Spatio–temporal variation in stable isotope signatures (δ^{13}C and δ^{15}N) of sponges on the Saba Bank

Fleur C. Van Duyl[1], Benjamin Mueller[1,2] and Erik H. Meesters[3]

[1] Department of Marine Microbiology and Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research and Utrecht University, Den Burg, The Netherlands
[2] Department for Freshwater and Marine Ecology, University of Amsterdam, Amsterdam, The Netherlands
[3] Wageningen Marine Research, Den Helder, The Netherlands

ABSTRACT

Sponges are ubiquitous on coral reefs, mostly long lived and therefore adaptive to changing environmental conditions. They feed on organic matter withdrawn from the passing water and they may harbor microorganisms (endosymbionts), which contribute to their nutrition. Their diets and stable isotope (SI) fractionation determine the SI signature of the sponge holobiont. Little is known of spatio–temporal variations in SI signatures of δ^{13}C and δ^{15}N in tropical sponges and whether they reflect variations in the environment. We investigated the SI signatures of seven common sponge species with different functional traits and their potential food sources between 15 and 32 m depth along the S-SE and E-NE side of the Saba Bank, Eastern Caribbean, in October 2011 and October 2013. SI signatures differed significantly between most sponge species, both in mean values and in variation, indicating different food preferences and/or fractionation, inferring sponge species-specific isotopic niche spaces. In 2011, all sponge species at the S-SE side were enriched in d^{13}C compared to the E-NE side. In 2013, SI signatures of sponges did not differ between the two sides and were overall lighter in δ^{13}C and δ^{15}N than in 2011. Observed spatio–temporal changes in SI in sponges could not be attributed to changes in the SI signatures of their potential food sources, which remained stable with different SI signatures of pelagic (particulate organic matter (POM): δ^{13}C −24.9‰, δ^{15}N +4.3‰) and benthic-derived food (macroalgae: δ^{13}C −15.4‰, δ^{15}N +0.8‰). Enriched δ^{13}C signatures in sponges at the S-SE side in 2011 are proposed to be attributed to predominantly feeding on benthic-derived C. This interpretation was supported by significant differences in water mass constituents between sides in October 2011. Elevated NO_3 and dissolved organic matter concentrations point toward a stronger reef signal in reef overlying water at the S-SE than N-NE side of the Bank in 2011. The depletions of δ^{13}C and δ^{15}N in sponges in October 2013 compared to October 2011 concurred with significantly elevated POM concentrations. The contemporaneous decrease in δ^{15}N suggests that sponges obtain their N mostly from benthic-derived food with a lower δ^{15}N than pelagic food. Average proportional feeding on available sources varied between sponge species and ranged from 20% to 50% for benthic and 50% to 80% for pelagic-derived food, assuming trophic enrichment factors of 0.5‰ ± sd 0.5 for δ^{13}C and 3‰ ± sd 0.5 for δ^{15}N for sponges. We suggest that observed variation of SI in

sponges between sides and years were the result of shifts in the proportion of ingested benthic- and pelagic-derived organic matter driven by environmental changes. We show that sponge SI signatures reflect environmental variability in space and time on the Saba Bank and that SI of sponges irrespective of their species-specific traits move in a similar direction in response to these environmental changes.

INTRODUCTION

The stable isotope (SI) approach is a widely used method to study sources of food and trophic transfer in food web studies. Application to sponges revealed a wide range in bivariate δ^{13}C and δ^{15}N signatures, illustrating interspecific differences in diet and isotopic niche space of sponges (*Van Duyl et al., 2011*; *Freeman, Easson & Baker, 2014*). In shallow water habitats, such as coral reefs, diets of sponges mainly comprise of pico- and nanophytoplankton, bacterioplankton, small detrital particles, dissolved organic matter (DOM) and metabolites provided by endosymbionts, which are hosted by various sponges (*Erwin & Thacker, 2007*; *Freeman & Thacker, 2011*; *Maldonado, Ribes & Van Duyl, 2012*). Therefore, coral reef sponges are considered as primary consumers living mainly on products of primary production, besides bacteria. Like other consumers, sponges are considered to reflect the SI signature of the ingested food mixture within 0–1‰ enrichment for carbon (δ^{13}C), and 1.5–3.5‰ for nitrogen (δ^{15}N) (*Fry et al., 1982*; *Vander Zanden & Rasmussen, 2001*; *Fry, 2006*). Endosymbiont communities also influence the SI signatures of their sponge hosts. Both sponge host and associated bacteria jointly determine the typical δ^{13}C and δ^{15}N signature of the sponge holobiont (*Thacker & Freeman, 2012*; *Freeman, Easson & Baker, 2014*). Sponges hosting phototrophic endosymbionts, which fix CO_2 tend to have lower δ^{13}C values than sponges without phototrophic endosymbionts and sponges hosting N_2 fixing endosymbionts tend to have lower δ^{15}N values (*Fiore et al., 2010*). Many sponges feed on DOM apart from plankton (*Yahel et al., 2003*; *De Goeij et al., 2008*; *Mueller et al., 2014a*; *McMurray, Pawlik & Finelli, 2017*; *Hoer et al., 2018*). It is widely recognized that sponges draw food from the pelagos to the benthos (benthic–pelagic coupling), but that sponges also ingest food derived from the benthos is less well-known (*Southwell, 2007*; *Van Duyl et al., 2011*; *Rix et al., 2016a*, *2016b*).

The current phase shift from scleractinian coral to macroalgal, turf algal and cyanobacterial mat dominated communities (*McCook, Jompa & Diaz-Pulido, 2001*; *Jackson et al., 2014*; *De Bakker et al., 2017*) is considered to lead to an increased supply of DOM and suspended detrital material from the benthos to the reef overlying water (*Haas et al., 2016*). These non-calcifying benthic primary producers produce more DOM than corals per unit surface (*Naumann et al., 2010a*; *Haas et al., 2010a*, *2010b*, *2011*; *Mueller et al., 2014b*, *2016*; *Mueller, Meesters & Van Duyl 2017*; *Brocke et al., 2015*). Moreover, there is increasing evidence that besides pelagic-derived food (mainly consisting

of microbial loop food, e.g., pico- and nano(phyto)plankton), benthic-derived DOM may be an important food source for sponges (*Van Duyl et al., 2011*; *Rix et al., 2016a*, *2016b*; *Pawlik, Burkepile & Thurber, 2016*). Additionally, suspended detrital material either derived from benthic or pelagic producers has also been identified as sponge food (*Maldonado, Ribes & Van Duyl, 2012*; *McMurray et al., 2018*).

The stable isotopic ratios (δ^{13}C and δ^{15}N) of benthic- and pelagic-derived food differ substantially (*Fry et al., 1982*; *Raven et al., 1995*). Pelagic primary producers (i.e., phytoplankton) in oligotrophic reef waters have usually lower δ^{13}C values than benthic primary producers (*Faganeli et al., 1988*; *France, 1995*; *Duarte, 1992*; *Van Duyl et al., 2011*). Contrary, δ^{15}N tends to be lower in benthic than in pelagic primary producers (*Naumann et al., 2010b*; *Van Duyl et al., 2011*; *Kolasinski et al., 2016*), particularly when N_2 fixing takes place by turf algae, cyanobacterial mats or nitrogen fixing bacteria associated with various reef organisms (e.g., corals and sponges) (*Lesser et al., 2007*; *Cardini et al., 2014*; *Den Haan et al., 2014*). DOM released by phytoplankton, macroalgae and corals is generally assumed to closely reflect the SI signature of its source with little isotopic fractionation during formation and degradation (*Williams & Gordon, 1970*; *Fry et al., 1998*; *Benner et al., 1997*). Depending on sponge species, food from these pelagic and/or benthic sources appears to be assimilated in different proportions leading to distinct SI signatures (*Van Duyl et al., 2011*). For sponge diet changes to be reflected in the SI signature a 1–2 months delay related to the integration time for new source information is required (*Freeman & Thacker, 2011*; *Simister et al., 2013*). Variations in hydrographic conditions may be drivers of such changes (current directions and velocities, upwelling) in the tropics. Besides variations in water mass constituents flowing toward coral reefs, water turbulence and vertical mixing affect the availability of benthic and pelagic food for sessile organisms (*Lowe & Falter, 2015*). Fine scale hydrodynamic conditions vary in geographic space and time around reefs, with reefs differently exposed to incoming waves and currents and different water mass properties. Whether such variations are reflected in sponge diets is still unknown.

The aim of this study was to explore and explain variations in δ^{13}C and δ^{15}N of sponges on fore reefs with different orientation toward incoming currents and waves (space) over time on the Saba Bank. It was hypothesized that sponges with different functional traits along these stretches (sides) of the Bank receive food from different sources over time. We sampled food reaching sponges from open water transported by currents, and local food derived from benthic sources on the Saba Bank to estimate the contribution of pelagic- and benthic-derived food to their diet under different hydrodynamic conditions. To identify different water masses and infer the source of its constituents, we measured concentrations of organic matter and inorganic nutrients in space and time.

MATERIALS AND METHODS

Fieldwork was performed under the research permits #WSH/2011/1400 and RWS-2013/42681 issued by Rijkswaterstaat, Dutch Ministry of Infrastructure and Environment and Ministry of Economic Affairs.

