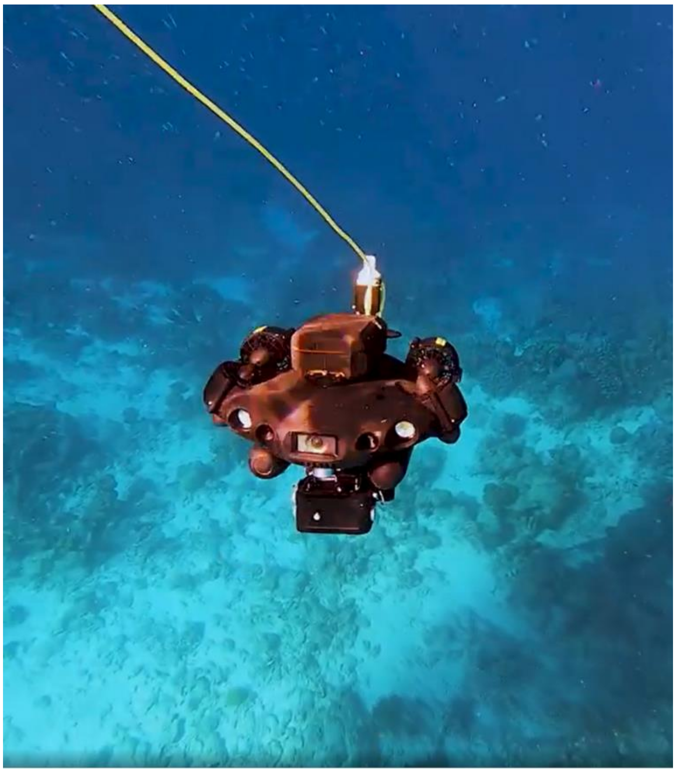
Discovering the Deep

ROV assisted data collection to understand the status of Mesophotic Coral Ecosystems around Bonaire



MSc Marine Sciences thesis by Rosanne Bartholomeus (4527496)

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Supervisors: Erik Meesters (WMR) & Gülşah Doğruer (WMR)
Examinator: Francesca Sangiorgi (UU)

Abstract

The coral reefs of Bonaire, providing resources and environmental services, are often ranked among the richest, most resilient and least degraded in the Caribbean, but they are not escaping the global degrading trend in coral reefs. Identifying and combatting local stressors, increases the resilience to global stressors. Research has shown that even the deeper, relatively unexplored reefs, mesophotic coral ecosystems (MCE), ranging from 30 to 150m in depth, are being impacted by anthropogenic disturbances. As the MCEs start where Scuba diving stops, and submersibles are often too costly, this study deployed an ROV to explore and monitor the shallow (5-20m) and the upper-mesophotic (40-60m) reefs at eight sites along the leeward coast of Bonaire. These sites were subdivided into different zones, showing a gradient in human impact and water quality. The imagery obtained by the ROV is of adequate quality, allowing for identification to genus level if not species level, and showed comparable results in estimated percentage coral cover with other recent studies. The benthic community composition changed along the vertical (depth) and horizontal (human impact and water quality) gradient. Benthic cyanobacterial mats were found around 40-60m depth, covering large parts of the ocean floor. Hard and soft corals, sponges, macroalgae and crustose coralline algae occurred at 40m depth at six of the eight monitored sites, indicating the presence of MCEs, and only at one site (Karpata), hard corals were present at 60m depth. Coral cover showed a clear increasing trend with decreasing human impact, addressing the need for a better understanding of heterogeneity among sites and local conservation measures. Developments in underwater robotics and machine learning enable more research on these hidden coral reefs and identification of the effect of local stressors on MCEs.

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1 Introduction

The coral reefs of Bonaire, providing resources and environmental services, are often ranked among the richest, most resilient and least degraded in the Caribbean (Jackson et al. 2014; Frade et al., 2019; IUCN 2011; Steneck et al., 2015), but they are not escaping the global degrading trend in coral reefs (Gardner et al., 2003; Eddy et al., 2021; Meesters et al., 2020; de Bakker et al., 2019). These reefs are also threatened by local and global stressors. Bonaire has experienced significant human population expansion, a 50% increase since 2001, as well as increased tourism, both increasing pressure on the environment (Verweij et al., 2020; CBS Dutch Caribbean 2021). Continuously occurring local stressors include overexploitation of fish populations, coastal development and environmental degradation and erosion due to free-roaming cattle, causing surface runoff resulting in sediments and excess nutrients, but also diseases and tourists trampling on the corals (Frade et al., 2019; Bak et al., 2005). Those local stressors weaken the coral reefs and make them less resilient to increasing global pressures, like sea level rise, ocean acidification and sea surface temperature rise, resulting in coral bleaching (de Bakker et al., 2019).

Bonaire knows the longest time series of a living coral reef, with the first scientific data collected in 1974. Information on the mesophotic coral ecosystems (MCEs), light-dependent coral communities ranging from 30 to 150 m, is however in general scarce, mainly because these depths are beyond the Scuba diving limits (Slattery & Lesser, 2012). Becking and Meesters (2014) mention that particular in the Dutch Caribbean the location and spatial extent of deep reef habitats are poorly known and how ecological processes change along depth gradients is not yet well understood. This study, being part of the larger multidisciplinary project "Resilience Restoration of Nature and Society in the Caribbean Netherlands", aims to contribute to filling in this critical knowledge gap, for developing future reef policies and management practices.

1.1 Background

A study from 2003 by Gardner and colleagues showed that coral cover in the Caribbean had decreased from approximately 50 to 10% over 30 years and several studies show similar degrading trends in coral cover in this area (Jackson et al., 2014; Bak and Nieuwland 1995; Bak et al., 2005; Bakker et al., 2017). According to Bakker and colleagues (2016), the total coral cover on the reef slopes of Curaçao and Bonaire is expected to have dropped below 1% by 2030 at the present rate of decline. The first study encompasses data on the shallow reefs in the whole Caribbean (<20m), whereas the second study included coral reefs down to 40m but only on 4 sites. Available information on what happens to coral reefs at greater depths, the mesophotic coral ecosystems (MCEs), is limited (Frade et al., 2019; Becking and Meesters, 2014; Menza et al., 2008).

MCEs include associated communities like algae, fish and sponges, that occur in de deepest half of the photic zone. Light penetration is influenced by the turbidity of the water and the distance from shore, thus the depth of the photic zone may vary accordingly. The Caribbean waters surrounding Bonaire have high visibility, which may cause the photic zone to reach deeper than in other coastal areas (Becking & Meesters, 2014). With increasing depth, ecological conditions change considerably: light attenuation, moderate temperature decrease, nutrient and particulate organic matter (POM) concentrations increase, turbulent flow decreases and more abiotic and biotic factors play a role at these depths (Lesser et al. 2009; Scott et all., 2019, fig.1). MCEs occur at the transition zones from shallow to deep reefs, supporting a combination of shallow- and deep-water benthic species, as well as some unique taxa (Scott et al., 2019).

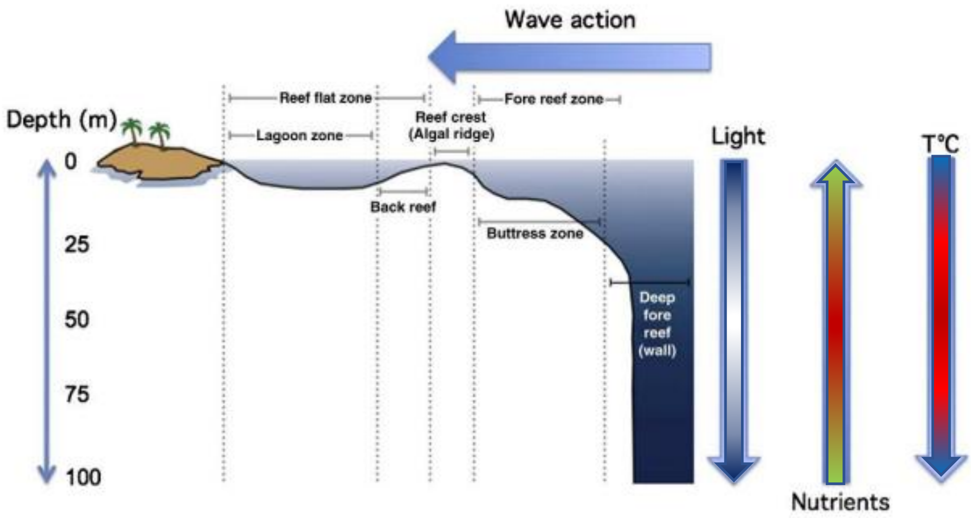


Figure 1: Coral reef zonation showing gradients of light, nutrients and temperature with changes in depth from shallow reefs to mesophotic depths. Figure derived from Lesser et al., (2009).

The first study conducted on deep coral reefs was the United States Exploring Expedition (1838-1842), where more than 200 species of coral were reported by Dana (1846) (Lesser et al., 2009). In the Caribbean region, MCE research started in the 1970s and ongoing research deploys manned submersibles, AUVs, mixed-gas, rebreather technology and ROVs (Frade et al., 2019). In 2000, the deep reefs of Bonaire, Curaçao and Aruba have been explored with the Johnson-sea-Link II Research submersible of Harbor Brach, USA, down to depths of 900 m (Reed and Pomponi, 2000). During this expedition, the focus was on biomedically interesting sponges and the biodiversity data has not been worked out (Becking and Meesters, 2014). Meanwhile, the Ministry of Economic Affairs commissioned the Institute for Marine Resources and Ecosystem Studies (IMARES) to investigate the deeper reef of Bonaire as part of the Exclusive Economic Zone (EEZ) management plan for the Dutch Caribbean. Therefore, in 2013 the deep reef of Bonaire was explored with the aid of the “Curasub” submarine of Substation Curaçao to depths of 140-250m, at three locations on the Southern coast of Bonaire: Kralendijk, Cargill and Statoil.

MCEs have gained more attention lately as it has been suggested that they are spared from some local and global stressors. Previous research (Bak et al., 2005) showed that deep reefs initially did not show the same degrading trend in coral cover as shallow reefs, as the impact of anthropogenic stressors appeared to be limited in the deeper reefs. The deeper reefs however are impacted by ecological processes, including cold water bleaching and storm-generated sedimentation (Bak et al., 2005). More recent research (de Bakker et al. 2017; de Bakker et al. 2016; Frade et al. 2019; Nagelkerken et al., 2005; Nugues and Bak 2008; Bongaerts et all, 2010) however, shows that the upper mesophotic reefs (30-40m depth) later also showed signs of degradation. Generally, natural and anthropogenic disturbances have the highest impact on the shallower reef and the coral reefs at greater depth (>20m), in particular at 40m, show lower levels of disturbance (Bakker et al., 2016). Bakker and colleagues (2016) observed an obvious degrading trend, especially since 1990, and found that the MCEs further away from urban areas were less degraded, suggesting anthropogenic stressors reach and affect corals at mesophotic depths (Bakker et al., 2016; Stefanoudis et al., 2019). This emphasizes the need to explore the distribution and status of coral reef assemblages at mesophotic depths.

Over the past 40 years, the information obtained about the distribution and ecology of MCEs has come mostly from surveys using deep advanced technical diving, drop-cameras, and manned submersibles (Armstrong et al., 2019; Frade et al., 2019). In the last two decades, development within the field of underwater robotics, including Remotely Operated Vehicles (ROVs) and Autonomous Underwater Vehicles (AUVs) as well as advanced acoustic and optical imaging techniques, allows for high-resolution benthic mapping and biological characterization of MCEs over large spatial scales (Armstrong et al., 2019). ROVs are tethered vehicles, allowing the operator to remotely control and supervise the vehicle from the surface, using live video and data streaming (Armstrong et al., 2019).

Generally, there is little difference between photos taken by an ROV and by a diver (Lam et al., 2006). Scuba-diving has been the main data collection method for reef monitoring, by means of photography or videotaping for visual documentation (e.g. Tratalos and Austin, 2001; Rogers and Miller, 2001; de Bakker et al., 2019; Lam et al., 2005). Scuba diving however is limited with regard to the range that can be covered (depth and horizontally), due to diving time and pressure, but also weather conditions and water temperature (Lam et al., 2005). Besides, inexperienced divers can cause damage to the reefs. ROVs and AUVs are increasingly being used in field surveys, mainly where divers are unable to collect data, like in deeper waters on continental shelves (Auster and Tusting, 1997) and deep seas ((Lauerman, 1998; Fijikura et al., 1999; Scoltwedel and Vopel, 2001; Lam et al., 2005). ROVs are also being used for environmental monitoring in shallow water habitats (e.g. Greene and Alevizon, 1989; Cook and Krumm, 1996;), and for coral reef surveys (Williams et al., 1999, 2011; Lam et al., 2005). For this study, an ROV (QYSEA FIFISH V6 Pro) was used to run transects at depths down to 100m and collect more imagery on the MCEs of Bonaire.

1.2 Objectives and Research Questions

This study aims to obtain experience in the deployment of an ROV and collect data on the distribution and status of MCEs, as well as the vulnerability of MCEs to anthropogenic stressors, thereby increasing the understanding of which local stressors influence the health of mesophotic corals most. Eventually, this study contributes to the evaluation of the effects of local stressors, and to the development of policy recommendations to increase the reef's resilience for global stressors. The following research questions will be answered within this study:

1. What is the technical approach to deploying an ROV and what are the pitfalls and benefits?
2. Where and till what depth do MCEs occur along the leeward coast of Bonaire?
3. What is the status of those MCEs?
   - How are diversity, richness, benthic cover and species composition influenced by the horizontal and vertical gradients?
4. Do these results show similar coral cover at 5 and 10 m depth compared to the data obtained by Bakker and colleagues (2020) with scuba diving, at similar sites?

Patterns in the abundance of corals, algae, and sponges with depth on mesophotic reefs around Bonaire will be quantified and described, thereby providing insight into the condition of these unexplored areas of Bonaire's coral reef. This study provides a baseline quantitative and qualitative characterization of Bonaire's upper mesophotic coral ecosystems (40-60m depth) by deploying an ROV.

The set-up of this research is further explained in the section Material & Methods, after which the Results are presented in the order of the research questions and elaborated on in the Discussion. A summary of this research and recommendations for further research are provided in the Conclusion.

2 Material & Methods

2.1 Site description

The Caribbean Netherlands, consisting of Bonaire, Saba and Sint Eustatius, are the three islands investigated within the overarching project. This study focuses on Bonaire, a special municipality of the Kingdom of the Netherlands, located in the Southern Caribbean (12°9'N, 68°16'W, fig.2), covering a surface area of 294 km^2. The narrow fringing reefs surrounding the island extend up to 150m offshore (Frade et al., 2019). Bonaire receives an average of 500mm of rainfall per year, mostly in short and heavy showers in the period between October and March (Van der Geest et al., 2020; Frade et al., 2019). Neogene limestone and basalt pillow lava make up the soil types of Bonaire (Van der Geest et al., 2020). These soil types, in combination with overgrazing mostly by goats, result in poor water retention capacity, causing most rainfall to run off into the ocean (Roos 1971, Van der Geest et al., 2020). The predominant water current on the leeward side of the island runs from south to north as a result of the prevailing easterly trade wind (Van der Geest et al., 2020).

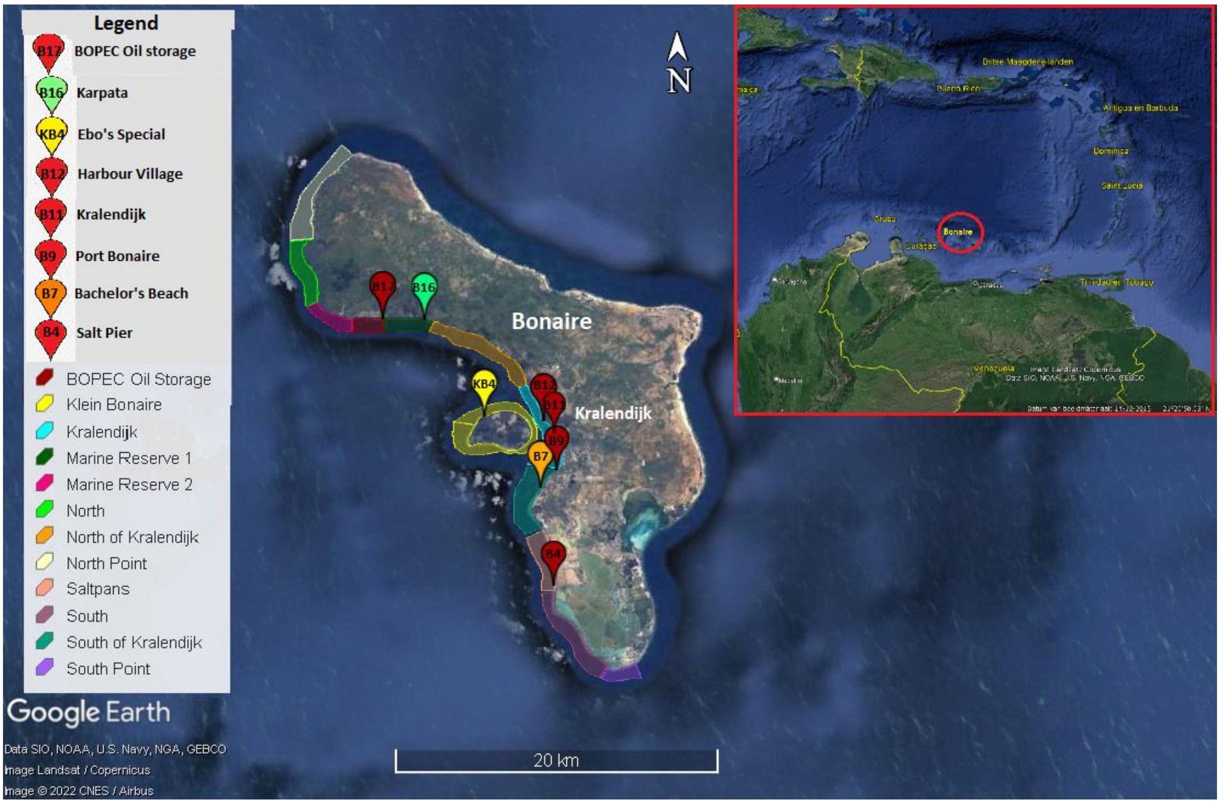


Figure 2: Location of Bonaire, Caribbean Netherlands. Google Earth Pro 2020 imagery date 14 December 2021. Bonaire sample locations along the leeward side of the island, located in certain impact zones defined by Bakker et al., 2019.

2.1.1 Human impact

Surveys to monitor the status of the coral reefs and MCEs were carried out on the leeward side of the island of Bonaire, including the smaller uninhabited island of Klein Bonaire (Fig.2), from January to April 2022. The west coast of Bonaire is exposed to smaller wave energy environments (van Duyl, 1985; Trembanis et al., 2017) compared to the east coast, resulting in better preserved and more researched reefs (Steneck and Mclanahan, 2004). The leeward side of the island is home to substantial reef formations and knows a strong gradient in the degree of direct human activity. Eight sites along the west coast of Bonaire were selected based on differences in human impact, where the major centers of local anthropogenic stress are Kralendijk, the BOPEC oil storage facility and the salt pans (De Bakker et al., 2019, fig.2). While less disturbed sites can be found closer to the in 1979 established no-dive marine reserves, where entrance by humans is prohibited. It is expected that the ecological status, here defined as coral cover, diversity and richness of a reef site, is affected by this variation in the degree of local anthropogenic impact along the west coast (Zanke & De Froe, 2015; De Bakker et al., 2019). De Bakker and colleagues (2019) identified a qualitative variable, ranging from minimal disturbance to extreme disturbance, based on among others distance to outflow or surface runoff, coastal development and activity, diving activity and conservation measures in place. Table 1 provides an overview of the impact factors on each site monitored within this study.

Table 1: Overview of the human impact and water quality risk on each site and the corresponding sites surveyed by De Bakker et al., (2019)

Study sites	Location	Dive buoy & coordinates	De Bakker sites	Impact	Source of Impact	Water quality risk assessment
B04	Salt Pier, Cargill outlet	Tori's Reef 12°4'15.7858" N 68°16'54.3402" W	14	Extreme (5)	Direct impact received from the Saltpan outlet	No-risk (1)
B07	Bachelor's Beach	Bachelor's Beach 12°7'32.7122" N 68°17'18.0432" W	24	High (4)	Substantial coastal development	No-risk (1)
B09	Port Bonaire	18th Palm 12°8'14.7912" N 68°16'42.06" W	27	Extreme (5)	Kralendijk, significant coastal development, high coastal run-off, sewage	Low-risk (2)
B11	Kralendijk	Personal buoys 12°9'24.0003" N 68°16'48.8745" W	31+32	Extreme (5)	Kralendijk, significant coastal development, high coastal run-off, sewage	No-risk (1)
B12	Harbour Village	From shore at Harbour Village 12°9'48.8448" N 68°17'13.9668" W	34	Extreme (5)	Kralendijk, significant coastal development, high coastal run-off, sewage	Moderate-risk (3)
KB4	Ebo's Special, Klein Bonaire	Ebo's Special 12°9'57.4091" N 68°19'10.8179" W	57+58	Moderate (3)	Coastal activity, diving	Low-risk (2)
B16	Karpata	Karpata 12°13'8.1901" N 68°21'9.0857" W	104+105	Marginal (1)	Dive site, near marine reserve 1, where entrance by humans prohibited	No-risk (1)
B17	BOPEC	BOPEC Jetty 12°13'10.9056" N 68°22'48.0648" W	99	Extreme (5)	Northern sites receiving impact of the BOPEC oil storage and pier	High-risk (4)

2.1.2 Water quality

Another factor that is likely to affect the status of the coral reefs is water quality, with inorganic nutrients and particulate material being the most important contaminants at local and regional scales (GESAMP, 2001; Fabricius, 2005). The report by Hoekema (2022) describes spatial and temporal monitoring of Bonaire's near-shore water quality, which includes measurements of dissolved inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) and physiochemical water quality parameters (chlorophyll-a and turbidity).

To compare different water quality zones with each other, a deterministic risk quotient-based analysis was conducted, providing a one-point evaluation of environmental risk (Mohtar et al., 2019). 'This approach is based on the ratio of measured or predicted environmental concentrations (MECs or PECs) to the predicted no-effect concentrations (PNECs), described as RQ = MEC (or PEC) / PNEC' (Mohtar et al., 2019, p.136). In this case, the water quality risk assessment is conducted for dissolved inorganic nitrogen (DIN) for the bi-weekly sampled temporal monitoring sites from Nov.2021- Feb.2022. The threshold value for DIN is 1µM, indicating the phase shift from coral to macroalgae-dominated coral reefs (Hoekema, 2022; Slijkerman et al., 2014). DIN consists of the sum of ammonia, nitrate and nitrite, and is used as an index of nutrient enrichment in marine waters. Nutrients need to be in the water, as they support the food chain, but when in excess, DIN is of environmental concern as it is responsible for eutrophication (Zhang et al., 2020).

