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# Changing benthic communities on Saba Bank
# Is Saba Bank becoming a 'Sponge reef'?

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*by*

**Mirthe Wiltink**
**10199179**

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*Supervisor / Assessor:*
dr. L. E. Becking
dr. H. W. G. Meesters

*Examiner:*
dr. ir. J. M. de Goeij

Wageningen University

Imares

***Abstract*** **-** Due to multiple stressors, many coral reefs are degrading. Shifts from a coral dominated system to an alternate phase are observed. For coral reefs, the shift described most in the scientific literature is a shift to an algae dominated system. However, shifts to other dominant species are observed as well, including sponge or cyanobacteria dominance. It is thought that sponges may become dominant when macroalgae or turf algae cover increases. Algae are producers of dissolved organic matter (DOM), a food source for sponges. Cyanobacteria are producers of DOM as well, although it remains unclear whether sponges can use this. Furthermore, the decrease in spongivore fish due to overfishing, and the increase in picoplankton can result in increasing sponge cover. In this study, benthic cover of eleven sites at Saba Bank in 2013 and 2015 is assessed. Corals and sponges were identified to species level, to get insight into assemblages. Using the program CPCe, photo quadrats (N=10) of two transects per site were analyzed. The same photo quadrats were used for analysis of sponge diversity, all sponges larger than 4 cm were identified to species level. Last, in 2015 92 specimens of sponges were sampled and identified using DNA barcoding and morphological analysis. Close-up photos of these specimens were made, so that sponge identification guide of Saba Bank can be developed. A shift from turf algae dominance to cyanobacterial dominance among the years 2013 and 2015 was observed. Possibly, Saba Bank experiences some influence from the nearby islands Saba, St. Eustatius, and St. Kitts and Nevis. Macroalgae cover was found to be higher in the northern and northeastern parts of the bank (closer to the islands), whereas coral cover was lower in these parts. The coral with highest cover in the benthic survey was *Montastraea faveolata* (34.7% and 45.5% of total coral cover in 2013 and 2015). Using multivariate analysis, year and water depth had a significant effect on coral composition, position of the site had no significant effect suggesting connectivity between sites for corals. The species *Xestospongia muta* and *Agelas sventres* contributed most to total sponge cover (*X.* muta: 11% and 12.9%; *A. sventres*: 10.4% and 15.4% of total sponge cover in 2013 and 2015). Water depth, northing and non living cover had a significant effect on sponge composition. Altogether, sponge cover was not high on Saba Bank and therefore it is not (yet) becoming a sponge reef. In 2015, Saba Bank was dominated by cyanobacteria, this may be beneficial to sponges, since cyanobacteria are producers of DOM.

# INTRODUCTION

### *Degradation of coral reefs*

Coral reefs all over the world are degrading, due to climate change, and natural and anthropogenic stressors. It is estimated that already 19% of the global reef area is lost, 15% of the reefs are critically endangered and are at risk of extinction between 10 and 20 years when no measures are taken (Wilkinson, 2008). As a result of climate change, sea surface temperature is increasing and the pH in the water is reduced, which is negatively affecting calcifying reef-building organisms, including several coral species (Baker *et al.,* 2008). Besides, more extreme weather events, such as hurricanes will occur more frequently, which can damage corals. Over the past few years, coral diseases have been documented more often, and the impacts of these diseases could be devastating to coral reefs as well (Harvell *et al.,* 2007; Gouezo *et al.,* 2015). Due to the increase of human populations inhabiting coastal areas, the environmental pressure on coral reefs is rising (Wilkinson, 1996; Oigman-Pszczol & Creed, 2011). The major local anthropogenic stressors on coral reefs are nutrients, runoff, and overfishing (Ferrier-Pagès *et al.,* 2000; Hughes *et al.,* 2007).

### *Scenarios for degrading reefs*

Increasing stress, may reduce coral growth and a system becomes more susceptible to become dominated by a different species (Anthony *et al.,* 2011). However, susceptibility to an alternative stable state depends on the resilience of coral reefs (Folke *et al*., 2004; Mumby *et al*., 2015). After a shift to another dominant species, community structure has changed and returning to the original state becomes challenging (Mumby, 2009). The phase-shift on coral reefs most described in literature is a shift from a coral dominated system to a macroalgae dominated system (Hughes, 1994; Nyström *et al.,* 2000). However, shifts to other alternative states are observed as well, including shifts to reefs that are dominated by corallimorpharia, soft corals, sea urchins, and sponges (Norström *et al*., 2009). In one study a shift to a cyanobacteria dominated system was observed (de Bakker *et al*., 2016). This long-term study of benthic community uses a dataset of permanent quadrants at Bonaire and Curacao over a time span of 40 years.

### *Algae dominated reefs*

Reefs dominated by macroalgae are mostly described, however it is expected that turf algae will become one of the most abundant benthic groups (Sandin *et al*., 2008). Algae benefit from eutrophication and decreased numbers of herbivorous fish due to overfishing (Hughes *et al*., 2007; Littler *et al.,* 2006). An increased algal cover may lead to decreased coral recruitment, and increased coral mortality due to shading, toxic metabolites or pathogens (Nugues *et al*., 2004; Kuffner *et al*., 2006; Titlyanov *et al*., 2007). Algae facilitate bacterial growth by producing dissolved organic matter (DOM), among these bacteria are coral pathogens that can cause coral disease (Kuntz *et al*., 2005; Haas *et al*., 2011). By producing DOM, algae are indirectly influencing oxygen availability in reef communities (Wild *et al*., 2010; Haas *et al*., 2011), because microbial activity is stimulated by DOM and consequently $O_2$ is depleted. This also is affecting corals, since their energy production in anaerobic conditions is less efficient (Murphy & Richmond, 2016).

### *Sponge reef hypothesis*

In the Caribbean basin, sponges are now equally abundant as corals, however, they were relatively under-investigated in the past (Loh *et al*., 2015). Sponges may become dominant on some coral reefs, since they are expected to be better competitors than corals for space under certain environmental conditions (Bell *et al*., 2013). Sponges are able to use DOM, and transform it into particulate detritus (de Goeij *et al.,* 2013). An increase in algal densities may be beneficial to sponges, since more DOM is released (Pawlik *et al.,* 2016). Cyanobacteria are able to produce DOM too, although it remains unclear whether sponges can use this as a food source (Brocke *et al.,* 2015).

There are more factors concerning chances in food chain dynamics that may initiate sponge dominance. The availability of picoplankton, the major food source for sponges, is important to the structure of sponge communities on Caribbean reefs (Lesser, 2006). Sponge growth was correlated with higher picoplankton availability on reefs (Lesser, 2006; Trussel *et al*., 2006). Besides, top-down processes may also play a role in the structure of sponge communities. Predatory fish prefer to feed on sponges that lack a chemical defense over sponges that produce secondary metabolites (Loh & Pawlik, 2014). When excluded from grazing, sponges without a chemical defense grew faster than defended sponges (Leong & Pawlik, 2010). It is predicted that overfished reefs, lacking spongivores, become dominated by undefended sponge species (Pawlik *et al.*, 2013).

A decrease in pH of seawater and an increase in temperature may be beneficial to boring sponges. Under predicted scenarios for acidification, calcification of corals was reduced, whereas bioerosion rates for sponges increased (Stubler *et al.,* 2014). Also, coral calcification was reduced when sea water temperatures increased, this was combined with increased bioerosion of sponges (Stubler *et al.*, 2015). It is thought that bioerosion depends on coral skeleton density, which in some species is lower at a lower pH (Hernández-Ballesteros *et al*., 2013). A coral skeleton that is less dense is easier to erode. Besides, reduced sea water pH is metabolically less costly for boring sponges, since less acidic compounds has to be excreted by the sponges to create a low pH at the site of erosion (Nava & Carballo, 2008; Wisshak *et al.*, 2012).

Sponges have dominated marine areas in the past. 200 million years ago, the end of the Triassic, a mass extinction took place which also affected calcifying marine invertebrates. The decrease in calcifying marine invertebrates, probably caused by ocean acidification, was followed by an increase of siliceous sponges (Delecat *et al.,* 2010). Nowadays, shifts to sponge dominated systems are observed in the reefs surrounding La Parguera (Puerto Rico), the Florida Keys National Marine Sanctuary (USA), and the Channel Cay reef complex (Belize) (Aronson *et al,.* 2002; Ward-Paige *et al.,* 2005; Williams *et al*., 1999). The sponges that became dominant in these shifts were coral-excavating sponges from the genera *Cliona* and *Chondrilla* (Norström *et al.,* 2009). It is thought that coral-excavating sponges will benefit from shifts to algae dominated systems, since they rely on dissolved organic carbon (DOC) to meet their carbon demand (Mueller *et al.,* 2014). Besides, new substrate will become available to these sponges as a result of coral decline (Ward-Paige *et al.,* 2005).

The main factors that may result in sponge dominance on reefs are:

- Increased DOM production by algae will result in increased food availability and facilitates sponge growth;
- Increased picoplankton availability by high nutrient input will result in increased food availability and facilitates sponge growth;
- Decrease in spongivore densities will result in reduced predation of undefended sponges that grow faster than sponges that produce secondary metabolites;
- Decreasing pH and increasing sea water temperatures will lead to decreased coral calcification and increased bioerosion of boring sponges.

***Saba Bank***

Saba Bank is part of the Caribbean Netherlands and Exclusive Economic Zone of the Netherlands. Saba Bank is a large coral bank with a total surface area of 2.200 $km^2$ shallower than 200 m, somewhat similar to an atoll. Most surface area of the bank is between 20 m and 30 m depth, which is deeper compared to most other Caribbean reefs. The coral reef community is highly divers and the sponge community is considered to be representative of the whole Caribbean Ocean (Thacker *et al.*, 2010). Saba Bank is removed from large landmasses, and therefore it appears that the reefs have suffered little from anthropogenic disturbances (de Bakker *et al.*, 2016). This makes Saba Bank an excellent case to study general processes that occur at coral reefs in the Caribbean. The bank is positioned upstream from Puerto Rico and the Meso-American Barrier Reef. Hypothetically, Saba Bank can serve as a reservoir for these surrounding reefs (Etnoyer *et al.*, 2010). Therefore, it is important to test connectivity of populations on Saba Bank first.

In this study, benthic cover and species diversity of corals and sponges on Saba Bank are assessed. Furthermore, I propose to test the sponge-reef hypothesis on the Saba Bank. Based on photographs of twenty-two transects of 50 m on 11 sites that were taken in 2013 and 2015, the changes in benthic cover will be examined. I want to find out whether benthic cover of Saba Bank changed within a timeframe from 2013 to 2015, and whether cover varies among sites on Saba Bank. Next, I look with more detail into coral and sponge cover in 2013 and 2015, and among the different sites. The assessment of cover among sites can give insight into connectivity on Saba Bank. An assessment of sponge diversity is performed to test whether diversity changed over time, diversity will be compared with data from previous studies in 1972, 1986, and 2006. If Saba Bank is becoming a sponge reef, then it is expected that sponge cover is increasing and coral cover is decreasing over the past years. In addition, the genera *Cliona* and *Chondrilla* would become more dominant, as is the case in reefs where a shift to a system dominated by sponges was observed (Norström *et al.*, 2009).

Research questions:

- What is the variation in benthic cover (i.e. corals, sponges, turf algae, macroalgae, cyanobacteria, and crustose coraline algae) among the years 2013 and 2015 and among sites along Saba Bank?
- Have sponge and coral cover changed over the past few years?

- What is the variation in benthic cover and species composition of sponges and corals among the years 2013 and 2015, and among sites along the bank?
- How is the variation in composition related to the years, presence of certain species, location of the sites or water depth?
- Which sponge species are dominant?
- Differs the sponge assemblage in 2013 and 2015 with sponge assemblages in 1972, 1986, and 2006?

# MATERIALS & METHODS

The Saba Bank research program 2011-2016 was conducted by IMARES to get insight into key ecological processes and determining the health status on Saba Bank. Data on benthic community was collected during expeditions to Saba Bank in 2011, 2013, and 2015. During the expedition in October 2015, 92 samples of sponges were collected from 12 different locations. Later on, samples were used for DNA analysis and morphological analysis. High definition photographs of sampled sponges were made, for visual identification. For each sample that was collected a photograph was made. Of these samples a DNA barcode was amplified, and a morphological slide and a spicule prep was made. After all analyzes were performed, a reference database for the identification of sponges on the Saba Bank was composed.

Transect data was collected on 11 different locations on Saba Bank in 2013, and 2015 (Figure 1; Table 1). On each location three 50 m transects were placed. All transects on a location started from the same point, the angle between the transects was 45° covering a large area, to minimize the effects of possible habitat heterogeneity. The transects were photographed from above, so that at least a square meter was visible. 50 photos per transect were made for identification of benthic cover. Since there are 3 transects per site, 150 photographs of quadrats per site were made. The photos were used to identify sponge abundance and cover benthic organisms.

Sponge abundance was compared with data from 1972 and 2010. In 1972, sponges were collected during a survey conducted by the Royal Dutch Navy for the Investigations of the Caribbean and Adjacent Regions Program (CICAR). The sponges collected during this survey were retained in Naturalis. In January 2010, a new survey was conducted to examine the sponge species composition on Saba Bank (Thacker *et al.,* 2010). A member of the dive team documented the presence of the sponges and photographed them. Samples were taken from specimens that represented a subset of the observed species for morphological analysis.


**Figure 1**. Map of Saba Bank, with the sites where samples and photo transects were taken.

**Table 1.** Sites that are sampled during the expeditions of 2013 and 2015.

| Site | Site code | Location (UTM) | Position on Bank | Depth 2013 | Depth 2015 |
|---|---|---|---|---|---|
| Dutch Plains | DP | 20 Q 452566 1905596 | South East | 27 | 21.5 |
| Scottish Hills | SH | 20 Q 456551 1909291 | South East | 17 | 17 |
| Gorgonian Delight | GD | 20 Q 463403 1908565 | South East | 29 | 19.5 |
| Paul's Cathedral | PC | 20 Q 470180 1909614 | South East | 26 | 24.5 |
| Coral Garden | CG | 20 Q 473320 1917831 | East | 23 | 23 |
| Tetre de Fleur | TdF | 20 Q 469207 1922078 | East | 16 | 18 |
| Erik's Point | EP | 20 Q 479140 1923479 | East | 30 | 25.75 |
| Twelve Monkeys | TM | 20 Q 476404 1930273 | East | 23 | 21.75 |
| Devils Corner | DC | 20 Q 473049 1935532 | North East | 33 | 23 |
| La Colline aux Gorgones | lCaG | 20 Q 471312 1937717 | North East | 25 | 23.5 |
| Rebecca's Garden | RG | 20 Q 469609 1941467 | North East | 25 | 24 |

***Photo analysis***

The sponges on the photographs, taken during the survey in 2015, were identified to species level using the website www.spongeguide.org (Zea *et al*., 2014). This website was consulted over a period of 8 months from January 2016 to August 2016. On spongeguide.org, more than

230 species morphs of Caribbean sponges are cataloged. It is possible to search on the physical characteristics of the sponges to find matching species. The identification of the photographed sponges was mainly to get to know the different sponge species present on Saba Bank, so that the sponges in the photo transects could be identified to species level. The identification of sponges by sight is considered to be difficult due to their high diversity and morphological variability, therefore DNA-barcoding was also performed on the sampled specimens (Diaz & Rützler, 2001; Erpenbeck *et al*., 2016).

***DNA-Barcoding***

Sponge samples were collected from 12 different locations during the 2015 Saba Bank survey. Samples were stored in 98% ethanol and kept in a -20°C freezer. DNA was extracted from the samples with the Qiagen DNeasy 96 kit using the animal tissue protocol (extraction protocol in Appendix; Qiagen, Hilden, Germany). This method was found to be successful in another study (Pöppe *et al*., 2010). Subsequently, the amount of DNA in the samples was quantified by using a spectrophotometer (TECAN, Männedorf, Switzerland). The quality of DNA was estimated by agarose gel electrophoresis (0.5% TBE buffer; 1.5% agarose; SERVA DNA stain G, SERVA, Heidelberg, Germany). Using a pipetting robot (TECAN Freedom EVO, Männedorf, Switzerland), new 96-well plates containing 50 µL DNA extract were prepared with DNA concentrations of 10 ng/µL. Samples that exceeded this concentration were diluted with MiliQ water by the TECAN robot.

Different PCR protocols were performed to find the method that allowed optimal amplification of the standard barcoding partition on the mitochondrial cytochrome oxidase subunit 1 (CO I) of the samples. In the end, a protocol using degenerate primers dgLCO1490 (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO2198 (5'-TAAACTTCAGGGTGACCAAARAAYCA-3') was found to be the most suitable method (Meyer *et al*., 2005; Eurogentech, the Netherlands). The primers are general CO I primers, which were used in barcoding of sponges before (Pöppe *et al*., 2010; Vargas *et al*., 2012).

Prior to PCR, a PCR cocktail was made containing 12.5 µL Master mix (OneTaq 2x Master Mix with Standard Buffer; New England BioLabs Inc., USA, MA), 0.5µL (10 µM) dgLCO1490, 0.5 µL (10 µM) dgHCO2198, 1.25 µL BSA (Bovine Serum Albumin; 10 mg/mL, Thermo Scientific, Maastricht, the Netherlands), 5.25 µL MilliQ, and 5 µL DNA template.

A standard three-step PCR program was used (thermocycler, Biometra, Göttingen, Germany), consisting of 3 minutes initial denaturation of 94°C, 34 cycles that consisted of 30 seconds denaturation of 94°C, 30 seconds annealing of 43°C, and 1 minute extension of 72°C. This was followed by a final extension step of 72°C for 5 minutes. During the PCR program, lid temperature was 105°C to prevent the formation of vapor on the lids of the PCR-tubes. After the program was finished, the PCR-machine switched to pause and the temperature was lowered to 4°C.

Amplification of DNA seemed to be difficult in some samples. For these samples, a PCR was performed with PCR-beads (illustra PuReTaq Ready-To-Go PCR Beads, GE Healthcare Life Sciences, Eindhoven, the Netherlands). These solid beads contain, polymerase, nucleotides, stabilizers, BSA, and reaction buffer. Only 20µL MilliQ and 5 µL DNA template have to be added. A three-step PCR program as mentioned before was used. When amplification failed after this, a new DNA extraction was performed using the QIAmp DNA mini kit (Qiagen, Hilden, Germany).

To see whether the PCR had succeeded and DNA was amplified, PCR products were visualized on a 1.5% agarose gel using electrophoresis. PCR products were stored in -20°C fridge prior to sequencing. Purification and sequencing was outsourced to Macrogen. A 96-well plate with 20 µL PCR product and primers were send to Macrogen, hereafter, results were send back via email. Sequences were analyzed in Geneious (trial version R9), first sequences were checked and if necessary, chromatograms were edited. Next, for each sequence a BLAST search in Genbank was performed to find similar sequences. Only matches with a similarity of at least 98% were used for identification of species, when similarity was lower the matches were used for identification of genus. For each sample, the 5 best matches were listed. Classification of species in Genbank is sometimes outdated, therefore species were checked in World Porifera Database (www.marinespecies.org/porifera/), this is a database where the most recent classification of sponges is incorporated.

### *Morphological analysis*

Slides of the sponge samples were made, in case that PCR would fail. Coupes were made of the sponge's ectosome and the choanosome. Tissue was placed on an object glass and covered with another object glass with some weight on it, so that the tissue stayed flat when the ethanol evaporated. Subsequently the sample was covered with a layer of Durcupan.

Durcupan is an epoxy for conserving tissues in microscopical slides, and should be prepared shortly before use (Sigma-Aldrich, Saint Louis, MO, USA). Multiple components are combined in order to get the embedding mixture (Supplementary). The object glass was placed on a heater on 50 C for at least 15 minutes, so that the Durcupan could spread through the tissue. Hereafter, a cover slip was placed over the sponge tissue. A little weight was placed on the cover slip, so that all of the sponge tissue was covered in Durcupan. Analysis of the slides was done after this master's thesis was finished.

Morphological analysis of sponge tissue is not often preformed on its own, but is carried out in combination with spicule analysis. Preparation of the spicules was performed after this master's thesis was finished. Sponge tissue (ectosome and choanosome) was placed in eppendorf tube and undiluted domestic bleach was added. After 60 min, as much bleach as possible was pipetted off. Demi water was added, and pipetted off after 15 min to let the spicules settle. Last ethanol was added, and spicules were stored until further identification later on.

***Cover identification***

Using the program CPCe, the cover of  different benthic groups (i.e. corals, sponges, turf algae, macro algae, cyanobacteria, crustose coralline algae) was analyzed. Corals and Sponges were identified up to species level. CPCe (Coral Point Count with Excel extensions) is software that is developed for determination of coral cover of transect photos. The program can distribute a number of random points on the image, species present under these points will be identified. Subsequently, species cover can be derived. In this study, two transects per site were analyzed. On these transects, every 5 m a quadrat was analyzed (i.e. on 5 m; 10 m; 15 m; 20 m; 25 m; 30 m; 35 m; 40 m; 45 m; 50 m). So 10 photos per transect were analyzed. In every quadrat 49 random points were analyzed.

The number of random points that was analyzed in each quadrat was 49 and thus 490 points per transect were identified, which is a sufficient number of points to get a good overview of the benthic cover. When the number of random points per plot increases, the number of species will increase following a saturation curve. Increasing the number of random points does not necessarily means that more species are found.

***Sponge abundance***

The same photos as in the cover identification were used. Thus, in the transects every 5 m a quadrat was analyzed. Presence and absence of all the Caribbean sponge species were assessed. Only sponges larger than 4 cm were scored, because it is very challenging to identify smaller specimens (de Voogd & Cleary, 2008). The website spongeguide.org was used to identify the sponges.

