Genetic diversity and connectivity of populations on the Sababank

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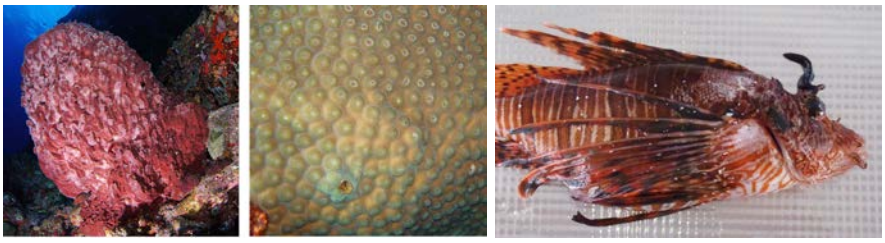

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Summary

From 19-27 October 2013, IMARES (Wageningen UR) organized a research expedition to the Saba Bank, to investigate the ecological functioning of the Bank. The expedition is a follow up of a survey of the bank in 2011 and is part of the "The Saba Bank Research Program 2011-2016" initiated by the Dutch Ministry of Economic Affairs (EZ). The bank is the largest submarine atoll in the Caribbean Sea, spanning an area of 2200km^2. It is a Marine Protected Area and is acknowledged by the Convention of Biological Diversity as an Ecologically and Biologically Significant Area. The project is part of the implementation of the Exclusive Economic Zone management plan for the Dutch Caribbean.

If the Saba Bank is to serve as a source of healthy larvae for the neighboring reefs, a key question is how populations of reef organisms on the bank are connected with populations in the region and in the Wider Caribbean. The aim of the current report is to investigate the health status and the population genetic structure of two common native benthic species, *Xestospongia muta* (giant barrel sponge) and *Montastrea cavernosa* (great star coral), and an invasive species, *Pterois volitans* (lionfish). With the aid of molecular techniques and species assessments, we aim to assess:

- the level of genetic diversity within the populations of two common benthic species (*X. muta* and *M. cavernosa*) on the Saba Bank;
- the degree of genetic connectivity between populations on Saba Bank and surrounding reefs, based on newly obtain genetic sequences and sequences obtained from GenBank from populations across the Wider Caribbean;
- the current density and health status of the populations of *X. muta* and *M. cavernosa* on Saba Bank.
- the genetic connectivity, population size and the dispersal direction of the invasion of the lionfish on the Saba Bank, in relation to the Eastern Caribbean populations.

For both the coral and sponge, gene flow was detected along the southeastern rim of Saba Bank, as well as between the populations on the bank and those at the nearby islands. The Saba Bank may therefore either be a source or sink of diversity to the reefs of nearby islands (Saba and St. Eustatius). There was no genetic differentiation between the populations of Saba Bank and multiple locations in the Wider Caribbean, This indicates that the Saba Bank populations likely have genetic connectivity with populations in locations ranging in distance of 100s – 1000s km.

The genetic diversity and of Saba Bank populations of *M.cavernosa* (π=0.055) and *X.muta* (π=0.0010) was similar to those in other regions in the Western Atlantic, indicating a genetically robust population. This was corroborated by our recorded densities of *M.cavernosa* (range 0.06-0.96 colonies m^{-2}) and *X.muta* (0.1-0.72 individuals m^{-2}), which were similar to, or in some locations even 2-3 times higher than, those recorded in any other region in the Caribbean. The population of *M. cavernosa,* furthermore, harbored unique genetic diversity on the Saba Bank which was not shared with any other locations.

No disease or bleaching was observed in any of the specimens of *M. cavernosa*. Nevertheless, the *M. cavernosa* colonies do appear to be under stress, displayed by old tissue loss in the majority of the colonies (78% of samples) and partial overgrowth by cyanobacteria, sponges or macroalgae (48%). The observed tissue loss might be the consequence of past mass bleaching events affecting reefs worldwide, including Saba Bank (*e.g.* Brandt 2009; Van Beek & Meesters 2013). The vast majority of *X. muta* (>80%) showed signs of bleaching in the form of circular shaped white spots. In fact, all observed larger individuals (diameter >50cm) had bleach spots. The prevalence of *X. muta* bleaching that we have recorded on the Saba Bank is 3-7 times higher than has been recorded anywhere in the Caribbean previously. One point of consideration is that our surveys took place in October which is the month that bleaching is generally known to be highest. Though the effect of bleaching on sponge survival seems to be variable (McMurray *et al.* 2011), the high prevalence of bleaching of *X. muta* on Saba Bank does raise

concern. Considering the key ecological role that sponges play in the reef and that they function as biotopes for a high diversity of symbiotic and commensal endofauna, this high prevalence of bleaching validates further research. We recommend that *X. muta* is monitored for bleaching during the next survey to the Saba Bank in 2015 and that its endobiont fauna are quantitatively described.

For the lionfish, the genetic diversity of the Saba Bank was low compared to locations closer to the presumed source of introduction (Florida), confirming its recent invasion of the bank. Neighbouring locations showed a similar diversity, suggesting gene flow among the eastern populations (Saba Bank & Saba, Martinique, Guadeloupe, St. Maarten, St. Eaustatius). In contrast, there was a strong genetic difference between the northern populations (North Carolina, Bahamas, Bermuda) and the Saba Bank (including the other eastern populations). Invasion did not likely occur from the North, but rather from the South (Bonaire or Curaçao) or the East.

The genetic connectivity of the populations of lionfish on Saba Bank and the nearby islands has serious implications for any potential management of lionfish. The population of lionfish, furthermore, appears to have increased in 2013 compared to 2011. Thorough control efforts on the Saba Bank are desirable in order to stabilize lionfish densities and prevent a further increase, otherwise the bank may be the source of constant recruitment to nearby located reefs on Saba Island, St. Eustatius, and St. Maarten.

Due to its remarkable biodiversity, upstream position with respect to the wider Western Atlantic, large area of mesophotic reef and relatively limited anthropogenic disturbance, the Saba Bank has the potential to harbor source populations to the wider Western Atlantic. The combined results of gene flow among bank populations and surrounding reefs, high abundance, and unique genetic diversity, means that Saba Bank could function as an important buffer for the region, either as a natural source of larvae to replenish genetic diversity in the region or as a storehouse of diversity that can be utilized if needed for restoration practices.

1. Introduction

1.1 Saba Bank Expedition

From 19-27 October 2013, IMARES (Wageningen UR), organized a research expedition to the Saba Bank to investigate the ecological functioning of the bank. On board of the "Caribbean Explorer II", an international team of marine scientists surveyed different aspects of the coral reef ecosystem. The expedition is a follow up of a survey of the Bank in 2011 and is part of the "The Saba Bank Research Program 2011-2016" initiated by the Dutch Ministry of Economic Affairs (EZ). Saba Bank (17° 25' N, 63° 30' W), is a large (2,200 km2) submerged carbonate platform (Macintyre *et al.* 1975; Van der Land 1977) (Fig. 1). The Saba Bank is a Marine Protected Area recognized by the SPAW Protocol as having particular importance for the Wider Caribbean Region, acknowledged by the Convention of Biological Diversity as an Ecologically and Biologically Significant Area (EBSA) and designated by the IMO as a Particularly Sensitive Sea Area (PSSA). The research is part of the implementation of the Exclusive Economic Zone management plan for the Dutch Caribbean.

The Saba Bank houses an expansive coral reef ecosystem with a rich diversity of species. Shallow reefs (to about 20 m) have been estimated at covering about 19000 ha, or just under 10% of the total surface area (Debrot and Sybesma 2000). Deeper, mesophotic reefs probably cover an even larger surface area. As such the bank is also a commercially important source of fish for the nearby islands. The Saba Bank furthermore forms the largest protected area of the Kingdom of the Netherlands, second only after the Dutch part of the Wadden Sea in Europe. As there are no large land masses nearby, Saba Bank reefs have suffered less from pollution, coastal development or run-off (Debrot and Sybesma 2000). However, the bank does remain vulnerable to many less-localized environmental threats such as climate change, sea surface temperature increase and acidification, atmospheric nutrient input and waterborne invasive species and diseases

A key question is how populations of reef organisms on the Saba Bank are connected with populations in the region and in the Wider Caribbean. The aim of the current report is to investigate the population genetic structure of two common benthic native species (barrel sponge and great star coral), and a rapidly spreading invasive species in the Caribbean (lionfish).

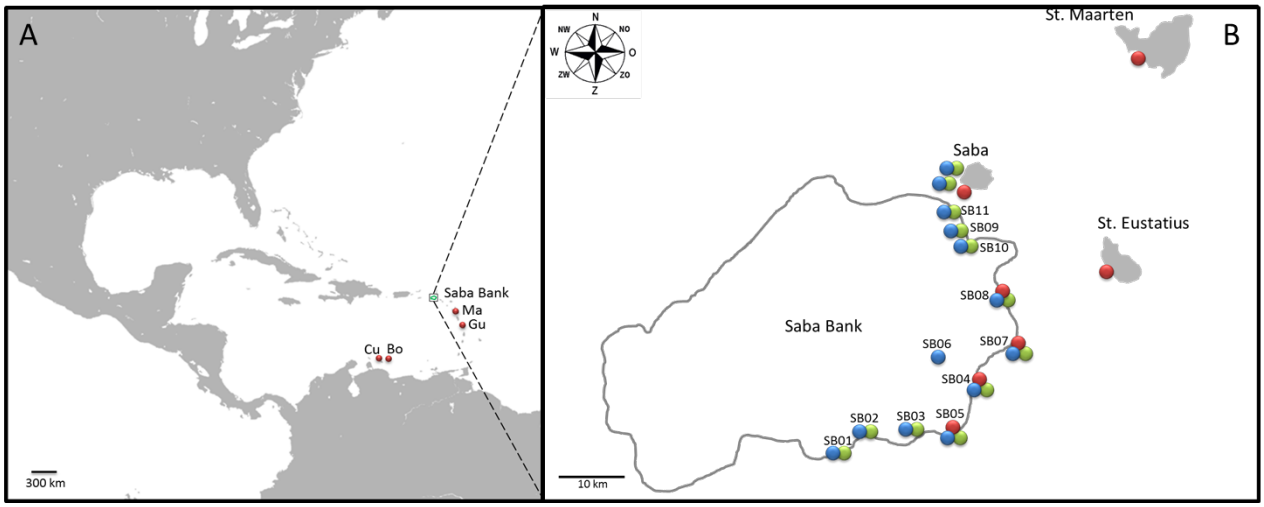

Figure 1 Saba Bank and other sample locations. *Blue dots represent Xestospongia muta, green dots Montastrea cavernosa and red dots lionfish (Pterois volitans).* ***A.*** *Lionfish sample locations in the wider*

Western Atlantic: Curacao (Cu), Bonaire (Bo), Martinique (Ma) and Guadeloupe (Gu). ***B.*** *SB01-SB11 correspond to all Saba Bank sample stations.*

1.2 Mesophotic reefs as refuge

Anthropogenic global rise in sea surface temperature (SST) is predicted to cause bleaching of many shallow coral reefs, placing increased importance on deeper reef habitats to maintain coral reef biodiversity and ecosystem function (Harris et al. 2013). It has been suggested that degraded shallow reefs (<20m) are increasingly reliant on recruitment of larvae from elsewhere, and that brood stocks in other habitats - such as mesophotic reefs (30 – 150 m) - could play a key role in the resilience of coastal seascapes (Slattery et al. 2011). Many coral and sponge species that are found in the shallow reefs are also found in the upper mesophotic zone. Serrano and colleagues (2014) studied vertical connectivity for *M. cavernosa* and their results indicate potential for recruitment from mesophotic to shallow reefs, although varying per location. Mesophotic reefs, such as those in the Saba Bank, may thus have the capacity to act as a refuge for endangered corals and sponges from which they could recolonize the shallow reefs and thus fulfill a key role in reef resilience (Bridge et 2013). Therefore, knowledge of marine population connectivity and larval dispersal between the Saba Bank and the surrounding region is critical to understanding its role in conservation, and management for all nearby coral reef systems (Slattery et al. 2011).