After dividing the measured DIN concentrations by the threshold for eutrophication that served as a surrogate for PNEC, a risk quotient (RQ) < 1 indicated that there is no adverse impact, the impact becoming more significant as the value of RQ equals 1. A range of 1 < RQ < 2 and 2 < RQ < 3 are considered as low and moderate, respectively, while RQ ⩾ 3 indicates a high and unacceptable impact (Mohtar et al., 2019).

Due to the varying range of temporal data, a baseline and worst-case scenario has been evaluated, which were based on the mean concentrations or the highest concentration over the sampling period. The categories of hazard were developed based on the frequency of occurrence (during the study period, therefore, considered as preliminary), where less than 30% of episodes is categorized as infrequent, 30%–70% is moderate, and more than 70% of episodes is categorized as frequent (Mohtar et al., 2019). The impact of DIN on the environment was determined based on four categories of hazards, i.e., green indicates no hazard, orange and yellow indicate moderate and low impact, respectively and red, is the highest level of hazard level with the most serious impact. Each sampling location was assigned by its associated hazard level. As this analysis was only carried out for depths of 5 and 10m, the hazard levels for 10m depth for the worst-case scenario have been used over all depth ranges combined (5-60m) within this study (Table 1).

2.1.3 Reef profile

Although the reef profiles along the coast vary, the general reef profile on this side of the island consists of a shallow terrace, including a lower terrace zone at about 3 to 7 m depth, stretching from the coast into the ocean over a distance of 20 to maximal 150 m (Van Duyl, 1985). The terrace gradually slopes from the shore to the upper drop-off zone, located around 10-15m depth, where the reef steeply runs down (angle between 20 and 50°) to a depth of 25-55m, where it gradually levels off to 6-12° (Van Duyl, 1985). Generally, a second vertical drop-off occurs at 70 to 80 m, ending at a sandy plain at 80 to 90 m (Fig.3) (Bakker et al., 2019; Bak, 1977; Van Duyl, 1985; Debrot et al., 2017). Over this profile a well-defined series of coral communities occur, each one representing a different response to environmental factors. Currently, a substantial part of the MCEs surrounding Bonaire is part of the Bonaire National Marine Park (BNMP) as this extends from the high-tide mark down to 60 m in depth (Steneck et al., 2015). With regards to Fig. 3, the *A. palmata* zone is nowadays unfortunately not as distinct anymore as back in the seventies, due to the white-band disease that killed nearly 90% of elkhorn and staghorn corals (Bak et al., 1984; Bowdoin & Wilson, 2005; Steneck, 2005).

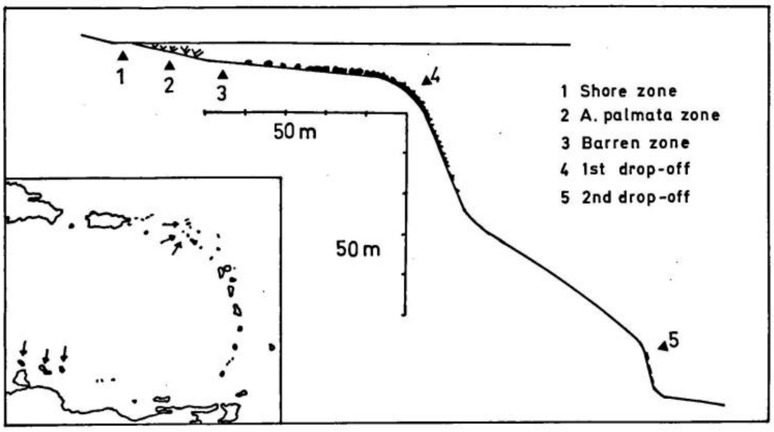


Figure 3: General reef profile of the S.W. Coast of Curaçao and Bonaire. The inset shows the geographical position of the Netherlands Antilles in the Caribbean. Figure derived from Bak 1977.

2.2 Procedure for Image Collection

An ROV (QYSEA Fifish V6 Pro), equipped with a GoPro Hero 10 camera pointed vertically down to take pictures of the reef floor, was deployed from a boat. Attached to the ROV was a GPS locator (Waterlinked), which sends acoustic signals to an antenna with 4 receivers, which is attached to the boat. To compensate for the weight of the GoPro and locator, a block of foam is attached on top of the ROV to create the right buoyancy. There are 6 thrusters, divided over the back, side and bottom of the ROV to give forward, side-way and downward movements, respectively, when in operation. The ROV was set at a fixed distance from the ocean floor (1-2m) and runs a transect of 25m parallel to the shore while taking pictures of the ocean floor at a time interval of 5 s. The maximum speed of the drone is 3 knots (1.5m/s). Practically, this very much depended on the currents underwater. Unfortunately at the time of conducting this research it was not possible to see the exact speed of the ROV. Therefore, we assumed the speed of the drone is approximately 1 m/s. As the GoPro takes pictures at a set interval, the pictures that are analyzed do not overlap and form each an individual sample, instead of being part of a continuous transect, increasing the sample size per transect. Parallel lasers, spaced 10 cm apart, are projected onto the field to provide scale in the images (Scott et al., 2019). It was at all times the goal to keep the GoPro perpendicular to the reef, to minimize parallax error and to keep it in focus. Most reefs are formed on a slight slope, where the coral colonies grow towards the light, this implies that 'perpendicular to the reef' entails that the ROV does not dive under an angle but stays straight above the coral reef.

Due to the relief of the ocean floor, the ROV had to move up and down following the contours of the corals, causing a variance in the height above the ocean floor at which the pictures have been taken. In general, the height above the bottom varied between 0.5m and 2.5m, depending on the ocean floor topography and the drone pilot, each picture therefore covers an area of approximately 1 to 4 m^2, resulting in a total covered area of about 10-15 m^2 per transect. This results in a total area of about 500 m^2 of ocean floor monitored by the ROV within this study. An overview of the exact heights and surfaces covered per picture and per transect is available as supplementary material.

Each survey started at 60 m depth, after which the ROV moved up to 40 m, where the above is repeated in the opposite horizontal direction, after which the same will be done for 20, 10 and 5 m depth. The battery lasts for about an hour, depending on current strength and whether the lights are switched on. This is enough time to run 5 transects at 5 depths.
The transects at 5 and 10m depth allow for a comparison with coral reef monitoring data obtained in 2020 by Bakker and colleagues by means of scuba diving. This way a comparison of two different data collection methods can be made. The transects at 5, 10 and 20 m provide data on the status of the reefs, whereas the transects at 40 and 60 m provide insights into the status of the upper MCEs. To continuously receive a position from the GPS system of where the ROV is located, a clear line of sight between the antenna on the boat and the ROV should be maintained (Fig.4), more on this topic can be found in the discussion section (4.1Technical approach ROV).

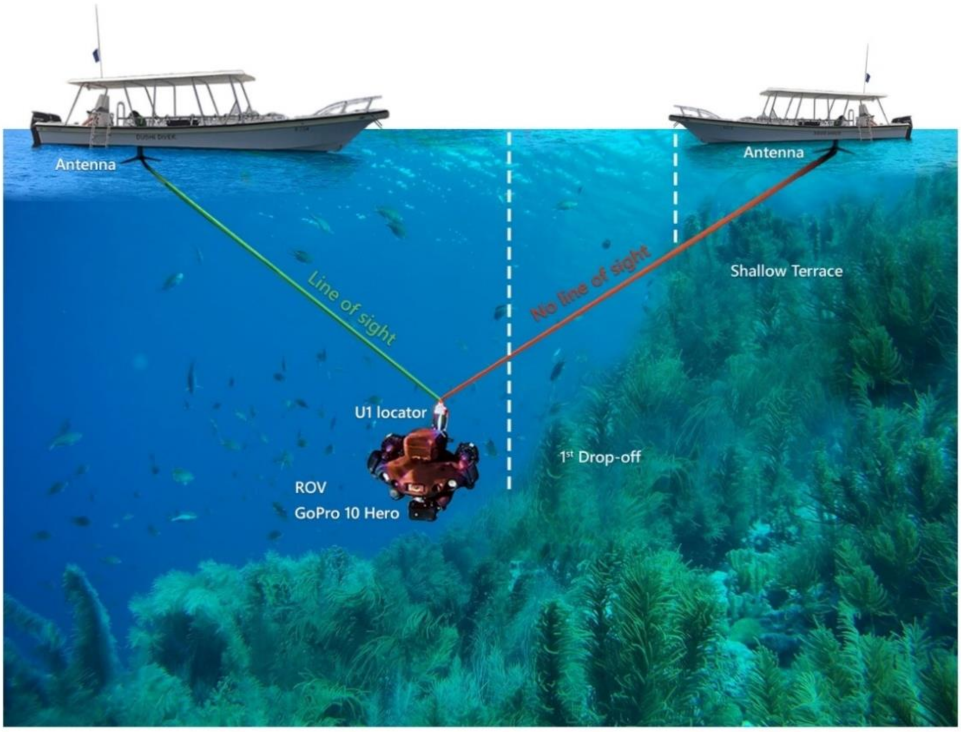


Figure 4: Set-up of the deployment of the ROV, left boat: above the deeper water with a clear line of sight and right boat above the shallow terrace with the reef blocking the line of sight. Illustration made by Lisette Woltjer.

2.3 Image selection & analysis

Five pictures (n=5) were chosen randomly along each 25 m transect (Fig.5). A set of selection criteria was applied as not every image from the surveys was suitable for analysis. Pictures were excluded when they were clearly non-random (e.g. photographs of specific objects), too close or too far away from the bottom or if the quality was too poor for accurate identification. Ideally, if the placement of the scaling lasers was not accurate, these pictures would be excluded as well. However, one laser did not have enough brightness to be always visible within the pictures, therefore the distance from the most bright and left laser point to the edge of the photo has been used to provide scale in the pictures.

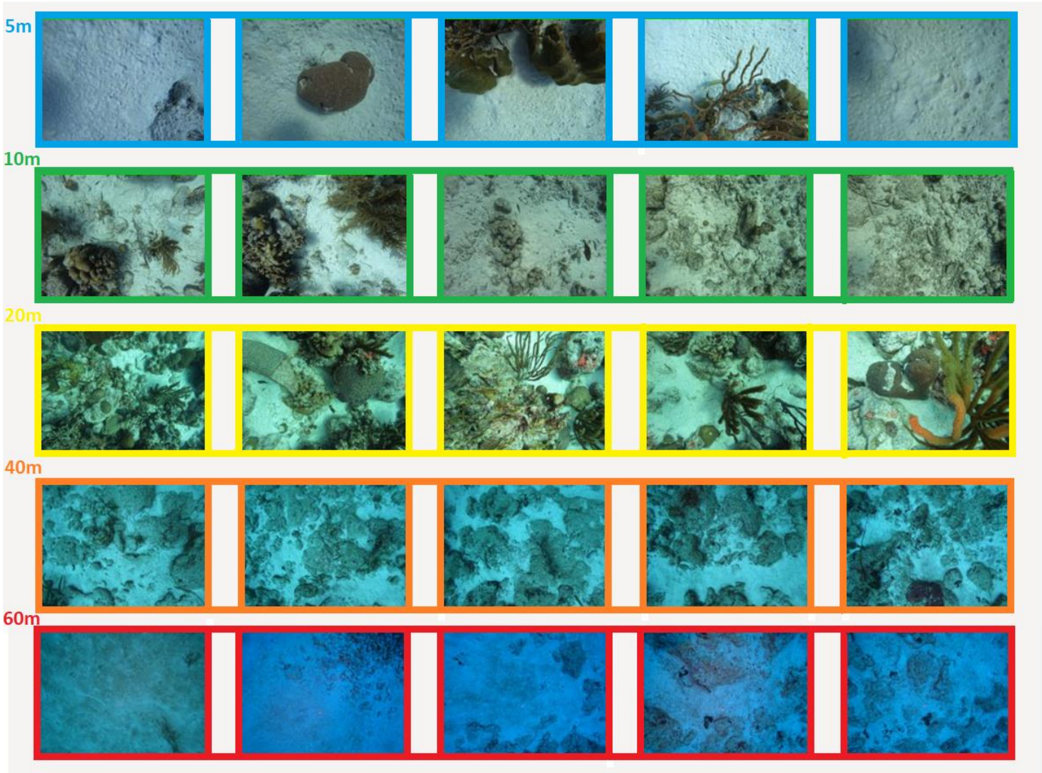


Figure 5: An example of site B04, Salt Pier. The pictures that are chosen per depth transect (5, 10, 20, 40, and 60m). Source material of the other sampling sites is provided in Appendix A.

The obtained images were analyzed using online image analysis software (CoralNet), where randomly stratified point annotation provides species count data per picture. The image annotation area was set to X: 15 – 85% / Y: 10 – 90% and 49 points were placed in a stratified random pattern on the image (a grid with seven rows and seven columns was established, after which a point was placed randomly within each grid cell; Figure 6). The organism or inorganic substratum beneath each point was identified to functional group (hard corals, soft corals, sponges, macroalgae, microalgae, cyanobacteria, crustose coralline algae (CCA), turf, rubble, or sand). The category of benthic macroalgae was subdivided into turf algae (turf), CCA and (fleshy) macroalgae, consisting mainly of Lobophora spp. and Dictyota spp. When distinction between species within a genus was possible, live hard (scleractinian) corals were visually identified to both genus and species level. Identification of scleractinian species followed the Atlantic and Gulf Rapid Reef Assessment (AGRRA). *M. complanata* and *M. alcicornis* were included in the group of hard corals. *Agaricia* is the dominant coral genus in Caribbean deep reefs, with *A. lamarcki* or *A. grahamae* as the dominant species (Frade et al., 2019; Bak et al., 2005). *A. lamarcki* being the most common and as distinction between these two species on pictures is challenging, as well as this distinction based on morphological data being debatable, these colonies were grouped as *A. lamarcki* (Bak et al., 2005).

Soft coral plumes, fish and other invertebrates, such as sea cucumbers, were not counted in the points, but rather the substratum or coral underneath. For groups overgrowing each other, only the overgrowing group was scored. Tunicates and other invertebrates were rare in the pictures and excluded from this analysis. The percentage cover of each benthic group was determined by dividing the number of points that fell on that type of organism by the total number of points in the quadrat that were identifiable. Points that were unidentified, due to poor image quality, e.g. due to shading or unknown organisms, were excluded (80 out of 9310 points, 0.85%, <1%) and relative percentages adjusted accordingly. The group cyanobacteria includes cyanobacterial mats, but distinguishing between these early mats and turf was challenging, which may have led in some cases to a minor overestimation of the fraction of those groups and should therefore be viewed as conservative.

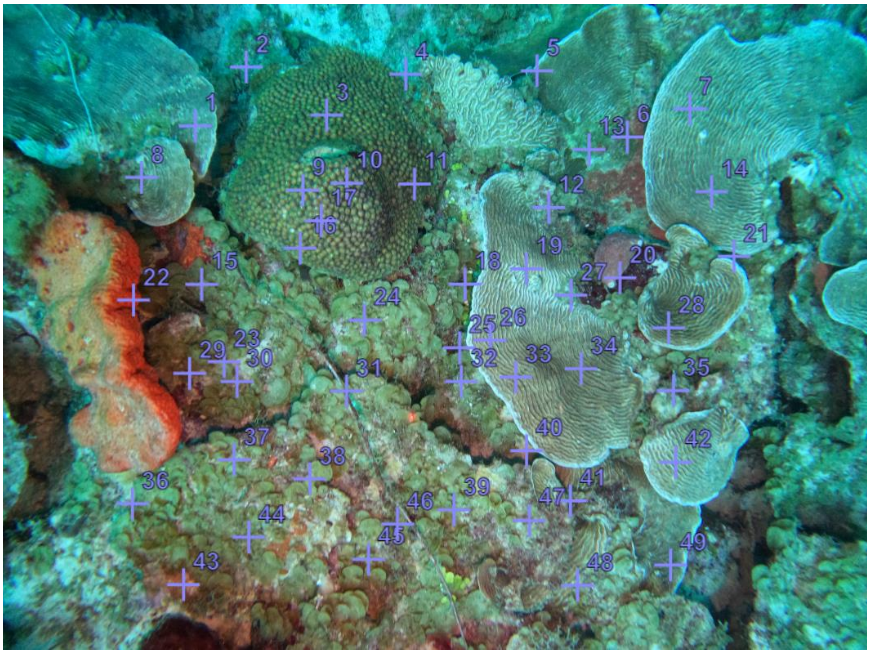


Figure 6: Example of stratified random points superimposed over a digital imagine from an ROV dive track used to estimate percentage cover. This picture is taken at 40 m depth at B16, Karpata.

2.4 Statistical analyses

Data processing and statistical analyses were conducted in the R programming environment v4.2.0 using the *vegan* package. Shannon-Weaver Diversity and Richness have been calculated over all benthic species identified, and the results have been analyzed in relation to depth, human impact and water quality risk zone. To distinguish community patterns, the species count data was turned into percentages and further analyzed using non-metric multidimensional scaling (nMDS), whereby a similarity matrix was computed between sites and depths. Ecological studies often use nMDS to visualise the discrimination of multivariate patterns (De Bakker et al., 2017). The species count data and benthic cover data were fourth root transformed after which this data was approximately normally distributed and to reduce the effect of high-cover groups (Bakker et al., 2019). Statistical assumptions of the multivariate analyses (multiple linear regression and analysis of variance) were assessed through graphical exploration of residual plots. Multivariate analysis techniques were applied to visualize changes in benthic group cover, diversity and richness with depth (5, 10, 20, 40, and 60m), human impact (Extreme, High, Moderate, Marginal) and water quality risk (High-, Moderate-, Low-, No-risk) as explanatory variables. The non-biotic categories bare substratum and rubble had low counts (as these were mostly overgrown by turf) and have been omitted to remove any collinearity among the remaining variables (Bakker et al., 2019).

To compare the data collected by the ROV with data obtained by divers, a dataset obtained while scuba-diving at similar sites has been compared to the ROV dataset. For both datasets, percentage coral cover was calculated, as well as 95% confidence limits and the results have been tested by a paired *t*-test.

3 Results

This chapter discusses the general findings encountered at mesophotic depths, to create a general idea of what the deeper areas look like. Thereafter the species count data are analysed to provide insights and answers to the research questions. The findings with regards to the deployment of the ROV are described in the discussion section, where limitations, advantages, improvements and comparisons with other studies are addressed.

Generally, around 30-45m the reef slope levels off into a sand plane (Fig.7). These sand planes are often colonized by Garden eels (*Heteroconger longissimus*) (Fig.7), but descending towards larger depths, benthic cyanobacterial mats (BCM) covered the sand, occurring around 40-60m depth (Fig.17; Appendix A, Fig.3). The sand planes are interchanged by occasional small rocks on which sponges and fan corals were present (Fig.8) and piles of coral rubble and stones were visible, which are likely constructed by sand tilefish, piskarai in the local language (*Malacanthus plumieri*) (Fig.8). Lionfish were especially found at larger depths, around 50 and 60m (Fig.8).

Within this study the ROV was able to reach a maximum depth of 90m. With increasing depth, mainly sand and gorgonians covered the ocean floor (Fig.9). Trash, such as tires, fishing lines, self-made ankers (stoneblocks), barrels and beer bottles, was observed among all depths, in front of the BOPEC Oil Storage, and during the dives in front of Kralendijk (Fig.9).

Figure 7: The transition from the reef onto the sandy plateau around 30m depth in front of Kralendijk. Right: Salt Pier, the presence of Garden eels around 30m depth.

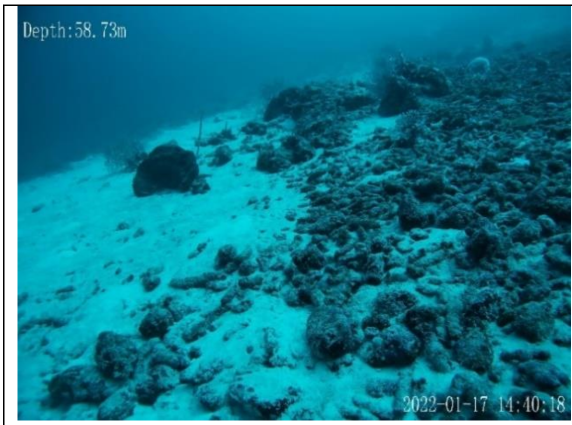


Figure 8: Carl's Hill, a lionfish present in the distance and the rocks and the presence of fan corals. Right: rubble mounds of Tilefish nests, piskarai (Malacanthus plumieri). Both pictures at 60m depth.

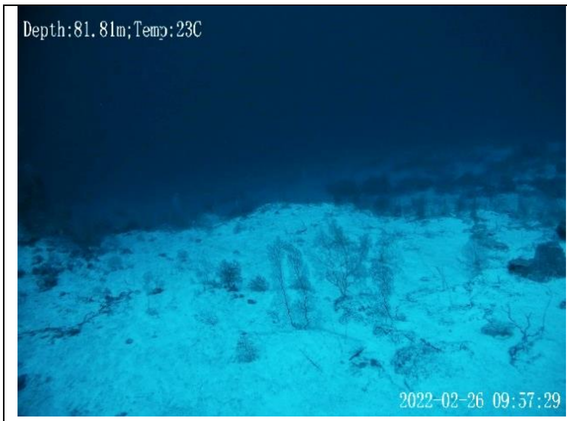


Figure 8: BOPEC, Gorgonians and sand visible. Right: Kralendijk, oil barrel on the reef.

3.1 Occurrence of MCEs

A general image of the depth-effect on coral cover over all 8 sites monitored on the leeward coast of Bonaire shows a clear pattern with depth, where the cover first increases towards 20m, after which it decreases going down to 40 m, reaching almost zero percent cover at 60m (Fig.10).

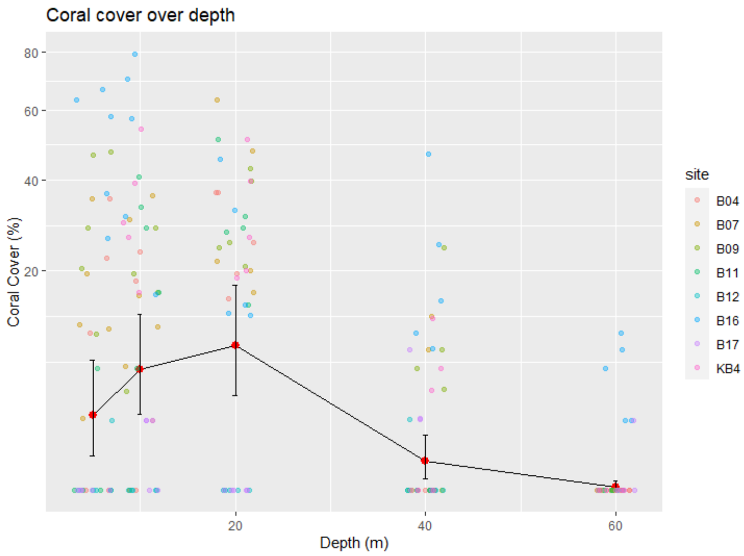


Figure 9: Percentage mean coral cover (±95% confidence limits) averaged over all 8 sites with 95% CI, showing in colours the coral cover data per site.

