THE EFFECTS OF SPONGES AND SPONGE METABOLITES ON THE SETTLEMENT, GROWTH AND ASSOCIATIONS OF SUBSTRATUM COMPETITORS

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ABSTRACT

The influence of three sponge species (*Acervochalina hooperi*, *Plakortis lita*, and *Xestospongia vansoesti*) on the settlement of a red encrusting algae (*Pneophyllum fragile*), a foraminiferan (*Planorbylinella* sp.) and a polychaete worm (family Spirorbidae) was investigated in two settlement studies in a coral reef off Mactan Island, Philippines. In the first study, settlement plates were exposed to intact sponges positioned adjacent to the plates. Foraminiferans were significantly smaller in the *X. vansoesti* treatment as compared to the control conditions. In the second settlement study, chemical extracts from each sponge species were incorporated into agar gels, which were separately affixed to acrylic settlement plates. Foraminiferans and algae experienced a reduction in size when exposed to extracts from *X. vansoesti* and *A. hooperi*, respectively. A positive association between algae and foraminiferans was also observed on the *P. lita* treatment plates.

KEY WORDS

Sponges, chemical ecology, allelochemical, settlement, competition.

INTRODUCTION

Chemical deterrents possessed or released by established, benthic coral reef organisms have been demonstrated to influence the settlement of substratum competitors (*e.g.,* allelochemicals released by soft corals) (BINGHAM & YOUNG, 1991; YOUNG & CHIA, 1981; COLL *et al.,* 1982; COLL & SAMMARCO, 1983; SAMMARCO *et al.,* 1983; BAK & BORSBOOM, 1984; BAKUS *et al.,* 1986). THOMPSON *et al.* (1985) isolated compounds from the sponge *Dysidea amblia* which inhibited the settlement of *Haliotis rufescens* larvae. Ethyl acetate extracts from the sponge *Lissodendoryx isodictyalis* were demonstrated by SEARS *et al.* (1990) to inhibit settlement of the barnacle *Balanus amphitrite.* In a more recent study by HENRIKSON & PAWLIK (1995), extracts from the sponge *Aplysilla longispina* incorporated in gels and set out into the field for almost a month had a negative effect on the settlement of invertebrate larvae.

The purpose of the present study was to investigate the effects of three sponge species (*Acervochalina hooperi*, *Plakortis lita*, *Xestospongia vansoesti*) and their metabolites on the settlement of substratum competitors. It was hypothesized that both the intact sponges and their crude extracts would either inhibit or enhance the settlement of particular fouling organisms onto settlement plates.

MATERIALS AND METHODS

Study Site

The study site was located on a flat, low limestone coral reef off Mactan Island, Philippines, approximately 400 m north of the University of San Carlos Maribago Marine Station at a depth of 11 m. A detailed description of the site is provided in BAKUS & NISHIYAMA (1999) and NISHIYAMA & BAKUS (1999).

Two settlement studies were conducted to investigate the influence of intact sponges and isolated sponge metabolites on the settlement of substratum competitors. With both studies, individuals of each sponge species, approximately 5 cm in diameter, were first collected at the study site by chiseling the dead coral the sponges grew on with a geological pick and removing the sponges. Care was taken not to damage the sponge during the removal process. The sponges were placed on dead coral at the study site for one week as a recuperation period. With the first settlement study, the sponges were used immediately after this one week period. Whereas with the second settlement study the sponges were kept in a –20° C freezer until needed.

