Symbionts and environmental factors related to deep-sea coral size and health

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Summer 2018

Keywords: deep-sea coral, epibionts, symbionts, ecology, Sur Ridge, white polyps

ABSTRACT

We analyzed video footage from a remotely operated vehicle to estimate the size, environmental variation, and epibiont community of three types of deep-sea corals (class Anthozoa) at Sur Ridge off the coast of central California. For all three of the corals, *Keratoisis*, *Isidella tentaculum,* and *Paragorgia arborea,* species type was correlated with the number of epibionts on the coral. *Paragorgia arborea* had the highest average number of symbionts, followed by *Keratoisis*. Epibionts were identified to the lowest possible taxonomic level and categorized as predators or commensalists. Around twice as many *Keratoisis* were found with predators as *Isidella tentaculum*, while no predators were found on *Paragorgia arborea*. Corals were also measured from photos and divided into size classes for each type based on natural breaks. The northern sites of the mound supported larger *Keratoisis* and *Isidella tentaculum* than the southern portion, but there was no relationship between size and location for *Paragorgia arborea*. The northern sites of Sur Ridge were also the only place white polyps were found. These polyps were seen mostly on *Keratoisis*, but were occasionally found on the skeletons of *Isidella tentaculum* and even *Lillipathes,* an entirely separate subclass of corals from *Keratoisis*. Overall, although coral size appears to be impacted by

environmental variables and location for *Keratoisis* and *Isidella tentaculum*, the presence of symbionts did not appear to correlate with coral size for any of the coral types.

INTRODUCTION

Deep-sea corals of the class Anthozoa can be found in every ocean in the world at a range of depths. Most are found between 200 and 1,000m, but they can range from 30 to 3,000m (Mortensen and Buhl-Mortensen, 2005). These suspension feeders may contain symbionts within their tissues as well as external epibionts (Bright-Diaz et al., 2011). The corals provide important spatial heterogeneity on the sea floor and act as microhabitats for hundreds of different types of deep-sea invertebrates and fish.

The extremely slow growth of these corals may limit their resiliency to disturbances and changes in environmental variables (Andrews et al, 2009). Despite this vulnerability, previous studies have shown that modular branching octocorals may exhibit a high potential for character lability due to the ability to respond to stressors at different morphological levels (polyps and branches) (Duenas and Sanchez, 2009). Thus far, few studies have explored the way environmental variation impacts deep-sea coral morphology and epibiont presence.

The study site, Sur Ridge, is a deep-sea ridge home to many different species of corals. Located off the coast of Big Sur, the ridge lies within the Monterey Bay National Marine Sanctuary (MBNMS), which was established by NOAA in 1992 (Taylor et al., 2014). Sur Ridge contains sites both within and outside of the oxygen minimum zone, which ranges from around 500-1000m in the sanctuary (Taylor et al., 2014). Sur Ridge has depths ranging from 800-1300m and temperature variation of around a degree and a half Celsius, making it an ideal site for studying the environmental variables that affect coral morphology. Previous studies have shown a decrease in community biomass and abundance with low oxygen levels (Mullins et al., 1985, Duffy et al., 2013). Building on

current knowledge, this project aims to assess potential relationships between environmental variables, external symbionts, and deep-sea coral size.

MATERIALS AND METHODS

VIDEO SURVEYS

Video footage taken by the ROV Doc Ricketts of corals at Sur Ridge was annotated using MBARI's Video Annotation and Reference System (VARS). Corals and their symbionts were identified to the lowest practical taxonomic level using conservative assignments. Symbionts were counted on each coral, and symbionts that could not be counted were estimated in order to keep overall symbiont load as accurate as possible. 10 symbionts were recorded for the presence of amphipod tubes, 15 for the presence of hydroids, 50 for the presence of gray polynoid worms, and 20 for the presence of zoanthids. The presence or absence of white polyps and the percent of the animal with dead, exposed white skeleton was also measured for each coral.
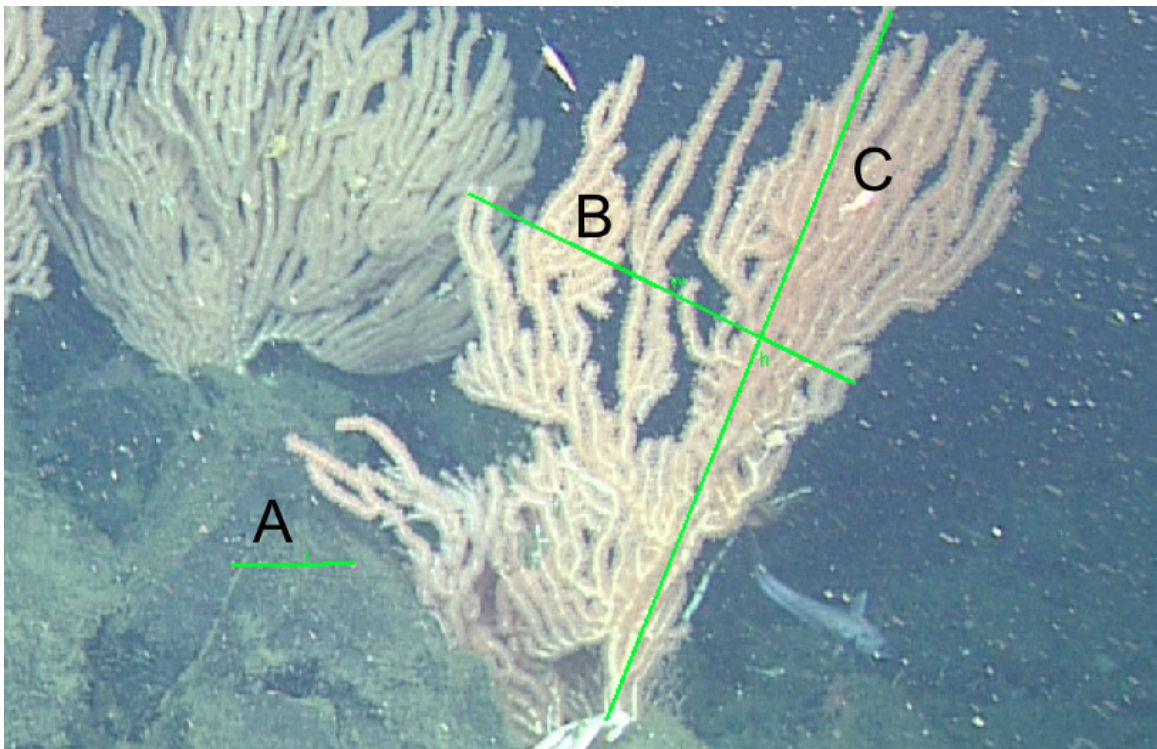
Figure 1. A section of a screen-grab showing the pixel measurement between lasers (A), the width measurement (B), and the height measurement (C) for a *Keratoisis*.

