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Dark Spots Disease Increase in Scleractinian Corals in Bonaire, D.C.

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Abstract

Pathogen-host relation dynamics have adjusted in the Caribbean due to increased epizootic events and decreased coral cover resulting from anthropogenic influences. Reef-building corals are being infected by numerous diseases including dark spots disease, a ubiquitous Caribbean disease of an unknown agent. The objectives of this study were to quantify the change in dark spots disease prevalence in *Siderastrea siderea* and *Stephanocoenia spp*. from 1998 to 2014 and determine influencing conditions on prevalence and infection severity of disease. The abundance of benthic organisms and substrate types were also quantified. A 1350 m^2 area between six sites on Bonaire was surveyed using belt and video transects to determine disease prevalence and benthic composition. Prevalence was compared temporally (1998 study to 2014 study) and spatially (Bonaire to Turks & Caicos, Grenada, and Bahamas). I found an increase in disease prevalence between 1998 and 2014 and moderate spatial variation between island sites. Site, colony size, spatial distribution, or coral density did not influence disease prevalence or infection severity of the disease. Substrate types varied between sites with live hard coral cover and sand and rubble cover. As dark spots disease did not have a positive correlation between coral density and prevalence, DSD does not follow a density- dependent model. Dark spots disease in these coral species most likely arises from opportunistic pathogens emerging from stressful environmental conditions due to lack of density-dependence. With the changing environment induced by anthropogenic consequences, it is prudent to monitor and quantify the status of reefs in terms of disease prevalence and its disease associated factors.

Keywords: Dark spots disease, Bonaire, coral density, prevalence**,** *Siderastrea siderea*, *Stephanocoenia spp*.

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Introduction

With loss of coral reefs amounting to more than 25% globally with an added 16% at risk (Sutherland et al. 2004), the increase in frequency of disease outbreaks is of great concern. The Caribbean, a hotspot for disease virulence and prevalence, has seen altering dynamics of pathogen-host relations as the ecosystem reconditions from outbreaks and less coral density as a result of climate change, human development, among other anthropogenic consequences. As established by Mora (2008), on small and large scales, humans have the strongest effect on the reef environment as human population size is positively correlated with macroalgae density and coral mortality and negatively correlated with biomass of herbivorous and carnivorous fish. Moreover, temperature was shown to correlate negatively with coral mortality and herbivorous fish biomass (Mora 2008). Similarly, Bruno et al. (2007) illustrated that thermal stress and coral density positively correlated with outbreaks of coral disease; therefore it seems likely that reefs with high human impacts and high coral cover will be affected by high outbreak frequency (Mora 2008, Bruno et al. 2007).

The alteration of the relationship between pathogen and host can be defined by the interaction of the host, in the form of the immune response, and pathogen as a reaction to the abiotic and biotic factors (Sutherland et al. 2004, Mydlarz et al. 2006, Patterson et al. 2002). As anthropogenic influences seep into the reef environment, abiotic conditions (i.e. nutrients, dissolved oxygen, temperature) and community assemblage are altered. This biotic and abiotic transformation of coral reef ecosystems is likely to have effects on the dynamics between corals and their pathogens. The simplistic immune system of corals, consisting of primarily mucus production and scattered phagocytic cells (Sutherland et al. 2004, Mydlarz et al. 2006), is often perturbed by wavering environmental conditions whereas the virulence of the pathogen is often heightened by the same conditions (Patterson et al. 2002). With the continual transformation of the reef environment, these factors are becoming increasingly pertinent to research as they offer the key to understanding the resulting dynamic between the coral host and its pathogen.

Dark spots disease (DSD) affects reef-building corals and is primarily a Caribbean coral disease. Dark spots disease is of concern as it is nearly ubiquitous and has an unconfirmed causative agent, suggested to be biotic in origin (Sutherland et al. 2004). Gil-Agudelo et al. (2007) identified a consortium of *Vibrio* related bacteria present in diseased colonies of *Orbicella annularis* (Ellis 1786), *Orbicella*

faveolata (Ellis 1786), and *Siderastrea siderea* (Ellis 1786), but not in healthy colonies from the same location, however inoculation processes were unsuccessful and Koch's postulates were not reached. Koch's postulates are measures for determining causative agents of disease. The process for obtaining Koch's postulates are as follows: the microbe is obtained from a diseased individual and cultured in lab. The cultured microbe is then inoculated into the same species infected by the disease and this individual must display symptoms of the disease. The newly diseased individual is sampled for microbes and the microbe must match the original inoculating microbe in order from Koch's postulates to be obtained. Due to the strenuous procedures of the postulates and the difficultly in inoculation of coral species in the lab, Koch's postulates are often unattainable for coral disease.

Characterized by irregular spots of purple to brown coloration, DSD can lead to tissue death and dimpling of the colony with its spread at a rate of four centimeters per month (Sutherland et al. 2004; Cervino et al. 2001). *Siderastrea siderea* and *Stephanocoenia spp*. (Lamarck 1816, Milne-Edwards &Haime 1848) are a few of the most susceptible massive corals (Cróquer & Weil 2009a, Cervino et al. 2001) whereas cases in *O*. *annularis*, *O*. *faveolata*, and *Montastraea cavernosa* (Linnaeus 1766) are sparse (Cróquer & Weil 2009a). The disease spreads in a clumped distribution (Gil-Agudelo and Garzón Ferreira 2001), leading to speculation of a correlation between spread and spatial distribution of susceptible corals for pathogen transmission. Positive correlations with high seasonal temperatures and shallower depths have been determined (Gil-Agudelo & Garzón Ferreira 2001); however no correlation has been established with other consequential abiotic conditions or anthropogenic influences. Borger (2005), though, has suggested that increased water temperature may be the agent of DSD in *S*. *siderea* as the coral produces a general stress response, triggering the blemishes.

Alarming levels of DSD were reached in 1998 with 53% of *S*. *siderea* infected in the reefs around Bonaire, but prevalence was not quantified in *Stephanocoenia spp*. (Cervino et al. 2001). In the last 15 years, Bonaire and its reefs have been subject to increased human impact as resident and tourist populations and, consequently, nutrient (nitrogen and phosphorus) production have risen (Van Kekem et al. 2006). The objectives of this study were to 1) determine differences in the prevalence of DSD on *S*. *siderea* and *Stephanocoenia spp*. from 1998 to 2014, 2) determine conditions (site, colony size, coral density and spatial

distribution) that may influence the prevalence or percent cover of DSD on these coral species and 3) quantify differences in substrate cover between sites to determine any role in DSD distribution.