1.3 Genetic connectivity

Degraded coral reefs and their associated species rely on re-colonization from less affected areas. A key question for this project is how populations of reef organisms on the Saba Bank are connected within the bank and with areas elsewhere in the Wider Caribbean.

Connectivity between and within coral reefs in different regions is an important determinant of coral-reef resilience. Larval-exporting or source reefs with diverse populations of healthy adult coral reef organisms are essential to maintain the genetic diversity and resilience of larval-importing or sink reefs in other locations. Obtaining direct estimates of connectivity by tracing small larvae (with high-mortality rates) through an expanse of sea is not feasible. Therefore we here make an assessment of larval exchange by indirect means, namely through inferences from genetics. Successful migrants should leave a genetic trail of their movements, offering an indirect means of estimating population connectivity (Hellberg et al. 2002). The amount of variation in an organism's DNA is the combined product of past and present population processes. Geographical surveys of genetic variation can thus provide a means of tracing dispersal patterns between marine populations by larvae and other dispersing life stages (Hellberg et al. 2002).

In aquatic systems, large part of gene flow is controlled by hydrological factors (*e.g.* currents), facilitating egg and larval dispersal over large distances. In the Caribbean Basin, the main flow direction is from southeast to northwest (Fig. 2).

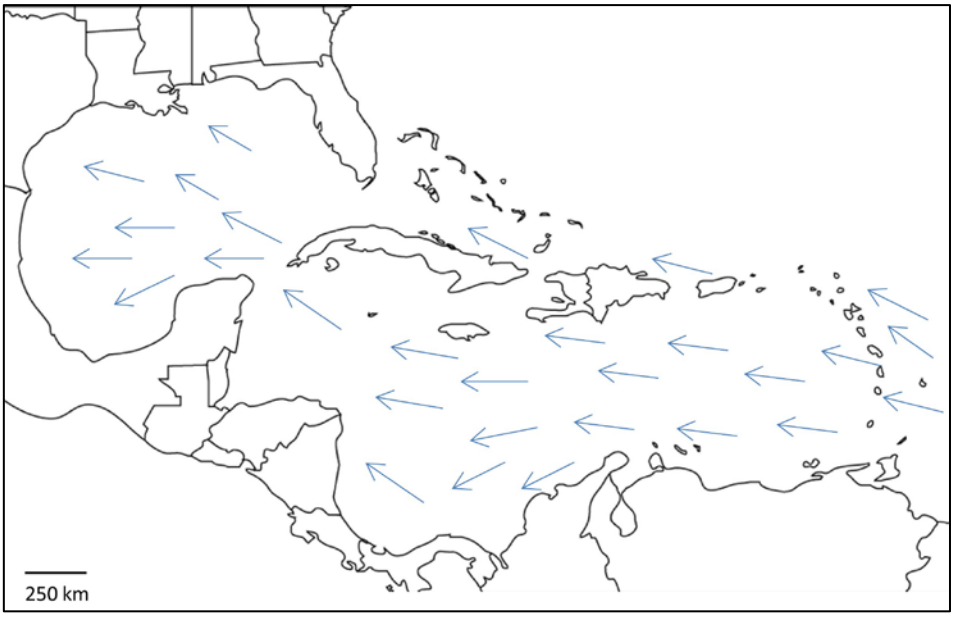

Figure 2. ***Direction of major currents in the Wider Caribbean.*** *Figure adapted from Miloslavich et al. (2010: Fig. 1)*

1.4 Target species

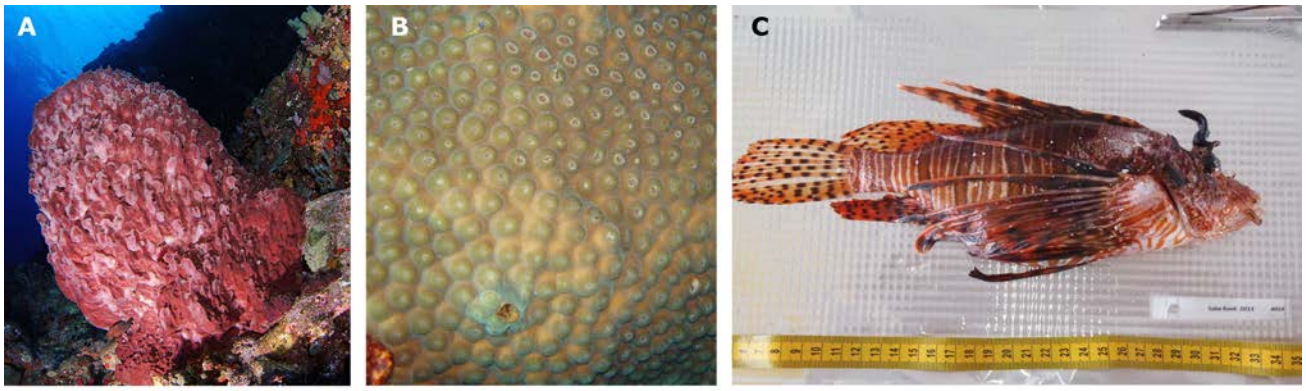

Figure 3 Study species. ***A.*** *Barrel sponge (Xestospongia muta) (photo Maggy Nugues),* ***B.*** *Great star coral (Montastrea cavernosa),* ***C.*** *Lionfish (Pterois volitans)*

Montastrea cavernosa (Star Coral)

Corals are facing massive worldwide decline due to a variety of natural and anthropogenic stressors (Bruno et al. 2007; Miller et al. 2009; Vega Thurber et al. 2014). Because corals are strongly dependent on recruitment after local disturbances, understanding patterns of connectivity is essential to implement effective conservation strategies. *M. cavernosa* (Scleractinia; Fig 3B) is a common reef building coral in the Western Atlantic (Szmant 1986; Veron 2000; Nunes et al. 2009). The species is a broadcast spawner, meaning that sperm and eggs are released into the water column where fertilization and development take place (Szmant 1986). Larvae of broadcast spawning corals are known to survive up to 100 days before final settlement, allowing them to disperse over very large distances (Wilson & Harrison 1998).

Nunes and colleagues (2009) were the first to look at the genetic structure of *M. cavernosa* using two fragments of the nuclear beta-tubulin gene. They found high levels of connectivity and gene flow among the majority of populations across the Caribbean. Similar results were found by Goodbody-Gringley *et al.* (2012), using beta(β)-tubulin, a fragment of the nuclear ribosomal internal spacer region (ITS) and a non-coding mitochondrial marker (IGR) to compare five Western Atlantic locations. They found moderate to high gene flow within and among these locations. Nevertheless, significant pairwise comparisons between several locations did suggest some restriction in gene flow.

Xestospongia muta (Giant Barrel Sponge)

Sponges are a prominent component of Caribbean coral reefs (Diaz & Rützler 2001) and the significant role they play in healthy reefs has become more apparent (Wilkinson 1983, 1990; Bell 2008; Wulff 2012; De Goeij et al. 2013). *X. muta* (Demospongiae: Haplosclerida; Fig 3A) is one of the largest and common members on Caribbean reefs (Armstrong et al. 2006; McMurray et al. 2008), reaching densities as high as 0.28 sponge/m2 (McMurray et al. 2010). *X. muta* contains high concentrations of cyanobacterial symbionts within its mesohyl and can be considered a high microbial abundance (HMA) sponge (Hentschel et al. 2006; Lopez-Legentil et al. 2010). Similar to corals, sponges are subject to bleaching as a consequence of increased water temperatures (Vicente 1990; Cowart et al. 2006; Lopez-Legentil et al. 2010). In *X. muta*, bleaching becomes apparent as the loss of its reddish-brown coloration, which is due to the presence of photosynthetic cyanobacteria (Vincente 1990; Cowart et al. 2006).

Within the Caribbean, many massive bleaching events have occurred, as reviewed by Angermeier et al. (2011). Cowart et al. (2006) described two types of bleaching in barrel sponges: cyclic bleaching, which seems to be temporary (affecting ± 25% of population) and fatal bleaching (affecting < 1% of population), which is synonymous with sponge orange band (SOB) disease and usually results in sponge mortality (Cowart et al. 2006; Lopez-Legentil et al. 2008; 2010). As pointed out by Lopez-Legentil et al. (2009), effective gene flow between populations might be essential to the recovery and ultimate survival of these sponges. To date, Lopez-Legentil et al. (2009) published the only study on the genetic structure of *X. muta* in the Western Atlantic. Using the mitochondrial I3-M11 partition of cytochrome oxidase subunit I (COI) (Erpenbeck et al. 2006), they found significant genetic divergence between most populations, probably caused by patterns of ocean currents and limited larval dispersal range. In their study, populations in Florida, the Bahamas and Belize were sampled. Only four different haplotypes were found for *X. muta* in the Western Atlantic (Lopez-Legentil *et al.* 2009). Swierts et al. (2013) found six haplotypes for the closely related *X. testudinaria* in Indonesia, of which two (haplotypes: C2 and C5) where shared with *X. muta* (haplotypes: H01 and H03). Results of both studies indicate that relatively little genetic variation was to be expected in the giant barrel sponge on Saba Bank, based on the I3-M11 partition fragment. The correlation between haplotype and morphology as described by Lopez-Legentil et al. (2009) in *X. muta* was also found by Swierts et al. (2013) in *X. testudinaria.*

Pterois volitans (Lionfish)

The two invasive Lionfish species (*P. volitans P. miles;* Fig. 3C), native to the Indo-Pacific, were introduced on the eastern coast of Florida around 1985 (Courtenay 1995; Semmens et al. 2004; Albins & Hixon 2008). After a temporal lag, *P. volitans* subsequently dispersed towards the Bahamas and northwards along the U.S. east coast. Within three decades *P. volitans* managed to successfully spread further throughout the Western Atlantic and is currently found from the northern part of the U.S. to the northern regions of South America (Schofield 2009; 2010; Betancur-R et al. 2011; Frazer et al. 2012). The efficiency of lionfish spreading, counter current, is remarkable. In its native range, lionfish reproduce seasonally, however, in the Western Atlantic reproduction seems to be year round. Currently, the population densities of Western Atlantic lionfish exceed those found in its native range by a factor of 13 to 15 (Darling *et al.* 2011; Kulbicki *et al.* 2011). Lionfish are thought to have a devastating effect on local (commercially important) fish populations, by predating on juveniles (Albins & Hixon 2008; Green *et al.* 2011; Valdez-Moreno *et al.* 2012). In addition, they are hardly predated on themselves (Morris 2009; Morris *et al.* 2011; Albins & Hixon 2013).

Lionfish removal strategies have successfully been implemented in many Western Atlantic location (Meesters et al. 2010; De León et al. 2013). Still, many Caribbean locations remain unfished, especially mesophotic reefs where the highest lionfish biomass can be found (White 2011; De León et al. 2013). This will allow continuous recruitment, reducing effectiveness of local removal efforts. In 2011 lionfish were first documented on the Saba Bank. Due to the largely mesophotic character, Saba Bank will possibly facilitate continuous lionfish recruitment to nearby locations (Personal comment: Kai Wulf, *Saba Conservation foundation*). Therefore, understanding patterns of dispersal in the Western Atlantic will be necessary for efficient lionfish control.

Hamner et al. (2007) used mitochondrial cytochrome b (cyt b) to compare Atlantic lionfish to native (Philippines, Western Indonesia) specimens. They defined 25 native, compared to three Western Atlantic haplotypes for *P. volitans* and 12 native, compared to a single Western Atlantic haplotype for *P. miles*. Similar results are described by Freshwater et al. (2009) for the mitochondrial control region (d-loop). Both studies indicate a strong founder effect and, together with Betancur-R et al. (2011) and Toledo-Hernández et al. 2014, have provided a first insight into lionfish population genetic structure in the Western Atlantic.

Here we analyze the genetic structures of the Eastern Caribbean populations and then, in combination with the previous data, construct a complete view on the population genetic structure and dispersal of lionfish in the Western Atlantic.

