Effects of Site and Sampling Time on Motile Cryptic Invertebrate Communities on Fringing Reefs

Los Efectos de Sitio y Tiempo de Muestreo en Comunidades de Invertebrados Mótiles Escondidos en Arrecifes de Franja

Les Effets de Terrain et Temps du Échantillonnage sur les Communautés des Motiles Invertébrés Énigmatique en les Récifs Fringents

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ABSTRACT

Cryptic invertebrates are a little studied part of tropical coral reef ecosystems, despite their significant contribution to coral reef biomass and biodiversity. In order to assess means of sampling these cryptofauna and begin a census of present and common taxa, coral rubble traps were deployed at three sites in St. Thomas, USVI for two durations (14 and 28 days) near the University of the Virgin Islands. Two sites were onshore fringing reefs, with one relatively flat and *Montastraea* sp. dominated (5 – 10 m deep) and the other of mixed coral and steeply sloping 10 – 15 m deep; a third site was a fringing reef on an offshore cay of mixed coral 10 – 15 m deep. We tested the hypothesis that invertebrate communities/relative abundances are different by site but not sampling time. From the 18 traps, 246 individual motile invertebrates were sampled, representing 31 Operational Taxonomic Units (OTUs). The ten most common OTUs accounted for 86.59% of specimens, with arthropods making up the majority of specimens (82.52%) and consisting of 13 OTUs. Shannon diversity index H' was calculated and then analyzed in a two-way crossed ANOVA for site and time; time was a significant factor ($p = 0.0146$). Average H' increased between 14 and 28 days (0.456 and 0.655, respectively). Results from PERMANOVA showed only site to have a significant effect on the number of specimens per taxon (permutation $p = 0.0435$). The significant effect of time on H' implies colonization new substrate to be on going within the time of deployment, while the difference among sites is evidence that different reefs, even in close proximity to one another, are affected differently by drivers of biodiversity and relative abundances of cryptic invertebrates.

KEY WORDS: Cryptic invertebrates, community ecology, rubble traps

INTRODUCTION

The inconspicuous cryptofauna may contribute more to total tropical coral reef biomass and biodiversity than conspicuous fauna (Bakus 1966, Enochs et al. 2011), but are often overlooked in research due to the practicalities of sampling (Carpenter 1997). There is relatively little known about their community structure in the Caribbean; published data is all but absent except in the form of some taxonomic works and species presence/absence biodiversity studies (e.g. (Choi and Ginsburg 1983, Collin et al. 2005, Gore and Abele 1976, Wagner 1990). Motile (i.e. movement due to volition and stochasticity) cryptic invertebrates encompass a diverse assortment of organisms, covering multiple taxa: the limited data on the subject on tropical coral reefs has revealed the most common encountered by research to be Annelida, Arthropoda, Echinodermata, and Mollusca (Carpenter 1997, Enochs et al. 2011, Gischler and Ginsburg 1996, Glynn and Enochs 2011, Zimmerman and Martin 2004).

As a preliminary study to assess invertebrate sampling techniques and suitability of fringing reefs near the University of the Virgin Islands Marine Science Center for further investigation, coral rubble traps were used. We hypothesized that site and sampling time would have significant effect on numbers of specimens, biodiversity, and community structure, as would interactions between site and time. This method allowed for analyzing how much community variation may be found among sites in a small area of similar reef types.

MATERIALS AND METHODS

Exploratory invertebrate sampling was conducted in the vicinity of Brewers Bay, St. Thomas, USVI to assess the homogeneity/heterogeneity of motile cryptic invertebrate communities at given sites and the effects of deployment time. Three fringing reef sites were chosen for deployment: Brewers Reef, Black Point, and Flat Cay (Figure 1). Brewers Reef is composed of predominantly *Montastraea annularis* colonies with a depth ranging 5 – 10 m; Black Point has a comparatively steeply sloped reef of evenly mixed abundance of coral species with a depth ranging 10 – 15 m; the Flat Cay is an offshore cay site which consists of evenly mixed coral species with a depth of 10 – 15 m.