Location and hydrography

The Saba bank (17°25′N; 63°18′W) is a 2,200 km^2 submerged carbonate platform as measured to the 200 m isobath in the northeastern Caribbean Sea. It is raised about 700–1,000 m above the general depths of the surrounding sea floor and lies 10–100 m below sea level with shallowest depths (~ 10 m) along the E-NE fringes. The nearest island is Saba (ca five km from the NE border of the Bank), which is separated from the Saba Bank by a 600 m deep channel. The Saba Bank is fringed by relatively undisturbed (negligible local human impact) coral reefs along its S-SE and E-NE side with an average stony coral and sponge cover of 7.1% and 7.8%, respectively, and a benthic algal cover of approximately 50% in the 15–32 m depth range (*Van Beek & Meesters, 2014*). The mainly wind-driven Caribbean Current flushes and envelopes the Saba Bank from the SSE to the WNW. The source water for the Caribbean Current is from the equatorial Atlantic Ocean (North Equatorial, North Brazil and Guiana Currents) and enters the Caribbean basin mainly through the southern part of the Antillean Arc. The Saba Bank also receives oligotrophic water from the Antillean Current flowing into the Caribbean Basin directly from the Atlantic (*Van Duyl, 2016*).

Fieldwork on the Saba Bank was conducted from the Caribbean Explorer II during two expeditions, the first from 22 to 28 October in 2011 and the second from 20 to 26 October in 2013. A total of 11 permanent coral reef stations, five stations along the S-SE side and six stations along the E-NE side of the bank were sampled, covering a distance of approximately 20 km along each side of the Bank (Fig. 1, see supplementary for coordinates and depth of stations). One site at the S-SE side (Dutch plains) was only sampled in 2013. Tidal amplitude is less than 10 cm per diurnal tidal cycle on the Bank, due to proximity of an amphidromic point (*Kjerfve, 1981*). Current speeds on the Bank in 2011 ranged from less than 0.25 up to 0.5 m/s (*De Graaf, 2012*). Water temperature varied between 29 and 30 °C in October 2011 and between 28 and 29 °C in October 2013.

Collection of samples

Sponges and benthic macroalgae were collected by SCUBA diving at stations varying in depth between 15 and 32 m on gently sloping fore reefs. At each station, up to seven commonly occurring sponge species (one to three specimen per species) were collected belonging to three different functional groups varying in microbial abundance and chlorophyll-a content (Table 1). Chlorophyll-a content in sponges is indicative of the presence and abundance of phototrophic endosymbionts (*Wilkinson, 1983*; *Erwin & Thacker, 2007*; *Freeman & Thacker, 2011*). As representatives of benthic food sources two common brown macroalgal species *Dictyota* spp. and *Lobophora* spp. were collected at each station (one to two samples per species). On a few stations six or less sponge species were found. Particularly at the shallowest site (15–17 m, Tertre de Fleur) only four of the sponge species and one macroalgal species (*Dictyota* spp.) were found. At this station some other benthic algae, *Sargassum* and filamentous cyanobacteria were sampled for SI values. Particulate organic matter (POM) and dissolved (in)organic nutrients were sampled with a six L niskin bottle between zero and two m depth from the research
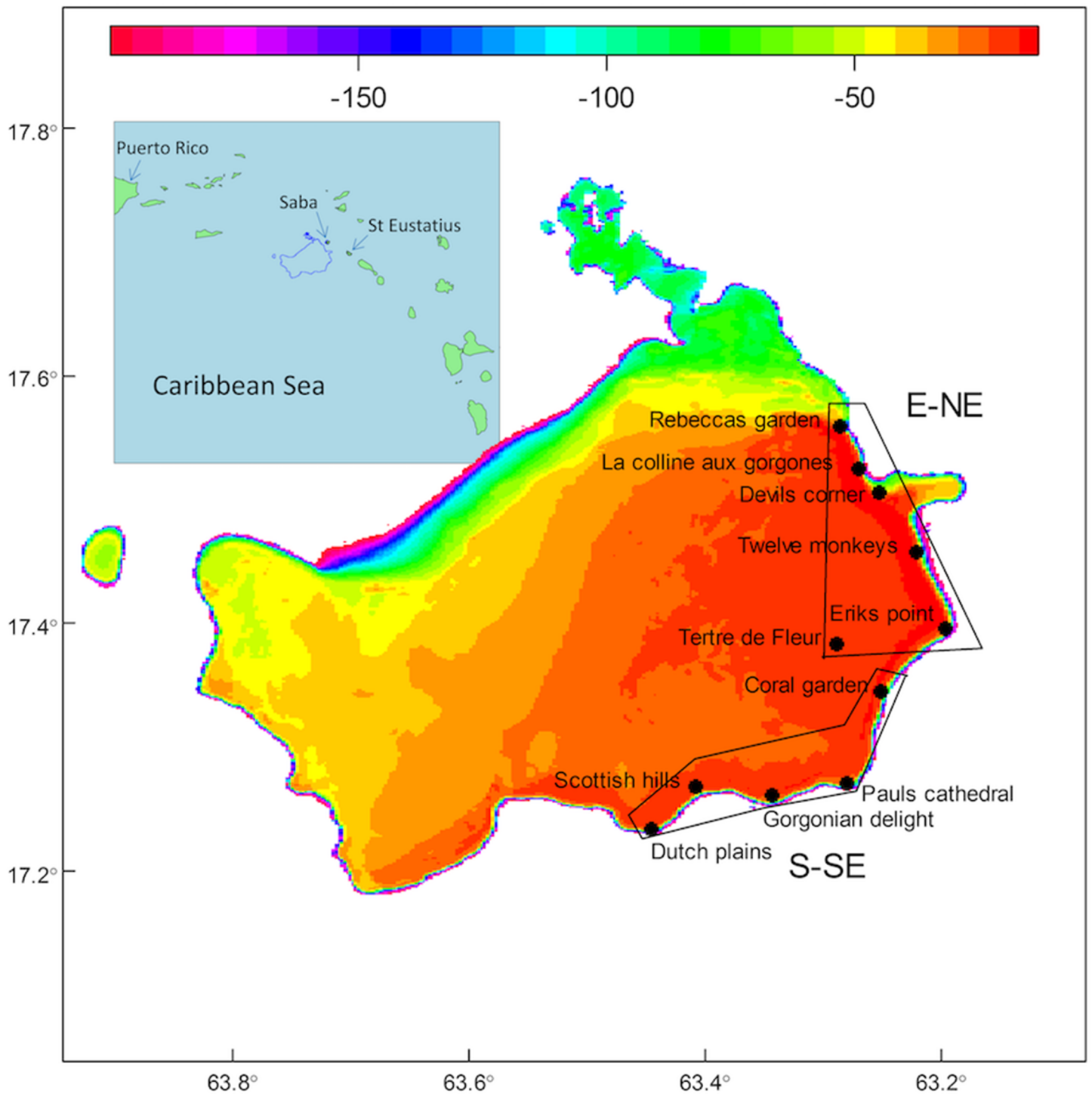
Figure 1 Map of the Saba Bank with inset. Map of the Saba Bank with depth contours (color scale in meter) and stations which were visited in October 2011 and October 2013. Fore-reef stations were in the 17–32 m depth range and the patch reef on top of the Saba Bank, Tertre de Fleur, was at 15 m depth. Stations Dutch plains until Coral garden were exposed to the S-SE and the rest of the stations had an E-NE exposition. Inset shows the position of the Saba Bank in the Caribbean Sea, with nearest islands Saba (~5 km NE of the Saba Bank) and St. Eustatius (~20 km East of the Saba Bank).   Full-size ⭐ DOI: 10.7717/peerj.5460/fig-1

Table 1 Sponge species. Collected sponge species with their functional traits, representing low microbial abundance sponges (LMA), high microbial abundance sponges with high chlorophyll-a (HMA-H) and low chlorophyll-a content (HMA-L). Selected sponge species were common on coral reefs of the Saba Bank (*Thacker et al., 2010*).

Sponge species	Functional trait	Chlorophyll-a (µg/g sponge tissue)	Reference
Amphimedon compressa	LMA	Negligible	*Erwin & Thacker (2007)* and *Gloeckner et al. (2014)*
Callyspongia plicifera	LMA	Negligible	*Erwin & Thacker (2007)* and *Gloeckner et al. (2014)*
Aplysina cauliformis	HMA-H	>125	*Gloeckner et al. (2014)*
Xestospongia muta	HMA-H	50–125	*Erwin & Thacker (2007)* and *Gloeckner et al. (2014)*
Plakortis spp.	HMA-H	50–125?	*Gloeckner et al. (2014)*
Agelas conifera	HMA-L	<50	*Freeman, Easson & Baker (2014)*
Aiolochroia crassa	HMA-L	<50	*Erwin & Thacker (2007)* and *Gloeckner et al. (2014)*

vessel at each station. In 2011, two water samples were taken per station and in 2013 one water sample. The suspended particulate matter obtained from the surface water was considered to represent pelagic food for sponges. Vertical temperature and salinity profiles over reefs down to 35 m depth showed that the water column was well mixed (temperature nor salinity changed with depth).

Sample processing

Water samples for inorganic nutrients were filtered over 0.2 µm acrodisc syringe filters (25 mm diameter). Filtrate was collected in pony vials, which were stored in a deep freezer ($-20\ ^{\circ}\mathrm{C}$) until analysis. Samples were processed on an autoanalyzer (TRAACS) for PO_4^{3-}, NH_4^+, NO_x, NO_2^-, ($NO_x - NO_2^- = NO_3^-$). Water samples for DOM were also filtered over 0.2 µm acrodisc filters (25 mm diameter) and fixed with concentrated (38%) HCl (six drops for fixing 20 mL seawater) in combusted EPA vials (40 mL). Analysis of samples was done on a TOC-VCPN, Shimadzu analyzer yielding total and dissolved organic carbon (TOC, DOC) and total and dissolved nitrogen (TN, TDN). TDN minus dissolved inorganic nitrogen (DIN) (DIN = $NH_4 + NO_2^- + NO_3^-$) renders dissolved organic nitrogen (DON).