1.5 Aims

With the aid of molecular techniques and species counts from transects we aim to address the following:

- The level of genetic diversity within the populations of two common benthic species (*X. muta* and *M. cavernosa*) on the Saba Bank;
- The degree of genetic connectivity between populations on Saba Bank and surrounding reefs, based on newly obtained genetic sequences and sequences obtained from GenBank from populations across the Wider Caribbean;
- The current density and health status of the populations of *X. muta* and *M. cavernosa* on Saba Bank.
- The genetic connectivity, population size and the dispersal direction of invasion of the lionfish on the Saba Bank, with a particular focus on the Eastern Caribbean populations.
- The potential of Saba Bank populations to serve as a source of unique genetic variation to the neighboring reefs of nearby islands.

2. Study site

Since the 10th of June 2010 an Exclusive Economic Zone (EEZ) has been declared within the former Netherlands Antilles (Meesters *et al.* 2010; Meesters 2010). In 2010, the Dutch Government declared the Bank as protected area and it has since been registered as such in the Specially Protected Areas and Wildlife (SPAW) protocol of the Cartagena Convention (for the Protection and Development of the Marine Environment of the Wider Caribbean) (Van Beek & Meesters 2013). In addition, the Dutch Ministry of Economic Affairs, Agriculture and Innovation developed "The Saba Bank Research Program 2011-2016" in order to assess the health status of Saba Bank, the carrying capacity for commercial (lobster) fishing and to gain insight in key ecological processes. As a consequence, Saba Bank received designation as a Particular Sensitive Sea Area and (PSSA) at the International Maritime Organization (IMO) in 2012. The bank also carries status of Ecologically or Biologically Significant Area (EBSA) at the Convention on Biological Diversity (CBD) (Van Beek & Meesters 2013). As part of the Saba Bank Research program, an expedition was undertaken towards the Saba Bank in 2011 and again in October of 2013. During both expeditions, data on coral cover and recruitment, fish standing stocks, hydrology and topography were collected. With the collection and population genetic analyses of the three selected species during the 2013 expedition, we also provide a first insight into the issues of population genetic structuring on the Saba Bank and its connectivity to the wider Western Atlantic.

Because the EEZ harbors exceptional biodiversity, a management plan was designed with the aim of integrally managing the biodiversity and fisheries of the EEZ and achieving a balance between sustainable use and preservation (Meesters *et al.* 2010). The bank receives special attention within the management plan to ensure the protection of its unique biodiversity (Hoetjes & Carpenter 2010; Meesters *et al.* 2010; Van Beek & Meesters 2013). Saba Bank is located approximately 5 km southwest of Saba Island (Fig. 1). It is the largest atoll in the Atlantic Ocean and one of the three largest atolls on earth (Van der Land 1977; Meesters *et al.* 1996 ; Hoetjes & Carpenter 2010). Except for the actively-growing, 55-km-long coral ridge on the Eastern and Southern edge, the majority of the bank is occupied by algal fields and sand-dominated patches (Macintyre *et al.* 1975; Meesters 2010). Due to its offshore location and the mesophotic reef system (overall 20-50 m deep), the coral reefs seem to have suffered relatively little anthropogenic disturbance compared to fringing reefs of the surrounding islands (Williams *et al.* 2010). This is indicated, for instance, by the relative absence of diseases (McKenna & Etnoyer 2010; Van Beek & Meesters 2013) and recent bleaching mortality, as well as by the presence of large predators (*e.g.* sharks, groupers and snappers) (Toller *et al.* 2010; Meesters 2010).

Although limited, Saba Bank is not free of harmful human influences, *e.g.* overfishing, anchoring and leakage of oil tankers and explorations for natural resources. Meesters (2010) notes in his report that, although the reefs seem to be recovering from a heavy bleaching event in 2005, a reef degrading trend is visible on the bank compared to previous observations (*e.g.* Van der Land 1977; Meesters *et al.* 1996).This is marked by a high algal cover (Littler *et al.* 2010; Toller *et al.* 2010) and limited presence of herbivores (black sea urchins, fish) and lobsters.

Table 1. *Coordinates and code names of sample locations*

Location	Site	Site	Latitude	Longitude	Date
Saba Bank	Dutch Plain	SB1	17.234898	-63.446182	20-10-13
	Scottish Hills	SB2	17.268384	-63.408775	20-10-13
	Gorgonian Delight	SB3	17.261937	-63.344296	20-10-13
	Coral Garden	SB4	17.345833	-63.251111	21-10-13
	Pauls Cathedral	SB5	17.271111	-63.280833	21-10-13
	Tertre des Fleurs	SB6	17.384167	-63.289883	21-10-13
	Eriks Point	SB7	17.396944	-63.196389	22-10-13
	Twelve Monkeys	SB8	17.458333	-63.222222	22-10-13
	La Colline aux Gorgones	SB9	17.525556	-63.270278	22-10-13
	Devils Corner	SB10	17.505833	-63.253889	23-10-13
	Rebeccas Garden	SB11	17.559434	-63.286377	23-10-13
Saba Island	Christal Rock	SI12	17.385125	-63.152146	24-10-13

3. Material and methods

3.1 Sample collection

Sponge (*Xestospongia muta)* and coral (*Montastrea cavernosa)* material was collected during the Saba Bank Expedition II 2013, organized by IMARES and commissioned by the Dutch Ministry of Economic Affairs (October 19th-26th 2013). Samples were collected at 11 sites on the eastern and southern ridge of the bank. In addition, two Saba Island sites where sampled (Fig. 1). As the majority of the bank is too deep or sand-covered, most coral reefs are found on the edge of the bank, hence determining the sample site distribution. All sampled sponges and coral colonies were id-labelled and extensively photographed for morphology and disease recognition (Fig. 2). Sponge tissue was obtained using an apple corer, in order to sample symbiont-rich surface as well as internal tissue. Coral samples were collected by chiseling of several polyps off the colonies' edge. To minimize the chance of sampling clones, a considerable distance (> 10 m) was kept between the individual sponges and coral colonies on each location. All collected samples werestored in individual zip lock bags. Location and depth where noted for each sample taken. Lionfish (*Pterois volitans*) were speared at several sites. Additional lionfish samples from Saba Island, St. Maarten, St. Eustatius, Bonaire, Curacao, Guadeloupe and Martinique were obtained via colleague researchers. All Saba Bank samples were collected by SCUBA diving within a depth range of 15 to 35 m (For details see van Beek & Meesters 2014).

For DNA analysis, a small piece (0.5 cm^3) of the *X. muta* internal tissue was cut off each sample using a razorblade and forceps and stored in individual 2 ml reaction tubes with RNA*later*^{tm} (QIAGEN). The remaining tissue was divided and kept on 96% ethanol for identification and microbe analysis. A similar-sized piece of the collected *M. cavernosa* was also stored in RNA*later*^{tm}-filled tubes for DNA analysis. Lionfish muscle tissue was collected from underneath the skin in order to minimize the possibility of contamination between fish. Lionfish DNA samples were stored in 96-99% ethanol. Each fish was measured and photographed. Razorblades and forceps were extensively rinsed in 70% ethanol and water between each individual sample. All samples were kept at 4°C directly after collection and during transport, and subsequently stored at -20°C.

3.2 Molecular labwork

Extractions

Total DNA was extracted from each individual sample using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma). Before extraction, remaining RNA*later* tm traces were removed from the sample by dabbing it on absorbing paper. For extraction, the manufacturers protocol was followed, with a supplementary step after Lyses 'T' solution addition, where the tissue was gently ground within the tube, using a plastic pestle. The solution was incubated for approximately 4 hours at 55°C, or until complete cell lysis. Furthermore, instead of 100 µL , 50 µL of elution buffer was pipetted on the column to achieve higher DNA concentrations. For the detailed protocol see Appendix 1.1. DNA was visualised by 1% agarose gel electrophoresis with a Bromo-Phenol Blue (BFB) loading buffer. All laboratory work was performed at the *Royal Netherlands Institute for Sea Research* (NIOZ), Texel, the Netherlands.

PCR amplification

All polymerase chain reaction amplifications were carried out in T-Gradient Thermo-block (Biometra) or Doppio Fuse 8.0A thermal cyclers at the NIOZ. Sequencing was done by BaseClear B.V Leiden, The Netherlands, using 30 µL of the each initial PCR product.

<u>Xestospongia muta</u>

The 544bp-long I3-M11 partition of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the universal metazoan primer *C1-J2165* (5'-GAA GTT TAT ATT TTA ATT TTA CCD GG-3') (Misof et al. 2000) and the reverse primer *C1-Npor2760* (5'-TCT AGG TAA TCC AGC TAA ACC-3') (Erpenbeck et al. 2002). Polymerase chain reaction (PCR) amplifications were carried out in a 50 µL reaction volume containing 5.0 µL 10x PCR buffer, 5.0 µL dNTP (2.5 mM), 0.5 µL (50 µM) of each Primer, 0.25 µL BiothermPlus Taq, 2.0 µL of DNA template and 36.74 µL H_2O. Following an initial denaturation soak at 95°C for 3 minutes, each reaction underwent 35 thermocycles of 95°C (30s), 42°C (30s) and 68°C (90s) followed by a final extension at 72°C for 10 minutes and 5 minutes at 4°C.

Montastrea cavernosa

Amplification of the 550bp-long nuclear β-tubulin region of nuclear DNA was achieved using the primers Tub-F (5'-GCATGGGAACGCTCCTTATTT-3') (Fukami et al. 2004) and Tub-Rjvb2 (5'-AGG AACCATGTTCACTGCCA-3'), newly developed in this study. The 50 µL reaction volume contained 35.76 µL H_2O, 5.0 µL 10x buffer, 5.0 µL dNTP (2.5 mM), 0.5 µL (50 µM) of each Primer, 0.25 µL Biotherm + Taq, 1.0 µL BSA and 2.0 µL of DNA template (10x diluted). The thermal cycler profile used was 94°C for 3 minutes followed by 36 cycles at 94°C for 30s, 50°C for 30s and 72°C for 45s. This was followed by a final extension at 72°C for 5 minutes and 4°C for 5 minutes. The 892bp-long internal transcribed spacer 1 – 5.8S ribosomal RNA – internal transcribed spacer 2 (ITS hereafter) was amplified as a second genetic marker for *M. cavernosa*. Primers used were 1S (5'-GGTACCCTTTGTACACACCGACCGTCGCT-3') and 2SS (5'-GCTTTGGGCGGC AGTCCCAAGC AACCCGACTC-3') (Odorico & Miller 1997; Goodbody-Gringley et al. 2012). PCR reaction volume and cycler profile were the same as for β-tubulin. However, only 0.25 µL (50 µM) was used for each primer to minimize primer dimer formation and 2 uL of undiluted template was used.

Lionfish

For lionfish (*P. volitans* and *P. miles*) the 679bp mitochondrial control region (d-loop) was amplified using LionA-H (5'-CCA TCT TAA CAT CTT CAG TG-3') and LionB-L (5'-CAT ATC AAT ATG ATC TCA GTAC-3') as primers (Freshwater et al. 2009). The 50 µL amplification reaction volume contained 5.0 µL 10x PCR buffer, 5.0 µL dNTP (2.5 mM), 0.5 µL (50 µM) of each Primer, 0.25 µL Biotherm + Taq, 2.0 µL of DNA template and 36.74 µL H_2O. The thermal cycler profile used was 94°C for 3 minutes followed by 36 cycles at 94°C for 30s, 50°C for 30s and 72°C for 45s followed by a final extension at 72°C for 5 minutes and 4°C for 5 minutes.

