinct zooid types in hydrozoans and in some bryozoans (Mills 1976, Conover 1978, Buss and Yund 1989), and have no known effect on the nature of symbiosis between epibionts and their host. Lastly, defensive zooids, also known as dactylozooids, take several forms including avicularia, tentaculozooids, and spiralzooids (e.g. Mills 1976, Harvell 1998, Langmead and Chadwick-Furman 1999, Damiani 2003, Lapid and Chadwick 2006). Most defensive zooids have been relatively well studied and contribute positive and negative components to the interaction with their hosts. Hydroid spiralzooids are a notable exception.

Spiralzooids consist of a long coiled shaft tipped with nematocysts. This zooid type has only been observed within the family Hydractiniidae, a group of common sessile hydrozoans found worldwide (e.g. McFadden *et al.* 1984, Folino and Yund 1998, Williams and McDermott 2004, Bumann and Buss 2008, Miglietta *et al.* 2009). The genus *Hydractinia*, one of the most diverse genera within the Hydractiniidae, contains over 30 species (Miglietta *et al.* 2009, Williams and McDermott 2004) that vary little in

morphology and behavior (Conover 1978, Buss and Yund 1989). In species that are commonly symbiotic with hermit crabs, such as *Hydractinia polyclina*, past authors have noted that spiralzooids appear to be associated with the aperture of the host's gastropod shell (Mills 1976, Conover 1978, Buss and Yund 1989, Damiani 2003), suggesting some role in mediating interactions between the epibiont and host. Nevertheless, there is still much to learn about spiralzooids and how they contribute to the overall interaction between colony and host.

Previously, spiralzooids have been hypothesized to contribute three possible negative interaction components that directly reduce the reproductive output of host hermit crabs (Damiani 2003). First, spiralzooid nematocysts were proposed to restrict host copulation (Damiani 2003). However, the presence of *Hydractinia symbiolongicarpus* colonies only weakly affected host ovigery, which was used as an indirect indicator for copulation (Damiani 2003). Second, female hermit crabs brood eggs against their abdomen within a gastropod shell, potentially exposing them to spiralzooids when crabs extend out of the shell to aerate their eggs. However, crabs in shells with colonies with and without spiralzooids did not differ in the number of eggs hatched (Damiani 2003), suggesting that any colony effect on brooded eggs is mediated through another zooid type. Lastly, spiralzooids have been hypothesized to capture newly hatched larvae as they are released from female hosts. However, the numbers of zoea released by female hosts inhabiting shells with and without spiralzooids did not differ (Damiani 2003). Consequently, no study to date has definitively demonstrated how spiralzooids affect the interaction between hydroid colonies and hermit crab hosts.

I propose that spiralzooids function in mediating predation on colonies by their own host crabs. Hermit crabs, such as *Pagurus* spp., are omnivorous scavengers that consume a wide range of detrital plant and animal particulate material (Roberts 1968, Caine 1975). While benthic diatoms may form the majority of their diet by volume, these prey have comparatively low caloric values and require extensive foraging time (Roberts 1968, Caine 1975). As a result, hermit crabs must consume large quantities in order to fulfill their metabolic requirements (Caine 1975). Although hermit crabs are active scavengers, those that are symbiotic with *Hydractinia* spp. have never been documented consuming their own epibiont colonies. Yet *Hydractinia* colonies could constitute a valuable prey source due to their potentially high caloric values from lipids stored in gonozooids and the minimal foraging time required for hermit crab access. I hypothesize that host *Pagurus longicarpus* crabs do not prey on their own *Hydractinia polyclina* epibionts because spiralzooids function to restrict host hermit crab foraging on colonies. If spiralzooids prevent hosts from foraging on hydroids, then the interaction component contributed by spiralzooids may be less negative than previously thought, and may shift the net interaction between colony and host towards a mutualism.

METHODS AND MATERIALS

I used a combination of experiments and surveys to investigate spiralzooid/host interaction components. The elements of my study can be grouped into three topics: distribution, structure, and function. Methods utilized in several sections are described in sections 1-3. Spiralzooid distribution experiments and surveys are described in sections 4-6, while structure surveys are described in sections 7-9. Spiralzooid function experiments and surveys are described in sections 10-13. Supporting experiments, surveys, and methods are described in appendices I - III.

(1) Specimen Collection Locations and Maintenance

Hydractinia polyclina colonies and *Pagurus* spp. hermit crabs were sampled at three sites within the Gulf of Maine (Johns Bay in Bristol, ME; Saco Bay in Saco, ME; and Portsmouth Harbor in Kittery, ME; Figure 1). These sites were chosen either because previous studies demonstrated that hydrozoan epibionts were exclusively *H. polyclina* and that the conspecific *H. symbiolongicarpus* was absent (Buss and Yund 1989, Yund and Parker 1989, Folino and Yund 1998), or because that distribution appeared likely from patterns among neighboring sites. Colonies were collected regardless of host hermit crab species, with both *P. longicarpus* and *P. acadianus* present at Site C (Johns Bay), and only *P. acadianus* present at the other sites. Specimens were repeatedly hand-collected at each sample site by SCUBA and/or snorkeling from August 2008-July 2010. All collected hydroid colonies were housed in a flowing seawater system and fed *Artemia* sp. nauplii every four days, while hermit crabs were fed herring pieces every six days.

(2) Image Analysis

To assess structure, spiralzooids were removed from colonies and flattened onto slides with cover slips then observed using a Nikon Eclipse E800 under total magnification of 1000X with oil emersion. This method allowed zooids to be viewed two-dimensionally and facilitated viewing of nematocysts and associated structures. Digital photos taken by a SPOT RTke camera were analyzed using Image-J software.

(3) Gastropod Shell Zones

Shell zones were used as a means of classifying relative distances on the shell from the aperture and were designated as percent areas to ensure that comparisons among different sized shells were not affected by absolute size. Shell zones were primarily used for quantifying spiralzooid location within a colony, and only *Littorina* gastropod shells were utilized in these surveys. I defined three zones (A-C) on the shells (Figure 2). Zone A consisted of the interior and exterior area of the shell extending 2mm from the shell aperture. Zone B consisted of the exterior area from zone A to $^{1}/_{3}$ the shell length on the exterior of the shell. Shell length was defined as the perpendicular distance from the outer aperture of a shell to its spire. Zone C consisted of the exterior area from zone B to the spire.

(4) Spiralzooid Distribution: Comparison Among Species

The goal of this survey was to test if a predominately shell-living existence is associated with the production of spiralzooids across diverse Hydractiniid species. A

literature review was utilized that covered all genera within the family Hydractiniidae. I analyzed published reports of the ability of colonies to form spiralzooids on varying substrates. In addition to the term spiralzooid, some past authors have used the term dactylozooid to refer to spiralzooids. However, other authors use the "dactylozooid" to refer to all defensive zooids (i.e., both spiralzooids and tentacular zooids, which are an un-coiled defensive zooid). Consequently, I only included species for which the literature specifically stated the presence or absence of spiralzooids, showed a clear representation of a spiralzooid, or stated the presence or absence of coiling dactylozooids.

(5) Spiralzooid Distribution: Crosses

Because the Gulf of Maine has two documented sibling species of *Hydractinia* (*H symbiolongicarpus* and *H. polyclina*), which are morphologically indistinguishable (Buss and Yund 1989), I conducted controlled crosses with colonies from the three sites to confirm hydroid species identification. The use of crosses for species identification, instead of genetic analysis, was considered sufficient because past studies found extremely low percentages of hybridization (in the range of 0-2.7% of eggs developing; Buss and Yund 1989, Folino and Yund 1998). Crosses within and among collecting sites were conducted following the methods of Buss and Yund (1989). Approximately 3-12 mature gonophores were removed from each colony and placed in sterile seawater in petri dishes, then kept in the dark at 12ºC for 12 hours. *Hydractinia* spp. are dioecious and spawn 1h after exposure to light (Bunting 1894, Ballard 1942), following a 12 hour period in the dark. Then gonozooids were exposed to light, initiating the release of gametes. I checked for development of planula larvae three days post-fertilization, with

development used as a proxy for successful fertilization. Replicates were performed for each cross. Although data expressed as percentages tend to fail normality tests, my cross data were found not to violate normality assumptions, and so were analyzed without transformation using a one-way ANOVA in SigmaPlot.

(6) Spiralzooid Distribution: Substrate Analysis

This survey documented the presence of spiralzooids in *Hydractinia polyclina* colonies occupying various substrates. Attachment substrata surveyed included: *Littorina* gastropod shells inhabited by either *Pagurus longicarpus* or *P. acadianus*, live *L. littorea* and *Buccinum undatum*, uninhabited *Littorina* gastropod shells, stones, and boulder/concrete. Stone substrates were defined as pieces of rock ≤30cm long, while boulder/concrete substrates were >30cm long. All colonies, except for those on boulder/concrete were analyzed in the lab for presence of spiralzooids as a function of substrate, collection site, colony gender, and substrate surface area. Boulder/concrete substrates were examined in situ by snorkel and/or SCUBA for the presence of spiralzooids with respect to collection site and approximate colony surface area.

(7) Spiralzooid Structure: Spiralzooid Morphology

The intent of this survey was to gain insight into the ability of spiralzooids to physically interact with surrounding surfaces by measuring zooid length and density, and documenting the type and organization of nematocysts present. Due to the rapid movement of the uncoiling zooids, image analysis was used for zooid length and density measurements. Zooid length was defined as the distance from the base of the zooid to the

end of the tip while the zooid was fully extended. Spiralzooid density was measured as the number of zooids within a 5mm long by 2mm wide section along the shell aperture. Next, nematocysts were evaluated using image analysis and nematocyst type was identified via published guidelines (Östman 2000). Nematocyst capsules are the spherical base of the nematocyst that contains nematocyst shafts and threads. Nematocyst capsule length, capsule width, and shaft length were measured. Capsule length was defined as the longest distance along the capsule, while width was defined as the distance perpendicular to the length of the capsule. Shafts were identified by the presence of barbs. Length of shaft was determined as the distance between the capsule and the thread. Dense spherical clumps of nematocysts that attach to zooid tips were defined as spiralzooid batteries. Battery widths and length were measured and these linear measurements were used to estimate cross-sectional surface area.

(8) Spiralzooid Structure: Spiralzooid Tip Condition

The objective of this survey was to investigate spiralzooid tip condition to gain insight into nematocyst dynamics. Zooid tips were defined as the end of a spiralzooid not attached to a colony's mat tissue, and condition levels were assessed via the number and arrangement of nematocyst batteries on a spiralzooid tip, as quantified with image analysis. Spiralzooid condition was classified in four levels. Level 1, postulated to be the least functional level, contained spiralzooids that had no nematocyst batteries present on their zooid tip (Figure 3A,B). This level was considered morphologically non-functioning due to a lack of nematocysts, although the spiralzooids were physically able to lash. A lash was defined as the forward movement of a spiralzooid, where the zooid uncoils from

the base to the tip towards the center of the shell aperture. Spiralzooids that contained single layers of nematocyst batteries with few sections of the zooid tip lacking nematocyst batteries were classified as level 2 (Figure 3C,D). This level was judged to be limited in function due to incomplete layers of nematocyst batteries. The level 3 classification included spiralzooids that had a uniform single layer of nematocyst batteries, with some sections containing a double layer (Figure 3E,F). This level was judged to be completely functional. Level 4, thought to be the highest functional level, contained spiralzooids with a uniform double layer of nematocyst batteries (Figure 3G,H).

(9) Spiralzooid Structure: Adhesion Properties of Nematocysts Batteries

This experiment investigated the capability of nematocyst batteries to adhere to different surfaces. Adhesion surfaces consisted of hermit crab abdomen, cephalothorax, *Pagurus arcuatus* cheliped, and *P. longicarpus* cheliped. The use of two species of hermit crab chelipeds allowed me to test the adhesion properties of nematocyst batteries to differing densities of decapod sensory hairs. *Pagurus arcuatus* do not occupy shells encrusted by any species of *Hydractinia*, but this species of hermit crab possesses more sensory hairs on their chelipeds than the two species occupied by hydroids. Colonies were mechanically stimulated in the mat tissue with forceps to initiate spiralzooid lashing. Each colony was stimulated twice and the number of adhered nematocyst batteries per zooid counted, and then analyzed in Sigma Plot with a one-way ANOVA.