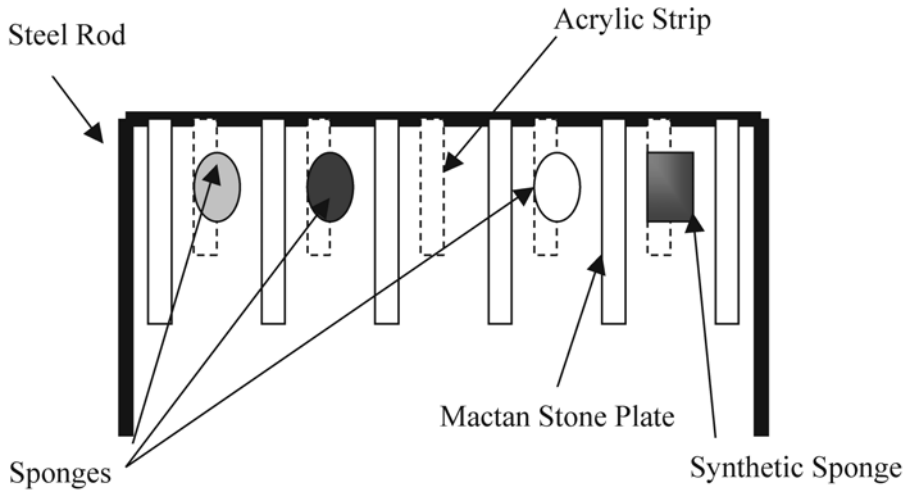

Fig. 1. Mactan stone settlement plate rack with treatment sponges, synthetic sponge control and acrylic strip control.

Intact Sponge Settlement Study

Settlement plates (10 x 10 x 1.4 cm) were custom cut at a masonry company in the Philippines from local limestone called Mactan Stone quarried from an area not far from the study site at Mactan Island, Philippines. Eight racks were constructed each with six Mactan stone plates hanging from a steel rod (Fig. 1). On acrylic strips either a sponge specimen (*Acervochalina hooperi*, *Plakortis lita* and *Xestospongia vansoesti*) or a synthetic control sponge (roughly the average dimensions of sponge specimens; 5 x 5.5 x 2 cm) was attached using plastic cable ties. The strips were positioned on the rack such that the sponges or control synthetic sponges were within 1 cm of each plate's surface (Fig. 1). As a second control, only an acrylic strip was placed between plates (Fig. 1). Each rack held six plates, three of which had a different sponge species adjacent to it, two with the synthetic sponge and acrylic strip controls and the sixth plate served to close the end of the rack. The positions of the sponges and controls on each rack were randomly selected using a table of random numbers. The rack

set-ups followed a randomized block design. The rods of each rack were bent at their ends such that they could be inserted into the sand and dead coral rubble to hold the rack upright with plates perpendicular to the substratum (Fig. 1). After a one-month exposure period (June 2 to July 2, 1996), the racks were removed from the study site, returned to the laboratory, air dried and analyzed.

The densities of three fouling organisms (a red encrusting algae, *Pneophyllum fragile*; a foraminiferan, *Planorbylinella* sp.; and a polychaete worm, family Spirorbidae) were determined using a dissecting microscope. Either a parametric ANOVA or non-parametric Friedman test (depending on the nature of the data), both incorporating randomized block design (ZAR, 1999), was used to analyze the data. A *post hoc* parametric multiple comparisons Tukey test or non-parametric multiple comparisons test (ZAR, 1999) was employed if a significant difference existed.

The size of fouling organisms was determined by measuring the diameters (using a vernier caliper) of all the individual colonies of each species found on each plate. An average of these diameters was determined for each organism. Colony diameters for the different treatments and control were compared with either an ANOVA or the Friedman test (depending on the nature of the data). A *post hoc* parametric Tukey multiple comparisons test or nonparametric multiple comparisons test was employed if the p-value was significant.

Sponge Metabolite Settlement Study

This settlement study investigated the effects of sponge metabolites in crude extracts on the settlement of substratum competitors. Crude sponge extracts were incorporated into an agar gel held within a container (plastic cup with holes) that was affixed to the middle of each settlement plate. The goal was to have the sponge extracts slowly leach out of the gel into the water surrounding the plates and observe any influences to the settlement of organisms. For all three sponge species the dominant metabolites found in the crude extracts were identical to allelochemicals found by NISHIYAMA & BAKUS (1999) to be released into the water by the sponges. This was confirmed by thin layer chromatograms. These allelochemicals were also found to be toxic to or cause tissue necrosis in hard corals (NISHIYAMA & BAKUS, 1999).