3.3 Sequences from GenBank

In order to compare our data to population data from the Wider Caribbean, sequences of previous studies (Lopez-Legentil et al. 2009, Montalvo & Hill 2011, Goodbody-Gringley et al. 2012, Freshwater *et al.* 2009, Betancur-R et al. 2011) were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

3.4 Analysis of molecular data

Sequence preparation

Consensus sequences were constructed from the forward and reverse sequences, using the software programs *Auto-assembler DNA Sequence Assembler* ver. 2.1 (Applied Bio-systems, Perkin-Elmer) and *Chromas Pro* ver. 1.7.5 (Technelysium Pty. Ltd, Tewantin, Queensland, Australia). Final consensus

sequences were loaded in Geneious® ver. 7.0.6 (Biomatters) and aligned (Geneious alignment, 93% similarity 5.0/-9.026186) with homologous sequences obtained from Genbank® (http://www.ncbi.nlm.nih.gov/genbank/). In Genbank, searches for each specific marker and species, as well as NCBI nucleotide BLAST (blastn) (www.ncbi.nlm.nih .gov/BLAST/) searches were conducted to recover all previously published sequences. Subsequently, nucleotides were added or removed on both trim-ends of the query sequences, to fit Genbank sequence lengths. If any nucleotides had to be added, non-consensus (only forward or reverse sequences) data was usedto achieve the desired length where possible. Where possible polymorphic sites were visually resolved using the original chromatogram files, only if one peak was clearly lower (< 80% of the highest peak). When multiple nucleotide ambiguities in one sequence could not be resolved or no consensus could be built, sequences were left out of the subsequent analyses (1 in I3-M11; 20 in ITS).

In the majority of β-tubulin sequences a multitude of ambiguities was found. As no further analyses were performed on these sequences.

Genetic variation and population structure

Genetic diversity on Saba Bank and populations in the wider Caribbean region was determined based on estimates of haplotype diversity (*h*, Nei 1987) and nucleotide diversity (*π*, Nei 1987) using the software Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010). The most suitable model (JC + G for ITS and K2 + G + I for I3-M11) was selected in jModelTest ver. 2.1.2 (Darriba *et al.* 2012), based on the Akaike Information Criterion (AIC). Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was conducted to determine presence of genetic population structuring on the Saba Bank. The bank was divided in three main geographical regions: North-East (NE), Middle-East (ME) and South-East(SE)(Fig. 1). Comparisons among Saba Bank populations (NE vs. ME vs SE) and among all sampled locations were conducted based on pairwise Φ_{ST} statistics (10 000 bootstrap permutations). All AMOVA, exact tests and Φ_{ST} statistics were conducted in Arlequin ver. 3.5.1.2. Maximum likelihood trees were constructed in MEGA ver. 6.06 (Tamura *et al.* 2013) including all sequences of all sampled location. Trees were subsequently also used to construct haplotype networks in HaplotypeViewer (http://www.cibiv.at/~greg/haploviewer).

For each individual genetic marker, haplotypes were given a specific color, corresponding to that specific haplotype. The maximum spanning network for each specific genetic marker was obtained from Arlequin and loaded in to Hapstar ver. 0.5 (Teacher & Griffiths, 2011) or FigTree ver. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree). Constructed networks were used as basis for the final haplotype networks.

3.5 Population density & health status

In order to estimate the densities of *X. muta* and *M. cavernosa*, at each station a 50 m transect lines was placed on the reef surface. Every meter, a high resolution picture was taken of a 1m^2 surface area(Fig. 3), resulting in 50 m^2 being surveyed per site. Transect pictures were taken by Erik Meesters, Jean-Philippe Maréchal and Franck Mazeas. Species were counted visually in the CPC (Coral Point Count with Excel extensions) software ver. 4.1 (Kohler & Gill 2006). Only sponges and corals present in a 1 m^2 section in the center of each picture were counted. Every square was aligned with each meter of transect to prevent repeated counting. Lionfish counts were obtained by Ingrid van Beek from video footage of the transects made by colleague expedition members Erik Bomans, Steve Piontek and Fleur Holtrop.

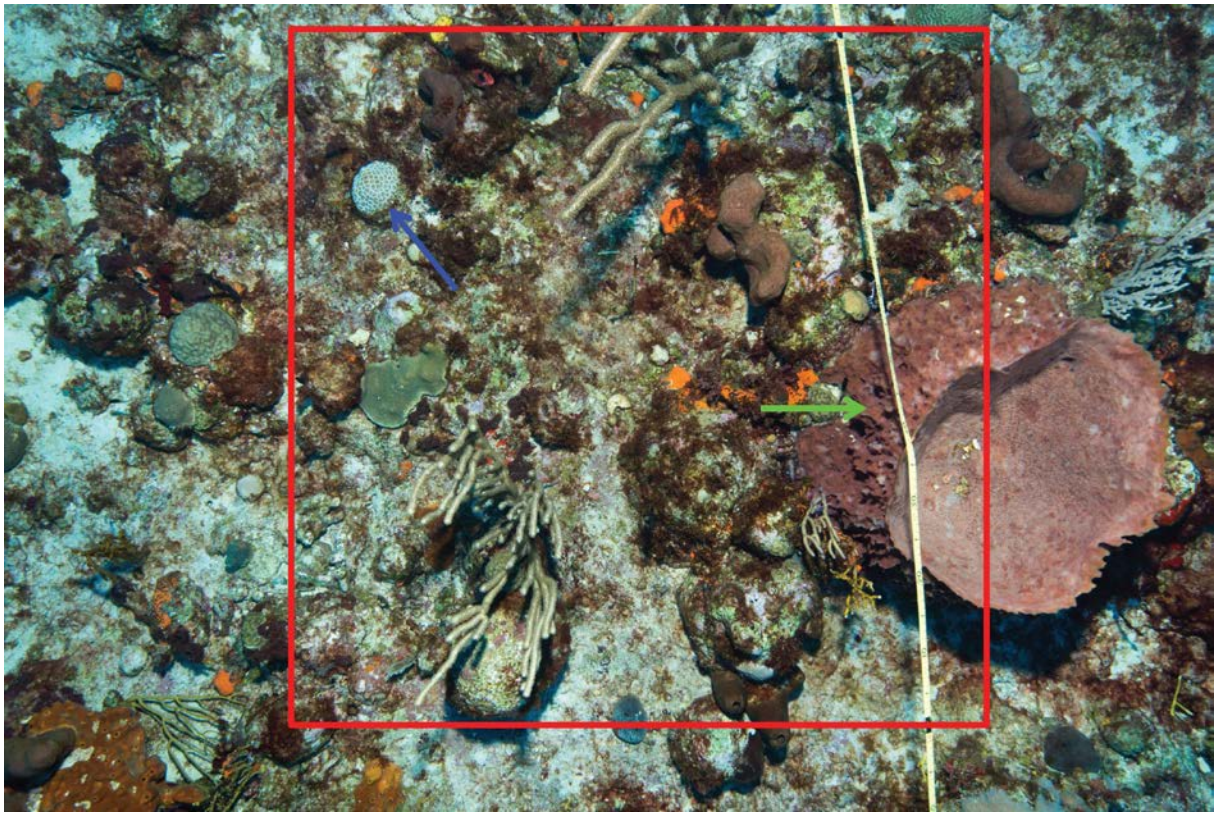

Figure 3. Transect picture from Saba Bank site 5 (SB5). *Red square (1 m2) overlaps with 1 m of the transect line (length between the two black dots. Only M. cavernosa (blue arrow) and X muta (green arrow) within the red square were counted.*

All collected colonies of *M. cavernosa* and individuals of *X. muta* were analyzed for signs of diseases and bleaching. In addition, transect-pictures covering 50 m^2 per site were analyzed for health status. *M. cavernosa* colonies were checked for white plague, black and orange band disease and dark spots. Previous mortality indicated by the loss of tissue – but still containing recognizable polyps – and partial algal overgrowth, was also recorded. Individual sponges of *X. muta* were examined for both types of assumed bleaching as described by Cowart *et al.* (2006) and McMurray et al. (2011). Cyclic bleaching can be recognized by the circular spots with loss of the typical brownish-red coloration in parts of the sponge tissue.

4. Results

4.1 Montastrea cavernosa

Genetic diversity

From Saba Bank and Saba island, 34 sequences of 832bp fragment length (ITS) were obtained, representing 13 haplotypes. Including sequences from previous studies from the Western Atlantic, a total of 46 haplotypes with 26 polymorphic sites (2.91% variation) were found. Haplotypes H01-H03 were dominant on Saba Bank and found throughout the Western Atlantic. Three new haplotypes were found for *M. cavernosa* in this study, two on Saba Bank (H44-45) and one on Saba Island (H46) (Fig.4). The

genetic diversity (indicated by haplotype- and nucleotide diversity) of *M. cavernosa* populations on the Saba Bank was high and comparable with other locations in the Western Atlantic (Table 2).

Table 2 Standard diversity measures for populations of M.cavernosa. *Including number of individuals sampled (n), number of haplotypes (with unique haplotypes in brackets), haplotype diversity with standard deviation (h), and nucleotide diversity with standard deviation (π).*

	n	**#haplotypes**	***h***	**π**
Saba Bank	30	12(2)	0.8828 ± 0.0360	0.005479 ± 0.003057
Saba Island	4	4(1)	1.0000 ± 0.1768	0.006913 ± 0.004966
Barbados[1]	14	9(3)	0.9011 ± 0.0624	0.005063 ± 0.002975
Bermuda[1]	30	15(9)	0.9287 ± 0.0247	0.006180 ± 0.003402
Flower Gardens[1]	18	13(7)	0.9608 ± 0.0301	0.005862 ± 0.003325
Jamaica[1]	18	13(4)	0.9542 ± 0.0335	0.005495 ± 0.003140
Panama[1]	21	15(8)	0.9667 ± 0.0236	0.005563 ± 0.003146

1. Data from Goodbody-Gringley et al. (2012)

Population structure

On the Saba Bank, sample locations were divided over three main eastern regions: South-East, Middle-East, North-East). There was no population genetic structuring between the three main regions on the Saba Bank (Table 3, Fig. 4), indicating unobstructed gene flow across the eastern rim of the Saba bank.

There was no significant population differentiation between the Saba Bank populations and the majority of populations from other regions in the Western Atlantic that have been sampled (Table 4, Fig. 4). There was only a significant, albeit weak (Φst<0.01), difference among Saba Bank and the populations in Panama, Barbados, and the Gulf of Mexico.

Table 3 Matrix of Pairwise population differentiation on Saba Bank. *pairwise Φ_{st} between the three Saba Bank regions for M. cavernosa (ITS). Diagional shows the within-region nucleotide diversity is shown in bold-italic.*

Group	South-East	Middle-East	North-East
South-East	***0.0059***		
Middle-East	0	***0.0048***	
North-East	0	0.0068	***0.0056***

Table 4. Matrix of Pairwise population differentiation of M. cavernosa on Saba Bank. *Pairwise population differentiation values (Φst) between Saba Bank and Western Atlantic locations. Bold indicates significant Φst values (p<0.05).*

	Saba Bank	Saba Island	Barbados	Bermuda	Gulf of Mexico	Jamaica	Panama
ITS							
Saba Bank	-						
Saba Island	0.0032	-					
Barbados	**0.0570**	-0.0644	-				
Bermuda	0.0238	-0.0602	0.0103	-			
G. Mexico	**0.0544**	-0.0672	0.0226	0.0099	-		
Jamaica	0.0340	-0.0644	-0.0072	-0.0170	0.0137	-	
Panama	**0.0973**	-0.0742	-0.0115	0.0066	0.0105	0.0156	-