(10) Spiralzooid Function: Spiralzooid Formation with Respect to Shell Zones and Host Density

Under in-situ conditions, hermit crab densities are relatively low and spiralzooid formation is assumed to be restricted to shell apertures (Yund and Parker 1989, Folino and Yund 1998). My objective was to determine if the distribution of spiralzooids on a shell is the result of low hermit crab densities, which largely limit contact to the host crab and zone A, or whether spiralzooid formation is morphologically constrained to zone A. This section consisted of two phases, a survey and an experiment. First, a population survey was conducted at Johns Bay, Bristol, ME (Site C), to establish spiralzooid densities within different shell zones under a natural hermit crab density. Randomly selected $1m^2$ quadrats were surveyed for *Pagurus longicarpus* abundance. From these quadrats, hermit crabs with 100% *H. polyclina* coverage on their gastropod shells were collected. Spiralzooid density was then quantified in each shell zone.

Second, an experimental approach was utilized to determine the effect of increased densities of hermit crabs on spiralzooid formation. Four treatments were constructed that tested crab density effects via a combination of physical and chemical crab cues: direct contact/host present, no contact/host present, direct contact/host absent, and no contact/host absent. All treatments utilized gastropod shells with 100% *H. polyclina* coverage and spiralzooids initially present only in shell zone A. Host absent treatments had shell apertures blocked to prevent subsequent inhabitation. Crabs, shells, and colonies from treatments with direct contact (both with host present and host absent) were placed in containers (8cm length x 8cm width x 5cm high), in flow through

seawater tanks, and an additional forty hermit crabs (in shells) were added to each container so that they were in physical contact with the experimental shells. Gastropod shells from treatments with no contact (for hosts both present and absent) were also divided into containers of the same size. An additional forty hermit crabs were placed below a mesh divider to physically isolate them from the experimental group, although water was allowed to circulate through both sections of the tank. The isolated crabs within these two treatments received chemical cues from high densities of hermit crabs in the absence of physical cues. Crabs and colonies in all treatments were allowed to interact for five days. Experimental colonies were evaluated for spiralzooid formation within shell zones B and C, then spiralzooid density data were analyzed using a three-way ANOVA in Sigma Plot with factors of shell zone (A, B, or C), nature of hermit crab cues (physical or chemical), and presence vs. absence of host crab.

(11) Spiralzooid Function: Mechanical Stimuli

Mechanical stimuli were imposed on colonies to determine if the location and intensity of physical contact affected the lashing response of spiralzooids. I investigated spiralzooid response to two levels of mechanical stimuli, strong and weak, in shell zones A-C. Pressure on colony mat tissue was considered a strong stimulus, while pressure on zooids was considered a weak stimulus. Stimuli were applied to either mat tissue or zooids with forceps five times per shell zone for a total of 30 stimulus events per crab. Responses were assessed for the presence or absence of spiralzooid lashing. The five observations within a crab and zone were then used to calculate a frequency of lashing, based on a 0-1 scale, for each level of stimulus applied to each of the three zones per

crab. This single frequency value per crab was replicated across the 50 crabs, then analyzed using a two-way ANOVA in Sigma Plot with main effects of stimulus and shell zone. Because each crab contributed five responses from this experiment, with six possible response variable levels (0, 20, 40, 60, 80, or 100%), the dependent variable was considered an acceptable approximation of a continuous variable and hence suitable for analysis via a parametric test.

(12) Spiralzooid Function: Effect of Spiralzooid Lashing on Crab Behavior

To test whether the lashing of spiralzooids modifies how hermit crabs behave, behavior was observed during induced lashing events and controls without hydroid colonies present. Two groups of hermit crabs (n=50 per group) were established. Group 1 consisted of hosts with 100% *H. polyclina* coverage on their gastropod shells, and was used to test the effects of probing the shell and subsequent spiralzooid lashing on crab behavior. Group 2 consisted of hermit crabs with bare gastropod shells (0% *H. polyclina* coverage), and served as a control to test the effect of probing the shell alone. Host gastropod shells were probed with forceps in shell zone A in both treatment groups, three times per host. Crab behavior was assessed as either continuing or disrupted, and the three observations were then used to calculate a frequency of disrupted behavior, based on a 0-1 scale. A single frequency value per crab was calculated for each crab in each group (n=50). Data from the replicate crabs were then analyzed using a two-way rank sum test. Because each crab contributed three responses from this experiment, with four possible response variable levels (0, 33.33%, 66.66%, 100%), the dependent variable was considered a discrete variable and thus best analyzed via a non-parametric test on ranks.

(13) Spiralzooid Function: Host Behavior Effects on Spiralzooid Lashing

Crab behaviors were investigated to determine whether spiralzooids lash defensively in response to particular host behaviors. Crab behaviors were classified into six categories: active foraging, foraging on colonies, feeding, fighting, zoea release, and shell swapping. Active foraging was defined as the constant forward movement of a host for at least 2 minutes. Foraging on colonies was defined as one hermit crab foraging on another crab's *H. polyclina* colony. Feeding was defined as at least two successful consumptions of prey (herring pieces) by the host. Fighting was the direct interaction between two crabs, where the cheliped of one crab directly contacted the aperture of another crab's gastropod shell. Zoea release was defined as the release of zoea from a female host. To initiate zoea release, female crabs with eggs were isolated until the eggs were fully matured, and then crabs were placed into an observation container with warm water (20ºC). Zoea release was directly observed. Shell swapping was defined as hosts exchanging their gastropod shell when provided with several unoccupied hydroid free shells. Hermit crabs were observed over a period of 30 minutes for each behavior type recorded. The lack of direct control over behavior type meant that there were 2-5 observations for each behavioral category. Single observations were excluded from statistical calculations. As zoea release was infrequent, the single observations for this behavior category were included in Table 4 to illustrate that the data did not support previous hypotheses. Crab behaviors were categorized as either continuing or disturbed, and the multiple observations were then used to calculate a frequency, based on a 0-1 scale. A single frequency of disturbed behavior was then calculated for each crab in each

behavior group. Data from the replicate crabs were then analyzed using a two-way rank sum test. Each crab contributed between two and six observations, with three (0, 50, 100%) to six (0, 20, 40, 60, 80, or 100%) possible response variable levels, and so the data was considered a discrete variable suitable for a non-parametric test on ranks.

(14) Spiralzooid Function: Prey Calorie Values

To establish the potential appeal of *H. polyclina* as prey for *P. longicarpus*, colony caloric values were assessed via bomb calorimetry and then compared to the published caloric values of other prey. *Hydractinia polyclina* zooids were removed from their mat tissue and dried in an oven at 65ºC for 24 hours, then ground into a fine powder. Colony mat tissue was not included in the caloric value assay because hermit crabs are not expected to consume mat tissue. Samples were compressed into 1g pellets and analyzed using a Parr 1672 Oxygen Bomb Calorimeter. Sample ash was not titrated for nitrogen and sulfur content due to the minimal caloric correction factor that this approach provides (plus 0 to 10 calories) and the broad nature of comparisons between prey values from the literature and *H. polyclina* values.

RESULTS

Spiralzooid Distribution: (1) Comparison among Species

I examined the scientific literature to test whether spiralzooids are only present in hermit-crab living species of *Hydractinia* (Table 1). Of the approximately 30 species in this genus that occur worldwide, clear statements about the presence or absence of spiralzooids are only available for fourteen. These species occupy five different attachment substrates, including other hydroids, live gastropods, bivalves, hermit crab occupied gastropod shells, and nonliving debris. Spiralzooids were documented only in hydroid species that live on hermit crab occupied gastropod shells (Table 1). These hydroid species occur in the Atlantic, Pacific, and Antarctic Oceans, so no geographic pattern is evident.

Spiralzooid Distribution: (2) Crosses

I conducted crosses to test whether all my experimental colonies belonged to a single species. All crosses (n=44) both within and among the collection sites (Pemaquid Beach in Bristol, ME; Saco Bay in Saco, ME; and Portsmouth Harbor in Kittery, ME; Figure 1) produced viable planulae. Egg release per cross ranged from 10 to 49 (Table 2). Fertilization levels were normally distributed and did not differ significantly among the cross types (ANOVA, $F=0.67$, $df=5$, $p=0.620$), with mean fertilization ranging from 52 to 69%.

Spiralzooid Distribution: (3) Substrate Analysis within a Species

Spiralzooid presence or absence was documented in relation to colony attachment substrate, collection site, colony gender, and substrate surface area for *H. polyclina*. Substrate type strongly affected spiralzooid presence. Colonies attached to stones, boulders/concrete, bivalve shells, live *Littorina littorea* and *Buccinum undatum*, and empty *Littorina littorea* shells all lacked spiralzooids, while 100% of colonies attached to *L. littorea* shells occupied by *Pagurus longicarpus* or *P. acadianus* possessed spiralzooids (Table 3). Collection site, colony gender, and substrate surface area appeared to have no effect on the presence of spiralzooids.

Spiralzooid Structure: (4) Spiralzooid Morphology

Mean spiralzooids length was 1.17 ± 0.62mm (n=822) and density was 1.91 ± 1.03 spiralzooids per mm^2 along the aperture edge (n=30). Spiralzooids contained only a single type of nematocyst, microbasic euryteles, which were organized into nematocyst batteries at zooid tips. Microbasic euryteles, which are considered a medium sized piercing nematocyst (Östman 2000), possessed mean dimensions of 10.48 ± 0.06µm capsule length, 4.48 ± 0.03µm capsule width, and a 9.15± 0.07µm shaft length (n=55). Nematocyst batteries were spherical in shape with mean dimensions of 38.65± 0.07µm length, 38.91 ± 0.07µm width and a cross-sectional area of 1,193.08 ± 3.71μm^2 (n=75).

Spiralzooid Structure: (5) Spiralzooid Tip Condition

Four levels of spiralzooid tip condition were identified in *H. polyclina* colonies (Figure 3). The most abundant levels in colonies were classes 2 and 3, with 38.2% of spiralzooids represented (Figure 4). The least common level was the nonfunctional level 1, which accounted for only 7.7% of spiralzooids (Figure 4).

Spiralzooid Structure: (6) Adhesion Properties of Nematocysts Batteries

Spiralzooid nematocyst batteries adhered to all tested substrate (Figure 5). Data were not normally distributed and were analyzed using a nonparametric test. Mean adhesion ranged from 0.19 ± 0.16 nematocyst batteries per spiralzooid lash for *P. longicarpus* cephalothorax, to 0.11 ± 0.13 nematocyst batteries per spiralzooid lash for *P. arcuatus* cheliped. However, the mean number of adhered nematocyst batteries per lash did not vary significantly among substrates (Kruskal-Wallis ANOVA on ranks, $H = 5.32$, $df = 3$, $p = 0.150$).

Spiralzooid Function: (7) Spiralzooid Formation in Respect to Shell Zones and Host Densities

The natural density of *P. longicarpus* at Site C (Pemaquid Beach, Bristol, ME) was 10.88 crabs per m^2. The manipulative experiment to test density effects on spiral zooid formation used *P. longicarpus* densities of approximately 200 crabs per m^2, over 100x greater than that of Site C. Four treatments tested crab density effects on the formation of spiralzooids via a combination of physical and chemical crab cues: direct

contact/host present, no contact/host present, direct contact/host absent, and no contact/host absent. Overall, mean spiralzooid densities in zones A and B were higher in treatments with hosts present than in the natural survey or host absent treatments (Figure 6), with the highest densities observed with hosts present and direct physical contact with other hermit crabs. Hermit crabs were commonly observed to feed on hydroid polyps in this treatment. Spiralzooid density varied significantly between host crab presence and absence treatments, but did not vary significantly with any other factors or interaction among factors (Table 4).

Spiralzooid Function: (8) Mechanical Stimuli

Mechanical stimuli generally elicited a lashing response in 60-90% of the events, regardless of the zone of stimulus application or the level of stimulus (Figure 7). However, the lashing response was reduced for zooid contact in zone A. Lashing response varied significantly among shell zones (ANOVA, $F=17.59$, $df=2$, $p<0.001$) and among level of stimulus (ANOVA, $F=15.542$, $df=1$, $p<0.001$). The interaction between these two factors was also significant (ANOVA, $F=58.49$, $df=2$, $p<0.001$), suggesting that *Hydractinia polyclina* are habituated to the host hermit crab contacting the colony along the shell aperture.

