

# Assessing the antipredatory defensive strategies of Caribbean non-scleractinian zoantharians (Cnidaria): is the sting the only thing?

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**Abstract** The relative importance of chemical, nematocyst, and nutritional defenses was examined for 18 species of Caribbean sea anemones (actinarians), zoanths, and mushroom polyps (corallimorpharians) from the Florida Keys and the Bahamas Islands, 2008–2010. Feeding assays were performed using the fish *Thalassoma bifasciatum* with artificial foods containing crude organic extracts of cnidarian tissues. A novel behavioral assay using brine shrimp nauplii was used to assess nematocyst defenses. The nutritional quality of cnidarian tissues was examined using bomb calorimetry and soluble protein assays. In general, actinarians invested in nematocyst defenses, zoanths in either nematocyst or chemical defenses, and corallimorpharians lacked both, except for 1 of 3 species that was chemically defended. Relative to other coral reef invertebrates, cnidarian tissues had similar caloric values but lower soluble protein concentrations. Trade-offs between chemical and nematocyst defenses were observed for 65% of species, while habitat and behavior provided a likely explanation for undefended species.

## Introduction

Resource trade-offs arise in nature because the energy available to organisms for key physiological tasks, including growth, reproduction, and defenses against predators

and pathogens, is finite (Stearns 1992). The role of resource trade-offs in ecology has been a subject of great interest to researchers for many years (reviewed by Zera and Harshman 2001) and has been particularly well documented in terrestrial plants (Felton and Korth 2000; Spoel et al. 2007; Kaplan et al. 2009). For example, trade-offs between anti-herbivore defenses and fungal resistance have been described for the lima bean, *Phaseolus lunatus*, which is able to resist fungal infection or produce hydrogen cyanide to deter herbivore grazing but cannot simultaneously do both (Ballhorn et al. 2010).

More recently, documentation of physiological resource trade-offs has been extended to sessile organisms in marine ecosystems. Coral reef sponges exhibit reduced healing and growth rates at the expense of producing secondary metabolites that deter predatory fishes (Walters and Pawlik 2005; Leong and Pawlik 2010a). Trade-offs between physical and chemical defensive mechanisms have also been demonstrated for hydroids (Stachowicz and Lindquist 2000). However, physiological resource trade-offs have not been observed in all benthic invertebrates. For example, no clear pattern between secondary metabolite and inorganic acid defenses was exhibited by ascidians (Pisut and Pawlik 2002). While some studies of sessile marine invertebrates have demonstrated evidence of physiological resource trade-offs, the importance of these trade-offs in the ecology of Caribbean non-scleractinian zoantharians, the sea anemones, zoanths, and corallimorpharians (Phylum Cnidaria: Orders Actiniaria, Zoanthidea, and Corallimorpharia), remains unexplored.

Non-scleractinian zoantharians can be an important component of the benthic community on some coral reefs (Work et al. 2008). Although coral reef ecosystems are known for high levels of predation pressure (Wood 1993), non-scleractinian zoantharians are rarely observed to be

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consumed by generalist predators (Randall 1967), implying the presence of defenses. Because non-scleractinian zoantharians lack a calcareous or proteinaceous endoskeleton common to scleractinian and antipatharian corals, respectively (Daly et al. 2003), alternative defensive mechanisms may be present. Moreover, because species within these taxa may employ a variety of defensive mechanisms, non-scleractinian zoantharians are a particularly relevant group for testing resource allocation theory. Historically, it has been presumed that many cnidarians rely on specialized stinging structures called nematocysts (a type of cnida) to inject venom as a defense against predation (Mariscal 1974; Basulto et al. 2006). Highly toxic compounds including actinoporins, equinatoxins, and sticholysins have been isolated and chemically characterized from inside the nematocysts of some non-scleractinian zoantharians in the families Aiptasiidae, Actiniidae, Stichodactylidae, and Metridiidae (Anderluh and Macek 2002; Martinez et al. 2002; Alvarez et al. 2009). However, other toxic compounds, most notably palytoxin, have been isolated from outside the nematocysts (Moore and Scheuer 1971; Fautin 2009), suggesting that their function is not linked to nematocyst firing. Furthermore, some cnidarians, such as gorgonians, rarely possess a heavy armament of nematocysts and rely instead on chemical defenses to deter predation (Pawlik et al. 1987; O'Neal and Pawlik 2002). Some non-scleractinian zoantharians can incorporate inorganic material such as sand into their bodies, but these structures likely provide little protection against predation because similar structures in sponges and gorgonians have little effect on predation (Chanas and Pawlik 1995; O'Neal and Pawlik 2002). As a result of the types of defensive mechanisms available to non-scleractinian zoantharians, these cnidarians may rely on chemical or nematocyst defenses, or a combination of both types of defense.

Another possible defensive strategy for non-scleractinian zoantharians is low nutritional quality. Poor food value has been proposed as a defensive mechanism in both plants and animals (Van der Meijden et al. 1984; Duffy and Paul 1992). For example, it has been proposed that the percentage of digestible tissue in some nudibranchs is lower than that of shelled mollusks of similar size, enhancing their chemical defense (Penny 2002). If the nutritional value of a prey species is very low, as in some gelatinous invertebrates, generalist predators may choose other prey items. As a result of these considerations, inclusion of nutritional analyses into studies of defensive strategies has become standard in recent years (Pawlik 2011).