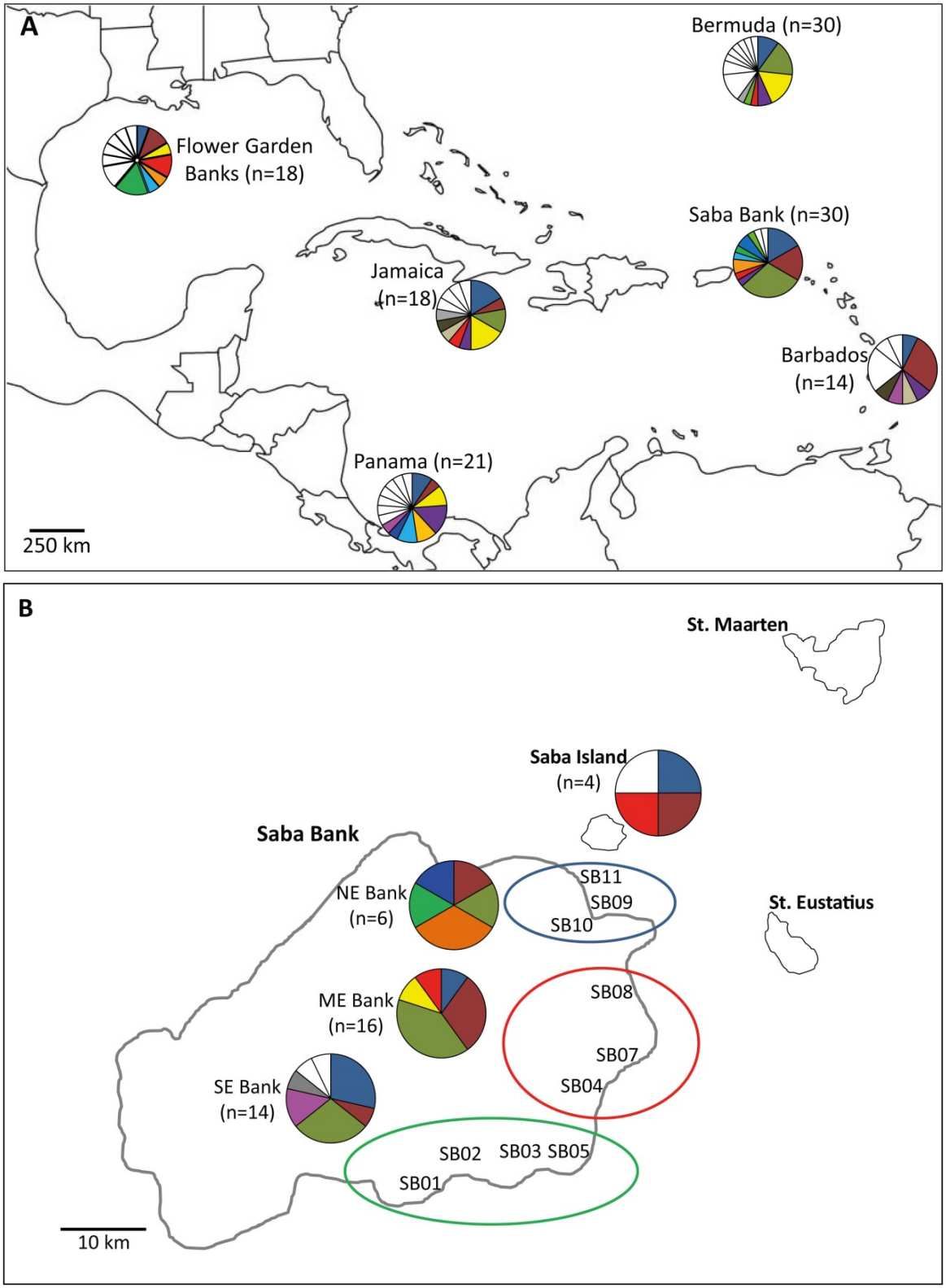

Figure 4. Frequency and distribution of haplotypes in populations of M. cavernosa **in the Wider Caribbean (above) and Saba Bank region (below).** *Haplotype frequencies provided in pie-chart per location, number of samples in brackets. Haplotype color-codes correspond to colors in Fig. 5.*

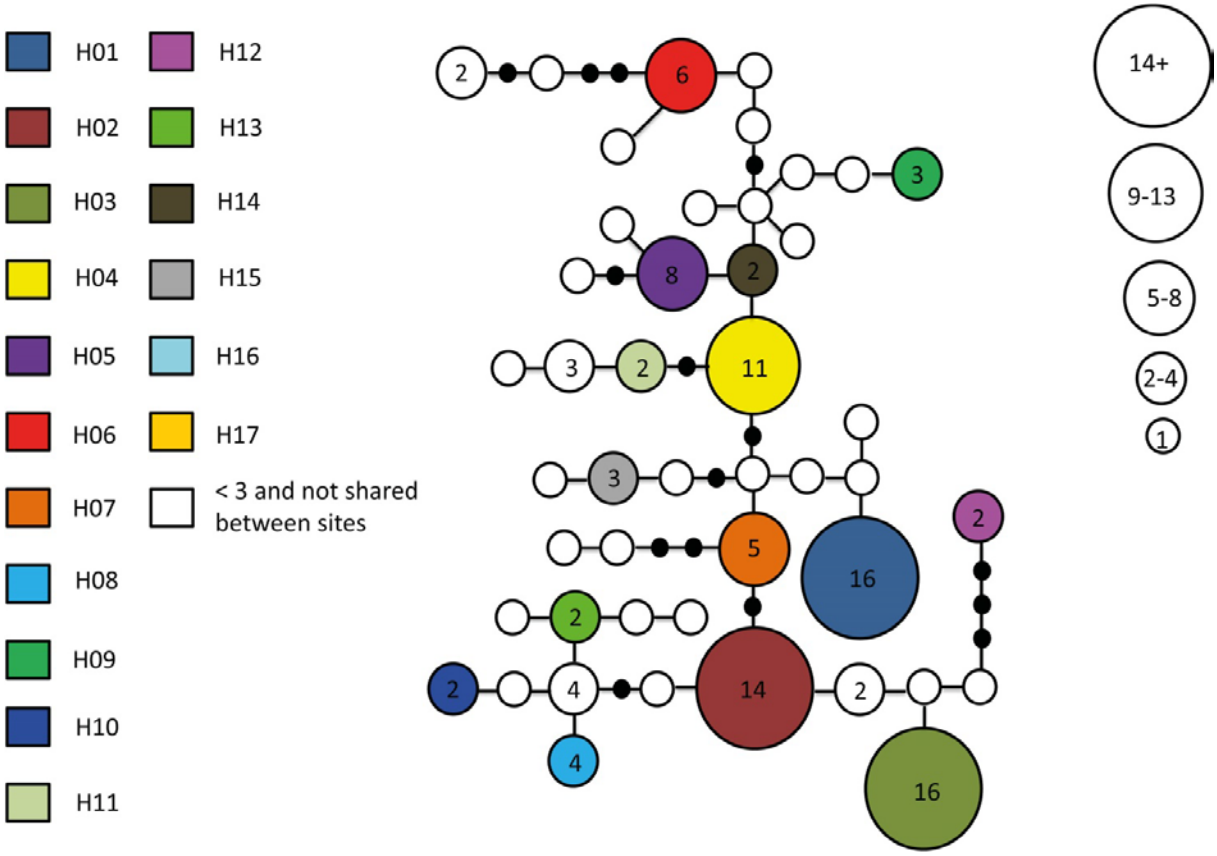

Figure 5. Haplotype network of M. cavernosa **populations in the Wider Caribbea*n.*** *Size of circle reflects number of individuals containing a specific haplotype (H01-H17). Each line represents the genetic distance between haplotypes. For distribution of haplotypes, see Figure 4.*

Population density

Based on our survey of 11 transects of 50 m^2 (1 per site, total area surveyed 550 m^2), the mean density of *M. cavernosa* was 0.38 ± 0.32 ind m^{-2}.

Table 5 Density of *M. cavernosa* on the Saba Bank in 2013. *Number of* M. cavernosa *along a transect (50 m^2) per location (n), tissue loss presumably due to past bleaching (t.l.), overgrowth by cyanobacteria, algae and/or sponges (o.g.). No disease detected in any of the recorded individuals.*

M. cavernosa	n	n/m^2	t.l.	o.g.
Dutch Plain	46	0.92	37	25
Scottish Hill	48	0.96	27	13
Gorgonian Delight	8	0.16	7	5
Coral Garden	9	0.18	9	7
Paul's Cathedral	23	0.48	20	14
Tertre des Fleurs	1	0.02	1	
Erik's Points	28	0.56	25	12
Twelve Monkeys	3	0.06	3	2
La Colline aux Gorgones	11	0.22	10	8
Devil's Corner	20	0.40	16	11
Rebecca's Garden	10	0.16	7	5

Disease

A total number of 261 *M. cavernosa* were photographed and assessed for bleaching, disease, and algae overgrowth. No disease was observed in any of the *M. cavernosa* colonies. However, previous tissue loss (78% of colonies) and current overgrowth of a part of the colony (48.08% of colonies) was observed. Cyanobacteria accounted for the vast majority of overgrowth (56.69%) followed by fleshy macroalgae or turf (23.62%), sponges (15.75%), and gorgonians (2.36%) and crustose coralline algae (1.57%).

4.2 Xestospongia muta

Genetic diversity

On Saba Bank and Saba Island, a total of 68 sequences of 544bp fragment length were obtained, representing 3 haplotypes. No new haplotypes were discovered. Combining sequences of this study with previous literature (Lopez-Legentil *et al.* 2009), a total of 4 haplotypes with 5 polymorphic sites (0.92% variation) was found for *X. muta*. Two haplotypes (H01, H02) were found to be the most dominant within the Saba Bank (Fig 6&7) and these are also common throughout the Western Atlantic. The genetic diversity (based on haplotype- and nucleotide-diversity) of the Saba Bank was comparable to the other locations that have been samples (Table 6)

Table 6. Standard diversity measures for populations of *X.muta*. *Number of samples collected (n), number of haplotype, haplotype diversity with standard deviation (h), and nucleotide diversity with standard deviation (π, pi).*

Location	n	#haplotypes	h	Π
Saba Bank	53	3	0.4579 ± 0.0634	0.001094 ± 0.000986
Saba Island	15	3	0.3619 ± 0.1448	0.000945 ± 0.000944
Florida, Key Largo[1,2]	21	3	0.6667 ± 0.0498	0.002136 ± 0.001601
Belize[1]	16	3	0.5750 ± 0.1120	0.003309 ± 0.002255
Bahamas, Sweetnings Cay[1]	19	3	0.6959 ± 0.0417	0.003612 ± 0.002389
Bahamas, Plana Cay[1]	15	2	0.1333 ± 0.1123	0.000490 ± 0.000636
San Salvador[1]	12	2	0.3030 ± 0.1475	0.001114 ± 0.001067
Ltl. San Salvador[1]	14	2	0.4396 ± 0.1120	0.003232 ± 0.002236
Bahamas, Stirrup Cay[1]	22	3	0.4805 ± 0.0935	0.003509 ± 0.002317

1. data from Montalvo & Hill (2011) **2.** data from Lopez-Legentil & Pawlik (2009)

Population structure

The absence of strong or significant Φ_{St} values among the populations on the Saba Bank (Table 7) indicates that there is little genetic structuring and thus likely unobstructed gene flow along the eastern rim of Saba Bank.

Between Saba Bank and Saba Island there was no significant genetic structuring, likewise indicating ample genetic connectivity. However, there was a strong and significant genetic differentiation between the Saba Bank and Belize and the Bahamas. This was due to the absence of haplotype H04 (yellow) which is present in Belize and the Bahamas but absent at the Saba Bank and Saba Island.