Suspended matter was collected on combusted glass fiber filters (Whatman GF/F, 47 mm diameter and 10 mm diameter). The volume of water filtered was noted and filters were folded with tweezers, wrapped in aluminum foil, labelled and stored in a freezer at ($-20\ ^{\circ}\mathrm{C}$). Subsequently, filters were fumed with acid vapor (HCl) in a desiccator to remove possible carbonates, and lyophilized.

Sponges and macroalgae were individually labelled and packed in aluminum foil immediately after the dive and stored in a freezer ($-20\ ^{\circ}\mathrm{C}$) until processing. Sponge and algal samples were treated with 2M HCl overnight on a shaking table to remove possible carbonates. This procedure was repeated if necessary. Afterward samples were washed

with UltraPure water until a pH between 5 and 6 was reached. Subsequently, samples were lyophilized.

Stable isotope analyses

For the SI analyses ($\delta^{13}C$ and $\delta^{15}N$) homogenized organic matter portions of sponges and macroalgae (0.8–1 mg) and pieces of GF/F filters with suspended matter were transferred to tin cups, which were closed with tweezers. The C and N content of the dry weight and the isotope composition of the samples was determined on a Flash 2000 elemental analyzer coupled online with a Delta V Advantage-isotope monitoring mass spectrometer (Thermo Scientific, Waltham, Bremen, Germany) relative to standards (acetanilide, urea and casein). Values were normalized to acetanilide. C and N isotope ratios were expressed as $\delta^{13}C$ to Vienna Pee Dee Belemnite standard and as $\delta^{15}N$ to air. Standard error of measurements of standards was ~0.15‰.

Statistical analyses

Bayesian techniques were adopted to analyze SI data (*Zuur, Hilbe & Ieno, 2013*). Bayesian approaches use statistical distributions to characterize the uncertainties in the data. Bayesian methods were solely applied for analysis of SI data of sponges and potential organic food sources for sponges. Means and credible intervals of the separate isotopes have been calculated using R (*R Core Team, 2017*) and R-INLA (*Rue, Martino & Chopin, 2009*). All models included a Gaussian error distribution that was allowed to differ per species because initial data explorations indicated heterogeneity between sponge species. R-INLA is used frequently for analyses that include spatial and temporal correlations (*Zuur, Ieno & Saveliev, 2017*), but can be used for any type of (Bayesian) analysis (see extensive model specification on www.r-inla.org). For model selection Watanabe–Akaike information criteria was used.

For bivariate isotopic niche space identification all sponge data collected on the Bank in 2011 and 2013 were pooled per sponge species. The isotope niche area of each sponge species was determined with Stable Isotope Bayesian Ellipses in R (*Jackson et al., 2011*). The method estimated the standard ellipse area (SEA_c) of the bivariate niche space of different sponges, after a correction for small sample size. Standard ellipses enveloped approximately 40% of the bivariate $\delta^{13}C$ and $\delta^{15}N$ data of the different sponge species. Means of isotopes with 95% credible intervals of different sponge species were also determined by Bayesian statistics using R-INLA.

Stable isotope samples of sponges and potential food sources collected at the S-SE were compared with samples at the E-NE of the Bank for 2011 and for 2013. Data analyses of $\delta^{13}C$ and $\delta^{15}N$ in sponges and potential food sources were conducted for each isotope separately because C and N metabolism and fractionation are not necessarily coupled (*Maldonado, Ribes & Van Duyl, 2012*).

An *isotope mixing model* was applied using R package SIAR, SI analysis in R, R package version 4.2 (*Parnell & Jackson, 2013*) to calculate the proportional contribution of benthic- and pelagic-derived food to the sponge diet. The model used the bivariate isotope data of sponges and potential food sources and fitted a Bayesian model to determine

the dietary proportions of the different sources (*Parnell et al., 2013*; *Phillips et al., 2014*). Suspended POM was used as pelagic-derived food and fleshy benthic macroalgae (SI data of *Dictyota* spp. and *Lobophora* spp. were combined) as representative for benthic-derived food. An average trophic enrichment factors (TEFs) of primary consumers (sponges) of 0.5 ± sd 0.5% for $\delta^{13}C$ and 3 ± sd 0.5 for $\delta^{15}N$ (*Peterson & Fry, 1987*) was assumed. The model was run for sponges at S-SE and the E-NE sides of the Bank and for the different years with vague priors using default settings.

A *non-metric multidimensional scaling approach* (nMDS) was applied to characterize water samples on the bank and to investigate whether there were differences between sides of the bank or between years. Concentrations of PO_4^{3-}, NH_4^+, NO_2^-, NO_3^-, DIN, DIN/PO_4^{3-} ratio, TOC, TON, DOC and DON and particulate organic carbon and nitrogen (POC, PON) and the ratio between POC and PON were used. The nMDS plot was generated after normalizing (scaling) values of environmental variables. Differences between samples were tested by Permanova (*Anderson, 2001*).

RESULTS

Isotopic bivariate niche space of sponge species

Combining data collected in this study over space and time, yielded ranges in $\delta^{13}C$ and $\delta^{15}N$ of sponges which showed considerable overlap between sponge species (Table 2). C/N ratios of sponges resembled those of bacteria with exception of the C/N ratio of *Plakortis* spp., which was surprisingly high for a High Microbial Abundance sponge (Table 2). Evident differences in the diet of sponges was best reflected in the isotopic bivariate niche spaces (Fig. 2). The placement, area and tilt of the SEAc around the different sponge species clearly varied (Fig. 2). HMA species were separated from Low Microbial Abundance species by differences in $\delta^{13}C$. Differences within functional groups (HMA-H, HMA-L and LMA) were mainly separated by differences in $\delta^{15}N$. The slight tilt of the ellipses of most sponge species indicated that SI ratios of C and N were mostly positively related.

Spatial and temporal variation in SI signatures of sponges

We found that $\delta^{13}C$ of the different sponge species varied in space and time, and that $\delta^{15}N$ only varied in time on the Saba Bank (Figs. 3A and 3B). The $\delta^{13}C$ values of the sponges were best described by a model that included species, sampling year, side of the bank, as well as an interaction between these latter two variables:

$$\delta^{13}C_{sponge} \sim Species + Side + Year + Side : Year$$

The best model for values of $\delta^{15}N$ included only the main effects, "species" and "year of sampling":

$$\delta^{15}N_{sponge} \sim Species + Year$$

"Species" refers to sponge species, "Side" to the E-NE facing or S-SE facing side of the Saba Bank and "Year" to October 2011 or October 2013. Raw data for Figs. 3A and 3B are presented in Supplementary Information.

Table 2 Sponge SI's. Ranges and (Bayesian) means of $\delta^{13}C$ and $\delta^{15}N$ of stable isotope signatures and averages of C:N ratio's (mol weight, raw data) of sponges with standard deviations. Sponges were collected between 15 and 32 m depth.

| Sponges	n	Range ‰ $\delta^{13}C$	$\delta^{13}C$ mean (sd)	Range ‰ $\delta^{15}N$	$\delta^{15}N$ mean (sd)	Average C/N ratio (sd)
Aplysina cauliformis	24	−20.11 to −17.36	−18.86 (0.58)	1.77–4.16	2.78 (0.69)	4.7 (0.39)
Plakortis sp.	17	−20.79 to −18.63	−19.49 (0.52)	−0.18–4.08	1.75 (1.41)	8.2 (1.80)
Xestospongia muta	16	−21.24 to −18.49	−19.74 (0.72)	3.45–6.65	4.82 (0.89)	4.9 (0.49)
Agelas conifera	17	−18.86 to −17.16	−17.86 (0.46)	3.92–6.44	4.77 (0.60)	4.3 (0.27)
Aiolochroia crassa	16	−18.85 to −17.19	−18.18 (0.51)	1.54–4.17	3.09 (0.70)	4.9 (0.32)
Amphimedon compressa	14	−18.49 to −16.31	−17.43 (0.67)	4.04–6.44	4.96 (0.66)	4.1 (0.52)
Callyspongia plicifera	16	−17.66 to −16.34	−17.12 (0.34)	2.46–3.71	3.19 (0.42)	3.6 (0.05)
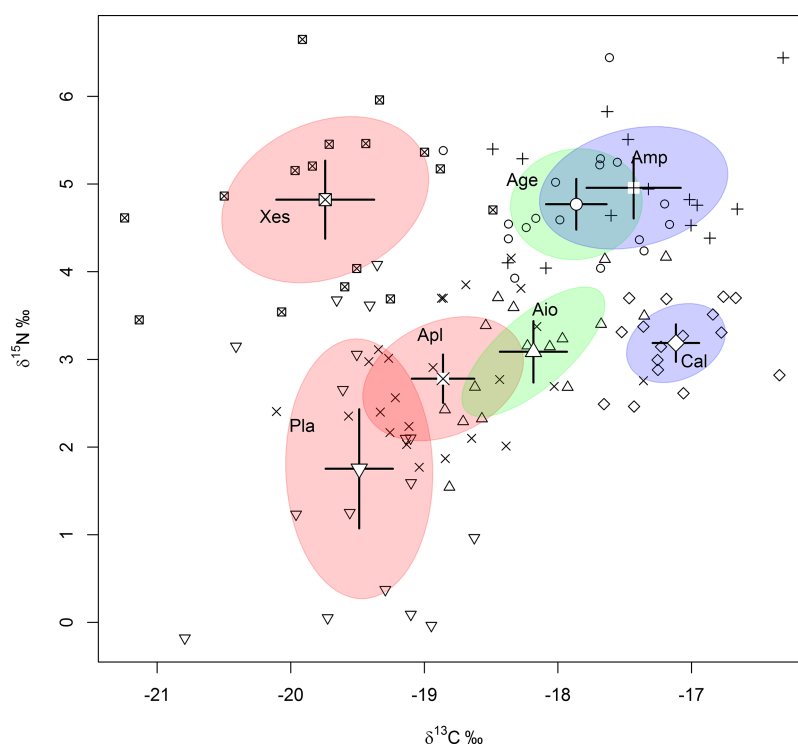
Figure 2 Sponge niche spaces. Isotopic bivariate niche space of seven sponge species on fore reefs of the Saba Bank, based on data collected in space and time. Standard ellipse areas of different sponge species were corrected for small sample size (SEAc). Bayesian means of different sponge species are shown within ellipses with the 95% credible intervals of the mean. HMA high chlorophyll-a sponges (HMA-H) are pink, HMA low chlorophyll-a sponges are green (HMA-L) and LMA sponges are purple. (+)Amp, *A. compressa*; (○)Age, *A. conifera*; (□)Xes, *X. muta*; (◇)Cal, *C. plicifera*; (△)Aio, *A. crassa*; (x)Apl, *A. cauliformis*; (▽)Pla, *Plakortis* spp. Full-size ☑ DOI: 10.7717/peerj.5460/fig-2