Coral rubble traps were chosen for sampling due to their efficacy as standard units of measure (Enochs 2012, Enochs et al. 2011, Takada et al. 2007, 2008, 2011, Valles et al. 2006) and simplicity. Traps act as new reef framework for colonization by fauna and the traps may be easily retrieved without detriment to the natural reef structure. Traps were constructed of vinyl-coated hexagonal wire of 3.81 cm mesh and were cylindrical with 16 cm diameter and 16 cm height. Once filled with

Figure 1. Map showing the three study sites, located on the southwest corner of St. Thomas, United States Virgin Islands.

coral rubble that was made to standard size (hexahedral and ≥ 0.2 kg to ≤ 0.44 kg) by physically breaking dead *Acropora palmata* fragments collected from nearby beaches. Total weight of traps varied 2.42 – 2.92 kg.

In November 2011, 6 traps were deployed by divers at each of the three sites, for a total of 18 traps. Traps were set as low as possible on flat substrate and between coral heads (i.e. not on top of coral heads) at a random point along each one of six 10 m permanent transects used by University of the Virgin Islands researchers.

At each of the three sites, six traps were deployed, for a total of 18 traps. Half of the traps at each site (n = 3 per site; n = 9 overall) were randomly selected to be retrieved after 14 days; the remaining traps were retrieved after 28 days. SCUBA divers retrieved traps by positioning a 2 gal (~7.6 L) plastic bucket over the trap, sliding a lid under the trap, and snapping on, thus encasing the trap and preventing motile fauna from escaping. Water from the buckets was run through a 20 µm sieve to capture animals suspended in the water, then traps were disassembled and clinging fauna were removed from the rubble with forceps. All animals larger than 2 mm were retained for analysis. All animals were preserved in 70% EtOH and subsequently photographed with either a RT KE Spot camera mounted on a Leica MZ FL III microscope or iPhone 4, depending on size of specimen; all had length measured (mm) and were wet weighed (g). Specimens were identified to lowest possible taxonomic level, or Operational Taxonomic Unit (OTU) (Enochs et al. 2011) by means of guide books, dichotomous keys, or expert opinion by means of distributing photographs. Data based on abundances of arthropods, gastropods, annelids, and echinoderms were analyzed with JMP (Version 9, SAS, Cary, NC) and PRIMER-E v6 (Clarke, KR, Gorley, RN, 2006. PRIMER-E, Plymouth) with the PERMANOVA+ add-on.

RESULTS

From the total 18 traps, 246 individual motile invertebrates were sampled, representing 31 OTUs. The ten most abundant OTUs accounted for 86.59% of specimens, with *Thor sp*. accounting for 52.44% (Figure 2). Arthropoda made up the majority of specimens (82.52%) and consisted of 13 OTUs.

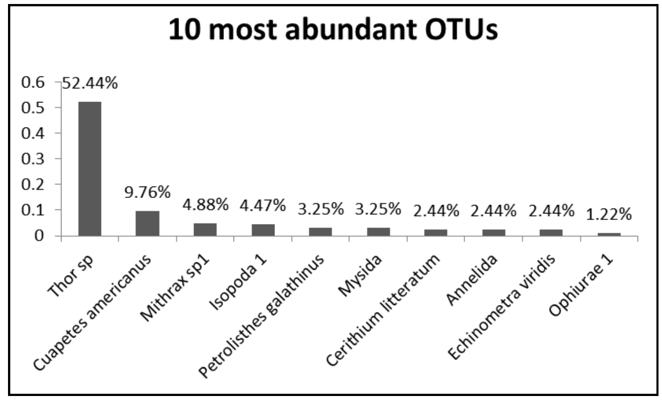

Figure 2. Composition of specimens identified to OTU in the study (n = 246).

After a square root transformation to normalize the data for the total number of specimens retrieved from each trap, a two-way crossed analysis of variance (ANOVA) testing for the influence of site, sampling time, and an interaction between the two showed site to be statistically significant ($p < 0.001$), while time and the interaction were not ($p = 0.847$ and $p = 0.792$, respectively) (Table 1). A Tukey Honestly Significant Difference analysis showed Flat Cay to be different from both Brewers Reef and Black Point by number of specimens per trap. On average for transformed data, each trap contained 4.84 specimens at Flat Cay, 3.2 at Brewers Reef, and 2.4 at Black Point. Species richness for each trap was compared for time and site in a two-way fully crossed ANOVA, resulting in time being significant ($p = 0.028$), while site ($p = 0.225$) and the interaction were not ($p = 0.225$) (Table 1). Mean species richness increased between 14 and 28 days (4.33 and 5.67, respectively). Shannon diversity index H' was calculated and then Box-Cox transformed for input in a two-way crossed ANOVA for site and time. Time was a significant factor ($p = 0.006$), while site ($p = 0.457$) and the interaction ($p = 0.529$) were not (Table 1). Average H' increased between 14 and 28 days (0.456 and 0.655, respectively).