Spiralzooid Function: (9) Spiralzooid Lashing Effects on Crab Behavior

Probing both bare gastropod shells and hydroid covered shells (thus causing spiralzooids to lash) tended to cause host hermit crabs to halt their current behavior (n=50). However, probing of hydroid covered shells halted host crab behavior a

significantly greater portion of the time than probes of bare shells (Figure 8; Mann-Whitney Rank Sum Test, T=3167.00, $p \leq 0.001$).

Spiralzooid Function: (10) Host Behavior Effect on Spiralzooid Lashing

Six crab behaviors were analyzed: foraging on hydroid colonies (n= 25), active foraging (n=50), feeding (n=50), fighting (n=50), zoea release (n=3), and shell swapping (n=35). Foraging on hydroid colonies induced the most spiralzooid lashing events and fighting behaviors produced the second largest response (Table 5). Active foraging, feeding, and zoea release did not stimulate spiralzooid lashing events. Variation in lashing response among different hermit crab behaviors was statistically significant (Kruskal-Wallis ANOVA on Ranks, $H = 80.23$, $df = 4$, $p \leq 0.001$). Because I only obtained one observation per crab, I excluded the effect of zoea release on spiral zooid lashing from the statistical analysis. Nevertheless, spiralzooids failed to lash during the three zoea releases that I observed (Table 5).

Spiralzooid Function: (11) Prey Calorie Values

Prey caloric values compiled from the literature were compared to *Hydractinia polyclina* values obtained in this study (Table 6). Samples of *H. polyclina* (n=5) had a calculated caloric value of 4,011.55 ± 65.47 cal/g dry wt. This value ranks *H. polyclina* within the top 10% of potential hermit crab prey, with a caloric value approximately 50% higher than that of algae and comparable to that of other animal prey (Table 5).

DISCUSSION

Spiralzooid Distribution:

In many colonial invertebrates with inducible polymorphism, the distribution of zooid types within a colony has long been used to infer their likely functions (Mills 1976, Harvell 1998, Langmead and Chadwick-Furman 1999, Damiani 2003, Lapid and Chadwick 2006). For example, tentaculozooids, although present throughout hydrozoan colonies, only form in high densities around other epibionts, suggesting a specialized defensive function against other encrusting invertebrates (Namikawa 1992, personal observation). Similarly, avicularia in bryozoans only form in heavily foraged areas of colonies, suggesting that they function defensively to reduce subsequent foraging (Harvell 1998). The distribution of spiralzooids can similarly be used to help infer function.

The genus *Hydractinia* contains a number of clades that are primarily associated with different geographic regions, and different species encrust a wide range of firm substrata including bivalves, other hydroids, and shells inhabited by hermit crabs (e.g. Cunningham *et al*. 1991, Folino and Yund 1998, Schuchert 2000, 2001). From a literature review, I was able to demonstrate that globally, only *Hydractinia* spp. that lives on gastropod shells occupied by hermit crabs have been documented to form spiralzooids (Table 2). Although past work suggested that the association between hermit crab living and presence of spiralzooids holds true for the North American clade of *Hydractinia* (*H. GM, H. symbiolongicarpus, H. polyclina, H. symbiopollicaris, H. milleri*; Buss and Yund

1989, Folino and Yund 1990, Miglietta *et al.* 2009), this pattern has not previously been documented to span multiple clades. The absence of a geographic or taxonomic pattern indicates that the presence or absence of spiralzooids does not reflect a phylogenetic constraint, and suggests that spiralzooids play a role in hermit crab/hydroid interactions.

Few *Hydractinia* spp. have been documented to encrust multiple substrates within the same geographical region. For instance, *H. polyclina* has previously been reported to encrust gastropod shells occupied by *Pagurus acadianus* or *P. longicarpus*, with spiralzooids distributed along shell apertures (Buss and Yund 1989, Folino and Yund 1990). Yet within my three collections sites in the Gulf of Maine, I found that *H. polyclina* encrusted eight different substrate types, five of which have not previously been reported (live *Buccinum undatum* and *Littorina littorea*, rocks, concrete/bolder, and bivalve shells; Table 3). The absence of spiralzooids on these alternative substrates extends the association with hermit crabs documented at the species level, and suggests that spiralzooid formation is inducible, rather than a fixed property of the species.

Spiralzooid Structure:

Nematocyst structure and organization can also shed light on polyp function. In Cnidarians, nematocysts are the primary organelles used for foraging and defense (Purcell and Mills 1988, Madin 1988, Östman 2000, Puce *et al.* 2010). Nematocysts are classified in two main groups; entangling, such as desmonemes, or piercing, such as microbasic euryteles, stenoteles, and mastigophores (Östman 2000). Entangling nematocysts tend to be used for foraging on fast moving, hard-bodied prey such as

copepods or crustacean larvae (Purcell and Mills 1988, Madin 1988). Piercing nematocysts tend to be used for both defense from predators, and foraging on soft bodied prey (Purcell and Mills 1988, Madin 1988). Previously, spiralzooids have been proposed to function as secondary gastrozooids to capture host zoea (Christensen 1967, Damiani 2003). However, due to a lack of entangling nematocysts, it is highly unlikely that spiralzooids could capture, retain, or transfer zoea to a feeding polyp. In contrast to spiralzooids, gastrozooids contain a combination of both entangling desmonemes and piercing microbasic euryteles (Mills 1976). This combination is more indicative of use in foraging on soft bodied or crustacean prey, such as zoea. The presence of only microbasic euryteles in spiralzooids is consistent with a defensive function for this zooid type.

Nematocyst organization also reflects how zooids interact with their surroundings (Puce *et al.* 2010). The arrangement of nematocysts into batteries tends to be associated with foraging on crustaceans (Puce *et al.* 2010). Although spiralzooid tips have previously been described as possessing "stubby" tentacles (Mills 1976), I have documented that these are detachable nematocyst batteries that vary in concentration on zooid tips. This organization is not common in hydrozoan polyps (Puce *et al.* 2010), though it is widespread in medusae (Purcell and Mills 1988, Madin 1988, Östman 2000, Puce *et al.* 2010). Even within the family Hydractiniidae, not all genera possess nematocyst batteries (Toth 1966, Mills 1976). For example, spiralzooids of *Podocoryne carnea*, a species that also predominantly forms on gastropod shells occupied by hermit crabs, do not contain nematocyst batteries (Toth 1966, Mills 1976). However,

Podocoryne also differs from *Hydractinia* in that colonies produce medusae instead of eggs (medusae subsequently develop gametes post-release), and hence colonies may be expected to have a lower caloric value.

Spiralzooids contain varying densities of nematocyst batteries on their tips, with all four tip levels occurring simultaneously within most colonies (Figure 3). This pattern is consistent with a regular use and hence loss of spiralzooid batteries. Undeveloped gonozooids also contain nematocyst batteries in an approximately level 4 state (personal observation, Mills 1976), suggesting that spiralzooids may form from this polyp type.

Individual nematocysts are not likely to be terribly effective against hermit crab exoskeleton. However, nematocyst batteries adhered at approximately the same rates to exoskeleton as to soft-bodied tissue. The porous nature of exoskeleton (Castro-Rosas and Escartin 2002) may permit nematocyst barbs to hook into pores. Batteries can continue to fire nematocysts after they detach from a spiralzooid; they also can potentially detach and roll back into the gastropod shell, where nematocysts might then contact the softer tissue of a crab abdomen. The combination of delivering multiple nematocysts in a small area and eventually reaching sites distant from the deployment location may make them a potent threat to hermit crabs.

Spiralzooid Function:

Hermit crabs are active mobile hosts, with the potential to frequently contact epibiont colonies. Colonies responded to weak and strong stimuli designed to mimic different levels of hermit crab contact by lashing with their spiralzooids (Figure 7).

However, in zone A, a weak stimulus produced significantly fewer lashing events than a strong stimulus (Figure 7). This two level response to stimuli near the aperture could help spiralzooids mediate interactions with hosts and conserve nematocyst batteries, which are lost during lashing events. Hermit crabs will contact colonies more frequently in colony zone A than in zones B & C, and contact in zone A is less likely to represent a threat to the colony.

Even though host hermit crabs are protected by exoskeletons, their behavior altered significantly during spiralzooid lashing events (Figure 8). Change in host behavior could be due to two main factors. First, hermit crabs are visual scavengers, with sophisticated eyes compared to many other invertebrates (Shaw 1969). Their visual acuity may permit detection of the uncoiling motion of spiralzooids and allow them to retreat into their shells. Alternatively, nematocysts may adhere to exoskeleton and directly alter behavior. Some combination of the two mechanisms is likely, with occasional nematocyst stings leading to learned avoidance.

Potentially because of this learned avoidance, *Pagurus longicarpus* forage on *Hydractinia polyclina* present on other crabs' shells, rather than their own. As a result of this behavior, foraging on other shells stimulated spiralzooid lashing 65% of the time. Other behaviors, such as feeding, foraging, and zoea release did not stimulate spiralzooid lashing (Table 5). This lack of response for these behaviors is not surprising, because they result in little contact and pose little threat to the colony.

Therefore, I suggest that spiralzooid formation is not mediated directly by position on the shell, but rather by the degree of direct contact with host hermit crabs, and may be less dependent on diffusible material produced by a host hermit crab than previously proposed (Braverman 1960). In the presence of high densities of hermit crabs, spiralzooids formed throughout the shell (Figure 6). All of the newly formed spiralzooids in this experiment were capable of lashing in co-ordination with the existing aperture spiralzooids, which suggests that these new zooids would function in a similar fashion. But their locations would render them ineffective for catching zoea and other previously hypothesized functions. Protecting the colony from foraging hermit crabs is the only function consistent with the location of these newly induced polyps.

Pagurus longicarpus are primarily deposit-feeders, but also act as scavengers, browsing on algae and even colonial invertebrates by slicing or plucking pieces off with the cheliped (Schembri 1982, Gherardi 1994). Calorically, *Hydractinia polyclina* are comparable to other prey available to host hermit crabs, probably due to the concentration of lipids in their gonozooids (Table 6). Captive hermit crabs are routinely observed foraging on the hydroids, primarily gonozooids, of other crabs. In habitats where competition for food resources is high, such as in many intertidal and subtidal zones, *P. longicarpus* might be expected to attempt to supplement their diets with their own *H. polyclina* epibionts. Although *P. longicarpus* have never been documented consuming their own *H. polyclina* epibionts, the potential role of spiralzooids in successfully preventing such foraging events deserves further consideration.

While this observation, and the effect of hermit crab density on spiralzooid formation, suggests an anti-foraging function for spiralzooids, I was not able to directly test hermit crabs foraging on their own epibiont. My expectation is that this behavior is limited by spiralzooids. Nevertheless, hermit crabs actively forage only on colonies on other shells. Since *Pagurus longicarpus* has never been documented foraging on colonies on their own shells, some aspect of hydroid morphology or behavior must prevent such actions.

Net Interaction:

Previously, under the hypothesis that spiralzooids consume host zoea, the net interaction between *Hydractinia polyclina* and *Pagurus longicarpus* has been suggested to be parasitic to the crab (Mills 1976, Conover 1978, Damiani 2003). Under this hypothesis, gastrozooids contribute positive and negative interaction components, gonozooids do not affect the interaction, tentaculozooids provide a positive interaction component, and spiralzooids contribute a strong negative interaction component. However, this hypothesis has overlooked several key aspects of spiralzooid biology and ecology. First, *H. polyclina* spiralzooids do not contain the desmonemes needed to capture crustacean prey. Next, if spiralzooids function to capture, retain, and transfer zoea to gastrozooids, then why are nematocyst batteries detachable? Most conclusively, spiralzooids did not lash during the release of zoea from the three female hermit crab hosts that I was able to observe. Consequently, my findings do not support the previous hypothesis for spiralzooid function.