***Data analysis***

Prior to data analysis, data was square root transformed, because data was not normally distributed. To visualize benthic community composition stacked bar graphs and bubble plots were made. Cover of benthic groups and cover of sponge and coral species was analyzed in R using multivariate analysis. Both constrained and unconstrained ordination methods were used, because unconstrained ordination is a good method to show variation in the data, constrained ordination is a method to display only the variation that can be explained with the constraining variables. Multivariate analysis based on Bray-Curtis similarity index was used, this is mostly used for datasets that contain species and site data. Since the benthic cover data includes null values, a Bray-Curtis similarity index is a suitable index to use (Legendre & Anderson, 1999). The best methods that meet the requirements for analysis of the data were non metric multidimensional scaling (NMDS) and distance based redundancy analysis (dbRDA). First, NMDS, is an unconstrained ordination method that handles non-linear species responses. NMDS is used to find compositional variation and to relate this variation to the observed environmental variation. dbRDA, on the other hand, is a constrained ordination method based on eigenvalue analysis, which can use continuous variables as explanatory variables. Results of dbRDA can reveal whether the explanatory variables have significant impact on the dissimilarities found in the data and shows to which extent these explanatory variables influence the ordination of the data. Significance of the total analysis is tested with ANOVA. In addition, significance of axis, and effects of the explanatory variables are tested with a structured ANOVA model (Legendre & Anderson, 1999).

Linear regression analysis is used to find direct effect of explanatory variables on particular benthic groups. The measure to which the explanatory variable contributes to the variation of a benthic group is indicated with adjusted $R^2$ ($R^2$adj). This is used because $R^2$adj corrects for the number of parameters in the model, and gives an estimate of the degree of the relationship in the underlying population. Significance is tested with an F-test.

# RESULTS

In this section the cover of benthic groups, and coral and sponges species on the transects at different sites in 2013 and 2015 will be assessed using multivariate analysis. To find out which factors influence the species assembly, non-metric multidimensional scaling (nMDS), linear regression analysis and distance based redundancy analysis (dbRDA) were performed. nMDS and dbRDA were used to find out how coral or sponge species influenced the distribution of sites. Last, the species that were present in the surveys of 2013 and 2015 were compared with the species observed by Thacker *et al.* (2010).

### *Cover of benthic species*

Cover of the benthic community (i.e. sponges, corals, turf algae, macroalgae, CCA, cyanobacteria) in 2013 and 2015 on the sites was analyzed using CPCe. The proportion of the species present on each site was calculated by averaging the species proportions of transect A and B of each site (Table 2 ; Figure 2).

Sponge cover ranged from 4.96% to 13.62% in 2013 and from 5.60% to 13.88% in 2015. Average sponge cover was 9.67% ± 1.40 (95% confidence interval) in 2013 and 9.62% ± 1.48 in 2015. The live coral cover ranged from 2.77% to 9.45% in 2013 and from 3.85% to 12.37% in 2015. Average coral cover was 7.11% ± 0.92 in 2013 and 7.82% ± 1.26 in 2015. The site with the lowest coral cover in both 2013 and 2015 was Tetre de Fleur. In contrast with the other sites, Tetre de Fleur is located in the middle of Saba Bank, whereas other sites are near the reef drop off. On average, coral cover was lower than sponge cover. Only, some sites (i.e. Devils Corner and Twelve Monkeys) had a higher cover in corals than sponges Macroalgal cover ranged from 0.91% to 22.59% in 2013 and from 0% to 20.44% in 2015. On average the macro algae cover decreased from 10.89% ± 2.88 in 2013 to 9.01% ± 2.64 in 2015. The highest cover in 2013 and 2015 was observed at Tetre de Fleur. Focusing on Figure 2, Tetre de Fleur seems to be different in species composition, compared to the other sites.

On all sites the cover of turf algae decreased from 2013 to 2015. Cover ranged from 17.97% to 25.40% in 2013 and from 3.29% to 17.40% in 2015. Turf algae cover decreased from 21.45% ± 1.32 in 2013 to 12.02% ± 1.78 in 2015. At Rebecca's Garden and Tetre de Fleur the turf algae cover decreased even with 14.00% and 19.34% respectively. On most sites, there was an increase in the cyanobacteria cover, except for Scottish Hills, Paul's Cathedral, and Rebecca's Garden. Overall the cover increased with 3.22%, with a cover of 19.80% ± 2.49 in 2013 to 23.02% ± 2.22 in 2015. Cyanobacteria cover ranged from 13.12%

to 29.01% in 2013 and from 19.21% to 34.60% in 2015. Sand cover ranged from 7.48% to 18.01% in 2013 and from 10.26% to 35.36% in 2015. Highest sand cover was observed in Rebecca's Garden, the site nearest to Saba.

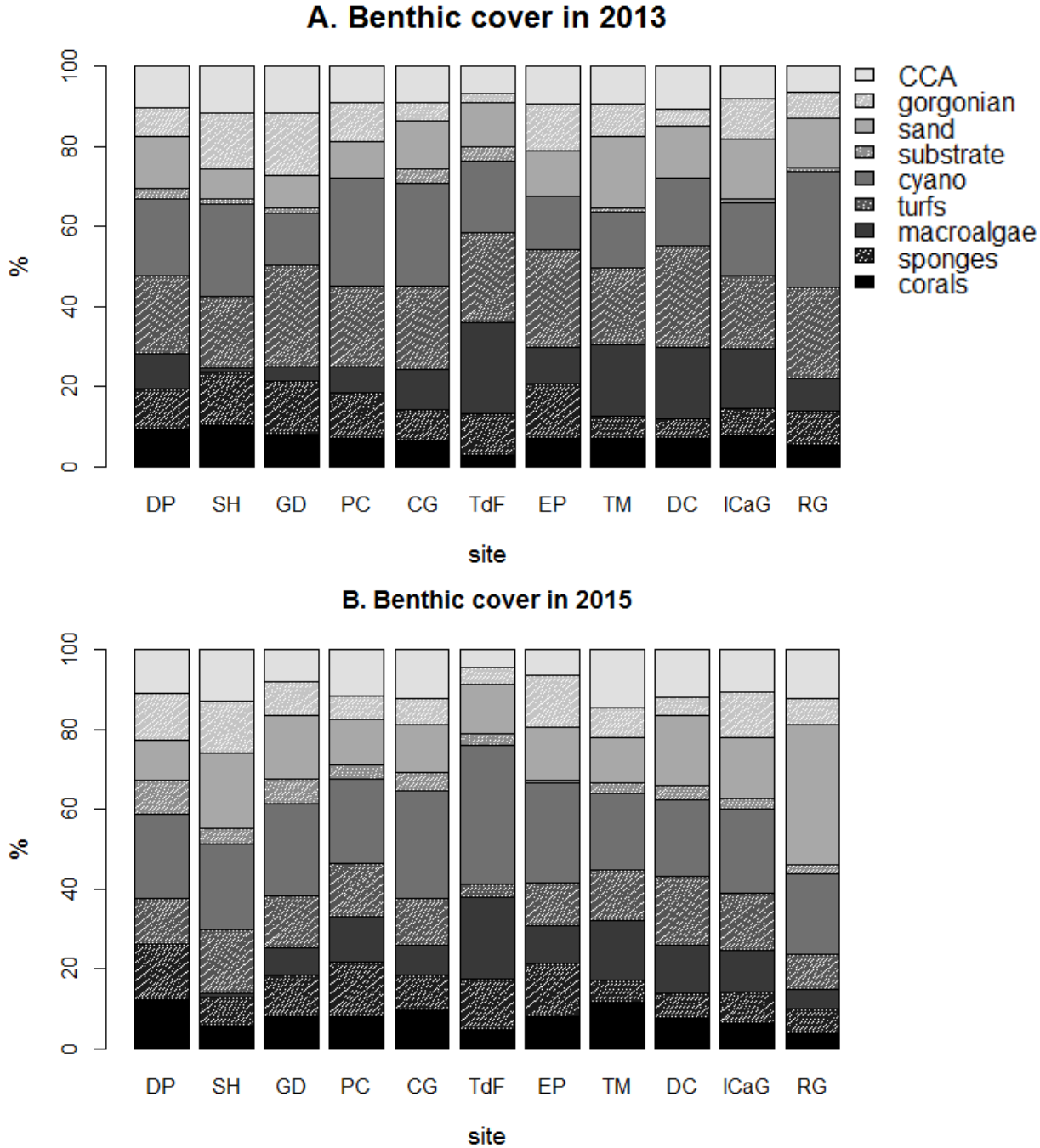
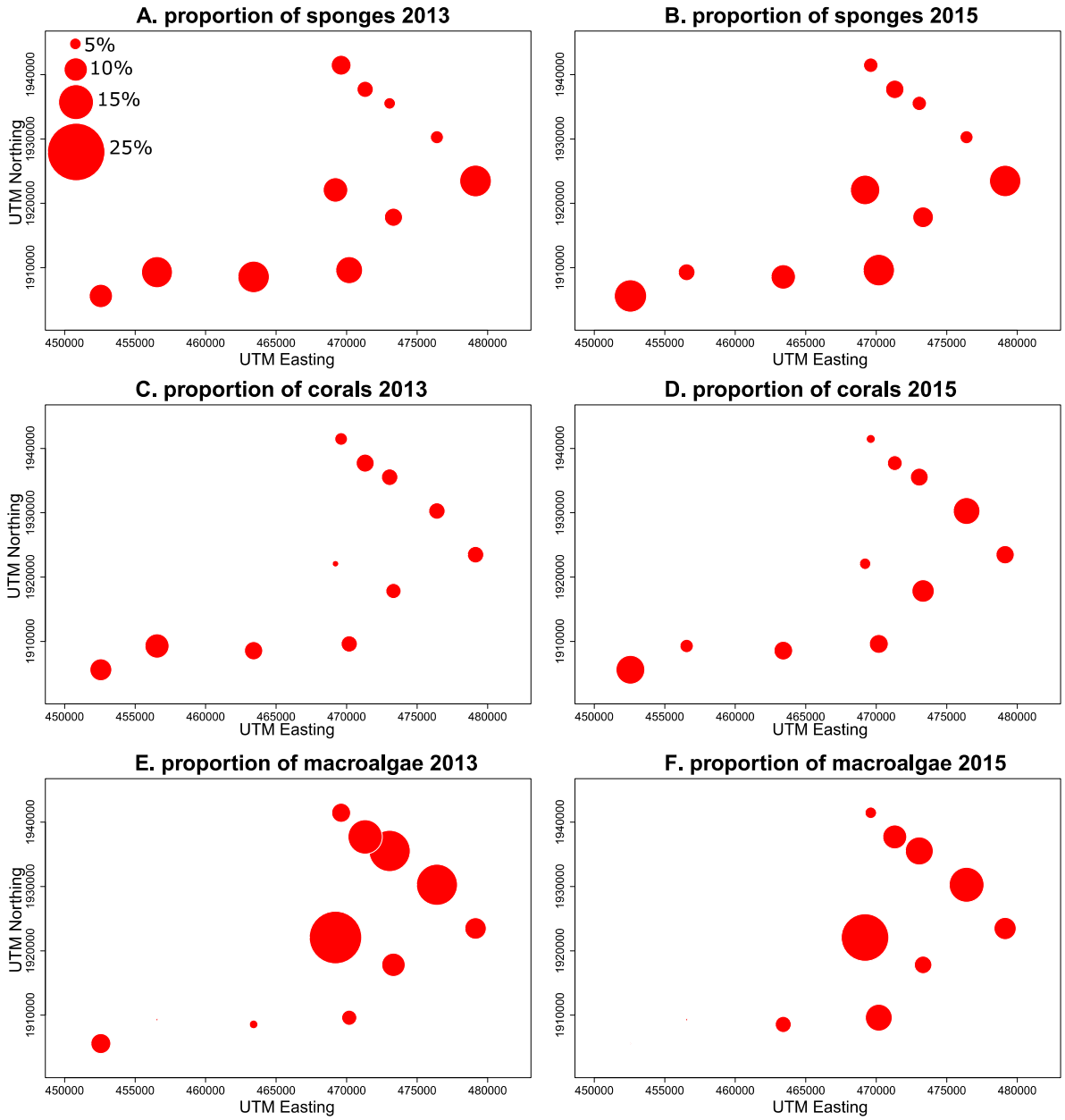



**Figure 2**. Proportions of groups present on different sites on Saba Bank, data is square root transformed. Sites are sorted from South to East; DP (Dutch Plains) is the most Southern site and RG (Rebecca's Garden) is located in the North East of Saba Bank, which is the site closest to Saba. **A**) Composition in 2013, a high turf algae cover is observed on all sites, macroalgal cover is high at TdF. **B**) Turf algal cover has decreased and cyanobacteria have become more abundant on all sites. A high sand cover is observed at RG

**Table 2**. Percentages of cover of benthic groups that were present on the different sites in 2013 and 2015, data was square root transformed. Cover of each benthic group per site is calculated by the average of two transects. Total mean is the average of all transects that were analyzed (n=22).

| Site | Site code | Year | Coral (%) | Sponge (%) | Macro algae (%) | Crustose coralline algae (%) | Turf algae (%) | Cyano bacteria (%) | Gorgonian (%) | Sand (%) | Substrate (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dutch Plains | DP | 2013 | 9.45 | 10.02 | 8.57 | 10.46 | 19.68 | 19.20 | 6.98 | 13.09 | 2.54 |
| | | 2015 | 12.37 | 13.88 | 0 | 11.06 | 11.31 | 21.35 | 11.49 | 10.26 | 8.29 |
| Scottish Hills | SH | 2013 | 10.39 | 13.33 | 0.91 | 11.64 | 17.97 | 22.96 | 14.03 | 7.48 | 1.30 |
| | | 2015 | 5.76 | 7.22 | 0.89 | 12.90 | 15.82 | 21.61 | 13.16 | 18.75 | 3.90 |
| Gorgonian Delight | GD | 2013 | 7.84 | 13.53 | 3.69 | 11.70 | 25.20 | 13.12 | 15.50 | 8.20 | 1.22 |
| | | 2015 | 8.03 | 10.48 | 6.88 | 7.95 | 12.91 | 22.95 | 8.73 | 15.81 | 6.26 |
| Paul's Cathedral | PC | 2013 | 6.98 | 11.58 | 6.50 | 8.97 | 19.96 | 27.17 | 9.86 | 8.97 | 0 |
| | | 2015 | 8.09 | 13.51 | 11.54 | 11.74 | 13.37 | 20.89 | 5.80 | 11.25 | 3.80 |
| Coral Garden | CG | 2013 | 6.50 | 7.78 | 10.05 | 8.97 | 20.68 | 25.76 | 4.77 | 12.07 | 3.47 |
| | | 2015 | 9.64 | 8.82 | 7.52 | 12.24 | 11.52 | 27.15 | 6.52 | 12.21 | 4.37 |
| Tetre de Fleur | TdF | 2013 | 2.77 | 10.53 | 22.59 | 6.66 | 22.63 | 17.93 | 2.23 | 11.28 | 3.38 |
| | | 2015 | 4.89 | 12.66 | 20.44 | 4.52 | 3.29 | 34.60 | 4.04 | 12.40 | 3.16 |
| Erik's Point | EP | 2013 | 7.07 | 13.62 | 9.23 | 9.43 | 24.23 | 13.45 | 11.49 | 11.48 | 0 |
| | | 2015 | 7.85 | 13.48 | 9.53 | 6.39 | 10.77 | 24.77 | 12.92 | 13.41 | 0.88 |
| Twelve Monkeys | TM | 2013 | 7.07 | 5.60 | 17.65 | 9.47 | 19.21 | 14.21 | 7.92 | 18.01 | 0.85 |
| | | 2015 | 11.49 | 5.60 | 14.99 | 14.63 | 12.66 | 19.36 | 7.31 | 11.46 | 2.51 |
| Devils Corner | DC | 2013 | 7.08 | 4.96 | 17.69 | 10.73 | 25.40 | 16.90 | 4.11 | 13.13 | 0 |
| | | 2015 | 7.64 | 6.17 | 12.04 | 11.84 | 17.40 | 19.21 | 4.76 | 17.65 | 3.29 |
| La Colline aux Gorgones | lCaG | 2013 | 7.68 | 6.98 | 14.75 | 8.18 | 18.33 | 18.07 | 10.04 | 14.81 | 1.17 |
| | | 2015 | 6.40 | 7.89 | 10.29 | 10.65 | 14.46 | 20.94 | 11.35 | 15.49 | 2.52 |
| Rebecca's Garden | RG | 2013 | 5.42 | 8.46 | 8.18 | 6.38 | 22.66 | 29.01 | 6.71 | 12.26 | 0.92 |
| | | 2015 | 3.85 | 6.15 | 4.93 | 12.31 | 8.66 | 20.36 | 6.34 | 35.36 | 2.05 |
| Mean ± 95% CI | | 2013 | 7.11±0.92 | 9.67±1.40 | 10.89±2.88 | 9.32±0.84 | 21.45±1.32 | 19.80±2.49 | 8.51±1.86 | 11.89±1.49 | 1.35±0.67 |
| | | 2015 | 7.82±1.26 | 9.62±1.48 | 9.01±2.64 | 10.57±1.37 | 12.02±1.78 | 23.02±2.22 | 8.40±1.55 | 15.82±3.12 | 3.73±1.10 |

Bubble plots show the same square root transformed benthic cover data as represented in Table 2. No large changes between coral cover in 2013 and 2015 are observed. However, sites in the northern parts seem to have a lower cover compared to sites that are more situated in the southern parts (Figure 3A+B). Also, sponge cover did not show large changes between 2013 and 2015 (Figure 3C+D). Macroalgae cover showed some changes over the years, the cover became lower in 2015. Besides, it seemed that the northern sites had a higher macroalgae cover (Figure 3E+F). Differences between years were clearly visible in turf algae cover and cyanobacteria cover. Turf algae cover decreased over time, whereas cyanobacteria cover increased over time (Figure 3G-J).



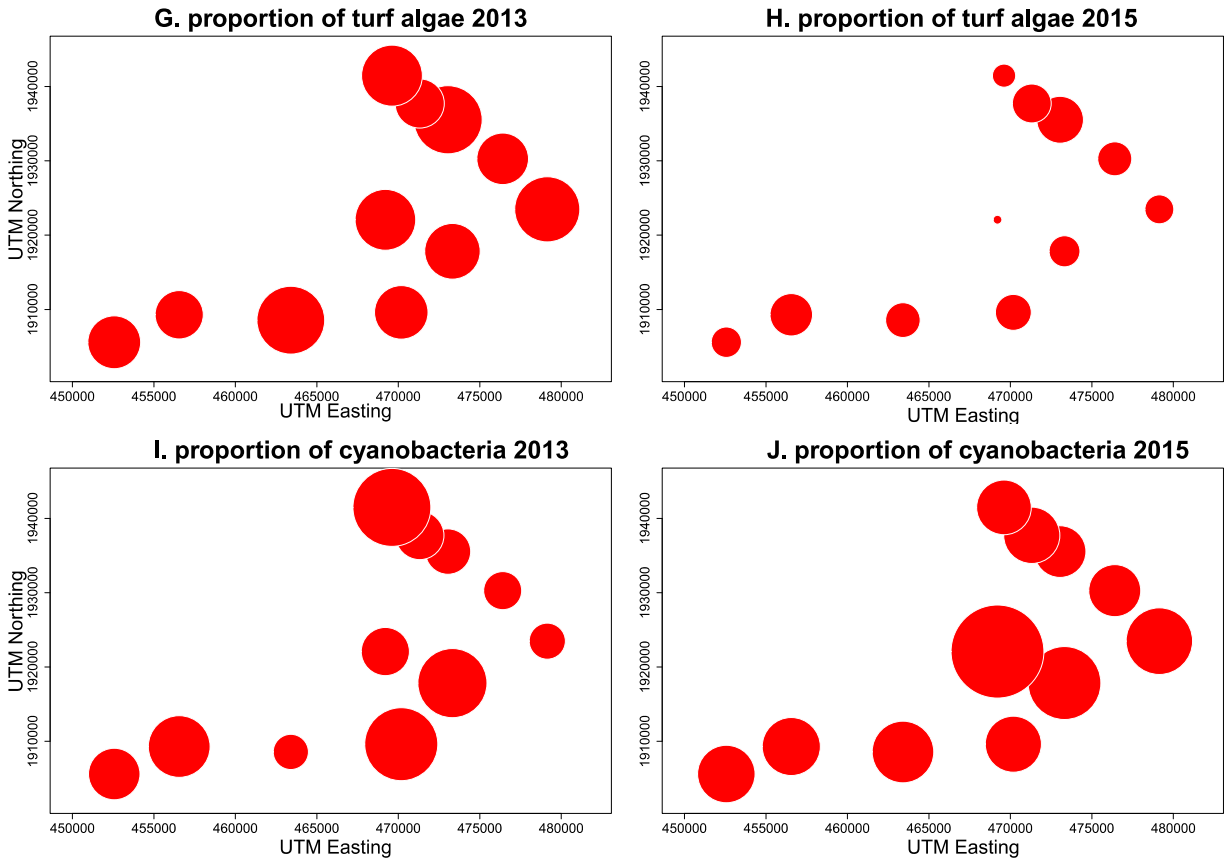



**Figure 3.** Percentages of benthic groups in 2013 and 2015, data was square root transformed. All plots were scaled in the same way, so that benthic groups and and years can be compared.

