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Cleaning mutualist or parasite? Classifying the association between the brittlestar *Ophiothrix lineata* and the Caribbean reef sponge *Callyspongia vaginalis*

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ABSTRACT

Symbioses often exist along a mutualism–parasitism continuum, and the classification of any given relationship requires a careful examination of costs and benefits for both symbiont and host. It has been proposed that deposit-feeding by the obligate sponge-dwelling brittlestar *Ophiothrix lineata* on the surface of the tube sponge *Callyspongia vaginalis* may increase filtration efficiency resulting in enhanced sponge growth or reproduction while providing protection and food for the brittlestar. However, *C. vaginalis* produces large (0.5–1.4 mm) larvae that are brooded in chambers and released into the interior of sponge tubes year-round, and these larvae could be consumed by *O. lineata*. In laboratory experiments, brittlestars readily consumed sponge larvae. When larval traps were placed over sponge tubes in the field, fewer larvae per brood chamber were collected from sponge tubes containing brittlestars than sponge tubes that lacked brittlestars, supporting the hypothesis that brittlestars consume sponge larvae under natural conditions. Sponges with brittlestars exhibited no difference in growth or number of brood chambers compared to sponges without brittlestars after 8 months, indicating no positive effect of symbiont on host. Spatial and temporal variations in larval release by *C. vaginalis* likely decrease encounter rates of brittlestars with sponge larvae, reducing the negative impact on the sponge and helping to maintain the association. The available evidence suggests that, depending on the reproductive status of the sponge, the association between *O. lineata* and *C. vaginalis* ranges from commensalism to larval parasitism.

1. Introduction

Facilitative interactions between organisms, in which one member of an association benefits and the other is helped or unaffected, can be important in determining community structure (Bruno et al., 2003; Stachowicz, 2001). However, determining whether interactions are facilitative is often difficult, because interspecific associations can fluctuate between commensalism, mutualism, and even parasitism (Hay et al., 2004; Hoeksema and Bruna, 2000; Thomson, 2003). For example, cleaning symbiosis, an often-cited example of mutualism on coral reefs, may become detrimental to client fishes when ectoparasite abundance on clients is low and cleaning fishes instead remove scales and mucus (Cheney and Cote, 2005). Additionally, client fishes may consume cleaner fishes, and the absence of cleaning stations may have little effect on the ectoparasite abundance of clients (Cote, 2000; Freckleton and Cote, 2003). While interspecific associations can be defined by the sum of the costs and benefits to participants (Bronstein, 1994; Hay et al., 2004), this requires a full understanding of the impacts of the association to each participant.

Sponges are often a dominant component of the marine benthos, and are well known as hosts to a taxonomically diverse population of organisms living on or inside them (Duffy, 1992; Hendler, 1984; Pawlik, 1983; Pearse, 1949; Ruetzler, 1975; Tyler and Bohlke, 1972). The tube sponge *Callyspongia vaginalis* is one of the most common sponges on Caribbean reefs (Pawlik et al., 1995) and provides habitat for shrimps, amphipods, and brittlestars (Henkel and Pawlik, 2005; Rhyne and Lin, 2006; Thomas and Klebba, 2006). In Belize and the Florida Keys, one of the most common inhabitants of *C. vaginalis* is the brittlestar *Ophiothrix lineata* which lives within the sponge tubes (Hendler, 1984; Henkel and Pawlik, 2005; Kissling and Taylor, 1977). With its central disk protected inside the sponge tube, the brittlestar deposit-feeds at night by extending its arms out over the outer surface of the sponge (Hendler, 1984). The relationship between *C. vaginalis* and *O. lineata* was hypothesized to be a mutualism by Hendler (1984), who proposed that the brittlestar gained refuge and a food source (detritus) from the sponge, and the sponge derived enhanced filtration efficiency from the cleaning activity of the brittlestar. Hendler (1984) noted, however, that direct evidence of an advantage to the sponge in filtration efficiency or increased growth rate remained to be demonstrated.

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Despite the presence of other species of tube sponges in the same coral reef environment, *O. lineata* has 99% fidelity in associating with *C. vaginalis* (Henkel and Pawlik, 2005) and uses chemical cues to detect this species in preference to others (Henkel and Pawlik, 2011). Growth of *O. lineata* is also greater for brittlestars living in *C. vaginalis* compared to other sponge species (Henkel and Pawlik, 2011). The high specificity of *O. lineata* for *C. vaginalis* suggests an obligate relationship. Survival of the sponge, however, is not dependent on the brittlestar, as 15% of *C. vaginalis* off Key Largo, FL did not contain any *O. lineata* (Henkel and Pawlik, 2005); and *O. lineata* is absent from *C. vaginalis* in other parts of its range, such as reefs around the Bahamas Islands (Henkel, pers. obs.).