I propose instead that spiralzooids function in *Hydractinia polyclina* to prevent host *Pagurus longicarpus* from foraging on epibiont colonies. This hypothesis is a better fit to my current data on spiralzooid ecology. Spiralzooids lashed 65% of the time in response to foraging on colonies (Table 5), and lashing terminated host behavior 43% of the time. Additionally, removable nematocyst batteries would appear to allow colonies to reach the soft-bodied abdomen tissue of hermit crabs. Lastly, foraging on *H. polyclina* stimulated spiralzooid formation throughout the shell. If my hypothesis is correct, I suggest that gonozooids actually have a weak positive interaction with the crab by providing desired caloric value and spiralzooids now have a weak negative interaction with the crab. Therefore, the resulting net interaction between *H. polyclina* and *P. longicarpus* would be considered commensal or weakly mutualistic and directly influenced by spiralzooids.

REFERENCES

Ballard, W. W. 1942. The mechanism of synchronous spawning in *Hydractinia* and *Pennaria*. Biological Bulletin (Woods Hole) 82: 329-339.

Bavestrello, G., Bianchi, C.N., Calcinai, B., Cattaneo-Vietti, R., Cerrano, C., Morri, C., Puce, S., and Sara, M. 2000. Bio-mineralogy as a structuring factor for marine epibenthic communities. Marine Ecology Progress Series. 193: 241-249.

Blackstone, N. W. 1985. Hermit crabs and carcinization: trends in the development and evolution of some decapod crustaceans. Dissertation. Yale University, New Haven, Connecticut, USA.

Bouillon, J., Medel, M.D., Pages, F., Gili, J.M., Beoro F., and Gravili, C. 2004. Fauna of the Mediterranean Hydrozoa. Scientia Marina. 68 (S2).

Brooks, W.R., and Mariscal, R.N. 1985. Protection of the hermit crab *Pagurus pollicaris* (Say) from predators by hydroid-colonized shells. Journal of Experimental Marine Biology and Ecology. 87 (2): 111-118.

Braverman, M.H. 1960.Differentiantion and commensalism in *Podocoryne carnea*. The American Midland Naturalist. 63 (1): 223-225.

Brawn, V.M., Peer, D.L., and Bentley, R.J. 1968. Caloric content of the standing crop of benthic and epibenthic invertabrates of St. Margaret's Bay, Nova Scotia. Journal of Fish Research and Biodiversity Canada. 25: 1803-1811.

Buss, L.W., and Yund, P.O. 1989. A sibling species group of *Hydractinia* in the northern United States. Journal of Marine Biological Association of the United Kingdom. 69: 857-874.

Bunting, M. 1894. The origins of sex-cells in *Hydractinia* and *Podocorvne*. Journal of Morphology 9: 203-236.

Bumann, D., and Buss, L.W. 2008. Nutritional physiology and colony form in *Podocoryna carnea* (Cnidaria: Hydrozoa). Invertebrate Biology. 127 (4): 368-380.

Caine, E.A. 1975. Feeding and masticatory structures of selected Anomura (Crustacea). Journal of Experimental Marine Biology and Ecology. 18 (3): 277-301.

Castro-Rosas, J., and Escartín, E.F. 2002. Adhesion and colonization of *Vibrio cholerae* (O1) on shrimp and crab carapaces. Journal of Food Protection. 65 (3): 492-498.

Cerrano,C., Puce, C., Chiantore, M., Bavestrello, G., and Cattaneo-Vietti, R. 2001. The influence of the epizoic hydroid *Hydractinia angusta* on the recruitment of the Antarctic scallop *Adamussium colbecki*. Polar Biology. 24 (8): 577-581.

Christensen, H. E., 1967. Ecology of *Hydractinia echinata* (Fleming) (Hydroidea, Athecata). I. Feeding biology. Ophelia, 4: 245-275.

Conover, M.R. 1978. The importance of various shell characteristics to the shell-selection behavior of hermit crabs. Journal of Experimental Marine Biology and Ecology. 32 (2): 131-142.

Cunningham, C.W., Buss, L.W., and Anderson, C. 1991. Molecular and geologic evidence of shared history between hermit crabs and the symbiotic genus Hydractinia. Evolution. 45 (6): 1301-1316.

Damiani, C.C. 2003. Reproductive costs of the symbiotic hydroid *Hydractinia symbiolongicarpus* (Buss and Yund) to its host hermit crab *Pagurus longicarpus* (Say). Journal of Experimental Marine Biology and Ecology. 288 (2): 203-222.

Dudgeon, S.R., and Buss, L.W. 1996. Growing with the flow; on the Maintenance and Malleability of colony form in the hydroid *Hydractinia*. The American Society of Naturalists. 147(5): 667-691.

Folino, N.C., and Yund, P.O. 1998. The distribution of hydroid sibling species on hermit crabs in estuaries in the Gulf Of Maine. Estuaries. 21(4b): 829-836.

Gherardi, F. 1994. Multiple feeding techniques in the sessile hermit crab *Discorsopagurus schmitti*, inhabiting polychate tubes. Oecologia 98: 139-146.

Harvell, C.D. 1991. Coloniality and inducible polymorphism. American Naturalist. 138 (1): 1-14.

Harvell, C.D. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution. 52 (1): 80-86.

Lamare, M.D., and Wing, S.R. 2001. Calorific content of New Zealand marine macrophytes. New Zealand Journal of Marine and Freshwater Research. 35: 335-341.

Langmead, O., and Chadwick-Furman, N.E. 1999. Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma*. 1. Role in competition for space. Marine Biology. 134 (3): 479-489.

Lapid, E.D., and Chadwick, N.E. 2006. Long-term effects of competition on coral growth and sweeper tentacle development. Marine Ecology Progress Series. 313: 115-123.

Madin, L.P. 1988. Feeding behavior of tentaculate predators: In situ observations and a conceptual model. Bulletin of Marine Science. 43 (3): 413-429.

McDermott, J.J. 2001. Symbionts of the hermit crab *Pagurus longicarpus* (Say, 1817) (Decapoda: Anomura): New observations from New Jersey waters and a review of all known relationships. Proceedings of the Biological Society of Washington. 114 (3): 624-639.

Mcfadden. C.S., Mcfarland, M.J., and Buss, L.W. 1984. Biology of Hydractiniid hydroids. 1. Colony ontogeny in *Hydractinia Echinata* (Fleming). Biological Bulletin.166: 54-67.

McLean, R. 1983. Gastropod shells; a dynamic resource that helps shape benthic community structure. Journal of Experimental Marine Biology and Ecology. 69: 151-174.

Miglietta, M.P., Schuchert, P., and Cunningham, C.W. 2009. Reconciling genealogical and morphological species in a worldwide study of the Family Hydractiniidae (Cnidaria, Hydrozoa). Zoologica Scripta. 38 (4): 403-430.

Mills, C.M. 1976. *Podocoryne Selena*, A new species of hydroid from the Gulf Of Mexico, and a comparison with *Hydractinia echina*. Biological Bulletin. 151: 214-224.

Namikawa, H. 1992. Role of the tentaculozooids of the polymorphic hydroid *Stylactaria conchicola* (Yamada) in interactions with some epifaunal space competitors. Journal of Experimental Marine Biology and Ecology. 162 (1): 65-75.

Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. Scientia Marina. 64 (S1): 31-46.
Peach, M.B., and Hoegh-Guldberg, O. 1999. Sweeper polyps of the coral *Goniopora tenuidens* (Scleractinia: Poritidae). Invertebrate Biology. 118 (1): 1-7.
Purcell, J.E., and Mills, C.E. 1988. The correlation between nematocyst types and diets in pelagic hydrozoa. In the biology of nematocysts. Pp. 463-485. Ed. By D.A. Hessinger and H.M. Lenhoff. Academic Press, Orlando.
Puce, S., Bo, M., Di Camillo, C.G., Paoli, L., Pica, D., and Bavestrello, G.. 2010. Morphology and development of the early growth stages of an Indonesian *Stylaster* (Cnidaria: Hydrozoa). Journal of the Marine Biological Association of the United Kingdom. 90 (6): 1145-1151.
Reiss, H., Knäuper, S., and Kröncke, I. 2003. Invertebrate associations with gastropod shells inhabited by *Pagurus bernhardus* (Paguridae) - Secondary hard substrate increasing biodiversity in North Sea soft-bottom communities. Sarsia. 88 (6): 404-414.
Roberts, M.H. 1968. Functional morphology of mouth parts of the hermit crabs, *Pagurus longicarpus* and *Pagurus pollicaris*. Chesapeake Science. 9 (1): 9-20.
Shaw, S.R. 1969. Optics of arthropod compound eye. Science. 164: 88-90.
Schembri, P.J. 1982. Functional morphology of the mouthparts and associated structures of *Pagurus rubricatus* (Crustacea: Decapoda: Anomura) with special reference to feeding and grooming. Zoomorphology. 101(1): 17-38.
Schuchert, P. 2000. Hydrozoa (Cnidaria) of Iceland collected by the BIOICE programme. Sarsia 85: 411- 438.
Schuchert, P. 2001. Hydroids of Greenland and Iceland (Cnidaria, Hydrozoa). Meddelelser Om Grønland, Bioscience. 53: 1–184.
Seipp, S., Schmich J., and Kehrwald, T. 2007. Metamorphosis of *Hydractinia echinata*-natural versus artificial induction and development plasticity. Development in Genetic Evolution. 217: 385-394.
Stampar, S.N., Tronolone, V.B., and Morandini, A.C. 2006. Description and life cycle of the hydrozoan *Hydracinia uniformis* spp.nov. (Cnidaria: Hydrazoa: Hydractiniidae),from the coast of southeastern Brazial. Zootaxa. 1200: 43-59.
Taylor, P.D. 1994. Evolutionary palaeoecology of symbioses between bryozoans and hermit crabs. Historical Biology. 9(3): 157-205.
Toth, S.E. 1966. An occurrence of nutritive to generative zooid transformation in the hydroid *Podocoryne carnea*. American Midland Naturalist. 76(1): 244-247.
Tyler, A.V. 1973. Caloric values of some North Atlantic invertebrates. Marine Biology. 19 (3): 258-261.
Williams, J.D., and McDermott, J.J. 2004. Hermit crab biocoenoses: A worldwide review of the diversity and natural history of hermit crab associates. Journal of Experimental Marine Biology and Ecology 305 (1): 1-128.
Yund, P.O., and Parker, H.M. 1989. Population structure of the colonial hydroid *Hydractinia* sp. nov. C in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology. 125: 63-82.