A video still was taken when each coral was in the vertical center of the frame and two horizontal sizing-lasers, which were positioned 29cm apart on the ROV, were clearly visible. The distance between laser points in each image was measured, along with the height and width of each coral. Height and width were measured diagonally for corals that were leaning over. Corals that could not be centered vertically in the frame with clear laser points were not used for statistical analysis. The pixel distance of height measurements were multiplied by 29 then divided by the pixel distance between lasers. The same equation was applied to width measurements before height and width were multiplied together to give a total area. For *Isidella tentaculum*, the total area was multiplied by 1.5 to correct for the candelabra shape. Size classes for each species of coral were determined based on the Jenks optimization method for finding natural breaks.

DATA ANALYSIS AND STATISTICS

Single factor ANOVAs and two-tailed T-tests assuming unequal variances were performed in Microsoft Excel with alpha values = 0.05. Figures were created in Excel, and maps were created using ArcGIS. PRIMER 7 (Clarke and Gorley, 2015) was used to develop a similarity index based on Euclidean distance and an associated metric multidimensional scaling (MDS) graph, overlaid with a segmented bubble plot showing the mean values for each variable. This was done using non-transformed data. Stress (a goodness of fit test) = 0.01.

RESULTS

ENVIRONMENTAL FACTORS

Consistent with oxygen patterns along rest of the California coast, there is an increase in oxygen and a decrease in temperature with increasing depth at Sur Ridge (**Figure 2**). Several of the corals analyzed in this project were within the oxygen minimum zone (OMZ), defined as <0.5 ml/l of dissolved O_2 (**Figure 2**).
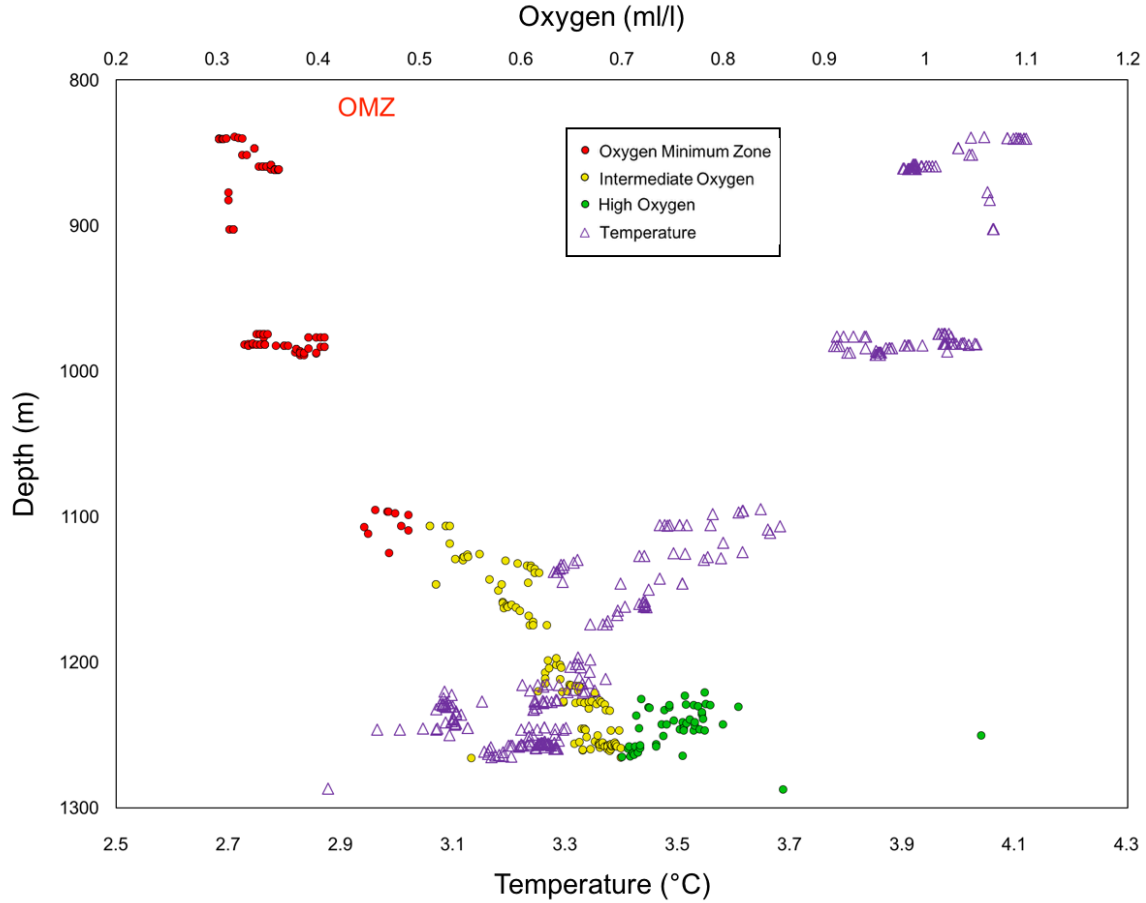
Figure 2. Temperature and oxygen levels for each site plotted against depth with points within the oxygen minimum zone (OMZ) in red. OMZ is defined as areas with <0.5 ml/l.