Table 1. Results from three two-way ANOVAs testing the effects of time, site, and the interaction on square root transformed total number of specimens, species richness, and Shannon H' per trap. Values are p-values, with asterisks denoting significant results.

Treatment/ Interaction	Square root of specimens	Species richness	Shannon H'
Time	0.847	0.028*	0.006*
Site	0.001*	0.225	0.457
Site x Time	0.792	0.225	0.529

A two-way crossed permutational multivariate analysis of variation (PERMANOVA) was conducted to analyze the effects of site, time, and the interaction on the cryptic invertebrate communities (number of specimens per OTU as the response variable) from each trap (Table 2). Count data of OTU abundance per trap were square root transformed to reduce the influence of highly abundant OTUs (Clarke and Green 1988, Clarke and Warwick 2001), then a Bray-Curtis similarity matrix created from which to conduct further analysis. The PERMANOVA showed only site to be significant (permutation p = 0.044). Pairwise test results showed statistically significant difference only between Flat Cay and Black Point (permutation p = 0.022). Analysis of Similarity (ANOSIM) revealed structure among samples (significance = 1.0%), with only nine permutations greater than the global R (Table 3). While Flat Cay was very distinct from Black Point (significance = 0.2%), and some distinction between Brewers Reef and Flat Cay (significance = 19.0%), Brewers Reef and Black Point were more similar (significance = 27.1%).

A non-metric Multidimensional Scaling (nMDS) multivariate analysis was performed with PRIMER-E to compare samples, as has been used in other studies researching cryptic motile invertebrates (Enochs et al. 2011, Takada et al. 2007, 2008, 2011). nMDS represents samples as points on a 2- or 3-dimentional rank ordination plot, where distance between points represents relative similarity-dissimilarity between samples. Operational Taxonomic Unit abundances for each trap were square root transformed and put into a Bray-Curtis similarity matrix which was used as the basis for the nMDS. Each sample represents an individual trap (n = 18). A 2-dimensional nMDS of all samples revealed adequate structure in the data for analysis (stress = 0.15), with Flat Cay showing a tight grouping of samples when compared to the other sites (Figure 3). Cluster analysis was used to create percent-similarity overlays for the nMDS plots to visualize groupings.

In order to further investigate which variables contributed to the similarity-dissimilarity among samples in the Bray-Curtis matrix, a two-way crossed Similarity Percentage (SIMPER) analysis of site and time was performed (Table 4). Average similarity within Sites was low, with Flat Cay having the greatest at 42.17%, with *Thor* sp. being the main contributor at Brewers Bay and Flat Cay and *Cuapetes americanus* at Black point. Time similarities were also low, with 14 days having the greater value at 37.14% with *Thor* sp. being the main contributor to 14 day similarity and *Cuapetes americanus* to 28 day similarity. Pairwise comparisons of dissimilarities between groups revealed *Thor* sp. abundance to be the most influential value in all comparisons (n = 4, 3 inter site comparisons and 1 inter time comparison).

Table 2. PERMANOVA results from OTU counts for site and time, with the interaction.

Treatment/ Interactions	df	Pseudo -F	P (permutational)	Unique permutations
Site	2	1.8949	0.045	9930
Time	1	1.5541	0.371	38
Site x Time	2	0.8894	0.569	9938

Table 3. ANOSIM results for global and pairwise tests of site similarities.

Groups	R statistic	Significance level	Permutations	# permutations ≥ observed
Global	0.186	1.0%	999	9
Brewers, Flat	0.078	19.0%	462	88
Brewers, Black	0.057	27.1%	462	125
Flat, Black	0.396	0.2%	462	1

Table 4. Results from two-way SIMPER analysis, where values within groups denotes average similarity and values between groups denotes average dissimilarity. OTU with greatest contribution to similarities or dissimilarities are listed for each comparison, with their quantified contribution.