Linear regression shows that coral cover significantly decreases with an increase in depth (*P*<.05) (Fig. 11), this concerns fourth root transformed data.

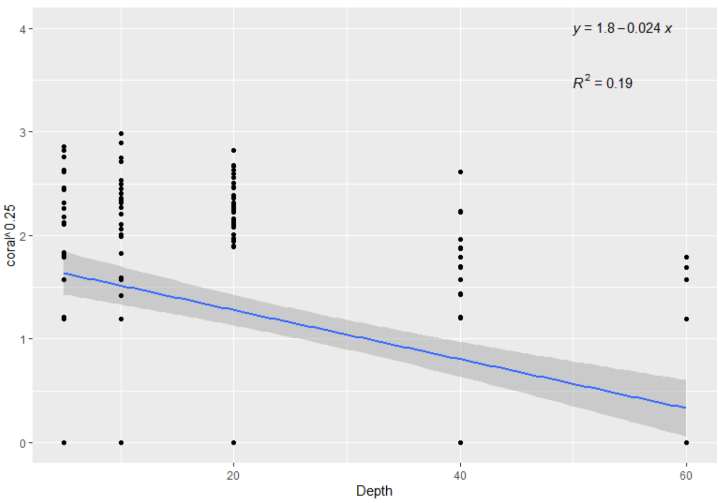


Figure 10: Linear regression, depth being negatively correlated to coral cover.

This model only explains 19% (adjusted $R^2 = 0.1851$) of the observed variation, emphasizing the need to include more explanatory variables to explain for the differences in coral cover. Multivariate analysis has been applied with the three explanatory variables, namely depth (5, 10, 20, 40 and 60m), impact zone (Extreme, high, moderate, marginal) and water quality risk (High-, moderate-, low-, no-risk).

First the depth-effect per site is discussed, to get a better understanding of the occurrence of MCEs along the leeward coast of Bonaire, the percentage hard coral cover gives an indication. As shown in

Fig. 12, the highest values for coral cover were found at Karpata (B16) in the shallow zone (5 & 10m), the site with marginal human impact. In addition, Karpata seems to be the only site where hard corals still occur at 60 m depth. The MCEs are present when there is still hard coral present below 30m depth. In the figure this is represented by a coral cover larger than zero percent at 40m depth, thus following from the figure and based on this data, MCEs occur at all sites except from sites B04, Salt Pier and B11, Kralendijk. Only sites B09, Port Bonaire and B16, Karpata have an estimated coral cover higher than 3% at 40m depth.

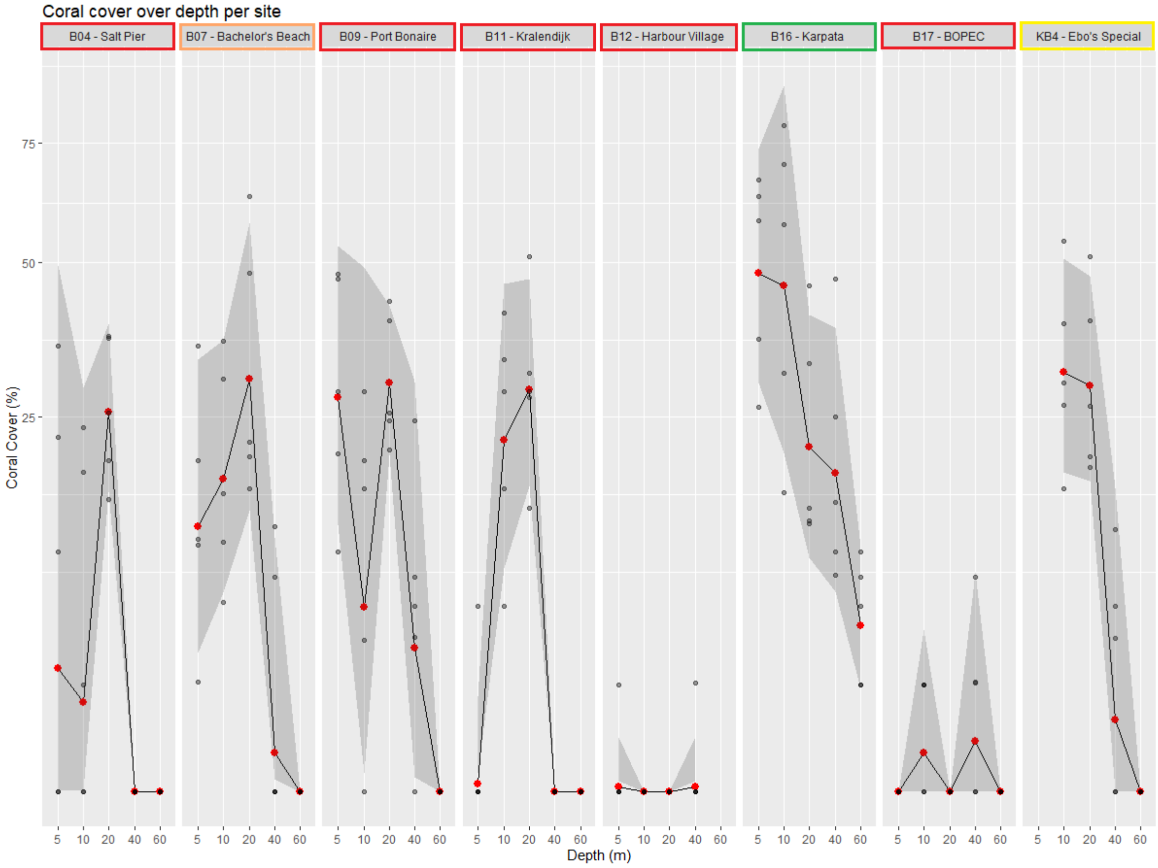


Figure 11: Percentage coral cover per sample site. Colours indicate the impact zone in which the site is located, with red=extreme, orange=high, yellow=moderate, green=marginal.

The different sites were chosen based on their differences in human impact and water quality, therefore in the remaining of this study there is looked at the differences in benthic cover per human impact and water quality zones.

3.2 Status of MCEs

To be able to say something about the status of the coral reefs and the mesophotic coral ecosystems, the percentage cover of main benthic groups, species richness and Shannon-Weaver diversity were calculated and analysed to get an idea of how speciose and diverse the communities are on different sites and at different depths, using multiple linear regression and analysis of variance.
In addition, nonmetric multidimensional scaling (NMDS) has been applied to get an idea of how different the communities are in species composition. Consequently, to check whether the observed differences are significant, a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) using distance matrices has been used (*adonis2)*.

3.2.1 Benthic group cover

Cover percentage per benthic group is analysed regarding depth, impact zone and risk level. How the benthic community composition changes over the depth gradient is shown in Fig. 13, where it is clearly visible that turf is the dominant benthic group and sand shows highest cover in the shallow (5m) and deeper (60m) parts of the reef. Macroalgae are nearly absent in the shallow and deepest parts of the reef, but make up for a large part of the cover from 10 to 40m depth.

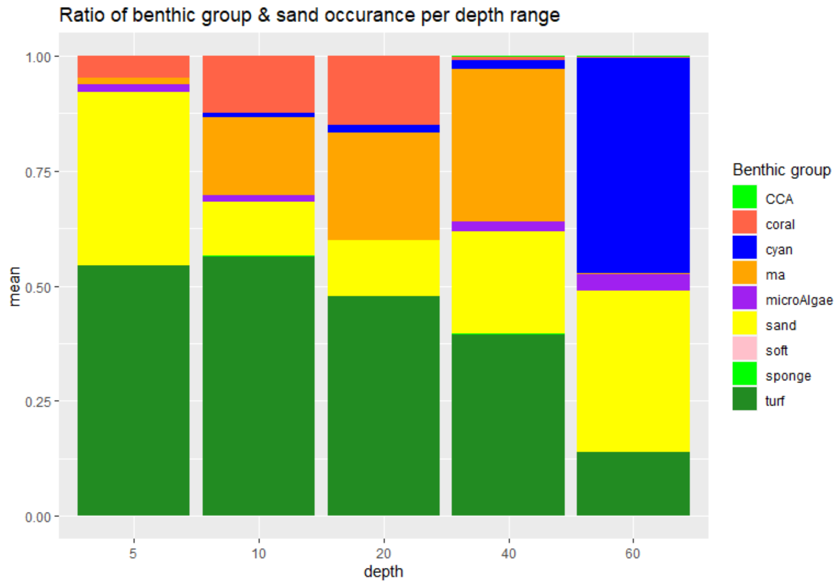


Figure 12: Benthic community composition and sand cover per depth range.

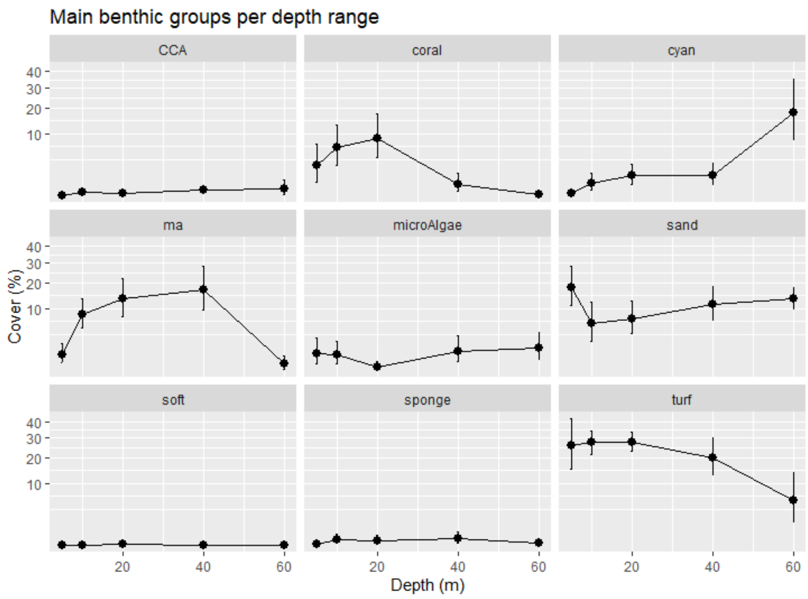


Figure 13: Main benthic groups per depth range, mean and 95% CI.

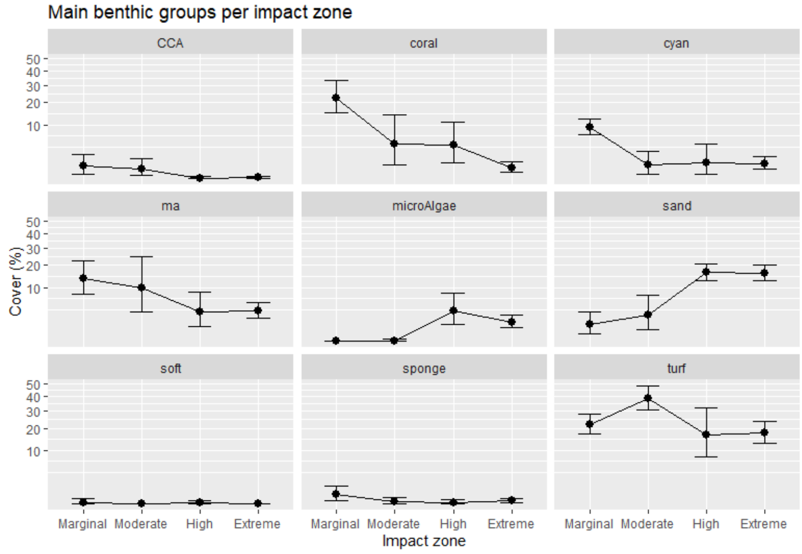


Figure 14: Main benthic groups per Impact zone, mean and 95% CI.

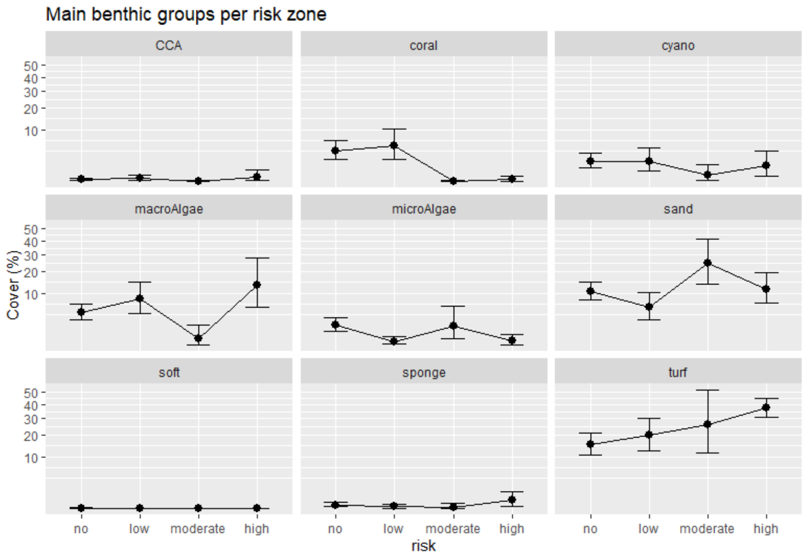


Figure 15: Main benthic groups per water quality risk zone, mean and 95% CI.

Coral Cover

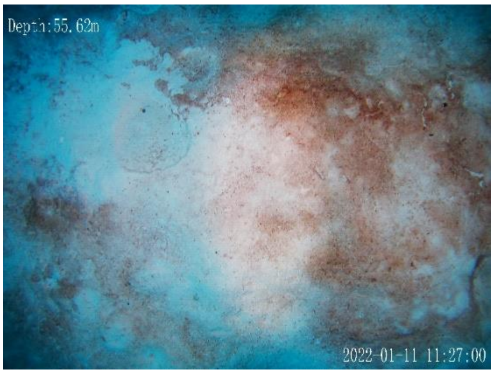
The interaction of explanatory variables has been tested with multiple linear regression (adjusted R^2 = 0.766, when looking at the explanatory variables as factors). Both impact zone and water quality risk significantly interact with depth (Appendix B, table 2), meaning that these variables are dependent on each other. With an increase in depth, human impact and water quality change accordingly, thus in explaining coral cover, the interaction between them should be taken into account.

Post-hoc tests showed significant differences between all impact zones, except for the moderate and high impact zone (Fig.15, TukeyHSD: *P*>.999). Significant differences were also observed between all the water quality risk zones (Fig.16) and between all depth groups, apart from the shallow reefs (Fig.14, 5 and 10m; TukeyHSD: *P*>.122 and 10 and 20m; TukeyHSD: *P*>.725), which apparently showed similarity in coral cover among these depths.

Coral cover varied initially from 0.14 to 0.95% in the extremely impacted zone, from 0.89 to 11.01% in the highly impacted zone, from 0.65 to 14.05% in the moderately impacted zone and from 15.02 to 33.24% in the marginally impacted zone, in case all depth ranges were taken together per impact zone (Fig.15).

Cyanobacteria

Cyanobacteria significantly increased with increasing depth from 40 (1.06% cover) to 60m (18.13%) depth (TukeyHSD: P<.001)(Fig.13 and 14). Cyanobacteria cover did not significantly change along the gradient in human impact zone or water quality risk (Fig.15 and 16). These fields with cyanobacteria extend for hundreds of meters, the only other organisms present within those fields were gorgonians (*Leptogorgia vigulata*) (Becking and Meesters, 2014) and sand eels (Fig.17). The white patches might be an indication of bioturbation, caused by polychaete worms living in the sand (Becking and Meesters, 2014).



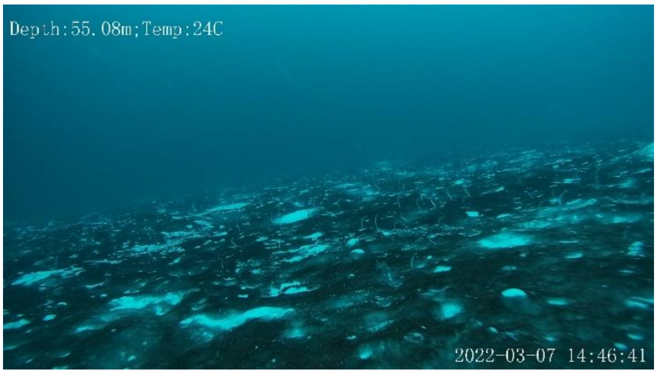


Figure 16: Cyanobacterial mats around 55m depth at Kralendijk (left) and Port Bonaire (right), colonized by sand eels.

Other benthic groups

Although CCA cover was generally low, CCA shows a slight increase in cover with an increase in depth, but not significantly (Fig.14). CCA does show a significant difference in cover between the marginally impacted site (B16, Karpata) and the extreme and high impact zones (Fig.15, TukeyHSD: P<.001). Macroalgae increase with increasing depth, but decrease in cover again at 60m depth (Fig.14). An insignificant increase in cover towards less impacted zones can be observed, whereas no clear trend is visible for water quality risk (Fig.15 and 16). Sand shows an opposite trend with depth opposed to coral cover, and significantly decreases going from extreme and high impacted sites towards moderate and marginal impacted sites (Fig.14 and 15). Soft corals and sponges had low cover among all sites and depths, but show a slight increase in cover towards less impacted zones (Fig.14 and 15). Turf shows an insignificant but clear decreasing trend with increasing depth and with a decrease in water quality risk (Fig. 14 and 16).

3.2.2 Diversity & Richness

Depth, risk and impact have a significant effect on species diversity (P<.001), post-hoc test showing significant differences between the deeper parts and the shallowest part of the reefs (TukeyHSD: 20-5m, 40-5m and 60-5m, P<0.05). Significant differences between all the risk and impact zones are observed, except for moderate-high risk and moderate-low risk (Fig.18 and 19, TukeyHSD, P>.071) and high-extreme and moderate-marginal human impact (TukeyHSD, P>.38), thus the zones that are closest to each other in terms of water quality and human impact.

The initial species richness, calculated over all living benthos identified within each picture, varied from 14 to 1, with the highest number found at Karpata (B16) and Bachelor's Beach (B07) at 10 and 20m depth, and the lowest numbers at 60m depth at both harbors in Kralendijk (B09 and B11), at 5, 10 and 60m depth. Species richness differed significantly at different depths and showed a slight increase in number in the direction of less human impact and less water quality hazard (lm, adj. R^2 = 0.8848, $P<.05$)(Fig.18 & 19). Post-hoc tests shows that there are significant differences between all depth ranges except for 20-10,40-20 and 60-40 (TukeyHSD: $P>.4$), the same goes for all the risk zones, except moderate- to low-risk (TukeyHSD: $P>.1$) and for all impact zones, apart from high-extreme and moderate-marginal (TukeyHSD: $P>.1$). For an overview of the test-output, see appendix B, tables 3,4,5.

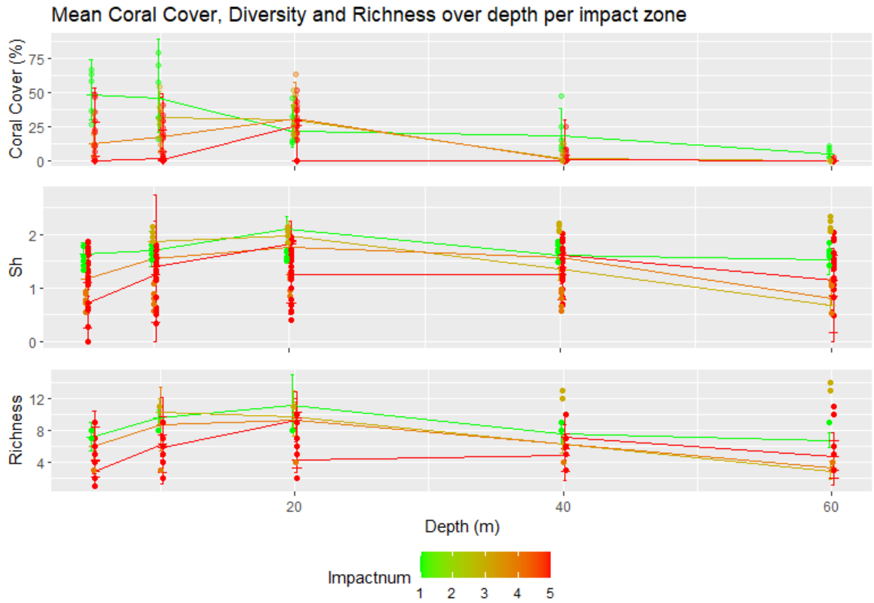


Figure 17: Mean Coral Cover, Diversity and Richness (with 95% confidence limits) over depth per Impact zone.

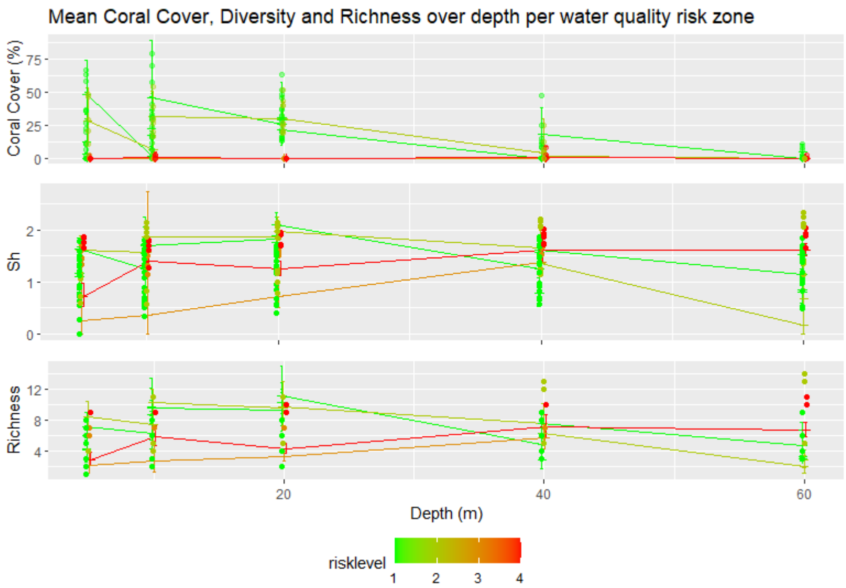


Figure 18: Mean coral cover, Diversity and Richness (with 95% confidence limits) over depth per water quality risk zone.

3.2.3 Species composition

The nMDS has been performed on species percentage data averaged per transect. According to how similar and dissimilar transects are from each other, clusters have been distinguished by cluster analysis. The clusters represent mainly similar depths among the different sites. The different cluster categories are plotted within the ordination graphs (Fig.20 and 21) and mainly connect the shallower sites with each other (5-20m) and the deeper sites (40-60m), with some exceptions. Especially the two smallest most left and most right clusters are the most delineated by depth group, but also by sites. The most left cluster only represents sites near Kralendijk at 60m depth, whereas the most right cluster only represents Klein Bonaire and Karpata (the two least impacted sites) in shallow waters.

To get a better overview of species occurance at the different transects, the species have been subdivided into the following benthos categories: turf, macro algae, hard corals, cyano bacteria, micro algae, sponges, crustose coraline algae (CCA) and soft corals. Sand is also added here, as this makes up a large part of the ocean floor, bare substrate and rubble (often overgrown by turf) were less present and excluded from this analysis.