Methods

Sampling design and study sites

Bonaire, a 294 km^2 island in the Dutch Caribbean 100 km off the northern coast of Venezuela, was chosen as the study site for the DSD research. The island has a sloping reef (~ 45°) to approximately 30 meters. Six sites on Bonaire were surveyed for DSD prevalence between June and August 2014 to determine any temporal or spatial differences (**Fig.1**). Only sites on the leeward side of the island were chosen due to difficulty in accessing sites on the windward eastern shore. The sites were chosen by a pairwise design in which the sites were either the same as the sites surveyed by Cervino et al. (2001) (Karpata, Buddy Dive, and Bari Reef) or similar in location (Punt Vierkant, Margate Bay, and Tolo).The non-identical sites were chosen by a stratified random design in which the location was separated into northern sites and southern sites, and one site from the northern site stratum and two sites from the southern site stratum were chosen at random.

Dark spots disease surveys

Disease prevalence was estimated at each of the sites using five 15 x 1m belt transects that were laid between 9 and 14 meters depth to create a 75 m^2 survey area. Each site was surveyed on three occasions on different days. A total number of 180 *S*. *siderea* and 470 *Stephanocoenia spp*. colonies were surveyed to estimate population parameters. All DSD infected colonies were noted with an estimation of infection severity (percent cover of the disease on colony), percent dead tissue on a single coral colony, and whether the colony was within one meter of another diseased colony in order to examine spatial distribution of DSD. Photos (Canon PowerShot G11camera with underwater housing) of all infected colonies were recorded in order to reference colony size and percent cover of disease using ImageJ Software.

Benthic composition analysis

Data on benthic composition was collected using video analysis in order to determine the mean percentage of live coral, pavement, sand/rubble, and other substrate. At each site, a video was taken at the each of the transects laid at 10, 12, and 14 meters depth using a Sony Handycam HDR-SR7 video camera

with underwater housing. The video recorded the substrate using a metal wand (50 cm) as a guide. Videos were assessed for cover of different biotic and abiotic substrates using Coral Point Count 4.1(Kohler and Gill 2006). Fifteen randomly selected points were analyzed and sorted into categories of live hard coral, live soft coral, recently dead coral, old dead coral, sponge, macroalgae, sand/rubble, other, or unknown.

Substrates were further condensed into four categories: live hard coral, pavement (Recently dead and old dead coral), sand/rubble, and other (Sponge, macroalgae, live soft coral, other, and unknown). Live hard coral was defined as any Scleractinian coral that was alive as seen by color in the colonies and having not undergone bleaching or purging of symbiotic algae. Any Scleractinian coral that was dead, either old or recently, was classified as pavement. Sand was loosely defined as small particles existing along the floor of the slope, whereas as rubble was loosely defined as rock and coral pieces not attached to the pavement. The other category was defined as anything not included in the aforementioned categories and contained live soft Alcyonacean corals, poriferans, macroalgae, other invertebrates (e.g. Echinodermata), vertebrates (e.g. Chordata), and unknown.

Data Analysis

One sample t-test hypothesis testing (α=0.05) of the previously known prevalences of *S*. *siderea* and *Stephanocoenia spp*. (H_0: μ= 53%, H_0: 0%) as derived from Cervino et al. (2001) were tested against the alternate hypothesis (H_1: μ >53%, H_1: μ >0%). Six one-factor ANOVAs (α=0.05) were used to examine the effect of site on mean disease prevalence (*S*. *siderea*, *Stephanocoenia spp*., & overall) and on mean infection severity of DSD (*S*. *siderea*, *Stephanocoenia spp*., & overall). A Tukey's multiple comparisons test was run on any ANOVA p-value under 0.05. Two Pearson's correlations were used to examine the effect of mean colony size on mean disease prevalence and on mean infection severity of DSD in order to determine any condition that may have affected prevalence and infection severity. Two one-factor ANOVAs (α=0.05) were run between site and percent of *S*. *siderea* and site and percent of *Stephanocoenia spp*. to determine any significant differences in the composition in the two coral species between sites. Two-sample t-test hypothesis testing (α=0.05) of the mean infection severity (H_0: $\mu_1 = \mu_2$) and mean colony size (H_0: $\mu_1 = \mu_2$) between *Stephanocoenia spp*. (μ_1) and *S*. *siderea* (μ_2) were tested against the alternative hypotheses of the mean infection severity (H_1: $\mu_1 > \mu_2$) and mean colony size (H_1: $\mu_1 \neq \mu_2$). For each site, I calculated mean percent cover of DSD and Pearson's correlation was calculated to determine

any significant interaction between total coral density and host prevalence to examine density-dependence of DSD. Correlations were based on means of prevalence, percent cover, colony size for each site. Four one-factor ANOVAs (α=0.05) were run to determine differences of live hard coral, pavement, sand/rubble, and other substrate percentages between individual sites. All hypothesis testing met the assumptions of normality using the Anderson-Darling test. ANOVA assumptions of normality (Anderson-Darling), homoscedasticity (Levene's Test), and independence (Residuals Versus Order) were met for most tests with the exception of the one-factor ANOVA testing site against sand/rubble percentages. Normality (Anderson-Darling) was not met for the sand/rubble ANOVA ($p<0.005$), but due to the robustness of the ANOVA test and its ability to withstand deviations from normality, it was chosen to be used. Furthermore, a Kruskal-Wallis Chi-Squared Test (α=0.05) indicated the same results for the sand/rubble ANOVA.

Results

Dark Spots Disease Prevalence in Bonaire

The ratio of diseased colonies to total colonies was quantified to calculate the prevalence of DSD on Bonaire for comparison with other surveys conducted in Bonaire or in other locales. A total of 180 *S. siderea* and 470 *Stephanocoenia spp*. colonies were surveyed in a total area of 1350 m^2 area over six sites on Bonaire. The mean DSD prevalence was 64% for *S. siderea* and 27% for *Stephanocoenia spp*. Hypothesis testing proved that there is enough statistical evidence to support the claim that mean prevalences of both *S. siderea* (two sample t-test, t= 10.02, p=0.00) and *Stephanocoenia spp*. (two sample t-test, t= 2.56, p=0.010) have increased since the Cervino et al. (2001) study. *Stephanocoenia spp*. had significantly higher mean infection severity (percent cover of DSD) on colonies by site (two sample t-test, t= 3.82, p=0.002) over *S. siderea* colonies.