While the sponge–brittlestar association is facultative for the sponge, to classify the association as mutualistic would require demonstrating that sponges with brittlestars have enhanced fitness, such as increased reproductive output or growth. *C. vaginalis* is dioecious and broods larvae in distinct chambers randomly distributed in the sponge tubes. Free-swimming, parenchymella larvae, 0.5–1.4 mm in length, are released during daylight hours throughout most of the year (Lindquist and Hay, 1996; Lindquist et al., 1997). Larval trapping studies have reported 3 to over 200 larvae released by tubes of *C. vaginalis* during the day (Lindquist et al., 1997). The size of the larvae of *C. vaginalis* is at the upper end of the range of the gut constituents of *O. lineata* examined by Hendler (1984), and considering the abundance of larvae in reproductive sponges, they could be an important source of food for *O. lineata*.

The potential for *O. lineata* to consume larvae from *C. vaginalis* prompts the question: is the association between *O. lineata* and *C. vaginalis* a mutualism, commensalism or parasitism? To answer this question, we examined growth and reproduction in *C. vaginalis* living with and without *O. lineata* for up to 8 months on coral reefs off Key Largo, FL. Reproductive output was quantified by collecting sponge larvae and assessing the number of brood chambers from sponges living with and without *O. lineata*. We conducted lab feeding assays in which brittlestars were presented with sponge larvae, as well as field-based experiments to assess differences in larval output from sponges with and without *O. lineata*. Further, we examined the growth of brittlestars confined to brooding and non-brooding *C. vaginalis* and confined to the brooding vase sponge *Niphates digitalis* to determine if the added larval food resource results in increased growth of *O. lineata*.

2. Materials and methods

2.1. Predation on sponge larvae by O. lineata

Laboratory feeding assays were conducted to assess predation on sponge larvae by *O. lineata*. Larvae were collected from *C. vaginalis* and another brooding vase sponge, *N. digitalis*, using the technique described below for larval trapping experiments. Between 20 and 30 free swimming larvae from each sponge species were placed into shallow dishes with ~250 ml seawater and a single *O. lineata*. Dishes were placed in the dark for 5 h, and the number of larvae remaining was compared to control dishes that did not have a brittlestar present. In addition, a video of *O. lineata* consuming larvae from *C. vaginalis* was taken using a Sony HandyCam and LED lighting from the camera.

The growth of *O. lineata* living in brooding and non-brooding *C. vaginalis* and *N. digitalis* was examined at North Dry Rocks, FL (25° 07.850′ N; 80° 17.521′ W), a shallow 10 m subtidal patch reef. The vase sponge *N. digitalis* is a common reef species that also broods larvae year round, but because this sponge species has a much larger tube opening (osculum) that permits predatory fishes to enter, it is not a suitable habitat for *O. lineata* (Henkel and Pawlik, 2005). To increase the sample size of brooding and non-brooding sponge tubes within a reasonable working area of the same reef, both tubes of *C. vaginalis* and modified vases of *N. digitalis* were used for this experiment. To modify vases of *N. digitalis*, the osculae were constricted using a monofilament line, so that the oscular diameter was similar to the average diameter of *C. vaginalis* (~2.5 cm). Brittlestars will not stay in unmodified *N. digitalis*, but will remain in modified vases, and show no difference in preference for modified vases of *N. digitalis* over tubes of *C. vaginalis* (Henkel and Pawlik, 2005). Growth of *O. lineata* was calculated as the percentage change in disk diameter and compared between brittlestars living in brooding and non-brooding sponges using a two-factor ANOVA. The presence of brood chambers and sponge species, *C. vaginalis* and *N. digitalis*, were treated as fixed factors.

In order to assess the presence or absence of brood chambers in sponges, individual tubes ~12 cm in height were collected and 4–6 longitudinal slices were made with a scalpel 5 cm from the top and bottom of each tube, ensuring that each end of the tube was not damaged. Once the reproductive state of each tube was checked, tubes were cable-tied upright to either bricks or acrylic plates secured to the substratum and placed haphazardly at least 2 m apart. Both of these sponge species heal rapidly (Walters and Pawlik, 2005), and sponges were allowed to heal for 2 weeks in the field. After the healing period, *O. lineata* were collected from *C. vaginalis* on the reef and brought back to the lab. The initial disk diameter of each brittlestar was measured using digital calipers and each was tagged with a spot of the histological dye Congo Red on their oral surface. Brittlestars were held overnight in a re-circulating aquarium before being transplanted singly to either brooding or non-brooding sponge tubes. After two months, brittlestars and sponges were collected. The final disk diameter of tagged *O. lineata* was measured and sponges were carefully dissected to determine the presence or absence of brood chambers at the end of the experimental period. Growth data were used in analyses upon verification that a tagged brittlestar had inhabited a sponge tube that maintained the same reproductive condition during the experimental period.