The function envfit is used for the different benthic groups (Fig.20), clearly showing that cyanobacteria increase with depth and decrease with distance to Kralendijk (B07,B09 and B11) and are negatively correlated with all the other benthic groups, as within the cyanobacterial fields the other benthic species are absent.

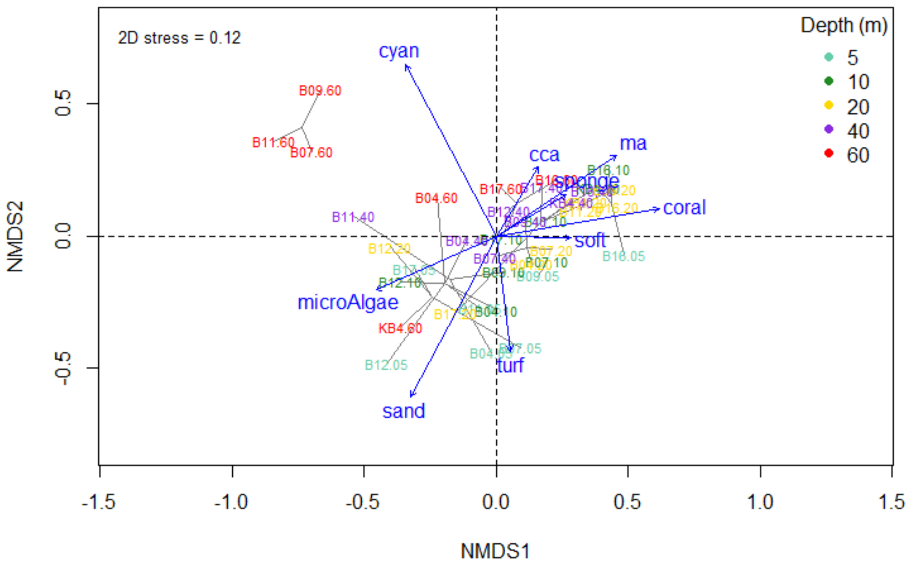


Figure 20: nMDS showing the different spider graphs and vectors indicating the correlation with the different benthic groups.

The nMDS clearly shows how microalgae, sand and turf are positively correlated and increase with an increase with human impact (Fig.20 and 21). Also, diversity increases in the same direction as CCA, macroalgae, hard and soft corals and sponges increase, which are all negatively correlated with sand, as these benthic groups cannot settle on sand, unlike microalgae.

The function *envfit* has also been used to visualize the effect of human impact, water quality risk, depth, diversity and occurrence of sand on species composition (Fig.21). Depth correlates positively in the direction of the arrow and corresponds with the position of many of the deeper sites, whereas diversity increases at sites around 20m depth. Sand occurs mostly at the shallower sites (5m) where sites also have lower diversity. At larger depth sand plains are also found, but mostly covered by cyanobacterial mats. Arrows pointing in opposite direction show a negative correlation, sand and impact being negatively correlated with depth and diversity.

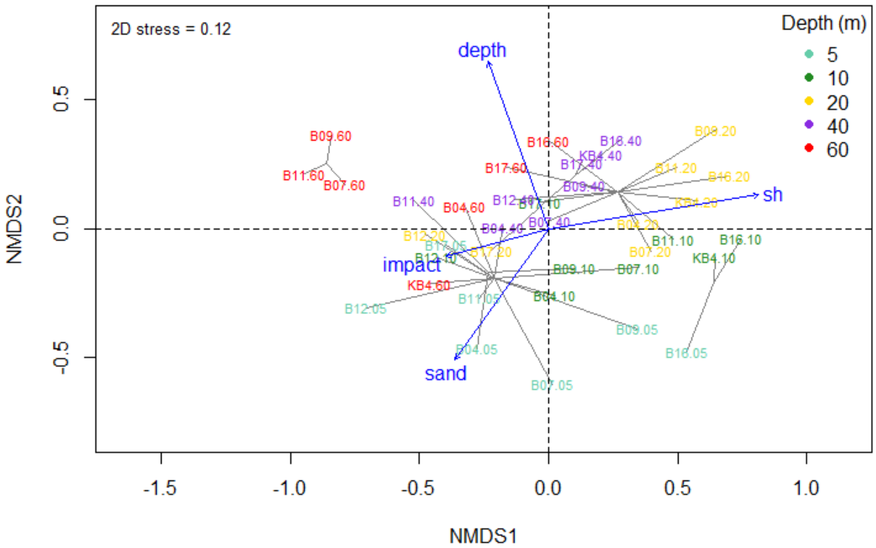


Figure 21: nMDS showing relative species composition of each site where sites in the same clusters have been connected by spider graphs. Colors distinguish the different depths.

The water quality risk is not displayed as an arrow here as this factor did not have a significant effect on the species composition, whereas depth and impact do, however, depth and impact, as well as depth and risk show a significant interaction (Appendix B, table 6).

In the heatmap (Fig. 22), the cover per benthic group becomes clearly visible, with turf being the most common and soft corals the least. Where hard coral cover is very low to non-existent, cyano bacteria and micro algae seem to take this place. The first cluster of deep sites is mainly covered by cyanos and microalgae, without corals, probably because of the sandy bottom, at depths of 40 and 60m. The second cluster is characterized by the absence of microalgae, and high coral cover, and includes all depth ranges (5-60m) but mainly consists of the least impacted sites, being Karpata and Klein Bonaire (B16 and KB4). The third cluster is the most diverse in occurance of benthic groups and consists of the sites around Kralendijk and BOPEC. The fourth cluster is characterized by the absence of both hard and soft corals, dominated by turf. The last cluster can be distinguished by the absence of CCA and the mainly shallow transects (5&10m).

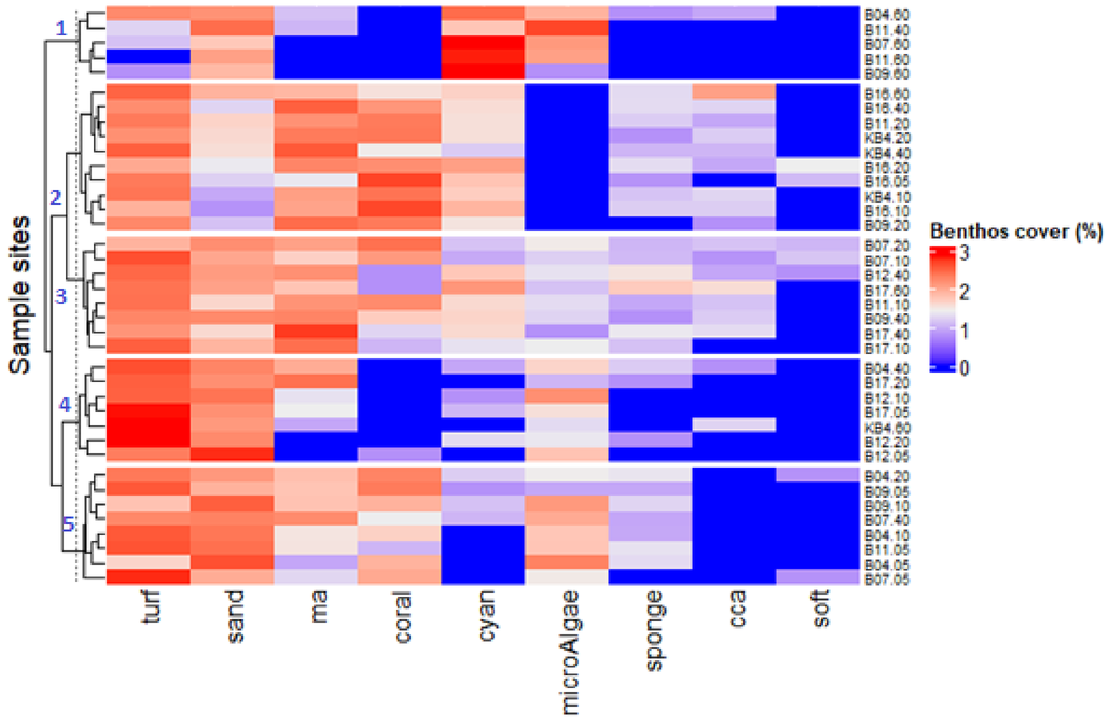


Figure 19: Heatmap showing the fourth root transformed benthos cover per transect (site and depth).

To visually show which benthic groups are most abundant at which depths, *ordisurf* is used, which fits smooth surfaces for continuous variables (depth) onto ordination, using thinplate splines with cross-validatory selection of smoothness (Wood, 2003). The lines are quite parallel, indicating a linear effect with depth (Fig.23). The fit shows an approximate significance of smooth terms (*P<.001)*(Appendix B, table 7). It is clearly visible that cyanobacteria occur at the deepest parts of the reefs, CCA are more abundant around 40m depth, soft corals are mostly present on the shallowest parts of the reefs, whereas hard corals occur a little bit deeper compared to the soft corals. The other benthic groups all occur mostly between 20 and 30m depth.

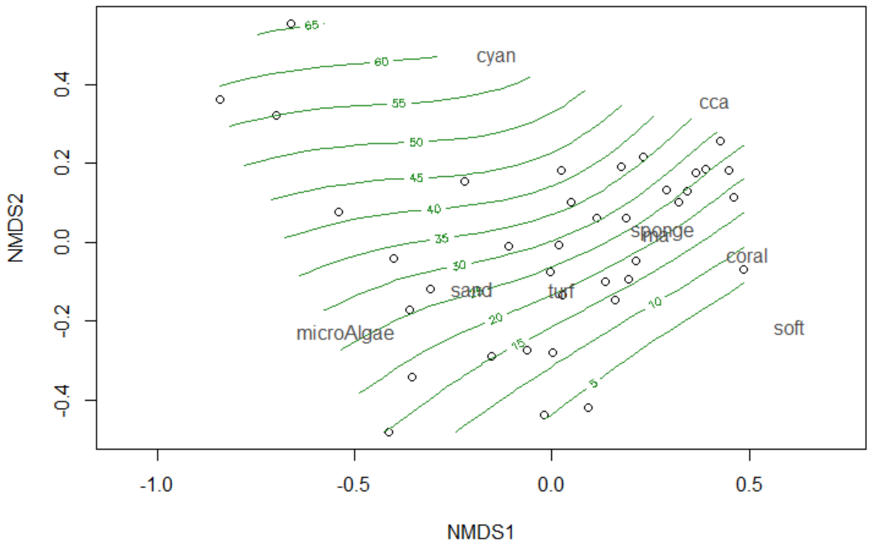


Figure 20: Ordisurf of the nMDS benthic group composition.

3.2.4 Shift in coral species composition

A total of 27 coral species were identified, of which the coral species with an occurrence of less than 1% per depth range were classified under the category 'other' for a better visualization of the data.

Karpata (B16), the site that is the least impacted by human disturbances, apart from diving activity, and has no risk regarding water quality, is the only site that still showed hard corals at a depth of 60m. The shape of the drop-off should however also be taken into account, as the steepness has an effect on the amount of sediment accumulation on the reef. Only two species were identified at this depth, namely *A. lamarcki* and *M. cavernoca* (Fig.24).

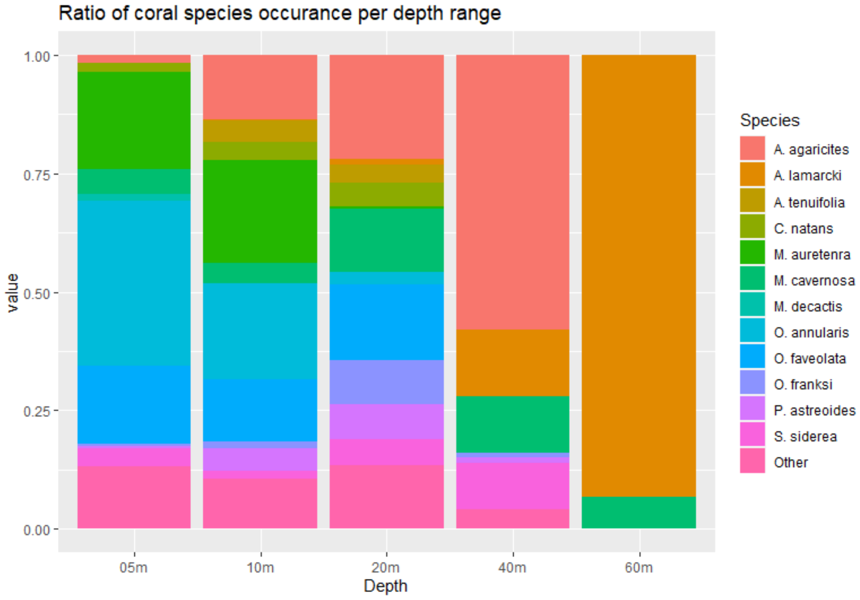


Figure 21: Ratio of occurrence of coral species per depth range.

The nMDS (Fig. 25) shows the coral species composition and how depth influences this distribution. Polygons for risk and impact all overlapped, but for the different depth groups there is a more clear distinction visible. *Adonis2* showed that only depth has a significant effect on coral species composition (*P<.05,* Appendix B, table 8).

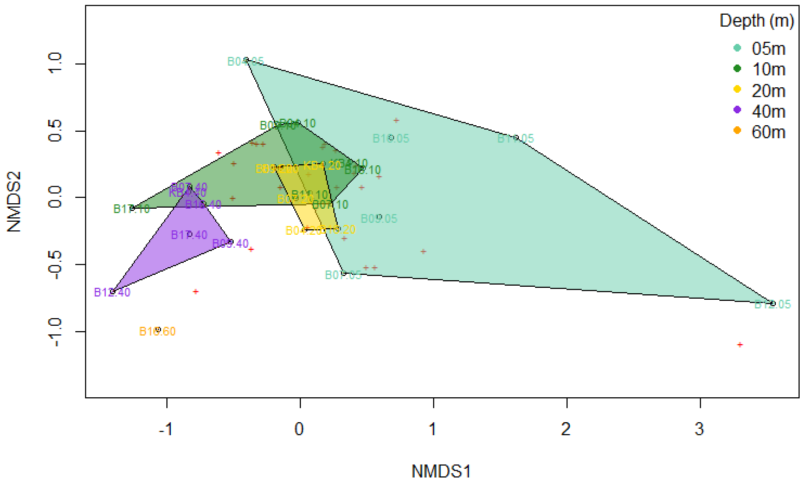


Figure 22: nMDS for coral species composition, clustered by depth ranges.

3.3 Data comparison

The most recent and thus comparable data of the shallow coral reefs comes from De Bakker and colleagues in 2020. By means of scuba diving they collected pictures of 25m transects, 2 transects per site, at 115 sites along the leeward side of the island. The eight sites from this study have been compared with the corresponding sites from De Bakker (Table 1 provides an overview of these sites). In case there was no corresponding site, the average of the two nearest sites was taken. The data collection methods differed, as De Bakker and colleagues did two 25m transect along a line, whereas the data in this study is based on 5 separate pictures per depth, covering an area of approximate 10-15 m^2 per depth range per site.

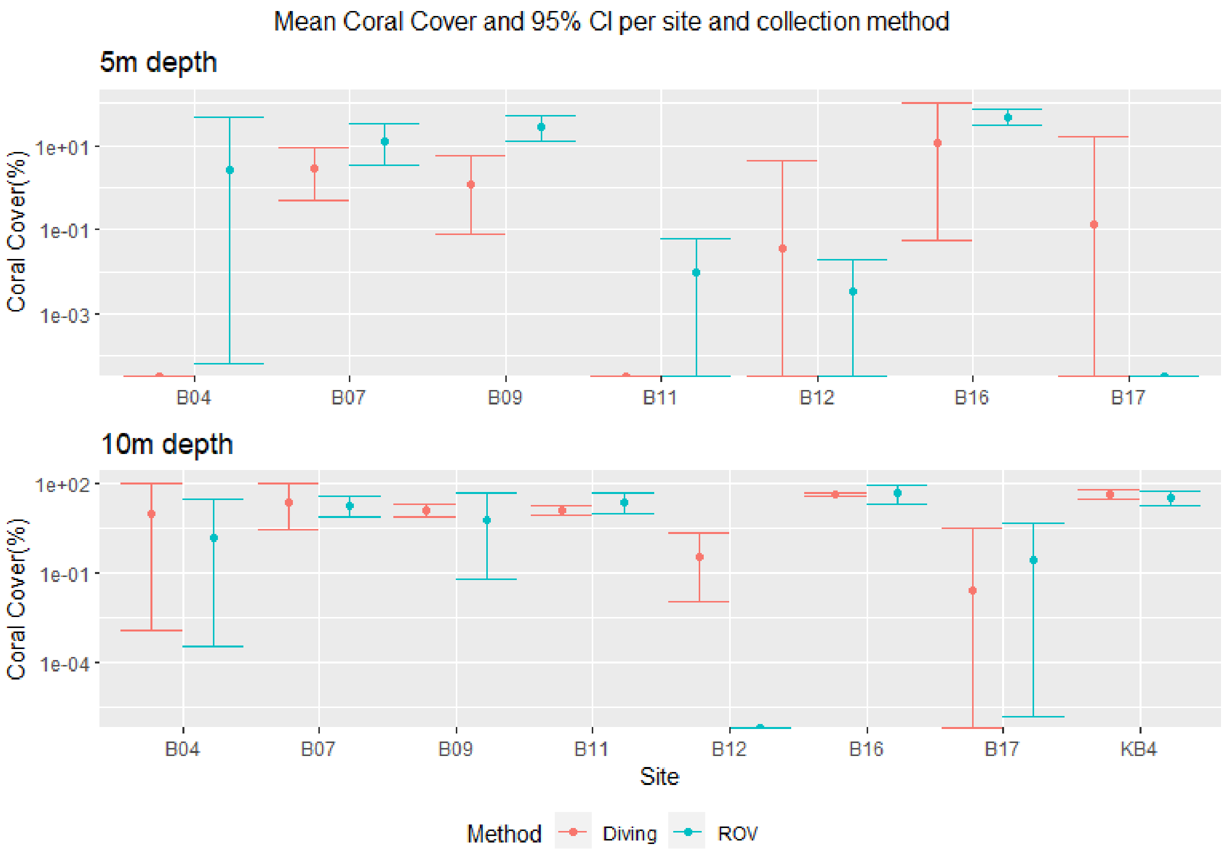


Figure 23: Mean percentage coral cover and 95% CI displayed for both data collection methods at 5 and 10m depth.

For both depth ranges (5 and 10 m), the means do not significantly differ from each other ($P > .05$)(Table 2, Fig.26), meaning that both collection methods show comparable results, despite the fact that there is 2 years in between the data gathering.

Table 2: Paired t-test output for two different data collection methods.

Paired t-test	t	Df	p-value	95%CI		Mean difference
5m	-1.9135	6	0.1042	-24.44482	2.99033	-10.72724
10m	0.78	7	0.4609	-3.833832	7.608074	1.887121

4 Discussion

4.1 Technical approach ROV

To obtain detailed biological characteristics of MCEs, high-resolution optical imaging from digital still and video cameras is required, for which ROVs and AUVs are ideally suited (Rivero-Calle, 2008; Lam et al., 2005). Areas with complex structure, steep slopes, vertical walls and high-bathymetric relief are most suitable to be mapped and surveyed with ROVs and manned submersibles. However, precise navigation for both ROVs and AUVs remains a challenge, especially in areas with complex terrain and/or strong currents.

The ROV deployed in this study (QYSEA V6 pro) weighs only 3.9 kg and has dimensions of 383 mm x 331 mm x 142 mm, this in contrast with for example the SeaBED-class AUV weighing 200kg. Such an AUV requires a ship with lifting gear for deployment, whereas this ROV can be deployed by one, preferably two people. This user-friendliness makes that such relatively small ROVs are increasingly being used for initial surveys of MCEs (Englebert et al., 2017).

Budget- and time-constraints are decisive, as well as the goal for which the underwater vehicle will be used. For biological characterization of the ocean floor at mesophotic depths, this ROV suffices, due to its great manoeuvrability with 6 degrees of freedom (DOF), sensor and led beams. For future research a robotic arm could even be added to the ROV for specimen collection. Due to its small size and quiet movements through the water, the ROV is ideal for viewing fish and other mobile species without disturbing them too much. Also, close examination of cryptic organisms living on vertical walls, in caves and overhangs, are easier to perform with an ROV than with AUVs (Armstrong et al., 2019).

However, as the images of the underwater world are viewed by humans through a screen, no robotic technology truly provides a replacement for divers in the water (yet) and their ability to study and collect fishes and benthos (Armstrong et al., 2019).

4.1.1 Picture quality

Due to the varying topography of the reef, the ROV functions to hold depth and dive at a fixed distance above the ocean, or when manoeuvring around everything manually, resulted in some variability in altitude at which the pictures were made.

Due to the strong light attenuation in water at mesophotic depths, adequate lights for close range (1-3 m) illumination are needed “to observe surface reflectance under the full visible spectrum and recover images that are close to ‘true’ colour”(Armstrong et al., 2019, p.980). Therefore, optical imaging is usually performed at 0.5-3m distance off the ocean floor, limiting the area covered by images to a few square meters (Armstrong et al., 2019). Even though the ROV stayed within this range of 0.5-3m distance from the ocean floor, the combination of the sometimes relatively high distance from the ocean floor and low light intensity at larger depths, resulted in imagery not always with sufficient resolution to identify corals and other organisms beyond the genus level. This was also observed by Trembanis et al., (2017), their photographs made by an AUV with increasing altitude above the seabed resulted in photographs covering an wider arrow, allowing to distinguish habitat type but not the species and genus level. It is important that in follow-up research pictures are made closer to the bottom, or that camera settings are changed, to deliver high resolution images, allowing for the detection of small changes in community structure, in order to be in time to undertake remedial actions to safeguard the coral communities (Lam et al., 2005). Turbidity has not been a problem in the Caribbean waters around Bonaire, but could cause a problem in other areas.

To achieve full coverage underwater is challenging, for which a sophisticated navigation suite is required. Thus far, robots that follow a “mow the lawn” pattern have been able to systematically cover an area without leaving gaps and providing adequate overlap, with a near-constant altitude off the seafloor (Bingham et al., 2010; Williams et al., 2010; Armstrong et al., 2019), ensuring image overlap across parallel track lines.

4.1.2 Battery duration

Both the remote controller, as well as the GoPro 10 Hero have a battery duration up to 4 hours and the GPS system, consisting of the U1 locator, the antenna and the topside box have a battery lifetime of 10 hours. The only limiting factor in monitoring more sites or longer transects is currently the ROV itself, with a battery lifetime of maximum 4 hours, but in the ocean, influenced by currents and with the lights turned on at larger depths, 1-1.5 hours is the maximum battery duration (Appendix B, table 1). To overcome this issue, there are several other versions of the QYSEA developed with an enhanced battery capacity. Another option would be to include an On-shore Power Supply System (OPSS) to provide power through the tether, which represents a major favour over data collection through scuba diving (Armstrong et al., 2019).