Table 7 Matrix of Pairwise population differentiation on Saba Bank. *For X. muta (I3-M11) pairwise Φ_{St} and P-values (blue) between the three Saba Bank regions are displayed. Diagional shows the within-region nucleotide diversity is shown in bold-italic.*

Xestospongia muta	Group	South-East	Middle-East	North-East
	South-East	***0.0023***		
	Middle-East	0	***0.0013***	
	North-East	0	0	***0.0008***

Table 8. Matrix of pairwise population differentiation of *X. muta* on the Saba Bank and locations in the Western Atlantic. *Pairwise population differentiation values (Φst) between Saba Bank and different Western Atlantic locations. Bold indicates significant Φst values (p<0.05)*

	Saba Bank	Saba Island	Florida	Belize	Bahamas (Sweeting s Cay)	Bahamas (Plana Cay)	Bahamas(San Salvador)	Bahamas (L. San Salvador)	Bahamas (Stirrup Cay)
I3-M11									
Saba B.	-								
Saba I.	-0.0212	-							
Florida	0.0557	0.0693	-						
Belize	**0.1981**	**0.1835**	0.0204	-					
Bahamas SC	**0.5031**	**0.3893**	**0.3321**	**0.2818**	-				
Bahamas PC	0.0673	-0.0216	**0.1756**	**0.2847**	**0.4065**	-			
Bahamas SS	0.0828	-0.0069	0.1120	**0.2210**	**0.2946**	-0.0277	-		
Bahamas LSS	**0.6975**	**0.6179**	**0.5445**	**0.4369**	0.0677	**0.6452**	**0.5535**	-	
Bahamas STC	**0.6387**	**0.5450**	**0.4906**	**0.3942**	0.0532	**0.5638**	**0.4836**	-0.0583	-

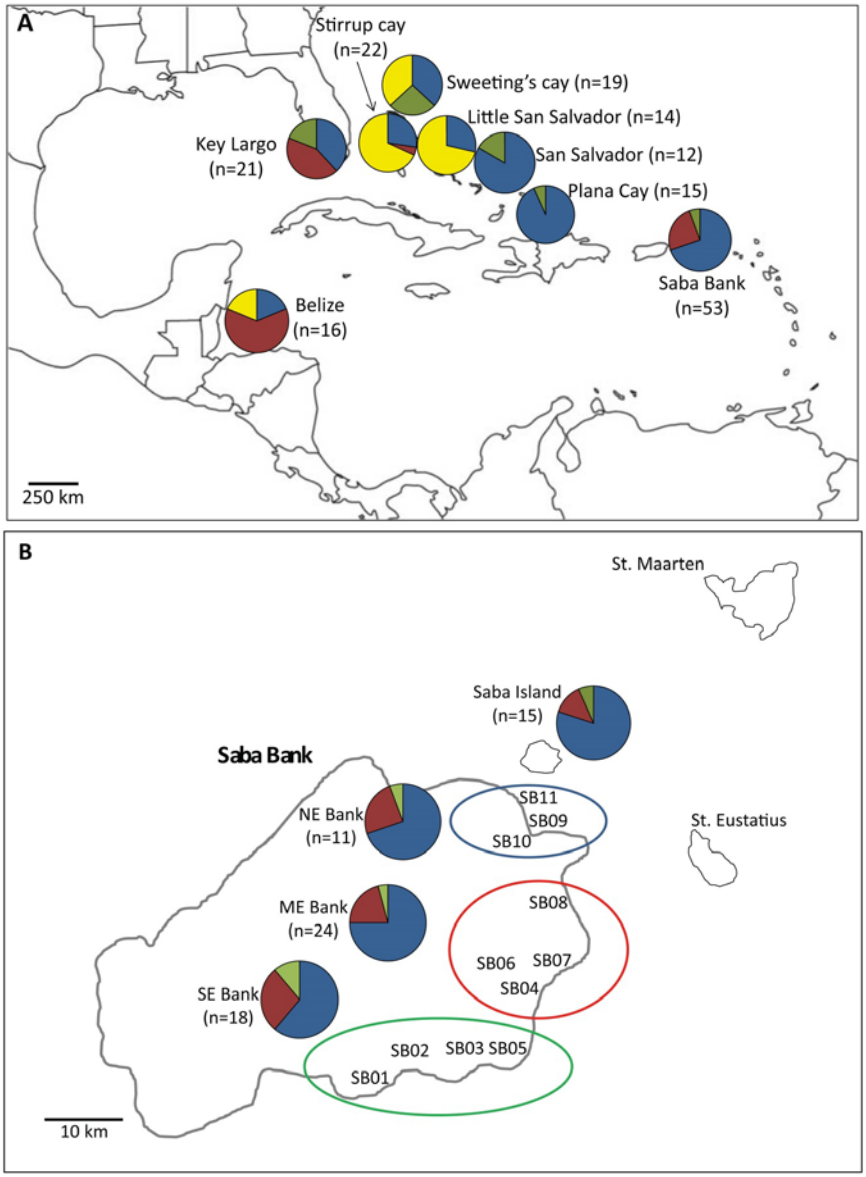

Figure 6. Frequency and distribution of haplotypes in populations of *X.muta* in the Wider Caribbean (above) and Saba Bank region (below). Haplotype frequencies provided in pie-chart per location, number of samples in brackets. Haplotype color-codes correspond to colors in Figure 7.

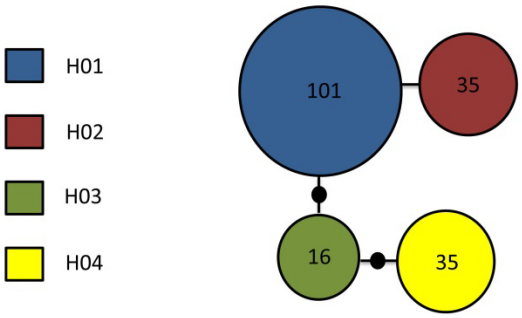

Figure 6. Haplotype network of *X.muta* populations in the Wider Caribbean. *Size of circle reflects number of individuals containing a specific haplotype (H01-H04). Each line represents the genetic distance between haplotypes. For distribution of haplotypes, see Figure 7.*

Population density

Based on our survey of 11 transects of 50 m^2 (1 per site, total area surveyed 550 m^2), the mean density *X. muta* was 0.23 ± 0.19 ind m^{-2}.

Table 9. Population density and number of individuals with bleaching and diseases in *X. muta* in 2013. *Number of X. muta along a transect line (50 m^2) per location (n). Number of X. muta with cyclic bleaching (After Cowart et al. 2006).*

X. muta	n	n/m^2	Cyclic bleaching
Dutch Plain	7	0.14	4
Scottish Hill	10	0.2	7
Gorgonian Delight	5	0.1	3
Coral Garden	0	0	0
Paul's Cathedral	23	0.46	12
Tertre des Fleurs	18	0.36	4
Erik's Points	36	0.72	24
Twelve Monkeys	0	0	0
La Colline aux Gorgones	5	0.1	4
Devil's Corner	3	0.06	2
Rebecca's Garden	11	0.22	10

Diseases

A total number of 186 *X. muta* were photographed and assessed for bleaching, disease, and algae overgrowth. We recorded extensive presence of presumable cyclic bleaching in *X. muta* (Cowart *et al.* 2006), which fits the description of 'spottily bleached', defined by McMurray *et al.* (2011) as 'numerous localized patches or spots of white tissue'. On almost all of the sampled sponges for DNA analysis (92%) and the majority of sponges on the transect pictures (75%), the sponge tissue was 'spottily bleached' (Table 1, Fig. 5). Many smaller *X. muta* (> 20% on the transect pictures) were overgrown by algae or other sponges. Microbial analysis of the bleached tissue will later be conducted by Detmer Sipkema (WUR).

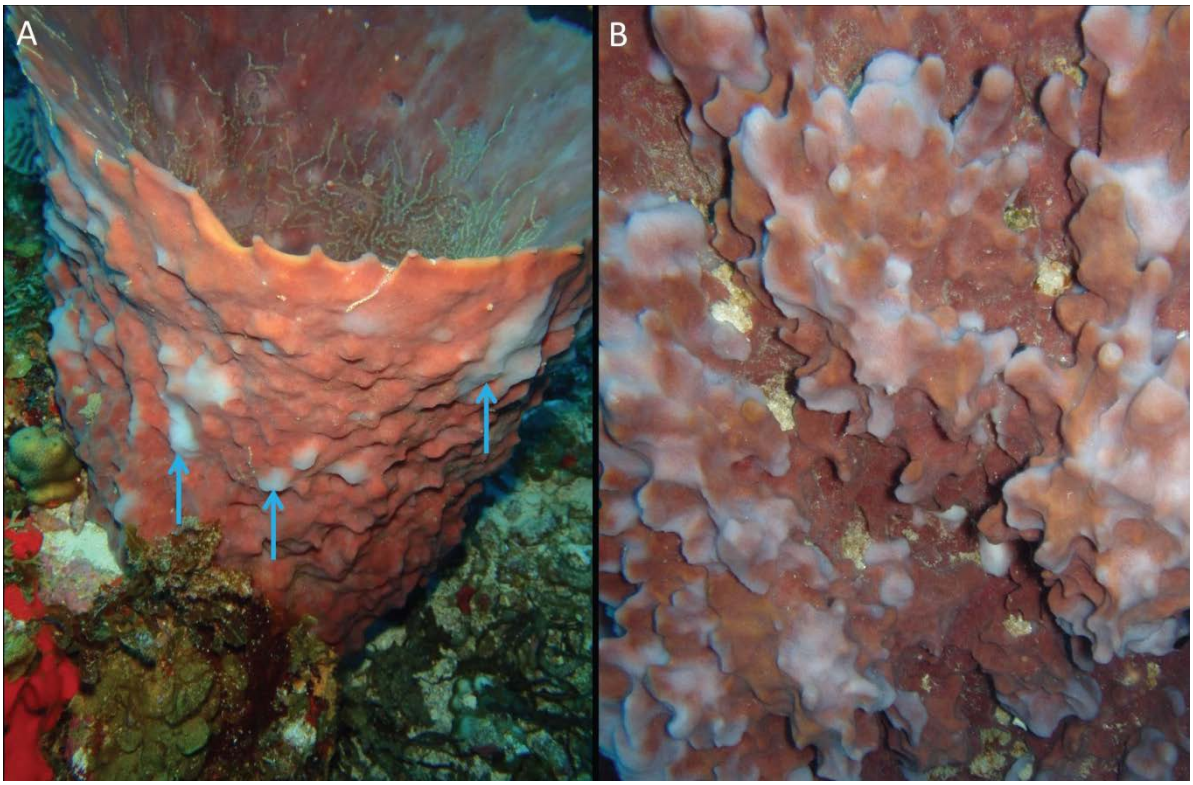

Figure 8. ***Diseases in Xestospongia muta on the Saba Bank*****.** ***A.*** *Cyclic bleaching (blue arrows).* ***B.*** *Detail of Cyclic bleaching.*

4.3 Lionfish

Genetic diversity

From Saba Bank and Saba Island, 41 sequences of 679bp fragment length were obtained, representing 3 haplotypes. Including sequences from previous studies, a total of 9 haplotypes were found. H01-H02 were dominant on Saba Bank and found throughout the Western Atlantic. The genetic diversity of the Saba Bank was among the lowest of all sampled locations, confirming a recent invasion of the bank (Table 10, Fig. 9). There are only three main haplotypes in the Wider Caribbean versus eight in the Northern region (Bahamas, North Carolina, Bermuda), which is the location of initial introduction of this invasive species. Diversity appears to diminish as locations become further removed from the location of initial introduction.