***Multivariate analysis benthic cover***

Sites are placed in a two-dimensional plane based on the benthic community that was present by using NMDS. Sites that are more similar in community composition are oriented closer to each other, while sites that differ are placed apart (Supplementary, Figure 15). To get a better visual overview, a cluster dendrogram was made of the same data used for the NMDS plot (x=0.6; Figure 4). In 2013 the transects were grouped into three clusters. Of the 2013 transect data, all transects from the same site were grouped in the same cluster, except for Dutch Plains (DP13.A and DP13.B). This means that DP13.A is more similar to Tetre de Fleur, la Colline aux Gorgones, Devils Corner, and Twelve Monkeys. Instead, DP13.B is more similar to Rebecca's Garden, Paul's Cathedral, Coral Garden, and Scottish Hills. In 2015 the transects were grouped in four clusters. Only the transects of Paul's Cathedral (PC15.A and PC15.B) were not grouped in the same cluster. PC15.A was more similar to Scottish Hills, la Colline aux Gorgones, Devils Corner, and Twelve Monkeys. PC15.B, however, was more similar to Dutch Plains, Gorgonian Delight, Erik's Point, and Coral Garden.

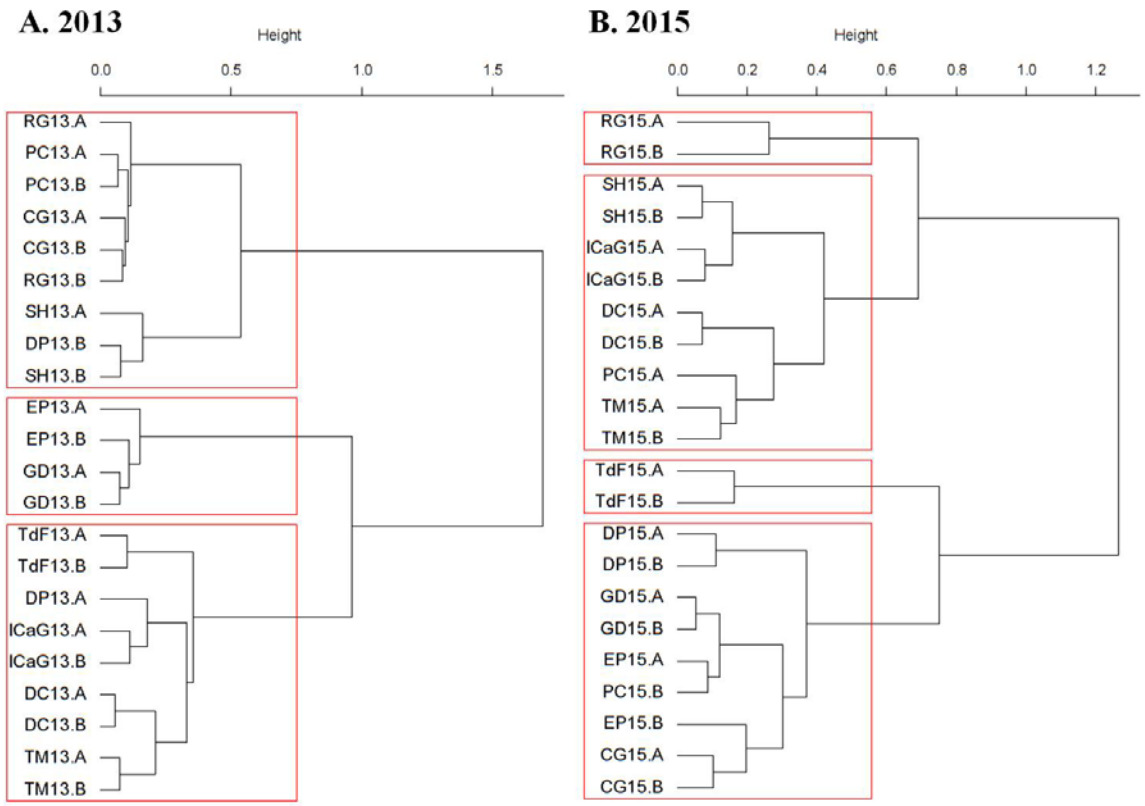



**Figure 4**. Cluster dendrograms were based on Bray-Curtis distances (x=0.6). **A)** In 2013, sites are clustered in three groups, both transects of each site are clusterd together in the groups, except for DP13.A and DP13.B (transects at Dutch Plains) **B)** In 2015, sites are clustered in four groups, transects from the same site are clustered into the same group, except for the transects PC15.A and PC15.B (transects at Paul's Cathedral)

When 2013 and 2015 benthic community data is combined, transects in 2013 are centered in the lower part of the graph and transects in 2015 are placed in the upper parts of the graph (Figure 5). This indicates that there is a distinction in community composition between the two years. The vectors of benthic groups give an indication how the distribution of sites is based on the presence of these groups. Sites that are centered in the lower parts of the graph are likely to have more macroalgae and turf algae, whereas sites that are in the higher parts of the graph contain more cyanobacteria (scores in supplementary, Table 5). To test which benthic groups are significantly important in the distribution of sites dbRDA is performed.

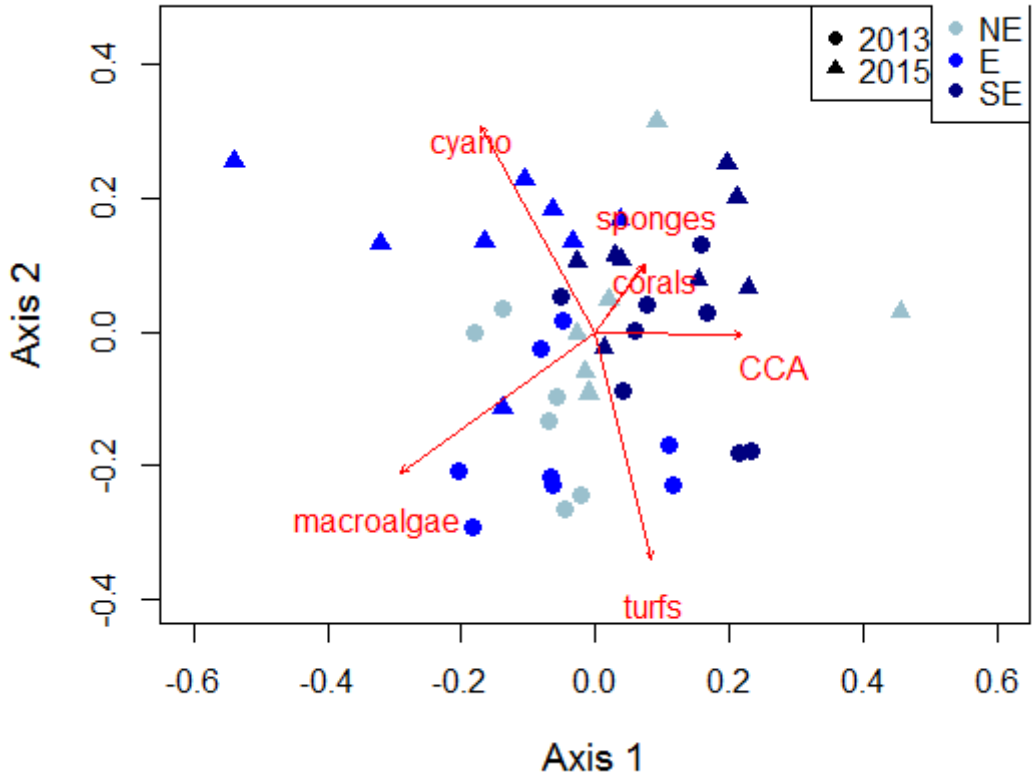



**Figure 5.** NMDS plot benthic community at sites in 2013 (circles) and 2015 (triangles). Transects in 2013 are centered in the lower part of the graph, whereas transects in 2015 are in the higher part of the graph (stress: 0.181). Vectors give an indication of which species could be important in the distribution of the sites.

Distance based redundancy analysis was performed in order to see how the environmental factors influenced the distribution of the sites in a two dimensional plot. The first two axis explain 75.2% of the cumulative variation in benthic community, the first axis explained 52.3% of this variation, and the second axis 22.9% (F=6.807, p=0.001). The third axis in the model was also significant and explained 18.9% of the variation in benthic community. Almost all environmental factors showed significant effect in the distribution of the sites, except for UTM_northing (depth: F=4.4386, p=0.002; UTM_northing: F=2.4080, p=0.038;

non living: F=4.7495, p=0.002; Year: F=11.2250, p=0.001). Depth and year were important in the distribution over the horizontal axis and UTM_easting and non living organisms played a significant role for the second axis.

Sites in 2015 are more clustered on the left side of the graph, whereas sites in 2013 are more clustered of the right side of the graph (Figure 6). The transects that were taken in 2013 were at a slightly greater depth than the sites in 2015. The sites on the right side of the diagram are associated with a relatively higher turf algae cover than the sites that are clustered on the left side of the diagram. Sites that are positioned in the east are more centered in the top part of the diagram, these sites are associated with a relatively higher macroalgae cover compared to the sites, which are more positioned in the lower parts of the graph.

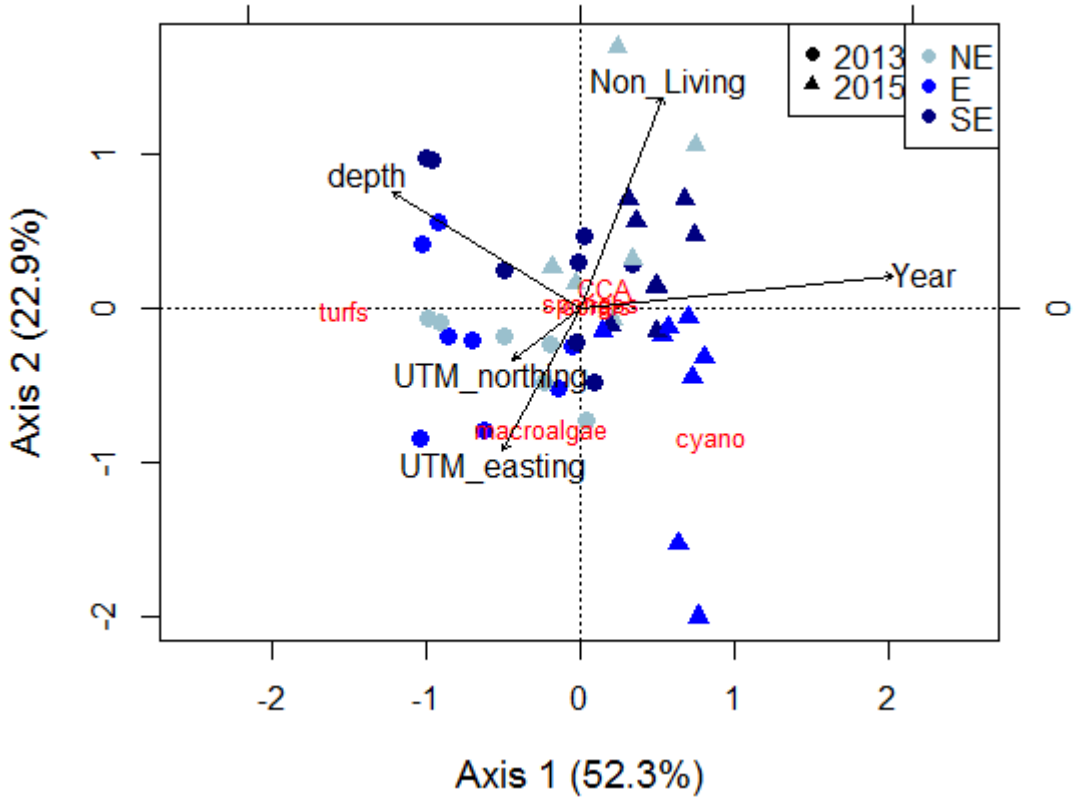



**Figure 6.** Distance-based redundancy analysis of benthic cover of sites at Saba Bank in 2013 (circles) and 2015 ( triangles). CAP1 explains 52.3% of the variation in benthic community between sites and CAP2 explains 22.9% of the variation. The clustering of sites is based on benthic community, the different benthic groups are showed i n red. Arrows in black indicate the contribution of environmental variables to the distributions of the site based on their benthic community. All environmental variables had a significant effect, except for UTM_Northing.

### *Linear regressions benthic cover*

Linear regression analysis in R was performed to test for correlation between different benthic groups (i.e. sponges, corals, macroalgae, turf algae, cyanobacteria or CCA) and environmental factors (i.e. depth, easting, northing, and year). Using the function lm, a linear model is fitted to the data, significance of the model is tested with F-statistics and a p-value. Four significance levels were distinguished; p≤ 0.001 (***), p≤0.01 (**), p≤0.05 (*), and p≤0.1 (‘). $R^2$adj indicates the proportion of variation in benthic groups that is explained by the independent variable (Table 3).

A significant positive correlation was found between depth and cover of turf algae, an increase in depth led to a higher cover of turf algae (Figure 7.A). Contrary, a weak negative correlation was found between cyanobacteria cover and the depth of the sites (Figure 7.B). A strong negative correlation was found between presence of turf algae and the year in which the benthic cover was assessed (Figure 7.C). The cover of turf algae significantly decreased over the years. In contrast to turf algae cover, cyanobacteria cover correlated positive with years, cover increased over time (Figure 7.D). However this correlation is not as strong as the correlation between turf algae and years. Thereby, it has to be taken into account that, on some sites, the transects were placed at different depths in 2013 and 2015. The depth of Gorgonian Delight and Devils Corner was 10 m deeper in 2013 compared to 2015. When these sites are excluded from analysis, the regressions of turf algae and depth and cyanobacteria and depth were not significant anymore ($R^2$adj: 0.0343; F= 2.243 & $R^2$adj: 0.01893; F=1.675, p>0.1). Also no significance was found for the regression analysis of cyanobacteria and year ($R^2$adj:0.02729; F=1.982, p>0.1). On the other hand, the regression analysis of turf algae and year, still turned out to be significant ($R^2$adj: 0.6596; F=68.83, p≤0.001).

**Table 3.** Results of the regression analysis between the environmental factors (i.e. depth, year, easting, and northing) and cover of benthic groups present on Saba Bank. $R^2$adj indicates the measure to which the environmental variable contributes to the variation of a benthic group. F-statistics were preformed to test for significance, significance level is indicated by asterisks.

| | **Depth** | | **Year** | | **Easting** | | **Northing** | |
|---|---|---|---|---|---|---|---|---|
| | **$R^2$adj** | **F** | **$R^2$adj** | **F** | **$R^2$adj** | **F** | **$R^2$adj** | **F** |
| Sponges | Ns | | Ns | | 0.05312 | 3.412‘ | 0.3795 | 27.29*** |
| Corals | Ns | | Ns | | 0.0455 | 3.05‘ | 0.1134 | 6.501* |
| Macroalgae | Ns | | Ns | | 0.2968 | 19.15*** | 0.1889 | 11.02** |
| Turf | 0.1894 | 11.05** | 0.6416 | 77.98*** | Ns | | Ns | |
| Cyanobacteria | 0.1165 | 6.672* | 0.06522 | 4‘ | Ns | | Ns | |
| Cca | Ns | | Ns | | Ns | | Ns | |
| *** p≤0.001; ** p≤0.01; * p≤0.05; ‘ p≤0.1; Ns=not significant | | | | | | | | |

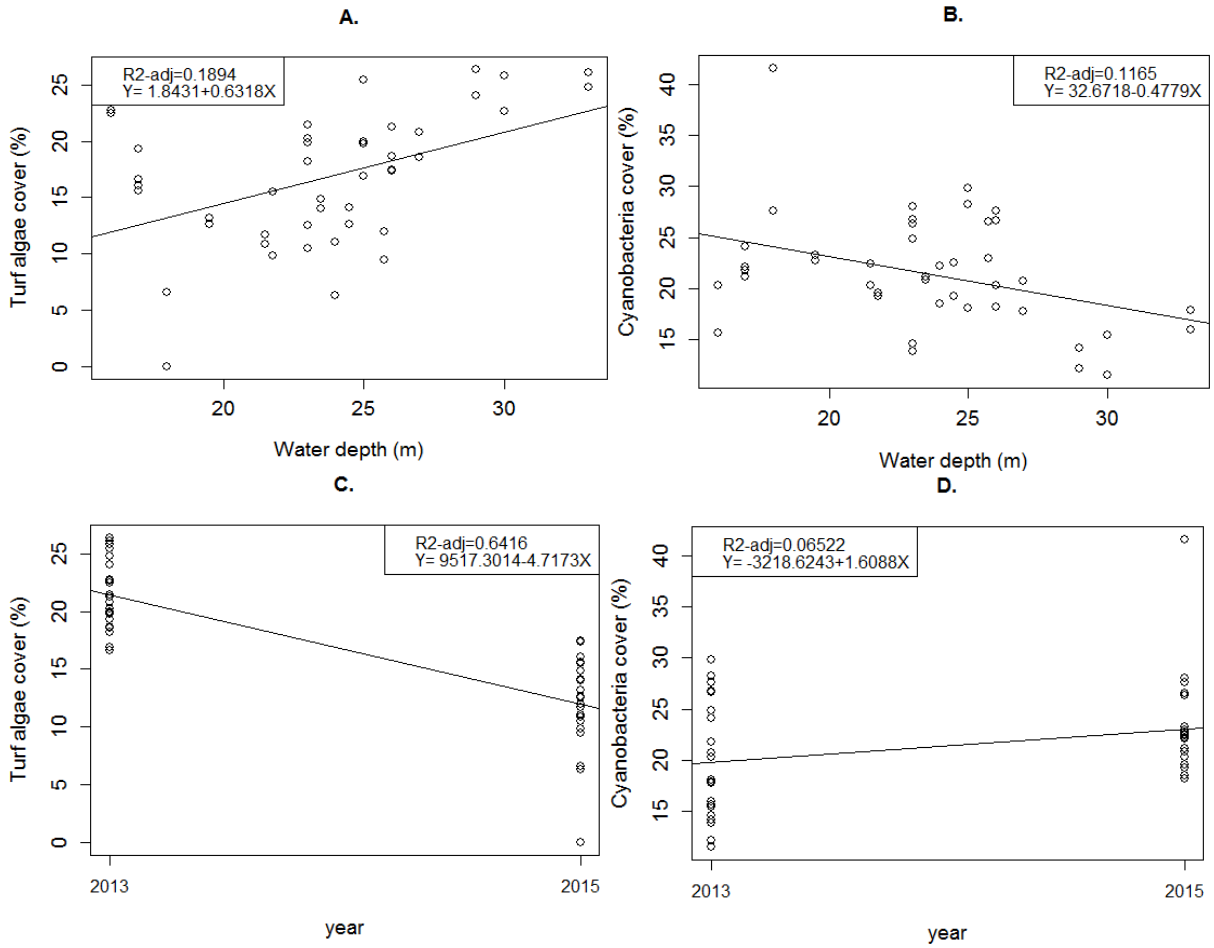



**Figure 7.** Linear regressions between water depth and turf algae (A), and cyanobacteria (B), together with linear regressions between year and turf algae (C), and cyanobacteria (D). For these plots, the sites Devils Corner and Gorgonian Delight were included. **A)** A positive regression between water depth and turf algae is observed. **B)** Regression between water depth and cyanobacteria cover was negative. **C)** Turf algae cover decreased significantly over the years, a negative regression was observed. **D)** Regression between cyanobacteria cover and year was positive, cover increased over time.

The location of the sites on the bank influenced the presence of some benthic groups. A positive correlation was found between macroalgae cover and easting of the site (Table 3; Figure 8.A). Thus when a site was located in the eastern part of the bank, the cover of macroalgae was higher. Also a significant positive correlation was found for northing and macroalgae cover, sites located in the northern parts of the bank had a higher macroalgae cover (Figure 8.B). Weak influences were found for easting in combination with coral or sponge cover, and northing with coral or sponge cover (Figures 8.C-F). When sites were more situated in the northern parts or the southern parts of the bank, coral and sponge cover were lower.

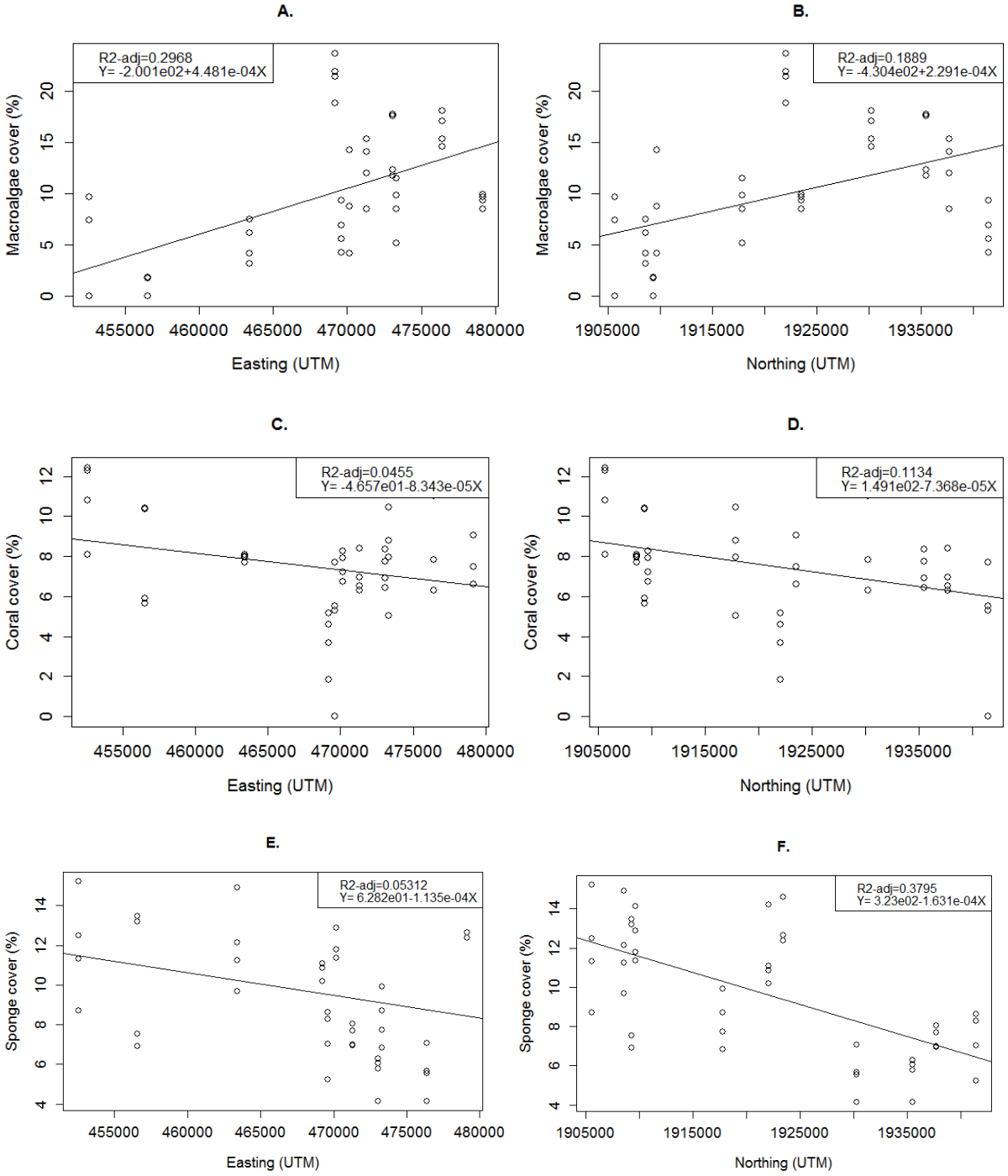



**Figure 8.** Linear regression analysis for location of the sites on Saba Bank and cover of macroalgae, corals, and sponges. **A)** Macroalgae cover increased at sites that were positioned in the eastern parts of the bank, a positive regression was found. **B)** Also, a positive regression was observed for northing and macroalgae cover. **C)** A negative regression was found for coral cover and easting, cover was lower at sites located in the east, compared to sites more situated in the western parts of the bank. **D)** Regression between northing and coral cover was tested to be negative as well. **E)** A significant negative regression was found between sponge cover and easting **F)** Last, a negative regression between sponge cover and northing was found, sponge cover in the northern parts of the bank was low and increased at sites more in the south.