4.1.3 Line of sight, tether & reflection

Except for one site (B12, Harbour Village), the data for the other 7 sites has been collected while deploying the ROV from a boat. This has three main reasons:

Firstly, the Waterlinked antenna needs to be positioned with a clear line of sight to the locator. When deploying the ROV from shore, the antenna would also be positioned on shore, resulting in a loss of clear line of sight as soon as the ROV dives past the first drop-off. As the ROV is attached to the controller by a cable, we did not dare to deploy the ROV from a sailing boat, to avoid the risk of the cable getting stuck in the running motors. This however did cause another problem: the boat being moored onto dive buoys was often located above the shallow terrace. As soon as the ROV disappeared into deeper waters, the reef slope came in between the ROV and the boat, thereby blocking the line of sight (Fig.4). In order to get around this problem, a kayak was introduced to the set-up. The topside box and the antenna were attached to the kayak whereafter the kayak was kept as close above the ROV as possible, to improve the line of sight between the locator and the antenna.

Secondly, the fact that the first drop-off occurs only after 20 to 150m from shore, this means that the ROV has to move over a long distance before it can descend to deeper waters. This brings along two problems: the first issue is that over such a distance in shallower waters, there is a higher risk that the ROV gets stuck behind rocks, mooring lines or other vehicles. In addition, the tether of the ROV is 200m long (this could be customized), meaning that depending on how wide the shallow terrace is, there is not enough cable left for the ROV to descend into the deeper waters. This is what happened during the transect from shore (B12, Harbour Village), where there was not enough cable left to descend deeper than 40m depth.

Lastly, the signal is disturbed by reflections from the bottom, so in case of shallow water, the antenna has more difficulty to receive a clean signal from the locator. How this exactly works is as follows: when the ROV is close to the bottom, the sound signals of the locator reverberate against the hard corals and interfere with the signal, causing inaccurate positioning of the ROV. The locator can be switched to other frequency channels in order to overcome this issue, but in our trials this did not solve the problem.

Most of the above pitfalls are covered by deploying the ROV from the boat, while simultaneously using the kayak to improve the determination of the GPS position. Another advantage of the kayak is that it can be used to mark the area where the ROV is diving and where the tether is drifting.

Armstrong and colleagues (2019) also mentioned that the particular concerns with ROVs are related to the management of the tether, to avoid entanglement and that the motions of the surface vessel can disturb the ROVs movements. With regard to the cable management and the risk of entanglement, Farr et al. (2010) refer to the use of optical modems, offering untethered high-bandwidth connections.

4.1.4 GPS-system

The topside unit determines the orientation, after a calibration, based on a gyroscope. This however turned out to be inaccurate, due to the fact that this drifts a number of degrees per hour. In addition, the topside unit had to be perfectly aligned with the antenna, which did not always worked out perfectly on a moving boat or kayak.

The six degrees of freedom of the ROV are necessary for georeferenced sensor data and due to the use of various instruments, the depth (vertical axis Z), vehicle velocities and orientation can be estimated. However, the determination of the horizontal position (XY) remains challenging, due to the inability of GPS signals to travel through water (Mücher et al., 2017). Typically, acoustic tracking systems are used as a solution, where the robotic platform is placed relative to a surface vessel, or to a network of beacons. These observations can be noisy (Armstrong et al., 2019), as also encountered within this study.

To overcome this, adjustments have been made to provide more accurate positions of the ROV. An external compass has been coupled to the antenna and topside unit and is being read out by an external raspberry pi. This external compass gives the absolute position and the orientation. This information is being passed on to the topside unit, which also receives the relative position (locator on ROV to antenna) and does the translation of absolute position (external compass) + orientation (external compass) + relative position (ROV) to an absolute position of the ROV.

A combination of acoustic position observations with precise and high-frequency velocity observations from a Doppler velocity log (DVL) could also improve the set-up. Kinsey and colleagues (2006) and Yoerger and colleagues (2007) provide a comprehensive discussion and application of the above mentioned technique. Thrun and Leonard (2008) mention simultaneous localization and mapping (SLAM) to handle drift in navigation sensors and improve the trajectory estimation.

To conclude, developments in underwater robotics, deep learning and online annotation and classification tools, such as Coralnet.org, enable the automation of image classification for scientific purposes and allow for analyses of much greater data volumes (Armstrong et al., 2019). The future of MCEs research seems promising; multiple semi disposable imaging floats could characterize MCEs at relatively low cost (Jaffe et al., 2017;), but also the increase in operation time of long-endurance AUVs (capable of operating for weeks), reduces operation costs (Furlong et al., 2012; Armstrong et al., 2019). In addition, arms and tailored manipulators will increasingly be used for sample collection, experiments and close range observations at greater depths (Armstrong et al., 2019).

4.2 Occurrence of MCEs

According to our data, where all sites were averaged and the benthic groups were analysed per depth range (Fig.13), hard coral (0.36%), CCA (0.07%), macroalgae (ma) (17%), soft corals (<0.01%) and sponges (0.02%) were still found at depths of 40m, representing MCEs. These findings are in line with the papers by Trembanis et al., (2019) and Becking & Meesters (2014), where they found that hard coral, soft corals and macroalgae still occurred at depths beyond 40-50m. At 60m, an extreme increase in cyanobacteria (mostly BCM) was observed (18.1%), hard coral cover decreased here to 0.03%, CCA showed a slight increase to 0.13%, macroalgae decreased to only 0.11%, soft corals disappeared from the pictures and sponges covered on average only 0.017% of the ocean floor.

As mentioned in the Results section, all sampling sites show presence of MCEs, except for Salt Pier (B04) and Kralendijk (B11). The Bonaire Deep Reef Expedition I (2013), described by Becking and Meesters (2014) investigated three sites along the leeward site of Bonaire, among which the Kralendijk pier (12.14692, -68.2821) and the Cargill pier (12.07996, -68.2938), where they did find live hard corals at Kralendijk till a depth of 44m and at Salt Pier till a depth of 30m and between 40-45m (Becking and Meesters, 2014). These different outcomes could be attributed to the fact that the research was conducted both at a different time as well as on a slightly different location. For the latter goes that much of the deeper ocean floor is dominated by sediment, with only patchy occurrences of MCEs reaching high percentages of coral cover locally (Trembanis et al., 2019; Frade et al., 2019). Frade et al., (2019) even state that in some locations coral assemblages can cover up to 100% of the seafloor down to depths of 70-85m, whereas other areas are solely covered by sand.

4.2.1 Improvements

Within this study, surveys have been taken till a depth of 60m, because in general after this depth a sandy plain with mostly black corals was present. Thus, surveying beyond the depth of 60m would not have resulted in meaningful data with regard to the occurrence and status of MCEs. At every site, whenever possible, the ROV descended beyond 60m, till about 80m, to visually check for MCE occurrence. Within this research, transects were taken at depth ranges between 37.5-42.5 (40m) and 57.5-62.5m (60m), gathering data about hard coral occurrence at these depth ranges and thereby excluding the depths in between (42.5-57.5m). This makes it hard to say till what maximum depths MCEs occurred per site. For future research it would be recommended to take more transects at the upper-mesophotic depths and/or visually explore till what maximum depth hard corals still occur.

4.2.2 Bioindicator

Data on the maximum depth of coral communities has been used as a bioindicator to infer changes in water quality on coral reefs (van Woesik et al. 1999; Cooper et al. 2009). The available irradiance determines coral growth and distribution (Yentsch et al., 2002; Cooper et al., 2009) and the maximum depth of coral reef development marks the transition zone from zooxanthellate hard corals to azooxanthellate filter feeders along a depth gradient (Cooper et al., 2009). An example comes from the Whitsunday region of the Great Barrier Reef, where the maximum depth of hard coral occurrence increased from 5m at coastal reefs with low irradiance and elevated nutrients, to 25m at offshore reefs with high irradiance and low nutrients at outer locations (Cooper et al., 2007). As changes in maximum depth of hard coral occurrence occur on a timescale of months to years, this approach is more suitable for monitoring over longer time periods (Cooper et al., 2009).

Due to the patchy distribution of the MCEs along the coast of Bonaire, it could be recommended to first explore the area by means of geoacoustic surveys, to identify the presence of MCEs and then explore them in more detail by means of an ROV. Trembanis and colleagues (2019) fused remote sensing information on morphology and class type, providing a quantitative way to identify areas of interest for follow-up research.

4.3 Status of MCEs

To assess the status of the coral reefs and the upper MCEs, this chapter discusses the effect of depth, human impact and water quality risk on benthic cover, species richness, diversity and species composition.

Within this study trash was observed among all depths at four sites, and other studies using submersibles in Bonaire and Curaçao reported relatively large amounts of human debris present on MCEs (Becking and Meesters 2014; Debrot et al. 2014; Bongaerts et al. 2015b; Hoeksema et al. 2017b; Frade et al., 2019). The upper MCEs (<60m) of Bonaire and Curaçao lie within the Marine Protected Areas (MPAs) and therefor experience some form of protection. This is not the case for the lower MCEs, which should be protected as well, to safeguard their structural complexity and associated biodiversity, including the ecological and economic services they provide (Frade et al., 2019). According to Trembanis et al. (2017), over half of all the observed reef structures occurred outside these designated MPAs.

4.3.1 Coral Cover, Diversity & Richness

The relationship between coral cover and depth showed a negative correlation, indicating that coral cover decreases with increasing depth, especially for mesophotic depths, coinciding with Rivero-Calle et al. (2008) and Frade et al., 2019. This is especially true for coral cover beyond 20-30m depth, as in the shallower water coral cover first increases from 5m towards 20m. As described by Frade et al., (2019), coral cover as well as diversity increases from the high-water mark toward the first drop-off. According to De Bakker et al., (2016), the highest diversity occurred at 20m depth in 1973 and 2014. The relatively lower diversity and coral cover at 5 and 10m depth, compared to 20m, is likely the consequence of higher natural and anthropogenic disturbances having larger impact on the shallow reefs (Frade et al., 2019; Bak, 1977).

Many zooxanthellate scleractinian corals occurring at mesophotic depths, have a plate-shape morphology or change shape with depth in order to increase the surface area for light capture (Lesser et al., 2009). The mounding coral, *M. cavernosa* is an example of a coral that develops such a flattened, plate-like morphology with depth (Lesser et al. 2009; Frade et al., 2019). The ability of coral species to photoacclimatize to low irradiances at depths below 30m can be attributed to the organization of the photosynthetic apparatus in their symbiotic zooxanthellae (Wyman, 1987; Falkowski et al., 1990; Lesser, 2000; Lesser et al., 2009).

The slope angle of the reefs is likely to play a role in the benthic community composition, in relation to sediment accumulation and solar irradiance (Scott et al., 2019). With an increase in depth, the angle of inclination decreases, resulting in an accumulation of sediments. Both coral cover and diversity decrease at mesophotic depths, where most of the surveyed substrate is covered by sediment, this was also observed by Bongaerts et al. (2013, 2015b), which probably limits the formation of MCEs (Bak 1977). Corals growing on steep vertical walls, despite the fact that they have a flattened surface to increase the light uptake, receive 25% less irradiance at any particular depth (Falkowski et al., 1990; Lesser et al., 2009). A disadvantage of this plate-shape morphology is that it makes the corals susceptible to sedimentation and prone to direct physical disturbances (Bongaerts et al. 2015b; Hoeksema et al. 2017b; Frade et al., 2019). Cold-water bleaching and sedimentation are among the potential major causes of coral mortality, disrupting space monopolization of the mesophotic reef by *agariciids* (Bak et al., 2005). A study by Bongaerts et al. (2015b) showed that growth rates of lower mesophotic *agariciid* communities have shown some potential recovery following damage, however, also these communities are impacted by anthropogenic activities.

4.3.1.1 Human impact

As depth and impact interact with each other, it would be wrong to solely look into the significant depth-effect on coral cover. The fact that coral cover first increases towards 20m, might be explained by the larger extent of human impact on the shallower reefs. Many coastal reefs around the world have suffered from increased levels of sedimentation from coastal erosion (Fabricius, 2005; Rogers, 1990). Coastal development and artificial beaches are causes of sedimentation resulting in chronic stress to corals, whereas acute stresses such as sediment “waterfalls” also affect corals at mesophotic depths (Bongaeats et al., 2015b; Waitt Institute 2017). The photosynthetic yields in corals is reduced at higher (<100mg cm^{-2}) sedimentation levels (Philip and Fabricius, 2003) and the metabolic costs increase due to the removal of settled particles from the surface of the coral (Telesnicki and Goldberg, 1995; Fabricius, 2005). Declining mean colony size, altered size frequencies and growth forms, and reduced growth and survival rates are associated with sedimentation stress (Fabricius, 2005).

With regard to Bonaire, Bakker et al. (2016) state that anthropogenic disturbances nowadays reach mesophotic depths and that reef degradation was less evident further away from urban areas. Van der Geest and colleagues (2020) assessed the link between watershed-specific erosion hazard and coral reef health on Bonaire and found a negative relationship between mean erosion hazard and coral cover at 5m depth, but no such effect at 10m depth. This suggests that the shallow reef is more prone to the impact of land-based run-off compared to the upper drop-off zone (10m depth) as the upper drop-off zone is further away from the terrestrial run-off point. Another explaining factor could be the stronger water currents at this depth, causing more water mixing and thus dilution of terrestrial run-off and nutrients (Van der Geest et al., 2020).

A clear decreasing trend in coral cover towards the more human impacted zones was observed within this study (Fig.15,18). Sedimentation stress in coral colonies increases linearly with the amount of sedimentation and the duration. The damage done to the coral then also depends on the sediment type, but varies among coral species. This has as a consequence, a decrease in biodiversity, as the sensitive species disappear due to higher sedimentation stress and the more tolerant species (such as *Porites* spp.) become more abundant (Fabricius, 2005). This is also what was observed within this study; diversity shows an increasing trend towards the least human impacted zones, a less pronounced trend is visible with regard to the water quality risk zones, this however varies greatly with depth range. Overall diversity shows lower values for larger human impact, water quality risk and larger depths.

Species with thin tissues and flat surfaces or small colonies appear to be more sensitive to sedimentation compared to corals with branching growth forms or thick tissues or large colonies. There are species (such as *P. astreoides and M. Cavernosa)* with thick tissues that are able to remove particles from their surfaces by ciliary movement (as found in Fungia), mucus production and tissue extension, which makes them more resilient and tolerant to sedimentation (Lasker, 1980; Rogers, 1990; Stafford-Smith and Ormond, 1992; Fabricius, 2005).

4.3.1.2 Water quality

With regard to the water quality risk zones, coral cover is significantly higher in the low- and no-risk zones, compared to the high- and moderate-risk zones (Fig. 19). It is suggested that coral growth (calcification) declines gradually with increasing dissolved inorganic nutrient availability (Fabricius, 2005). In upwelling regions calcification can be reduced by 50%, caused by the elevated nutrients as well as the colder temperatures (Kinsey and Davies, 1979; Fabricius, 2005). Upwelling occurs along the coast of Venezuela and affects Bonaire, primarily between December and April due to the increase in trade winds, as well as mid-year upwelling around June-August (Frade et al., 2019). Contradictory, upwelling and internal waves supply nutrients, enabling coral growth in these low light conditions due to the use of mixotrophic strategy (shift from autotrophic to heterotrophy for carbon requirements (Muscatine et al., 1989)) of corals (Leichter and Genovese, 2006).

The overall consequences of terrestrial run-off and elevated levels of DIN on coral reefs are shallower photosynthetic compensation points, reduced reef calcification, changed community structure and largely reduced species richness (Fabricius, 2005). This has as a result that with increasing exposure to terrestrial run-off, reef ecosystems simplify in order to keep their essential ecosystem functions while coping with the increasing local and global stresses (Fabricius, 2005). Generally, species richness showed lower values for the upper-mesophotic reef compared to the shallower reef and insignificant higher mean values for the marginally impacted sites and the sites with low- to no-risk. According to Duprey et al., (2016), eutrophication (measured as chlorophyll-a concentration) is negatively correlated with species richness and coral cover, this is also being measured within the overarching project and will be analysed in a later stadium. They also found that particulate suspended matter, DIN, and dissolved inorganic phosphorus (DIP) had a negative effect on species richness and coral cover, with the effect of nutrients being 1.5-2 times larger. As heavy metals, pesticides, hydrocarbons and other human-made pollutants can also significantly affect the status of coral reefs at local scales (Guzman and Holst, 1993), it is recommended to include such measurements as well in follow-up research.

4.3.2 Benthic cover

So far the effects of changes in the vertical (depth) and horizontal (human impact and water quality) gradient have been discussed for coral cover, diversity and richness. These are not the only proxies for the status of coral reefs, environmental gradients impact the abundances of the other benthic groups, which on their turn affect the health and abundance of corals directly or indirectly (Fabricius, 2005). The effects of those gradients on other benthic groups is discussed below.

4.3.2.1 Coral settlement - Crustose Coralline Algae

The substratum availability, thus the absence of sand and the presence of crustose coralline algae, is essential for coral settlement (Fabricius, 2005). Sand does not offer a hard substrate which corals need to settle on and generally shows an opposite trend with coral cover (Fig.20 and 21). Mücher and colleagues (2017) compared the amount of sand with the underwater atlas of Bonaire and Curaçao (van Duyl, 1985) and observed that sand has increased largely since the early eighties, replacing hard corals. Over thirty years the area of sand increased with 58%. The Northern region, the least impacted by anthropogenic activities, showed an increase by a factor 10. With regard to Klein Bonaire, the situation had improved concerning the area covered by sand.

CCA has been low throughout all transects, but did show a slight increasing pattern with an increase in depth and a decrease in human impact and water quality risk (Fig.14,15,16). The low cover of CCA is in line with findings from De Bakker and colleagues (2017), where they indicated a decrease in CCA from 6.4 to 1% over the period of 1973 to 2014 across all sites and depths (10,20,30 and 40m). There are experiments and field data suggesting that sedimentation is an important factor in CCA cover, stating that CCA is negatively related to sedimentation (Kendrick, 1991; Fabricius, 2005). Data from the Great Barrier Reef showed that CCA cover decreased from 30% in some low sedimentation habitats to 1% at areas with high sedimentation (Fabricius and Death, 2001b).

CCA interact with turf algae, as these trap sediments (Purcell, 2000) and smother and replace CCA (Steneck, 1997). Simultaneously turf makes the area less suitable for coral settlement (Fabricius, 2005), which complicates the response of CCA to sedimentation. The effect of light-availability on CCA cover is species-specific, resulting in high-irradiance species being replaced by low-irradiance species as light availability decreases. This could explain the results that CCA does increase with depth, where human impact and thus sedimentation is less pronounced, as long as the reef slope is steep enough to prevent sediment accumulation. Enrichment with dissolved inorganic nutrients did not affect CCA or turf algae in field experiments (Koop et al., 2001; Fabricius, 2005).

4.3.2.2 Competitive interactions – Macroalgae & turf algae

Macroalgae and turf algae have a negative effect on corals as they slow down coral growth, make corals less resilient to diseases and constrain recruitment and survival (Jackson et al., 2014; Fabricius, 2005; D'eath & Fabricius, 2010; Duprey et al., 2016). A shift from calcifying organisms, i.e., hard corals and CCA, to turf algae and macroalgae has been observed in the period 1973 to 1990 by De Bakker and colleagues (2017) for Curacao and Bonaire. Algal turf increased from 24.5 to 38% and fleshy macroalgae were still absent in 1973 but covered 12% of the substratum in 1990. Macroalgae and algal turfs benefit from eutrophication (Vermeij et al. 2010; Bakker et al., 2017; Cooper et al., 2009).

Our results show that turf algae cover decreased over the full depth range (Fig.14), similar as to what Scott and colleagues (2019) observed at Puerto Rico. Turf algae decreased towards lower water quality risk, and it varied around similar mean values with regard to human impact (Fig.15,16). Similar to findings of Sandin et al., (2008) turf algae were the most abundant benthic type (Fig.13). Turf has become the dominant cover on many coral reefs worldwide, due to their opportunistic life-history characteristics enabling them to rapidly occupy substratum (Sandin et al., 2008; Bakker et al., 2017). Turf algae consists of many species, including cyanobacteria, and can undergo successional patterns (Connell et al., 2014). Depending on local and global conditions, they can be supplanted by macroalgae or BCM. Elevated water temperature, high grazing pressure and reduced water quality stimulate the growth of BCM over macroalgae (Bender et al., 2014).

Macroalgae showed a slight increase towards less human impact, and towards lower water quality risk levels (Fig.15,16). With regard to depth, macroalgae cover first increased towards 40m after which it became almost absent again (Fig.14). Without grazing control, slight increases in dissolved inorganic nutrients and POM lead to an increase in the growth and productivity of certain groups of macroalgae (Fabricius, 2005). Van der Geest et al. (2020) found a small but significant quadratic effect of mean erosion hazard on algae cover at the shallow reef (5m), which had a negative effect on macroalgae cover, likely caused by the decreased light availability. Lobophora spp. is the dominant macroalgae on the MCEs of Bonaire and has been increasing over the last decades, overgrowing corals (De Bakker et al., 2017; Frade et al., 2019). The salt industry is causing high concentrations of ammonium (NH_4^+) in the coastal waters of Bonaire and *Lobophora variegata* has a high NH_4^+ uptake capacity, this could potentially explain the dominance of *Lobophora* on the upper MCEs (Vermeij 2011; Lapointe and Malin 2011; Slijkerman et al., 2014) .

The macroalgae *Dictyota* spp. interact with BCM, as the groups overgrow each other; BCM seemed to stabilize the sandy substratum and thereby facilitated growth of *Dictyota* spp., but was also observed to smother and overgrow *Dictyota* spp. (De Bakker et al., 2017).

4.3.2.3 Harmful bacteria - Cyanobacteria

Cyanobacteria are considered harmful bacteria, as they inhibit coral recruitment, produce chemicals that are toxic for coral and fish, overgrow and smother reef benthos, act as pathogens and are often a sign of nutrient enrichment (Cooper et al., 2009; Kuffner et al., 2006; de Bakker et al., 2017; Fabricius, 2005; Becking & Meesters, 2014). Cyanobacteria use oxygen during the night, and create anoxic layers where only some organisms are able to survive (Becking & Meesters, 2014).

As described in the Results section, and in the paper by Becking and Meesters (2014), fields of BCM were observed around 40-90m depth. Cyanobacteria show an increase in occurrence with increasing depth (Fig.14). There were also higher abundances of cyanobacteria at the marginally impacted site (B16, Karpata), when it comes down to water quality risk, the cover stayed more or less the same among all risk level zones (Fig.15,16).

In general, cyanobacteria are in favour of nitrogen rich environments, the occurrence of cyanobacteria thus suggests for a source of nitrogen. Around Bonaire this could be the down-welling of heavy nutrient rich water from the island itself, or upwelling of cold and nutrient rich water from the deeper parts of the ocean (Becking & Meesters, 2014). A decline in water quality and increased water temperature could be plausible causes for the shift to BCM on the reefs (Brocke et al., 2015a; Bakker et al., 2017), as the local sewage systems are overall not working properly, untreated sewage water reaches the coral reefs via discharge and/or groundwater (Lapointe and Mallin 2011; Bakker et al., 2017). Another reason for the rise of BCM is that they are not so tasty for herbivores due to their production of nitrogenous secondary metabolites (Thacker et al., 1997; Bakker et al., 2017).