Table 10. Standard diversity measures for populations of Lionfish. *Number of samples collected (n), number of haplotype, haplotype diversity with standard deviation (h), and nucleotide diversity with standard deviation (π).*

Location	region	n	# haplotypes	h	π
Saba Bank	EAST	25	3	0.3967 ± 0.1027	0.002081 ± 0.001468
Saba Island	EAST	16	3	0.4917 ± 0.1174	0.002676 ± 0.001820
St. Maarten	EAST	31	3	0.5591 ± 0.0735	0.003395 ± 0.002126
St. Eustatius	EAST	29	2	0.3399 ± 0.0897	0.001502 ± 0.001153
Guadeloupe	EAST	24	2	0.2899 ±0.1028	0.001281 ± 0.001040
Martinique	EAST	178	4	0.4409 ± 0.0314	0.002152 ± 0.001452
Curacao	SOUTH	25	3	0.4767 ± 0.0855	0.002425 ± 0.001647
Bonaire	SOUTH	49	2	0.5102 ± 0.0149	0.002254 ± 0.001527
Santa Marta[1]	SOUTH	166	3	0.5196 ± 0.0328	0.002787 ± 0.001770
North Carolina[1]	NORTH	267	8	0.7037 ± 0.0176	0.003746 ± 0.002235
Bahamas[2]	NORTH	127	8	0.6477 ± 0.0284	0.003257 ± 0.002005
Bermuda[2]	NORTH	45	8	0.6273 ± 0.0413	0.002978 ± 0.001895
Grand Cayman[1]	WEST	79	4	0.4320 ± 0.0488	0.002074 ± 0.001423
San Andres[1]	WEST	47	3	0.5550 ± 0.0406	0.002918 ± 0.001863
Puerto Rico[3]	WEST	118	4	0.4492 ± 0.0371	0.002160 ± 0.001461

1. Betancur-R et al. (2011); **2**. Freshwater et al. (2009); **3.** Toledo-Hernández et al. (2014)

Genetic structure

We found no population genetic differentiation between the eastern populations (Saba Bank & Saba, Martinique, Guadeloupe, St. Maarten, St. Eustatius) (Table 11, Fig. 9 & 10). However, there was a strong genetic structuring between the northern populations (North Carolina, Bahamas, Bermuda) and the Saba Bank (including the other eastern populations) (Table 11, Fig. 9 &10). Invasion thus did not likely occur from the North, but rather from the South (Bonaire or Curacao) or East (Puerto Rico or Grand Cayman), which is opposite from the direction of the major currents (Fig. 11).

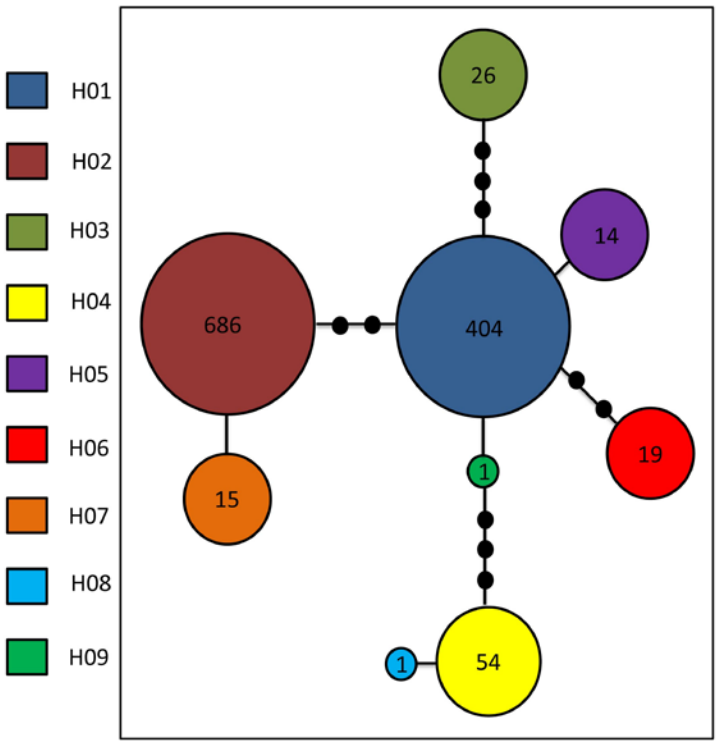

Figure 9. Haplotype network of Lionfish populations in the Wider Caribbean. *Size of circle reflects number of individuals containing a specific haplotype (H01-H09). Each line represents the genetic distance between haplotypes. For distribution of haplotypes, see Figure 10.*

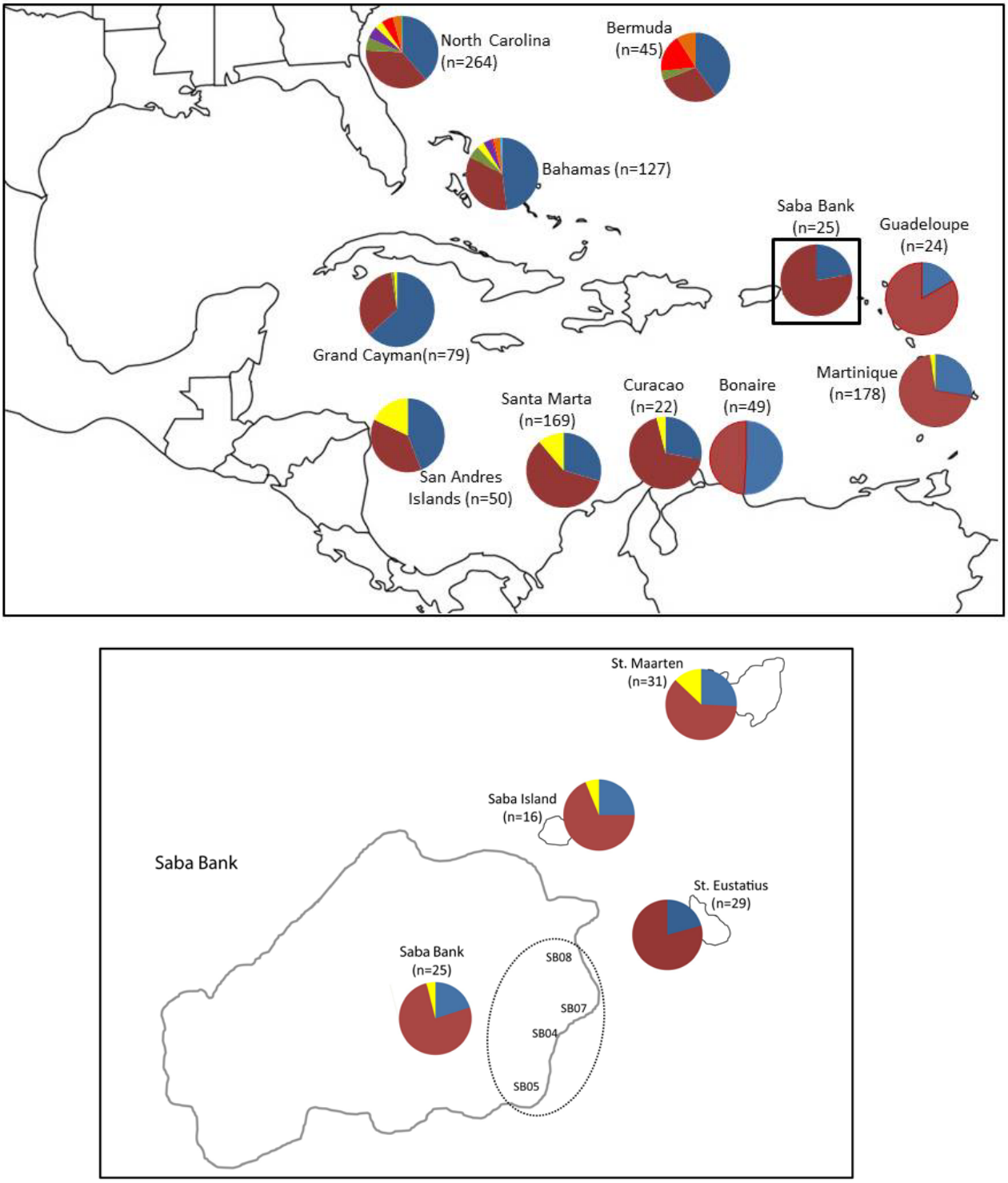

Figure 10. Frequency and distribution of haplotypes in populations of lionfish in the Wider Caribbean (above) and Saba Bank region (below). Haplotype frequencies provided in pie-chart per location, number of samples in brackets. Haplotype color-codes correspond to colors in Figure 11.

Table 11 Matrix of Pairwise population differentiation of Lionfish on Saba Bank and Western Atlantic locations. *Pairwise population differentiation values (Φst) and P -values (Blue) between Saba Bank and different Western Atlantic locations. Bold indicates significant Φst values (p<0.05)*

	Saba Bank	Saba Island	St Eustatius	Guadeloupe	Martinique	St Maarten	Bonaire	Curacao	Santa Marta	Bahamas	North Carolina	Bermuda	Puerto Rico	San Andres
Saba Island	-0.043													
St Eustatius	-0.031	-0.018												
Guadeloupe	-0.026	0.000	-0.034											
Martinique	-0.013	-0.030	-0.002	0.013										
St Maarten	0.005	-0.035	0.044	0.061	0.018									
Bonaire	**0.120***	0.056	**0.151***	0.192	**0.077***	**0.058***								
Curacao	-0.028	-0.052	-0.009	0.011	-0.022	-0.016	0.051							
Santa Marta	0.012	-0.012	0.027	**0.042***	**0.016***	0.019	**0.063***	-0.003						
Bahamas	**0.147***	**0.085***	**0.175***	**0.204***	**0.130***	**0.072***	0.010	**0.087***	**0.093***					
North Carolina	**0.098***	**0.047***	**0.123***	**0.146***	**0.088***	**0.037***	0.013	**0.053***	**0.061***	0.001				
Bermuda	**0.078***	0.024	**0.106***	**0.137***	**0.059***	0.032	-0.004	0.026	**0.039***	0.015	0.003			
Puerto Rico	-0.010	-0.030	0.001	**0.018***	-0.007	0.016	**0.067***	-0.023	0.012	**0.116***	**0.078***	**0.049***		
San Andres	0.036	-0.014	0.068	**0.095***	**0.024***	-0.008	0.002	-0.007	0.022	**0.029***	**0.013***	-0.002	0.018	
Grand Cayman	-0.018	-0.032	-0.009	0.006	-0.009	0.020	**0.084***	-0.024	0.011	**0.128***	**0.086***	**0.060***	-0.010	0.027

Population density

During the 2011 expedition to Saba Bank 4 lionfish were counted (0.004 per m2) along 150m transect lines per location. In 2013 a total of 21 lionfish were found (0.023 per m2) along the same transect length, suggesting a 5.75-fold increase in two years (Table 12).

Table 12 Density of lionfish in 2011 and 2013 on the Sababank. *Number of lionfish along a transect line (50 m^2) per location (n).*

Pterois volitans	2011	2013
Dutch Plain		
Scottish Hill		
Gorgonian Delight	1	
Coral Garden		4
Paul's Cathedral		6
Tertre des Fleurs		2
Erik's Points		9
Twelve Monkeys		
La Colline aux Gorgones		
Devil's Corner		
Rebecca's Garden	3	

5. Discussion

5.1 Genetic connectivity and diversity

The populations of the common benthic species *Montastrea cavernosa* (coral) and *Xestospongia muta* (sponge) appear to be connected along the eastern and southern rim of the Saba Bank (4 - 10 km between sites). There is, furthermore, genetic connectivity between these populations on the Saba Bank and the nearby island of Saba. The observed genetic connectivity indicates that there is an exchange of larvae between these locations.

The genetic diversity of the populations of *M. cavernosa* (π=0.055, h=0.8828) and *X. muta* (π=0.0010, h=0.3619-0.4579) on the Saba Bank are comparable to the ranges of diversity found for these species in other Caribbean locations (*M. cavernosa*: π=0.0051-0.0062, h=0.9011-0.9667, Goodbody-Gringley et al. (2012); *X. muta*: π=0.0005-0.0036, h=0.1333-0.6959, Lopez-Legentil & Pawlik (2009)). Our findings would imply that the populations are genetically robust and viable with high population densities.

Montastrea cavernosa

Our findings show that exchange of genetic material occurs between Saba Bank and the most nearby and distant populations (Saba Island, Jamaica and Bermuda). Differentiation values between the more distant location of Flower Gardens Bank and the closer island Barbados can also be considered relatively low, but were nevertheless significant. A relatively high degree of gene flow appears to be present in this species throughout the region. The seemingly substantial exchange of genetic diversity is likely caused by the potential of *M. cavernosa* larvae to disperse over distances up to 3000 km (Nunes et al. 2009). Despite the potential of larvae to spread over great distances, hydrological features, such as currents or marine barriers, still can cause limitations in connectivity and thus population differentiation between certain locations, even in relative proximity to each other. The combination of both regional and local patterns of recruitment is common among many Western Atlantic populations of *M. cavernosa* (Goodbody-Gringley et al. 2012).