2.2. Effect of O. lineata on C. vaginalis

Three long-term experiments were conducted to examine the effects of the brittlestar *O. lineata* on the host sponge *C. vaginalis*. The first experiment was conducted at North Dry Rocks and then two additional experiments on the upper deck of the *Aquarius* undersea habitat at Conch Reef (24° 56.965′ N; 80° 27.224′ W). Tubes of *C. vaginalis*, 12 cm in height, were collected from 8 m depth on a patch reef at North Dry Rocks and brought back to the lab in containers of seawater. The wet mass of sponge tubes was determined by briefly weighing them on an electronic balance, after which the tubes were returned to seawater containers and then returned to the field within ~3 h. Individual tubes were reattached upright using cable ties to either bricks or acrylic plates that were previously attached to the substratum. A single *O. lineata* was haphazardly placed in half of the sponge tubes to create two treatments, sponge tubes with and without associated *O. lineata*.

During the first experiment on the reef, sponges were inspected every 2 weeks to ensure the presence or absence of *O. lineata*. Immigrant brittlestars were occasionally found in the “without” treatment sponge tubes, and these brittlestars were removed by prodding them with a long, hooked stick. The two subsequent experiments were conducted on the upper deck of the *Aquarius* habitat to reduce the effort spent monitoring for immigrant brittlestars. The *Aquarius* habitat, with its base in ~20 m seawater and the top deck at 9 m depth, provided a substratum that prevented *O. lineata* from invading the experimental treatments. During the second *Aquarius* experiment, sponges were enclosed in 4 separate 1.5 × 0.75 m cages made from 1.7 cm plastic mesh to reduce the effects of fish predation on sponge growth (Leong and Pawlik, 2010a, 2010b). Both treatments were equally distributed in each cage. In addition to the caged treatments, 4 tubes of *C. vaginalis* with *O. lineata* present were placed outside the cage to assess the possible effects of sponge-eating fishes on sponge growth. The effect of *O. lineata* on growth of *C. vaginalis* was examined by comparing

percentage change in mass per day for sponges with and without *O. lineata* using ANOVA for each long-term experiment.

To assess larval output from sponge tubes, larval traps were constructed in a manner similar to those employed in Lindquist et al. (1997) using fine nylon mesh (pantyhose) placed over a ~12 × 8 cm cylindrical 1.7 cm plastic mesh frame. A 50 ml plastic centrifuge tube with its tip excised was attached to one end of the larval trap to create a funnel. A 200 ml plastic collection bottle, with two windows cut into its side and covered by 50 μm mesh to permit some water flow through the bottle, was fitted over the plastic funnel. Larval collection bottles were centered over the osculum of the sponge, with the plastic mesh frame preventing direct contact of the larval traps with the sponge surface and thereby minimizing any effects on the pumping of *C. vaginalis* or deposit-feeding by *O. lineata*. For each long-term experiment, larval traps were placed over sponges for 2–5 days and bottles were collected daily in the afternoon (Table 1). Larvae were quantified by counting them using a dissecting microscope.

After at least 6 months, sponge tubes were collected, brought back to the lab in a seawater container and the final wet mass determined using an electronic balance. Oscular diameter and tube height were also measured; and inner tube surface area was calculated using the equation for a cylinder. The presence or absence of brood chambers was determined by carefully slicing sponge tubes longitudinally in ~7 mm strips. This distance was enough to ensure that brood chambers were only counted once. For sponges from the 2006 and 2007 experiment, photographs were taken of brood chambers and cross sectional area was measured using the image analysis program ImageJ v1.41h.

Three short-term experiments were conducted to examine larval predation in the field (Table 1). For the experiments conducted at Dixie Shoals (25° 04.66′ N; 80° 18.74′ W) and Pickles Reef (24° 59.286′ N; 80° 24.600′ W), sponge tubes, ~12 cm in height, were collected and attached as described previously. Sponge tubes were allowed to heal for 7 days, and then a single *O. lineata* was haphazardly placed into half of the sponge tubes and larval traps were placed over all tubes. The third experiment was conducted on the shipwreck *USS Spiegel Grove* (25° 4.000′ N; 80° 18.651′ W) to examine larval output from whole, intact sponges, as opposed to the manipulated tubes in the previous two experiments. The shipwreck provided a high density of *C. vaginalis* in a localized area. The multi-tubed growth of *C. vaginalis* allows *O. lineata* to move between tubes of an individual sponge. To prevent movement of the brittlestar inside the sponge, a single tube (~12 cm tall) was selected and a piece of fiberglass window screen was placed at the base of the selected tube, by slicing the tube base with a scalpel, placing the screen across the cut surface, and closing the cut surface with a cable tie. For half of the selected sponges, a single *O. lineata* was placed inside the screened tube which prevented the brittlestar from moving to other tubes within the multi-tubed sponge. Larval traps were placed over the mesh-bottomed sponge tube and bottles were collected daily in the afternoon with larvae quantified as previously described.