Correlation between Prevalence & Percent Cover and Site & Colony Size

All sites surveyed were similar in DSD prevalence (percentage of colonies infected) and infection severity (percent cover of disease on colony) for both *S. siderea* and *Stephanocoenia spp*. There was no significant effect of site on the prevalence of DSD on *S. siderea* (one way ANOVA, $F_{5, 12} = 0.41$, p=0.834), *Stephanocoenia spp*. (one way ANOVA, $F_{5, 12} = 1.22$, p=0.359), or both species combined (one way ANOVA, $F_{5, 12} = 2.52$, p=0.088). Moreover, there was no significant effect of site on mean infection

severity of DSD on *S. siderea* (one way ANOVA, $F_{5,12}$= 1.65, p=0.220), *Stephanocoenia spp*. (one way ANOVA, $F_{5,12}$= 0.80, p=0.569) or both species combined (one way ANOVA, $F_{5, 12}$= 1.57, p=0.241).

Colony size was assessed as a correlative factor using Pearson's correlation with the prevalence and infection severity of DSD. There was no correlation between mean colony size and DSD prevalence on *S. siderea, Stephanocoenia spp*., or both species combined (all p> 0.05). There was no correlation between colony size and infection severity of DSD on *S. siderea* or *Stephanocoenia spp*. (all p>0.05). There was no significant difference in colony size between *S. siderea* and *Stephanocoenia spp*. (two sample t-test, t-value= 0.31, p=0.767). There was a no significant correlation (r=-0.748, p=0.087) between mean colony size and mean DSD infection (severity) of combined species. Prevalence and infection severity were similar between each site and colony size was not identified as an influencing condition for either prevalence or percent cover of disease.

Distribution of S. siderea and Stephanocoenia spp.

Siderastrea siderea and *Stephanocoenia spp*. density was calculated for the total area of 225 m^2 per site to evaluate differences in coral composition and density. The distribution of *S. siderea* and *Stephanocoenia spp*. was significantly different between Bari Reef and Punt Vierkant (**Fig. 2** one-way ANOVA, $F_{5, 12}$ = 3.93, p=0.024) in which Bari Reef had a higher percentage of *Stephanocoenia spp*. colonies and lower percentage of *S. siderea* colonies than Punt Vierkant. There was a greater percentage of *Stephanocoenia spp*. colonies than *S. siderea* colonies at all sites. Coral colony size was not significantly different between any of the sites (one way ANOVA, $F_{5,12}$ = 0.84, p=0.545).

Coral density was calculated by dividing the total number of *S. siderea* and *Stephanocoenia spp*. colonies by the 75 m^2 area to determine differences in density between sites. Bari Reef had significantly higher coral density (0.8 colonies m^{-2}) than Punt Vierkant (0.4 colonies m^{-2}), Karpata (0.3 colonies m^{-2}), and Tolo (0.3 colonies m^{-2}) (**Fig. 3**, one-way ANOVA, $F_{5, 12}$= 7.04, p=0.003). There was no significant effect of site on diseased coral density (one-way ANOVA, $F_{5,12}$=2.05, p=0.143), but Bari Reef had the highest mean diseased density (0.2 colonies m^{-2}) whereas Tolo had the lowest (0.1 colonies m^{-2}).

To analyze influencing conditions, Pearson's correlation was run between total coral density (total, *S. siderea*, and *Stephanocoenia spp*.) and disease prevalence (combined, *S. siderea*, and *Stephanocoenia spp*.). No significant trends were identified for *S. siderea*, *Stephanocoenia spp*., or both species combined

(all p> 0.05) Moreover, visual inspection of the survey area showed that 42% of diseased colonies were within one meter of another diseased colony.

Substrate covers

There were significant differences in coral cover among sites (**Fig. 4** one-way ANOVA, $F_{5,12}$ =4.95, p=0.011). Bari Reef had significantly lower mean coral cover (8%) than Karpata (30%), Tolo (26%), and Buddy Dive (28%). There were significant differences in sand/rubble among sites (**Fig. 4** one-way ANOVA, $F_{5,12}$ =17.17, p=0.000). Bari Reef had significantly higher sand and rubble cover (54%) than all of the other sites. There was no significant difference in pavement cover between sites (**Fig. 4** one-way ANOVA, $F_{5,12}$ =3.01, p=0.054). Other substrata was significantly different between Punt Vierkant and Buddy Dive and Bari Reef (**Fig. 4** one-way ANOVA, $F_{5,12}$ =4.63, p=0.014).

Discussion

The objectives of this study were to 1) determine differences in the prevalence of DSD on *S. siderea* and *Stephanocoenia spp*. from 1998 to 2014, 2) determine conditions (site, colony size, coral density and spatial distribution) that may affect the prevalence or percent cover of DSD on these coral species and 3) quantify differences in substrate cover between sites to determine any role in DSD distribution. The results indicate that DSD prevalence of both *S. siderea* and *Stephanocoenia spp*. has increased from 1998 to 2014 and did not have any significant influencing conditions. Moreover, the central difference in substrate cover was in the comparison between Bari Reef, with low live hard coral and high sand and rubble, and the other five sites. Bari Reef also exhibited higher density of *S. siderea* and *Stephanocoenia spp*. colonies.

Dark Spots Disease prevalence

The prevalence of DSD has increased on Bonaire from 53% of *S. siderea* in 1998 to 64 % in 2014 and from unquantifiable to 27 % in *Stephanocoenia spp*. Moreover, Stephanocoenia *spp*. colonies exhibited a greater mean infection severity (% of outer surface infected) than *S. siderea* colonies It is clear that the prevalence varies spatially due to the high range of prevalence from across the Caribbean in 1998 in which the lowest record was less than 1% in Lee Stocking Island, Bahamas (Voss & Richardson 2003) and the highest record was 56% in Turks and Caicos (Cervino et al. 2001). Due to imperfect matching of sites and missing sites, specifically on Klein and far north Bonaire, prevalence may be slightly lower than previously

indicated due to reduced diver impact from inaccessible or hard to access dive sites (far north) and distance from Kralendijk impact (Klein, far north) (Slijerkman et al. 2014). Variation temporally for the rest of the Caribbean could not be confirmed due to lack of recent studies on the prevalence of DSD.