Keratoisis and *Isidella tentaculum* that are located in shallower, warmer sites within the OMZ tend to be smaller in area, while depth, oxygen, and temperature do not appear to correlate with the size of *Paragorgia arborea.* (**Figure 3**). The P-values for coral size-class in relation to depth were found to be 0.526 for *Paragorgia arborea*, 4.543E-11 for *Isidella tentaculum*, and 0.002 for *Keratoisis*. Locational data is consistent with these trends, as the northern, deeper part of the ridge supports the largest *Keratoisis* and *Isidella tentaculum* specimen (**Figures 4 & 5**).
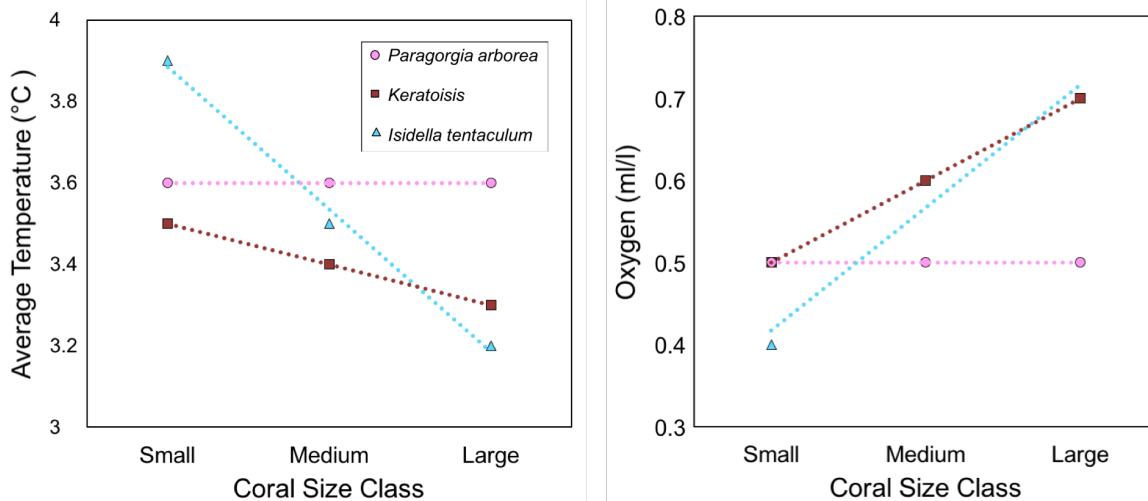
Figure 3. Large *Keratoisis* and *Isidella tentaculum* were most likely to be found in colder, higher oxygen environments, while the size of *Paragorgia arborea* did not appear to change with differences in temperature or oxygen.
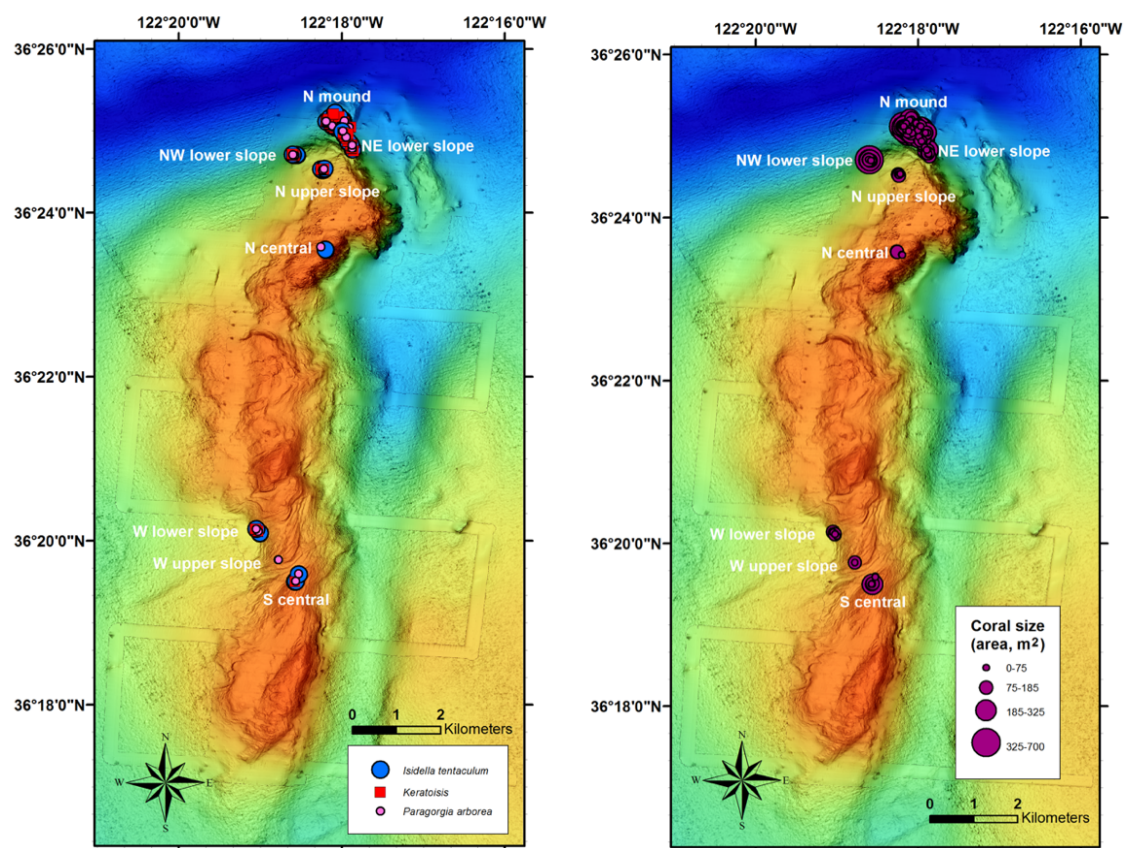
Figure 4. Locations of different coral types (left) and sizes (right).
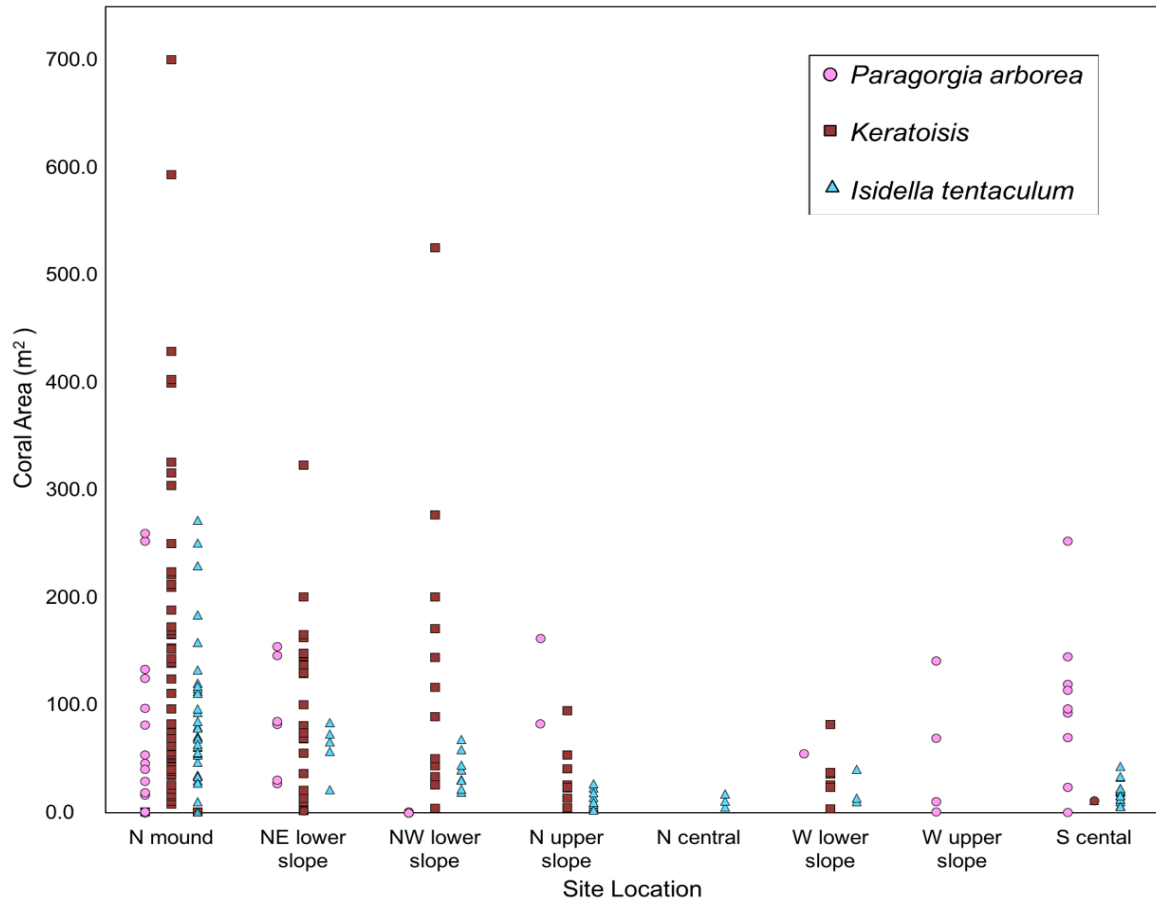
Figure 5. Coral area measurements (m^2) at different areas.