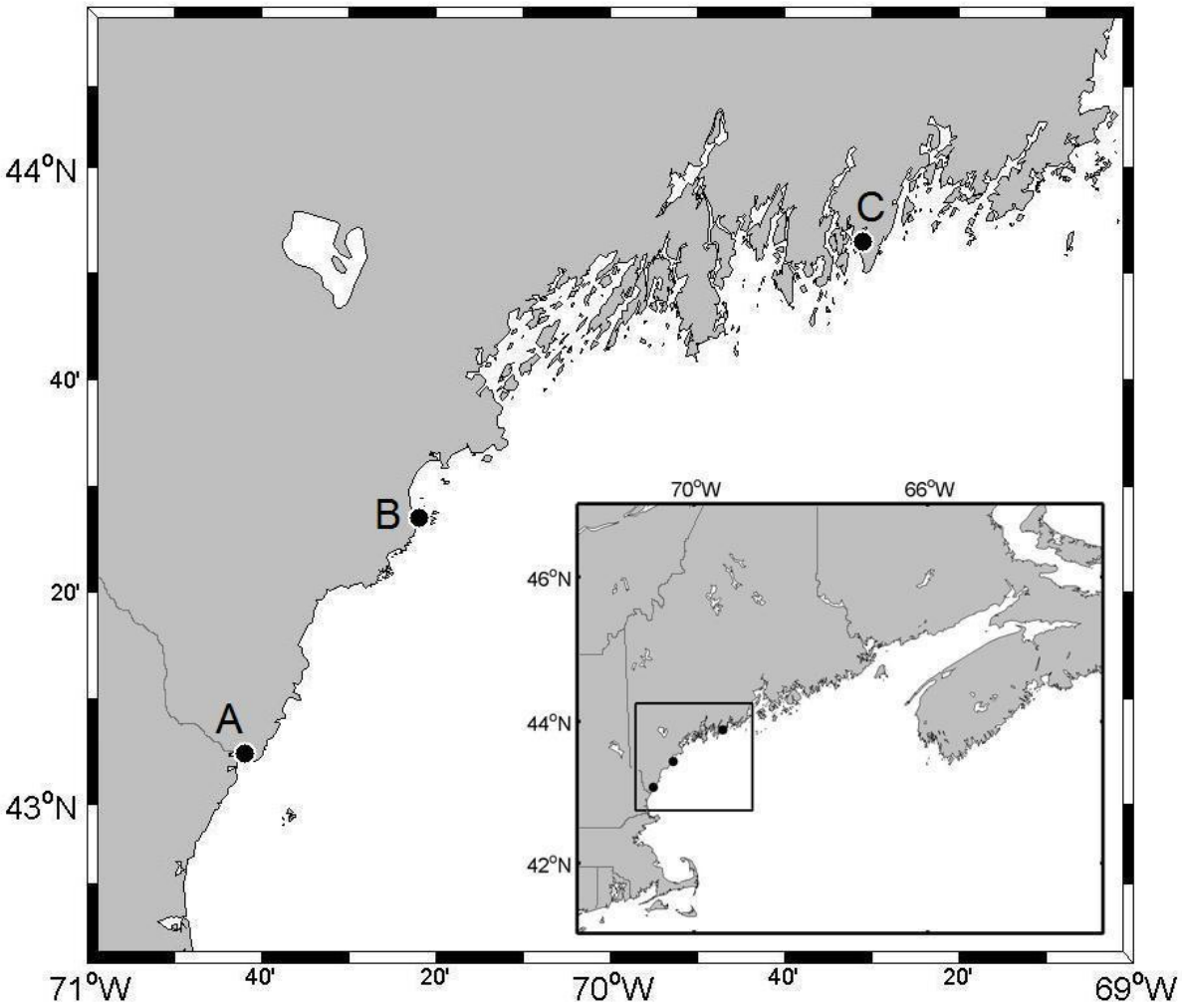

Figure 1: Study location within the Gulf of Maine; (A) Portsmouth Harbor at Kittery, ME; (B) The mouth of the Saco River at Saco, ME; (C) Pemaquid Beach at Bristol, ME.

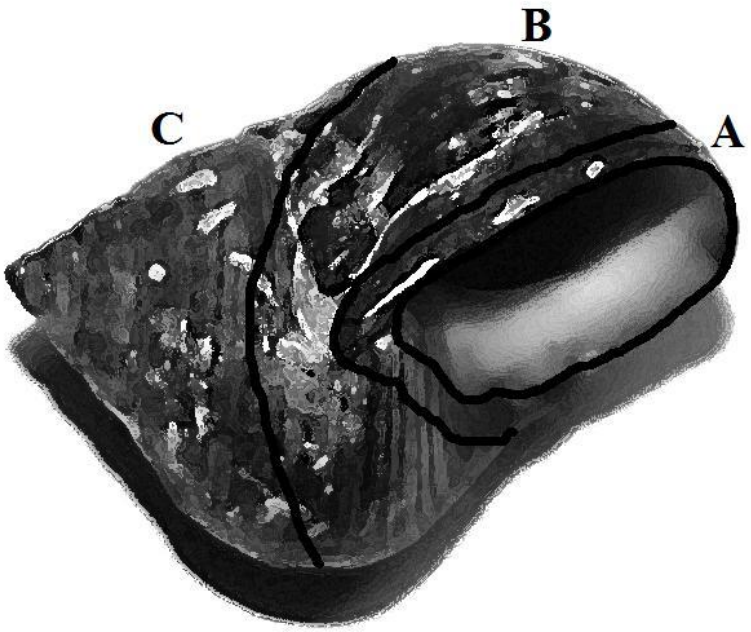

Figure 2: Shell Zones; A – leading edge of shell by the aperture, B – one-third of shell closest to the aperture, C – two-thirds of shell furthest from the aperture.

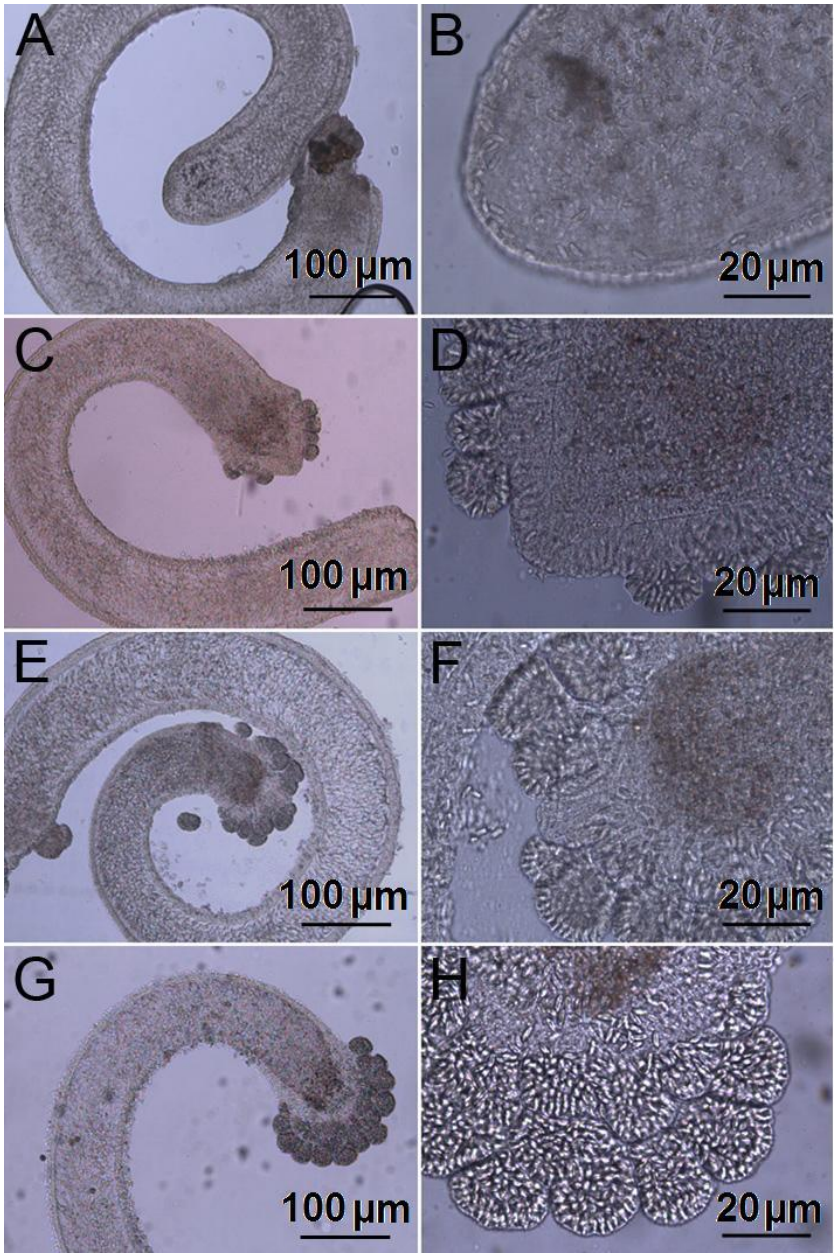

Figure 3: The four levels of spiralzooid condition. Panels A, C, E, and G are macroscopic views of spiralzooids, while panels B, D, F, and H are microscopic views. (A, B) Level 1 spiralzooid with no nematocyst batteries present. (C, D) Level 2 spiralzooid with some batteries, but bare sections of zooid tip. (E, F) Level 3 spiralzooid with complete single layer of nematocyst batteries on zooid tip. (G, H) Level 4 spiralzooid with complete double layer of nematocyst batteries on zooid tip.

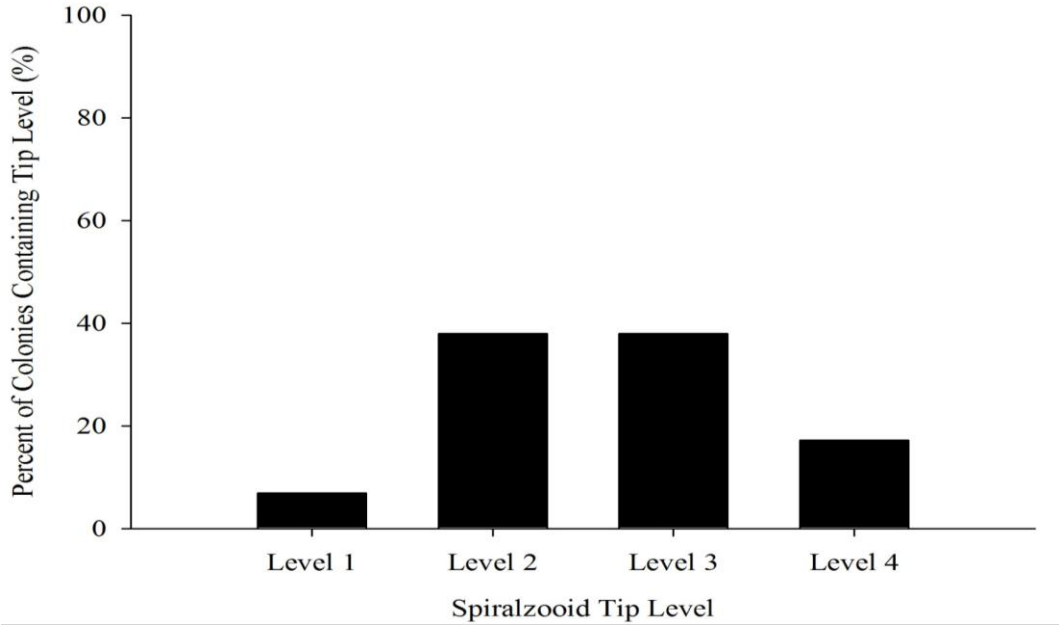

Figure 4: Percent of spiralzooids in each condition level; n (colonies)=30.

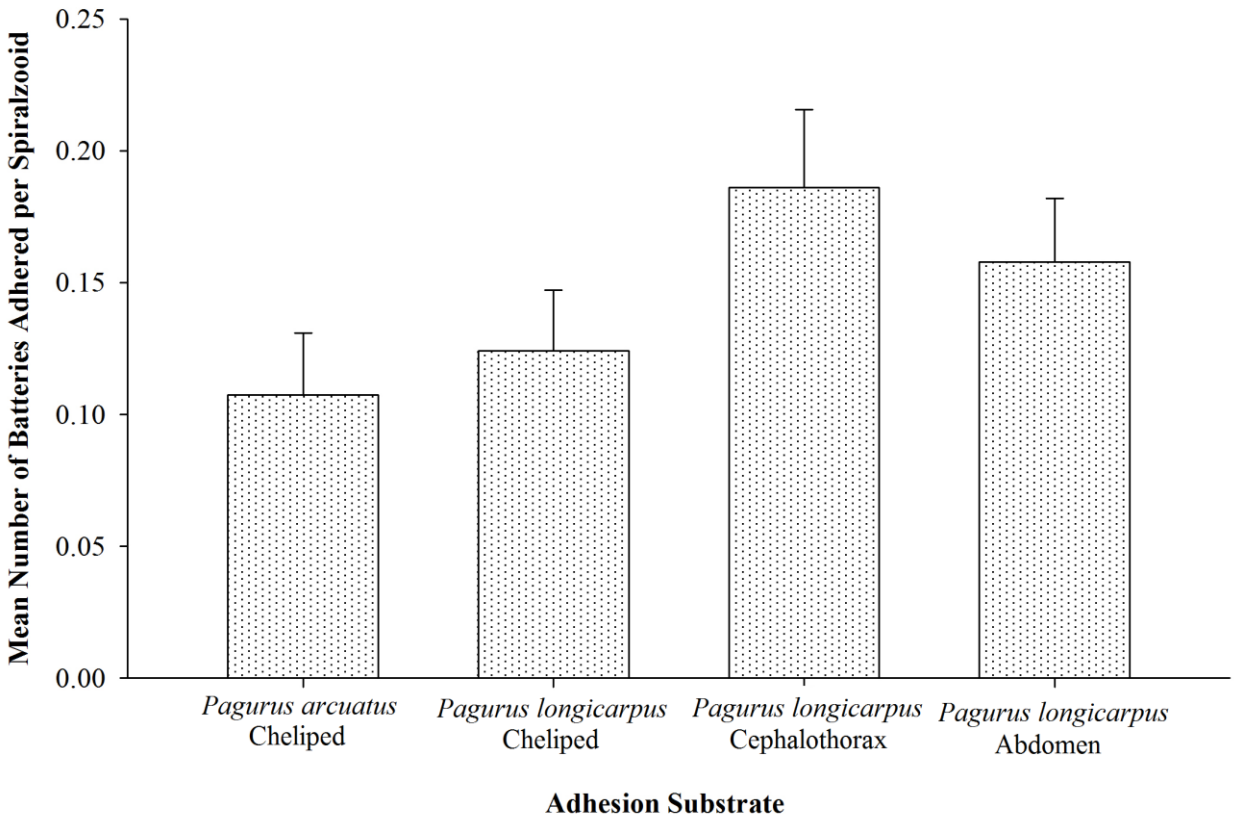

Figure 5: Mean number of spiralzooid batteries adhered per spiralzooid for different substrates; n=30 for each substrate. Error bars represent one standard error.