***Multivariate analysis coral assemblages***

Species with highest cover in 2013 were *Montastraea faveolata* (34.7% of total coral cover) and *Porites asteroides* (14.6% of total coral cover). In total, 20 different coral species were observed in the quadrats. In 2015, *Montastraea faveolata* had the highest cover (45.5% of total coral cover), and a total of 24 species was found (Figure 9). Using NMDS, sites were grouped in a two dimensional plot based on Bray-Curtis similarities (Figure 10). For displaying species in the graph a cutoff point of $r^2>0.200$ was used. Species that contributed to distribution of sites were *Montastrea faveolata* (MFAV; $r^2=0.5018$), *Siderastrea siderea* (SS; $r^2=0.4716$), *Madracis decactis* (MD; $r^2=0.2411$), *Porities divaricata* (PD; $r^2=0.2210$), *Stephanocoenia michelinii* (SM; $r^2=0.2109$), and *Agaricia agaricites* (AA; $r^2=0.2066$). All species scores are showed in the supplementary (Table 6). Sites were clustered into two groups (x=2), however no patterns could be observed. Transects at the same site that were analysed in the same year were not nescessary clustered into the same group.

To get insight into the extent to which environmental factors (i.e. depth, year, easting, and northing) contributed to the distribution of sites, dbRDA was performed (Figure 11). The horizontal axis explained 52.62% of the variation between sites, and the vertical axis 25.48% ($F=2.3774$, $p=0.001$). Only year ($F=2.0895$; $p<0.05$) and depth ($F=4.1348$; $p\leq0.001$) had a significant effect on the coral assemblage of the sites. One of the species that was influenced by depth was *Montastraea faveolata* (MFAV), sites at a greater depth were associated with this species (Supplementary, Figure 16.A)

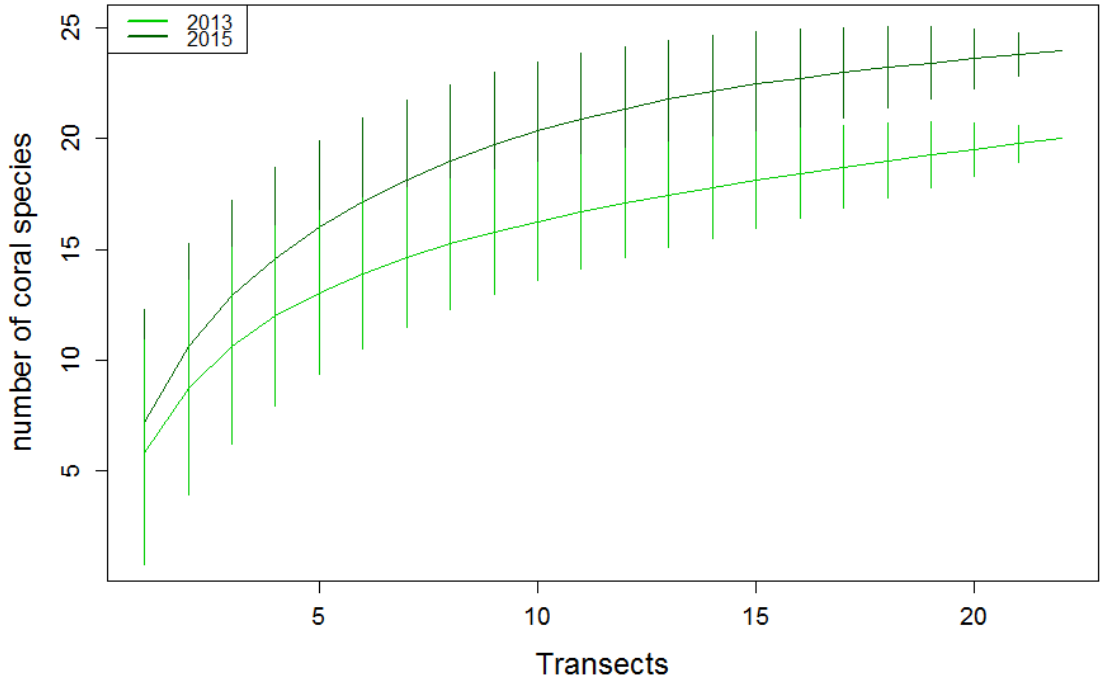



**Figure 9.** Species accumulation curves for coral species in 2013 and 2015 found in the CPCe benthic cover photo analysis. In 2013, a total of 20 species was found. In 2015, 24 different species were found.

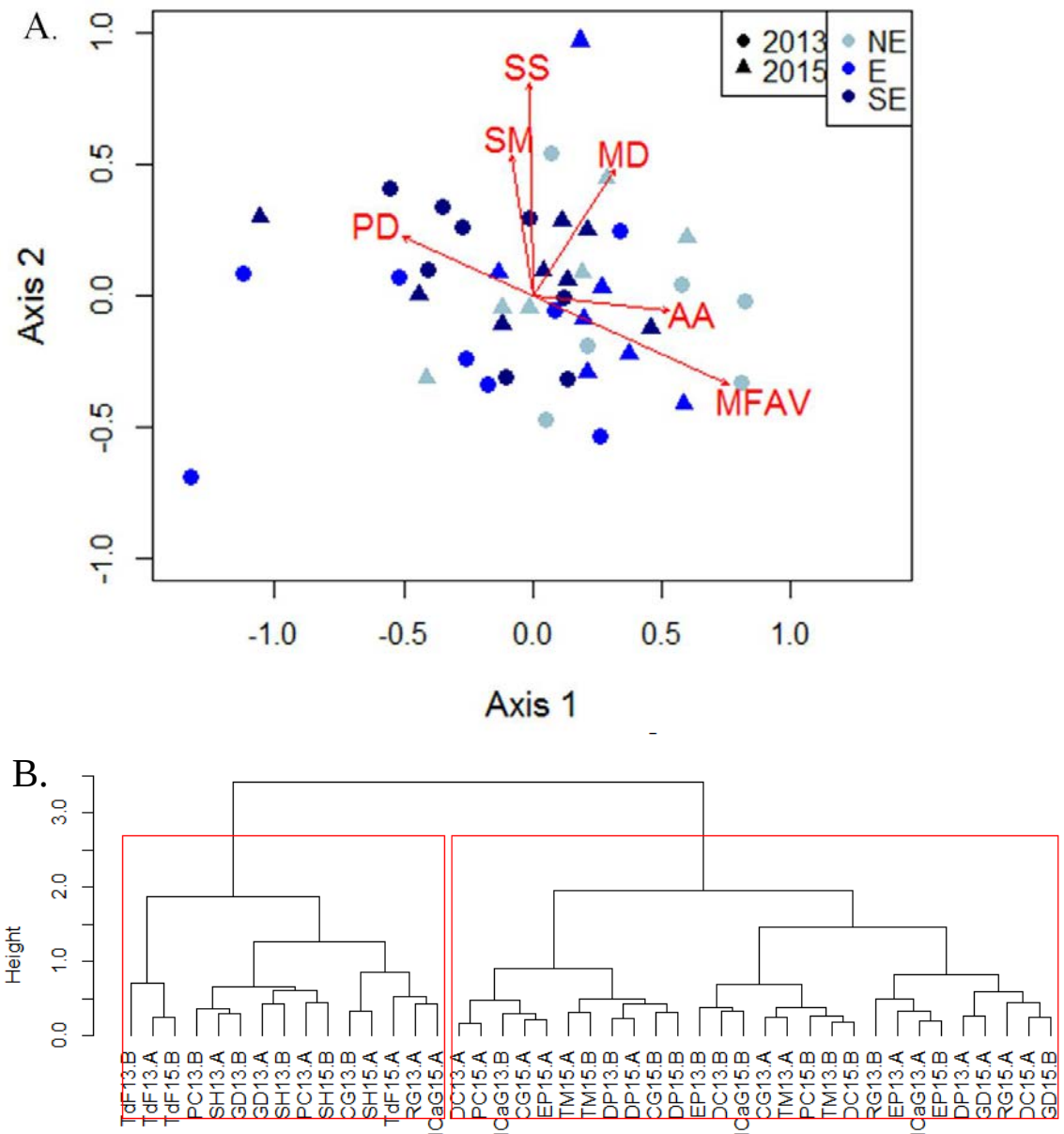



**Figure 10. A)** NMDS plot based on Bray-Curtis distances of coral species (stress=0.2020076). Sites in 2013 are represented as circles, sites in 2015 as triangles. A subdivision of sites in the Northeast (light blue), East (blue), and Southeast (dark blue) was made. The cutoff point for representing species was $r^2$>0.200. *Montastraea faveolata* (MFAV), *Siderastrea siderea* (SS), *Madracis decactis* (MD), *Porities divaricata* (PD), *Stephanocoenia michelinii* (SM), and *Agaricia agaricites* (AA) seemed to play a role in the clustering of sites. **B)** Sites are clustered into two groups (x=2) based on their Bray-Curtis distances. A and B are indicating transect A and transect B, and 13 and 15 are indicatin the years 2013 and 2015 in which the transect photos are taken.

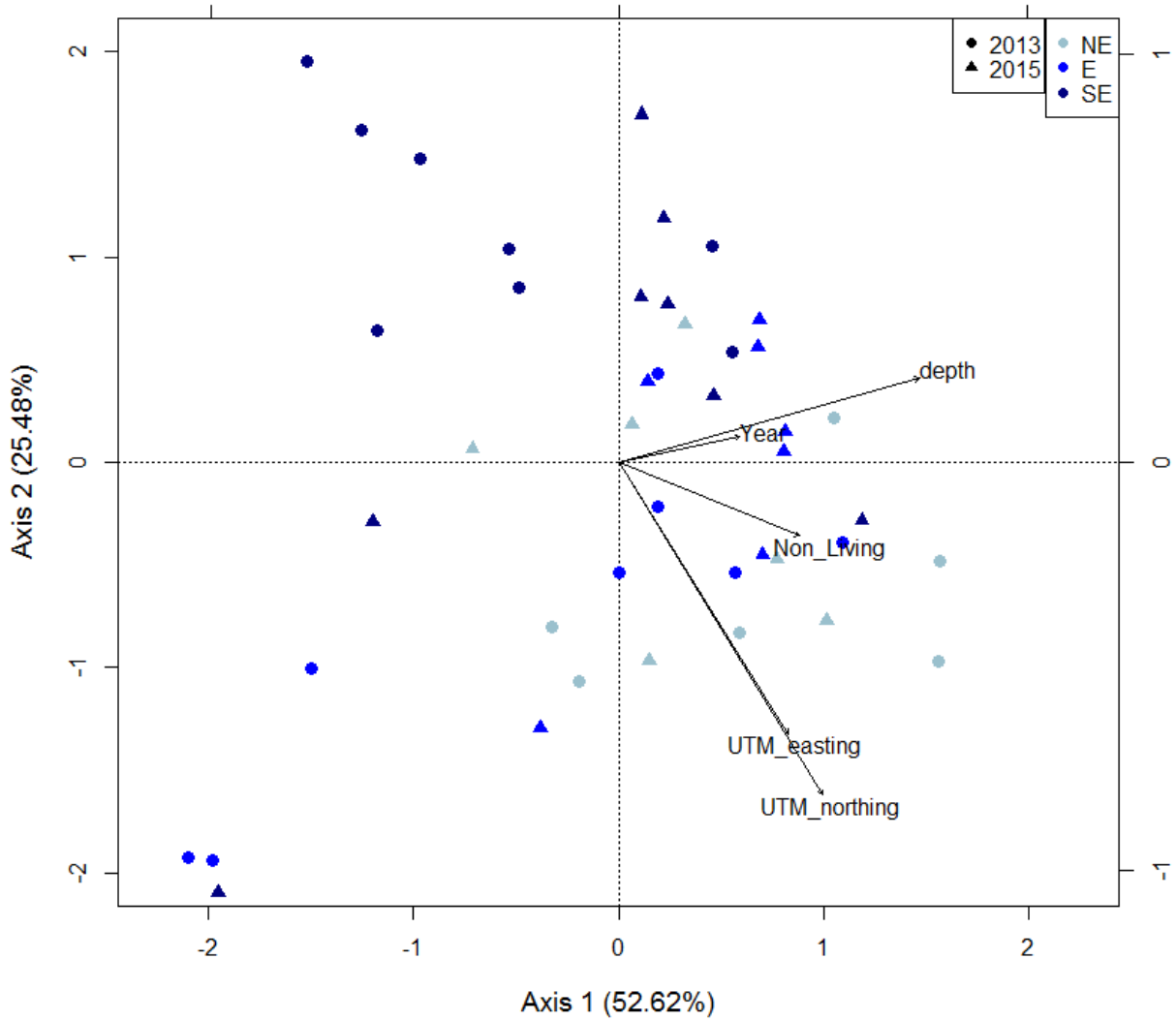



**Figure 11.** dbRDA of coral species on sites at Saba Bank in 2013 (circles) and 2015 (triangles). A division of the sites was made base on position on the bank (northeast = light blue; east = blue; southeast = dark blue). Axis 1 explains 52.62% of the variation in coral assemblage between sites and Axis 2 explains 25.48% of the variation. Environmental variables that had a significant effect on the coral assemblage of the sites were year and depth.

***Multivariate analysis sponge assemblages***

In 2013, highest sponge cover was observed by *Xestospongia muta* (11% of total sponge cover) and *Agelas sventres* (10.4%). The total number of sponge species found in the CPCe photo analysis in 2013 was 51 (Figure 12). Highest sponge cover in 2015 was again reached by *Agelas sventres* (15.4% of total sponge cover) and *Xestospongia muta* (12.9% of total sponge cover). Total number of sponge species counted in the CPCe photo analysis in 2015 was 56 (Figure 12). Sites were ordered using NMDS based on Bray-Curtis distances (Figure 13). For representing the most important sponge species a cutoff point of $r^2>0.200$ is used, otherwise the graph would be unclear. The species that contribute to the ordination of sites were *Cliona caribbea* (CC; $r^2=0.4984$), *Aplysina cauliformis* (ACa; $r^2=0.3476$), *Spirastrella coccinea* (SC; $r^2=0.3234$), *Monanchora arbuscula* (MA; $r^2=0.3229$), *Xestospongia muta* (XM; $r^2=0.2893$), *Niphates caribica* (NC; $r^2=0.2191$), *Agelas tubulata* (AT; $r^2=0.2130$), and *Amphimedon compressa* (ACo; $r^2=0.2070$). All species scores are showed in Table 7, supplementary. After clustering the sites based on their Bray-Curtis similarity (x=2), 3 clusters were formed. Transects at the same sites in both years were grouped together in a cluster. DC13 and DC15 (Devils Corner in 2013 and 2015) were not grouped in the same cluster as well as RG13 and RG15 (Rebecca's Garden 2013 and 2015). No patterns concerning position of the sites on the Bank or year are found.

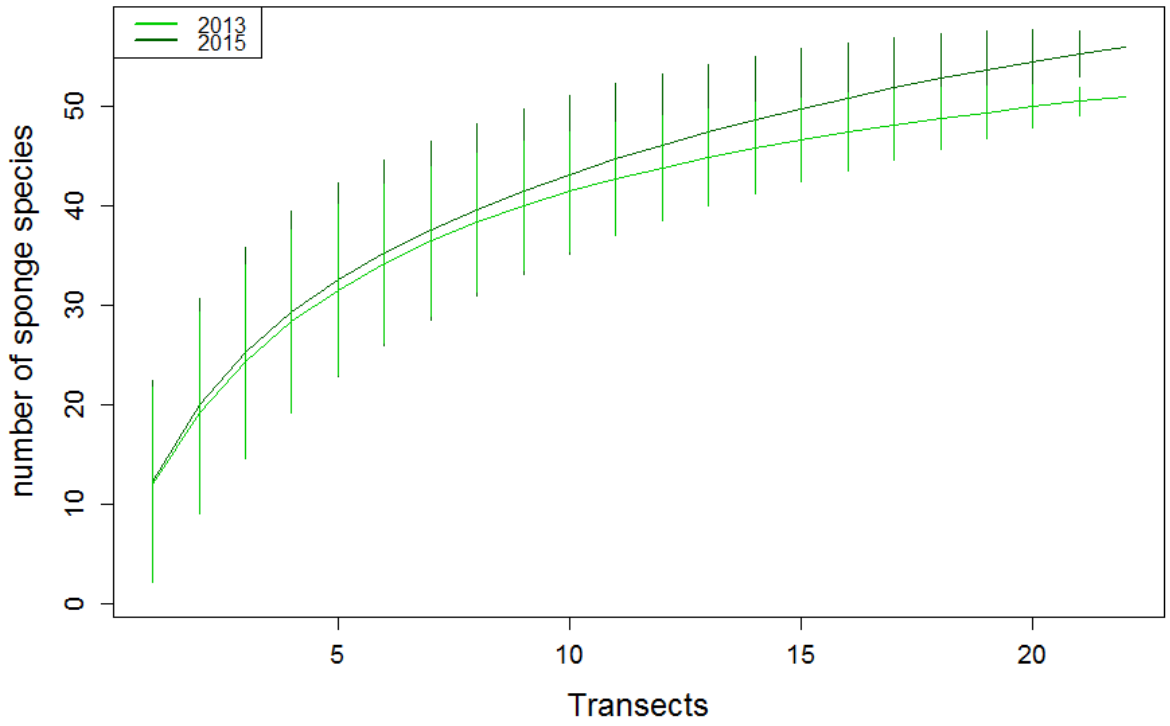



**Figure 12.** Species accumulation curves for sponge species in 2013 and 2015 found in the CPCe benthic cover photo analysis. In 2013, a total of 51 species was found. In 2015, 56 different species were found.