Settlement plates (11.5 x 11.5 x 0.25 cm) were cut from transparent acrylic. Plastic cups were cut such that the bottom portion had the dimensions: 4.7 cm for the top diameter, 3.8 cm for the bottom diameter and 1.5 cm in height (Fig. 2a). One 0.6 cm hole was punched on the bottom and twelve 0.6 cm holes punched into the sides of each cup with a paper hole puncher (Fig. 2a). Transparent tape was placed over these holes. These cups formed the containers which would hold the agar with extracts onto the plates.

An agar mixture containing sponge extract was made for each sponge. Extracts from all three sponge species were obtained by grinding 10 g of each sponge separately in a mortar and pestle with 40 ml of acetone, filtering the extract through filter paper (Whatman #1) and air drying for 72 h. Forty ml of acetone was also filtered and air dried as a control. Each extract was then re-suspended separately in 35 ml of deionized water. Agar gel was then prepared by mixing 4 g agar powder with 130 ml of deionized water and heating the mixture in a microwave oven for 1 minute and 15 seconds and then setting it aside to cool. When the temperature of the agar reached approximately 30° C (the agar was a thick liquid that was warm to the touch), 10 ml of agar was poured into four beakers. To each beaker either one of the sponge extracts or the acetone control was added to the agar and stirred. Each extract-agar mix and the control were then poured into separate sets of six plastic cups (prepared earlier) and allowed to solidify. The metabolites were not altered by either the acetone treatment or by the earlier freezing as was also verified by thin layer chromatograms. The transparent tape on the cups was then removed. These cups were then inverted and one agar cup was attached to each acrylic plate using plastic cable ties passed through holes punched into the cups and drilled through the plates (Fig. 2b). The plates were attached to plastic mesh (1 cm^2 mesh size) using plastic cable ties and set out flat on dead coral at the study site

(Mactan Island, 11 m depth) in a randomized block design (six blocks each containing a control and one of each sponge extract treatment) for a three month period (August 20, 1998 to November 22, 1998). The mesh itself was affixed to the substratum using cable ties. In total, there were six plates for each extract-gel type (three sponges) and six plates for the acetone gel controls.