Bonaire has seen an increase in the number of residents (~78 persons/ year) and tourists (~39 persons/year) yearly between 1970 and 2005 (Van Kekem et al. 2006). Due to the status of Bonaire as a "Diver's Paradise", more than 50% of its tourists are divers (TCB 2010) and contribute to increased diver traffic along the reefs. Sites that are frequented by divers (high impact sites) are categorized by coral damage, including tissue mortality, colony abrasion, and broken skeletons, as well as sedimentation (Krieger & Chadwick 2013). Injured colonies are more susceptible to disease infection (Peters 1997, Lamb et al. 2014), therefore diver damage from abrasion and stress-causing sedimentation may increase the likelihood of infection (Hawkins 1999). One study found a 3-fold increase in disease prevalence on corals at high impact sites over low impact sites (Lamb et al. 2014). Moreover, abrasion by divers also removes the thin surface mucopolysaccharide layer (SML) composed of coral-species specific bacteria which serves as a first line of defense (Ritchie & Smith 2004). It has been reported that 25% to 70% of the cultivable mucus-associated bacteria display antibacterial activity with massive, solitary corals, such as *S. siderea* and *Stephanocoenia spp*., at the higher end of the spectrum as compared to branching and soft corals (Shnit-Orland & Kushmaro 2009). The removal of this layer decreases the antibacterial activity and increases susceptibility for infection by pathogens. Other types of physiological stress that may increase susceptibility to disease are thermal anomalies and nutrient enrichment of the marine environment. Dark spots disease has been reported to vary seasonally with greater prevalence in warm water months (Gil-Agudelo & Garzón Ferreira 2001) and other studies have illustrated disease frequency correlated with elevated temperatures (Harvell et al. 1999, Sutherland et al. 2004) and bleaching events. Cróquer and Weil (2009b) illustrated that sites with a large percentage of bleached corals were likely to have a large mean prevalence of yellow band disease, white plague, Caribbean ciliate infections, and multiple diseases on *Montastraea spp*. and *Diploria spp*. Similarly, Brandt and McManus (2009) reported that *S. siderea* colonies affected with DSD showed higher bleaching activity than the unaffected counterparts. Moreover, increased nutrient concentrations in the environment can increase the severity of diseases due to nitrogen limited nature of many marine fungi and bacteria (Bruno et al. 2003). With the increased of nitrogen

concentrations in the environment, nitrogen limited bacteria and fungi have an excess of nutrients and can increase their virulence and severity due to the lack of hindrance from available nitrogen. Production of nitrogen and phosphorus has increased on Bonaire at a rate of ~510 kg/ year and ~84 kg/year, respectively, and higher nutrient concentrations in the seawater have been found on the inhabited west coast as compared to the uninhabited east coast (Van Kekem 2006). Bruno et al. (2003) subjected corals to higher levels of nutrients that were within the range of anthropogenic enrichment and inoculated the corals with *Vibrio spp*., the putative cause of yellow band disease. The results indicated tissue loss at 1.8 times the control with advancement of yellow band disease front. Although DSD has not been associated with nutrient enrichment, it may react similarly to yellow band disease provided that it is caused by a *Vibrio* related bacteria as hypothesized by Gil-Agudelo et al. (2007).

The number of diseases described has been increasing exponentially since 1965 when the first coral disease was described (Sutherland et al. 2004). Spatial variation exists among common diseases (White Plague II, Yellow Band Disease, Black Band Disease, DSD, and Aspergillosis) and mean disease incidence increased significantly from northern Caribbean to southern Caribbean (Weil et al. 2000). As for temporal changes in disease frequency, Ward et al. (2004) analyzed and normalized scientific literature containing disease reports and showed significant increases in coral disease and bleaching since 1993. Disease prevalence in this study seems to follow the same trend (Ward et al. 2004), however influencing conditions for this increase cannot be determined due to lack of data on synergistic effects of biotic and abiotic sources including diver impact, nutrient production, and site differences.

Influencing conditions

Conditions (site, colony size, and spatial distribution) were surveyed to determine influence on prevalence and infection severity of DSD. Site and colony size had no effect on prevalence or infection severity of DSD and distribution of *S*. *siderea* and *Stephanocoenia spp*. did not differ between sites. Colony size could not be determined as influencing condition for either prevalence or infection severity, although studies have shown correlation between colony size and susceptibility to disease. Nugues (2002) determined that larger colonies (greater mean surface area) were more likely to be infected by the disease and were less likely to face tissue mortality as compared to their smaller counterparts. In this study, larger colonies seemed to be correlated with lower infection severity of DSD ($r=-0.748$, $p=0.087$), similar to the

study by Nugues (2002). Larger colonies, although infected by DSD, suffered less tissue mortality due to lower infection severity of the disease. Larger colonies would seem to be older due to greater time allotment for growth. The use of model invertebrate organisms (*Drosophila melanogaster* (Meigen 1830) & *Caenorhabditis elegans* (Maupus 1900)) has indicated an immunosenescence in which immunity is impaired with increasing age due to increased susceptibility for infection (*C. elegans)*, although in *D. melanogaster* anti-microbial peptides increased with age (Müller et al. 2013). Therefore, coral immunity may be impaired with increasing age and colony size as immunoscence reduces its ability to prevent infection, but increase of motile phagocytic cells that aid in wound healing and tissue reorganization may reduce the tissue mortality and with it, the infection severity in terms of percent cover of the disease (Mydlarz et al. 2006). Moreover, coral age is not easily determined due to reports of individuals of same ages that a different sizes and vice versa (Hughes and Connell 1987 & Hughes 1984). Meesters et al. (2001) also noted that sensitivity to environmental conditions (urban coastal vs. upstream control) was species dependent, therefore certain species may be more prone to disease infection based on resilience and tolerance to environmental conditions rather than immunosenescence. Age and size may contribute to increased immunological functions and decreased infection severity, but species tolerance to stress inducing conditions has also proved to be a factor in reducing infection severity.