Group	Average similarity/ dissimilarity	Greatest contribution	Contribution
Brewers	29.93%	*Thor* sp.	62.58%
Flat	42.17%	*Thor* sp.	69.08%
Black	28.00%	*C. americanus*	31.44%
Brewers & Flat	61.68%	*Thor* sp.	19.11%
Brewers & Black	72.20%	*Thor* sp.	21.12%
Flat & Black	78.59%	*Thor* sp.	27.27%
14 days	37.14%	*Thor* sp.	57.96%
28 days	29.59%	*C. americanus*	35.88%
14 & 28 days	65.99%	*Thor* sp.	13.9%

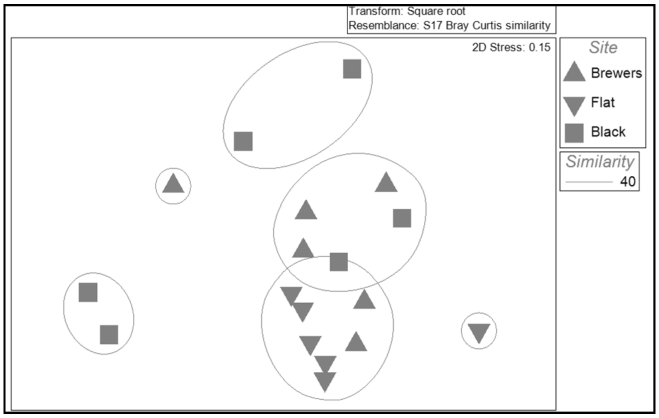

Figure 3. nMDS plot of samples (traps) with Site symbolized. The data shows adequate structure with a 2D stress of 0.15, with overlay of similarity from cluster analysis. The tight grouping of Flat Cay indicates high relative similarity between traps and similar sampled invertebrate communities.

DISCUSSION

This study aimed to investigate the influence of site and time (duration) for sampling of nearshore reefs for cryptic invertebrates with coral rubble traps; it was the first of its kind in the in the Tropical Atlantic. Although artificial reef frameworks have been used in previous studies in the region (Valles et al. 2006, Zimmerman and Martin 2004), this is first to both approximate natural substrate and focus specifically on variance among invertebrate communities at different sites.

The abundance of arthropods in this study compared to other phyla has been shown before in rubble trap experimentation (Enochs et al. 2011), suggesting the ability of coral rubble traps to provide suitable habitat and thus allow passive sampling of locally abundant and diverse species. The number of specimens per traps did not significantly change over duration between 14 and 28 days, while Takada et al. (2007) in a similar study did see a slight increase in invertebrate specimens and number of species between 1 and 2 weeks of deployment, and over a period of 1 to 8 weeks a greater increase. The analyses of species richness and Shannon index H', however, did show a significant effect of time, with richness and Shannon diversity both increasing between 14 and 28 days; this may be due to continual community development after initial colonization. There is a paradox which must be kept in mind regarding newly available substrate: the duration of initial colonization and community succession may overlap with seasonal changes in the dispersal methods and behavior of invertebrates. A study of community development that lasts too long will be confounded by seasonal changes at the study site. The lack of apparent nMDS pattern between relative abundances in the samples deployed for 14 and 28 days in this study may be indicative of rapid random initial colonization before the 14 day mark and before patterned subsequent succession could take place by 28 day mark.

Site had a significant effect on total specimens per sample and on all multivariate tests conducted. Flat Cay stood out as being significantly different from the other two sites on multiple occasions. Despite the differences in reef structure between Brewers Reef and Black Point, the was no statistical difference between the sampled cryptic invertebrate communities, implying that proximity to one another (0.2 km) is important and that other factors may be driving community structure in sites. This relative similarity between the two sites is further confirmed by the ANOSIM results. The within site similarity of samples from Flat Cay, apparent from the relatively tight grouping in the nMDS (Figure 3) and SIMPER analysis (Table 4), is further confirmed by the significant PERMANOVA results. The within site variation of OTU counts is less than among site variation implies that the separation between Flat Cay and the other sites is well established and consistent over the two time periods. The relatively similar of cryptic invertebrate communities sampled at Flat Cay make this site the most appropriate of the three for hosting a manipulative field experiment designed to investigate the drivers affecting community composition.