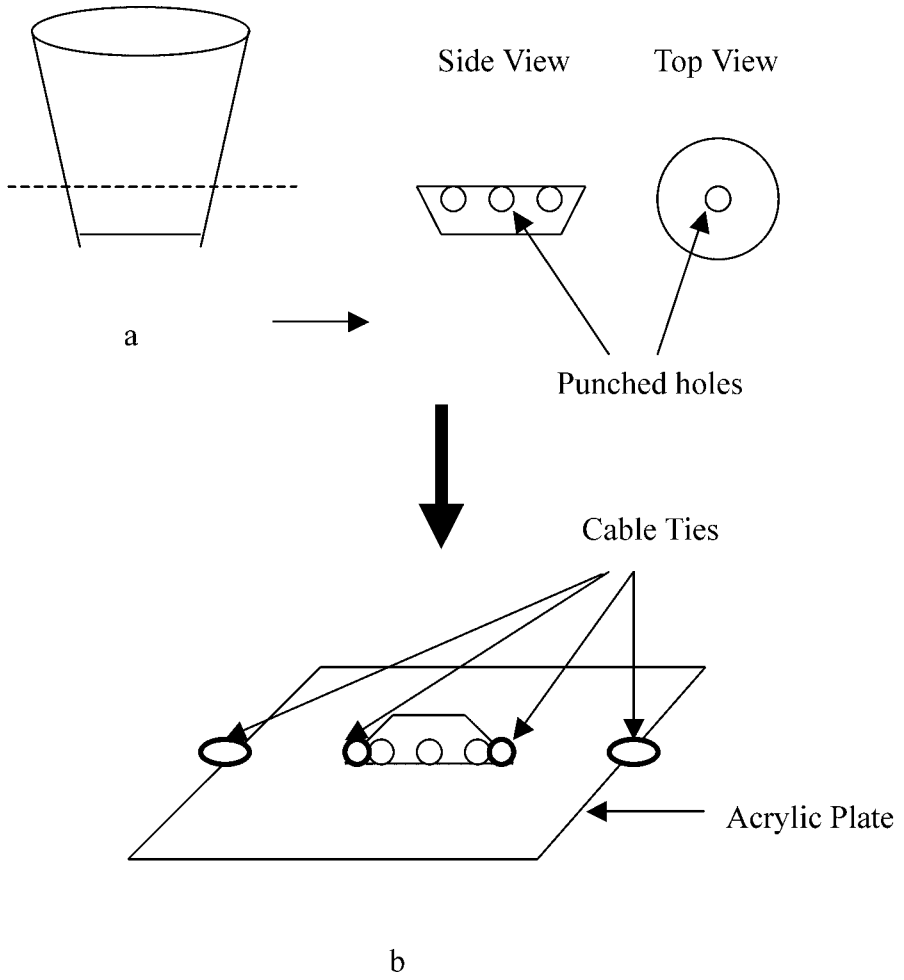

Fig. 2. Construction of acrylic plates with agar containing extracts. **a,** Cutting the lower portion of a cup. **b,** Attaching cup with agar and extract onto acrylic plate.

The densities of algal colonies, foraminiferans, and polychaete worms were determined for a 3 cm wide annulus extending from each cup's edge (0 - 3 cm) on the acrylic plates. Either a parametric ANOVA or non-parametric Friedman test (depending on the nature of the data), both incorporating randomized block design, was conducted to determine if any significant difference existed between the settlement densities in the different treatments and control. A *post hoc* parametric (Tukey test) or non-parametric multiple comparisons test (ZAR 1999) was conducted if a significant difference existed.

The size of fouling organisms was determined by measuring the diameters of twenty individuals of each species found within a 1 cm wide annulus (1 - 2 cm interval from each cup's edge) using a vernier caliper and an average of these diameters was determined for each organism. Colony diameters for the different treatments and control were compared with either an ANOVA or the Friedman test (depending on the nature of the data). A *post hoc* parametric Tukey multiple comparisons test or nonparametric multiple comparisons test was employed if the p-value was significant.

Associations

A mathematical solution to ascertain the degree of association between two organisms was developed earlier by NISHIYAMA & BAKUS (unpubl. data). This model, called the Encounter Index, was found to be more accurate and precise than methods developed by earlier workers such as DICE (1945), JACCARD (1908), OCHIAI (1957) and DE JONG *et al.* (1983). The Index was determined by comparing the number of observed contact encounters between two organisms with the number expected if no association existed.

$$\text{Encounter Index} = \frac{\text{Observed Number of Encounters}}{\text{Expected Number of Encounters}}$$

The expected number of contact encounters if a random association existed was obtained by determining the probability that each individual would encounter another if both were randomly distributed. The number of random contact encounters is contingent upon the sizes and densities of both organisms.

$$\text{Expected Number of Encounters} = \frac{2(\pi)(r_A + r_B) \times (D_B)^2 \times \text{Dens}_A \times \text{Dens}_B}{D_B}$$

r_A = Radius of species A
r_B = Radius of species B
D_B = Diameter of species B
Dens_A = Density of species A
Dens_B = Density of species B

Note: Species A is the species with the larger diameter, whereas species B has the smaller diameter.