SYMBIONTS

Species type was found to be correlated with symbiont load. *Paragorgia arborea* was most likely to contain symbionts and also had the highest average number of symbionts per coral, followed by *Keratoisis* and *Isidella tentaculum* (**Table 1**). A single factor ANOVA comparing symbiont loads for the three coral types resulted in a P-value of 1.023E-21, and two-sample T-tests comparing the types one at a time each resulted in significant results. The T-test P-value that resulted from comparing *Isidella tentaculum* symbiont load to that of *Keratoisis* was 0.0003, while for *Isidella tentaculum* and *Paragorgia arborea* the P-value was 2.726E-8, and for *Keratoisis* and *Paragorgia arborea* it was P = 1.268E-6. These values show that *Paragorgia arborea* contains on average, a significantly larger number of symbionts. When predators were isolated from other symbionts, however, *Keratoisis* became the most likely to be "infected", followed by *Isidella*

tentaculum (**Table 2**). None of the *Paragorgia arborea* analyzed contained any predators.

Table 1. *Paragorgia arborea* corals were most likely to have symbionts, as almost 75% of the corals examined hosted epibionts of some type. This species also supported the highest average number of symbionts per coral, followed by *Keratoisis* and *Isidella tentaculum.*

| Species	Symbionts present	Average Number	Standard Deviation
Isidella tentaculum	30.77%	2.9	6.4
Keratoisis	67.96%	7.5	10.5
Paragorgia arborea	74.47%	32.0	29.7

Table 2. *Keratoisis* individuals were almost twice as likely to be found with predators on them as *Isidella tentaculum*, while no predators were seen on any of the *Paragorgia arborea* sampled.

Species	Predators present	Average Number	Standard Deviation
Isidella tentaculum	6.59%	0.120879121	0.554480033
Keratoisis	11.65%	0.147368421	0.397258835
Paragorgia Arborea	0.00%	0	0

Coral size was found to be a poor predictor of symbiont load (**Figure 6**), but location was statistically significant (P=0.00016), with the highest numbers of symbionts per coral found on the North Mound and the Northeast Lower Slope (**Table 3**).

Table 3. Summary statistics for symbionts in relation to location. The single factor ANOVA for this data resulted in a statistically significant difference between the average symbiont load per coral at the sites (P=0.00016).

Location	Count	Sum	Average	Variance
North central	19	27	1.4	20.7
North mound	114	1222	**10.7**	302.5
North upper slope	36	160	4.4	230.4
Northeast lower slope	41	794	**19.4**	588.7
Northwest lower slope	31	145	4.7	126.5
South central	30	167	5.6	180.7
West lower slope	26	155	6.0	213.8
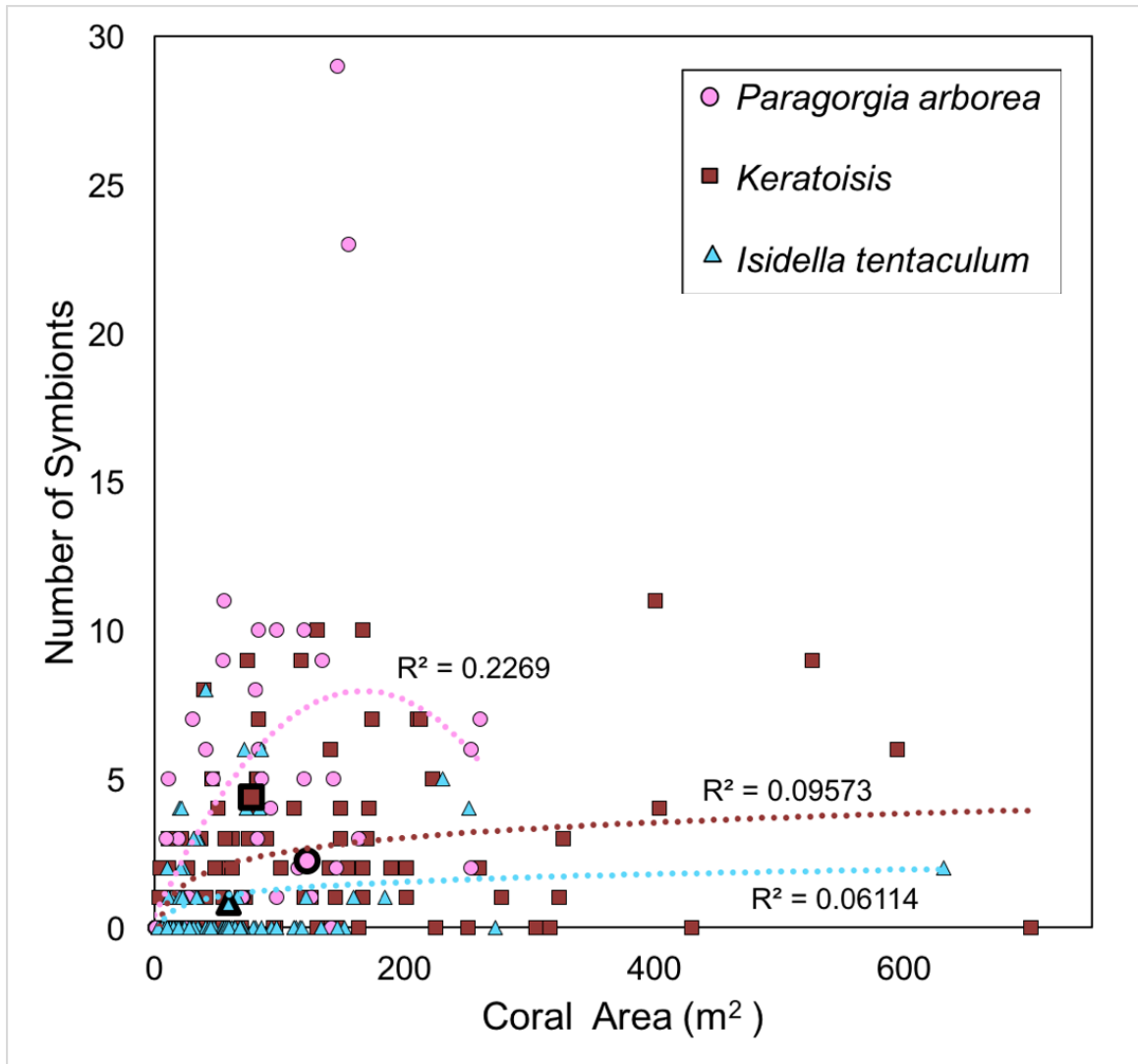
Figure 6. Number of symbionts plotted against coral area with best fit lines determined by highest correlation coefficients (R^2 value).