Spatial distribution in terms of distribution of targeted coral species and coral density did not have significant evidence of influence on disease prevalence. Coral composition was similar at each site in which *Stephanocoenia spp*. had a greater distribution than *S. siderea*. Bari Reef had the highest density of targeted species (*S. siderea* at 0.14 colonies m^{-2}, *Stephanocoenia spp*. at 0.65 colonies m^{-2}) and the lowest overall coral cover (8.18%). Alternatively, Karpata and Tolo had the lowest density of targeted species (*S. siderea* at 0.11 colonies m^{-2} & 0.08 colonies m^{-2}, *Stephanocoenia spp*. at 0.19 colonies m^{-2} & 0.16 m^{-2}) and the highest coral cover (30.20% & 28.22%). Both coral species were in lower abundance in areas of high coral cover which may be a result of their weak aggressive nature (Logan 1984). *Stephanocoenia spp*. and *S. siderea* are more likely to survive in areas that are not overrun by competing species. In 1998, *S. siderea* was higher in abundance than the rare *Stephanocoenia spp*., but the results in our study indicated that *S. siderea* has declined while *Stephanocoenia spp*. has risen (Cervino et al. 2001). *Siderastrea siderea* has the ability to survive in adverse conditions, such as extreme salinity changes, due to phenotypic plasticity

(Muthiga & Szmant 1987, Foster 1979, Foster 1980), but it is affected by six coral diseases and is not a good competitor (Sutherland et al. 2004, Logan 1984), thus populations are likely to decline due to disease infection and are unable to recover due to high competition with continued infection.

Dark spots disease has proven to correlate with seasonal high temperatures and shallow depths based on the distribution of susceptible species (Gil-Agudelo and Garzón Ferreira 2001). Furthermore, Gil-Agudelo and Garzón Ferreira (2001) produced evidence of clumped distribution of DSD, although it may have been related to the clumped distribution of *O.annularis* and *S. siderea*. Dark spots disease prevalence did not correlate with total coral density, thus suggesting that it is not density dependent. Density-dependence models though have been observed with coral disease (white syndrome) and are often characteristic of horizontally transmitted infectious diseases (Bruno et al. 2007). The clumped distribution and hypothesized causative agent as *Vibrio*-related (Gil-Agudelo et al. 2007) are consistent with a horizontally transmitted, vectored pathogen, but other hypotheses suggest coral disease arises from opportunistic pathogens after environmental stress (Lesser et al. 2007). Microorganisms are already extant in the mucus layer and may emerge as pathogenic due to a weakened immune defense from a changing and stress inducing environment. Borger et al. (2005) proposed that DSD was a general stress response in *S. siderea* that was provoked by thermal stress, but due to the lack of density dependence of DSD, it is uncertain if DSD is vectored or an opportunistic pathogen. Previous studies have shown correlation between environmental changes (nutrient enrichment, increased temperature) and virulence of biotic pathogens (Sutherland et al. 2004, Bruno et al. 2003), therefore vectored pathogens may act similarly to opportunistic pathogens due to increased virulence from correlative factors that also impose stress upon the coral colony.

Substrate cover

Bari Reef had significantly higher sand and rubble cover than all other sites and significantly lower coral cover than Karpata, Tolo, and Buddy Dive. Bari Reef which is the "house reef" of the Den Laman Resort, in Kralendijk, is likely to have high diver impact because of its house reef status in which guests of the resort dive frequently on the reef. Additionally, Bari Reef attracts fish identification enthusiasts as a large number of century (100-fish) REEF surveys have been completed there. Higher diver traffic has been seen to significantly increase the number of damaged and partially dead coral colonies

(Hawkins et al. 1999), increase sedimentation, and significantly reduce coral cover (Hawkins et al. 1999, Hasler & Ott 2008, Lyons et al. in preparation). Common forms of damage caused by divers include sedimentation, tissue mortality, colony abrasion and broken skeleton which all occurred at significantly higher rates at sites with more buoys for diver entry (Krieger & Chadwick 2013). Therefore, the significantly higher sand and rubble cover and significantly lower live hard coral cover at Bari Reef could be attributed to the greater influx of divers that damage coral colonies and cause dead coral rubble and sedimentation.

Coral cover in this study was lower than previous studies (22.8% ± 3.3SE) due to the sampling of only six sites which were all of the leeward side of Bonaire and did not include sites on Klein Bonaire. Steneck et al. (2013) reported that Bonaire's reefs averaged above 40% live coral cover for studies done between 1999 and 2009, but had a 10% loss in live coral due to the 2010 bleaching event and had a slight recovery for 2013 (36.2% ± 1.9 SE). The reports were long-term monitoring projects that included eleven sites, including a no-dive reserve and sites on Klein Bonaire, and covered a much larger area of Bonaire's reefs, therefore incorporating diversified environmental conditions. Similarly, Bruckner et al. (2010) surveyed 25 sites on Bonaire in 2010, ranging from far north to far south and Klein Bonaire, that averaged 40% to 60% live coral cover, with the exception of two northern sites, Weber's Joy/ Witches' Hut and Jeff Davis, with ranges from 30-35% live coral. Klein Bonaire often has higher coral cover than coastal Bonaire sites (Bruckner et al. 2010), therefore, since this did not include Klein Bonaire sites and had an very low coral cover at Bari Reef (8.2% ± 2.8 SE), the mean of coral cover was curtailed. In contrast to both Steneck et al. (2013) and Bruckner et al. (2010), a study by Wieggers et al. (2007) surveyed ten sites on Bonaire, including Klein Bonaire, and determined an mean of 29.6% live coral cover, which more closely coincides with the results of this study. The three previous studies and present study used different sites which may account for the differences; however, since information for the site Karpata was included in all of these studies with the exception of Wieggers et al. (2007), it can be used as a comparison site. I observed a lower coral cover for Karpata in 2014 (30.2% ± 3.4 SE) than in 2009 (40.5% ± 3.61 SE) (Steneck et al. 2013) and in 2010 (~ 43%) (Bruckner et al. 2010). Karpata has seen a decline in coral cover (~10%) similar to the rest of the Caribbean which has flattened as a result of a decline in rugosity from 1969 to 2008 (Alvarez-Filip et al. 2009) and region-wide declines in coral cover (Gardner et al. 2003).