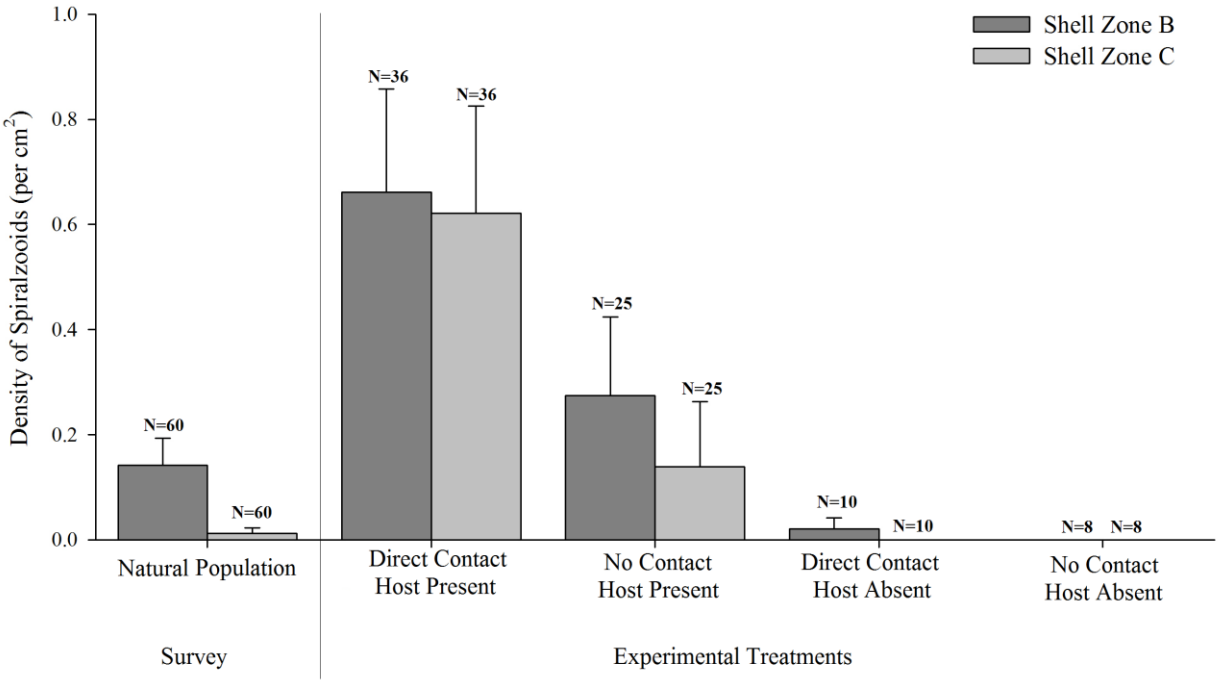

Figure 6: Mean spiralzooid density in shell zones B and C as a function of crab access and density manipulations. Densities in nature are depicted on the left for comparison. Error bars represent one standard error.

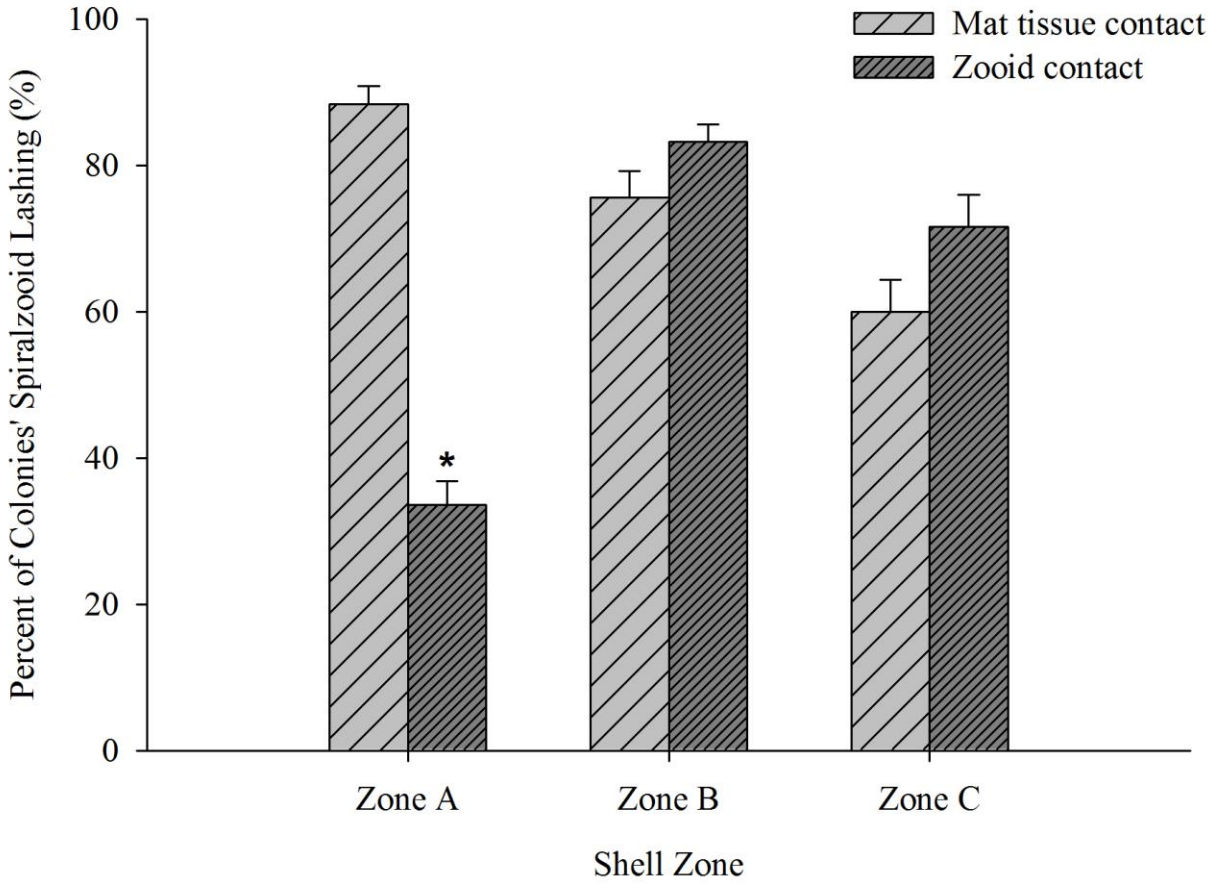

Figure 7: Mean percent of spiralzooids lashing in response to mat tissue contact or zooid contact in shell zones A-C; n=50 for each shell zone and tissue type. Error bars represent one standard error; * denotes the single treatment significantly different from the others at $p<0.05$.

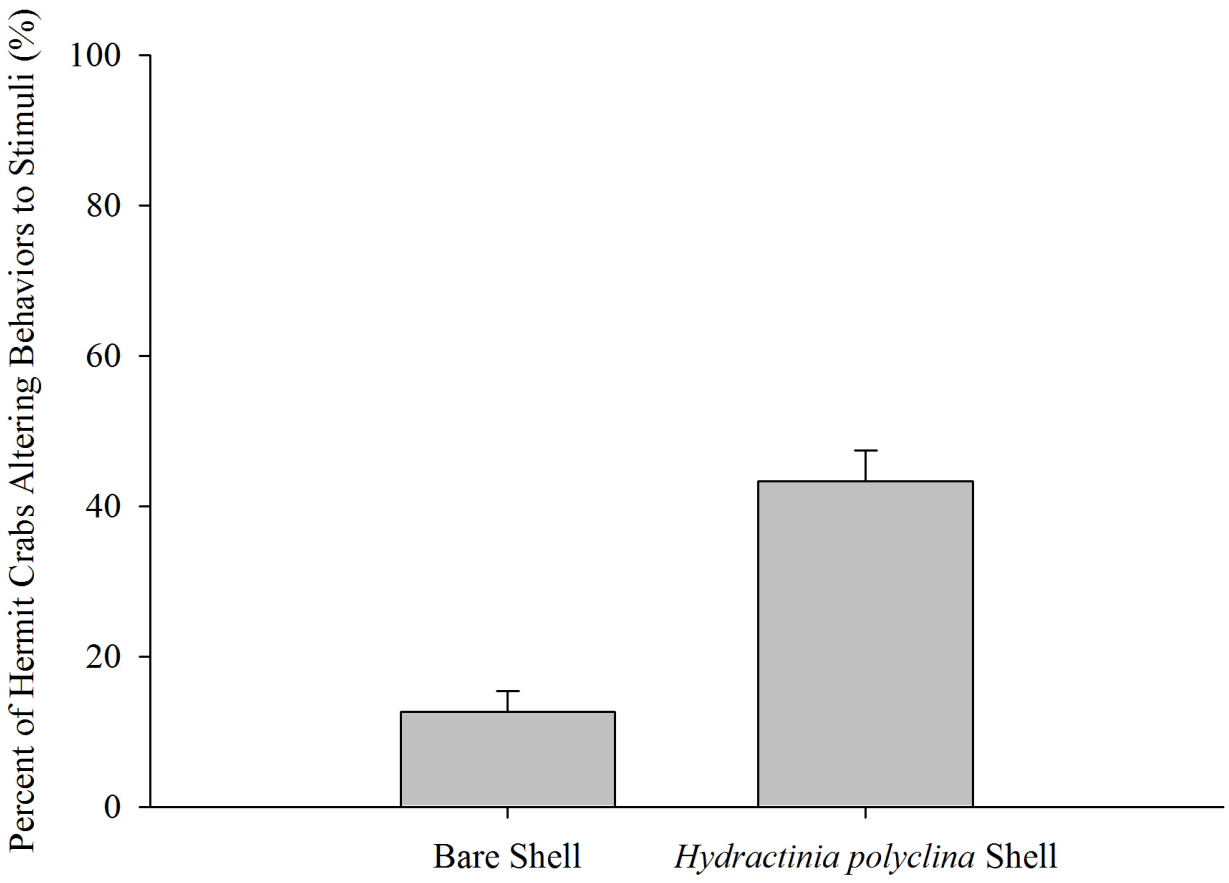

Figure 8: Mean percent of hermit crabs altering their behavior in response to probing, which caused spiralzooids on hydroid-covered shells to lash; n=50 in both treatments.

Table 1: *Hydractinia* spp. literature review of colony attachment substrate type and presence of spiralzooids; * represents colonies that are monomorphic; + represents the presence of spiralzooids; - represents the absence of spiralzooids.