Index values between 0.00 - 0.49 signify negative associations, values between 0.50 - 2.49 represent random associations and those values above 2.50 signify positive associations. In the present study, Encounter Index values were determined for contact encounters between algae and foraminiferans on each acrylic plate. The average index value for the plates in each treatment or control was then determined.

RESULTS

Intact Sponge Settlement Study

The number of algae, foramininferans and polychaete worms settling on the Mactan Stone plates was not significantly different between the three sponge treatments and the two controls (synthetic sponge and no-sponge) (Friedman test was used for the two former organisms and randomized block ANOVA used for the later).

No significant difference was observed between the sizes of algal colonies on the treatment and control Mactan stone plates (randomized block ANOVA). With the foraminiferans, a significant difference was observed between the conditions (non-parametric Friedman test; df = 4; $p < 0.05$). Foraminiferans on the *X. vansoesti* plates had smaller colony diameters (Tab. I) than colonies on the synthetic sponge and acrylic strip controls (non-parametric multiple comparisons Test, Tab. II).

Tab I. Average densities (m^{-2}) of the algae (*Pneophyllum fragile*), foraminiferan (*Planorbylinella* sp.) and polychaete worm (family Spirorbidae), and average colony diameters (m) of the algae and foraminiferan on the Mactan Stone settlement plates exposed to different sponges or controls in the intact sponge settlement study. Standard errors are shown in parentheses. (*Xesto* = *Xestospongia vansoesti*; *Plakor* = *Plakortis lita*; *Acervo* = *Acervochalina hooperi*).

	Treatment				
Organism	*Xesto*	*Plakor*	*Acervo*	Synthetic Sponge Control	Acrylic Strip Control
Densities (m^{-2})					
Algae	843.7 (163.2)	1062.5 (509.9)	1156.25 (264.9)	562.5 (160.2)	953.1 (284.4)
Foraminiferan	234.4 (79.9)	562.5 (179.9)	234.4 (49.8)	375.0 (81.8)	281.3 (128.9)
Polychaete	1546.9 (216.4)	2656.3 (297.6)	2453.1 (451.9)	1687.5 (255.5)	2671.9 (299.7)
Diameters (m)					
Algae	0.00137 (0.0001)	0.00121 (0.0001)	0.00157 (0.0001)	0.00117 (0.0001)	0.00130 (0.0001)
Foraminiferan	0.00169 (0.0001)	0.00245 (0.0025)	0.00239 (0.0002)	0.00289 (0.0005)	0.00261 (0.0003)

Tab. II. Results of non-parametric multiple comparisons test between foraminiferan (*Planorbylinella* sp.) colony sizes on the different Mactan Stone settlement plates. (*Xesto* = *Xestospongia vansoesti*; *Plakor* = *Plakortis lita*; *Acervo* = *Acervochalina hooperi*; Cont 1 = Synthetic Sponge Control; Cont 2 = No Sponge Control) (df = 4 for each test).

Comparison	q_{calc}	q_{crit}	Conclusion
Cont 1 vs. *Xesto*	4.47	4.10	**Difference**
Cont 1 vs. *Acervo*	1.34	4.10	No Difference
Cont 1 vs. *Plakor*	0.45	4.10	No Difference
Cont 1 vs. Cont 2	0.22	4.10	No Difference
Cont 2 vs. *Xesto*	4.25	4.10	**Difference**
Cont 2 vs. *Acervo*	1.12	4.10	No Difference
Cont 2 vs. *Plakor*	0.22	4.10	No Difference
Plak vs. *Xesto*	4.03	4.10	No Difference
Plak vs. *Acervo*	0.89	4.10	No Difference
Acer vs. *Xesto*	3.13	4.10	No Difference

Sponge Metabolite Settlement Study

With the acrylic, sponge extract settlement plates, no significant difference was detected between any of the densities of fouling organisms (algae, foraminiferans, and polychaetes) for the three treatments and control (acetone gel plates) (randomized block ANOVA test was used for the two former organisms and Friedman test used for the later organism). These were the same results observed for the Mactan Stone plates.