Conclusions

The increase in DSD prevalence is not correlated with a density-dependence model, thus it cannot be determined if the causative agent is a vectored pathogen or an opportunistic pathogen. Previous studies have correlated DSD with clumped distribution, but due to the difficulty in obtaining Koch's postulates, the proposed causative agent as *Vibrio* related has not yet been confirmed. Moreover, increasing anthropogenic influences on coral reefs are changing the dynamic between pathogenic organisms and corals. A majority of disease surveyed was found on reefs expected to have medium to high human impacts (Green & Bruckner 2000), therefore it is important to monitor the influences on the reefs to prevent further disruptive changes of the environment. For DSD, correlations have only been confirmed between prevalence and high temperatures and prevalence and depth. *Ex situ* causation experiments would enlighten the status of disease on the reefs; therefore it is prudent to examine further effects on prevalence and infection severity, including nutrient enrichment, anthropogenic toxins (e.g. sunscreen), climate change, etc. Moreover, to gain the greatest understanding of DSD, it is crucial to determine the causative agent and to quantify the prevalence and infection severity throughout the Caribbean. Although the acquisition of data is challenging and limited by time and resources, it is needed to develop effective management plans. Enhancing comprehension of the marine system and the role of disease on survivorship of coral will further management plans in preventing the progression of reef degradation.

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Appendix 1: Figures

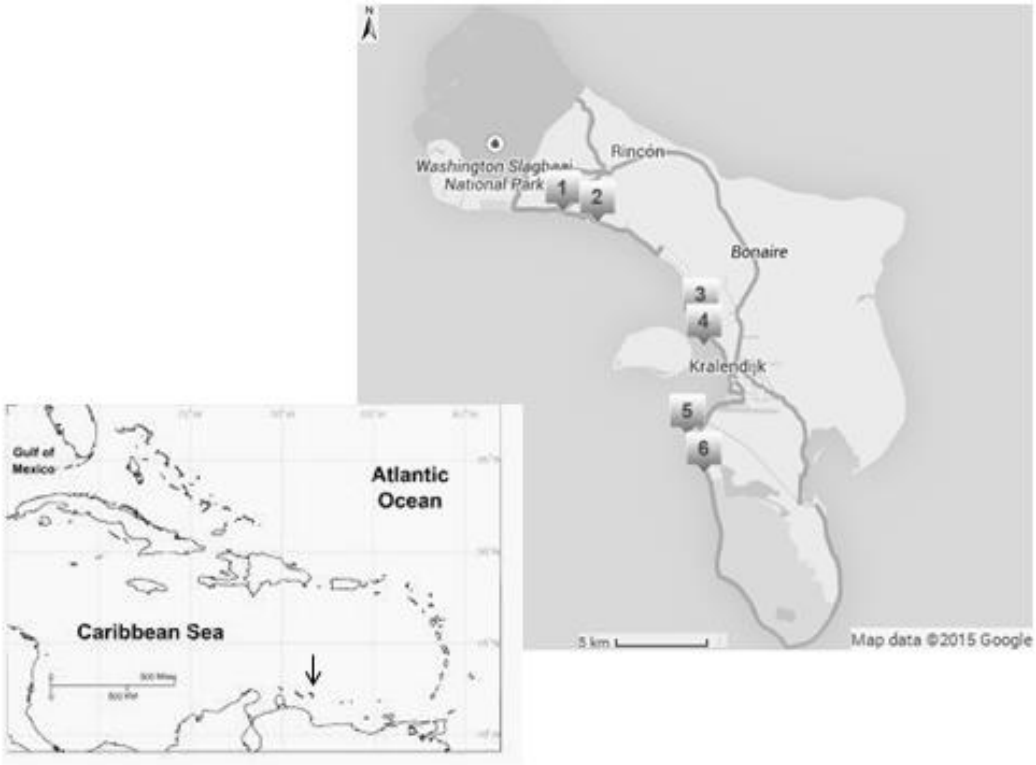

Fig. 1 Study sites surveyed on Bonaire in 2014. Identical sites include Karpata(1), Buddy Dive(3), and Bari Reef(4) whereas Tolo(2), Punt Vierkant(5), and Margate Bay(6) were randomly selected from the northern region (Tolo) or the southern region (Punt Vierkant and Margate Bay). Arrow indicates the location of Bonaire in reference to the entire Caribbean.

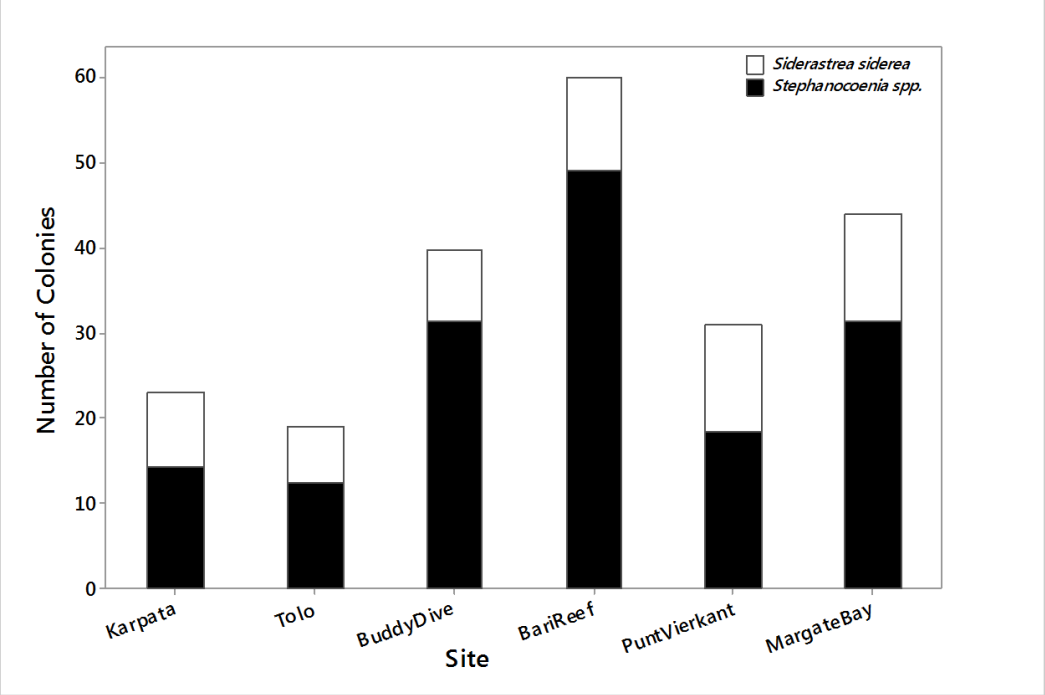

Fig. 2 Distribution of *S*. *siderea* and *Stephanocoenia spp*. between sites. *Stephanocoenia spp*. had more colonies than *S*. *siderea* at all sites and only Bari Reef and Punt Vierkant differed in distribution. Punt Vierkant had a higher percentage of *S*. *siderea* and lower percentage of *Stephanocoenia spp*. than Bari Reef.