Linear regression analysis was used to assess the relationship between the number of brood chambers in *C. vaginalis* and the total cross sectional area of brood chambers in a sponge tube. The number of brood chambers per 10 cm^2 sponge tissue was also compared between treatments using ANOVA on log-transformed data. The total number of larvae collected per day from sponges with and without associated *O. lineata* was compared for each long- and short-term larval trapping experiment using ANOVA on log-transformed data. Predation on sponge larvae by *O. lineata* would reduce not only the total number of larvae collected, but also the variation in the number of larvae collected between sponges with brittlestars present compared to sponges without brittlestars. This hypothesis was examined by comparing the coefficient of variation of the average larvae collected per day from the 6 field experiments using a one-tailed paired *t*-test.

Larval output relative to the number of brood chambers per sponge surface area was also compared between *C. vaginalis* with and without *O. lineata* using least squares regression and ANCOVA, as well as quantile regression. Quantile regression is a useful statistical method for complex ecological data that have unequal variance (Cade and Noon, 2003). Quantile regression estimates linear coefficients across the distribution of the response variable, allowing for comparison at upper and lower bounds. Larval output data were compiled from 4 experiments conducted at 3 sites for which the corresponding number of brood chambers in the sponges was known. The number of larvae collected per day was the response variable and the number of brood chambers per 10 cm^2 sponge tissue was the covariate. Brood chambers were quantified at the end of the experiments; therefore, only larval counts from the same time-period were used in analyses, as the abundance of brood chambers may have changed over time and may not be related to previous larval collections. In addition, only brooding sponges or sponges that had produced larvae were included in larval and brood chamber analyses. Quantile regressions were calculated for the 10%, 50%, and 90% quantiles using the quantreg package v4.53 (Koenker) in R. All other statistics, including tests for the assumptions of ANOVA and ANCOVA (Sokal and Rohlf, 1981) were calculated using JMP 7.0 (SAS Institute).

3. Results

In laboratory experiments, a mean of 53 ± 10% SE of sponge larvae placed in a dish with an individual of the brittlestar *O. lineata* (n = 13) were consumed (disappeared) within 5 h. There was no loss of sponge larvae in dishes without a brittlestar present. Videography with infrared illumination of *O. lineata* revealed that brittlestars trap sponge larvae with their tube feet, pass them along an arm to the mouth and then consume them, often several larvae at a time. Sponge larvae were observed escaping tube feet and occasionally swimming out of the mouth of brittlestars; however, sponge larvae did not appear to avoid *O. lineata*.

In field experiments, growth of *O. lineata* did not differ when brittlestars were placed in either of the two different sponge species (Table 2). Although mean growth of brittlestars was greater in brooding sponges than in non-brooding sponges, the variance in these experiments was high, and there was no significant difference between the means (Fig. 1, $p = 0.065$, Table 2). Growth in brooding individuals of *C. vaginalis* and *N. digitalis* was 18.2 ± 6.0% and 29.0 ± 7.7% SE,

Table 1
Timing of long and short term experiments assessing the effect of the brittlestar *Ophiothrix lineata* on the sponge *Callyspongia vaginalis*. Sponge larvae were collected every day during the short term experiments. Metrics: sponge growth (SG); larval counts (LC); brood counts (BC); brood chamber cross-sectional area (BA).

Location	Depth	Dates	Metrics	Larval traps
Long term				
North Dry Rocks	10 m	Feb 3–Oct 4, 2005	SG, LC, BC	Jun 20–25, Jul 5–6, Aug 8–14
Aquarius, Conch Reef	10 m	May 31–Nov 12, 2006	SG, LC, BC, BA	Jul 2–6, Nov 8–12
Aquarius, Conch Reef	10 m	Nov 17, 2006–Jul 6, 2007	SG, LC, BC, BA	Jul 2–6
Short term				
Dixie Shoals	12 m	May 20–25, 2005	LC, BC	
Spiegel Grove	25–30 m	Oct 3–5, 2007	LC	
Pickles Reef	12 m	Dec 4–8, 2007	LC	

Table 2
Results of two-factor ANOVA comparing percentage change in disk diameter of the brittlestar *Ophiothrix lineata* living in two species of sponge, *Callyspongia vaginalis* and *Niphates digitalis*. The presence of brood chambers in sponges was determined prior to the start of the experiment.

Source	DF	F ratio	p
Brood chambers	1	4.406	0.065
Species	1	0.840	0.383
Brood chambers × species	1	0.580	0.466

respectively, compared to growth in non-brooding *C. vaginalis* and *N. digitalis* of 9.5 ± 6.9% and 10.5 ± 4.7%, respectively (Fig. 1, Table 2).

There was no statistical difference in the percentage change in wet tissue mass of *C. vaginalis* after at least 6 months living with and without associated *O. lineata* (Fig. 2, Table 3). Sponge tubes had an overall loss of tissue during the uncaged 2006 *Aquarius* experiment (Fig. 2). In the 2007 *Aquarius* experiment, the 4 uncaged tubes of *C. vaginalis* with associated *O. lineata* grew to a similar extent as caged sponges (8.9 ± 4.8% and 9.5 ± 3.4% SE, respectively).