Sponge species was the main driver of variations in $\delta^{13}C$ and $\delta^{15}N$. In space and time, however, the $\delta^{13}C$ of sponge species varied in concert irrespective of their different functional traits (Fig. 3A). Mean $\delta^{13}C$ was higher at the S-SE than the E-NE side in 2011 and decreased from 2011 to 2013 in all sponge species. An evident interaction effect between Side and Year for variations in $\delta^{13}C$ was observed, implying that spatial differences in $\delta^{13}C$ in sponges between sides with different orientation toward incoming

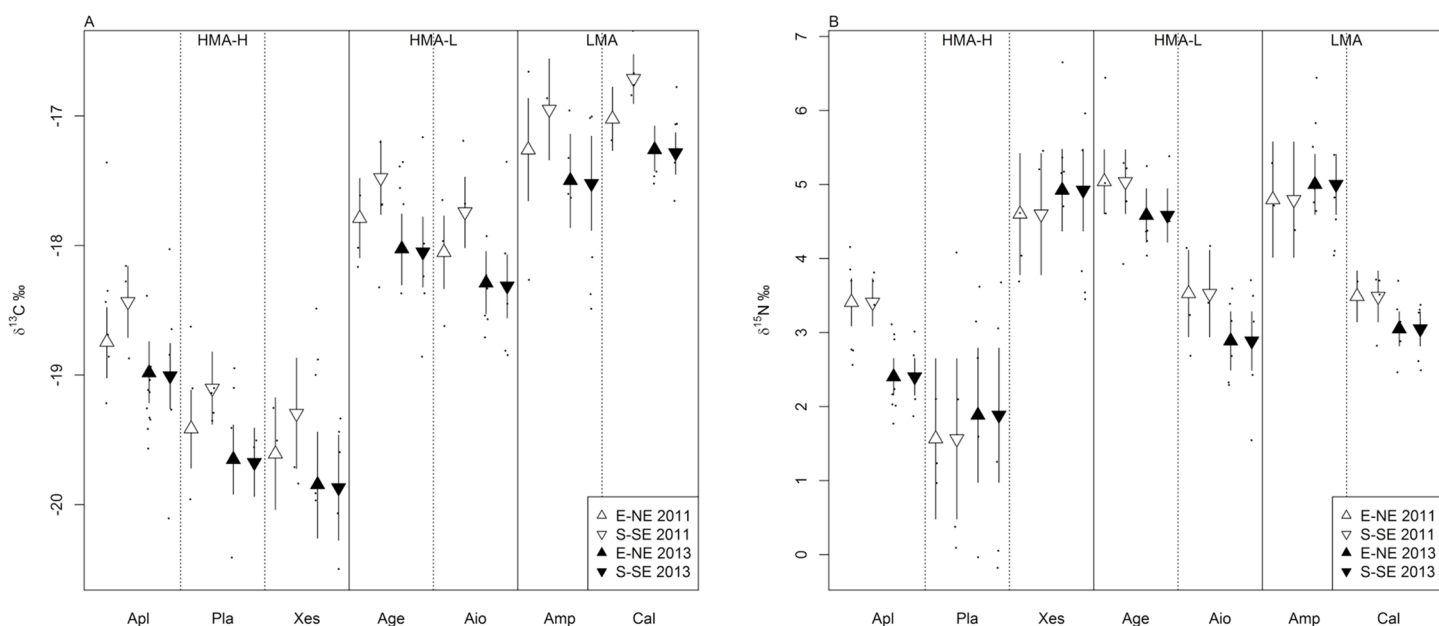**Figure 3 Variation in δ^{13}C (A) and δ^{15}N (B) of different sponge species along the S-SE and E-NE side of the Saba Bank in 2011 and 2013.** Means and 95% credible intervals are based on Bayesian statistics. Black dots represent the raw data of δ^{13}C (A) and of δ^{15}N (B), respectively, of the different sponge species. Sponges are grouped according to their functional traits (HMA-H, HMA-L and LMA). For sponge species abbreviations see Fig. 2. Full-size ⬛ DOI: 10.7717/peerj.5460/fig-3

currents and waves occurred, but varied in time. In 2011, the δ^{13}C signatures in sponges was on average 0.31‰ lower at the E-NE side than at the S-SE side (mean 0.31, 95% credible interval 0.04–0.59). From 2011 to 2013 δ^{13}C signatures decreased in all sponges species on average with 0.23‰, but sponges at the S-SE side decreased even further than at the E-NE side with 0.34‰ (median 0.34, 95% credible interval −0.67 to −0.01). This decrease of δ^{13}C in sponges at the S-SE side compensated the difference that existed in 2011 between sides, making sides again comparable in 2013.

Overall the δ^{15}N in sponges (Fig. 3B) showed an evident temporal effect with a mean decrease of 0.51‰ (95% credible interval 0.27–0.75) from 2011 to 2013. Spatial variations were not found and variations in δ^{15}N of sponges in time were not consistent for species within HMA-H and LMA groups. Only *A. cauliformis*, *A. conifera* (Age), *A. crassa* and *C. plicifera* showed a clear decrease in δ^{15}N from 2011 to 2013 with little or no overlap of 95% credible intervals. The mean δ^{15}N of other sponge species (*Plakortis* spp., *X. muta* and *A. compressa*) did not clearly change in time. No relations were found between sponge SI signatures and depth.

Stable isotope signatures of potential food sources for sponges

Stable isotope signatures of benthic genera of fleshy brown algae and cyanobacterial mats largely overlapped and were evidently higher in δ^{13}C and lower in δ^{15}N than the pelagic-derived food, POM (Table 3). *Sargassum* spp. and cyanobacteria were only collected at up to three stations. Therefore, only SI data of *Dictyota* spp. and *Lobophora* spp. were used and pooled as benthic-derived food representative, and compared with the pelagic food source (POM). SI signatures of suspended POM (δ^{13}C: −24.91‰ [95%

Benthic algae and POM	n	Range in ‰ δ^{13}C	δ^{13}C mean (sd)	Range in ‰ δ^{15}N	δ^{15}N mean (sd)	Average C/N ratio (sd)
Dictyota spp.	15	−18.44 to −15.04	−16.60 (1.17)	−0.26–1.19	0.47 (0.60)	18.7 (4.08)
Lobophora spp.	16	−18.97 to −11.21	−14.23 (2.10)	−0.19–3.38	1.12 (0.93)	33.6 (6.00)
Sargassum spp.	3	−19.50 to −14.62	−16.31 (2.36)	−0.41–2.52	1.31 (1.32)	35.4 (3.80)
Filamentous benthic cyanobacteria	1		−15.07		0.00	8.1
POM in surface water	19.8	−27.67 to −17.78	−24.91 (1.77)	2.92–6.25	4.33 (1.24)	12.9 (5.52)

credible interval −24.20‰ to −25.63‰]; δ^{15}N: 4.33‰ [95% credible interval 3.66–4.99‰]) clearly differed from the benthic source (δ^{13}C: 15.38‰ [95% credible interval −14.69‰ to−16.07‰] and δ^{15}N: 0.80‰ [95% credible interval 0.47–1.14‰]), with lower mean δ^{13}C (9.5‰ lower) and higher δ^{15}N values (3.5‰ higher). The C/N ratio of pelagic POM was lower compared to that of benthic macroalgae, but overlapped with benthic cyanobacteria (Table 3). No differences in geographic space or time were observed for δ^{13}C and δ^{15}N SI signatures of benthic food sources. The same was found for the δ^{13}C in pelagic food (POM). For δ^{15}N in POM insufficient data were available to test its variation in space and time (only one observation in 2011 and seven in 2013). Values obtained in 2013, however, did not show spatial variation and covered the single value available for 2011 assuming that also temporal variation was negligible.