Tab. III. Average densities (m^{-2}) of the algae (*Pneophyllum fragile*), foraminiferan (*Planorbylinella* sp.) and polychaete worm (family Spirorbidae), and average colony diameters (m) of the algae and foraminiferan on the acrylic settlement plates exposed to different sponge extracts or control in the sponge metabolite settlement study. Standard errors are shown in parentheses. (*Xesto* = *Xestospongia vansoesti*; *Plakor* = *Plakortis lita*; *Acervo* = *Acervochalina hooperi*)

	Treatment			
Organism	*Xesto*	*Plakor*	*Acervo*	Acetone Control
Densities (m^{-2})				
Algae	25846.3 (2610.8)	26257.0 (2528.6)	24728.5 (2153.9)	25116.3 (1772.6)
Foraminiferan	21124.2 (1986.2)	17246.1 (2167.9)	19687.0 (2353.1)	20531.1 (1911.7)
Polychaete	45.6 (28.9)	410.6 (383.9)	22.8 (22.8)	593.1 (511.7)
Diameters (m)				
Algae	0.00167 (0.0001)	0.00176 (0.0002)	0.00146 (0.00004)	0.00204 (0.0001)
Foraminiferan	0.00069 (0.00004)	0.0008 (0.00004)	0.00076 (0.00004)	0.00087 (0.00004)

Tab. IV. Results (p values) of a Tukey multiple comparisons test (ZAR, 1999) between average algal (*Pneophyllum fragile*) diameters on the acrylic settlement plates. (*Xesto* = *Xestospongia vansoesti*; *Plakor* = *Plakortis lita*; *Acervo* = *Acervochalina hooperi*). Bold numbers represent significant differences.

Mean Diameter (m)		*Xesto*	*Plakor*	*Acervo*	Control
0.00167	*Xesto*	-----	0.85	0.94	0.13
0.00176	*Plakor*	0.85	-----	0.52	0.52
0.00146	*Acervo*	0.94	0.52	-----	**0.03**
0.00204	Control	0.13	0.52	**0.03**	-----

With the algae, a significant difference in colony size was observed between treatment and control conditions (Tab. III) (randomized block ANOVA; $df = 3$; $p < 0.05$). A Tukey multiple comparisons test showed that algae colonies were smaller on *A. hooperi* extract plates than on control plates (Tab. IV).

Tab. V. Results (p values) of a Tukey multiple comparisons test (ZAR, 1999) between the average diameters of foraminiferans (*Planorbylinella* sp.) on the acrylic settlement plates. (*Xesto* = *Xestospongia vansoesti*; *Plakor* = *Plakortis lita*; *Acervo* = *Acervochalina hooperi*). Bold numbers represent significant differences.

Mean Diameter (m)		*Xesto*	*Plakor*	*Acervo*	Control
0.00069	*Xesto*	-----	0.06	0.35	**0.03**
0.00080	*Plakor*	0.06	-----	0.82	1.00
0.00076	*Acervo*	0.35	0.82	-----	0.71
0.00087	Control	**0.03**	1.00	0.71	-----

With the foraminiferan, a randomized block ANOVA showed that a significant difference existed between the treatments and control ($df = 3$; $p < 0.05$). Foraminiferans were smaller on the *X. vansoesti* treatment plates than the control (Tukey multiple comparisons test) (Tab. V). This was the same result observed for the Mactan Stone plates.

Due to the irregular shapes of the polychaete worms the sizes for the different treatments and control were not compared.

Associations

Association analyses between the algae and foraminiferan colonies on the acrylic settlement plates using the Encounter Index were conducted separately for each treatment (three sponge species and control). The average Encounter Index values for each treatment were as follows: *A. hooperi* 1.85 (Random association), *P. lita* 2.76 (Positive association), *X. vansoesti* 1.92 (Random association), control 1.59 (Random association). Only the *Plakortis lita* treatment deviated from a random association. With this treatment both the algae and foraminiferan had a strong positive relationship (2.76) with each other.