Finally, both behavior and distribution may influence the defensive strategies used by non-scleractinian zoantharians. Unlike some sessile organisms, soft-bodied, benthic cnidarians may be able to avoid predation by holding themselves close to the substratum (Rupert et al. 2004). Furthermore,

differences in predation pressure between habitats may alter the selective pressure for producing defensive mechanisms. Therefore, when considering possible trade-offs in resource investment, it is important to consider whether antipredatory defenses are necessary at all, given the behavior or distribution of the organism of interest relative to potential predators.

In this study, we evaluated chemical, nematocyst, and nutritional defenses of 18 species of non-scleractinian zoantharians from the Florida Keys and the Bahamas Islands with the objective of determining whether resource trade-offs exist among these defensive mechanisms. In addition to performing relevant assays to assess defensive capabilities, the habitats of each species were observed and recorded. Under the assumption that resources are limited and both chemical and nematocyst defensive mechanisms are similarly effective, we hypothesized an inverse relationship between nematocyst and chemical defenses in Caribbean non-scleractinian zoantharians. In accord with other studies (Penny 2002), we hypothesized no relationship between nutritional quality and defensive strategies, as lowered food value has been hypothesized to contribute to other defensive mechanisms rather than supplant them.

## Materials and methods

### Cnidarian collections

Specimens of 18 species of non-scleractinian zoantharians were collected from shallow reefs and mangroves during six expeditions to the Florida Keys and the Bahamas Islands between June 2008 and July 2010 (Table 1). All species that were encountered during sampling, and could be positively identified according to the guidelines described in Humann and DeLoach (2002), were included in this study. Samples from the Florida Keys were collected at Bird Sanctuary (N 25°1'58.54" W 080°30'19.09"), Carysfort Reef (N 25°14'54.72" W 080°11'47.46"), Conch Reef (N 24°56'59.76" W 080°27'13.38"), Harry Harris Park (N 25°1'25.36" W 080°29'35.99"), Jewfish Creek (N 25°10'58.59" W 080°23'25.99"), North Dry Rocks (N 25°7'51.00" W 080°17'31.26"), and Thurmond Street (N 25°5'22.62" W 080°26'59.36"). Collection sites in the Bahamas were Bimini (N 25°42'52.86" W 079°17'36.90"), Cat Cay (N 25°31'51.30" W 079°16'16.62"), Compass Cay (N 24°16'17.46" W 076°30'20.22"), Little San Salvador (N 24°34'16.85" W 075°55'57.77"), Northern Exumas (N 24°46'27.60" W 076°49'2.76"), San Salvador (N 24°9'4.66" W 074°28'25.94"), Stirrups Cay (N 25°49'45.51" W 077°54'49.80"), and Sweetings Cay (N 26°33'39.84" W 077°52'32.28"). Careful records were kept of the habitats in which species were collected, specifically regarding the

**Table 1** Summary of cnidarian species name, collection habitat, and defensive strategies for 18 species of non-scleractinian zoantharians examined in this study

Habitats were classified as mangrove (*M*), reef (*R*), seagrass bed (*S*), and intertidal (*I*). The presence and absence of chemical and physical defenses is indicated by a + or –, respectively. A ± symbol indicates a variable defense. *ND* indicates no data. Animals were classified as having both chemical and nematocyst defenses (Both), nematocyst defenses but no chemical defenses (Nem.), chemical defenses but no nematocyst defenses (Chem.), or neither type of defense (Neither)

	Habitat collected	Mean depth collected (m)	Chemical defenses	Nematocyst defenses	Defensive strategy
Order Actiniaria					
<i>Actinia bermudensis</i>	R	7.6	+	+	Both
<i>Actinostella flosculifera</i>	R	7.6	±	+	Both
<i>Aiptasia</i> sp.	M	1.1	–	+	Nem.
<i>Bartholomea annulata</i>	M, R, S	2.8	–	+	Nem.
<i>Bunodeopsis antillensis</i>	S	0.9	–	+	Nem.
<i>Condylactis gigantea</i>	M, R, S	3.2	–	+	Nem.
<i>Epicystis crucifer</i>	R	3.4	–	+	Nem.
<i>Lebrunia danae</i>	R	4.3	–	+	Nem.
<i>Ragactis lucida</i>	R, S	5.9	–	+	Nem.
<i>Stichodactyla helianthus</i>	I, M, S	1.7	–	+	Nem.
Order Zoanthidea					
<i>Palythoa caribbaeorum</i>	R	8.6	±	+	Both
<i>Palythoa mammilosa</i>	R	7.6	+	+	Both
<i>Zoanthus pulchellus</i>	R	12.2	+	–	Chem.
<i>Zoanthus sociatus</i>	R	7.6	+	–	Chem.
<i>Zoanthus</i> sp.	R	4.6	–	–	Neither
Order Corallimorpharia					
<i>Discosoma sanctithomae</i>	R	2.0	+	–	Chem.
<i>Pseudocorynactis caribbaeorum</i>	R	6.1	–	ND	ND
<i>Ricordea florida</i>	R	8.4	–	–	Neither

presence of predatory fishes and whether specimens were exposed or in cryptic locations. Whole, undamaged, living animals were collected and transported to the laboratory for nematocyst experiments and then frozen and stored at –20°C before tissue extraction for chemical defense and nutritional assays. Because many non-scleractinian zoantharians can reproduce asexually, specimens for each replicate experiment were obtained from sites that were at least 1 km apart to prevent the use of clones. When necessary to obtain sufficient tissue mass, multiple specimens of a species collected from a single site were pooled and treated as a single replicate.