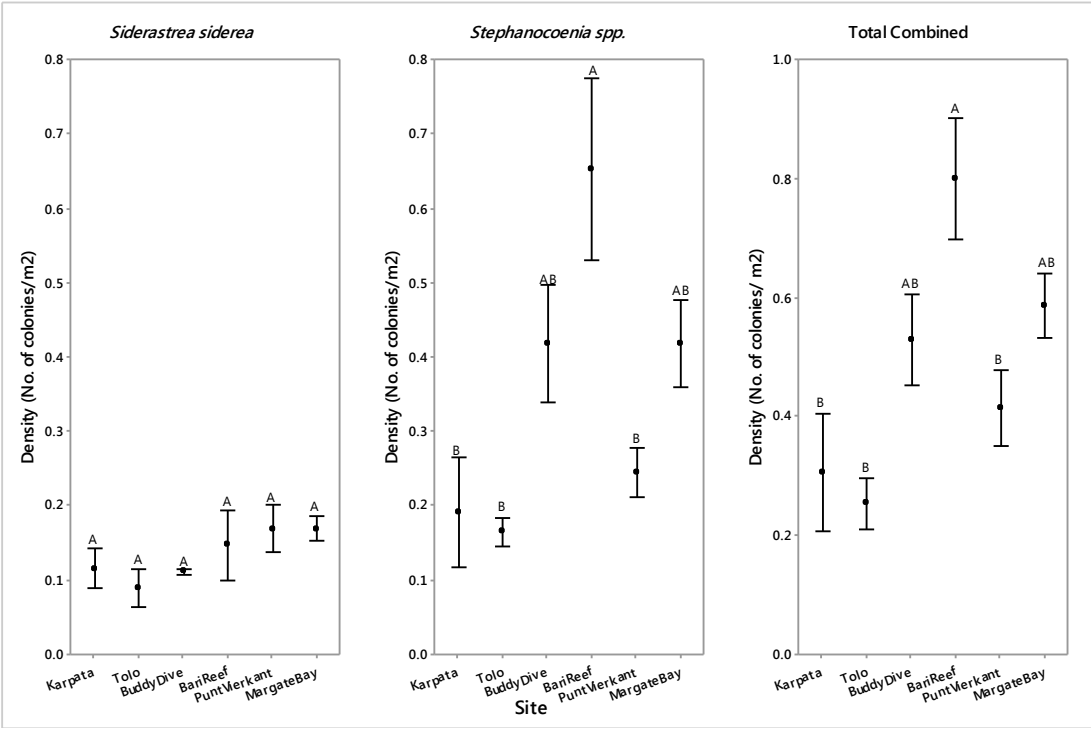

Fig. 3 *Siderastrea siderea*, *Stephanocoenia spp*., and total combined density between sites. Same letters indicate no significant difference between sites and bars represent standard error of the mean density (No. of coral colonies per m^2) (n=3 surveys per site).

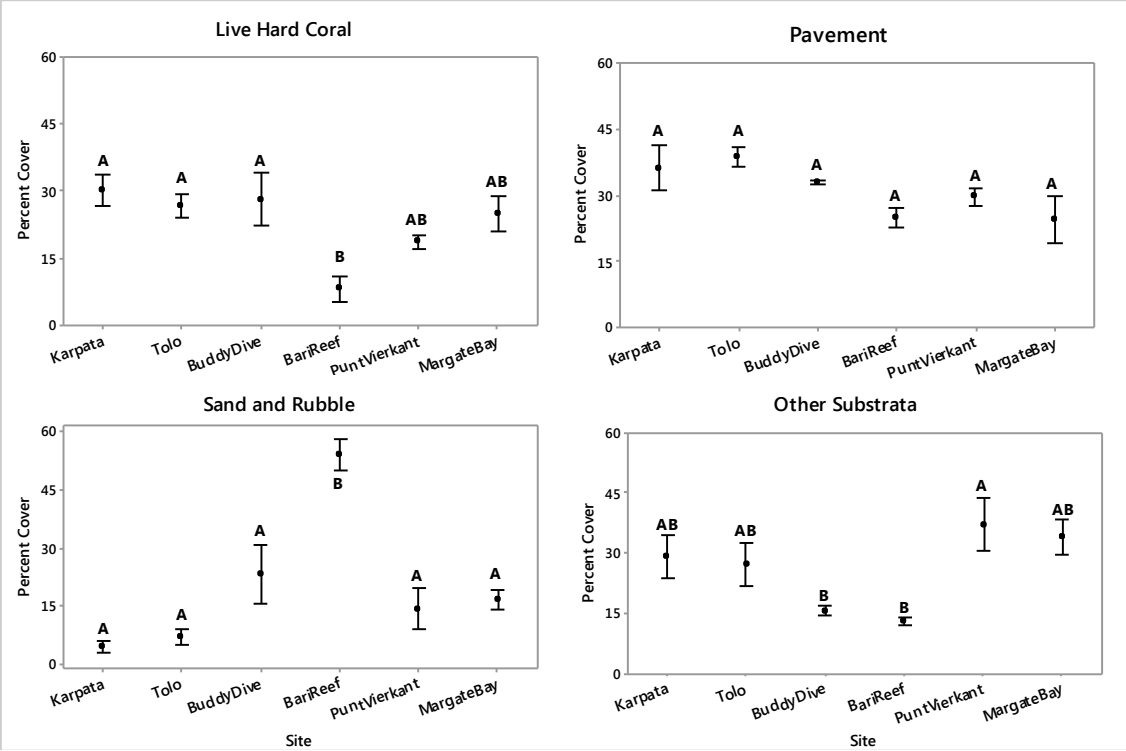

Fig. 4 Substrate covers categorized into live hard coral, pavement, sand and rubble, and other (Alcyonacean corals, poriferans, macroalgae, other invertebrates and vertebrates). Same letters indicate no significant difference between sites and bars represent standard error of the mean percent cover of substrate (n= 3 video transects per site).