Source partitioning

The proportional contribution of pelagic- and benthic-derived food to the diet of the seven different sponge species revealed that based on the assumed TEFs of C (0.5‰ ± sd 0.5) and N (3.0‰ ± sd 0.5), diets varied depending on sponge species from 51%/49% (LMA sponge *C. plicifera*) to 21%/79% (HMA-H sponge *X. muta*) with regard to benthic/pelagic feeding. LMA sponges appeared to rely more on benthic-derived food than HMA sponges. Five (*X. muta*, *A. conifera*, *A. crassa*, *A. compressa* and *C. plicifera*) of the seven sponge species were estimated to consume relatively more benthic food at the S-SE side than at the E-NE side in 2011 based on the means of the 95% credibility internals (Table 4). In 2013, *X. muta* and *Plakortis* spp. were estimated to consume on average relatively less benthic food and thereby more pelagic-derived food than their conspecifics in 2011. Results showed that average shifts in benthic–pelagic feeding of ~1 (*A. crassa*) to ~13% (*A. compressa*) would be sufficient to cover the recorded differences in bivariate isotope signatures of sponges in space in 2011. To cover changes in time in sponge SI's along the S-SE and E-NE side, shifts of up to 19% (*Plakortis* spp.) in benthic–pelagic feeding were estimated. It should be noted here that the 95% credibility internals were large (Table 4), which made it impossible to assess evident differences in proportional feeding of sponges between different sides of the Bank in 2011 and between years.

Table 4 Contribution of benthic food to sponge diet. Bayesian means with 95% credibility internals (between brackets) of benthic food contribution (%) to the diet of different sponge species in space and time according to the Stable Isotope mixing model (*Parnell et al., 2013*). Complementary % to reach 100% is ascribed to pelagic food (not shown).

| Sponges	S-SE 2011 (%)	E-NE 2011 (%)	S-SE 2013 (%)	E-NE 2013 (%)
Aplysina cauliformis	38.7 (20–57)	40.7 (29–53)	45.2 (26–64)	40.5 (28–53)
Plakortis spp.	36.4 (14–58)	53.0 (15–91)	30.3 (11–50)	33.9 (15–53)
Xestospongia muta	29.1 (0–59)	25.7 (4–47)	20.7 (7–34)	22.9 (5–41)
Agelas conifera	37.6 (17–58)	35.5 (10–61)	34.2 (18–51)	39.0 (24–54)
Aiolochroia crassa	43.3 (15–71)	42.7 (24–61)	44.3 (30–58)	44.2 (32–56)
Amphimedon compressa	48.8 (5–93)	36.5 (2–71)	37.4 (21–45)	45.2 (30–61)
Callyspongia plicifera	50.9 (37–65)	49.1 (4–94)	51.2 (40–62)	49.1 (37–61)
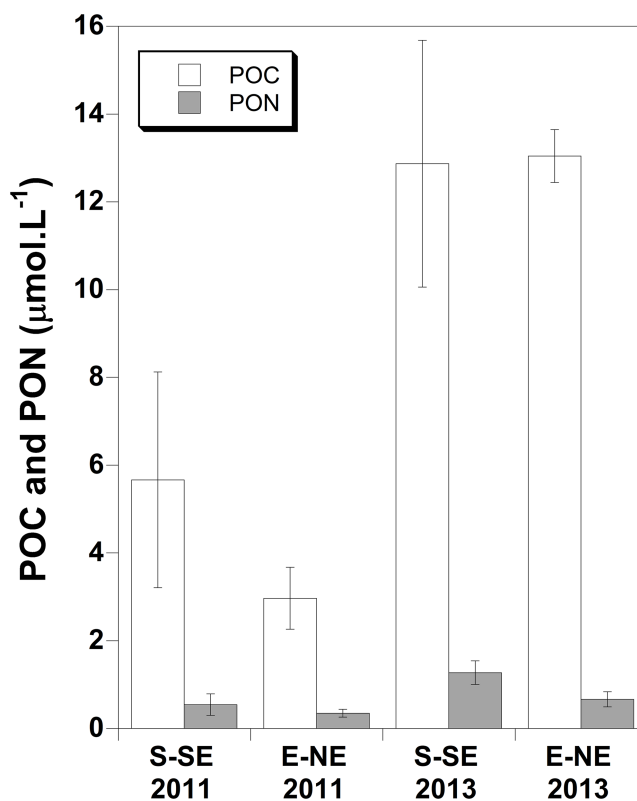
Figure 4 POC and PON concentrations on the Saba Bank. Variations in particulate organic carbon (POC) and nitrogen (PON) concentrations in surface water along the S-SE and E-NE side of the Saba Bank in 2011 and 2013 with standard deviations. Full-size ◱ DOI: 10.7717/peerj.5460/fig-4

Water mass nutrient concentrations

In October 2013, POM concentrations in surface waters were significantly higher along both sides of the Bank than in 2011 (Fig. 4). POC/PON ratios differed between sides in 2013 with a ratio of 20.5 ± sd 4.8 at the E-NE and 10.2 ± sd 2.0 at the S-SE side. The latter ratio was comparable to the ratios found in 2011 along both sides (10.1 ± sd 0.8
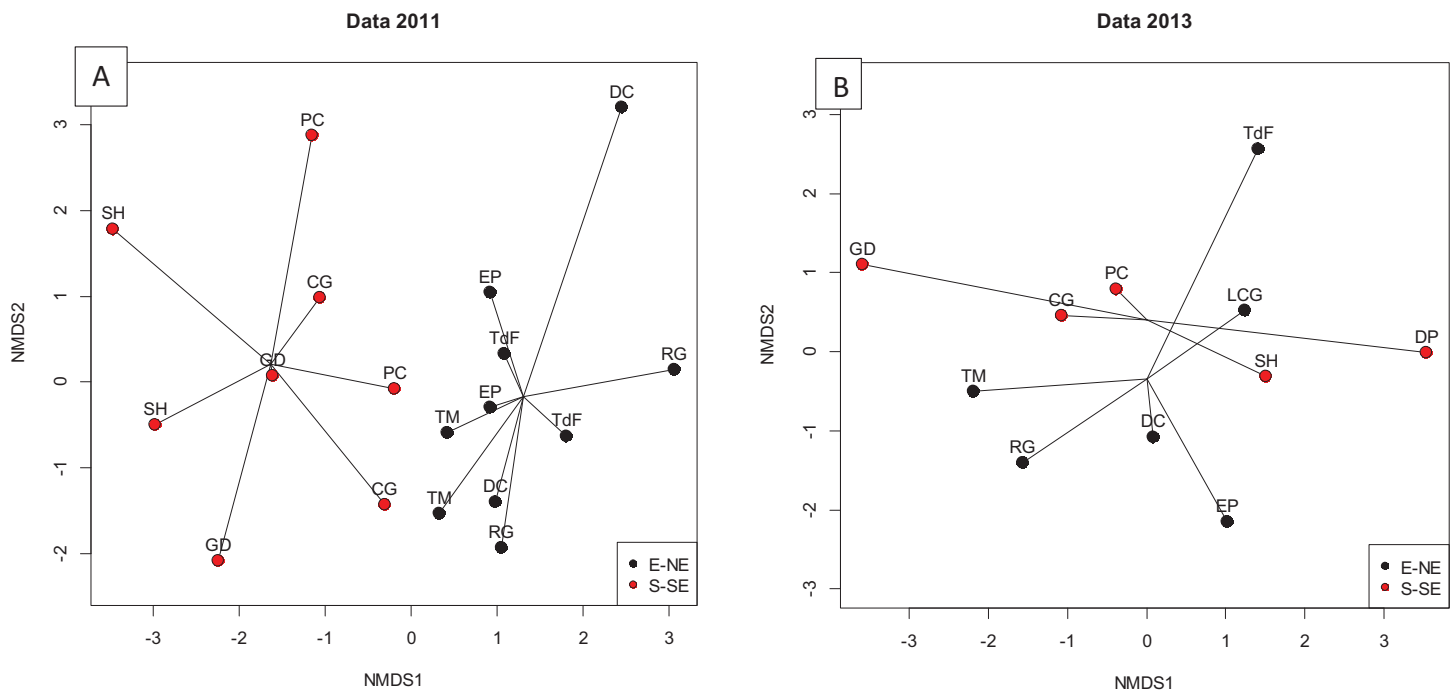
Figure 5 Water mass characterization. Numerical dimensional scaling plots with water mass differences on basis of the following variables: average PO_4^{3-}, NH_4^+, NO_2^-, DIN, DIN/PO_4^{3-}, ratio, DOC, DON, TOC, TON, POC and POC/PON ratio at different sides of the Saba Bank (S-SE and E-NE) in (A) October 2011 and (B) October 2013. Samples along the S-SE side were collected at stations DP, SH, GD, PC, CG and along the E-NE side at EP, TdF, DC, TM, LCG and RG (i.e., abbreviations of station names, see Fig. 1 or Supplemental Data for full names of locations).