For experiments in which tubes of *C. vaginalis* had brood chambers present, the number of brood chambers per tube was positively correlated with total brood chamber cross-sectional area of the sponge tube ($\log_{10}$ number of brood chambers = 1.35 $\log_{10}$ total brood cross-sectional surface area + 0.165; $R^2 = 0.9223$ $p < 0.001$). There was no difference in the number of brood chambers per 10 cm^2 sponge tissue between sponges living with and without *O. lineata* (Fig. 3, Table 3).

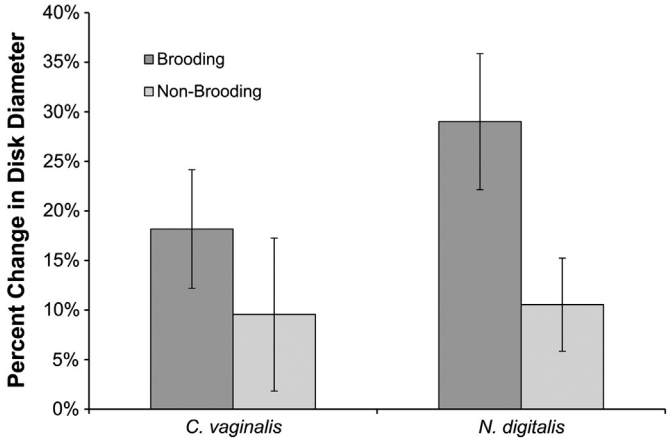

Fig. 1. Percentage change in disk diameter (±SE) of the brittlestar *Ophiothrix lineata* living in brooding and non-brooding tubes of the sponges *Callyspongia vaginalis* (n = 4, 3 respectively) and *Niphates digitalis* (n = 3, 3 respectively) after a 2 month period in the field.

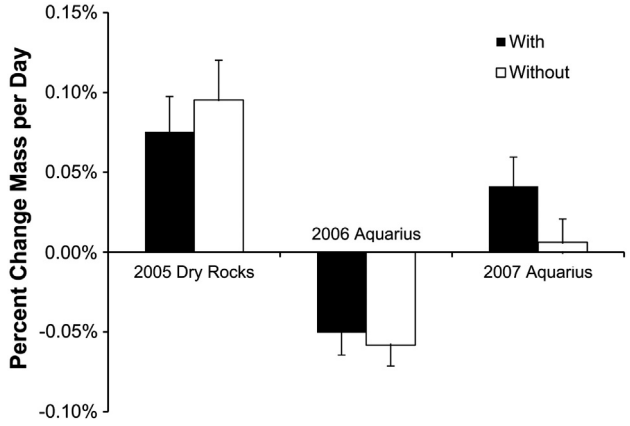

Fig. 2. Mean percentage change in the wet mass of the sponge *Callyspongia vaginalis* (±SE) living with and without associated brittlestar *Ophiothrix lineata*. There was no significant difference in any of the three trials (Table 3).

Table 3
Results of ANOVA comparing growth, number of brood chambers, and number of larvae collected from the sponge *Callyspongia vaginalis* living with and without associated brittlestar *Ophiothrix lineata*. n is the number of sponge tubes with and without *O. lineata* for each experiment.

Treatment	Experiment	n	df	F	p
Percentage change in mass of *C. vaginalis*	North Dry Rocks	16/12	1,27	0.356	0.556
	2006 *Aquarius*	22/24	1,45	0.168	0.068
	2007 *Aquarius*	15/10	1,24	1.890	0.183
Log (number of brood chambers per 10 cm^2 tissue)	North Dry Rocks	15/11	1,25	0.995	0.329
	2006 *Aquarius*	17/22	1,38	0.011	0.919
	2007 *Aquarius*	2/2			
Log (number of larvae collected per day)	Dixie Shoals	18/18	1,35	0.421	0.521
	North Dry Rocks	18/17	1,34	1.617	0.212
	2006 *Aquarius*	21/27	1,47	1.427	0.238
	2007 *Aquarius*	8/3	1,10	0.738	0.413
	Spiegel Grove	16/18	1,33	0.447	0.508
	Pickles Reef	18/22	1,39	0.521	0.475

For larval trapping experiments, the number of larvae collected per day increased with increasing number of sponge brood chambers, and the number of larvae collected per day was significantly greater in sponges without brittlestars compared to sponges with brittlestars based on ANCOVA of least squares regression (Tables 4 and 5, Fig. 4). Linear coefficients of quantile regressions varied between sponges with and without brittlestars (Table 4, Fig. 4). In the 10% quantile, no larvae were collected from sponges with *O. lineata* while larvae were collected from sponges without brittlestars in the same quantile. Sponges within the 50% quantile had a greater increase in larvae collected with increasing brood chambers without a brittlestar present compared to sponges with a brittlestar present. In addition, sponges in the upper 90% quantile differed in the number of larvae collected with respect to the presence of *O. lineata*, with more larvae being collected from sponges without brittlestars compared to sponges with a brittlestar.