The differences among cryptic motile invertebrate communities sampled on these three inshore fringing reefs, even in close proximity, warrants further investigation. The next step in this project is to determine what physical drivers may be affecting these communities on nearshore fringing reefs in the northeast Caribbean. The recent rapid changes in coral reefs in the area (Miller et al. 2006) encourage research to begin to understand:

i) What drivers are shaping community structure,
ii) What and how global-scale drivers are likely to impact communities, and
iii) How local management/issues will affect invertebrate ecology and broader coral reef ecology.

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LITERATURE CITED

Bakus, G.J. 1966. Some relationships of fishes to benthic organisms on coral reefs. *Nature* **210**:280-284.

Carpenter, R.C. 1997. Invertebrate predators and grazers. Pages 198-229 in: C. Birkeland (ed.) *Life and Death of Coral Reefs*. Chapman and Hall, New York, New York USA.

Choi, D.R. and R.N. Ginsburg. 1983. Distribution of coelobites (cavity-dwellers) in coral rubble across the Florida reef tract. *Coral Reefs* **2** (3):165-172.

Clarke K. and R. Green. 1988. Statistical design and analysis for a "biological effects" study. *Marine Ecology Progress Series* **46** (1):213-226.

Clarke K.R. and R.M. Warwick. 2001. *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*. Primer, Plymouth, United Kingdom.

Collin, R., M.C. Diaz, J.L. Norenburg, R.. Rocha, J.A. Sanchez, A. Schulz, et al. 2005. Photographic identification guide to some common marine invertebrates of Bocas Del Toro, Panama. *Caribbean Journal of Science* **41**(3):638-707.

Enochs, I.C. 2012. Motile cryptofauna associated with live and dead coral substrates: implications for coral mortality and framework erosion. *Marine Biology* **159**(4):709-722.

Enochs, I.C., L.T. Toth, V.W. Brandtneris, J.C. Afflerbach, and D.P. Manzello. 2011. Environmental determinants of motile cryptofauna on an eastern Pacific coral reef. *Marine Ecology Progress Series* **438**:105-118.

Gischler, E., and R.N. Ginsburg. 1996. Cavity dwellers (coelobites) under coral rubble in southern Belize barrier and atoll reefs. *Bulletin of Marine Science* **58**(2):570-589.

Glynn, P.W. and I.C. Enochs. 2011. Invertebrates and their roles in coral reef ecosystems. Pages 273-325 in: Z. Dubinsky and N. Stambler (eds.) *Coral Reefs: An Ecosystem in Transition*. Springer Verlag, New York, New York USA.

Gore, R.H., and L.G. Abele. 1976. Shallow water porcelain crabs from the Pacific coast of Panama and adjacent Caribbean waters (Crustacea, Anomura, Porcellanidae): Smithsonian Institution Press, Washington, D.C. USA.

Miller, J., R. Waara, E. Muller, and C. Rogers. 2006. Coral bleaching and disease combine to cause extensive mortality on reefs in US Virgin Islands. *Coral Reefs* **25**(3):418-418.

Takada, Y., O. Abe, and T. Shibuno. 2007. Colonization patterns of mobile cryptic animals into interstices of coral rubble. *Marine Ecology Progress Series* **343**:35-44.

Takada, Y., O, Abe, and T. Shibuno. 2008. Cryptic assemblages in coral-rubble interstices along a terrestrial-sediment gradient. *Coral Reefs* **27**(3):665-675.

Takada Y., Abe O., Shibuno T. 2011. Variations in cryptic assemblages in coral-rubble interstices at a reef slope in Ishigaki Island, Japan. *Fisheries Science* **78**(1):91-98.

Valles, H., D.L. Kramer, and W. Hunte. 2006. A standard unit for monitoring recruitment of fishes to coral reef rubble. *Journal of Experimental Marine Biology and Ecology* **336**(2):171-183.

Wagner, H. 1990. The genera *Mithrax* Latreille, 1818 and *Mithraculus* White, 1847 (Crustacea: Brachyura: Majidae) in the western Atlantic Ocean. *Zoologische verhandelingen* **264**(1):1-65.

Zimmerman, T.L. and J.W. Martin. 2004. Artificial reef matrix structures (ARMS): an inexpensive and effective method for collecting coral reef-associated invertebrates. *Gulf and Caribbean Research* **16**:59-64.