Full-size ◩ DOI: 10.7717/peerj.5460/fig-5

and 9.2 ± sd 0.7 at the S-SE and E-NE side, respectively). Concentrations of all other organic and inorganic nutrient variables were not significantly different between sides in 2011 or in 2013. However, taking all nutrient variables in surface water into account (except the ones showing high autocorrelation, e.g., NO_3^-, TN, TOC), a clear difference in water mass constituent proportions (NH_4^+, NO_2^-, PO_4^{3-}, DOC, DON, POC concentrations and DIN/PO_4^{3-} and POC/PON ratio's) between the S-SE and E-NE side was found in 2011 (Fig. 5A). Testing the distance matrix ~ side of the nMDS results, yielded a p-value of <0.001 (Permanova test). The difference in water masses between sides in 2011 was characterized by higher average DIN (notably NO_3^-), DOC, POC and PON concentrations and DIN/PO_4^{3-} ratio along the S-SE than E-NE side of the Bank, and lowest average PO_4^{3-} values at the S-SE side in 2011. Particularly the higher concentrations of NO_3^- of 0.197 ± sd 0.15 at the S-SE vs 0.111 ± sd 0.04 μmol/L at the E-NE (Fig. S1) and lower concentrations of PO_4^{3-} (~0.013 μmol/L, Fig. S2) at S-SE side in combination with higher DOC concentrations (Fig. S3) and DIN/PO_4^{3-} ratios (Fig. S4) than the E-NE side pointed to a reef signal in the water mass in 2011. In 2013, the water mass at the S-SE was not significantly different from the water mass at the E-NE side (Fig. 5B, Permanova test, p = 0.596). See Supplemental Information for concentrations of all nutrients.

DISCUSSION

We show that carbon and nitrogen SI compositions of sponges on the Saba Bank vary in geographic space and through time. Trajectories of change in SI signatures were comparable between most sponge species irrespective of different functional traits. Variations in sponge $\delta^{13}C$ signature in geographic space concurred with differences in water mass constituents. Differences in the composition of these constituents suggest different exposure times to reef waters due to differences in hydrodynamic conditions between the E-NE and S-SE side of the Bank in 2011. Observed lower $\delta^{13}C$ and $\delta^{15}N$ values of sponges in 2013 compared to 2011 was suggested to be induced by an increase in pelagic food, which might have influenced the proportion of C and N absorbed from pelagic- and benthic-derived food by sponges differently.

Variation in bivariate isotopic niche space in sponges

Sponge species in this study showed ranges in SI signatures, which agree well with ranges for similar sponge species in the Caribbean (*Southwell, 2007*; *Weisz et al., 2007*; *Fiore, Baker & Lesser, 2013*; *Freeman, Easson & Baker, 2014*). This might imply that bivariate isotope niche space of these sponge species persisted in space and time and that sponges maintained their trophic position relative to other sponge species. The placement of the niche spaces (SEAs$_c$) in bivariate plots showed clear differences between sponge species indicating distinct diets. Larger niche spaces suggest that species have a broader access to food sources and might be more apt to maintain themselves under varying trophic conditions than species with relatively small niche spaces (*Freeman, Easson & Baker, 2014*, *2016*). The observation that the ellipses of most sponges slightly tilted to the right indicate that the $\delta^{13}C$ and $\delta^{15}N$ tended to follow comparable trajectories of change irrespective functional traits (HMA vs LMA sponges). This suggested that the basic sponge metabolism overruled the contribution of its endosymbionts.

Interestingly, the dichotomy of HMA and LMA sponges (*Gloeckner et al., 2014*) was mainly covered by differences in $\delta^{13}C$. HMA-H sponges had lower $\delta^{13}C$ values than LMA sponges. This difference might be attributed to the presence of phototrophic endosymbionts, which preferably fix inorganic ^{12}C over ^{13}C (*Freeman, Easson & Baker, 2014*) and supply HMA-H sponges with photosynthates. Niche spaces of HMA-L sponges were found to be in between HMA-H and LMA sponges, which suggest that they rely more on external food sources and to a lesser part on food supplied by their endosymbionts Patterns for $\delta^{15}N$ were unresolved with respect to HMA–LMA dichotomy, suggesting that N might be obtained from a different source and/or that fractionation varies between sponge species. The division we found between the low <3.5 and high >4‰ $\delta^{15}N$ values in HMA sponges has also been reported by others, although at a slightly different separation of the low $\delta^{15}N$ group (*Weisz et al., 2007*; *Mohamed et al., 2010*). The LMA group mainly resides in the high $\delta^{15}N$ group (*Freeman, Easson & Baker, 2014*), although in our study the LMA sponge *C. plicifera* was part of the low group. Complex biochemical N cycling pathways involving different N fractionation have been reported for both HMA (*Weisz et al., 2007*; *Southwell et al., 2008*; *Fiore et al., 2010*; *Fiore, Baker &*

Lesser, 2013) and LMA sponges (*Schläppy et al., 2010*; *Cardoso et al., 2013*; *Diaz & Ward, 1997*; *Jiménez & Ribes, 2007*; *Southwell, Popp & Martens, 2008*; *Southwell et al., 2008*; *Van Duyl et al., 2008*). Nitrification and other microbial metabolic pathways (e.g., denitrification, anammox, N_2 fixation) probably influenced the $\delta^{15}N$ signature of the sponge holobiont (*Weisz et al., 2007*; *Southwell et al., 2008*; *Mohamed et al., 2010*). Nitrification and denitrification increased the $\delta^{15}N$ values in sponges (*Southwell et al., 2008*), while sponges harboring N_2 fixing microbes tended to decrease in $\delta^{15}N$ (*Wilkinson & Fay, 1979*; *Southwell, Popp & Martens, 2008*; *Southwell et al., 2008*; *Taylor et al., 2007*; *Weisz et al., 2007*; *Mohamed et al., 2008*; *Ribes et al., 2015*). To what extent N_2 fixation contributed to the differences in $\delta^{15}N$ of the sponge holobiont is still questionable (*Southwell et al., 2008*) and might depend on ambient nutrient conditions. Overall these and our results suggest that the carbon and nitrogen incorporated in the sponge holobiont were not necessarily retrieved from the same source.

Variations in sponge SI signature in space and time

To date it is still poorly understood to what extent hydrodynamic conditions in geographic space and/or time affect variations in SI signature of different sponge species. Variations in space of $\delta^{13}C$ and $\delta^{15}N$ of corals, macroalgae, suspended matter, (in)organic nutrients and sponges on coral reefs have mostly been described in relation to onshore–offshore distances and depth (*Risk, Sammarco & Schwarcz, 1994*; *Risk et al., 2009*; *Southwell, 2007*; *Lapointe et al., 2010*; *Wyatt et al., 2013*). On the Saba Bank the distance from shore did not play a role and the depth gradient from 15 to 32 m, in which sponges were collected, did not influence the spatial variation in SI signatures of sponges on the Saba Bank. Nevertheless, in 2011 clear differences in SI signatures were found between the E-NE and the S-SE sides of the Bank. All sponges irrespective of species or functional traits had higher $\delta^{13}C$ values at the S-SE side than at the E-NE side in 2011. Each species was on average (raw data) 0.01–0.64‰ higher in $\delta^{13}C$ than its conspecifics at the other side. With Bayesian statistics the best estimate for all sponges yielded a difference of 0.31 (95% credible interval of 0.04–0.59) in $\delta^{13}C$ between sides. This was comparable to ranges found for $\delta^{13}C$ signatures of *X. muta* between different sites in the NE and W Caribbean (*Fiore, Baker & Lesser, 2013*; *Freeman, Easson & Baker, 2014*), but smaller than differences found for onshore–offshore gradients of up to 5‰ (*Southwell, 2007*). The fact that all seven sponge species had higher $\delta^{13}C$ values at the S-SE-side than the E-NE side implied that this phenomenon was likely to be caused by general differences in organic matter supply of pelagic and/or benthic sources, rather than by species-specific functional traits, such as the presence and abundance of (phototrophic) endosymbionts. As sponges only differed in $\delta^{13}C$, but not in $\delta^{15}N$ signatures, it further appeared that N-metabolism of sponges, irrespective of sponge species, was not affected by space on the Saba Bank. The effect of spatial differences in hydrodynamic conditions concerning pelagic food supply were apparently more important for the $\delta^{13}C$ than for the $\delta^{15}N$ in sponges, which is in accordance with other studies (*Southwell, 2007*; *Freeman, Easson & Baker, 2014*).

Spatial differences in δ^{13}C of sponge species were only found in 2011, but not in 2013. This indicated that the difference was not determined by the fixed position of the Saba Bank toward incoming currents and waves. The environmental conditions were apparently different between sides (S-SE and E-NE side) in 2011 (see below), which lasted over a long enough period for sponges to obtain a distinct δ^{13}C signal. Integration time for a distinct signal has been estimated on 1–2 months (*Freeman & Thacker, 2011*; *Simister et al., 2013*). Thus, it is likely that divergent environmental conditions led to the increased δ^{13}C signature in sponges at the S-SE side and not at the E-NE side, where variations in δ^{13}C in time were smaller than at the S-SE side.

Time affected both the δ^{13}C and the δ^{15}N values of sponges on the Saba Bank. The sponge SI signatures decreased between 2011 and 2013 with 0.23‰ for δ^{13}C and with 0.51‰ for δ^{15}N. That decreases in δ^{13}C in time at the S-SE side (0.34‰) were larger than at the E-NE side suggested that the uptake of pelagic-derived food was higher, purportedly due to an increased supply rate at the S-SE than E-NE side preceding our sampling in 2013. The variability in hydrodynamics in time might have been larger at the S-SE than E-NE side of the Bank supporting the higher fluctuation in δ^{13}C at the S-SE side. Comparable trajectories of excursions of bulk δ^{13}C and δ^{15}N in sponges in time were also observed by *Simister et al. (2013)*. They (ibid) reported several significant excursions from the mean δ^{13}C and δ^{15}N values in the LMA sponges *Ancorina alata* and *Tethya stolonifera* without a significant change over a 2-year period. Interestingly the trajectories of the excursions were comparable between the sponge species irrespective species-specific differences in SI signatures, which is in accordance with our SI changes for most sponge species in space and time. Comparable patterns of change in the δ^{13}C and δ^{15}N of most sponges appeared particularly driven by temporal variation in dietary food sources.