WHITE POLYPS

> White polyps were usually found on corals with dead sections and unhealthy polyps. Of the corals with white polyps, only 4.9% of them appeared completely healthy otherwise. These polyps were usually found on *Keratoisis*, but were occasionally observed on *Isidella tentaculum* and *Lillipathes*. All observances of white polyps were on corals living in the northern sites of Sur Ridge (**Figure 7**).
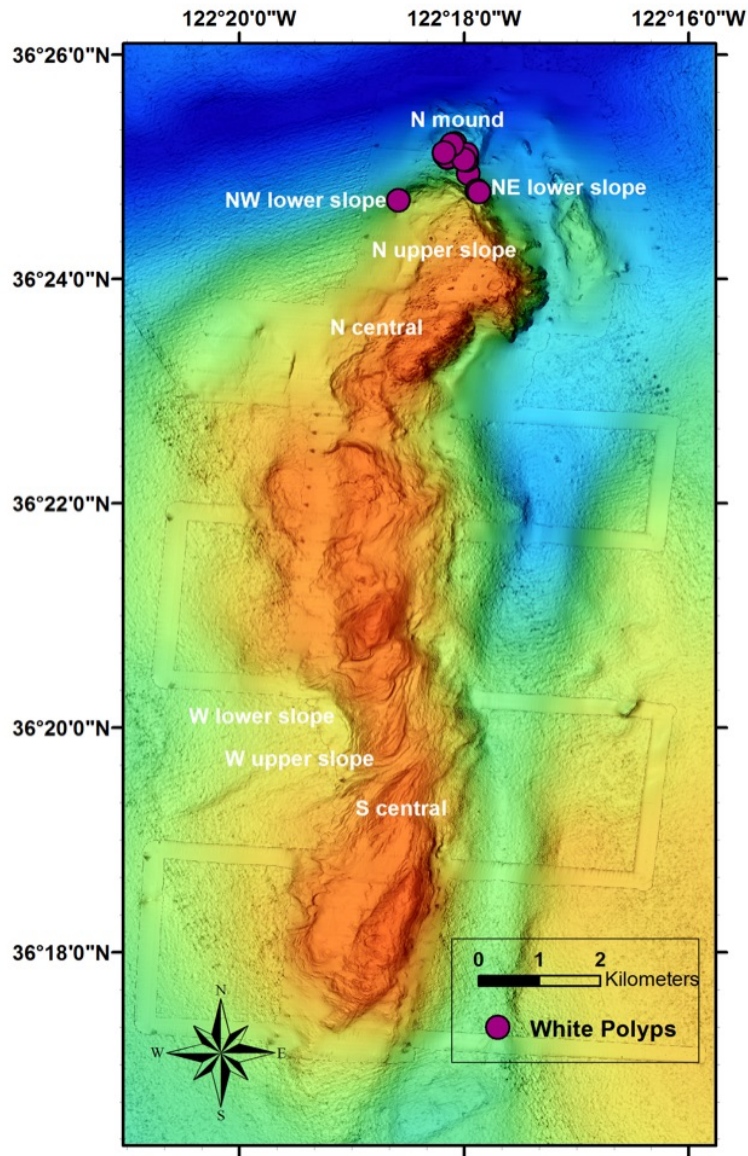
Figure 7. Location of corals in which white polyps were present.

SUMMARY

Small *Isidella tentaculum* were differentiated from the rest of the coral types and size classes based on their likelihood to be found in locations with higher temperatures. Large *Isidella tentaculum* and *Keratoisis* could be grouped together in the MDS based on their affinity for deeper, higher oxygen sites (**Figure 8**).