#### Chemical defense assays

Frozen cnidarian tissue was thawed to room temperature and then measured volumetrically by displacement of extraction solvents. Extraction and feeding assay techniques were performed as described in Pawlik et al. (1995). Briefly, freshly thawed wet tissue was extracted twice with agitation for 24 h at 4°C, first in 1:1 dichloromethane/methanol (DCM:MeOH) and then in 100% MeOH, which extracted secondary metabolites from the tissues without potentially extracting proteinaceous venoms from nematocysts. Both extracts were combined after rotary evaporation and vacuum centrifugation. The resulting extract was mixed at its natural volumetric concentration into an arti-

cial food matrix made of alginic acid, powdered squid mantle, and water, extruded through a syringe into a solution of calcium chloride to form a string from which food pellets were cut, and the pellets presented to *Thalassoma bifasciatum* (bluehead wrasse), a generalist predator common on Caribbean coral reefs. Artificial food pellets without tissue extracts were used as controls. Each assay consisted of ten trials, in which extract-treated pellets were either accepted or rejected. Extract-treated pellets were considered to be rejected if they were mouthed and spit out by the fish three times or spit out and subsequently ignored. Trials were considered to be valid only if fish consumed control pellets immediately after rejecting extract-treated pellets, indicating that they were not satiated.

#### Cnida assays

A novel assay was developed to assess the severity of the sting delivered by the nematocysts of non-scleractinian zoantharians. Live cnidarians were placed in glass observation dishes and allowed to acclimate for several hours in a well-illuminated coral reef display aquarium until their pedal disks had attached to the glass or the polyps were fully emergent. The observation dishes were then placed under a dissecting microscope and viewed at 10–20 times magnification, depending on the size of the animal. Live brine shrimp nauplii (*Artemia* sp.), 1–3 days post-hatching,

**Table 2** Ranking system for the three reaction variables used for cnida assays: tentacle reaction, tentacle stickiness, and reaction by nauplii of *Artemia* (brine shrimp)

	Criteria
Tentacle reaction	
0	Tentacle has no reaction to <i>Artemia</i>
1	Tentacle reacts to <i>Artemia</i> briefly by moving, but no stinging is observed
2	Tentacle reacts to <i>Artemia</i> by moving with apparent stinging
3	Tentacle quickly jerks toward <i>Artemia</i> with clear stinging
Tentacle stickiness	
0	Tentacle does not adhere to <i>Artemia</i>
1	Tentacle adheres to <i>Artemia</i> for fewer than 3 s; <i>Artemia</i> escapes
2	Tentacle adheres to <i>Artemia</i> for 3 s or longer; <i>Artemia</i> escapes
3	Tentacle adheres to <i>Artemia</i> indefinitely; <i>Artemia</i> does not escape
Reaction by <i>Artemia</i>	
0	<i>Artemia</i> does not react to contact with tentacle
1	<i>Artemia</i> slows pulsing of antenna or alters swimming pattern
2	<i>Artemia</i> experiences immediate paralysis of antenna, antennula, or mandible
3	<i>Artemia</i> dies immediately

were introduced to the dishes one at a time. Nauplii of *Artemia* were chosen to gauge nematocyst effects because they provide the simultaneous mechanical and chemical stimuli required by most nematocysts to fire (Thorington and Hessler 1998; Watson et al. 1998) and can be introduced to the tentacles of cnidarians in a controlled manner. For each experiment, three response metrics were recorded using standardized qualitative scales: (1) the intensity of the tentacle reaction to contact with nauplii, (2) the duration of adherence of nauplii to the tentacle (tentacle stickiness), and (3) the reaction of nauplii to contact with the tentacle. The ranking system for each scale is detailed in Table 2. Ten interactions in each category were scored and averaged for each animal tested. As a control, identical assays were run using an artificial anemone made from inert twine ( $N = 3$ ). Cnidarian species were considered to possess nematocyst defenses if the score for at least two of the three response metrics (tentacle reaction, tentacle stickiness, and reaction by *Artemia*) was significantly greater than the score obtained using the control artificial anemone, which was always zero (Dunn's two-tailed analysis, see "Data Analyses").

The ecological relevance of results from laboratory cnida assays was further tested with field assays conducted in San Salvador, Bahamas Islands. Three species (actinians *C. gigantea* and *L. danae*, and corallimorpharian *R. florida*) were selected for testing because it was determined that they lacked chemical defenses but possessed varying nematocyst defenses. Freshly cut pieces of each species, collected on reefs of the same island, were presented to a group of reef fishes by a diver kneeling on the reef at 5 m depth. Several species of common reef fishes from the families Balistidae, Carangidae, Haemulidae, and Labridae were present. Control food pieces, composed of alginic acid

and powdered squid mantle matrix and similar in size to tissue pieces, were presented to fish immediately before and after cnidarian tissue pieces to verify that the natural aggregation of reef fishes was feeding. Pieces of cnidarian tissue were considered to be rejected if they were mouthed and spit out by the fish three times or spit out and subsequently ignored. Assays were performed haphazardly with shifting groups of fishes as divers moved from place to place on the same reef. Four to six replicate tissue samples were assayed for each species tested.

#### Nutritional analyses

Tissues of selected cnidarian species were subjected to two analyses of nutritional quality: one to determine total energy content and one to determine soluble protein content. Eight species, representing each of the three orders of non-scleractinian zoantharians, were tested because sufficient tissue mass for nutritional analyses was available: *B. annulata*, *C. gigantea*, *E. crucifer*, *L. danae*, *R. lucida*, *S. helianthus*, *P. caribbaeorum*, and *R. florida*.