Food sources contributing to sponge SI signatures

Contrasting to the spatio–temporal variations in sponge SI signatures, SI signatures of pelagic POM and the benthic macroalgae, as well as their difference remained stable in both space and time. Pelagic-derived food was ~9.5‰ lower in δ^{13}C and ~3.5‰ higher in δ^{15}N compared to these values in benthic-derived food. Comparable differences in SI signatures between pelagic and benthic primary producers in coral reef ecosystems have been reported by others (*Granek, Compton & Phillips, 2009*; *Van Duyl et al., 2011*; *Wyatt et al., 2013*; *Kürten et al., 2014*; *Kolasinski et al., 2016*). We therefore, propose that shifts in benthic vs pelagic feeding occurred to explain variations in SI signatures in space and time. On average 25–50% of the sponge diets on the Saba Bank consisted of benthic-derived food. The HMA-H sponges *X. muta* relied the least on benthic food (25% ± sd 4%) and the LMA sponge *C. plicifera* the most (50% ± sd 1%). Shifts of less than 10% in the diet of sponges (Table 4) would be sufficient to explain the recorded spatio–temporal patterns in most sponge bivariate SI signatures. Such shifts were likely triggered by local environmental (hydrodynamic) conditions influencing the availability of benthic- and pelagic-derived food.

Effect of environmental conditions on stable isotopic signatures in sponges

The spatial enrichment of $\delta^{13}C$ in sponges in 2011 along the S-SE side of the Bank concurred with a small, but significant difference in water mass constituent proportion between sides (Fig. 5A). Although upwelling and different water mass intrusions occur along the Saba Bank and Saba (*Van Duyl, 2016*; *De Nooijer & Van Heuven, 2016*; Van Heuven et al., submitted), no clear indications were found to support that such events occurred during the sampling in October 2011 and October 2013. Therefore, water masses at the different sides of the bank might have come from different sources or the retention time of the water on the fore reefs along the S-SE and E-NE side of the Bank differed. The general trend is that the main surface flow approaching the Saba Bank comes from the SSE and slightly bends toward more westerly directions over and along the Bank to continue to the NW (*Gordon, 1967*). However, source water reaching the Bank and flow patterns along and over the Bank can be highly variable (*Andrade & Barton, 2000*). Model simulations of source, track and speed of water parcels reaching the Saba Bank in October 2011 and October 2013 were quite different (A. Candy, 2017, unpublished data). This might also have affected water mass constituents and SI signatures of sponges found between sides and years at the Saba Bank. Increased average NO_3^- concentrations, decreased PO_4^{3-} and slightly enhanced DOM concentrations pointed to an elevated reef benthic influence on water mass constituents at the S-SE compared to the E-NE side in 2011. This implied that the retention time of water might have been longer on the reefs along the S-SE than E-NE side of the Bank. Sponge and coral holobionts are known for their potential to nitrify leading to increased nitrate concentrations above reefs (*Webb et al., 1975*; *Corredor et al., 1988*; *Wafar, Wafar & David, 1990*; *Diaz & Ward, 1997*; *Gast et al., 1999*; *Southwell et al., 2008*). Also increased DOC concentrations have been reported for reef overlying waters (*Van Duyl & Gast, 2001*; *Dinsdale et al., 2008*; *Mueller et al., 2014b*; *Mueller, Meesters & Van Duyl, 2017*). Sponges obtaining food from this water mass might therefore, have been higher in $\delta^{13}C$ than sponges at the E-NE side in 2011. The fact that the $\delta^{15}N$ signatures did not differ spatially in 2011 nor in 2013 might indicate that sponges obtain N for a large part from benthic-derived organic matter. Organic N requirements of sponges are high considering the low C:N ratio of most sponges in this study and the generally high inorganic N exudation rates (*Maldonado, Ribes & Van Duyl, 2012*).

The decrease of $\delta^{13}C$ in sponges from 2011 to 2013 is most likely driven by higher POC concentrations in October 2013 than in October 2011 which might have affected the proportional feeding of benthic- and pelagic-derived food in favor of pelagic-derived food. The $\delta^{13}C$ in phyto-, bacterioplankton comprising most of the POM is much lower than in benthic-derived organic matter, which might have been reflected in the SI signature of sponges at the S-SE as well as the E-NE side in 2013. That several sponge species had lower $\delta^{15}N$ values (*A. cauliformis*, *A. conifera* and *C. plicifera*) in 2013 than 2011 is more difficult to explain considering the fact that plankton derived PON was higher in $\delta^{15}N$ (mean $\delta^{15}N$ is 4.3‰) compared to benthic-derived food (0.8‰).

It suggests that also feeding on benthic-derived nitrogen was intensified by increasing the proportion of benthic N in the diet of these sponges. Additionally, the lower C/N ratio of POM along the E-NE side in 2013 than in 2011 potentially contributed to this proportional shift. Furthermore, it is well-known that cyanobacterial mats and turf algae on coral reefs fix N_2 and that $\delta^{15}N$ signatures of cyanobacterial mats are lower than of other benthic primary producers (Table 3; *Kayanne et al., 2005*; *Kolasinski et al., 2016*). Released products from these mats contain N compounds lower in $\delta^{15}N$ than N released by macroalgae or pelagic primary producers (*Kayanne et al., 2005*; *Thibodeau et al., 2013*; *Brocke et al., 2015*). In the Caribbean significant increases in cyanobacterial mat cover on coral reefs were reported from less than 10 to more than 20% since ~1990 (*De Bakker et al., 2017*). Such shifts in cover might have occurred on the Saba Bank as well, considering the fact that in October 2013 ca 30% of the cover of benthic primary producers and 20% of total cover on reefs of the Saba Bank was occupied by cyanobacterial mats (*Wiltink, 2016*). In case sponges feed on this cyanobacterial mat derived nitrogen, it is likely that $\delta^{15}N$ in their bulk tissue further decreased in time. It is evident that various nitrogen sources contributed to the observed patterns of $\delta^{15}N$ in sponges in time.

CONCLUSION

Bivariate SI signatures of sponges ($\delta^{13}C$, $\delta^{15}N$) on coral reefs of the Saba Bank differed between species with different functional traits. Orientation of reefs toward incoming currents and waves (space) influenced the variation of $\delta^{13}C$ in sponges but not the $\delta^{15}N$. Bulk $\delta^{13}C$ and $\delta^{15}N$ in sponges moved in a comparable direction in time, while the $\delta^{13}C$ appeared more responsive to changes in pelagic food concentrations than the $\delta^{15}N$. The variable supply of pelagic-derived organic carbon to sponges and the accumulation of benthic-derived food in reef overlying waters was ascribed to variations in hydrodynamic conditions in time and space (currents and retention time of water over reefs). Variations in SI signatures of sponges were most likely realized by mainly unselective shifts in proportional feeding on pelagic- and benthic-derived food sources, which differed significantly in SI composition. Shifts in diet were likely influenced by spatial differences in retention time of water over reefs and concentration of pelagic food relative to benthic-derived food.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Fleur C. Van Duyl conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Benjamin Mueller performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Erik H. Meesters analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft, statistics.

Field Study Permissions
The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Research permits for conducting research on the Saba Bank in 2011 and 2013 were issued by Rijkswaterstaat, Dutch Ministry of Infrastructure and Environment and Ministry of Economic Affairs (No. WSH/2011/1400 and No. RWS-2013/42681).

Data Availability
The following information was supplied regarding data availability:

The raw data are provided in a Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5460#supplemental-information.

REFERENCES

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26(1)**:32–46 DOI 10.1111/j.1442-9993.2001.01070.pp.x.

Andrade CA, Barton ED. 2000. Eddy development and motion in the Caribbean Sea. *Journal of Geophysical Research: Oceans* **105(C11)**:26191–26201 DOI 10.1029/2000jc000300.

Benner R, Biddanda B, Black B, McCarthy M. 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Marine Chemistry* **57(3–4)**:243–263 DOI 10.1016/s0304-4203(97)00013-3.

Brocke HJ, Wenzhoefer F, De Beer D, Mueller B, Van Duyl FC, Nugues MM. 2015. High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef ecosystem. *Scientific Reports* **5(1)**:8852 DOI 10.1038/srep08852.

Cardini U, Bednarz VN, Foster RA, Wild C. 2014. Benthic N_2 fixation in coral reefs and the potential effects of human induced environmental change. *Ecology and Evolution* **4(9)**:1706–1727 DOI 10.1002/ece3.1050.

Cardoso JFMF, Van Bleijswijk JDL, Witte H, Van Duyl FC. 2013. Diversity and abundance of ammonia oxidizing Archaea and Bacteria in tropical and cold-water coral reef sponges. *Aquatic Microbial Ecology* **68(3)**:215–230 DOI 10.3354/ame01610.

Corredor JE, Wilkinson CR, Vicente VP, Morell JM, Otero E. 1988. Nitrate release by Caribbean reef sponges. *Limnology and Oceanography* **33(1)**:114–120 DOI 10.4319/lo.1988.33.1.0114.

De Bakker DM, Van Duyl FC, Bak RPM, Nugues MM, Nieuwland G, Meesters EH. 2017. 40 years of benthic community change on the Caribbean reefs of Curacao and Bonaire: the rise of slimy cyanobacterial mats. *Coral Reefs* **36(2)**:355–367 DOI 10.1007/s00338-016-1534-9.