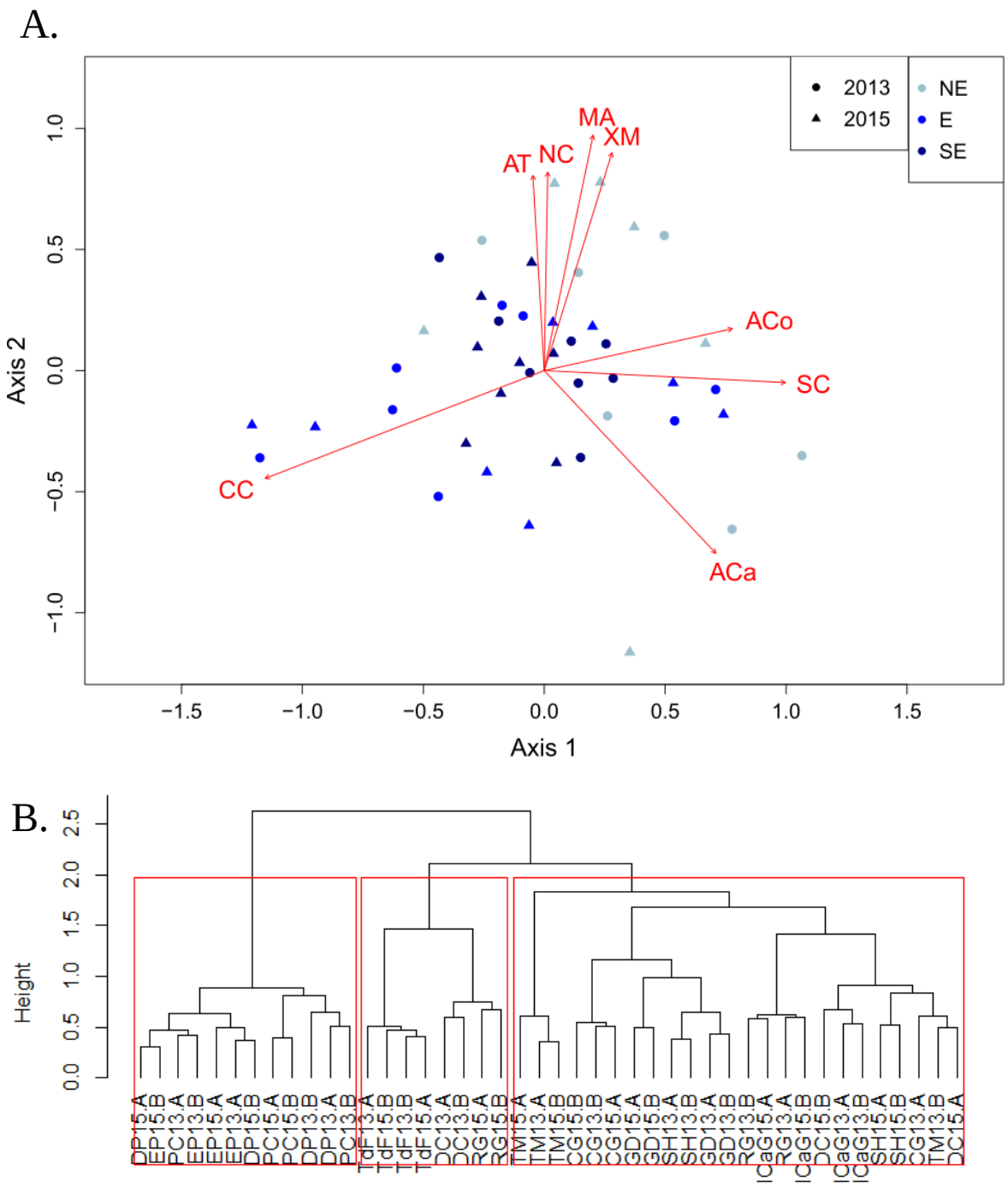



**Figure 13. A)** NMDS plot based on Bray-Curtis distances of sponge species present on the sites (stress=0.2136637). Sites in 2013 are represented as circles, sites in 2015 as triangles. A subdivision of sites in Northeast (lightblue), East (blue), and Southeast (darkblue) was made. The cutoff point for displaying species was $r^2$>0.200. *Cliona caribbea* (CC), *Aplysina cauliformis* (ACa), *Spirastrella coccinea* (SC), *Monanchora arbuscula* (MA), *Xestospongia muta* (XM), *Niphates caribica* (NC), *Agelas tubulata* (AT), and *Amphimedon compressa* (ACo) seemed to play a role in clustering of the sites. **B)** Sites are clustered into three groups (x=2) based on their Bray-Curtis distances. A and B are indicating transect A and transect B, and 13 and 15 are indicatin the years 2013 and 2015 in which the transect photos are taken.

Influence of environmental factors was tested using dbRDA (Figure 14). 60.3% of the variation between sites was explained by the axis, the horizontal axis explained 38.3% and the vertical axis 22.0% (F=2.0769; p=0.001). Environmental factors that had a significant effect on sponge assemblage at Saba Bank were depth (F=2.1149; p<0.005), UTM_northing (F=2.9554; p=0.001), and non-living (F=1.6205; p<0.05). No differences in sponge assemblage in 2013 and 2015 were found. The sites situated in the northeastern part of the bank are clustered in the bottom left part of the graph, this is associated with a higher cover of non-living things such as sand, rubble or bare substrate. The species *Spirastrella coccinea* seemed to be dependent of depth, sites at a shallower depth were associated with this species (Supplementary figure 16.B)

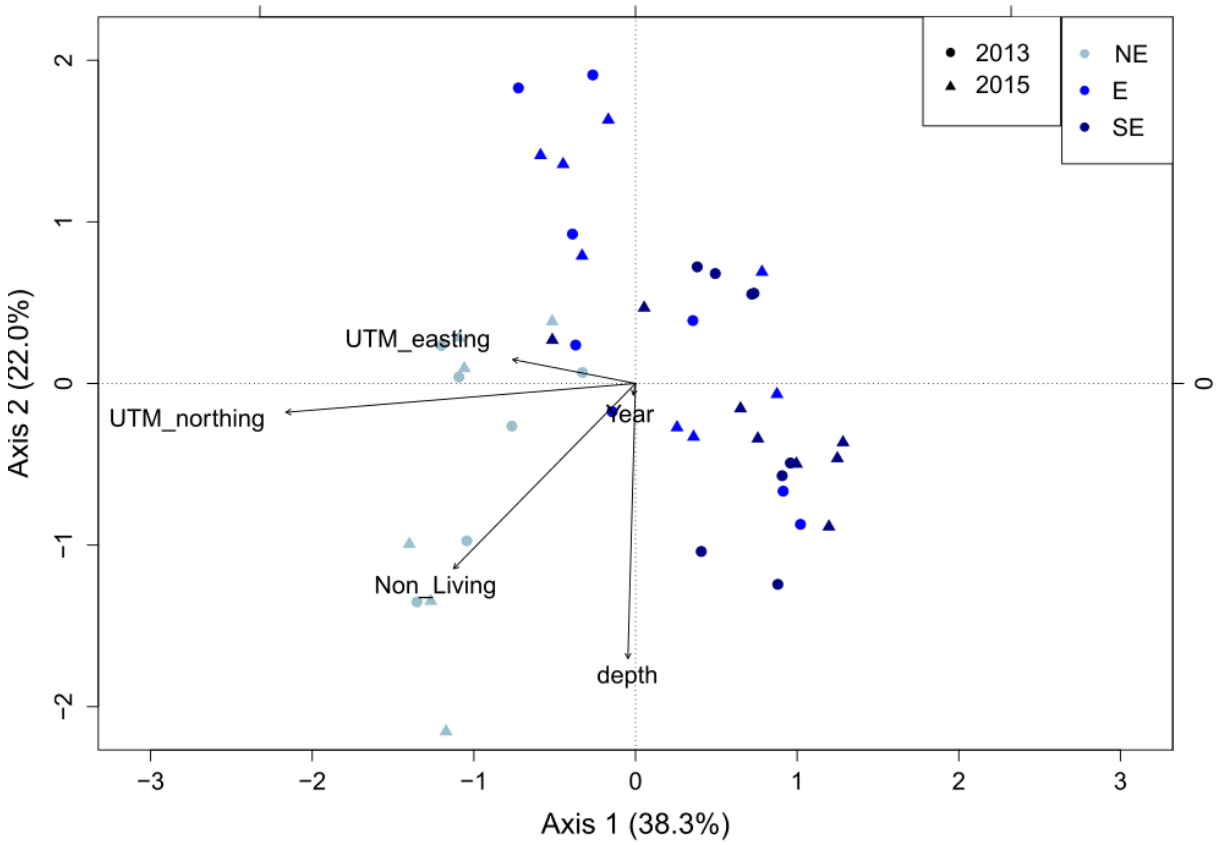



**Figure 14.** dbRDA of sponge species on sites at Saba Bank in 2013 (circles) and 2015 (triangles). A division of the sites was made base on position on the bank (northeast = light blue; east = blue; southeast = dark blue). Axis 1 explains 38.3% of the variation in sponge assemblage between sites and Axis 2 explains 22.0% of the variation. Environmental variables that had a significant effect on the sponge assemblage of the sites were depth, UTM_northing, and Non_Living.

***DNA barcoding***

DNA extraction succeeded for most of the 92 samples, average concentration was 29,15 ng/µL and ranged from 0.07 to 158.42 ng/µL. The DNA concentration of 11 samples was below 5 ng/µL, nevertheless PCR was performed on these samples. In 78 samples DNA was amplified after PCR, and after a second attempt with PCR-beads, DNA was amplified in two more samples. After sequencing, in 67 of the samples sponge DNA was amplified and a match was found in GenBank (Supplementary, Table 8). In 11 other samples, other DNA was amplified, including that of bacteria, annelids, crane-fly, diatoms, and cnidarians. Three of these samples were lionfish, it turned out that these specimens were collected during the expedition.

***Sponge assemblages***

Data on sponge abundance at Saba Bank was already collected prior to the most recent expeditions of the Saba Bank Research Program 2011-2016. In 1972 during the CICAR program, 57 different species were collected. In 1986, J. J. Vermeulen collected 29 different species from Saba Bank. During the expedition of Thacker *et al.* in 2006, 45 different species were observed (Thacker *et al.* 2010). Here, surveys of 20 to 45 min were done at six different sites during eight dives in the eastern part of the bank. In 2013 and 2015, benthic cover surveys were done for transects at 11 sites. At each site, 20 photos of a square meter were analyzed in CPCe for benthic cover with 49 random points, sponges were scored up to species level. In 2013, 49 different species were observed and in 2015 54 species. The photos of 2015 that were analyzed in CPCe were also used for analysis of total sponge abundance. Here, all species bigger than 4 cm were scored, here 79 different species were counted. There were 98 specimens that could not be identified using reference photos. Specimens were grouped based upon appearance, 57 groups were formed of sponges that looked the same. The sponges that were not identified were not included in Table 4. The species will be included after identification by a sponge-expert. Due to the morphological variability in a species, it is possible that some species are already included in table 4.

**Table 4.** Species observed during surveys on Saba Bank (edited from Thacker et al. 2010). Table is ordered by order, family, genus. For each expedition it is indicated which species were present (in green). 1972 indicates CICAR program; 1986 is the collection of J. J. Vermeulen; 2006 indicates the survey of Thacker *et al.*; 2013 CPCe indicates sponge species that are found in benthic community survey 2013; 2015 CPCe indicates sponge species that are found in benthic community survey 2015; 2015 abundance shows the species that were found in the total sponge abundance survey.

| Order | Family | Genus-species | Year of Expedition | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1972 | 1986 | 2006 | 2013 CPCe | 2015 CPCe | 2015 abundance |
| **Total number of sponge species observed** | | | **72** | **29** | **45** | **49** | **54** | **79** |
| **Calcarea** | | | | | | | | |
| Clathrinida | Leucettidae | *Leucetta floridana* | | | | | | |
| **Demospongiae** | | | | | | | | |
| Agelasida | Agelasidae | *Agelas cervicornis* | | | | | | |
| | | *Agelas cerebrum* | | | | | | |
| | | *Agelas citrina* | | | | | | |
| | | *Agelas clathrodes* | | | | | | |
| | | *Agelas conifera* | | | | | | |
| | | *Agelas dispar* | | | | | | |
| | | *Agelas sceptrum* | | | | | | |
| | | *Agelas sventres* | | | | | | |
| | | *Agelas tubulata* | | | | | | |
| Astrophorida | Ancorinidae | *Ancorina* sp. | | | | | | |
| | | *Stelletta stenospiculata* | | | | | | |
| Astrophorida | Geodiidae | *Erylus formosus* | | | | | | |
| | | *Geodia gibberosa* | | | | | | |
| | | *Geodia neptuni* | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| Chondrosida | Chondrillidae | *Chondrilla caribensis* | | | | | | | |
| Dendroceratida | Dictyodendrillidae | *Igernella notabilis* | | | | | | | |
| Dictyoceratida | Dysideidae | *Dysidea etheria* | | | | | | | |
| | | *Dysidea fragilis* | | | | | | | |
| | Irciniidae | *Ircinia sp.* | | | | | | | |
| | | *Ircinia campana* | | | | | | | |
| | | *Ircinia Felix* | | | | | | | |
| | | *Ircinia strobilina* | | | | | | | |
| | Spongiidae | *Spongia "obscura"* | | | | | | | |
| | | *Spongia (Spongia) pertusa* | | | | | | | |
| | Thorectidae | *Hyrtios proteus* | | | | | | | |
| | | *Hyrtios violaceus* | | | | | | | |
| | | *Smenospongia aurea* | | | | | | | |
| | | *Smenospongia conulosa* | | | | | | | |
| Hadromerida | Clionaidae | *Cervicornia cuspidifera* | | | | | | | |
| | | *Cliona caribbaea* | | | | | | | |
| | | *Cliona delitrix* | | | | | | | |
| | | *Cliona varians* | | | | | | | |
| | | *Cliona sp. "amber papillae"* | | | | | | | |
| | | *Spheciospongia vesparium* | | | | | | | |
| | | *Spheciospongia* sp. | | | | | | | |
| | Spirastrellidae | *Spirastrella coccinea* | | | | | | | |
| | Suberitidae | *Prosuberites laughlini* | | | | | | | |
| | | *Terpios fugx* | | | | | | | |
| | Tethyidae | *Tectitethya crypta* | | | | | | | |
| | Timeidae | *Timea unistellata* | | | | | | | |
| Halichondrida | Axinellidae | *Axinella morchella* | | | | | | | |
| | | *Dragmacidon explicatum* | | | | | | | |
| | | *Dragmacidon lunaecharta* | | | | | | | |
| | | *Dragmacidon reticulatum* | | | | | | | |
| | | *Phakellia folium* | | | | | | | |
| | | *Ptilocaulis walpersi* | | | | | | | |
| | Dictyonellidae | *Dictyonella funicularis* | | | | | | | |
| | | *Scopalina ruetzleri* | | | | | | | |
| | | *Svenzea zeai* | | | | | | | |
| | Halichondriidae | *Halichondria* | | | | | | | |
| | | *Halichondria (Halichondria) magniconulosa* | | | | | | | |
| | | *Halichondria (Halichondria) melanodocia* | | | | | | | |
| | | *Topsentia bahamensis* | | | | | | | |
| | Heteroxyidae | *Myrmekioderma gyroderma* | | | | | | | |
| | | *Myrmekioderma rea* | | | | | | | |
| Haplosclerida | Callyspongiidae | *Callyspongia (Callyspongia) fallax* | | | | | | | |
| | | *Callyspongia (Cladochalina) armigera* | | | | | | | |
| | | *Callyspongia (Cladochalina) plicifera* | | | | | | | |
| | | *Callyspongia (Cladochalina) vaginalis* | | | | | | | |
| | Niphatidae | *Amphimedon caribica* | | | | | | | |
| | | *Amphimedon complenata* | | | | | | | |
| | | *Amphimedon compressa* | | | | | | | |
| | | *Amphimedon erina* | | | | | | | |

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| | | *Amphimedon viridis* | | | | | | |
| | | *Cribrochalina dura* | | | | | | |
| | | *Niphates amorpha* | | | | | | |
| | | *Niphates caribica* | | | | | | |
| | | *Niphates digitalis* | | | | | | |
| | | *Niphates erecta* | | | | | | |
| | Petrosiidae | *Neopetrosia carbonaria* | | | | | | |
| | | *Neopetrosia proxima* | | | | | | |
| | | *Neopetrosia rosariensis* | | | | | | |
| | | *Neopetrosia subtriangularis* | | | | | | |
| | | *Petrosia (Petrosia) weinbergi* | | | | | | |
| | | *Xestospongia muta* | | | | | | |
| | Phloeodictyidae | *Aka xamaycaensis* | | | | | | |
| | | *Calyx podatypa* | | | | | | |
| | | *Oceanapia bartschi* | | | | | | |
| | Phloeodictyidae | *Oceanapia peltata* | | | | | | |
| Homosclerophorida | Plakinidae | *Plakortis angulospiculatus* | | | | | | |
| | | *Plakortis halichondrioides* | | | | | | |
| | | *Plakortis sp.* | | | | | | |
| Lithistida | Desmanthidae | *Petromica (Chaladesma) ciocalyptoides* | | | | | | |
| Poecilosclerida | Chondropsidae | *Batzella rubra* | | | | | | |
| | | *Batzella sp. "creamy salmon"* | | | | | | |
| | Crambeidae | *Monanchora arbuscula* | | | | | | |
| | Desmacididae | *Desmapsamma anchorata* | | | | | | |
| | Desmacellidae | *Neofibularia nolitangere* | | | | | | |
| | Hymedesmiidae | *Phorbas amaranthus* | | | | | | |
| | Iotrochotidae | *Iotrochota birotulata* | | | | | | |
| | Microcionidae | *Artemisina melana* | | | | | | |
| | | *Clathria bulbotoxa* | | | | | | |
| | | *Clathria calla* | | | | | | |
| | | *Clathria curacaoensis* | | | | | | |
| | | *Clathria faviformis* | | | | | | |
| | | *Clathria (Thalysias) juniperina* | | | | | | |
| | | *Clathria spinosa* | | | | | | |
| | | *Clathria virgultosa* | | | | | | |
| | | *Clathria sp.* | | | | | | |
| | | *Pandaros acanthifolium* | | | | | | |
| | Mycalidae | *Mycale (Arenochalina) laxissima* | | | | | | |
| | | *Mycale (Mycale) laevis* | | | | | | |
| | Raspailiidae | *Ectyoplasia ferox* | | | | | | |
| | Tedaniidae | *Tedania (Tedania) ignis* | | | | | | |
| | | *Tedania (Tedania) klausi* | | | | | | |
| Spirophorida | Tetillidae | *Cinachyrella alloclada* | | | | | | |
| | | *Cinachyrella arenosa* | | | | | | |
| | | *Cinachyrella kuekenthali* | | | | | | |
| Verongida | Aplysinellidae | *Suberea sp. "soft Aplysina lacunosa"* | | | | | | |
| | Aplysinidae | *Aiolochroia crassa* | | | | | | |
| | | *Aplysina archeri* | | | | | | |
| | | *Aplysina cauliformis* | | | | | | |

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|---|---|---|---|---|---|---|---|
| | *Aplysina fistularis* | | | | | | |
| | *Aplysina fulva* | | | | | | |
| | *Aplysina insularis* | | | | | | |
| | *Aplysina lacunosa* | | | | | | |
| | *Aplysina sp. "long branchelets"* | | | | | | |
| | *Verongula gigantea* | | | | | | |
| | *Verongula reiswigi* | | | | | | |
| | *Verongula rigida* | | | | | | |
| | *Verongula sp.* | | | | | | |

# DISCUSSION

In this study, benthic community was assessed using CPCe in order to find out whether community structure was changing from 2013 to 2015. Benthic community on Saba Bank gives insight into its health status. Using multivariate analysis, the effects of environmental factors such as water depth, year, easting, northing, and non living cover, were tested for their influence on community structure. Besides, this study was focusing on coral and sponge community structure in particular. Both, corals and sponges were identified to species level in CPCe, and drivers for their assemblages were examined. Total sponge abundance was compared with surveys preformed in 1972, 1986, and 2006. Since visual identification of sponges is considered to be difficult, DNA barcoding was preformed for 92 specimens sampled in 2015. High resolution photos of the same specimens were used, in combination with morphological analysis, to make a reference guide for identifying sponges present at Saba Bank.

### *Changes in benthic cover among 2013 and 2015*

There is variation between sites and between the years 2013 and 2015. Part of the variation in benthic cover between sites can be explained by the spatial variation on the sites. However, depth was varying between the sites and also between years, this may also result in differences in benthic cover. In future, there should be aimed to place all the transects at the same depth.

For sponge cover and coral cover, no changes were found among the years 2013 and 2015. Strikingly, a strong decrease for turf algae cover was observed, whereas cyanobacteria cover increased. dbRDA and regression analysis both showed that the changes in turf algae cover en cyanobacteria cover were significant. Only when the sites Gorgonian Delight and Devils Corner were excluded, due to their great difference (10 m) in water depth in 2013 and 2015, regression of year and cyanobacterial cover was not significant anymore. Depth is known to be a driver for reef organisms including corals, this is caused by decreasing in irradiance with increasing water depth (Huston, 1985; Roberts *et al.*, 2015). However, a study showed that low light availability is not affecting cyanobacteria (Bell *et al.*, 2005). It seems more likely that the loss of eight data points influenced significance after excluding these sites.

Coral cover at Saba Bank is low compared to the mean Caribbean cover of 16% (Schutte *et al.*, 2010). In the study of Schutte *et al.* (2010), data is used from 3777 coral cover

studies at different reefs in the Caribbean. Average water depth of the combined studies was 8.2 m, whereas water depth in this study was 23.4 m on average. Corals are limited by decreasing irradiance when depth increases, however, disturbances including storms or bleaching are less severe in deeper waters (Roberts *et al.*, 2015). This results in changes in communities along depth gradients.

Also, sponge cover was not considered to be high, although it was higher than coral cover. Aronson *et al.* (2002) found a strong increase in sponge cover to 43% after a bleaching event in Belize. In this case the reef was shifting to a sponge dominated system. The average cover of 7% in this study seems nowhere near a shift to a sponge dominated reef.

On the other hand, cyanobacteria cover of 26% in 2013 and 36% in 2015 is high compared to other studies in the Caribbean basin. In Grenada, a cover up to 8.8% was found, and in the Florida Keys cover was even lower than 3% (Kuffner *et al.,* 2006; Anderson *et al.,* 2014). Even in the study of de Bakker *et al.* (unpublished), where cyanobacteria were observed to be the most dominant benthic component in 2013, cover reached only 22%. Turf algal cover on other reefs at Bonaire and Curacao ranged from 22% to 45% (Sandin *et al*., 2008; de Bakker *et al*., unpublished). Turf algal cover observed in 2013 is in line with these other studies, however, the 11% cover observed in 2015 is rather low.

Shifts to high algal cover and decreasing coral cover are observed throughout the whole Caribbean basin (Hughes, 1994; Gardner *et al.,* 2003). Increasing nutrient conditions, reduced grazing in combination with disturbances, and other perturbations such as coral diseases or bleaching events are drivers for shifts from coral dominated systems to another dominant species like macroalgae or turf algae (McManus & Polsenberg, 2004; Vermeij *et al*., 2010). Shifts towards cyanobacteria on coral reefs are not extensively described in literature yet, but there is a study that describes cyanobacterial dominance in the Caribbean. De Bakker *et al.* (unpublished) found in their long term study a shift from stony coral to turf algae and fleshy macroalgae to cyanobacterial dominance now. Besides, periodic blooms of cyanobacteria are observed on reefs (Albert *et al.,* 2005; Paul *et al*., 2005).