Appendix 2: Dark spots disease prevalence

Siderastrea siderea

Site	Transect 1(%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	50	89	40	60	12.7
Tolo	50	75	67	64	7.9
Buddy Dive	75	63	78	72	4.8
Bari Reef	39	38	86	54	8.7
Punt Vierkant	88	33	67	63	19.8
Margate Bay	75	73	73	74	4.4

Stephanocoenia spp.

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	36	18	43	32	25.8
Tolo	42	27	30	33	12.7
Buddy Dive	28	19	25	24	8.1
Bari Reef	30	28	14	24	27.4
Punt Vierkant	35	53	13	34	27.7
Margate Bay	15	21	12	16	1.2

Combined Prevalence

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	41	50	42	44	5.2
Tolo	45	29	38	38	8.0
Buddy Dive	35	51	39	42	8.4
Bari Reef	33	29	24	29	4.4
Punt Vierkant	55	48	33	46	11.1
Margate Bay	29	35	35	33	3.6

Appendix 3: Mean infection severity (Percent cover of the disease)

Siderastrea siderea

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	17	17	48	27	17.9
Tolo	18	19	4	14	8.4
Buddy Dive	25	14	6	15	9.5
Bari Reef	11	5	6	7	3.2
Punt Vierkant	9	13	5	9	4.0
Margate Bay	20	14	9	14	5.5

Stephanocoenia spp.

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	40	12	33	28	14.6
Tolo	38	21	25	28	8.9
Buddy Dive	19	31	17	22	7.6
Bari Reef	16	21	11	16	5.0
Punt Vierkant	16	30	31	26	8.4
Margate Bay	22	29	19	23	5.1

Combined Mean Infection Severity

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	31	16	39	28	11.7
Tolo	29	20	16	22	6.7
Buddy Dive	21	22	13	19	5.2
Bari Reef	14	19	8	14	5.1
Punt Vierkant	12	24	12	16	6.9
Margate Bay	21	21	10	17	6.0

Appendix 4: Mean colony size

Siderastrea siderea

Site	Transect 1 (cm^2)	Transect 2 (cm^2)	Transect 3 (cm^2)	Mean (cm^2)	Std. Dev.
Karpata	294.8	108.2	59.2	172.0	175.2
Tolo	1384.9	597.4	1118.3	1063.2	1510.6
Buddy Dive	365.6	297.2	265.0	307.5	317.7
Bari Reef	403.0	68.3	707.5	454.4	578.8
Punt Vierkant	319.8	293.8	1455.2	587.1	841.9
Margate Bay	370.1	282.5	688.5	470.2	565.2

Stephanocoenia spp.

Site	Transect 1 (cm^2)	Transect 2 (cm^2)	Transect 3 (cm^2)	Mean (cm^2)	Std. Dev.
Karpata	372.7	342.6	170.1	325.0	349.4
Tolo	234.0	474.3	920.6	485.8	498.0
Buddy Dive	525.6	377.4	340.6	445.1	417.8
Bari Reef	248.3	499.5	697.6	455.8	468.0
Punt Vierkant	432.1	734.0	653.1	598.4	643.1
Margate Bay	252.7	553.8	884.4	499.5	501.1

Combined Colony Size

Site	Transect 1 (cm^2)	Transect 2 (cm^2)	Transect 3 (cm^2)	Mean (cm^2)	Std. Dev.
Karpata	341.5	155.1	142.9	213.2	111.3
Tolo	745.5	527.1	142.9	471.8	305.1
Buddy Dive	472.3	337.3	299.9	369.8	90.7
Bari Reef	308.5	440.7	702.5	483.9	200.5
Punt Vierkant	576.8	360.6	195.2	377.5	191.4
Margate Bay	323.2	398.8	730.5	484.2	216.7

Appendix 5: Distribution of *S. siderea* and *Stephanocoenia spp*.

Total Coral Density

Site	Transect 1 (colonies/m^3)	Transect 2 (colonies/m^3)	Transect 3 (colonies/m^3)	Mean (colonies/m^3)	Std. Dev.
Karpata	0.5	0.3	0.2	0.3	0.17
Tolo	0.3	0.3	0.2	0.3	0.07
Buddy Dive	0.7	0.5	0.4	0.5	0.13
Bari Reef	0.7	1.0	0.7	0.8	0.18
Punt Vierkant	0.5	0.4	0.3	0.4	0.11
Margate Bay	0.7	0.5	0.5	0.6	0.09

Diseased Coral Density

Site	Transect 1 (colonies/m^3)	Transect 2 (colonies/m^3)	Transect 3 (colonies/m^3)	Mean (colonies/m^3)	Std. Dev.
Karpata	0.2	0.1	0.07	0.1	0.07
Tolo	0.1	0.06	0.07	0.08	0.03
Buddy Dive	0.2	0.1	0.2	0.2	0.05
Bari Reef	0.2	0.3	0.2	0.2	0.07
Punt Vierkant	0.3	0.2	0.1	0.2	0.09
Margate Bay	0.2	0.2	0.2	0.2	0.01

Appendix 6: Substrate Covers

Live Hard Coral

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	27	26	37	30	6.0
Tolo	28	21	30	26	4.7
Buddy Dive	37	30	17	28	10.2
Bari Reef	14	7	4	8	4.9
Punt Vierkant	22	18	17	19	2.6
Margate Bay	27	31	17	25	7.1

Pavement

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	26	43	40	36	8.8
Tolo	39	35	43	39	4.1
Buddy Dive	34	32	33	33	0.9
Bari Reef	29	22	24	25	3.7
Punt Vierkant	29	27	34	30	3.5
Margate Bay	14	33	27	25	9.5

Sand and Rubble

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	8	3	3	5	2.6
Tolo	3	8	9	7	3.4
Buddy Dive	11	22	37	23	13.0
Bari Reef	46	58	58	54	7.0
Punt Vierkant	25	7	11	14	9.1
Margate Bay	20	11	19	17	4.8

Other (Live soft coral, macroalgae, poriferans, other invertebrates, vertebrates, unknown)

Site	Transect 1 (%)	Transect 2 (%)	Transect 3 (%)	Mean (%)	Std. Dev.
Karpata	39	28	20	29	9.5
Tolo	29	35	17	27	9.3
Buddy Dive	17	16	13	15	2.3
Bari Reef	11	14	14	13	1.4
Punt Vierkant	25	47	39	37	11.5
Margate Bay	39	25	38	34	7.6