Total energy content of cnidarian tissues was determined using bomb calorimetry. Whole specimens were dried in an oven for 5 days at 60°C and the dry tissue mass determined. The resulting dried tissue was powdered with a mortar and pestle and combusted in an IKA-WERKE C2000 bomb calorimeter to measure caloric value. Replicate analyses for samples collected from 1 to 6 geographically separate sites were conducted for each species.

To examine soluble protein content, a colorimetric protein assay was conducted using a Pierce bicinchonic acid protein assay kit (Thermo Scientific). As with the bomb calorimetry protocol, whole specimens were dried in an oven for 5 days at 60°C and powdered with a mortar and pestle.

Powdered dry tissue (0.25 g) from each specimen was rehydrated with 5 ml of deionized water for 2 h. The tissue was homogenized with deionized water and incubated at 4°C for 24 h, and then the homogenization and incubation processes were repeated. The resulting solution, which contained the soluble proteins, was filtered through Celite. A Thermo Multiskan Ascent plate reader and Ascent software were used to measure the absorbance of the extracts at 540 nm in a 96-well plate. Measurements were compared to a standard curve created using bovine serum albumin (BSA). Replicate analyses for samples collected from 1 to 4 geographically separate sites were conducted for each species.

Because predators consume prey volumetrically rather than gravimetrically (Pawlik 2011), conversion factors for dry mass to wet volume were generated for each species (Pawlik et al. 1995). The volumes of cnidarian tissue were measured by water displacement. Samples were then dried and weighed to calculate dry mass to wet volume ratios. Both total energy content and soluble protein content were converted to units per wet volume.

#### Data analysis

For feeding experiments to assess chemical defenses, Fisher's exact test (one-tailed) was used to determine the palatability of each extract from 10 trial assays (Pawlik et al. 1995). An extract was considered to be deterrent if six or fewer pellets were accepted ( $p \leq 0.0433$ ). If the mean assay result for a species was six or fewer pellets eaten out of ten, a species was considered to be chemically defended (Pawlik et al. 1995).

For cnida assays, mean scores for tentacle reaction, tentacle stickiness, and the reaction by nauplii of *Artemia* were compared using linear regression analysis. Among-species comparisons were made with a Kruskal–Wallis test. Post hoc multiple comparisons were performed with a Dunn's two-tailed analysis (Zar 1999).

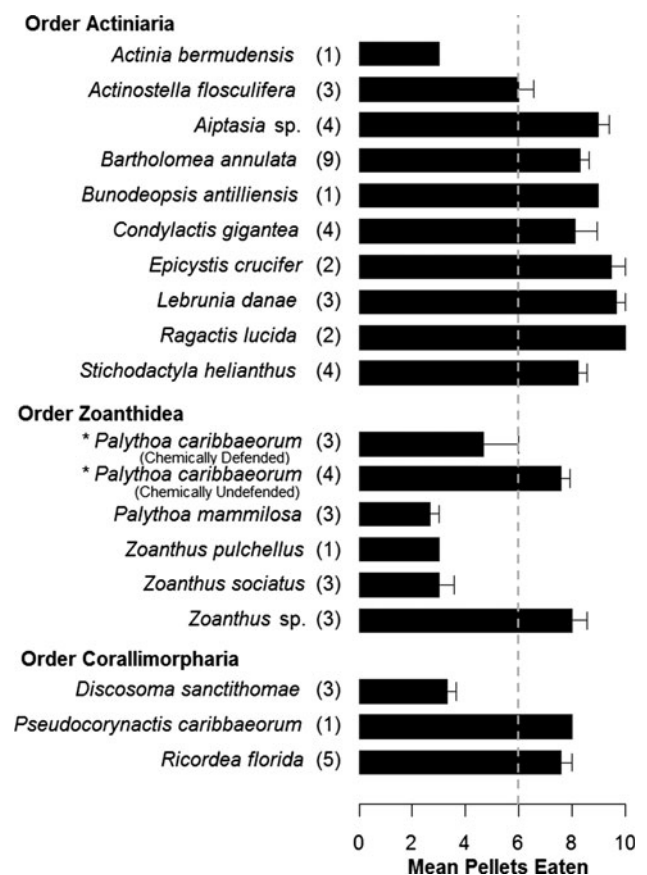
Total energy content and soluble protein content were compared among species with one-way ANOVA. Post hoc multiple comparisons were made with a Tukey multiple comparisons test with unequal sample sizes. Analyses were conducted with XLSTAT version 2010.5.01.