With regard to sponges and soft corals, this cover has been overall low according to our data and there were no patterns observed.

4.3.3 Species composition

At mesophotic depths, the most common corals are *Agaricia* spp. and *M. cavernosa* (Fig.24), which is supported by literature on other sites in the Caribbean (Rivero-Calle et al., 2008; Lesser et al., 2009) and on the deeper reefs of Curaçao and Bonaire (Frade et al., 2019; Bak et al., 2005; Sandin et al., 2008; Van Duyl, 1985). *Agariciids* are typically dominating the upper MCEs due to their efficiency at capturing light in low-light environments (Frade et al., 2019). Other species that occur both in the shallows as well as on the upper MCE and down to 80m depth are *Stephanoceoenia intersepta*, and *Madracis* spp. (Bak 1975; Bongaerts et al. 2013), these could be considered depth generalists (Bak, 1977; Bongaerts et al., 2013). Communities occurring below 60m depth consist mainly of deep-water specialists, like *A. undata* and *A. Grahamae* (Bongaerts et al., 2010). *Scolymia cubensis (*identified only once within this study*)* and *Madracis Formosa* (not identified within this study) are deep water specialists present, but never dominant, in the upper and lower mesophotic reef (Bak 1977; Bongaerts et al., 2010).
The shallow-water coral community is dominated by *M. auretenra*, *O. annularis, O. faveolata* and *A. agaricites* (Fig.24), which is exactly in line with other papers on the reefs of Bonaire (Bak 1975; Bak 1977; Waitt Institute 2017; Frade et al., 2019).

Within this study, 27 scleractinian species were identified in contrast to the study by Bak in 1977 where they identified 50 hard coral species. Bak & Niewland (1995) observed that there is a consistent decrease in species richness over the time interval of 1972-1992. Currently 65 hard coral species are recognized for Bonaire and Curaçao (IUCN 2011; Frade et al., 2019), in the upper MCEs 25 species were found and this number decreased down to 7 species at 80-90 m depth (Bongaerts et al. 2015a, b; Vermeij and Bak 2003; Frade et al., 2019).

With depth, the photosynthetically active radiation (PAR) decreases exponentially at an attenuation coefficient (Kd) of 0.06-0.07 m^{-1} (Vermeij and Bak, 2002; Frade et al., 2008b). This results in a light irradiance of ~0.18-0.45% of surface irradiance at 90m depth, which corresponds to the maximum depth at which zooxanthellate corals are still observed on the reefs of Curaçao (Bongaerts et al., 2015b). As mentioned before, the lower limit of MCEs is not only determined by light, but also by other environmental factors such as sedimentation, substrate and nutrient availability and temperature (Bak 1977; Frade et al., 2019).

With regard to temperature, upwelling along the coast of Venezuela is causing striking differences in temperature along 10 to 60m depth throughout the year (Frade et al., 2019; Rueda-Roa, 2012). In spring (April-June) there are generally small differences in temperature between the upper mesophotic region and the shallow reefs, but during the warmer half of the year (from August onward), the warming of the surface water results in stratification of the water column. A steep depth-temperature gradient during late summer (October) is the result of this, with more than 8 degrees difference between surface waters and the lower mesophotic. Temperatures decrease again in winter (December-April), coinciding with the upwelling season in the southern Caribbean, due to the strengthening of the trade winds (Varela et al., 2015; Frade et al., 2019; Rueda-Roa, 2012). The cold-water fluxes accompanying this extend to upper mesophotic depths (40m) and are described as breaking internal waves (Leichter et al., 2006; Frade et al., 2019). Temperature drops of 1-3°C every 10-30 min can have negative effects on the MCEs, as such low temperatures (~22°C) have been linked to deep water bleaching of *Agaricia* in Curaçao (Bak et al., 2005; Nugues and Bak 2008; Frade et al., 2019) and Bonaire (Kobluk and Lysenko 1994), resulting in mortality of *Agaricia* colonies. These results underline the role of temperature in shaping mesophotic reef communities (Frade et al., 2019).

Inland pollution and soil erosion, resulting in sedimentation, are large threats to coral reefs around the world, especially in countries with widespread land clearing (Fabricius, 2005). Models of global scale of pollution around coral reefs estimated that 30% of reefs are threatened from coastal development (distance to cities, mines and resorts), and 12% at threat from marine pollution (proximity to ports, oil wells; Bryant et al., 1998). Marine pollution is therefore of a similar threat to coral reef health as bleaching, overfishing and destructive fishing, on a global scale (Frabricius, 2005). The problem of pollution however can be addressed on local scales. In addition, several studies mention that excess nutrients negatively affect thermal tolerance and thus bleaching resistance (Cunning & Baker, 2012; Wiedenmann et al., 2012; Duprey et al., 2016), again underlining the importance of limiting local stressors in order to amplify the impact of global stressors on coral reefs.

Our results confirm that human impact and water quality affect the status of the coral reefs. As mentioned by De Bakker et al., (2016), the highly impacted region in front of Kralendijk is an extreme example of this, showing a near-complete functional collapse of the reef system in this area, especially on the lower terrace. These reefs are supposed to contribute to diminishing wave energy, especially in the anticipation of rising sea level predictions, as the southern shore of Bonaire, including Kralendijk, only rises 1m or less above mean sea level (Mücher et al., 2017; Van Duyl, 1985). This underlines how local adaptations, that conserve and restore coral reefs, can improve an area's resilience to global stresses. The results obtained within this study confirm that the understanding of the heterogeneity in local conditions is important for implementation of local conservation and management (Knowlton & Jackson, 2008). The extension of proper sewage treatment and run-off prevention are likely to benefit the general health and survival of coral reef communities (De Bakker et al., 2016). In addition, more awareness on the vulnerability of these pristine ecosystems in combination with recreational regulations would also be recommended, as tourists, which are largely divers, have a local impact on the reef as well (Lamb et al., 2014).

4.4 Data comparison: ROV & Diving

The 8 monitored sites along the leeward coast of Bonaire resulted in an average coral cover of 17.65% for 5-10m depth, which is in line with the estimate of De Bakker et al., (2019) of 19% coral cover measured over 115 sites and the results of Sommer et al., (2011), who found as estimate of 9.5% for 5m depth and 19.7% for 10m depth, measured over 12 sites.

For both depth ranges (5 and 10 m), the means of the two methods do not significantly differ from each other (P>.05, table 2), meaning that both collection methods show comparable results, despite the fact that there is 2 years in between the data collection. These findings correspond to the paper by Lam and colleagues (2005), where they found that there was no significant difference between the by ROV and Diver collected data. Also Armstrong et al. (2019) mention that imaging obtained with ROV research is at extents and resolution similar to pictures taken by divers.

In order to compare the results obtained by diving and the ROV, it would have been best if all settings were similar to each other. Unfortunately the execution of the surveys differed from each other and the sites that are being compared are approximately similar to each other, but the exact transect locations might differ in the order of tens of meters from each other. These facts should be taken into account when comparing these two datasets.

A drawback of deploying an ROV can be the costs that come along with the investment and maintenance. However, as described in 4.1 Technical approach ROV, the developments within this field allow for a decrease in costs and also the operational costs are expected to decrease. To deploy an ROV, some practice with how to use a controller might be required, but apart from that, compared to Scuba-diving data collection, there is no need for diving certificates and as the pictures can be analysed by experienced marine biologists, the ROV pilot does not need to have expertise in the identification of marine life (Hill and Wilkinson, 2004; Lam et al., 2005).

Something that both scuba-diving and ROV data collection have in common when collecting pictures of the benthic cover to analyze later, is that permanent visual records are available for more information. This footage could for example be used to show the status of the reefs to policy makers and develop outreach products to increase awareness.

For follow-up research, it is recommended to increase the statistical power of the transects, first of all in the field, by collecting more suitable photographs per transect and thus by increasing the number of frames analysed, or by increasing the number of points analysed per frame. Increasing the number of frames (n) analysed will be more effective (Hill and Wilkinson, 2004), as this decreases the minimum detectable change *(δ).* Monitoring programs have as major aim to detect temporal change, thus a method with a higher chance to detect such changes, i.e., producing a low δ from two datasets obtained at different times, is preferred (Lam et al., 2005).

Whether ROV or Scuba-diving based data collection would be preferred depends on what kind of data needs to be collected, but also on the conditions of the site, such as the currents underwater and weather conditions (as it's easier for divers to swim against a current than it currently is for some ROVs). In case of an ROV, where power is supplied via the tether, the ROV has as main advantage that there is no time limit for how long the vehicle can stay submerged, resulting in a wider horizontal and vertical range. In case of unfavourable diving conditions, like currents, limited visibility and adverse weather, Lam and colleagues (2005) argue that an ROV would be more useful. In terms of diver safety, this is true, but the ROV deployed in this study would not have been happy with unfavourable diving conditions either. Other models of ROVs might be more suitable for this. As encountered in this study and appointed by Lam et al. (2005) Scuba divers can attain better balance in high current areas, to stabilize the pictures and swim along the required transect lines. Also dives that are possible from shore are more suitable for scuba-diving, which saves on the use of a vessel (Lam et al., 2005).

Mücher et al. (2017) state that new monitoring programs should not solely be based on data gathered by means of diving, as this is too time-consuming and the delineation of classes is not always accurate (if this is done during the dive instead of afterwards). Simultaneously, airborne imagery with high spatial resolution does not function as alternative as it has limited amount of benthic habitat classes, individual species cannot be detected and it is bound to the first 20m (Rivero-Calle et al., 2008). Therefore, a combination of multispectral imagery corrected by a bathymetric model and in-situ data would provide for a future coral reef classification. They also mentioned that the in-situ measurements should be geotagged, but that it is challenging to have accurate GPS coordinates under water, although underwater GPS positioning systems are available. Our experiences with such a GPS positioning system were discussed in 314.1.4 GPS-system.

4.5 Methodological considerations

First of all, it is important to mention that this study is conducted within the first phase of the project and performed the first trial in deploying an ROV in coral reef monitoring. Therefore, the data obtained within this study is very experimental and due to shortcomings and errors observed within this study, the interpretation of the results should be done conservatively. The relatively small sample size and the fact that at KB4 and B12 data is missing at respectively 5 and 60m depth, are examples of this. Within the overarching project, data collection and improvements regarding the technical set-up are ongoing and this study may serve as a baseline for coral reef monitoring with an ROV within this project and beyond.

As can be observed in chapter 3 Results, the data shows a high variance, addressing the need for a larger sample size. In addition, the altitude of the ROV above the bottom should be smaller and more consistent along the transect, resulting in higher resolution and better quality imagery. For example, Lam and colleagues (2005) deployed an ROV for coral reef monitoring, where they monitored five 50 m transects, at 0.4m above the substratum. Each obtained frame was equivalent to an area of 0.6x0.44 m, i.e., ~0.26m^2 on the seabed. This is expected to result in higher resolution imagery, which in turn is likely to result in more accurate genus and species identification and a lower number of unknown points. With regard to the human impact zones (defined by De Bakker et al., 2019), it would be recommended that more sites are monitored within each zone, for a larger sample size and a more balanced comparison, as the current dataset only consisted of 1 site per impact zone, and 6 sites within another impact zone.

5 Conclusion

This study describes the technical set-up of the use of an ROV in coral reef monitoring on the shallow (5 - 20m) and upper-mesophotic (40- 60m) reefs at 8 sites along the leeward coast of Bonaire. These 8 sites have been subdivided into different zones, showing a gradient in human impact and water quality, and have been analyzed in relation to vertical and horizontal gradients.

The mesophotic coral ecosystems (MCE) are relatively unexplored compared to the shallow reefs, as MCEs range from 30 to 150m in depth, which coincides with the Scuba diving limit. The rapid developments within underwater robotics have made the use of remotely operated vehicles (ROVs) accessible for deployment in for example coral reef surveys. This study showed that the imagery obtained by the ROV is of adequate quality, allowing for hard coral identification to genus level if not species level, and showed comparable results in estimated percentage coral cover with other recent studies. To improve the data collection by the ROV, it is recommended that the ROV preserves at a fixed and smaller altitude (0.5-1m) above the ocean floor. In addition, it is suggested to collect more pictures per transect, to increase the sample size. The accurate determination of the coordinates of the ROV remained challenging. The addition of a kayak and an external compass to the set-up slightly improved the GPS positions.

Generally, around 30-45m the reef slope levels off into a sand plane, often colonized by garden eels and from about 40m covered by benthic cyanobacterial mats. Down to about 80-90m, mainly sand and gorgonians were present on the ocean floor. Trash was observed among all depths at BOPEC (B17) and Kralendijk (B09, B11, B12). All sites, except for Salt Pier (B04) and Kralendijk (B11), showed the presence of hard coral, crustose coralline algae (CCA), macroalgae, soft corals and sponges at 40m depth, indicating the occurrence of MCEs. Only at Karpata (B16) also hard coral cover was observed at 60m depth. This site generally showed the highest percentage of coral cover and was determined to be the furthest away from human impact and also being indicated to have no-risk with regard to water quality. As MCEs appear to occur in patchy distributions, these results are very location specific and it can very well be that at a certain distance from our sampling locations MCEs do occur at and even beyond 40m.

Environmental factors affecting the benthic communities, such as light availability, temperature, sedimentation, nutrient availability and wave energy change along the depth gradient and also the effect of human impact and water quality on the status of the reef interact with the depth-effect. Overall, coral cover was negatively correlated with increasing depth and showed an increase in cover with decreasing human impact and decreasing risk in water quality. Significant differences in coral cover were observed between the extremely impacted zone and the marginal impacted zone. Also, CCA and macroalgae showed a slight increase in cover towards the least human-impacted sites, whereas sand cover decreased in this direction. Trends in benthic cover related to human impact were observed, but to make stronger statements about the effect of human impact and water quality, a larger sample size and more quantitative data are necessary.

Our results contribute to findings by other researchers that the status of the reef is affected by both the vertical and horizontal gradients. Anthropogenic activities reach mesophotic depths affecting the MCEs, addressing the need for local conservation measures to increase resilience to global stresses. The rapid development in machine learning, computer vision and robotics enable sophisticated and more in-depth (literally!) exploration, mapping, monitoring and research of these interconnected systems.

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7 References

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*(1), 32-46.

Armstrong, R. A., Pizarro, O., & Roman, C. (2019). Underwater robotic technology for imaging mesophotic coral ecosystems. In *Mesophotic Coral Ecosystems* (pp. 973-988). Springer, Cham.

Auster, P.J., Tusting, R.F., 1997. Scientific sampling systems for underwater vehicles. Mar. Technol. 31, 3–4.

Bak, R. P. (1975). Ecological aspects of the distribution of reef corals in the Netherlands Antilles. *Bijdragen tot de Dierkunde*, *45*(2), 181-190.

Bak, R. P. (1977). Coral reefs and their zonation in Netherlands Antilles: modern and ancient reefs.

Bak, R. P. M., & Nieuwland, G. (1995). Long-term change in coral communities along depth gradients over leeward reefs in the Netherlands Antilles. *Bulletin of Marine Science*, *56*(2), 609-619.

Bak, R. P., Nieuwland, G., & Meesters, E. H. (2005). Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curacao and Bonaire. *Coral reefs*, *24*(3), 475-479.

Becking, L. E., Cleary, D. F. R., de Voogd, N. J., Renema, W., de Beer, M., van Soest R. W. M., et al. (2006). Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Marine Ecology 27: 76-88

Becking, L. E., & Meesters, H. W. G. (2014). *Bonaire deep reef expedition I* (No. C006/14). IMARES.

Bender D, Diaz-Pulido G, Dove S (2014) Warming and acidification promote cyanobacterial dominance in turf algal assemblages. Mar Ecol Prog Ser 517:271–284

Bingham B, Foley B, Singh H, Camill R, Delaporta K, Eustice R, Mallios A, Mindell D, Roman C, Sakellariou D (2010) Robotic tools

Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. Coral Reefs 29:309–327

Bongaerts P, Frade PR, Hay KB, Englebert N, Latijnhouwers KR, Bak RP, Vermeij MJ, Hoegh-Guldberg O (2015b) Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coralendosymbiont community. Sci Rep 5:7652

Bongaerts P, Frade PR, Ogier JJ, Hay KB, van Bleijswijk J, Englebert N, Vermeij MJA, Bak RPM, Visser PM, Hoegh-Guldberg O (2013) Sharing the slope: depth partitioning of agariciid corals and associated Symbiodinium across shallow and mesophotic habitats (2–60 m) on a Caribbean reef. BMC Evol Biol 13:205

Brocke HJ, Polerecky L, De Beer D, Weber M, Claudet J, Nugues MM (2015a) Organic matter degradation drives benthic cyanobacterial mat abundance on Caribbean coral reefs. PLoS One 10:e0125445

Bryant, D.G., Burke, L., McManus, J., Spalding, M., 1998. Reefs at Risk: a Map-based Indicator of Threats to the Worlds Coral Reefs. World Resources Institute, Washington, DC

Connell SD, Foster MS, Airoldi L (2014) What are algal turfs? Towards a better description of turfs. Mar Ecol Prog Ser 495:299–307

Cook, S.B., Krumm, K.K., 1996. From submersibles to seagrass: educational programmes at Harbor Branch Oceanographic Institution. In: Oceans '96 MTS/IEEE Conference Committee (Ed.), Prospects for the 21st Century, OCEANS '96 MTS/IEEE Conference Proceedings. The Marine Technology Society and the Institute of Electrical and Electronics Engineer and Oceanic Engineering Society, Washington, D.C., pp. 1412–1413.

Cooper TF, Uthicke S, Humphrey C, Fabricius KE (2007). Gradients in water column nutrients, sediment parameters, irradiance and coral reef development in the Whitsunday Region, central Great Barrier Reef. Estuar Coast Shelf Sci 74:458–470

Cooper, T. F., Gilmour, J. P., & Fabricius, K. E. (2009). Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. *Coral reefs*, *28*(3), 589-606.

Cunning R, Baker AC (2012) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3, 259–262.

Dana, J. D. (1846). *Structure and classification of zoophytes* (Vol. 7). Lea and Blanchard.

De'ath, G., & Fabricius, K. (2010). Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. *Ecological Applications*, *20*(3), 840-850.

De Bakker, D. M., Meesters, E. H., Bak, R. P., Nieuwland, G., & Van Duyl, F. C. (2016). Long-term shifts in coral communities on shallow to deep reef slopes of Curaçao and Bonaire: are there any winners?. *Frontiers in Marine Science*, *3*, 247.

De Bakker, D. M., Van Duyl, F. C., Bak, R. P., Nugues, M. M., Nieuwland, G., & Meesters, E. H. (2017). 40 Years of benthic community change on the Caribbean reefs of Curaçao and Bonaire: the rise of slimy cyanobacterial mats. *Coral Reefs*, *36*(2), 355-367.

De Bakker, D. M., van Duyl, F. C., Perry, C. T., & Meesters, E. H. (2019). Extreme spatial heterogeneity in carbonate accretion potential on a Caribbean fringing reef linked to local human disturbance gradients. *Global change biology*, *25*(12), 4092-4104.

Debrot AO, Vinke E, van der Wende G, Hylkema A, Reed JK (2014) Deepwater marine litter densities and composition from submersible video-transects around the ABC-islands, Dutch Caribbean. Mar Pollut Bull 88(1):361–365

Den Haan J (2015) Effects of nutrient enrichment on the primary producers of a degraded coral reef. Ph.D. thesis, University of Amsterdam, p 38–58

Duprey, N. N., Yasuhara, M., & Baker, D. M. (2016). Reefs of tomorrow: eutrophication reduces coral biodiversity in an urbanized seascape. *Global change biology*, *22*(11), 3550-3565.

Eddy, T. D., Lam, V. W., Reygondeau, G., Cisneros-Montemayor, A. M., Greer, K., Palomares, M. L. D., ... & Cheung, W. W. (2021). Global decline in capacity of coral reefs to provide ecosystem services. *One Earth*, *4*(9), 1278-1285.

Englebert, N., Bongaerts, P., Muir, P. R., Hay, K. B., Pichon, M., & Hoegh-Guldberg, O. (2017). Lower mesophotic coral communities (60-125 m depth) of the northern Great Barrier Reef and Coral Sea. *PloS one*, *12*(2), e0170336.

Fabricius, K., De'ath, G., 2001b. Environmental factors associated with the spatial distribution of crustose coralline algae on the Great Barrier Reef. Coral Reefs 19, 303–309.

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine pollution bulletin*, *50*(2), 125-146.

Falkowski, P.G., Jokiel, P.L., Kinzie III, R.A., 1990. Irradiance and corals. In: Dubinsky, Z. (Ed.), Ecosystems of the World 25: Coral Reefs. InElsevier, Amsterdam, pp. 89–107.

Farr N, Bowen A, Ware J, Pontbriand C, Tivey M (2010) An integrated, underwater optical/acoustic communications system. In: OCEANS 2010 IEEE-Sydney 2010 May 24. IEEE, pp 1–6

Fijikura, K., Kojima, S., Tamaki, K., Hunt, Y., Okutani, J., 1999. The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan Trench. Mar. Ecol. Prog. Ser. 190, 17–26.

Frade, P. R., Bongaerts, P., Baldwin, C. C., Trembanis, A. C., Bak, R. P., & Vermeij, M. J. (2019). Bonaire and Curaçao. In *Mesophotic Coral Ecosystems* (pp. 149-162). Springer, Cham.

Frade PR, De Jongh F, Vermeulen F, Van Bleijswijk J, Bak RPM (2008b) Variation in symbiont distribution between closely related coral species over large depth ranges. Mol Ecol 17(2):691–703

Furlong ME, Paxton D, Stevenson P, Pebody M, McPhail SD, Perrett J (2012) Autosub Long Range: A long range deep diving AUV for ocean monitoring. In: Autonomous underwater vehicles (AUV). Institute of Electrical and Electronics Engineers, New York, 1–7

Gardner TA, Cote IM, Gill JA, Gant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

GESAMP, 2001. Protecting the oceans from land-based activities. Land-based sources and activities affecting the quality and uses of the marine, coastal and associated freshwater environment. United Nations Environment Program, Nairobi.

Greene, L.E., Alevizon, W.S., 1989. The comparative accuracies of visual assessment methods for coral reef fishes. Bull. Mar. Sci. 44, 899–912.

Guzman, H.M., Holst, I., 1993. Effects of chronic oil-sediment pollution on the reproduction of the Caribbean reef coral Siderastrea siderea. Marine Pollution Bulletin 26, 276

Hill, J., Wilkinson, C., 2004. Methods for Ecological Monitoring of Coral Reefs. Australian Institute of Marine Science, Townsville, Australia.

Hoekema, L., 2022. Spatial & Temporal monitoring of Bonaire's near-shore water quality. Wageningen Marine Research.

Hoeksema BW, Reimer JD, Vonk R (2017b) Editorial: biodiversity of Caribbean coral reefs (with a focus on the Dutch Caribbean). Mar Biodivers 47(1):1–10

Hogg, M. M., Tendal, O. S., Conway, K. W. , Pomponi, S. A. , van Soest, R. W. M., Gutt, J. (2010). Deep-sea sponge grounds: reservoirs of biodiversity. Cambridge, UK.