When comparing larvae released during the 6 larval trapping experiments, the mean number of larvae collected per day from sponges without *O. lineata* was consistently greater than the mean number of larvae collected per day from sponges with *O. lineata*, although this difference was not significant (Table 3; Fig. 5A). The coefficient of variation of larvae collected per day was high (>1) for all treatments and significantly less in sponges with *O. lineata* present compared to sponges without *O. lineata* present (Fig. 5B; one-tailed paired *t*-test: $t_{15} = 2.018$ $p = 0.0498$).

4. Discussion

The results of this study suggest that the association between the brittlestar *O. lineata* and the tube sponge *C. vaginalis* is not a mutualism,

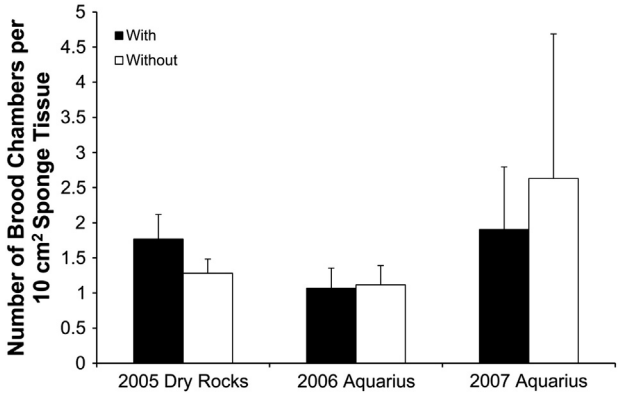

Fig. 3. Mean number of brood chambers per 10 cm^2 of tubes of the sponge *Callyspongia vaginalis* (+ SE) after living with and without associated brittlestar *Ophiothrix lineata*. There was no significant difference in any of the three trials (Table 3).

Table 4
Regression coefficients from least squares regression and quantile regressions. Comparison of number of larvae collected from the sponge *Callyspongia vaginalis* with and without associated brittlestar *Ophiothrix lineata*.

	10th quantile	50th quantile	90th quantile	Least squares
Log total larvae per day + 1 (Y) vs. log brood chambers per cm sponge tissue + 1 (X)				
With *O. lineata*	$Y = 0$	$Y = 3.890\,X + 0.146$	$Y = 5.560\,X + 0.466$	$Y = 4.832\,X + 0.261$
Without *O. lineata*	$Y = 4.810\,X + -0.115$	$Y = 6.497\,X + 0.118$	$Y = 4.184\,X + 0.719$	$Y = 2.811\,X + 0.227$

but can vary from a simple commensalism with the brittlestar living in, and feeding on, the surface of the sponge with no apparent effect on the sponge, to a parasitism with the brittlestar consuming larvae produced by the sponge. The presence of a brittlestar did not result in increased growth or reproductive potential of the sponge, and the brittlestar readily ate larvae produced by the sponge. While other sponge-dwelling fauna directly consume sponge tissue (Pawlik, 1983; Rios and Duffy, 1999), this study is the first to describe predation on sponge larvae by a sponge-dwelling symbiont. Previous studies have found larvae of *C. vaginalis* to be chemically defended against planktivorous predators (Lindquist and Hay, 1996). The present study, however, suggests that larval predators may be dwelling within the sponge itself.

This study can be added to a growing list that have re-assessed symbiotic associations once thought to be mutualistic and found that the net effects of the relationship on the host are neutral, negative, or context-dependent. For example, the sponge *Mycale laevis*, which often grows between or around stony corals on Caribbean reefs, was thought to provide a benefit to adjacent corals (Goreau and Hartman, 1966), but was subsequently found to smother corals in the absence of sponge-eating fishes, which grazed the sponge down to the cracks between coral branches (Loh and Pawlik, 2009, 2012). In a system that is in some ways analogous to the one described in the present study, annelid worms that were thought to be mutualist cleaners of crayfish gills would consume gill tissue when present at high densities (Brown et al., 2012). Density-dependent interactions may be important in driving the shift along the mutualism–parasitism continuum (Stoll et al., 2013).

In three separate field experiments, there was no effect of *O. lineata* on the growth (Fig. 2) or reproductive potential of *C. vaginalis* (Fig. 3). Observed growth rates of *C. vaginalis* were similar to previous reported growth rates of the sponge (Leong and Pawlik, 2010b). Sponge growth can be affected by water flow (Kaandorp, 1999), and the high variation observed in the growth of *C. vaginalis* may be a function of transplantation and small scale differences in flow for each sponge tube. The loss of sponge tissue during the 2006 *Aquarius* experiment could have been due to a variety of factors including food availability or predation by fishes (Leong and Pawlik, 2010a). Four tubes of *C. vaginalis* left uncaged on the *Aquarius* in 2007 had similar growth to caged sponges, suggesting predation pressure was not responsible for the loss of tissue in 2006. However the two experiments were conducted during different seasons (2006 Winter–Spring, 2007 Spring–Summer) and may represent seasonal variation in predation pressure or sponge growth (Leong and Pawlik, 2010a). Interestingly, larval output and brood chamber abundance were similar in 2006 as in other experiments, despite the loss of sponge mass (Figs. 3 and 5A).