DISCUSSION AND CONCLUSIONS

The present study represents a preliminary work that investigated the influences of three sponge species (*Acervochalina hooperi*, *Xestospongia vansoesti*, *Plakortis lita*) on the settlement of common foulers. These three sponge species were the same species demonstrated by NISHIYAMA & BAKUS (1999) to release allelochemicals that were toxic to hard coral species (*Acropora formosa*, *Millepora dichotoma*, *Pocillopora damicornis*, *Porites andrewsi*). The intact sponge settlement study addressed the influence of entire, living sponges on the settlement of foulers. However, in order to determine the influence of only sponge metabolites on the settlement of foulers, sponge metabolites were isolated and incorporated separately into agar gels, which were then attached to acrylic settlement plates and placed into the field.

The results show that all three sponge species could influence either the size or associations of benthic spatial competitors (algae, foraminiferans and polychaetes). *X. vansoesti* was found to significantly inhibit the growth (or size) of the foraminiferan on both Mactan stone and acrylic plates. Foraminiferans were approximately 38 % and 20 % smaller when settling next to *X. vansoesti* on Mactan plates and acrylic plates, respectively, than on control plates. Likewise, *A. hooperi* extracts were shown to reduce the size of algae on acrylic plates by 29 % relative to those on control plates. The reduction of growth of substratum competitors around

a sponge would be of benefit to the sponge. Such inhibition would help to prevent spatial competitors from growing into spaces adjacent to the sponge or even overgrowing the sponge, as well as maintaining open space where the sponge can grow.

Although particular concentrations of sponge extracts were used in the acrylic sponge extract settlement study, these were not necessarily the natural concentrations of metabolites found within the sponges. However, the metabolite concentration of concern is not that which is found within the sponge but the amount of metabolites released by the sponge into the surrounding water. In a separate preliminary study of the concentration of allelochemicals released by *X. vansoesti*, *P. lita*, and *A. hooperi*, the concentrations were all found to be much lower than that found within the sponges (NISHIYAMA, unpubl. data). The authors are currently constructing a system that will release sponge allelochemicals in natural concentrations over settlement plates for prolonged periods.

In the present study, acetone was used to extract sponge metabolites because this solvent is neither very polar nor non-polar. However, the use of this solvent may not have properly extracted all the metabolites. Air-drying of extracts may also have led to a loss of the activity of metabolites through oxidation. These issues will be addressed in the future settlement study.

Although sponge metabolites most likely influence the growth of adjacent organisms, other factors such as the effect of the sponge bodies on water flow around it and the influence of sponge feeding may also affect the settlement of organisms. These factors may also have a synergistic effect which would influence the level of spatial competition around the sponge. Further investigation is needed to ascertain the importance of these other factors.

Using the neighbourhood Encounter Index it was determined that algae colonies and foraminiferans had a positive association with each other (Encounter Index = 2.78) when metabolites from *P. lita* were present. Thus, this sponge appears to influence local, small scale community structure by influencing the interactions between two neighbourhood spatial competitors. Though the *P. lita* extract was the influencing factor, whether the relationship between the two organisms was a benefit to the sponge or to either the algae or foraminiferan is unknown. An increase in the number of contacts between these two organisms would create larger patches of free space available for the sponge (that is, the more the substratum competitors are aggregated the larger the open spaces between these aggregates). The organisms may be in greater competition with each other and possibly less so with the sponge. It is suggested that this experiment be repeated but attention be given to the initial organization of the micro-community as well as observing changes in the spatial relationships between the fouling organisms over time.

The present study demonstrates that sponge metabolites can influence the growth and associations of spatial competitors. This study provides some of the first evidence that sponges can influence small-scale community structure.

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