***Hydractinia* spp.**	**Substrate Type(s)**	**Presence of Spiralzooids**	**Location(s)**	**Reference(s)**
H. allmani	*Colus* spp. Gastropod	-	Atlantic Ocean, Greenland Atlantic Ocean, Iceland	Schuchert 2001
H. antonii *	Rock cobble and Shell debris	-	Gulf of Alaska, USA	Miglietta *et al.* 2009
H. angusta	*Adamussium colbecki* Shell bivalve	-	Ross Sea, Antarctica	Cerrano *et al.* 2001
H. areolata	Gastropod shells occupied by Hermit crabs	+	Mediterranean Sea	Bouillon *et al.* 2004
H. borealis	*Tubularia indivisa* Hydroid *Pagurus bernhardus* Hermit crab	- +	Atlantic Ocean, Greenland Atlantic Ocean, Iceland	Schuchert 2000
H. carica	*Buccinium groenlandicum* Shell Gastropod	-	Arctic Ocean, Russia	Schuchert 2001
H. echinata	*Pagurus bernhardus* Hermit crab *Pagurus pollicaris* Hermit crab Rock cobble and Shell debris	+ + -	Gulf of Mexico, USA	Cunningham *et al.* 1991 Mills 1976 Mills 1976
H. milleri *	Rock cobble and Shell debris	-	Pacific Ocean, USA	Cunningham *et al.* 1991
H. polyclina	*Pagurus longicarpus* Hermit crab	+	Gulf of Maine, USA	Folino and Yund 1998
H. pruvoti	*Clibanarius erythropus* Hermit crab	+	Atlantic Ocean	Bavestrello *et al.* 2000
H. sarsii *	Rock cobble and Shell debris	-	Atlantic Ocean, Greenland Atlantic Ocean, Iceland Atlantic Ocean, Norway	Schuchert 2001
H. symbiolongicarpus	*Pagurus longicarpus* Hermit crab	+	Gulf of Maine, USA	Folino and Yund 1998
H. symbiopollicaris	*Pagurus pollicaris* Hermit crab	+	Gulf of Maine, USA	Buss and Yund 1989
H. uniformis *	Rock cobble and Shell debris	-	Atlantic Ocean, Brazil	Stampar *et al.* 2006

Table 2: Results of *Hydractinia polyclina* crosses from within and among the three collection sites: Saco River (SR), Portsmouth Harbor (KH) and Pemaquid Beach (PB).

Hydroid Cross	**Number of Crosses**	**Eggs Released per Cross**	**Mean Percent Fertilization ± Standard Error**
SR vs SR	14	3-36	61.15 ± 1.91%
PB vs SR	7	7-18	69.29 ± 3.58%
PB vs PB	13	3-31	53.14 ± 1.94%
KH vs KH	6	3-21	51.95 ± 3.95%
KH vs PB	4	3-49	67.99 ± 7.76%

Table 3: Percentage of *Hydractinia polyclina* colonies containing spiralzooids across different substrate types and sizes, collection sites, and colony gender (n=212).

Substrate Type	Number of Colonies within Substrate Type	Percent Containing Spiralzooids	Collection Site			Colony Sex			Mean Surface Area (cm^2) ± Standard Error
			PB	KH	SR	♀	♂	NA	
Stones	58	0%	0	52	5	18	17	23	58.62 ±1.68
Boulder/Concrete	5	0%	0	5	0	0	0	5	3251.61 ± 328.26
Bivalve Shell	3	0%	2	1	0	1	0	2	120.04 ± 41.36
Buccinum undatum Shell with Gastropod	2	0%	0	2	0	0	0	2	105.65 ± 10.32
Littorina littorea Shell	23	0%	1	0	22	5	3	8	44.71 ± 0.53
Littorina littorea Shell with Gastropod	11	0%	1	0	10	4	5	2	50.94 ± 2.16
Littorina littorea Shell with *Pagurus longicarpus*	51	100%	51	0	0	29	21	1	11.75 ± 0.06
Littorina littorea Shell with *Pagurus acadianus*	59	100%	51	8	0	22	14	21	13.89 ± 0.09

Table 4: ANOVA results for spiralzooid formation with respect to shell zones and host density DF = degrees of freedom, SS = sum of squares, bold values are significant ($p \leq 05$).

Source of Variation	DF	SS	F	P
host crab (presence and absence)	1	1.372	5.960	**0.016**
shell zone (A, B, or C)	2	4.881	0.0806	0.777
hermit crab cues (physical or chemical)	1	0.066	1.675	0.198
hermit crab cues X host crab	1	1.256	1.534	0.217
hermit crab cues X shell zone	1	0.001	0.0121	0.913
host crab X shell zone	1	0.043	0.0520	0.820
hermit crab cues X host crab X shell zone	1	0.022	0.0274	0.869
Residual	151	123.655	0.819	

Table 5: Mean percent of *Hydractinia polyclina* spiralzooids lashing during six *Pagurus longicarpus* behaviors.

***Pagurus* Behavioral Event**	**N_{crabs}**	**Range of $N_{Behavior\ Events}$ per Crab**	**Mean Percent Spiralzooid Lashing**
Foraging on hydroids	27	2-5	60.23 ± 0.20%
Active Foraging	50	2	0%
Feeding	50	2	0%
Zoea Release	3	1	0%
Fighting	28	2-5	50.08 ± 0.18%
Shell Swapping	8	2-3	16.63 ± 0.61%

Table 6: Calorific values (cal/g dry weight) of potential prey of *Pagurus* hermit crab.
* represents cal/g dry weight of prey that were averaged over several genera and species.

Organism(s)		Cal/g dry wt	Reference(s)
Cnidaria			
Snail Fur	*Hydractinia polyclina*	4003	Current Study
Arthropoda			
Amphipods	Multiple Species*	3761*	Brawn *et al.* 1968
Barnacle	*Balanus balanoides*	4746	Tyler 1973
Mollusca			
Whelk	*Thais lapillus*	4595	Brawn *et al.* 1968
Moon Snail	*Natica clausa*	4392	Brawn *et al.* 1968
Crepidula	*Crepidula convexa*	2908	Tyler 1973
Crepidula	*Crepidula fornicata*	4066	Tyler 1973
Chordata			
Ascidian	*Mogula manhatienis*	3002	Tyler 1973
Chlorophyta			
Green Algae	Multiple Species*	2650*	Lamare and Wing 2001
Phaeophyta			
Brown Algae	Multiple Species*	2610*	Lamare and Wing 2001
Rhodophtya			
Red Algae	Multiple Species*	2460*	Lamare and Wing 2001

APPENDICES

Appendices I-III report preliminary experiments and surveys that were performed as this thesis evolved. However, they represent directions that were ultimately abandoned and hence not integrated into the main methods and results sections of the thesis. Although the work reported in Appendices I and II resulted in significant outcomes, these results do not directly address the broader question of spiralzooid function within *Hydractinia polyclina* colonies. Additionally, the work reported in Appendix III provided only qualitative data. However, the data in these 3 sections do provide potentially useful information on the fundamental biology of spiralzooids.

APPENDIX I: HOST FIT AND SPIRALZOOID LENGTH/DENSITY

Introduction

Hydractinia polyclina have been documented encrusting shells of both *Pagurus longicarpus* and *P. acadianus*, although *H. polyclina* are predominantly found to encrust *P. acadianus* gastropod shells (Yund and Parker 1989, Folino and Yund 1998). Populations of hermit crabs naturally exhibit variation in shell fit due to differing body sizes and variation in shell sizes within a habitat (Conover 1978, McLean 1983). As a host's fit to its gastropod shell becomes tighter, the distance between the hermit crab's cephalothorax and the edge of the aperture of its shell decreases, resulting in the potential for increased contact between spiralzooids and hosts. Findings from this (Figure 6) and previous studies (Braverman 1960), show that contact from host hermit crabs, or any other hermit crab, induces spiralzooid growth at the location of contact.

This appendix expanded upon the idea of hermit crab contact as a stimulus for spiralzooid growth by determining if the fit of a hermit crab to its shell dictates spiralzooid growth characteristics, such as length and/or density. Spiralzooid length and density are important characteristics of colonies that regulate to what extent the polyps are able to interact with their surrounding environments and hosts. In the work reported in this appendix, my goal was to determine if the fit of a *Pagurus* host, due to crab species and/or size relative to its shell, affected spiralzooid length and/or density.

Methods and Materials

Host Fit:

Hermit crabs were measured according to Blackstone's shell fit score (Blackstone 1985) in order to remove potential biases created by comparing different absolute sized hosts. Crabs were probed until they retreated as far into their shell as physically possible. Crabs with the ability to retreat fully into their shells were scored as small relative to their shells. Crabs whose cheliped acted as a tight, but uniform, operculum in the aperture of their shell were scored as a medium fit. Crabs whose cheliped extended past the aperture of their shells were scored as large for their shells.

Spiralzooid Length and Density:

Image analysis was used for zooid length and density measurements. Length data were collected to assess the ability of the spiralzooids to reach various surfaces. Density data were collected to assess how many zooids can contact surfaces. Length data and density data were analyzed separately using two two-way ANOVAs with main factors of host hermit crab species (*Pagurus longicarpus* or *P. acadianus*) and host fit (small, medium, or large) in Sigma Plot. The interaction between the two main effects was also included in the models.

Results

Length and density data were analyzed separately (Figure A1). Spiralzooid density did not vary significantly among fit categories (ANOVA, $F=0.57$, $df=2$, $p=0.567$), but did vary significantly between host species (ANOVA, $F=10.55$, $df=1$, $p=0.001$). The interaction between these two factors was not significant (ANOVA, $F=0.34$, $df=2$, $p=0.713$). Spiralzooid length varied significantly among both fit (ANOVA, $F=4.69$, $df=2$, $p=0.009$) and host species (ANOVA, $F=10.66$, $df=1$, $p=0.001$), and the interaction of these two factors was also significant (ANOVA, $F=7.66$, $df=2$, $p\leq0.001$). Spiralzooids were generally denser on shells occupied by *P. longicarpus* (Figure A1).

APPENDIX II: APERTURE POSITION AND SPIRALZOOID LENGTH/DENSITY

Introduction

Variation in macrohabitat, or physical environment, can have drastic effects on the morphology of sessile invertebrates (Bravermann 1960, Harvell 1984, 1990). Zooid formation can be affected by several external factors including increases in dissolved carbon dioxide (Bravermann 1960), contact with competitors (Harvell 1990), waterborne cues (Harvell 1984, 1990), or attachment substrate (Bravermann 1960). Findings from this study (Tables 1 and 3) show variation in spiralzooid density can occur even within one macrohabitat. This variation suggests that spiralzooid formation is not only affected by the physical environment, but could also potentially be influenced by microhabitats created by the shape of the colony's attachment substrate. *Hydractinia polyclina* that encrusts hermit crab (*Pagurus longicarpus* or *P. acadianus*) occupied gastropod shells appear to experience small-scale variation in microhabitat on different portions of the shell (personal observation).

No previous study has assessed whether variation in microhabitat (i.e., proximity to host or the benthos) affects spiralzooid structure. In the work reported in this appendix, my goal was to determine if the relative position of the host and benthos to the aperture, and the resulting differences in host and benthos contact, affected spiralzooid length and/or density. Spiralzooid length and density are important characteristics of colonies that regulate to what extent zooids are able to interact with their surrounding environment and hosts.

Methods and Materials

Aperture positions:

All *Littorina littorea* shells were dextral (i.e., had right-hand apertures). Four aperture positions were defined with respect to the host hermit crab and benthos: top, right, bottom, and left. The segment of aperture edge that was furthest from the benthos and directly above the host's carapace was classified as the top position. Right positions were defined as segments of the aperture edge that were opposite the spire and not in direct contact with the host's cheliped. Bottom positions were defined as segments of the shell that were closest to, and generally rubbed against, the benthos. Left positions were defined as segments of the aperture edge that were adjacent to the spire and came into direct contact with the host's cheliped. Aperture positions were classified on shells in order to help identify changes in spiralzooid length/density measurements that could be caused by varying contact with the host's cheliped and/or the benthos (i.e., to address whether proximity to the benthos inhibits spiralzooid growth, or if spiralzooids on the top of the aperture are longer or occur at a higher density).

Spiralzooid Length and Density:

Photographic analysis was used for both zooid length and density measurements. Length data were collected to assess whether position affects the ability of the spiralzooids to reach various surfaces. Density data were collected to assess whether position affects how many zooids contact surfaces. Length data and density data were

analyzed separately using two-way ANOVAs in Sigma Plot with main factors of host hermit crab species (*Pagurus longicarpus* or *P. acadianus*) and aperture positions (Bottom, Left, Right, Top). The interaction between the two main effects was also included in the models.

Results

Spiralzooid density varied significantly among aperture positions (Figure A2; ANOVA, F=4.36, df=3, p=0.005) and between host species (ANOVA, F=10.07, df=1, p=0.002). However, the interaction between these two factors was not significant (ANOVA, F=0.16, df=3, p=0.922). Spiralzooid length varied significantly among aperture positions (ANOVA, F=22.13, df=3, $p \leq 0.001$) and between host species (ANOVA, F=12.15, df=1, $p \leq 0.001$). The interaction of these two factors was also significant (ANOVA, F=4.483, df=3, p=0.004). Spiralzooids were generally longer and denser on *P. longicarpus* than on *P, acadianus* crabs, while the length and density patterns among positions were more complex (Figure A2).