## Results

### Chemical defense assays

Tissue volumes of the cnidarians used for organic solvent extraction ranged from 1 to 10 mL. At natural volumetric concentrations, crude extracts from 5 of the 18 species examined (*A. bermudensis*, *D. sanctithomae*, *P. mammil-*

*osa*, *Z. pulchellus*, and *Z. sociatus*) exhibited consistent feeding deterrent activity in assays with *Thalassoma bifasciatum* (Fig. 1). For two species, *A. flosculifera* and *P. caribbaeorum*, extracts were deterrent in 66 and 43% of assays, respectively. Assay results for *A. flosculifera* were analyzed as a single category because the number of pellets consumed in trials was similar (mean  $\pm$  SD,  $6 \pm 1$ ,  $N = 3$ ), while those for *P. caribbaeorum* were analyzed in two categories (chemically defended and chemically undefended) because extracts were markedly different, either strongly deterrent or exhibiting little impact on fish consumption (Fig. 1). No relationship was observed between collection depth and the presence of chemical defenses (Table 1). All of the species with deterrent extracts were found in reef habitats, while none of the species found in other habitats possessed deterrent extracts. Of the surveyed species found only on reefs, 58% had deterrent extracts (Table 1). No



**Fig. 1** Mean ( $\pm$ standard error) number of pellets containing a natural concentration of crude organic tissue extract that were eaten by *Thalassoma bifasciatum* in 10 trials for the 18 species of cnidarians surveyed in this study. Extracts were deterrent if six or fewer pellets were eaten (dotted line;  $p \leq 0.0433$ , Fisher's one-tailed exact test). Replication indicated in parentheses, showing total number of geographically distant tissue samples prepared and tested in assays. Error bars represent standard error. Asterisk represents single species split into two categories due to clear differences seen in defensive chemistry among individual samples

relationship was observed between deterrent activity and taxonomy across the orders examined (Actinia, Zoanthidea, and Corallimorpharia); both chemically defended and chemically undefended species were observed in all three orders, but a higher proportion of chemically defended species was found in the Zoanthidea.

Cnida assays

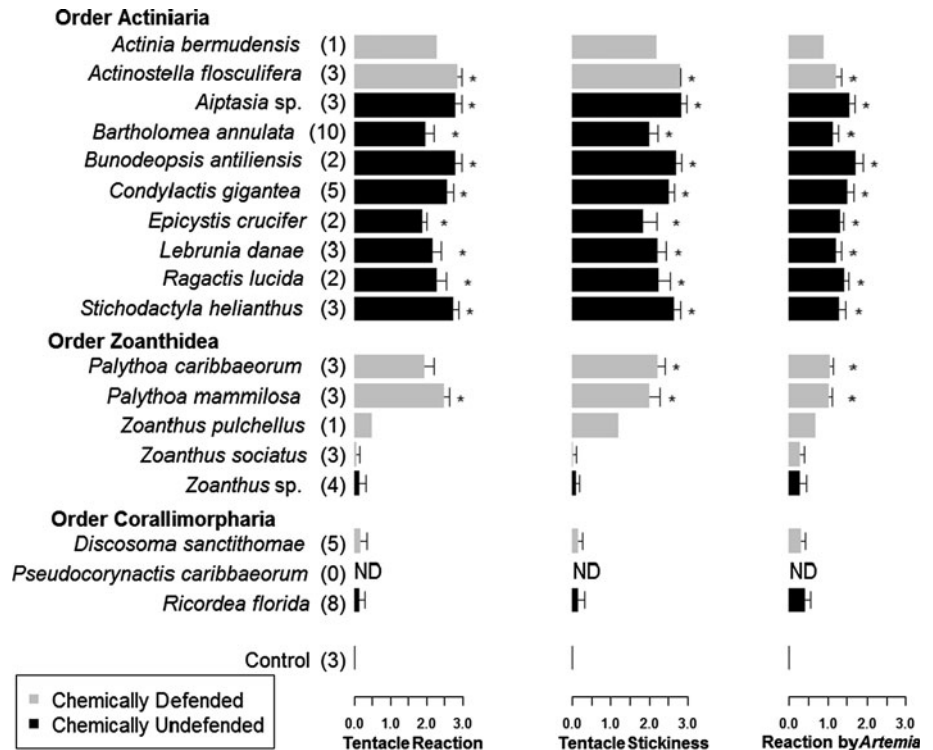
All three cnida assay response metrics were strongly correlated with one another. Tentacle reaction and tentacle stickiness showed the strongest correlation (linear regression,  $r^2 = 0.95$ ,  $F_{(1,15)} = 319.1$ ,  $p < 0.0001$ ), while tentacle reaction and reaction by nauplii of *Artemia* (linear regression,  $r^2 = 0.84$ ,  $F_{(1,15)} = 84.57$ ,  $p < 0.0001$ ) and tentacle stickiness and reaction by nauplii of *Artemia* (linear regression,  $r^2 = 0.86$ ,  $F_{(1,15)} = 106.7$ ,  $p < 0.0001$ ) displayed slightly weaker relationships. The strongest interactions were observed for actinarians, and the weakest interactions were observed for corallimorpharians. Kruskal–Wallis test performed on among species results for each response metric yielded highly significant results in all cases (tentacle reaction Kruskal–Wallis test,  $H_{13} = 50.59$ ,  $p < 0.001$ , tentacle stickiness Kruskal–Wallis test,  $H_{13} = 51.01$ ,  $p < 0.001$ , reaction by nauplii of *Artemia* Kruskal–Wallis test,  $H_{13} = 51.92$ ,  $p < 0.001$ ). Multiple comparisons indicated that, when statistics could be conducted, all actinarians examined had at least two response metrics that grouped

significantly higher than the corresponding metrics of the artificial control anemone (Dunn's two-tailed analysis,  $p \leq 0.05$ ). Response metrics for corallimorpharians were not significantly different than those of the artificial control anemone, while those for zoanthideans varied by genus. Members of the genus *Palythoa* possessed at least one interaction metric that grouped significantly higher than those of the artificial control anemone (Dunn's two-tailed analysis,  $p \leq 0.05$ ) and members of the genus *Zoanthus* did not (Fig. 2).