Absence of differentiation between Saba Bank and Jamaica might point towards gene flow facilitated by the main Caribbean current (SE-NW). This implies that populations between both tested locations be genetically linked to Saba Bank as well.

Xestospongia muta

For *X. muta,* there is more genetic structure among the populations of the Saba Bank and in the Wider Caribbean, indicating limited larval dispersal. The pattern of the genetic structure appear to be most strongly related to patterns of currents. Restricted larval dispersal is a common feature in sponges (reviewed by Maldonado, 2005) this might explain the observed limited recruitment of *X. muta* over large distances (Montalvo *et al.* 2005 &2011). Lopez-Legentil & Pawlik (2009) found significant Φst values between most populations of *X.muta* that they studied in Florida, Bahama's and Belize, ranging in distance between 100-1000km. Yet the authors did not see any evidence of isolation-by-distance, per se. It is important to note that due to the low number of I3-M11 haplotypes (n = 4) found in *X. muta*, the presence or absence of one specific haplotype can have a large impact on the Φst values.

Using the same genetic marker in a closely related species, *X.testudinaria*, genetic divergence over small spatial scales of 2-100 km has been detected in Indonesia (Bell et al. 2013, Swierts et al. 2013). *X.testudinaria* has short dispersal distances and seems to rely largely (up to 80%) on self-recruitment

(Bell *et al.* 2014). The genetic diversity found on Saba Bank could be the result of a combination of influx from nearby reefs as well as self-recruitment.

5.2 Population Density

M. cavernosa colony densities on Saba Bank were found to be highly variable between sites (range 0.02 – 0.96 colonies m^2), but fit within the range of densities described by Porter et al. (1987) for southern Florida around the mid 1980's at a depth range of 10-40m (0.14-1.09 colonies m^{-2}). However, higher densities (up to 6.32 colonies m^{-2}) can also be found in the Caribbean region (Rose and Risk 1985; Chiapone and Sullivan 1996). The rather atypical flat reef character on Saba Bank, as a consequence of continuous hydrologic and wind (including hurricanes) stress, compared to the more common massive reef structures on fringing reefs around nearby islands might explain the lower densities at several sites. Also, at some sites the dominant benthic cover was sand which likely restricts coral recruitment(*e.g.* SB06 with densities of 0.02 colonies m^{-2}). This is in line with the general low coral cover at these locations. The density of *X.muta* on the Saba Bank (0-0.72 individuals m^{-2}) was generally comparable to previous recordings in Florida with mean densities of 0.186- 0.277 in m^{-2} at depth ranges between 15-30m (McMurray *et al.* 2010 &2011). In three locations on the Saba Bank (SB05, SB06, SB07) the densities of *X.muta* were 2-3 times higher than elsewhere, and then previously recorded in the Caribbean.

5.3 Health status of Saba Bank

The absence of any diseases in *M. cavernosa* colonies confirms previous accounts (*e.g.* McKenna & Etnoyer 2010, Meesters 2010 and Van Beek and Meester 2013) of the relatively high health status of corals on the Saba Bank. In particular in comparison to other Western Atlantic locations where the presence of Black Band and White Plague Disease are common (*e.g.* Bruckner *et al.* 1997; Croquer *et al.* 2003 and Kaczmarsky *et al.* 2011). Nevertheless, the *M. cavernosa* colonies do appear to be under stress, displayed by old tissue loss in the majority of the colonies and partial overgrowth of cyano's, sponges or macroalgae. The observed tissue loss might be the consequence of past mass bleaching events affecting reefs worldwide, including Saba Bank (*e.g.* Brandt 2009; Van Beek & Meesters 2013). *M. cavernosa* appears to be highly susceptible to bleaching, affecting up to 80% of colonies (Leão *et al.* 2003; Miranda *et al.* 2013).

The vast majority of *X. muta* (>80%) showed signs of "spotted bleaching" in the form of circular shaped white spots, where tissue had lost its color. In fact, all observed larger individuals (diameter >50cm) had bleach spots. In comparison the proportion of bleached *X. muta* on Saba Bank was 4-7 times higher than in Florida, with 16-21 % bleaching at depths of 15-30m (McMurray *et al.* 2011). Our observations are also considerably higher than reports by Cowart *et al.* (2006), who found cyclic bleaching in approximately 25% of the sponge population in Florida. The high proportion of bleached sponges is concerning given the fact that no bleached sponges were recorded on Saba Bank in 2006, during a study specifically aimed to document bleaching and disease in *X. muta* on the bank (Thacker *et al.* 2010). Bleaching is known to be seasonal in *X. muta* with a peak during the fall (McMurray *et al.* 2011), which might partly explain the high levels observed during our study in October. Though the effect of bleaching on sponge survival seems to be variable (McMurray *et al.* 2011), the high prevalence of bleaching of *X. muta* on Saba Bank does raise concern and validates further research. We recommend that *X.muta* is monitored for densities and bleaching during the next survey to the Saba Bank in 2015.

At present the recorded densities and genetic diversity of *X.muta* on the Saba Bank indicate a solid population, yet there is a risk of a reduction in population size due to the high prevalence of bleaching.

X.muta plays a crucial role in the coral reef ecosystem providing habitat complexity (Humann 1992; Buettner 1996) and biotope for symbionts from microbes (*e.g.* Hentschel *et al.* 2006; López-Legentil *et al.* 2008; Montalvo *et al.* 2014) to invertebrates (*e.g.* crustaceans and brittlestars) (Wilkinson 1983; Duffy 1992; Henkel & Pawlik 2005). Furthermore, populations of this sponge species can filter tremendous amounts of water, for example a water column of 30m deep every 2.3 – 18 days (McMurray *et al.* 2014). A loss of *X.muta* would thus likely cause a significant change to ecosystem.

5.4 Lionfish genetic structure

This is the first study of lionfish population genetic structure in the eastern Caribbean. All lionfish caught for this study (all from the eastern Caribbean) were *P. volitans.* The absence of *P. miles* coincides with the findings by Betancur-R *et al.* (2011). To date, invasive *P. miles* have only been found in North Carolina and the Bahamas (Hamner et al. 2007; Freshwater et al. 2009), indicating *P. miles* disperses much less efficiently than the closely related *P. volitans.* The substantial genetic distance between several *P. volitans* haplotypes in the Western Atlantic could point towards multiple introductions. However, as previously pointed out by Betancur-R et al. (2011), the genetic pattern suggests dispersal from a single source. Another hypothesis could be that a considerable number of *P. volitans*, from differentiated native regions, were introduced simultaneously.

The genetic diversity of the *P. volitans* seems to mirror its pattern of dispersal over time through the Western Atlantic. Highest haplotype and nucleotide diversity was found in North Carolina and Bermuda (Freshwater et al. 2009; Betancur-R et al. 2011), which were invaded almost simultaneously in 2000 (Whitfield et al. 2002). Subsequently, first lionfish were sighted in the Bahamas around 2004 (Schofield 2009), where high levels of diversity were found as well. Locations situated in the southern Caribbean were invaded more recently (Grand Cayman, the San Andres Islands and Puerto Rico in 2008; Santa Marta and Curacao and Bonaire in 2009. Source: Schofield 2009) and displayed a clear cline in genetic diversity. This decreasing trend continues towards the eastern Caribbean (including the Saba Bank) where the lowest diversity can be found on St. Eustatius (first documentation in 2010), Saba Bank and Guadeloupe (first report late 2011). Although lionfish seem to have penetrated the wider eastern Caribbean only very recently, there have been early reliable reports of lionfish sightings in 2008 and 2009 (Schofield 2009) for the islands St. Croix and St. Maarten, respectively. These seem to have failed to result in rapid dispersal to most nearby islands, but this might explain the relatively high genetic diversity documented for St. Maarten. Significant differentiation between the three main Western Atlantic regions might be the result of the recent introduction and subsequent dispersal through the Western Atlantic. Betancur-R and colleagues (2011), pointed out that over time subsequent waves of dispersal from the north may eventually lead to the genetic homogenization of the Western Atlantic populations.

5.5 Lionfish densities

Lionfish densities were obtained from the line transect monitoring all sampled locations of the 2011 (Van Beek & Meesters 2013) and 2013 (unpublished data of Van Beek) Saba Bank research expeditions. Within the two years between both expeditions, a 5.75-fold increase in lionfish densities was observed on Saba Bank. Population outbursts are commonly seen in biological invasions and indicate the relatively early stage of the process for the Saba Bank. In time, the population densities of most biological invaders to a healthy ecosystem can be expected to eventually stabilize at much lower densities. However, as the reefs of the Caribbean are almost universally highly stressed and in decline (Jackson et

al. 2014), it remains to be seen how soon and to what densities lionfish populations will eventually equilibrate.

Experiments with lionfish removal by means of spearfishing have proven successful locally in reducing the number of lionfish on Bonaire and Curacao (De León et al. 2013). However, due to the relatively harsh conditions, offshore location and large (mesophotic) reef area, controlling lionfish population on Saba Bank through spearfishing will be close to impossible. The non-significant differentiation between Saba Bank and all other eastern and southern locations (excl. Bonaire), confirms the absence of strong genetic structure in these region, thus allowing for continuous exchange of *Pterois volitans* larvae. As a consequence, Saba Bank populations might continuously resupply lionfish to nearby Islands and thereby offset any local removal efforts.

Recently a Saban Fisherman reported finding lionfish remains in the stomach of a snapper caught on Saba Bank, indicating successful predation (http://www.saba-news.com/lionfish-may-natural-predator-caribbean/), however this could not be verified after the fact, and despite a request to all fishermen to bring in any other such cases for documentation this has as yet not resulted in other such cases. Similarly, low lionfish densities around Saba Island, despite the absence of large scale removal efforts, could also be attributed to control by natural predation. The presence of large predators (*e.g.* groupers and snappers) might allow for top-down regulation of lionfish populations on Saba Bank (Meesters 2010; Diller et al. 2014). The fact remains however, that such natural predation has as yet not been convincingly documented anywhere in the region.

6. Conclusions & Recommendations

Conclusions

- Genetic analyses of two common and one invasive species revealed genetic connectivity among populations along the south eastern rim of the Saba Bank, indicating free larval transport.
- We also demonstrate genetic connectivity between the populations of the Saba Bank and the nearby islands of Saba and St. Eustatius, as well as multiple Western Atlantic locations at distances up to 3000km.
- The presumed genetic connectivity among populations in the Western Atlantic, high species diversity, large mesophotic reef area, relatively pristine condition and upstream position with respect to the wider Western Atlantic, indicate that Saba Bank reefs could serve an essential role in buffering the degradation of both nearby and more distant reefs.
- For the coral *Montastrea cavernosa* the Saba Bank can be a source of genetic diversity to the wider Western Atlantic.
- The lack of genetic structuring in *M. cavernosa* between Saba Bank and the nearby islands of Saba and St. Eustatius indicate that there is gene flow among these locations and that the Saba Bank can function as a buffer for the region.
- There is a relative absence of coral disease on the Saba Bank.
- Saba Bank is not free of harmful stressors, as indicated by common overgrowth of *M. cavernosa* and high prevalence bleaching in *Xestospongia muta.*

Recommendations

- If ever feasible, thorough control efforts are desirable in order to stabilize lionfish densities and prevent a further increase on Saba Bank, otherwise the Bank will be a source of constant recruitment to nearby located reefs, as this population can also replenish Saba Island and St. Eustatius.
- As *X. muta* likely is an important harbor of a biodiverse endobiotic fauna, but clearly is also vulnerable, studies are recommended to further document this fauna.
- The likely important role of the Saba Bank in terms of genetic and ecological resilience of reef communities in the region (as suggested by our results) should to be acknowledged in individual island coral reef management plans and needs to be further studied and documented.

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8. Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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10. Justification

Report number: C015/15
Project Number: *BO-11-011.05-033.*

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved:	HWG Meesters Researcher
Signature:	
Date:	11 February, 2015
Approved:	Dolfi Debrot Researcher
Signature:	
Date:	11 May, 2015
Approved:	Floris Groenendijk Head Maritime Department
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Date:	11 May, 2015