APPENDIX III: CHEMICAL/MECHANICAL STIMULI

Introduction

Organisms rely on environmental stimuli in order to interpret and interact with their surroundings. Interacting species, such as prey, predators, and intra- and inter-specific competitors (Madin 1988) provide one class of environmental stimuli. Stimuli from different species are distinguished from one another based upon specific combinations of chemical and mechanical signals. Chemical signals are formed from biochemical compounds such as bio-films or hormones that are presented on an organism's outer surface. Mechanical stimuli are formed from the actual movement of the organism, and vary with intensity of the contact.

Within natural environments, chemical and mechanical stimuli tend to elicit varying responses, depending upon the species providing the stimulus. Many scyphozoans and hydrozoans respond to these combined stimuli by discharging nematocysts and/or to altering zooid behavior (Madin 1988, Östman 2000, Puce *et al.* 2010). These responses initiate foraging in response to prey stimuli and defensive actions in response to predator stimuli (Östman 2000).

However, alteration of zooid behavior in response to different stimuli has never been tested specifically within *H. polyclina*. In this appendix, my goal was to determine if the type of stimulus and the intensity of mechanical stimuli affected the lashing response of spiralzooids. I tested the basic stimulus categories of prey, predators, and competitors. Since *H. polyclina* is a colonial epibiont, I also tested stimuli by polyps from the same

colony to determine zooid behavior in response its own colony, and stimuli from *Pagurus* hermit crabs, such as female egg clutches or the hosts' exoskeleton to determine zooid behavior in response to hosts.

Methods and Materials

Stimuli:

Five categories of combined mechanical and chemical stimuli were tested: host, prey, predator, colony, and competing epibionts. Host cues included contact from exoskeleton and abdomen tissue, eggs, and zoea from *Pagurus longicarpus*. Prey cues included isopods, *Artemia* spp. and fish particulates. Predator cues were from naturally occurring predators of *H. polyclina* such as nudibranchs (*Flabellina* spp.). Colony cues included gastrozooids, gonozooids, spiralzooids, and mat tissue from other *H. polyclina* colonies. Competing epibiont cues were from naturally occurring epibiont competitors of *H. polyclina* including *Crepidula plana* and crustose algae. Stimuli from each class were presented to spiralzooids in zone A. I investigated spiralzooid response to two levels of mechanical/chemical stimuli, strong and weak. Pressure on colony mat tissue was considered a strong stimulus, while pressure on zooids was considered a weak stimulus. Stimuli from all classes were presented in a random order until each stimulus class had received three replicate treatments of approximately 30 seconds of contact. Spiralzooid responses were measured as percent of zooid lashing events per stimuli.

Results

Overall, there were no apparent trends in lashing response among the five classes of stimuli tested (Table A1). However, there were clear differences between mat tissue and zooid contact (corresponding to strong and weak stimuli, respectively) within many of the stimulus categories. Contact with mat tissue tended to provoke more lashing events then zooid contact.

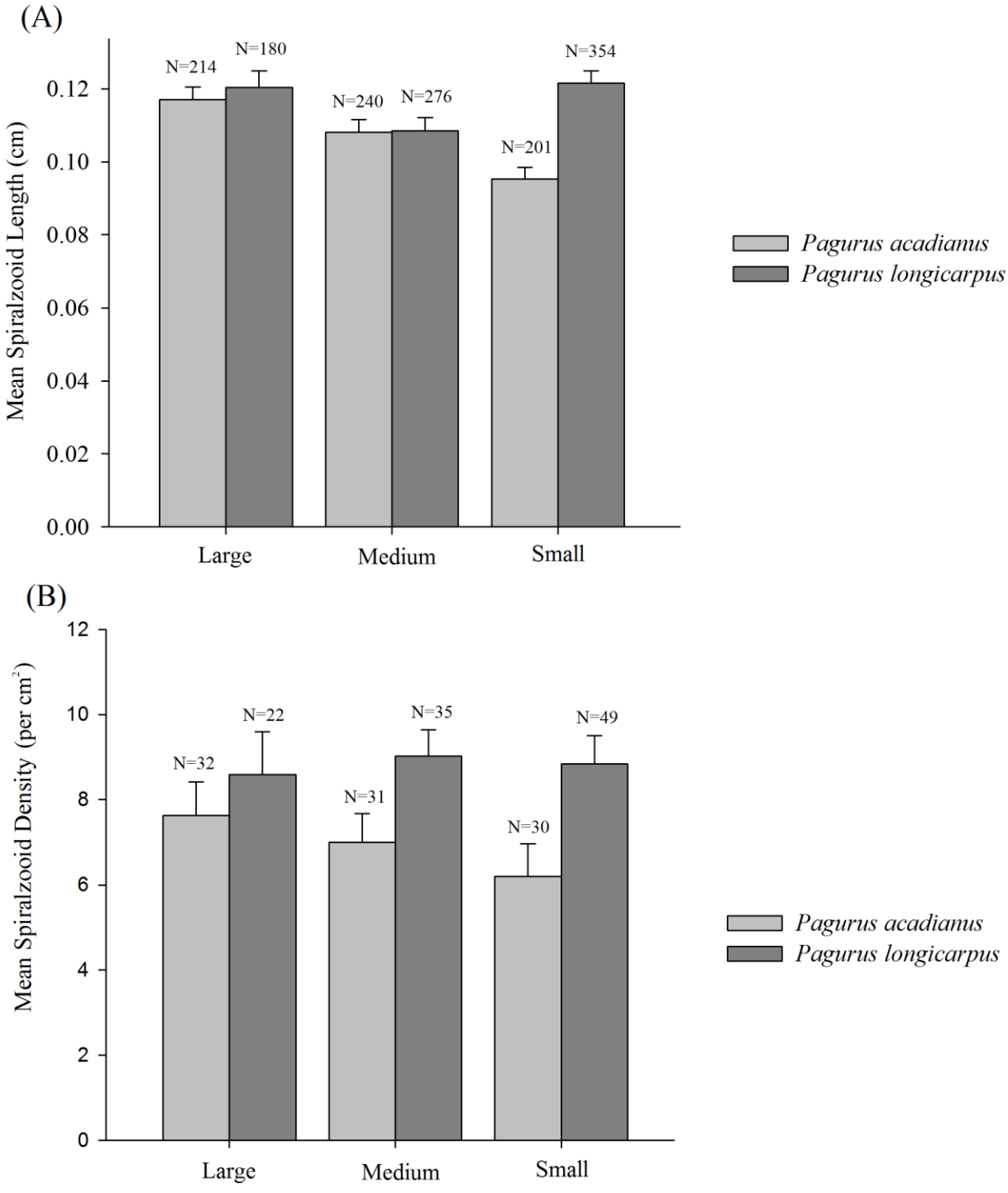

Figure A1: (A) Mean spiralzooid length (cm) and (B) density (per cm^2) with respect to host species and host/shell fit. Error bars represent one standard error.

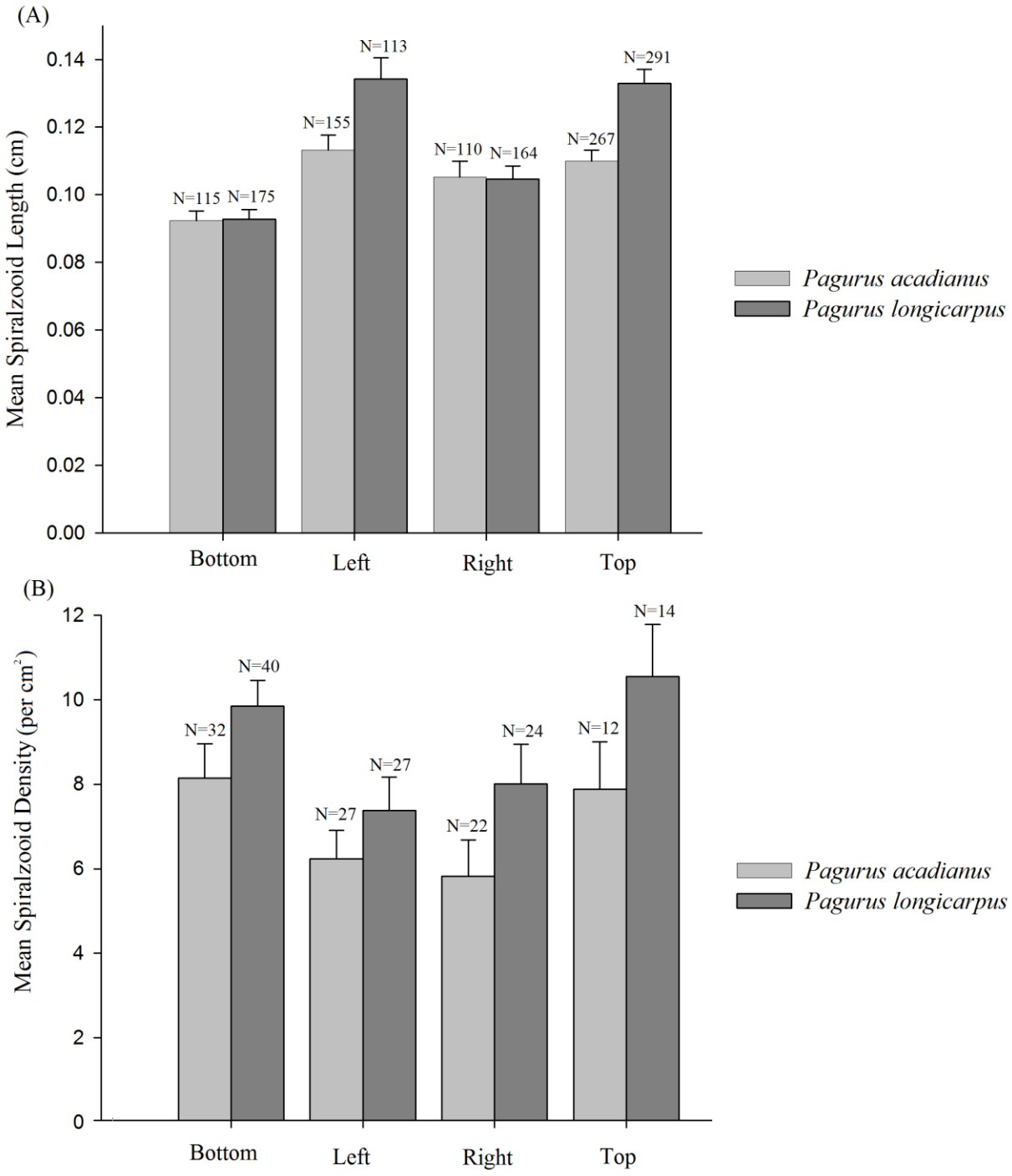

Figure A2: (A) Mean spiralzooid length (cm) and (B) density (per cm^2) with respect to host species and aperture position. Error bars represent one standard error.

Table A1: Mechanical/chemical stimuli effect on *Hydractinia polyclina* spiralzooid lashing.

Mechanical/Chemical Stimuli		(N_{crabs})	($N_{Behavior\ Events}$)	Mean Percent Lashing With Zooid Contact	Mean Percent Lashing With Mat Tissue Contact
Host Stimuli					
	Hermit Crab Exoskeleton	30	3	8.89%	65.52%
	Hermit Crab Abdomen	30	3	3.33%	15.54%
	Un-hatched Eggs	30	3	0.00%	3.33%
	48 Hour Zoea	30	3	2.22%	NA
Prey Stimuli					
	Artemia spp.	30	3	1.11%	8.88%
	Decomposing Fish	30	3	8.89%	25.56%
Predator Stimuli					
	Nudibranch	30	3	1.11%	9.52%
Colony Stimuli					
	Gastrozooids	30	3	8.89%	40.00%
	Gonozooids	30	3	6.24%	41.11%
	Spiralzooids	30	3	5.55%	38.89%
	Mat Tissue	30	3	6.24%	56.21%
Epibiont Stimuli					
	15 scale Worm	30	3	0.00%	1.11%
	Crepidula spp.	30	3	29.97%	77.44%
	Crustose Algae	30	3	7.78%	27.78%