Paragorgia arborea of all size classes appeared in a similar location on the MDS due to their high numbers of epibionts and larger temperature range (**Figure 8**).
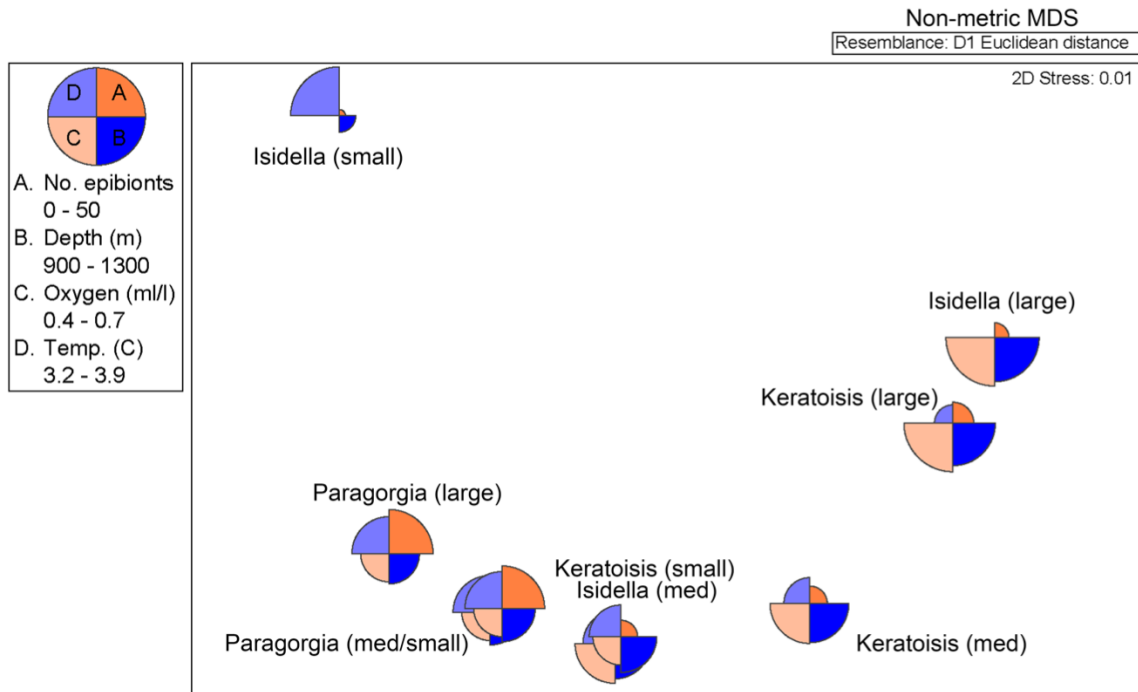
Figure 8. A representation of non-metric multidimensional scaling based on the number of epibionts (A), depth (B), oxygen (C), and temperature (D). The distance between coral types represents overall difference, and the sizes of the wedges illustrate the degree of influence each of the variables have on the size of that distance.

DISCUSSION

ROV cameras allow for detailed analysis of deep-sea communities but still involve certain limitations and challenges. The oblique view of the science camera, while advantageous for identifying organisms, distorts the field of view in the image and makes it more difficult to obtain accurate measurements of area. A previous study of the sizing laser technique showed no significant difference in accuracy of horizontal and vertical measurements but does show an underestimation for measurements taken at a diagonal orientation (Dunlop et al., 2014).

The relationship between coral size and oxygen levels supports previous findings of a decrease in diversity at submarine canyon depths considered "low oxygen environments" (Duffy et al., 2013). While *Keratoisis* and *Isidella tentaculum* appeared to respond similarly to environmental variables, *Paragorgia arborea* size did not appear to be determined by depth, oxygen, or temperature changes. A previous study in the Atlantic correlated *Paragorgia arborea* colony height with the size of the boulder it was attached to (Mortensen and Buhl-Mortensen, 2005).

The predator defense mechanisms of different types of corals may play an important part in determining symbiont load. *Paragorgia arborea*, for example, contains a lipophilic chemical compound which has been shown to repel pinfish (Bright-Diaz et al., 2011). This may help explain the absence of predators on the species. The high number of average symbionts can be attributed to the gray polynoid worms that often inhabit *Paragorgia arborea*. *Isidella tentaculum's* predation avoidance involves long, stinging sweeper tentacles that project out from the base of the colony and prevent predators such as *Hippasterea* and *Tritonia* from climbing up to the branches of the coral (Etnoyer 2008).

It is unclear whether the white polyps appearing at the northern sites of Sur Ridge are a response to predation by *Keratoisis*. The white polyps can be seen taking over bare skeleton in time sequence photos, but whether these polyps are new growth from the original colony or settlement from a new colony is still uncertain. Previous genetic analysis of normally colored polyps from a different seamount has shown that "morphologically-cryptic species variation" may exist among bamboo corals (family Isididae), which can make it more difficult to categorize polyp type (France, 2007). It appears that at least in certain instances, these white polyps are settling rather than growing from the same colony, as they were observed on a *Lillipathes* which does not share the same subclass as *Keratoisis* and *Isidella tentaculum* (**Figure 9**).
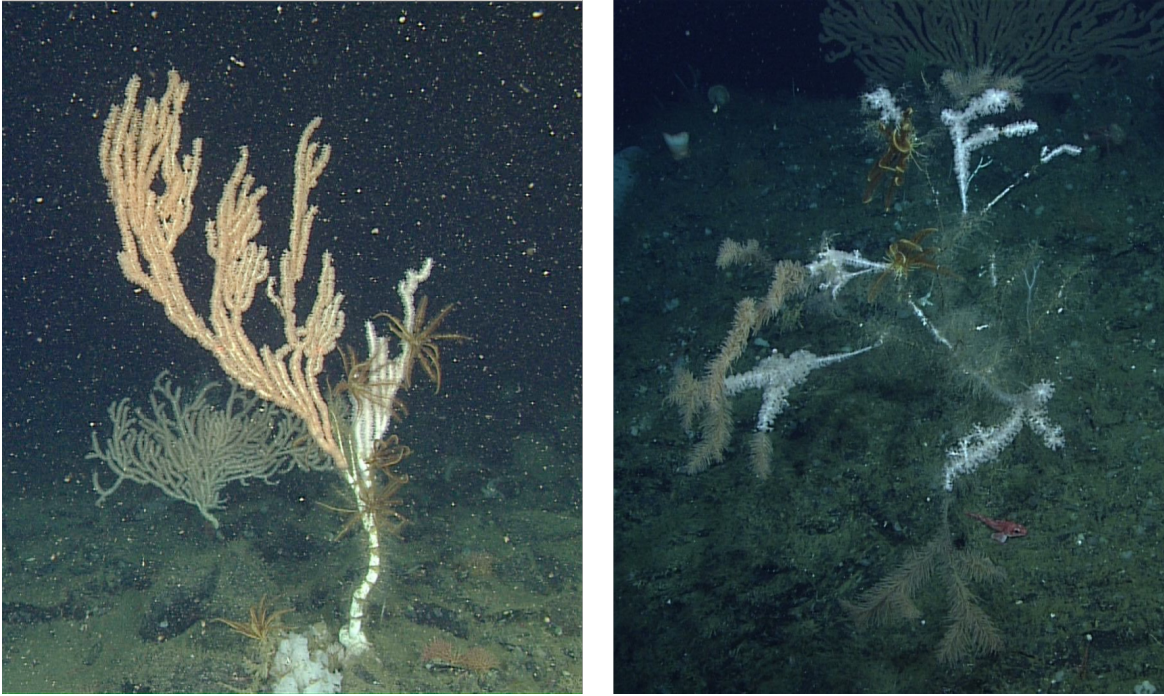
Figure 9. White polyps on the skeletons of *Isidella tentaculum* (left) and *Lillipathes* (right).