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547–1551

IUCN (2011) Coral reef resilience assessment of the Bonaire National Marine Park, Netherlands Antilles. Gland

Jackson JBC, Donovan MK, Cramer KL, Lam W (2014) Status and trends of Caribbean coral reefs: 1970–2012. Global Coral Reef Monitoring Network, IUCN, Gland

Jaffe JS, Franks PJ, Roberts PL, Mirza D, Schurgers C, Kastner R, Boch A (2017) A swarm of autonomous miniature underwater robot drifters for exploring submesoscale ocean dynamics. Nat Commun 8:14189

Kendrick, G.A., 1991. Recruitment of coralline crusts and filamentous turf algae in the Galapagos archipelago: effect of simulated scour, erosion and accretion. Journal of Experimental Marine Biology and Ecology 147, 47–63.

Kinsey, D.W., Davies, P.J., 1979. Effects of elevated nitrogen and phosphorus on coral reef growth. Limnology and Oceanography 24, 935–940.

Kinsey, J. C., Eustice, R. M., & Whitcomb, L. L. (2006, September). A survey of underwater vehicle navigation: Recent advances and new challenges. In *IFAC conference of manoeuvering and control of marine craft* (Vol. 88, pp. 1-12).

Knowlton, N., & Jackson, J. B. C. (2008). Shifting baselines, local impacts, and global change on coral reefs. PLoS Biology, 6(2), e54. https://doi. org/10.1371/journal.pbio.0060054

Kobluk DR, Lysenko MA (1994) Ring bleaching in southern Caribbean Agaricia agaricites during rapid water cooling. Bull Mar Sci 54:142–150

Koop, K., Booth, D., Broadbent, A., Brodie, J., Bucher, D., Capone, D., Coll, J., Dennison, W., Erdmann, M., Harrison, P., HoeghGuldberg, O., Hutchings, P., Jones, G.B., Larkum, A.W.D., ONeil, J., Steven, A., Tentori, E., Ward, S., Williamson, J., Yellowlees, D., 2001. ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Marine Pollution Bulletin 42, 91–120

Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Mar Ecol Prog Ser 323:107–117

Lam, K., Shin, P. K., Bradbeer, R., Randall, D., Ku, K. K., Hodgson, P., & Cheung, S. G. (2006). A comparison of video and point intercept transect methods for monitoring subtropical coral communities. *Journal of Experimental Marine Biology and Ecology*, *333*(1), 115-128.

Lamb, J. B., True, J. D., Piromvaragorn, S., and Willis, B. L. (2014). Scuba diving damage and intensity of tourist activities increases coral disease prevalence. Biol. Conserv. 178, 88–96. doi: 10.1016/j.biocon.2014.06.027

Lapointe BE, Mallin M (2011) Nutrient enrichment and eutrophication on fringing coral reefs of Bonaire and Curaçao, Netherlands Antilles. Florida Atlantic University and University of North Carolina Wilmington

Lasker, H. R. (1980). Sediment rejection by reef corals: the roles of behavior and morphology in Montastrea cavernosa (Linnaeus). *Journal of Experimental Marine Biology and Ecology*, *47*(1), 77-87.

Lauerman, L.M.L., 1998. Diet and feeding behaviour of the deepwater sea star Rathbunaster californicus (Fisher) in the Monterey submarine canyon. Bull. Mar. Sci. 63, 523–530.

Leichter, J.J., Genovese, S.J., 2006. Intermittent upwelling and subsidized growth of the scleractinian coral Madarcis mirabilis on the deep fore reef slope of Discovery Bay, Jamaica. Mar. Ecol. Prog. Ser. 316, 95–103.

Lesser, M.P., 2000. Depth-dependent effects of ultraviolet radiation on photosynthesis in the Caribbean coral, Montastraea faveolata. Mar. Ecol. Prog. Ser. 192, 137–151.

Lesser, M. P., Slattery, M., & Leichter, J. J. (2009). Ecology of mesophotic coral reefs. *Journal of experimental marine biology and ecology*, *375*(1-2), 1-8.

Locke, J. M., Weil, E., & Coates, K. A. (2007). A newly documented species of Madracis (Scleractinia: Pocilloporidae) from the Caribbean. *Proceedings of the Biological Society of Washington*, *120*(2), 214-226.

Meesters, E. H., Bak, R. P., & Van Duyl, F. C. (2020). Commentary: Managing recovery resilience in coral reefs against climate-induced bleaching and hurricanes: a 15 year case study from Bonaire, Dutch Caribbean. *Frontiers in Marine Science*, *7*, 346.

Menza, C., Kendall, M., & Hile, S. (2008). The deeper we go the less we know. *Revista de Biología Tropical*.

Mohtar, W. H. M. W., Maulud, K. N. A., Muhammad, N. S., Sharil, S., & Yaseen, Z. M. (2019). Spatial and temporal risk quotient based river assessment for water resources management. *Environmental Pollution*, *248*, 133-144.

Mücher, S., Suomalainen, J., Stuiver, J., & Meesters, E. (2017). *Hyperspectral Coral Reef Classification of Bonaire* (No. C062/17). Wageningen Marine Research.

Muscatine, L., Porter, J.W., Kaplan, I.R., 1989. Resource partitioning by reef corals as determined from stable isotope composition I. δ13C of zooxanthellae and animal tissue versus depth. Mar. Biol. 100, 185–193.

Nagelkerken I, Vermonden K, Moraes OCC, Debrot AO, Nagelkerken WP (2005) Changes in coral reef communities and an associated reef fish species, Cephalopholis cruentata (Lacepede), after 30 years on Curac¸ao (Netherlands Antilles). Hydrobiologia 549:145–154

Nugues MM, Bak RPM (2008) Long-term dynamics of the brown macroalga Lobophora variegata on deep reefs in Curac¸ao. Coral Reefs 27:389–393

Pawlik JR, Burkepile DE, Vega-Thurber RL (2016) A vicious circle? Altered carbon and nutrient cycling may explain the low resilience of Caribbean coral reefs. BioScience

Philipp, E., Fabricius, K., 2003. Photophysiological stress in scleractinian corals in response to short-term sedimentation. Journal of Experimental Marine Biology and Ecology 287, 57–78.

Purcell, S.W., 2000. Association of epilithic algae with sediment distribution on a windward reef in the northern Great Barrier Reef, Australia. Bulletin of Marine Science 66, 199–214.

Reed, J. K. and S. A. Pomponi (2000). Final cruise report. Submersible and SCUBA collections in the Netherlands Antilles (Curacao, Bonaire) and Aruba: biomedical and biodiversity research of the benthic communities with emphasis on the Porifera and Gorgonacea. Harbor Branch Oceanographic Institution, Ft. Pierce, FL. 183 pp.

Rivero-Calle, S., Armstrong, R. A., & Soto-Santiago, F. J. (2009). Biological and physical characteristics of a mesophotic coral reef: Black Jack reef, Vieques, Puerto Rico. In *Proc 11th Int Coral Reef Symp* (Vol. 1, pp. 567-571).

Rogers, C.S., 1990. Responses of coral reefs and reef organisms to sedimentation. Marine Ecology Progress Series 62, 185–202.

Rogers, C.S., Miller, J., 2001. Coral bleaching, hurricane damage, and benthic cover on coral reefs in St. John, US Virgin Islands: a comparison of surveys with the chain transect method and videography. Bull. Mar. Sci. 69, 459–470.

Roos PJ (1971) The Shallow-Water Stony Corals of the Netherlands Antilles. Studies on the Fauna of Curaçao and other Caribbean Islands 37:1 – 108

Rueda-Roa, D. T. (2012). *On the spatial and temporal variability of upwelling in the southern Caribbean Sea and its influence on the ecology of phytoplankton and of the Spanish sardine (Sardinella aurita)*. University of South Florida.

Sandin, S. A., Sampayo, E. M., & Vermeij, M. J. (2008). Coral reef fish and benthic community structure of Bonaire and Curaçao, Netherlands Antilles. *Caribbean Journal of Science*, *44*(2), 137-144.

Scott, A. R., Battista, T. A., Blum, J. E., Noren, L. N., & Pawlik, J. R. (2019). Patterns of benthic cover with depth on Caribbean mesophotic reefs. *Coral Reefs*, *38*(5), 961-972.

Scoltwedel, T., Vopel, K., 2001. Bacterial abundance and biomass in response to organism-generated habitat heterogeneity in deep-sea sediments. Mar. Ecol. Prog. Ser. 219, 291–298.

Slattery, M., & Lesser, M. P. (2012, July). Mesophotic coral reefs: a global model of community structure and function. In *Proceedings of the 12th International Coral Reef Symposium* (Vol. 1, pp. 9-13). Cairns: ICRS.

Slijkerman D, de León R, de Vries P (2014) A baseline water quality assessment of the coastal reefs of Bonaire, southern Caribbean. Mar Pollut Bull 86:523–529

Sommer, B., Harrison, P. L., Brooks, L., & Scheffers, S. R. (2011). Coral community decline at Bonaire, southern Caribbean. Bulletin of Marine Science, 87(3), 541–565. https://doi.org/10.5343/bms.2010.1046

Stafford-Smith, M.G., Ormond, R.F.G., 1992. Sediment-rejection mechanisms of 42 species of Australian scleractinian corals. Australian Journal of Marine and Freshwater Research 43, 683– 705.

Stefanoudis, P. V., Gress, E., Pitt, J. M., Smith, S. R., Kincaid, T., Rivers, M., ... & Rogers, A. D. (2019). Depth-dependent structuring of reef fish assemblages from the shallows to the rariphotic zone. *Frontiers in Marine Science*, *6*, 307.

Steneck, R., 1997. Crustose corallines, other algal functional groups, herbivore and sediments: complex interactions along reef productivity gradients. In: Lessios, H.A., Macintyre, I.G (Eds.), Proceedings of the Eighth International Coral Reef Symposium. Smithsonian Institute Press, Balboa, Panama, pp. 695–700

Steneck, R. S., McClanahan, T., Arnold, S. N., & Brown, J. B. (2005). A Report on the Status of the Coral Reefs of Bonaire in 2005 with Advice on a Monitoring Program. *Unpublished report to the Bonaire Marine National Park (STINAPA): University of Maine Darling Marine Center*.

Steneck RS, Arnold SN, de León R, Rasher DB (2015) Status and trends of Bonaire's coral reefs in 2015: slow but steady signs of resilience. University of Maine, School of Marine Sciences

Telesnicki, G.J., Goldberg, W.M., 1995. Effects of turbidity on the photosynthesis and respiration of two south Florida reef coral species. Bulletin of Marine Science 57, 527–539.

Thacker RW, Nagle DG, Paul VJ (1997) Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. Mar Ecol Prog Ser 147:21–29

Thrun S, Leonard J (2008) Simultaneous localization and mapping. Springer Handbook of Robotics; Siciliano, Khatib Editors. ISBN:978-3-540-23957-4, pp 871–886

Tratalos, J.A., Austin, T.J., 2001. Impacts of recreational SCUBA diving on coral communities of the Caribbean islands of Grand Cayman. Biol. Conserv. 102, 67–75.

Trembanis, A. C., Forrest, A. L., Keller, B. M., & Patterson, M. R. (2017). Mesophotic coral ecosystems: a geoacoustically derived proxy for habitat and relative diversity for the leeward shelf of Bonaire, Dutch Caribbean. *Frontiers in Marine Science*, *4*, 51.

Van Duyl, F. C. (1985). Atlas of the Living Reefs of Curaçao and Bonaire (Netherlands Antilles). *Foundation for Scientific Research in Surinam and the Netherlands Antilles*, *117*.

Van der Geest, M., Meesters, E., & Mücher, S. (2020). *Impact of terrestrial erosion on coral reef health at Bonaire: a plea for nature-inclusive" watershed-to-reef" based coastal management* (No. C055/20). Wageningen Marine Research.

Van Woesik R, Tomascik T, Blake S (1999) Coral assemblages and physico-chemical characteristics of the Whitsunday Islands: evidence of recent community changes. Mar Freshw Res 50:427–440

Varela R, Alvarez I, Santos F, deCastro M, Gomez-Gesteira M (2015) Has upwelling strengthened along worldwide coasts over 1982–2010? Sci Rep 5:10016

Vega-Thurber, R., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly, F., ... & Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. *Environmental microbiology*, *11*(8), 2148-2163.

Vermeij MJA, Bak RPM (2002) How are coral populations structured by light? Marine light regimes and the distribution of Madracis. Mar Ecol Prog Ser 233:105–116

Vermeij MJA, Bak RPM (2003) Species-specific population structure of closely related coral morphospecies along a depth gradient (5–60 m) over a Caribbean reef slope. Bull Mar Sci 73(3):725–744

Vermeij MJA, van Moorselaar I, Engelhard S, Hornlein C, Vonk SM, Visser PM (2010) The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. PLoS ONE 5(12):e14312

Vermeij MJA (2011) Inventory of Bonaire's coral reefs, with particular attention to previously undescribed phenomena illustrating the ongoing decline of Bonaire's reefs. CARMABI Foundation

Verweij, P., Cormont, A., Nel, J., de Rooij, B., Jones-Walters, L., Slijkerman, D., ... & Teles, I. D. (2020). *A nature inclusive vision for Bonaire in 2050* (No. 3023). Wageningen Environmental Research.

Waitt Institute (2017) The state of Curaçao's coral reefs: marine scientific assessment. May 2017

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2012) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3, 160–164

Williams, S.B., Newman, P., Majumder, S., Rosenblatt, J., DurrantWhyte, H.F., 1999. Autonomous transect surveying of the Great Barrier Reef. In: Roberts, J., Wyeth, G. (Eds.), Proceedings of the 1999 Australian Conference on Robotics and Automation, Brisbane, 1999. Australian Robotics and Automation Association, Brisbane, Australia, pp. 128–132.

Williams, S. B., Pizarro, O., Webster, J. M., Beaman, R. J., Mahon, I., Johnson-Roberson, M., & Bridge, T. C. (2010). Autonomous underwater vehicle–assisted surveying of drowned reefs on the shelf edge of the Great Barrier Reef, Australia. *Journal of Field Robotics*, *27*(5), 675-697.

Wood, S.N. (2003) Thin plate regression splines. J. R. Statist. Soc. B 65, 95–114.

Wyman, K.D., 1987. Light absorption and utilization among hermatypic corals: a study in Jamaica, West Indies. Mar. Biol. 96, 283–292.

Yentsch CS, Yentsch CM, Cullen JJ, Lapointe B, Phinney DA, Yentsch SW (2002) Sunlight and water transparency: cornerstones in coral research. J Exp Mar Biol Ecol 268:171–183

Yoerger, D. R., Jakuba, M., Bradley, A. M., & Bingham, B. (2007). Techniques for deep sea near bottom survey using an autonomous underwater vehicle. *The International Journal of Robotics Research*, *26*(1), 41-54.

Zanke, F., & de Froe, E. (2015). Small-scale variation in coral reef quality on the leeward side of Bonaire. *IMARES Wageningen University and Research*.

Zhang, P., Chen, Y., Peng, C., Dai, P., Lai, J., Zhao, L., & Zhang, J. (2020). Spatiotemporal variation, composition of DIN and its contribution to eutrophication in coastal waters adjacent to Hainan Island, China. *Regional Studies in Marine Science*, *37*, 101332.

8 Appendix A – Source material

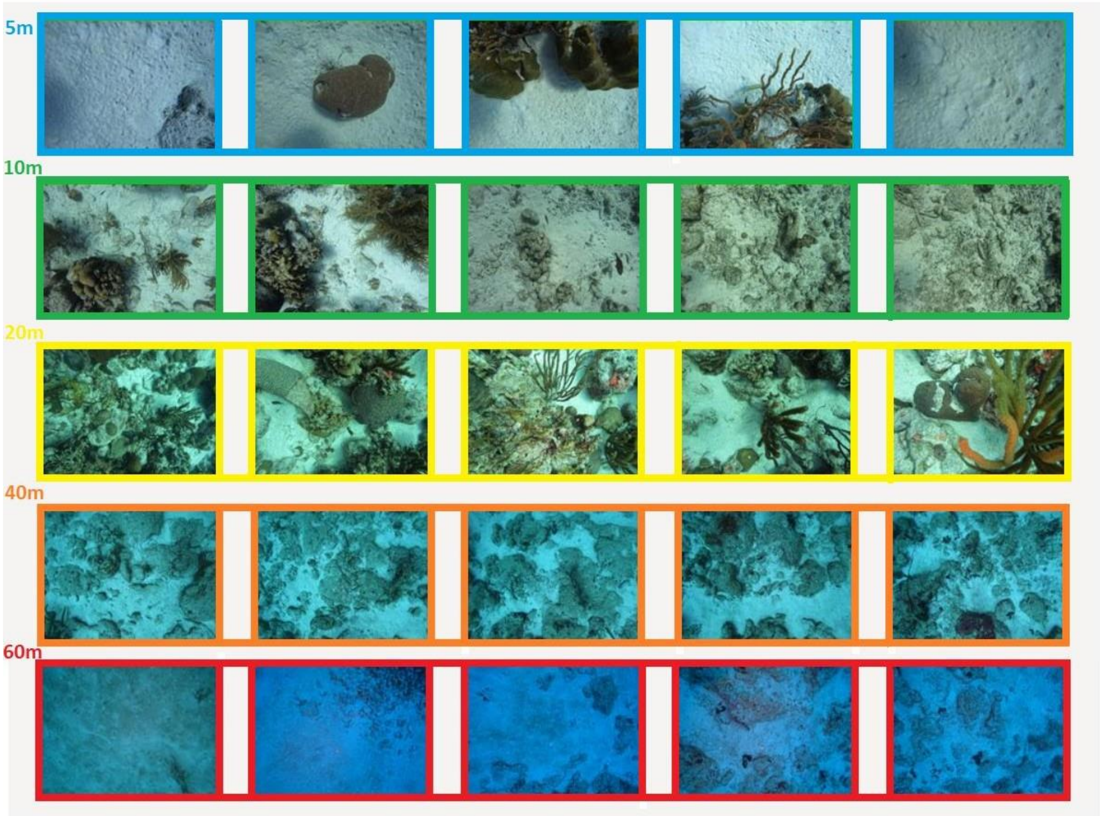

Figure 1: B04, Salt Pier.

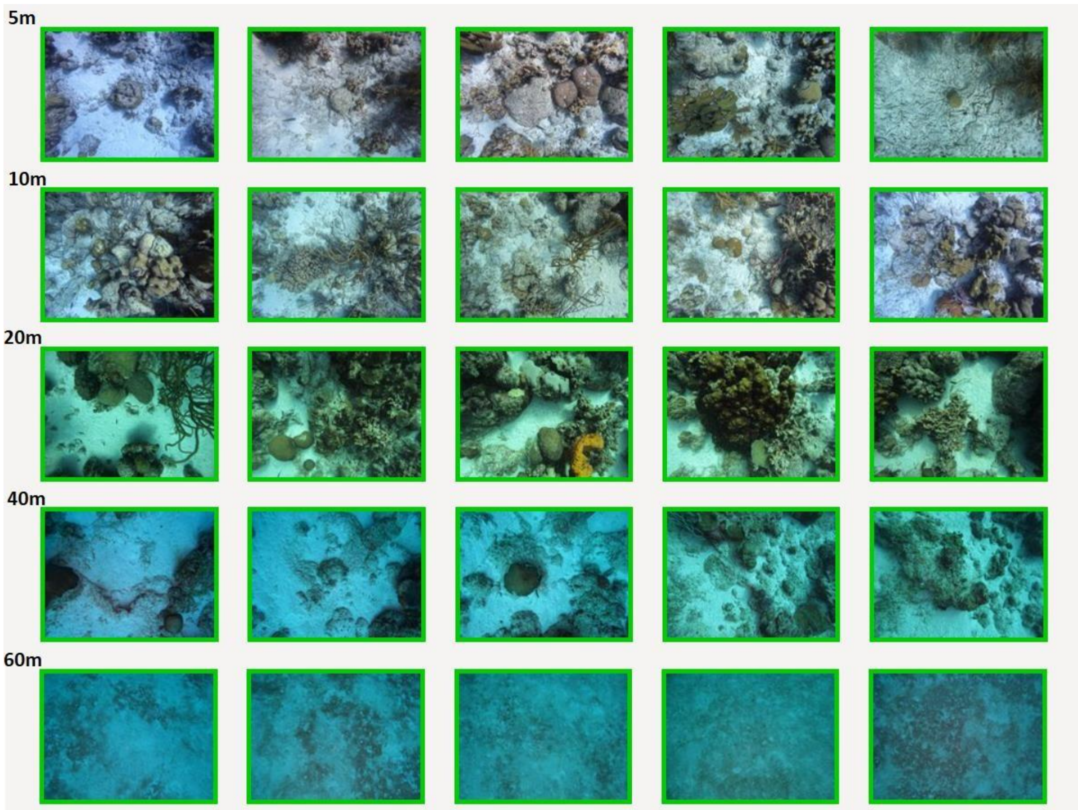

Figure 2: B07, Bachelors Beach.

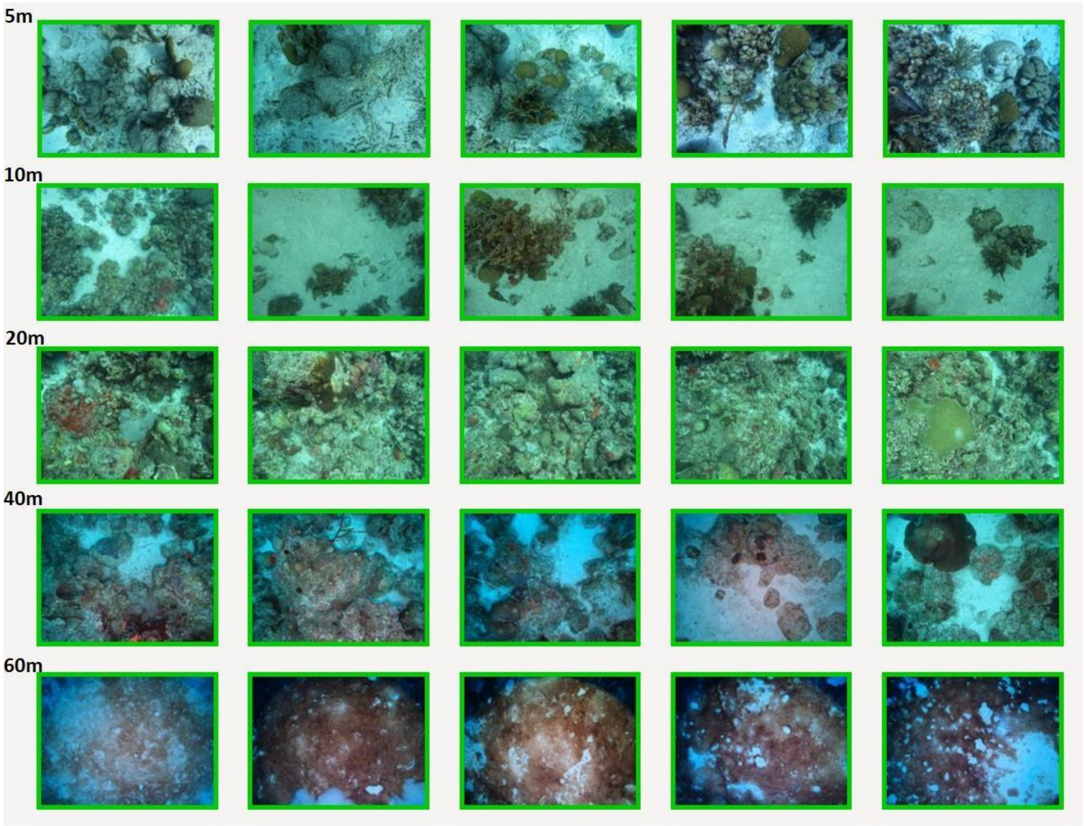

Figure 324: B09, Port Bonaire.