The role of *O. lineata* as a parasite, consuming larvae of the *C. vaginalis*, was supported by direct observation in laboratory experiments, and more equivocally supported by field experiments. Because field experiments were performed under conditions in which larval production by sponge tubes was highly variable, and experiments were performed on reefs subjected to storms that resulted in losses of replicates, the statistical power of comparisons was often greatly reduced. For example, the mean growth of *O. lineata* was greater in brooding vs. non-brooding *C. vaginalis* and *N. digitalis* after 2 months in the field, but this difference was not significant ($p = 0.065$; Fig. 1). Post-hoc power analysis revealed that a significant result would have been detected at an $n = 22$, while the number of replicates retrieved in this experiment was only 7 brooding and 6 non-brooding sponges (Fig. 1). Despite the loss of replicates due to storm events, the trend in the data suggests that the additional larval food resource provided by brooding sponges enhanced brittlestar growth during a period as short as 2 months. Next, there were consistent differences in the number of larvae collected in larval traps from sponges with and without associated brittlestars in the field across multiple field experiments. Although the large variation in larval output among sponge tubes resulted in no statistical difference between treatments, on average more larvae were collected from *C. vaginalis* without *O. lineata* in 5 of the 6 larval trapping experiments, with means ranging from 24 to 241% more larvae collected per day relative to sponges with *O. lineata* (Fig. 5A). The pattern of lower numbers of larvae collected from sponges with associated brittlestars is based on data collected across three seasons with samples from 99 sponges with *O. lineata* and 105 without associated brittlestars. More convincing, however, is the analysis of a subset of these experiments for which data on the abundance of brood chambers was also available, and for these, the number of larvae collected per day was significantly greater in sponges without brittlestars (Fig. 4). Given the large amount of variation in larval output observed, quantile regressions were examined in addition to typical least squares regression models. While least squares regressions estimate the slope based on the entire distribution, quantile regressions allow examination of patterns at different points in the distribution of the response variable (Cade and Noon, 2003). There was no significant difference between the slopes calculated using least square means (Table 5), however the change in larvae collected per day relative to increasing brood chambers was greater in the 50% quantile of sponges without associated brittlestars compared to sponges with a brittlestar (Table 4). Slopes and intercepts also varied between the two groups of sponges in the 90% and 10% quantiles, with fewer larvae collected from sponges with *O. lineata* present. Comparison of the 90% quantile suggests that larval output is constrained because of larval predation by *O. lineata*. Additionally, sponges with brittlestars had a lower coefficient of variation compared to sponges without brittlestars (Fig. 5B), suggesting that larval predation by *O. lineata* reduced the variability in the number of larvae released and may restrict the maximum larval output by the sponge. Overall then, while more equivocal than the direct evidence of brittlestars feeding on sponge larvae in laboratory experiments, the patterns from field experiments of reduced larval output and lower variation in larval production by sponges with associated brittlestars, along with the higher mean growth of *O. lineata* living in brooding sponges (Fig. 1), support the hypothesis that *O. lineata* consumes *C. vaginalis* larvae in the field.

The discovery that *O. lineata* could be a larval parasite of *C. vaginalis* raises interesting questions about the fitness impact of the brittlestar on its host sponge. Based on data from all 6 larval trapping experiments, there was an average of 5.4 larvae released per day from sponge tubes

Table 5
Analysis of covariance (ANCOVA) using least squares means comparing number of larvae collected from the sponge *Callyspongia vaginalis* with and without associated brittlestar *Ophiothrix lineata*.