In field assays, freshly cut pieces of *R. florida* ( $n = 4$ ) were readily consumed by reef fish predators, but pieces of *C. gigantea* ( $n = 6$ ) and *L. danae* ( $n = 4$ ) were not. Tissue for a given species was either always consumed or always rejected. Consumption by reef fishes was inversely related to the presence of nematocyst defenses determined by laboratory assays (Fig. 2).

Of the 17 species for which both chemical defense and nematocyst data were collected, 8 (*Aiptasia* sp., *B. annulata*, *B. antillensis*, *C. gigantea*, *E. crucifer*, *L. danae*, *R. lucida*, and *S. helianthus*) exhibited nematocyst defenses but not chemical defenses. Three species (*D. sanctithomae*, *Z. pulchellus*, and *Z. sociatus*) exhibited chemical defenses but not nematocyst defenses, four species (*A. bermudensis*, *A. flosculifera*, *P. caribbaeorum*, and *P. mammilosa*) exhibited both types of defensive strategies, and two species (*R. florida* and *Zoanthus* sp.) exhibited neither strategy (Table 1).

**Fig. 2** Mean ( $\pm$ standard error) rank for tentacle reaction, tentacle stickiness, and reaction by nauplii of *Artemia* for behavioral assays with each of the 18 species of non-sclereactinan zoatharians surveyed in this study. Black bars indicate chemically undefended species, and gray bars indicate species with chemical defenses. Error bars show standard error. Geographically distant replicate specimens shown in parentheses. Species with no nematocyst data marked ND. Significant differences from artificial control anemone marked with asterisk (Dunn's two-tailed  $p \leq 0.05$ )



Nutritional assays

Total energy content of the species examined ranged from 1.6 to 4.2 kJ mL<sup>-1</sup> with a mean (±SD) of 2.9 ± 0.7 kJ mL<sup>-1</sup>, N = 22. Significant differences were detected among the means (ANOVA,  $F_{(5,14)} = 16.0$ ,  $p < 0.0001$ ), and 4 species (*B. annulata*, *R. lucida*, *S. helianthus*, and *R. florida*) had significantly higher mean total energy content than that of *L. danae* (Tukey's HSD,  $p < 0.05$ ; Fig. 3). *C. gigantea* had significantly higher energy content than all other species except for *R. lucida*, and *L. danae* had significantly lower energy content than all other species except for *E. crucifer* (Tukey's HSD,  $p < 0.05$ ; Fig. 3). The zoanthidean *P. caribbaeorum* was excluded from total energy content analysis because inorganic material in the colony matrix did not combust completely.

The soluble protein content of species examined ranged from 0.6 mg to 8.4 mg mL<sup>-1</sup> with a mean (±SD) of 4.4 ± 2.2 mg mL<sup>-1</sup>, N = 16 (Fig. 4). Significant differences were observed among the mean soluble protein content of the species tested (ANOVA,  $F_{(4,9)} = 4.71$ ,  $p = 0.025$ ). Soluble protein content was significantly higher in *S. helianthus* compared to *P. caribbaeorum* (Tukey's HSD,  $p = 0.004$ ). No significant differences were detected between the other species tested.

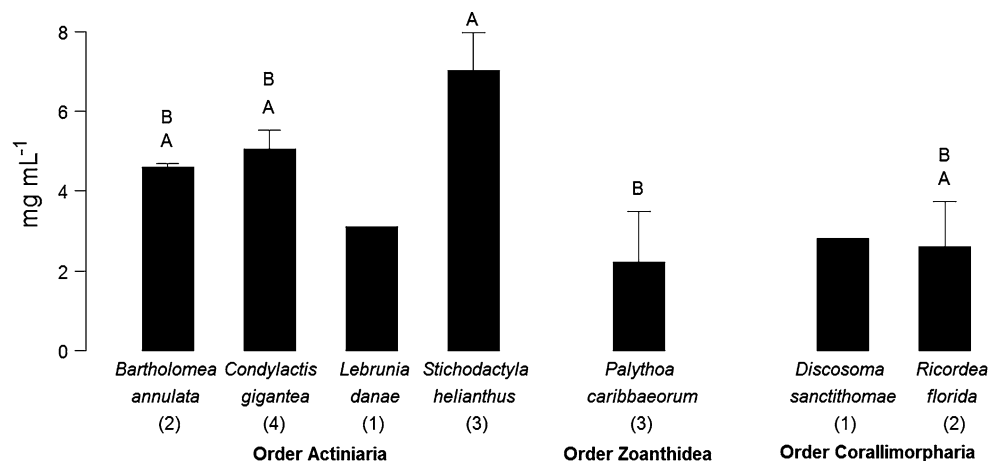
Discussion

Is there evidence for resource trade-offs in the defenses of non-scleractinian zoantharians?