A shift from turf algae to cyanobacteria can be initiated by several drivers. Turf algae consist of multiple species, including chlorphyta, phaeophyta, and rhodophyta species, but also cyanobacteria (Connel *et al*., 2014). Cyanobacteria become dominant in late stages of succession of algal turfs (Fricke *et al.,* 2011). Due to their high content of secondary metabolites, cyanobacteria experience less grazing and are protected against potential competitors (Thacker *et al*., 2001; Paul *et al*., 2005). Under elevated temperatures and ocean acidification the cyanobacteria species, *Lyngbya*, started to dominate in turf communities and

had the potential to outcompete the turf algae (Bender *et al.*, 2014). Last, increasing input of nutrients can play a role in flourishing of cyanobacteria. Normally, cyanobacteria limited by low levels of phosphorus, iron, and DOM. Increase of these nutrients can lead to proliferation of cyanobacteria (Albert *et al*., 2005, Brocke *et al*., 2015). The nutrient input from nearby islands is considered to be low at Saba Bank, therefore it seems more likely that increase in cyanobacteria cover is caused by increasing water temperatures or grazers that are avoiding the cyanobacteria present in turf algae. However, this could not be tested in this study, since no data was available.

***Position of sites on Saba Bank influencing benthic community***

Significant regressions were found for position of the site on the bank and macroalgae, corals and sponges. A positive correlation was found for macroalgae and easting and northing, the other way around, a negative correlation was found for coral cover and easting and northing. For sponges, only a significant linear regression was found for northing. The gradients in macroalgae, sponge, and coral cover may be caused by influences of nearby islands. Saba is located north of the Saba Bank and St. Eustatius and Saint Kitts and Nevis are located east of Saba Bank. Potentially, nutrient runoff will cause an increased algal growth at sites that are situated closer to this islands. The effects of nutrient runoff are mostly described to reefs immediately adjacent to islands (Reopanichkul et al. 2010; Brocke et al. 2015). Saba is less than 10 km away from the nearest site, whereas the distance between St. Eustatius and St. Kitts and Nevis and the nearest site on Saba Bank are 25 km or more apart. Because the distances from landmasses are large, it was always thought that Saba Bank was relatively free of problems concerning sedimentary and nutrient runoff (Littler et al. 2010). However, it would be interesting, whether there is a connection between the islands and the bank caused by oceanic currents. If this is the case, there is a possibility that the currents transport nutrients towards Saba Bank. McLaughlin et al. (2003) suggested that at some reefs a long-range connection between runoff and reef habitat exist.

***Coral assemblages***

In 2013, a total of 20 different species was found on Saba Bank. Two years later, in 2015, 24 species were found. In a previous assessments of stony corals on Saba Bank a total of 33 species was documented, combined with other studies a total of 43 species was found (McKenna & Etnoyer, 2010). In the study of McKenna & Etnoyer, species abundance of stony corals on 18 sites was reported using rapid assessment techniques. The main difference

between both studies is the sampling method. Two roving scuba divers identified corals during a dive in the study of McKenna & Etnoyer (2010), whereas in this study, photo transects were analyzed after the dives. It is expected that more coral species are present at Saba Bank, common species are likely to be found, to find rare species, more sites should be examined and more effort in searching has to be taken. The number of species found on Saba Bank is low compared to other Caribbean reefs. This is cause by the average depth of Saba Bank, which is deeper than in most studies. Besides, the sites that are studied on Saba Bank are not all located on a slope, whereas most Caribbean reefs are situated on a slope from shallow to deep, resulting in higher species abundance.

In this study, *Montastraea faveolata* contributed most to the total coral cover of Saba Bank. *M. faveolata* is the most abundant reef-building coral throughout the Caribbean (Aronson *et al*., 2008). Abundance of this species was associated with depth, at a greater depth the cover of *M. faveolata* is higher (Supplementary, Figure 14.A). This species is the most abundant coral between 10 m and 20 m and its lower depth limit is considered to be 40 m (Aronsen *et al*., 2008).

Coral assemblage was driven by year and water depth. Positioning of the sites on Saba Bank was not a driver, since no differences in coral assemblages between sites was found. This shows that there is a possibility of connectivity between sites on Saba Bank, a connection between sites can exist through movement of water masses (Mumby, 1999). De Bakker *et al*. (2016) already showed that there was genetic connectivity between populations on Saba Bank, for *Xestospongia muta* and *Montastraea cavernosa.*

***Sponge assemblages***

Sponge assemblage was influenced by water depth, easting and non living cover. In 2013, 51 different sponge species were observed in the cover analysis in CPCe, in 2015, this were 56 species. Assessment of all sponges in 2015, resulted in 79 different sponge species that were observed. In 2006, Thacker *et al*. found 45 different species during their surveys. The species data was combined with historical collections from 1972 and 1986, a total of 81 different sponge species was found. This was low in comparison with other biodiversity surveys for sponges (Rützler *et al.,* 2000; Alcolado, 2002; Diaz, 2005). However, these studies were executed in different habitat types, namely mangrove cays (Rützler *et al.,* 2000), and at shallow reefs (Diaz, 2005), whereas this study was done at deeper sites on a reef flat. In the period from 1972 to 2015, 122 different species were counted on Saba Bank. This is more in

line with the survey of Diaz in the Bocas del Toro region, Panama, where 120 species were counted.

Another reason for the difference in sponge biodiversity in the Caribbean can be explained by the sampling methods that were used. Thacker *et al.* (2010) documented sponges for 20 to 45 min at six sites, the survey was carried out by only one person. In the study of Rützler *et al.* (2000), sponges were collected from seven sites by six collectors. Diaz (2005) collected the sponges on his own from 14 sites for approximately one hour per site. The study of, Alcolado (2002) consists of multiple studies of the past 140 years that were executed by multiple researchers. More effort is needed to find rare species (i.e. longer searching time, more people that are performing the surveys, more sites.

The species contributing most to the total sponge cover were *Xestospongia muta* and *Agelas sventres. X. muta* is the largest sponge species in the Caribbean, when a specimen is found in a CPCe photo quadrat, it often contributes to a considerable amount of the cover. In other studies, where reefs were dominated by sponges, cover which mainly consisted of *Chondrilla* cf. *nucula*, an encrusting species. In Puerto Rico, were a shift to a sponge dominated system was observed, the boring sponge *Cliona langae* covered 10.8% of the total transect area (Williams *et al.*, 1999). In this study, mainly *Cliona delitrix* and *Cliona caribbaea* were present, however their cover was not very high. This may indicate that Saba Bank is healthier than the reefs where boring sponges became dominant, since boring sponges profit from increased ocean acidification and increased sea water temperature (Stubler *et al.*, 2014; Stubler *et al.*, 2015). Also, boring sponges grow on bare substratum as a result of coral decline, this is probably not the case on Saba Bank. The assessment of sponge cover using CPCe is not the best method. Sponges often have multidimensional structures (i.e. branching or tubes), and thus have a larger surface area than is visible on a photo. Using species specific models for estimating sponge cover would be a better method, however these models are not extensively developed yet.

High cover of turf algae could be beneficial to sponges. DOM, produced by corals, macroalgae and turf algae are a food source for sponges (Rix *et al*., 2016). Sponges possibly can profit from increased algal densities, since more DOM is released (Pawlik *et al*., 2016). Thus the shifts to algal dominance, which occurred throughout the whole Caribbean, are beneficial to sponges. Cyanobacteria are able to produce DOM as well, although it remains unclear whether sponges can use this as a food source (Brocke *et al*., 2015). If sponges are able to use DOM produced by cyanobacteria, it would be possible that sponges on Saba Bank benefit from the increased food availability and increase in cover or abundance. The sponge

reef hypothesis mostly applies to reefs in the Atlantic ocean, sponge cover in the Atlantic ocean is higher than the cover in Pacific reefs (Loh & Pawlik, 2014). Besides, Atlantic sponges are heterotrophic and are able to use DOM, whereas most Pacific sponges are foliose phototrophic and cannot use DOM (Pawlik *et al*., 2016). If DOM is an important driver for sponge dominance, it is not likely that this will occur on Pacific reefs.

***Difficulties during this research and future directions***

Visual sponge identification is often difficult, especially for the encrusting species (Diaz & Rützler). It is only possible to identify species bigger than 4 cm, otherwise structures of the sponges and osculi are not visible properly (de Voogd & Cleary, 2008). It is very important to extent the current reference databases based on photos of sponges. Now, some species lack photos or are underrepresented, therefore, sponge identification by the use of photo databases may be biased towards species that have more photos in the database. During this research, a reference guide of sponges on Saba Bank is created. Specimens that were photographed were identified using DNA barcoding, and morphological analysis, so that there is a higher certainty that the photos are referring to the correct species.

Water depth on Saba Bank was a driver in benthic composition, however some sites differed 10 m in their water depth in 2013 and 2015. Therefore, it is important to collect transect data from the same depth, in order to compare the sites in different years. It is difficult to collect transect data from the exact same location every year. Divers go into the water at the right position that is set by GPS-coordinates. However, due to currents, divers will not always reach the exact same location on reefs as before and the transect will be placed on a other part of the reef. Maybe it is inevitable to reach the exact same site every year, at least, divers should try to place the transects at a fixed depth in subsequent surveys.

This study only covers benthic community data of a period of 2 years, so it is impossible to study long term effects and long term benthic community changes. However, in this short period of time, significant differences were found in benthic community. Turf algae and cyanobacteria are fast growing, opportunistic species, that can colonize reefs rapidly especially during nutrient surges (Russell & Connell, 2007; Palinska et al. 2012). For corals and sponges, it is possible that a two year timescale is too short, since they grow slower. It is important to continue benthic surveys on the Saba Bank, because there is little knowledge of reefs dominated by cyanobacteria. Also it would be good to find out how coral and sponge cover will react on a high cyanobacterial cover, and if this cyanobacteria dominance is indeed beneficial to sponges.

***Conclusion***

To conclude, a shift from a turf algae dominated system to a cyanobacteria dominated system was observed on Saba Bank. In 2015, cyanobacteria were the most dominant benthic species, whereas turf algae were most dominant two years earlier. Small influences of Saba, St. Eustatius, and possibly Saint Kitts and Nevis are observed. Sites closer by these islands had higher macroalgae cover and lower sponge and coral cover. It is unclear how the cyanobacteria will develop in the future, since cyanobacteria dominated systems are scarcely described in literature. Possibly, Saba Bank is shifting to a cyanobacteria reef rather than a sponge reef. Since only one year of cyanobacteria dominance is observed, further research has to prove whether this is really a phase shift. Last, sponges may profit from the cyanobacteria dominance on Saba Bank, and increase in abundance.

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# SUPLEMENTERY

***Qiagen DNeasy 96 blood & tissue kit extraction protocol***

- Add 180 µL Buffer ATL to microtubes
- 20 mg of sponge tissue in each well (sample has to be cut into small pieces)
- **!** It took more than a day to cut all samples, samples were stored in a fridge at 4°C
- Add 20 µL proteinase K, triturate to mix, seal microtubes with caps
- Centrifuge at 3000 rpm, samples has to be submerged in working solution
- Incubate at 56°C overnight, after two hours samples were mixed by shaking the tubes
- **!** After incubation, it seemed that samples were not fully lysed
- Shake tubes and centrifuge at 3400 rcf for 1 minute
- Add 410 µL Buffer AL and shake tubes
- Transfer lysate to DNeasy 96 plate on top of S-Block
- Seal DNeasy 96 plate with AirPore Tape and centrifuge at 3800 rcf for 10 min
- Add 500 µL Buffer AW1, seal with AirPore Tape, centrifuge at 3800 rcf for 5 min
- Add 500 µL Buffer AW2 and centrifuge at 3800 rcf for 15 min
- Place DNeasy 96 plate on top of a rack of elution microtube RS
- Add 150 µL Buffer AE and seal with AirPore tape sheet
- Incubate 1 min at room temperature, and centrifuge at 3800 rcf for 2 min
- Add 150 µL Buffer AE and seal with AirPore tape sheet
- Incubate 1 min at room temperature, and centrifuge at 3800 rcf for 2 min

***Qiagen QIAamp DNA mini and blood mini kit***

- Add 180 µL Buffer ATL to 1.5 mL microcentrifuge tube
- 20 mg of sponge tissue in each well (sample has to be cut into small pieces)
- Add 20 µL proteinase K, mix by vortexing
- Incubate overnight at 56°C, centrifuge tubes briefly after incubation
- Add 200 µL Buffer AL, mix by pulse-vortexing
- Incubate at 70°C for 10 min, centrifuge tubes briefly after incubation
- Add 200 µL ethanol (98%), mix by vortexing, centrifuge briefly
- Transfer from microcentrifuge tubes to Mini spin column with collection tube
- Centrifuge at 8000 rpm for 1 min and place spin column in new collection tube
- Add 500 µL Buffer AW1 and centrifuge at 8000 rpm for 1 min
- Place spin column in new collection tube and add 500 µL Buffer AW2
- Centrifuge at 14000 rpm for 3 min
- Place spin column in 2 mL eppendorf with push cap
- Add 150 µL Buffer AE
- Incubate 1 min at room temperature and centrifuge at 8000 rpm for 1 min
- Add 150 µL Buffer AE
- Incubate 1 min at room temperature and centrifuge at 8000 rpm for 1 min

***Preparation Durcupan***

Component A: Epoxy resin 5 mL

Component B: 964 hardener 11 mL

Component C: 964 accelerator 1 mL

Component D: Dibutyl phthalate 0.2 mL (reducing brittleness)

All components were mixed using a glass Pasteur pipette. A total volume of 17 mL was made. However, I would recommend to prepare 4.25 mL. Durcupan can be stored in a freezer, but after defrosting, it is more viscous, contains some small bubbles, and it harder to use to prepare tissue slides.

## *$R^2$ values in NMDS for groups or species*

**Table 5.** $R^2$ values for benthic groups observed in 2013 and 2015 used in NMDS for analysis total benthic community

| | NMDS1 | NMDS2 | R2 |
|---|---|---|---|
| Corals | 0.42154 | 0.9068115 | 0.1016 |
| Sponges | 0.30381 | 0.9527317 | 0.1084 |
| Macroalgae | -0.77448 | -0.6326039 | 0.7717 |
| Turf algae | 0.22807 | -0.9736444 | 0.6874 |
| Cyanobacteria | -0.50718 | 0.8618410 | 0.8067 |
| CCA | 0.99997 | 0.0083216 | 0.2896 |

**Table 6.** $R^2$ values for observed coral species in 2013 and 2015 used in NMDS for coral assemblages

| | NMDS1 | NMDS2 | R2 |
|---|---|---|---|
| MFAV | 0.91627 | -0.400561 | 0.5018 |
| SS | -0.01908 | 0.999818 | 0.4761 |
| MD | 0.54955 | 0.835459 | 0.2411 |
| PD | -0.9126 | 0.408864 | 0.221 |
| SM | -0.15024 | 0.988649 | 0.2109 |
| AA | 0.99492 | -0.100695 | 0.2066 |
| MC | 0.86705 | -0.498217 | 0.1581 |
| MILA | -0.99568 | 0.092833 | 0.1147 |
| ML | 0.85493 | -0.518744 | 0.1121 |
| DS | -0.74903 | 0.662531 | 0.1042 |
| CORAL | 0.93207 | -0.36229 | 0.0913 |
| PP | -0.14686 | 0.989158 | 0.0896 |
| DL | -0.16599 | 0.986127 | 0.089 |
| MFRN | 0.83804 | -0.545614 | 0.0619 |
| CN | 0.5171 | 0.855925 | 0.0618 |
| SR | -0.53335 | -0.845894 | 0.0605 |
| DSO | 0.18137 | -0.983416 | 0.0594 |
| PF | 0.79152 | 0.611142 | 0.0521 |
| MM | 0.91722 | -0.398392 | 0.0413 |
| DC | 0.32811 | 0.944638 | 0.0235 |
| MILC | 0.45471 | -0.890642 | 0.0143 |
| EF | -0.48719 | -0.873297 | 0.0132 |
| MME | 0.15298 | 0.988229 | 0.01 |
| PA | -0.98786 | -0.155359 | 0.0084 |
| AL | 0.97566 | 0.219302 | 0.0083 |
| SB | -0.54368 | 0.839296 | 0.0038 |

**Table 7.** $R^2$ values for observed sponge species in 2013 and 2015 used in NMDS for sponge assemblages**.**

| | **NMDS1** | **NMDS2** | **r2** |
|---|---|---|---|
| CCar | -0.93922 | -0.343317 | 0.4984 |
| ACau | 0.68587 | -0.727723 | 0.3476 |
| SCoc | 0.99886 | -0.047687 | 0.3234 |
| MArb | 0.20437 | 0.978893 | 0.3229 |
| XMut | 0.29528 | 0.95541 | 0.2893 |
| NiCar | 0.01769 | 0.999844 | 0.2191 |
| ATub | -0.05796 | 0.998319 | 0.213 |
| ACom | 0.97588 | 0.218308 | 0.207 |
| ADis | -0.59711 | 0.802159 | 0.1984 |
| ASve | -0.69282 | 0.721108 | 0.1742 |
| ACon | -0.26608 | 0.96395 | 0.1447 |

| | | | |
|---|---|---|---|
| CVag | 0.99634 | 0.085528 | 0.1413 |
| CFal | 0.89203 | -0.45198 | 0.1313 |
| NRos | 0.89203 | -0.45198 | 0.1313 |
| AFul | 0.95724 | -0.289284 | 0.131 |
| NEre | -0.00362 | -0.999993 | 0.1234 |
| EFer | 0.0543 | 0.998525 | 0.1182 |
| NPro | 0.97563 | 0.219429 | 0.1018 |
| CKue | 0.86504 | 0.501701 | 0.0917 |
| ACit | -0.68636 | 0.727259 | 0.0898 |
| AIns | 0.95998 | -0.280059 | 0.0884 |
| ASce | -0.13374 | 0.991016 | 0.0834 |
| DFun | -0.08339 | -0.996517 | 0.074 |
| IFel | 0.94296 | -0.332912 | 0.0729 |
| PAng | 0.06354 | 0.997979 | 0.0671 |
| AArc | 0.93071 | -0.365762 | 0.0652 |
| AMel | -0.99648 | -0.08383 | 0.063 |
| PWal | -0.47548 | 0.879728 | 0.0613 |
| VRei | -0.47629 | -0.879286 | 0.0604 |
| SPNG | -0.11959 | 0.992823 | 0.0603 |
| HPro | 0.00333 | 0.999994 | 0.0584 |
| CCur | 0.66998 | 0.742382 | 0.0583 |
| SSPP | -0.41477 | 0.909927 | 0.0544 |
| NDig | 0.61097 | 0.791652 | 0.0521 |
| PHal | -0.4397 | 0.898147 | 0.0464 |
| CSpi | -0.6385 | 0.769623 | 0.0462 |
| CFav | -0.36317 | 0.931724 | 0.0429 |
| ISPP | 0.77981 | -0.626013 | 0.0427 |
| NNol | -0.98322 | 0.182444 | 0.0335 |
| ACer | -0.07309 | 0.997325 | 0.0308 |
| VGig | -0.07309 | 0.997325 | 0.0308 |
| BRub | -0.45991 | 0.887964 | 0.0252 |
| MRea | -0.56787 | -0.823117 | 0.0243 |
| ACra | 0.50482 | 0.863224 | 0.024 |
| ACla | -0.47394 | 0.880557 | 0.0217 |
| AMor | -0.47394 | 0.880557 | 0.0217 |
| CPod | -0.47394 | 0.880557 | 0.0217 |
| COCar | -0.3861 | 0.922456 | 0.0188 |
| SZea | -0.93378 | -0.357845 | 0.0177 |
| CPli | -0.0057 | 0.999984 | 0.0158 |
| PAma | 0.88942 | -0.457091 | 0.0149 |
| CDel | 0.98477 | 0.173845 | 0.0147 |
| SRue | 0.1882 | -0.982131 | 0.0145 |
| NCar | -0.1934 | -0.98112 | 0.0115 |
| AFis | -0.51521 | 0.857066 | 0.0113 |
| SAur | 0.99683 | 0.079569 | 0.0104 |
| ICam | -0.24138 | 0.97043 | 0.0089 |
| VSPP | -0.88846 | 0.458957 | 0.0088 |
| ClSPP | 0.76797 | -0.640483 | 0.0076 |
| BSPP | 0.86191 | 0.507056 | 0.0048 |
| NAmo | 0.504 | 0.863705 | 0.0035 |
| IBir | -0.99536 | -0.096183 | 0.0028 |
| CBul | -0.87747 | 0.479632 | 0.0013 |
| CSPP | -0.87747 | 0.479632 | 0.0013 |
| IStr | 0.09328 | 0.99564 | 0.0003 |

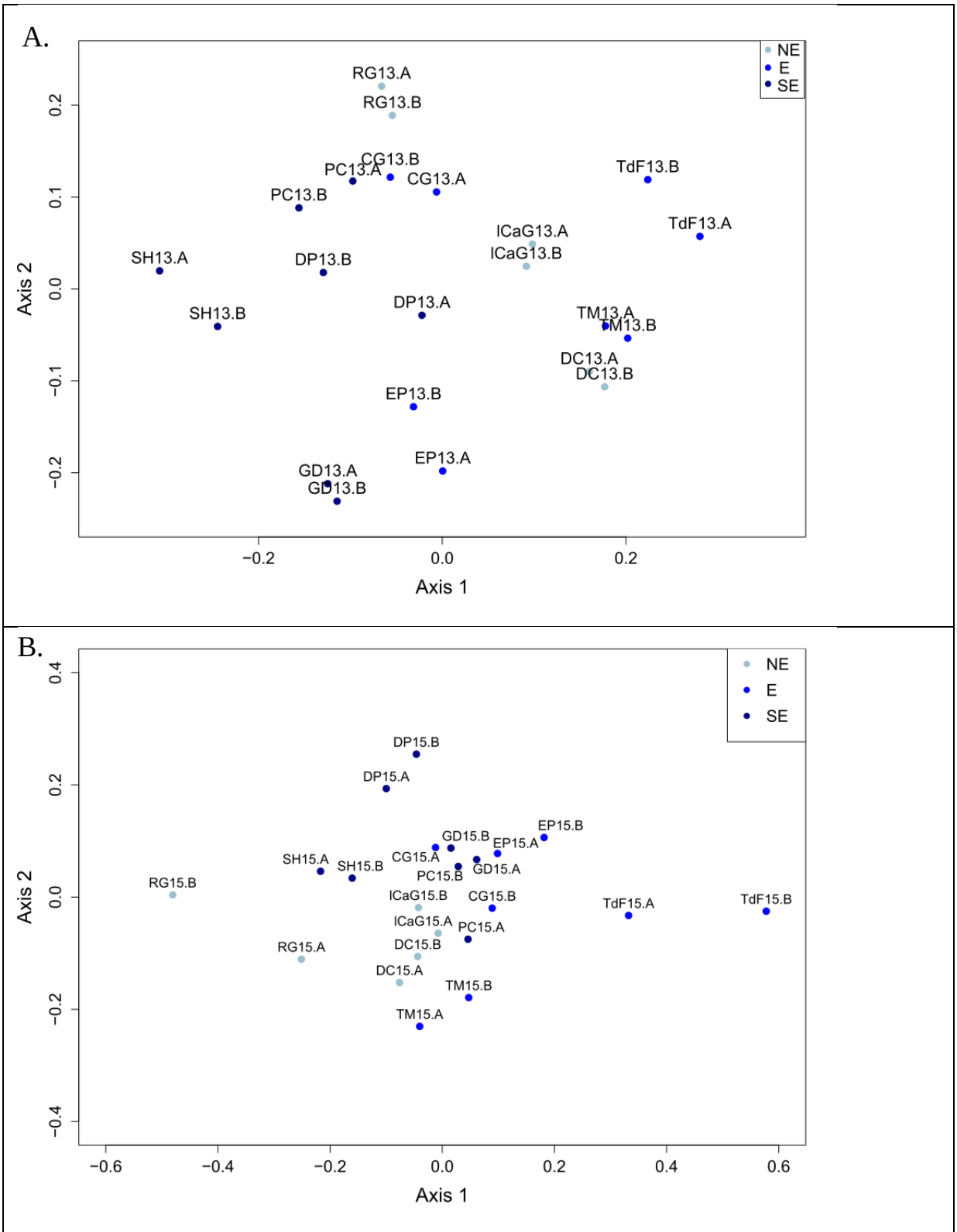


**Figure 15.** NMDS plots based on total benthic cover in 2013 (A) and 2015 (B). A division of sites was made based on orientation on Saba Bank: sites located in the northeastern parts of the bank (light blue), sites located in the eastern parts of the bank (blue), and sites located at the southeastern parts of the bank (dark blue).