Source	df	F	p
Treatment	1	3.943	0.0498
Slope	1	1.491	0.2250

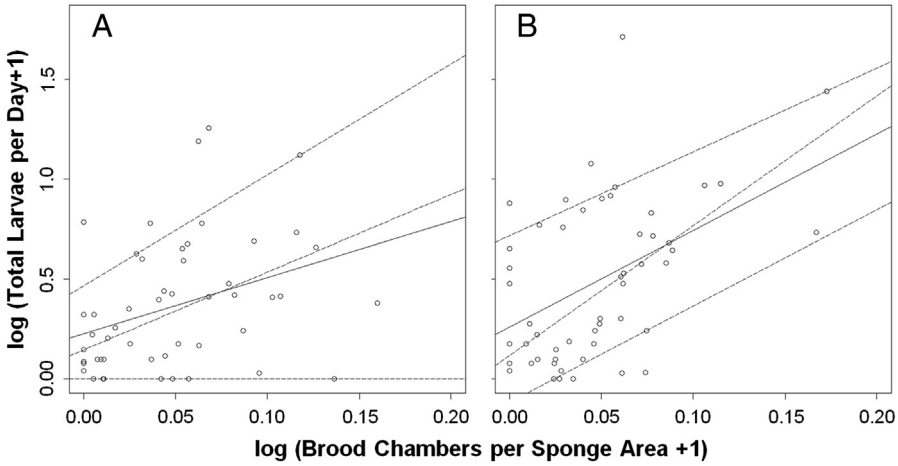

Fig. 4. Log–log plot of total larvae collected per day as a function of the number of brood chambers per cm^2 of the sponge *Callyspongia vaginalis*. Larvae were collected from sponge tubes (A) with brittlestars and (B) without associated brittlestars. The solid line represents least squares regression, and dashed lines represent the 10%, 50% and 90% quantile regression.

with associated brittlestars (n = 99) compared to 7.4 larvae per day from sponges without brittlestars (n = 105). Thus, sponge tubes without brittlestars may release an average of 27% more larvae relative to sponges with an associated brittlestar. The overall effect on populations of *C. vaginalis* would be determined by the relative population density of *O. lineata*. Densities of *O. lineata* increase with the size of individual *C. vaginalis* (Henkel and Pawlik, 2011), increasing the probability that a single *O. lineata* will encounter sponge larvae. A previous survey in the Florida Keys found that 85% of *C. vaginalis* had *O. lineata* present, with only 58% of sponges containing brittlestars with a disk diameter ≥5 mm and no preferential occurrence in sponges that were brooding (61% of *C. vaginalis*; Henkel and Pawlik, 2005). If *O. lineata* is associated with 58% of *C. vaginalis* and 61% of inhabited sponges are brooding, then larval predation could occur in 35% of *C. vaginalis*. While the factors limiting the population size of *O. lineata* are unclear, the presence of large *O. lineata* in only 58% of *C. vaginalis* suggests that sponge habitat is not limiting (Henkel and Pawlik, 2005). Based on the data presented here, we would expect that any increase in populations of *O. lineata* could have a negative impact on *C. vaginalis*, and the factors currently limiting the population size of *O. lineata* may have an indirect positive effect on *C. vaginalis*.

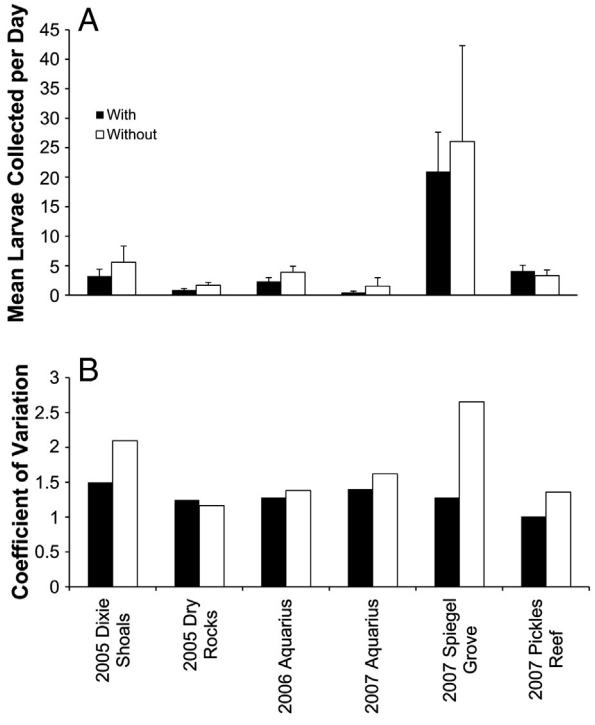

Fig. 5. (A) Mean number of larvae collected from sponge tubes of *Callyspongia vaginalis* with and without associated *Ophiothrix lineata* per day (+SE) and (B) coefficient of variation for the same data for each of the 6 experimental iterations. There was no significant difference in the number of larvae collected per day between the two treatments in any of the 6 experiments (Table 3); however, the coefficient of variation was significantly less in sponges with *O. lineata* compared to sponges without *O. lineata* (one-tailed paired *t*-test: $t_{15} = 2.018$ $p = 0.0498$).

The present study provides further support for the context dependent, mutualism–parasitism continuum view of symbiotic associations. Considering that *O. lineata* exhibits strong host specificity for *C. vaginalis* (Henkel and Pawlik, 2005, 2011), has no positive effect on the growth of the sponge (this study), and may reduce sponge fitness by consuming sponge larvae (this study), the association between *O. lineata* and *C. vaginalis* is either a commensal relationship if the sponge is not producing larvae, or the relationship is a parasitism if the sponge is producing larvae that are being captured and eaten by the brittlestar.

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