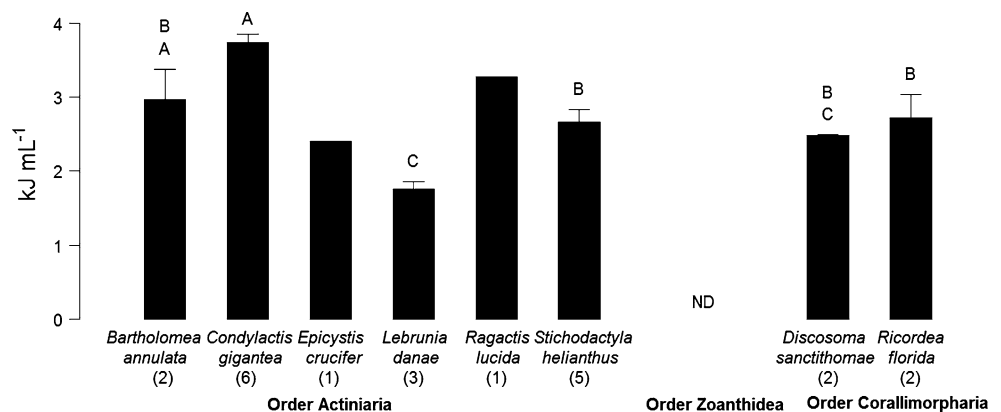
Chemical defenses were present in at least one species of all three orders of cnidarians examined in this study, and the presence of chemical defenses was most frequently associated with a lack of nematocyst defenses. The majority (65%) of the species examined had either chemical or nematocyst defenses, but not both, indicating that resource trade-offs may be present in some Caribbean non-scleractinian zoantharians (Table 1). However, unlike many other benthic invertebrates, the majority of Caribbean non-scleractinian zoantharians surveyed in this study did not rely primarily on chemical defenses as a means to deter predation. While prior studies have shown that 69% of surveyed sponges (Pawlik et al. 1995), 66% of surveyed hydroids (Stachowicz and Lindquist 2000), and 100% of surveyed gorgonians (O'Neal and Pawlik 2002) possess deterrent secondary metabolites, only 28% of the cnidarians surveyed in this study had extracts with consistent feeding deterrent activity (Fig. 1).

Nematocyst defenses were common in species examined, with 71% exhibiting defensive nematocyst activity in

**Fig. 3** Mean (±standard error) total energy content in kJ mL<sup>-1</sup> of wet tissue for eight species of non-scleractinian zoantharians. Error bars represent standard error. ND represents no data. Geographically distant replicate tissue samples indicated in parentheses. Significant differences indicated by different letter groups (Tukey's HSD,  $p < 0.05$ )



**Fig. 4** Mean (±standard error) soluble protein content in mg mL<sup>-1</sup> of wet tissue for seven species of non-scleractinian zoantharians. Error bars represent standard error. Geographically distant replicate tissue samples indicated in parentheses. Significant differences indicated by different letter groups (Tukey's HSD,  $p < 0.05$ )



the brine shrimp nauplii assay (Table 1). All of the actinarians tested had strong interactions with nauplii of *Artemia*, while none of the corallimorpharians tested had strong nematocyst defenses. Zoanthideans were varied in their responses with 40% of the species tested possessing nematocyst defenses (Fig. 2). The ecological relevance of the nematocyst defenses detected in the laboratory experiments was confirmed by field observations: species that lacked chemical defenses but exhibited nematocyst defenses were not consumed by reef fish predators, but the same reef fish predators consumed species that lacked chemical and nematocyst defenses.

Differences in the cnidom (combination of nematocysts and spirocysts, as ptychocysts are not present) among the cnidarians surveyed provide a plausible explanation for the diversity of reactions observed in the cnida assays. Nematocyst size and type are known to vary across the subclass Zoantharia to the extent that the cnidom has been used as a diagnostic tool for taxonomic identification (Fautin 1988; England 1991). Different types of cnidae may serve different functions. For example, cnidae are typically classified as being used for capture, defense, or locomotion, although some may serve multiple functions (Fautin 2009). Of the more than 25 varieties of cnidae recognized, at least six different types have been identified in the Anthozoa (Mariscal 1974). Furthermore, nematocyst composition has also been shown to affect tentacle adhesion (Thorington and Hessinger 1996). Intraspecific variation in the location of nematocysts could further affect their defensive capabilities. For example, corallimorpharians often lack nematocysts on their distal tentacles (Hamner and Dunn 1980), which is consistent with the lack of activity observed in cnida assays in this study. Although interspecific variation in the cnidom may explain the observed differences in nematocyst defensive capabilities, further investigation is required to confirm this hypothesis.

Although 65% of cnidarians species surveyed showed evidence of resource trade-offs, the remaining 35% of species possessed either both chemical and nematocyst defenses or neither type of defense, suggesting that resource trade-offs are not the only factor influencing defensive strategy. As a result, the present study provided limited support for the hypothesis that there is an inverse relationship between chemical and nematocyst defensive strategies in Caribbean non-scleractinian zoantharians, a finding that is only partially consistent with other studies. Analyses of chemical and nematocyst defenses in subtropical hydroids indicated that species possessed either one type of defense or the other in all cases (Stachowicz and Lindquist 2000). The presence of both strategies among some species in the present study suggests that resources are not necessarily limiting for those species, or that nematocyst and chemical defensive strategies are not similarly

effective against all predators. Alternatively, trade-offs may be present between defense and either growth or reproduction, as has been recently demonstrated for sponges on Caribbean coral reefs (Walters and Pawlik 2005; Pawlik et al. 2008; Leong and Pawlik 2010a; Leong and Pawlik 2010b).

Are non-scleractinian zoantharians a valuable food source?