CONCLUSIONS/RECOMMENDATIONS

Environmental parameters such as depth, oxygen, and temperature were found to influence size in *Keratoisis* and *Isidella tentaculum*, but there are several other factors that could be considered, such as coral orientation, current flow, and substrate size. Quantitative transects would allow for biomass estimates to accompany area estimates, giving a better overall picture of the community structure of Sur Ridge. Information was gathered for eight other coral types, but more data is required for each before statistical analysis can take place. Analysis of the DNA of white polyps and fully-pigmented polyps from the same skeleton could help determine whether these polyps are growing from the same colony or are settling on the dead skeletons built by other colonies. Future studies may also compare the accuracy of polygonal area measurements to traditional length and width measurements.

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SUPPLEMENTAL INFORMATION

Table 4. Average number of each type of symbiont for each type of coral analyzed. The highest average of each type of symbiont is bolded.

Symbiont type	Acanthogorgia	Corallium	Isidella tentaculum	Isididae	Keratoisis	Lepidisis	Lillipathes	Paragorgia arborea	Parastenella	Sibogagorgia cauliflora	Swiftia kofoidi
Aeolid nudi	0.000	0.000	**0.044**	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
amphipod tubes (10=y, 0=n)	0.000	0.000	0.659	0.000	0.680	0.000	0.000	**1.064**	0.000	0.000	0.000
amphipods (caprellid)	**19.000**	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500
amphipods (gammarid) (1=y, 0=n)	0.000	0.000	0.011	0.000	**0.058**	0.000	0.000	0.000	0.000	0.000	0.000
anemone	1.000	**2.750**	0.044	0.000	1.291	0.000	0.200	2.277	0.000	0.045	0.000
Asteronyx	0.000	0.000	0.000	0.000	0.019	0.000	0.000	**0.277**	0.000	0.000	0.000
Careproctus kawakami	0.000	0.000	**0.033**	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Chorilia	0.000	0.000	0.000	0.125	0.019	0.000	0.000	**0.277**	0.250	0.045	0.000
Crinoid	0.000	**0.250**	0.209	0.000	0.233	0.000	0.200	0.404	0.000	0.000	0.000
Hippasteria	0.000	0.000	**0.022**	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Hydroids (15=y, 0=n)	0.000	**11.250**	1.198	1.875	3.786	0.000	0.000	0.638	0.000	0.000	0.000
Galathea (wht)	0.000	**0.250**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gastroptychus	0.000	0.000	0.022	0.000	0.010	0.000	0.000	**0.128**	0.000	0.000	0.000
Gastropoda	0.000	0.000	**0.022**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gorgonocephalus	0.000	0.000	0.000	0.000	0.010	0.000	0.000	**0.106**	0.000	0.000	0.000
lithodid	0.000	0.000	0.000	0.000	**0.029**	0.000	0.200	0.021	0.000	0.000	0.000
Mysids (1=y, 0=n)	1.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000
Neolithodes	0.000	0.000	0.022	0.000	0.000	0.000	0.000	**0.064**	0.000	0.000	0.000
Ophiuroidea	0.000	0.000	0.000	0.000	0.010	0.000	**2.200**	0.021	2.000	0.000	1.250
Pandalopsis	0.000	0.000	0.000	0.000	0.019	0.000	0.000	**0.064**	0.000	0.000	0.000
Shrimp (red)	0.000	0.000	**0.077**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Paralomis	0.000	0.000	0.000	0.000	0.010	0.000	0.000	**0.043**	0.000	0.000	0.000
Gray Polynoids (50=y, 0=n)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	26.596	0.000	0.000	0.000
Poraniopsis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.250**
Psolus	0.000	0.000	0.000	0.000	**0.010**	0.000	0.000	0.000	0.000	0.000	0.000
Pycnogonida	0.000	0.000	**0.033**	0.000	0.010	0.000	0.000	0.021	0.000	0.000	0.000
Tritonia	0.000	0.000	0.055	0.000	**0.087**	0.000	0.000	0.000	0.000	0.000	0.000
Zoanthids (20=y, 0=n)	0.000	0.000	0.440	0.000	**1.165**	0.000	0.000	0.000	0.000	0.000	0.000