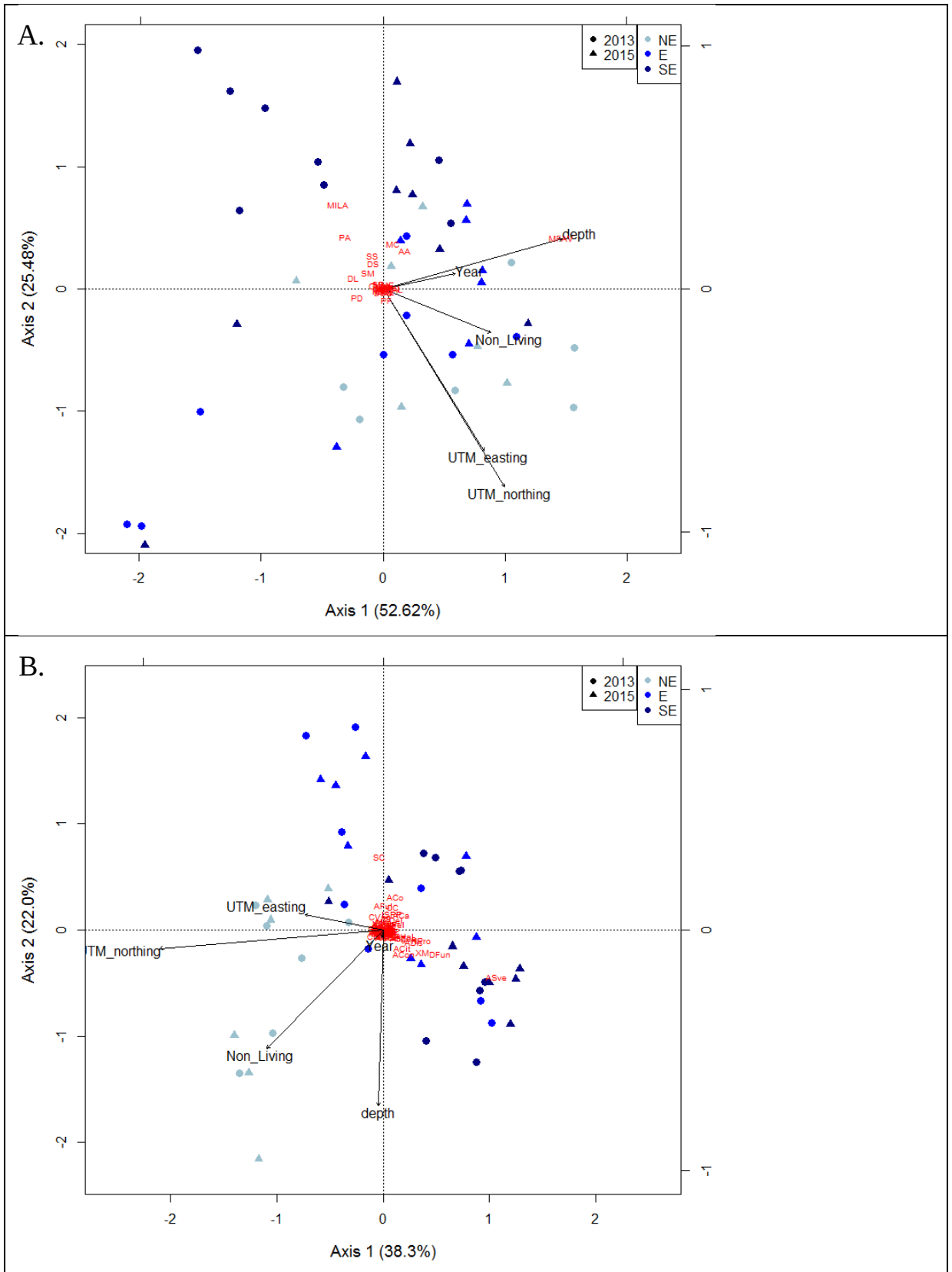


**Figure 16.** dbRDA of sponge coral assemblages (A) and sponge assemblages (B). Species are indicated in red, species that are further away from the center of the plot are more likely to influence clustering of sites in the plot.

**Table 8.** Results from DNA barcoding, best 5 matches found in GenBank are represented. The species that were found in GenBank were verified for their most recent name and classification in World Porifera Database. Samples highlighted in yellow were not sequenced, sequencing failed or, other DNA than sponge DNA was amplified during PCR. In blue are species that are not accepted in World Porifera Database. Sample SB_124, SB_125, and SB_126 turned out to be lionfish, these were actually sampled during the survey in 2015.

| sample name | Identified on photo | Top 5 result genbank | Accesion | Grade | Order | Family |
|---|---|---|---|---|---|---|
| SB_60 | | Aplysina lacunosa | AM076985 | 99,60% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034569 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034568 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034567 | 99,50% | Verongiida | Aplysinidae |
| SB_61 | Agelas dispar | Agelas schmidti | EU237475 | 97,70% | Agelasida | Agelasidae |
| | | Agelas dispar | DQ075707 | 96,80% | Agelasida | Agelasidae |
| | | Agelas dispar | DQ075736 | 96,70% | Agelasida | Agelasidae |
| | | Agelas cervicornis | DQ075753 | 96,60% | Agelasida | Agelasidae |
| | | Agelas dispar | DQ075715 | 96,60% | Agelasida | Agelasidae |
| SB_62 | Ectyoplasia ferox | Not sequenced | | | | |
| SB_63 | | Ceratoporella nicholsoni | DQ075775 | 71,10% | Agelasida | Astroscleridae |
| | | Epipolasis | JQ034572 | 71,00% | Suberitida | Halichondriidae |
| | | Saprolegnia delica | HQ709018 | 23,10% | Protozoan | |
| | | Saprolegnia delica | HQ709017 | 23,10% | Protozoan | |
| | | Aphanomyces sp. | HQ708196 | 23,10% | Oomycetes (mould) | |
| SB_64 | | Ircina oros | JN655186 | 96,40% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | KC510274 | 96,30% | Dictyoceratida | Irciniidae |
| | | Ircinia fasciculata | JN655174 | 96,30% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | HE591459 | 96,30% | Dictyoceratida | Irciniidae |
| | | Ircinia strobilina | GQ337013 | 96,30% | Dictyoceratida | Irciniidae |
| SB_65 | | Plakortis halichondrioides | HQ269359 | 98,30% | Homosclerophorida | Plakinidae |
| | | Plakortis angulospiculatus | EF519536 | 93,30% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 96,40% | Homosclerophorida | Plakinidae |
| | | Plakinastrella cf. onkodes | EU237487 | 94,60% | Homosclerophorida | Plakinidae |
| | | Plakortis halichondrioides | KP972554 | 88,20% | Homosclerophorida | Plakinidae |
| SB_66 | Agelas sventres | Agelas sventres | DQ075735 | 93,10% | Agelasida | Agelasidae |
| | | Agelas clathrodes | DQ075743 | 93,00% | Agelasida | Agelasidae |
| | | Agelas sventres | DQ075696 | 93,00% | Agelasida | Agelasidae |
| | | Agelas sventres | DQ075758 | 92,90% | Agelasida | Agelasidae |
| | | Agelas clathrodes | DQ075740 | 92,90% | Agelasida | Agelasidae |
| SB_67 | | Hyrtios proteus | JQ082820 | 92,60% | Dictyoceratida | Thorectidae |
| | | Sarcotragus spinosulus | HE591460 | 96,00% | Dictyoceratida | Irciniidae |
| | | Sarcotragus spinosulus | HE797930 | 95,20% | Dictyoceratida | Irciniidae |
| | | Ircinia oros | JN655186 | 95,60% | Dictyoceratida | Irciniidae |
| | | Hippospongia lachne | EU237484 | 95,40% | Dictyoceratida | Spongiidae |
| SB_68 | Niphates amorpha | Niphates erecta | EF519660 | 94,50% | Haplosclerida | Niphatidae |
| | | Niphates alba | EF519654 | 93,90% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519655 | 91,20% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519656 | 91,10% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519657 | 91,00% | Haplosclerida | Niphatidae |
| SB_69 | Amphimedon compressa | Not sequenced | | | | |

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|---|---|---|---|---|---|---|
| SB_70 | | Cinachyrella kuekenthali | EU237479 | 99,80% | Tetractinellida | Tetillidae |
| | | Cinachyrella kuekenthali | FJ711646 | 99,50% | Tetractinellida | Tetillidae |
| | | Cinachyrella kuekenthali | HM032743 | 99,10% | Tetractinellida | Tetillidae |
| | | Cinachyrella kuekenthali | JX177902 | 99,00% | Tetractinellida | Tetillidae |
| | | Amphitethya cf. microsigma | JX177910 | 98,30% | Tetractinellida | Tetillidae |
| SB_71 | Niphates amorpha | Niphates erecta | EF519660 | 95,30% | Haplosclerida | Niphatidae |
| | | Niphates alba | EF519654 | 94,70% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519655 | 91,90% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519656 | 91,90% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519657 | 91,80% | Haplosclerida | Niphatidae |
| SB_72 | | Fail | | | | |
| SB_73 | Neopetrosia proxima | Amphimedon compressa | EU237474 | 93,60% | Haplosclerida | Niphatidae |
| | Amphimedon compressa | Petrosia ficiformis | KR911863 | 91,70% | Haplosclerida | Petrosiidae |
| | | Halicnemia sp. | HQ379423 | 89,20% | Axinellida | Stelligeridae |
| | | Halicnemia patera | HQ379422 | 89,10% | Axinellida | Stelligeridae |
| | | Paratimea constellata | HQ379419 | 89,00% | Axinellida | Stelligeridae |
| SB_74 | Spirastrella coccinea | Clionaopsis platei | HM999042 | 94,80% | Clionaida | Clionaidae |
| | Cliona sp. | Clionaopsis platei | HM999043 | 94,80% | Clionaida | Clionaidae |
| | | Cliona chilensis | HM999018 | 94,60% | Clionaida | Clionaidae |
| | | Cliona chilensis | HM999014 | 94,60% | Clionaida | Clionaidae |
| | | Placospongia sp. | AY094604 | 94,60% | Clionaida | Placospongiidae |
| SB_75 | | Crambe crambe | JX999091 | 95,70% | Poecilosclerida | Crambeidae |
| | | Monanchora arbuscula | EF519645 | 93,60% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611612 | 96,30% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611613 | 96,30% | Poecilosclerida | Crambeidae |
| | | Monanchora sp. | HE611610 | 95,70% | Poecilosclerida | Crambeidae |
| SB_76 | | Aplysina fistularis | AY561987 | 99,60% | Verongiida | Aplysinidae |
| | | Aplysina lacunosa | AM076985 | 99,30% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 99,20% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034569 | 99,20% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034568 | 99,20% | Verongiida | Aplysinidae |
| SB_77 | | Verongula gigantea | AM076984 | 98,30% | Verongiida | Aplysinidae |
| | | Verongula reiswigi | KT921334 | 97,70% | Verongiida | Aplysinidae |
| | | Amphimedon compressa | EF519560 | 93,60% | Haplosclerida | Niphatidae |
| | | Verongula reiswigi | EF519691 | 93,60% | Verongiida | Aplysinidae |
| | | Amphimedon compressa | EF519558 | 93,60% | Haplosclerida | Niphatidae |
| SB_78 | | Plakinastrella cf. onkodes | EU237487 | 96,10% | Homosclerophorida | Plakinidae |
| | | Plakortis albicans | KJ162931 | 91,90% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 94,60% | Homosclerophorida | Plakinidae |
| | | Plakina trilopha | HQ269356 | 94,50% | Homosclerophorida | Plakinidae |
| | | Plakinastrella sp. | KU674380 | 91,00% | Homosclerophorida | Plakinidae |
| SB_79 | Callyspongia vaginalis | fail | | | | |
| SB_80 | Agelas conifera | Agelas dilatata | DQ075693 | 97,30% | Agelasida | Agelasidae |
| | Agelas tubulata | Agelas repens | DQ075757 | 97,20% | Agelasida | Agelasidae |
| | | Agelas repens | DQ075756 | 97,20% | Agelasida | Agelasidae |
| | | Agelas sceptrum | DQ075744 | 97,20% | Agelasida | Agelasidae |

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|---|---|---|---|---|---|---|
| | | Agelas sceptrum | DQ075739 | 97,20% | Agelasida | Agelasidae |
| SB_81 | | Agelas sventres | DQ075735 | 82,20% | Agelasida | Agelasidae |
| | | Agelas sventres | DQ075696 | 80,30% | Agelasida | Agelasidae |
| | | Agelas clathrodes | DQ075743 | 82,10% | Agelasida | Agelasidae |
| | | Agelas clathrodes | EF519540 | 80,20% | Agelasida | Agelasidae |
| | | Agelas clathrodes | EF519538 | 80,20% | Agelasida | Agelasidae |
| SB_82 | | fail | | | | |
| SB_83 | | | | | | |
| SB_84 | | Plakortis halichondrioides | HQ269359 | 98,30% | Homosclerophorida | Plakinidae |
| | | Plakortis angulospiculatus | EF519536 | 92,70% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 96,20% | Homosclerophorida | Plakinidae |
| | | Plakinastrella cf. onkodes | EU237487 | 93,20% | Homosclerophorida | Plakinidae |
| | | Plokortis halichondrioides | KP972554 | 88,10% | Homosclerophorida | Plakinidae |
| SB_85 | Oceanapia bartschi | Paracornulum dubium | HE611605 | 96,40% | Poecilosclerida | Acarnidae |
| | | Paracornulum sp. | HE611606 | 96,10% | Poecilosclerida | Acarnidae |
| | | Acantheurypon pilosella | JF440337 | 93,00% | Axinellida | Raspailiidae |
| | | Clathria rugosa | HE611604 | 92,50% | Poecilosclerida | Microcionidae |
| | | Negombata magnifica | AM420314 | 94,00% | Poecilosclerida | Podospongiidae |
| SB_86 | | Aiolochroia crassa | KT921333 | 97,80% | Verongiida | Aplysinidae |
| | | Aiolochroia crassa | AJ843885 | 97,70% | Verongiida | Aplysinidae |
| | | Aiolochroia crassa | KX034574 | 95,90% | Verongiida | Aplysinidae |
| | | Aiolochroia crassa | KX034573 | 95,90% | Verongiida | Aplysinidae |
| | | Aiolochroia crassa | KX034572 | 95,90% | Verongiida | Aplysinidae |
| SB_87 | | Scopalina ruetzleri | AY561976 | 94,20% | Scopalinida | Scopalinidae |
| | | Scopalina ruetzleri | AM498648 | 92,90% | Scopalinida | Scopalinidae |
| | | Scopalina ruetzleri | JX999075 | 90,90% | Scopalinida | Scopalinidae |
| | | Scopalina ruetzleri | EF519669 | 90,40% | Scopalinida | Scopalinidae |
| | | Halicnemia sp. | HQ379423 | 84,00% | Axinellida | Stelligeridae |
| SB_88 | Callyspongia plicifera | Annelida sp. | KP254496 | 91,80% | Segmented worms | |
| | | Rhysipolinae | KX058578 | 83,30% | Hymenoptera (wasps) | |
| | | Rhysipolis sp. | KX058590 | 82,60% | Hymenoptera (wasps) | |
| | | Lispe orientalis | EU627716 | 86,30% | Diptera | |
| | | Aphidiinae sp. | KR418568 | 82,40% | Hymenoptera (wasps) | |
| SB_89 | | Chelonaplysilla erecta | EF519582 | 94,30% | Dendroceratida | Darwinellidae |
| | | Chelonaplysilla sp. | KU060584 | 89,30% | Dendroceratida | Darwinellidae |
| | | Dictyodendrilla cavernosa | JQ082807 | 91,80% | Dendroceratida | Dicyodendrillidae |
| | | Dictyodendrilla elegans | JQ082808 | 91,70% | Not in World Porifera Database | |
| | | Chelonaplysilla delicta | JQ082800 | 89,90% | Dendroceratida | Darwinellidae |
| SB_90 | Xestospongia muta | Xestospongia testudinaria | HQ452961 | 98,60% | Haplosclerida | Petrosiidae |
| | | Xestospongia testudinaria | HQ452959 | 98,60% | Haplosclerida | Petrosiidae |
| | | Xestospongia muta | HQ452958 | 98,60% | Haplosclerida | Petrosiidae |
| | | Xestospongia muta | HQ452957 | 98,60% | Haplosclerida | Petrosiidae |
| | | Xestospongia muta | EU237490 | 98,60% | Haplosclerida | Petrosiidae |

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|---|---|---|---|---|---|---|
| SB_91 | | Geodia vosmaeri | HM592711 | 97,80% | Tetractinellida | Geodiidae |
| | | Geodia neptuni | AY320032 | 98,10% | Tetractinellida | Geodiidae |
| | | Geodia vosmaeri | HM592722 | 97,30% | Tetractinellida | Geodiidae |
| | | Geodia gibberosa | EU44209 | 96,70% | Tetractinellida | Geodiidae |
| | | Sidonops neptuni | EF519673 | 93,30% | Tetractinellida | Geodiidae |
| SB_92 | Amphimedon compressa | Timea sp. | AY561968 | 80,70% | Tethyida | Timeidae |
| | | Geodia media | AY561962 | 81,50% | Tetractinellida | Geodiidae |
| | | Scorpio maurus | KF997866 | 72,60% | Scorpion | |
| | | Scorpio fuscus | KT188287 | 73,60% | Scorpion | |
| | | Scorpio fuscus | KT188219 | 73,70% | Scorpion | |
| SB_93 | | Crambe crambe | JX999091 | 94,30% | Poecilosclerida | Crambeidae |
| | | Monanchora arbuscula | EF519645 | 92,60% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611612 | 94,70% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611613 | 94,60% | Poecilosclerida | Crambeidae |
| | | Monanchora sp. | HE611610 | 94,00% | Poecilosclerida | Crambeidae |
| SB_94 | | Aplysina fistularis | AY561987 | 98,90% | Verongiida | Aplysinidae |
| | | Aplysina lacunosa | AM076985 | 98,90% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034569 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034568 | 98,80% | Verongiida | Aplysinidae |
| SB_95 | | Clathria armata | KC869418 | 96,20% | Poecilosclerida | Microcionidae |
| | | Microciona prolifera | DQ087475 | 96,30% | Poecilosclerida | Microcionidae |
| | | Ophlitaspongia papilla | KF225485 | 95,50% | Poecilosclerida | Microcionidae |
| | | Ophlitaspongia papilla | KF225484 | 95,50% | Poecilosclerida | Microcionidae |
| | | Ophlitaspongia papilla | KF225483 | 95,60% | Poecilosclerida | Microcionidae |
| SB_96 | | Cf. Hippospongia sp. | JQ082792 | 93,00% | Dictyoceratida | Spongiidae |
| | | Dactylospongia elegans | JQ082802 | 92,90% | Dictyoceratida | Thorectidae |
| | | Hippospongia lachne | EU237484 | 96,30% | Dictyoceratida | Spongiidae |
| | | Ircinia oros | JN655186 | 96,30% | Dictyoceratida | Irciniidae |
| | | Hyattella sinuosa | JX535019 | 96,20% | Dictyoceratida | Spongiidae |
| SB_97 | | fail | | | | |
| SB_98 | Polymastia tenax | Polymastia littoralis | KJ129611 | 96,80% | Polymastiida | Polymastidae |
| | | Sphaerotylus borealis | HG423725 | 96,00% | Polymastiida | Polymastidae |
| | | Polymastia euplectella | HG423710 | 96,00% | Polymastiida | Polymastidae |
| | | Polymastia penicillus | KF225486 | 96,00% | Polymastiida | Polymastidae |
| | | Polymastia penicillus | LN606464 | 95,90% | Polymastiida | Polymastidae |
| SB_100 | | Prosuberites laughlini | AY561960 | 97,40% | Agelasida | Hymerhabdiidae |
| | | Axinella sp. | JX915786 | 97,40% | Axinellida | Axinellidae |
| | | Axinella sp. | KJ008097 | 97,30% | Axinellida | Axinellidae |
| | | Axinella sp. | JX915787 | 97,30% | Axinellida | Axinellidae |
| | | Eurypon cf. clavatum | AJ843893 | 95,90% | Axinellida | Raspailiidae |
| SB_101 | Callyspongia vaginalis | Callyspongia vaginalis | EF519579 | 88,10% | Haplosclerida | Callyspongiidae |
| | | Callyspongia vaginalis | GQ304704 | 87,50% | Haplosclerida | Callyspongiidae |
| | | Callyspongia vaginalis | LK026376 | 87,40% | Haplosclerida | Callyspongiidae |
| | | Callyspongia vaginalis | GQ304697 | 87,40% | Haplosclerida | Callyspongiidae |
| | | Callyspongia vaginalis | GQ304613 | 87,30% | Haplosclerida | Callyspongiidae |
| SB_102 | | Crella sp. | HE611614 | 96,60% | Poecilosclerida | Crellidae |