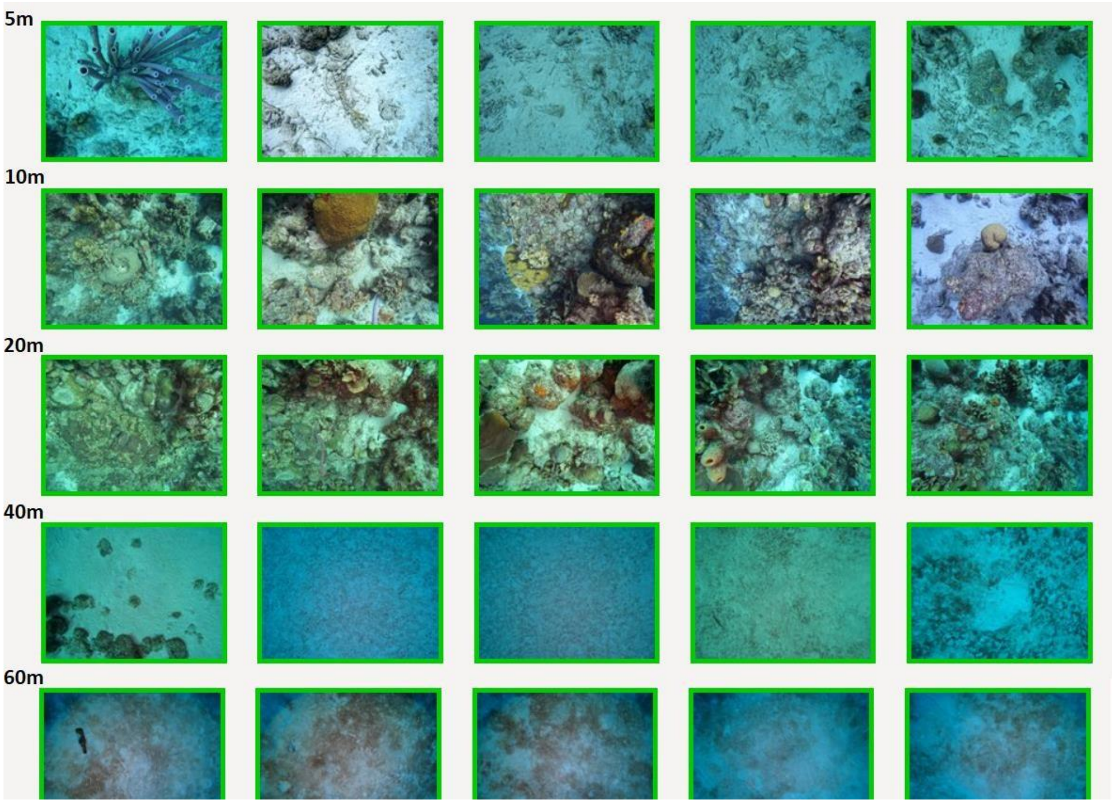

Figure 4: B11, Kralendijk

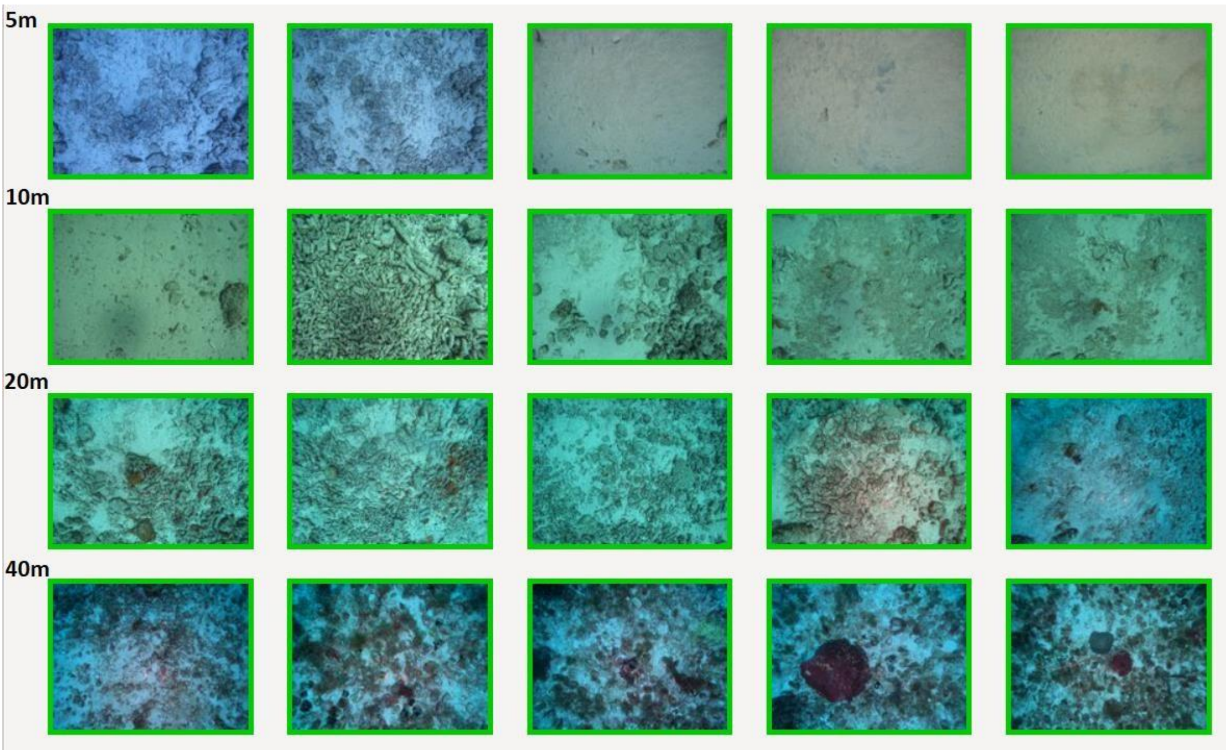

Figure 5: B12, Harbour Village.

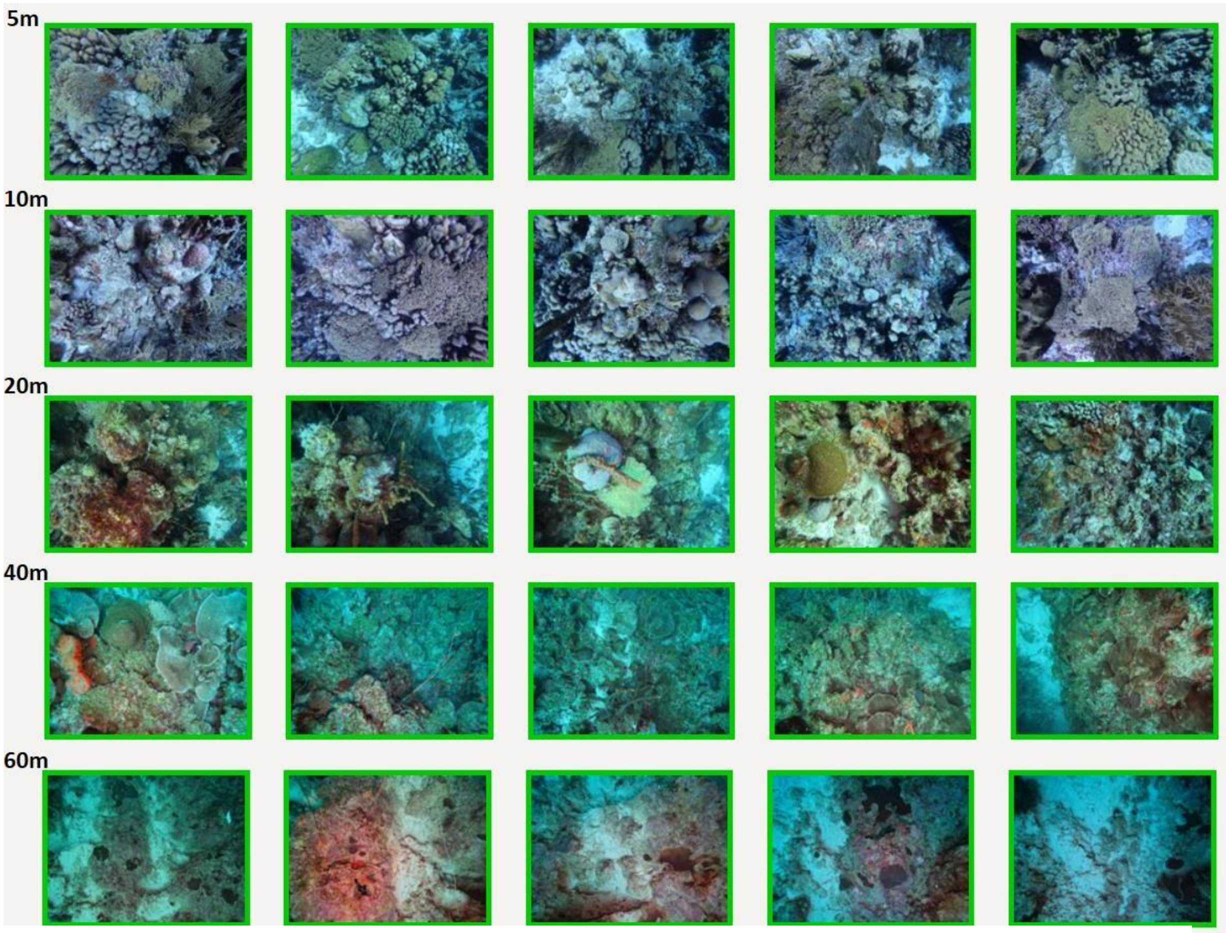

Figure 6: B16, Karpata

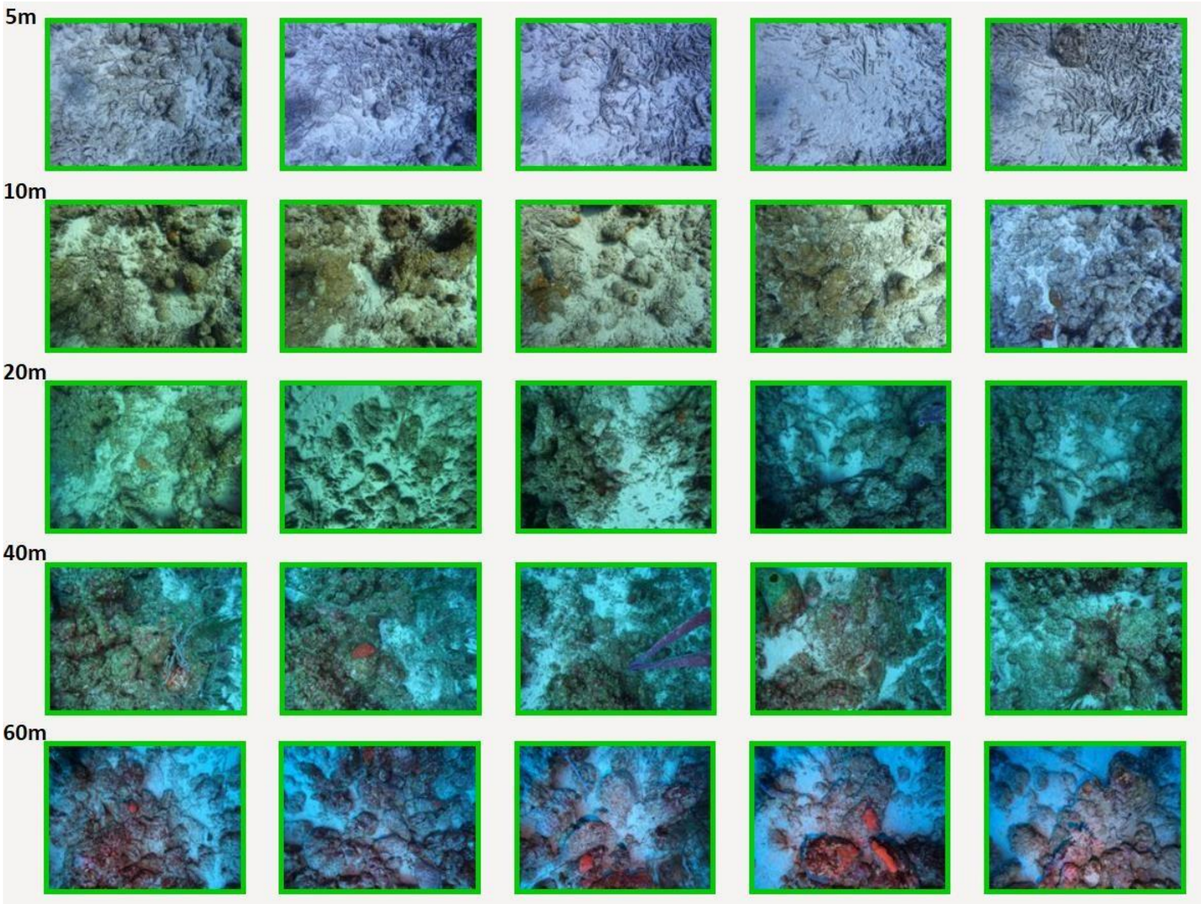

Figure 7: B17, BOPEC.

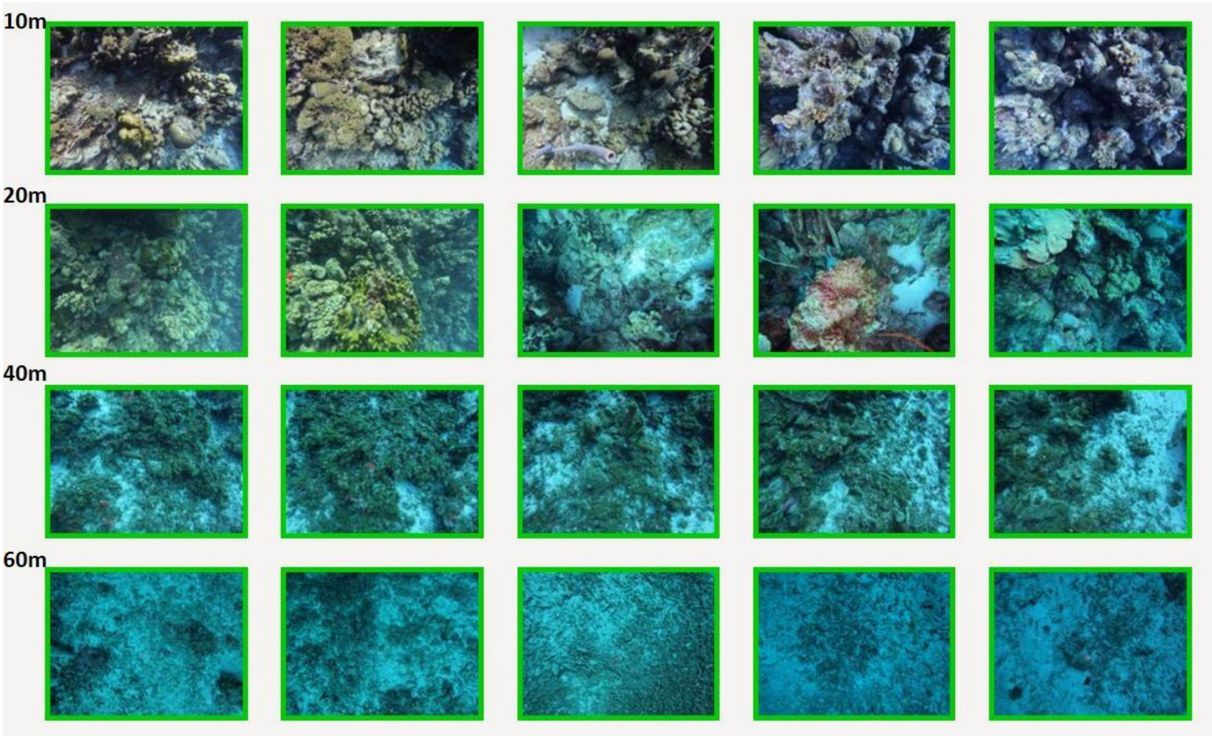

Figure 8: KB4, Klein Bonaire, Ebo's Special.

9 Appendix B – Tables

9.1 ROV battery life-time

Table 3: Battery life-time of the QYSEA Fifish V6 in different conditions. Obtained from: Qysea.com customer service.

v6	Condition		LED Level	Throttle	Battery Last
	Still water	Pool	OFF	Auto Holding (Depth & Orientation Auto correction)	around 5 hours
	Still water	Pool	2	Full	50 min
	Still water	Pool	2	Occasional Full (shooting films and objects)	1.5 hours
	Still water	Reservoir	2	Occasional Full (shooting films and objects)	1.5 hours
	Open water	Shallow Sea	1	Occasional Full (shooting films and objects)	about 2 hours
	Open water	Shallow Sea	2	Occasional Full (shooting films and objects)	1.5 hours

9.2 Analysis of variance

Table 4: Analysis of Variance (ANOVA) explaining variation in coral cover by Depth, Impact and Risk zone.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Impact	3	44.91	14.969	53.955	2e-16	***
Depth	4	59.91	14.977	53.986	2e-16	***
Risk	3	36.83	12.276	44.251	2e-16	***
Impact:Depth	11	8.79	0.799	2.879	0.00179	**
Depth:Risk	11	30.06	2.733	9.851	1.86e-13	***
Residuals	157	43.56	0.277			

9.3 Tukey HSD

Table 5: Adjusted p-values retrieved from the TukeyHSD test for significance difference in coral cover among impact zones, depth ranges and water quality risk zones.

Impact zone	Extreme	High	Moderate	Marginal
Extreme	-	**0,00**	**0,00**	**0,00**
High	-	-	0,99	**0,00**
Moderate	-	-	-	**0,00**
Marginal	-	-	-	-

Depth	5	10	20	40	60
5	-	0,12	0,00	0,00	0,00
10	-	-	0,73	0,00	0,00
20	-	-	-	0,00	0,00
40	-	-	-	-	0,00
60	-	-	-	-	-

Risk	High-risk	Moderate-risk	Low-risk	No-risk
High-risk	-	0,04	0,00	0,00
Moderate-risk	-	-	0,00	0,00
Low-risk	-	-	-	0,00
No-risk	-	-	-	-

Table 6: Adjusted p-values retrieved from the TukeyHSD test for significance difference in diversity among impact zones, depth ranges and water quality risk zones.

Impact zone	Extreme	High	Moderate	Marginal
Extreme	-	0,40	**0,00**	**0,00**
High	-	-	**0,00**	**0,00**
Moderate	-	-	-	0,99
Marginal	-	-	-	-

Depth	5	10	20	40	60
5	-	0,08	**0,00**	**0,00**	**0,00**
10	-	-	0,40	0,24	0,09

20	-	-	-	1,00 (0,9983)	0,92
40	-	-	-	-	0,98
60	-	-	-	-	-
Risk	High-risk	Moderate-risk	Low-risk	No-risk	
High-risk	-	0,07	**0,00**	**0,00**	
Moderate-risk	-	-	0,73	**0,00**	
Low-risk	-	-	-	**0,00**	
No-risk	-	-	-	-	

Table 7: Adjusted p-values retrieved from the TukeyHSD test for significance difference in species richness among impact zones, depth ranges and water quality risk zones.

Impact zone	Extreme	High	Moderate	Marginal	
Extreme	-	0,41	**0,00**	**0,00**	
High	-	-	**0,00**	**0,00**	
Moderate	-	-	-	0,13	
Marginal	-	-	-	-	
Depth	5	10	20	40	60
5	-	**0,00**	**0,00**	**0,00**	**0,00**
10	-	-	0,75	**0,03**	**0,00**
20	-	-	-	0,41	**0,03**
40	-	-	-	-	0,70
60	-	-	-	-	-
Risk	High-risk	Moderate-risk	Low-risk	No-risk	
High-risk	-	**0,00**	**0,00**	**0,00**	
Moderate-risk	-	-	0,14	**0,00**	
Low-risk	-	-	-	**0,00**	
No-risk	-	-	-	-	

9.4 Adonis

*Table 8: Adonis2 p-values for nMDS on benthic species composition. ((adonis2(formula = benthostrans^0.25 ~ depth * impact * risk, data = env2, permutations = 10000)*

	Df	Sum of Sqs	R2	F	Pr(>F)
Depth	1	0.5946	0.13928	7.8986	9.999e-05 ***
Impact	1	0.5691	0.13331	7.5598	0.00020 ***
Risk	1	0.1795	0.04204	2.3840	0.05809
Depth:Impact	1	0.2221	0.05203	2.9504	0.02660 *
Depth:Risk	1	0.2352	0.05510	3.1247	0.02010 *
Impact:Risk	1	0.0632	0.01481	0.8396	0.48745
Depth:Impact:Risk	1	0.1470	0.03443	1.9524	0.09349 .
Residual	30	2.2584	0.52901		
Total	37	4.2691	1.00000		

Table 9: Ordisurf output, family: faussin, link function: identity, formula: y~s(x1,x2,k=10, bs="tp", fx=F)

Parametric coefficients	Estimate std. error	T value	Pr(>|t|)
Intercept	26.711	2.244	11.9 1.45e-13 ***
Approx. sign. Of smooth terms	Edf Ref.df	F	p-value
S(x1,x2)	3.585	9 4.634	2.98e-06 ***
R-sq.(adj) = 0.53	Deviance explained = 57.5%		
-REML = 155.67	Scale est. = 191.39	N=38	

*Table 10: Adonis2 p-values for nMDS on benthic species composition.(adonis2(formula = coralspecies^0.25 ~ Depth * Impact * Risk, data = envcoralspecies, permutations = 10000)*

	Df	*Sum of Sqs*	*R2*	*F*	*Pr(>F)*
Depth	*4*	*2.3786*	*0.37554*	*2.3690*	*0.0406 **
Impact	*3*	*0.6820*	*0.10768*	*0.9057*	*0.5675*
Risk	*3*	*0.9114*	*0.14390*	*1.2103*	*0.3306*
Depth:Impact	*8*	*0.8287*	*0.13084*	*0.4127*	*0.9958*
Depth:Risk	*4*	*0.7801*	*0.12316*	*0.7770*	*0.7242*
Residual	*3*	*0.7530*	*0.11889*		
Total	*25*	*6.3339*	*1.00000*		