While total energy content in Caribbean non-scleractinian zoantharians was variable, all species tested had ecologically valuable quantities of energy in their tissues when compared with other benthic invertebrates (mean =  $2.9 \pm 0.7$  kJ mL<sup>-1</sup>,  $N = 22$ , Fig. 3). For example, sponges are now known to be an important food source for coral reef fishes and invertebrates (Sloan 1980; Dunlap and Pawlik 1996; 1998; Hill 1998) and had a similar range of total energy content to non-scleractinian zoantharians (mean =  $2.0 \pm 0.9$  kJ mL<sup>-1</sup>, Chanas and Pawlik 1995). Gorgonians also had similar total energy content to non-scleractinian zoantharians ( $4.0 \pm 2.0$  kJ mL<sup>-1</sup>, O'Neal and Pawlik 2002). Although statistically significant differences were detected among the species examined, it is unlikely that these differences are ecologically significant in coral reef habitats where predation is intense.

On a volumetric basis, the soluble protein content of cnidarian tissues varied only slightly among species (Fig. 4). The mean soluble protein content in the organisms studied ( $4.4 \pm 2.2$  mg mL<sup>-1</sup>,  $N = 16$ ), however, was notably less than that of other tropical benthic invertebrates such as sponges (mean =  $20.7 \pm 11.6$  mg mL<sup>-1</sup>, Chanas and Pawlik 1995) and gorgonians ( $17 \pm 8$  mg mL<sup>-1</sup>, O'Neal and Pawlik 2002). It has been suggested that protein content is the most important factor when considering the value of food to reef fishes (Millikin 1982). Therefore, although the tissues of non-scleractinian zoantharians possess ecologically valuable levels of energy, they may not represent a high-quality food source for all potential predators. Poor nutritional quality may explain the scarcity of non-scleractinian zoantharians observed in the guts of Caribbean fishes (Randall 1967); however, some predators including hawksbill turtles (*Eretmochelys imbricata*) and butterfly fishes (family Chaetodontidae) have diets containing large proportions of cnidarians (Parker 1984; Stampar et al. 2007). For example, the corallimorpharian *Ricordea florida* made up 59% of the total volume of gut contents from lavage samples in Hawksbill turtles from the Dominican Republic (León and Bjorndal 2002). Although some specialist predators consume non-scleractinian zoantharians, it is possible that the lower protein content of these cnidarians in comparison with other available food sources may reduce the evolutionary pressure on non-scleractinian zoantharians to invest resources in antipredatory defenses.



One possible explanation for the discrepancy between ecologically valuable total energy content and relatively low soluble protein content in non-scleractinian zoantharians, as well as the variation observed among species, is that interspecific differences in the reproductive cycles of these cnidarians may affect their nutritional values. Some sea anemones display a strong periodicity in their reproductive cycles (Ford 1964), while other species reproduce throughout the year (Dunn 1982). The nutritional analysis conducted in this study was performed on specimens collected in the summer months, allowing for the possibility that some species may have been at different stages in their reproductive cycles. Although little variation was observed between specimens collected, the degree to which reproductive periodicity affects the nutritional value of non-scleractinian zoantharians remains unclear and warrants further investigation.

Is there a relationship between nutritional quality and defensive strategy?

No evidence of a relationship between the presence of either defensive strategy (chemical or nematocyst) and nutritional quality could be found for the cnidarians species investigated in this study. Defensive strategies were variable for all three orders, and each had at least one member classified as chemically defended (Table 1). In contrast, the nutritional quality of species tested in all three orders was relatively consistent in terms of both total energy content and soluble protein content (Figs. 3, 4). As described above, the soluble protein content of cnidarian tissues examined in this study was consistently lower than that of other benthic invertebrates surveyed in earlier studies, which may result in decreased predation pressure on non-scleractinian zoantharians. However, the lack of a relationship between nutritional quality and other defensive strategies (chemical or nematocyst) suggests that nutritional quality is not a stand-alone defense mechanism for these cnidarians. This finding is consistent with the results of previous surveys of other marine organisms (Chanas and Pawlik 1995; O'Neal and Pawlik 2002; Penny 2002) and suggests that low nutritional value alone is not a common defensive strategy in benthic marine invertebrates.

Resource trade-offs and the distribution of Caribbean non-scleractinian zoantharians

A clear relationship was observed between the habitat of the cnidarians examined in this study and the defensive strategies they employed. Of the 18 species surveyed, 58% of those found only on coral reefs possessed chemical defenses, while chemical defenses were absent in species found in other habitats (Table 1). This pattern suggests that higher levels of predation drive the evolution of chemical

defense, a conclusion that has been advanced in many studies of coral reef invertebrates and plants (Pawlik et al. 1995; Dunlap and Pawlik 1996; Pawlik 1998; Pawlik et al. 2008; Harborne et al. 2009; Loh and Pawlik 2009).

The habitat of cnidarians examined in this study may also explain the within-species variability observed in chemical defenses. Tissue from two species had a high degree of intraspecific variation in chemical defense (*A. flosculifera* and *P. caribbaeorum*), suggesting that some individuals may produce secondary metabolites, while others do not. Palytoxins produced by species of zoanthids in the tropical Pacific are found only in individuals from specific locations (Moore and Scheuer 1971). Furthermore, variations in location may affect the presence of photosymbionts within cnidarian tissues that may contribute to the production of chemical defenses.

Behavior may be an important consideration in explaining discrepancies between the results of the present study and other work conducted on cnidarians (Stachowicz and Lindquist 2000; O'Neal and Pawlik 2002). Sea anemones, zoanthids, and corallimorpharians possess longitudinal retractor muscles that run the length of the polyp column. These muscles enable the polyps to reduce their size when threatened by predators (Rupert et al. 2004). This ability to avoid predation by retreating into substratum interstices may reduce the need for alternative defense mechanisms in some species, particularly when combined with a distribution in predator-poor habitats.

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