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|---|---|---|---|---|---|---|
| | | Phorbas fictitius | LT160715 | 97,00% | Poecilosclerida | Hymedesmiidae |
| | | Phorbas fictitius | LT160716 | 96,80% | Poecilosclerida | Hymedesmiidae |
| | | Phorbas fictitius | LT160723 | 96,40% | Poecilosclerida | Hymedesmiidae |
| | | Phorbas fictitius | LT160722 | 96,40% | Poecilosclerida | Hymedesmiidae |
| SB_103 | | Predicted: Pantholops hodgsionii | XM_005964 | 85,80% | Tibetan antelope | |
| | | Sinorhizobium meliloti | CP009144 | 80,90% | Bacteria | |
| | | Sinorhizobium meliloti | CP004140 | 80,90% | Bacteria | |
| | | Sinorhizobium meliloti | CP002740 | 80,90% | Bacteria | |
| | | Sinorhizobium meliloti | AL591688 | 80,90% | Bacteria | |
| SB_104 | Callyspongia fallax | Haliclona amboinensis | KR707689 | 97,00% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707686 | 96,50% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707688 | 96,90% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707687 | 96,90% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707685 | 96,30% | Haplosclerida | Chalinidae |
| SB_105 | | Ircinia oros | JN655186 | 99,50% | Dictyoceratida | Irciniidae |
| | | Ircinia fasciculata | JN655174 | 99,20% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | HE591459 | 99,20% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | KC510274 | 99,10% | Dictyoceratida | Irciniidae |
| | | Ircinia strobilina | GQ337013 | 99,10% | Dictyoceratida | Irciniidae |
| SB_106 | | Ptilocaulis walpersi | EU237488 | 99,00% | Axinellida | Axinellidae |
| | | Ptilocaulis marquezi | EF519668 | 94,90% | Axinellida | Axinellidae |
| | | Hymeraphia breeni | KC869421 | 93,00% | Axinellida | Raspailiidae |
| | | Thrinacophora cervicornis | JQ034586 | 89,60% | Axinellida | Raspailiidae |
| | | Thrinacophora cervicornis | JQ034585 | 89,60% | Axinellida | Raspailiidae |
| SB_107 | | Not sequenced | | | | |
| SB_108 | Callyspongia plicifera | Callyspongia plicifera | EU237477 | 99,40% | Haplosclerida | Callysponiidae |
| | | Haliclona elegans | JX999087 | 95,00% | Haplosclerida | Chalinidae |
| | | Haliclona sp. | JN242203 | 92,90% | Haplosclerida | Chalinidae |
| | | Haliclona implexiformis | EF519623 | 93,00% | Haplosclerida | Chalinidae |
| | | Haliclona oculata | HQ379430 | 94,80% | Haplosclerida | Chalinidae |
| SB_109 | ?? | Stenotrophomonas maltophilia | CP011306 | 75,70% | Bacteria | |
| | | Stenotrophomonas maltophilia | CP011305 | 75,70% | Bacteria | |
| | | Stenotrophomonas maltophilia | CP011010 | 75,50% | Bacteria | |
| | | Stenotrophomonas maltophilia | HE798556 | 75,20% | Bacteria | |
| | | Xanthomonas translucens | CP008714 | 76,50% | Bacteria | |
| SB_110 | | Crambe crambe | JX999091 | 95,30% | Poecilosclerida | Crambeidae |
| | | Monanchora arbuscula | EF519645 | 93,30% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611612 | 96,10% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611613 | 96,00% | Poecilosclerida | Crambeidae |
| | | Monanchora sp. | HE611610 | 95,40% | Poecilosclerida | Crambeidae |

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|---|---|---|---|---|---|---|
| SB_111 | | Aplysina fistularis | AY561987 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina lancunosa | AM076985 | 98,90% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034569 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034568 | 98,80% | Verongiida | Aplysinidae |
| SB_112 | | Dercitus bucklandi | HM592674 | 95,30% | Tetractinellida | Ancorinidae |
| | | Stryphnus fortis | HM592697 | 94,40% | Tetractinellida | Ancorinidae |
| | | Stryphnus ponderosus | HM592685 | 94,40% | Tetractinellida | Ancorinidae |
| | | Stelletta normani | EU442193 | 94,00% | Tetractinellida | Ancorinidae |
| | | Stelletta clarella | HM592736 | 93,70% | Tetractinellida | Ancorinidae |
| SB_113 | | Lysobacter gummosus | CP011131 | 89,30% | Bacteria | |
| | | Pseudoxanthomonas suwonensis | CP011144 | 84,30% | Bacteria | |
| | | Lysobacter capsici | CP011130 | 88,00% | Bacteria | |
| | | Lysobacter antibioticus | CP013141 | 89,00% | Bacteria | |
| | | Lysobacter enzymogenes | CP013140 | 85,90% | Bacteria | |
| SB_114 | | | | | | |
| SB_115 | | Aplysina fistularis | AY561987 | 99,80% | Verongiida | Aplysinidae |
| | | Aplysina cauliformis | EU518938 | 99,60% | Verongiida | Aplysinidae |
| | | Aplysina fulva | EU237476 | 99,60% | Verongiida | Aplysinidae |
| | | Aplysina lacunosa | AM076985 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 99,20% | Verongiida | Aplysinidae |
| SB_116 | | Not sequenced | | | | |
| SB_117 | | Amphimedon compressa | EU237474 | 95,70% | Haplosclerida | Niphatidae |
| | | Eunapius fragilis | DQ176779 | 90,50% | Spongillida | Spongillidae |
| | | Hemiasterella sp. | AY561977 | 90,60% | Tethyida | Hemiasterellidae |
| | | Haliclona amphioxa | AJ843892 | 88,90% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707688 | 90,30% | Haplosclerida | Chalinidae |
| SB_118 | | Analges sp. | | | | |
| SB_119 | Agelas citrina | Agelas clathrodes | DQ075703 | 95,50% | Agelasida | Agelasidae |
| | | Agelas citrina | DQ075741 | 95,40% | Agelasida | Agelasidae |
| | | Agelas clathrodes | DQ075726 | 95,40% | Agelasida | Agelasidae |
| | | Agelas citrina | DQ075701 | 95,40% | Agelasida | Agelasidae |
| | | Agelas cf. clathrodes | DQ075729 | 95,30% | Agelasida | Agelasidae |
| SB_120 | Niphates erecta | Niphates alba | EF519654 | 94,00% | Haplosclerida | Niphatidae |
| | | Niphates erecta | EF519660 | 93,40% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519655 | 90,80% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519656 | 90,70% | Haplosclerida | Niphatidae |
| | | Niphates digitalis | EF519657 | 90,60% | Haplosclerida | Niphatidae |
| SB_122 | Neopetrosia carbonaria | Geodia californica | EU442200 | 85,50% | Tetractinellida | Geodiidae |
| | Amphimedon compressa | Geodia media | AY561962 | 87,00% | Tetractinellida | Geodiidae |
| | | Stelletta clarella | HM592736 | 84,90% | Tetractinellida | Ancorinidae |
| | | Geodia gibberosa | EU442209 | 85,40% | Tetractinellida | Ancorinidae |
| | | Geodia gibberosa | HM592723 | 84,50% | Tetractinellida | Ancorinidae |
| SB_123 | Neopetrosia carbonaria | Plakinastrella cf. onkodes | EU237487 | 96,00% | Homosclerophorida | Plakinidae |
| | Plakortis halichondrioides | Plakortis albicans | KJ162931 | 91,60% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 94,80% | Homosclerophorida | Plakinidae |
| | | Plakina trilopha | HQ269356 | 94,50% | Homosclerophorida | Plakinidae |
| | | Plakinastrella sp. | KU674380 | 90,60% | Homosclerophorida | Plakinidae |
| SB_124 | | Pterois volitans | KJ739816 | 99,20% | Red lionfish | |

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|---|---|---|---|---|---|---|
| | | Pteriois volitans | KM488633 | 99,00% | | |
| | | Actinopterygii | JQ843265 | 97,90% | | |
| | | Actinopterygii | JQ843273 | 97,70% | | |
| | | Actinopterygii | JQ843149 | 97,80% | | |
| SB_125 | | Pterois volitans | KJ739816 | 98,90% | Red lionfish | |
| | | Pterois volitans | KM488633 | 98,70% | | |
| | | Actinopterygii | JQ843265 | 96,90% | | |
| | | Actinopterygii | JQ843273 | 96,70% | | |
| | | Actinopterygii | JQ843149 | 96,90% | | |
| SB_126 | | Pterois volitans | KJ739816 | 98,70% | Red lionfish | |
| | | Pterois volitans | KM488633 | 98,60% | | |
| | | Actinopterygii | JQ843265 | 97,40% | | |
| | | Actinopterygii | JQ843273 | 97,30% | | |
| | | Actinopterygii | JQ843149 | 97,40% | | |
| SB_127 | | Plakortis halichondrioides | HQ269359 | 97,20% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 96,30% | Homosclerophorida | Plakinidae |
| | | Plakortis angulospiculatus | EF519536 | 91,30% | Homosclerophorida | Plakinidae |
| | | Plakina jani | HQ269360 | 95,10% | Homosclerophorida | Plakinidae |
| | | Plakina trilopha | HQ269356 | 94,70% | Homosclerophorida | Plakinidae |
| SB_128 | | Cliona chilensis | HM999018 | 75,30% | Clionaida | Clionaidae |
| | | Cliona chilensis | HM999014 | 75,30% | Clionaida | Clionaidae |
| | | Clionaopsis platei | HM999042 | 74,50% | Clionaida | Clionaidae |
| | | Clionaopsis sp. | LC126251 | 73,60% | Clionaida | Clionaidae |
| | | Cliona celata | HM999029 | 75,10% | Clionaida | Clionaidae |
| SB_129 | | Iotrochota acerata | HE611625 | 86,80% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota birotulata | EU237486 | 86,80% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota coccinea | HE611623 | 86,70% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota baculifera | HE611621 | 86,70% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota birotulata | AY561963 | 84,20% | Poecilosclerida | Iotrochotidae |
| SB_130 | Ectyoplasia ferox | Ectyoplasia ferox | HE591462 | 98,60% | Axinellida | Raspailiidae |
| | | Ectyoplasia ferox | EU237480 | 98,60% | Axinellida | Raspailiidae |
| | | Ectyoplasia ferox | EF519612 | 93,90% | Axinellida | Raspailiidae |
| | | Ectyoplasia sp. | KU060764 | 88,70% | Axinellida | Raspailiidae |
| | | Ectyoplasia sp. | KU060627 | 88,50% | Axinellida | Raspailiidae |
| SB_131 | Xestospongia muta | Xestospongia testudinaria | KC763778 | 98,40% | Haplosclerida | Petrosiidae |
| | | Xestospongia testudinaria | HQ452961 | 98,40% | Haplosclerida | Petrosiidae |
| | | Xestospongia testudinaria | HQ452960 | 98,40% | Haplosclerida | Petrosiidae |
| | | Xestospongia testudinaria | HQ452959 | 98,40% | Haplosclerida | Petrosiidae |
| | | Xestospongia muta | HQ452958 | 98,40% | Haplosclerida | Petrosiidae |
| SB_132 | Niphates caribica | Tipula coloradensis | KR441661 | 25,50% | Crane-fly | |
| | | Tipula coloradensis | KR440460 | 33,90% | | |
| | | Tipula coloradensis | KM570688 | 25,00% | | |
| | | Pascula darrosensis | HE584057 | 24,90% | | |
| | | Diplopoda sp. | KP421790 | 24,30% | | |
| SB_133 | | Crambe crambe | JX999091 | 95,30% | Poecilosclerida | Crambeidae |
| | | Monanchora arbuscula | EF519645 | 93,60% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611612 | 95,70% | Poecilosclerida | Crambeidae |
| | | Monanchora clathrata | HE611613 | 95,60% | Poecilosclerida | Crambeidae |
| | | Monanchora sp. | HE611610 | 95,10% | Poecilosclerida | Crambeidae |
| SB_134 | Halichondria? | Stellettinopsis megastylifera | AY561980 | 99,50% | Tetractinellida | Ancorinidae |

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| | | Stellettinopsis megastylifera | FJ711642 | 98,50% | Tetractinellida | Ancorinidae |
| | | Geodia macandrewi | EU442198 | 96,00% | Tetractinellida | Geodiidae |
| | | Geodia cydonium | EU442199 | 95,90% | Tetractinellida | Geodiidae |
| | | Geodia megastrella | HM592741 | 95,70% | Tetractinellida | Geodiidae |
| SB_135 | | Clionaopsis sp. | LC126251 | 94,10% | Clionaida | Clionaidae |
| | | Clionaopsis sp. | LC126252 | 90,20% | Clionaida | Clionaidae |
| | | Demospongiae sp. | KU060720 | 87,90% | | |
| | | Clionaopsis platei | HM999043 | 94,20% | Clionaida | Clionaidae |
| | | Clionaopsis platei | HM999042 | 94,10% | Clionaida | Clionaidae |
| SB_136 | | Verongula gigantea | AM076984 | 85,20% | Verongiida | Aplysinidae |
| | | Verongula reiswigi | KT921334 | 85,20% | Verongiida | Aplysinidae |
| | | Pseudoceratina sp. | KJ546361 | 83,60% | Verongiida | Pseudocertinidae |
| | | Pseudoceratina sp. | EF043378 | 83,50% | Verongiida | Pseudocertinidae |
| | | Pseudoceratina sp. | KJ546363 | 82,50% | Verongiida | Pseudocertinidae |
| SB_137 | Siphonodictyon coralliphagum | Verongula gigantea | AM076984 | 96,90% | Verongiida | Aplysinidae |
| | | Verongula reiswigi | KT921334 | 96,10% | Verongiida | Aplysinidae |
| | | Pseudoceratina sp. | KJ546361 | 95,30% | Verongiida | Pseudocertinidae |
| | | Pseudoceratina sp. | EF043378 | 95,70% | Verongiida | Pseudocertinidae |
| | | Amphimedon compressa | EF519560 | 92,10% | Haplosclerida | Niphatidae |
| SB_140 | Callyspongia plicifera | Annelida sp. | KP254496 | 91,30% | Segmented worms | |
| | | Rhysipolinae gen. | KX058578 | 84,30% | | |
| | | Rhysipolis sp. | KX058590 | 83,60% | | |
| | | Drosophila bocki | AB669730 | 84,80% | | |
| | | Dinotrema sp. | FJ414051 | 83,90% | | |
| SB_142 | | fail | | | | |
| SB_143 | | Aplysina fistularis | AY561987 | 99,50% | Verongiida | Aplysinidae |
| | | Aplysina lacunosa | AM076985 | 98,90% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034570 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034569 | 98,80% | Verongiida | Aplysinidae |
| | | Aplysina sp. | KX034568 | 98,80% | Verongiida | Aplysinidae |
| SB_144 | Artemisina melana | Iotrochota birotulata | EU237486 | 97,00% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota acerata | HE611625 | 96,30% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota coccinea | HE611623 | 96,30% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota baculifera | HE611621 | 96,20% | Poecilosclerida | Iotrochotidae |
| | | Iotrochota birotulata | AY561963 | 95,00% | Poecilosclerida | Iotrochotidae |
| SB_146 | | Halisarca sp. | HQ606142 | 96,30% | Chondrillida | Halisarcidae |
| | | Chondrilla aff. Nucula | EU237478 | 94,50% | Chondrillida | Chondrillidae |
| | | Chondrilla aff. Nucula | FR819682 | 94,40% | Chondrillida | Chondrillidae |
| | | Chondrilla nucula | AJ843887 | 92,50% | Chondrillida | Chondrillidae |
| | | Chondrilla australiensis | JX999064 | 91,60% | Chondrillida | Chondrillidae |
| SB_147 | Niphates erecta | Plakinastrella cf. onkodes | EU237487 | 91,60% | Homosclerophorida | Plakinidae |
| | | Plakortis albicans | KJ162931 | 87,60% | Homosclerophorida | Plakinidae |
| | | Plakinastrella sp. | KU674380 | 86,80% | Homosclerophorida | Plakinidae |
| | | Plakina trilopha | HQ269356 | 89,30% | Homosclerophorida | Plakinidae |
| | | Plakortis simplex | HQ269362 | 90,50% | Homosclerophorida | Plakinidae |
| SB_148 | | Ircinia oros | JN655186 | 99,50% | Dictyoceratida | Irciniidae |
| | | Ircinia fasciculata | JN655174 | 99,10% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | HE591459 | 99,10% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | KC510274 | 99,10% | Dictyoceratida | Irciniidae |
| | | Ircinia strobilina | GQ337013 | 99,10% | Dictyoceratida | Irciniidae |
| SB_149 | Verongula | Verongula gigantea | AM076984 | 99,50% | Verongiida | Aplysinidae |

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| | | Verongula reiswigi | KT921334 | 98,90% | Verongiida | Aplysinidae |
| | | Verongula reiswigi | EF519691 | 94,40% | Verongiida | Aplysinidae |
| | | Amphimedon compressa | EF519560 | 94,30% | Haplosclerida | Niphatidae |
| | | Amphimedon compressa | EF519558 | 94,20% | Haplosclerida | Niphatidae |
| SB_150 | | Topsentia ophiraphidites | EU237482 | 99,00% | Suberitida | Halichondriidae |
| | | Petromica pacifica | LN624193 | 88,80% | Bubarida | Desmanthidae |
| | | Petromica sp. | LN624195 | 89,00% | Bubarida | Desmanthidae |
| | | Petromica pacifica | LN624194 | 85,70% | Bubarida | Desmanthidae |
| | | Ciocalypta sp. | JQ034562 | 87,60% | Suberitida | Halichondriidae |
| SB_152 | | | | | | |
| SB_153 | | | | | | |
| SB_162 | | Invertebrate environmental sample | GU071901 | 53,10% | | |
| | | Sellaphora pupula | KC911839 | 53,20% | Diatoms | |
| | | Sellaphora pupula | HQ317106 | 53,20% | | |
| | | Thalassionema nitzschioides | AB020228 | 52,50% | | |
| | | Pseudo-nitzschia hasleana | JN050310 | 53,10% | | |
| SB_163 | | Cliona delitrix | HM999041 | 96,10% | Clionaida | Clionaidae |
| | | Clionaopsis platei | HM999042 | 97,30% | Clionaida | Clionaidae |
| | | Clionaopsis platei | HM999043 | 97,30% | Clionaida | Clionaidae |
| | | Cliona delitrix | EF519609 | 93,50% | Clionaida | Clionaidae |
| | | Cliona delitrix | EF519610 | 93,40% | Clionaida | Clionaidae |
| SB_166 | | Geryonia proboscidalis | KT809331 | 22,50% | Cnidaria | |
| | | Geryonia proboscidalis | GQ120078 | 22,50% | | |
| SB_167 | Neopetrosia carbonaria | Haliclona amboinensis | KR707689 | 94,80% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707688 | 94,80% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707687 | 94,80% | Haplosclerida | Chalinidae |
| | | Haliclona amboinensis | KR707686 | 94,80% | Haplosclerida | Chalinidae |
| | | Petrosia sp. | JN242216 | 90,90% | Haplosclerida | Petrosiidae |
| SB_168 | | Ircinia oros | JN655186 | 94,50% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | KC510274 | 94,10% | Dictyoceratida | Irciniidae |
| | | Ircinia fasciculata | JN655174 | 94,10% | Dictyoceratida | Irciniidae |
| | | Ircinia sp. | HE591459 | 94,10% | Dictyoceratida | Irciniidae |
| | | Ircinia strobilina | GQ337013 | 94,10% | Dictyoceratida | Irciniidae |