De Goeij JM, Van den Berg H, Van Oostveen MM, Epping EHG, Van Duyl FC. 2008. Major Bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series* **357**:139–151 DOI 10.3354/meps07403.

De Graaf N. 2012. Wind waves and tidal controls over flow processes in the Saba Bank. MSc thesis research, Hydrology and Quantitative Water Management, Wageningen University.

Den Haan J, Visser PM, Ganase AE, Gooren EE, Stal LJ, Van Duyl FC, Vermeij MJA, Huisman J. 2014. Nitrogen fixation rates in algal turf communities of a degraded versus less degraded coral reef. *Coral Reefs* **33(4)**:1003–1015 DOI 10.1007/s00338-014-1207-5.

De Nooijer LJ, Van Heuven SMAC. 2016. NIOZ Cruise report *RV Pelagia* 64PE414 Saba Bank: 19th of August—8th of September 2016 Guadeloupe—Curaçao. *Available at* http://www.dcbd.nl/document/cruise-report-rv-pelagia-64pe414-saba-bank.

Diaz MC, Ward BB. 1997. Sponge-mediated nitrification in tropical benthic communities. *Marine Ecology Progress Series* **156**:97–107 DOI 10.3354/meps156097.

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis BL, Azam F, Knowlton N, Rohwer F. 2008. Microbial ecology of four coral atolls in the northern line Islands. *PLOS ONE* **3(2)**:e1584 DOI 10.1371/journal.pone.0001584.

Duarte CM. 1992. Nutrient concentration of aquatic plants: patterns across species. *Limnology and Oceanography* **37(4)**:882–889 DOI 10.4319/lo.1992.37.4.0882.

Erwin PM, Thacker RW. 2007. Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. *Journal of the Marine Biological Association of the United Kingdom* **87(6)**:1683–1692 DOI 10.1017/s0025315407058213.

Faganeli J, Malej A, Pezdic J, Malacic V. 1988. C:N:P ratios and stable C isotope ratios as indicators of sources of organic matter in the Gulf of Trieste (Northern Adriatic). *Oceanologica Acta* **11**:377–382.

Fiore CL, Baker DM, Lesser MP. 2013. Nitrogen biogeochemistry in the Caribbean sponge, *Xestospongia muta*: a source or sink of dissolved inorganic nitrogen? *PLOS ONE* **8(8)**:e72961 DOI 10.1371/journal.pone.0072961.

Fiore CL, Jarett JK, Olson ND, Lesser MP. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. *Trends in Microbiology* **18(10)**:455–463 DOI 10.1016/j.tim.2010.07.001.

France RL. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine Ecology Progress Series* **124**:307–312 DOI 10.3354/meps124307.

Freeman CJ, Easson CG, Baker DM. 2014. Metabolic diversity and niche structure in sponges from the Miskito Cays, Honduras. *PeerJ* **2**:e695 DOI 10.7717/peerj.695.

Freeman CJ, Easson CG, Baker DM. 2016. Niche structure of marine sponges from temperate hard-bottom habitats within Gray's Reef National Marine Sanctuary. *Journal of the Marine Biological Association of the United Kingdom* **96(2)**:559–565 DOI 10.1017/s0025315415000363.

Freeman CJ, Thacker RW. 2011. Complex interactions between marine sponges and their symbiotic microbial communities. *Limnology and Oceanography* **56(5)**:1577–1586 DOI 10.4319/lo.2011.56.5.1577.

Fry B. 2006. *Stable Isotope Ecology.* New York: Springer, 297.

Fry B, Hopkinson CS Jr, Nolin A, Wainright SC. 1998. 13C/12C composition of marine dissolved organic carbon. *Chemical Geology* **152(1–2)**:113–118 DOI 10.1016/s0009-2541(98)00100-4.

Fry B, Lutes R, Northam M, Parker PL, Ogden JC. 1982. A 13C/12C comparison of food webs in Caribbean seagrass meadows and coral reefs. *Aquatic Botany* **14**:389–398 DOI 10.1016/0304-3770(82)90112-7.

Gast GJ, Jonkers PJ, Van Duyl FC, Bak RPM. 1999. Bacteria, flagellates and nutrients in island fringing coral reef waters: influence of the ocean, the reef and eutrophication. *Bulletin of Marine Science* **65**:523–538.

Gloeckner V, Wehrl M, Moitinho-Silva L, Gernert C, Schupp P, Pawlik JR, Lindquist N, Erpenbeck D, Worheide G, Hentschel U. 2014. The HMA-LMA dichotomy revisited: and electron microscopical survey of 56 sponge species. *Biological Bulletin* **227(1)**:78–88 DOI 10.1086/bblv227n1p78.

Gordon AL. 1967. Circulation of the Caribbean Sea. *Journal of Geophysical Research* **72(24)**:6207–6223 DOI 10.1029/jz072i024p06207.

Granek EF, Compton JE, Phillips DL. 2009. Mangrove-exported nutrient incorporation by sessile coral reef invertebrates. *Ecosystems* **12(3)**:462–472 DOI 10.1007/s10021-009-9235-7.

Haas AF, Fairoz MF, Kelly LW, Nelson CE, Dinsdale EA, Edwards RA, Giles S, Hatay M, Hisakawa N, Knowles B, Lim YW, Maughan H, Pantos O, Roach TNF, Sanchez SE, Silveira CB, Sandin S, Smith JE, Rohwer F. 2016. Global microbialization of coral reefs. *Nature Microbiology* **1(6)**:16042 DOI 10.1038/nmicrobiol.2016.42.

Haas AF, Jantzen C, Naumann MS, Iglesias-Prieto R, Wild C. 2010a. Organic matter release by the dominant primary producers in a Caribbean reef lagoon: implication for in situ O_2 availability. *Marine Ecology Progress Series* **409**:27–39 DOI 10.3354/meps08631.

Haas AF, Naumann MS, Struck U, Mayr C, El-Zibdah M, Wild C. 2010b. Organic matter release by coral reef associated benthic algae in the northern Red Sea. *Journal of Experimental Marine Biology and Ecology* **389(1–2)**:53–60 DOI 10.1016/j.jembe.2010.03.018.

Haas AF, Nelson CE, Wegley KL, Carlson CA, Rohwer F, Leichter JJ, Wyatt A, Smith JE. 2011. Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. *PLOS ONE* **6(11)**:e27973 DOI 10.1371/journal.pone.0027973.

Hoer DR, Gibson PJ, Tommerdahl JP, Lindquist NL, Martens CS. 2018. Consumption of dissolved organic carbon by Caribbean reef sponges. *Limnology and Oceanography* **63(1)**:337–351 DOI 10.1002/lno.10634.

Jackson J, Donavan M, Cramer K, Lam V. 2014. Status and Trends of Caribbean Coral Reefs: 1970–2012. *CEP Technical Report* **71**:330.

Jackson AL, Inger R, Parnell AC, Bearhop S. 2011. Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* **80(3)**:595–602 DOI 10.1111/j.1365-2656.2011.01806.x.

Jiménez E, Ribes M. 2007. Sponges as a source of dissolved inorganic nitrogen: nitrification mediated by temperate sponges. *Limnology and Oceanography* **52(3)**:948–958 DOI 10.4319/lo.2007.52.3.0948.

Kayanne H, Hirota M, Yamamuro M, Koike I. 2005. Nitrogen fixation of filamentous cyanobacteria in a coral reef measured using three different methods. *Coral Reefs* **24(2)**:197–200 DOI 10.1007/s00338-004-0465-z.

Kjerfve B. 1981. Tides of the Caribbean Sea. *Journal Geophysical Research* **86(C5)**:4243–4247 DOI 10.1029/jc086ic05p04243.

Kolasinski J, Nahon S, Rogers K, Chauvin A, Bigot L, Frouin P. 2016. Stable isotopes reveal spatial variability in the trophic structure of a macro-benthic invertebrate community in a tropical coral reef. *Rapid Communications in Mass Spectrometry* **30(3)**:433–446 DOI 10.1002/rcm.7443.

Kürten B, Al-Aidaroos AM, Struck U, Khomayis HS, Gharbawi WY, Sommer U. 2014. Influence of environmental gradients on C and N stable isotope ratios in coral reef biota of the Red Sea, Saudi Arabia. *Journal of Sea Research* **85**:379–394 DOI 10.1016/j.seares.2013.07.008.

Lapointe BE, Langton R, Bedford BJ, Potts AC, Day O, Hu C. 2010. Land-based nutrient enrichment of the Buccoo Reef Complex and fringing coral reefs of Tobago, West Indies. *Marine Pollution Bulletin* **60(3)**:334–343 DOI 10.1016/j.marpolbul.2009.10.020.

Lesser MP, Falcon LI, Rodriguez-Roman A, Enriquez S, Hoegh-Guldberg O, Iglesias-Prieto R. 2007. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastrea cavernosa*. *Marine Ecology Progress Series* **346**:143–152 DOI 10.3354/meps07008.

Lowe RJ, Falter JL. 2015. Oceanic forcing of coral reefs. *Annual Review of Marine Science* **7(1)**:43–66 DOI 10.1146/annurev-marine-010814-015834.

Maldonado M, Ribes M, Van Duyl FC. 2012. Nutrient fluxes through sponges: biology, budgets, and ecological implications. *Advances in Marine Biology* **62**:113–182 DOI 10.1016/B978-0-12-394283-8.00003-5.

McCook LJ, Jompa J, Diaz-Pulido G. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* **19(4)**:400–417 DOI 10.1007/s003380000129.

McMurray SE, Pawlik JR, Finelli CM. 2017. Demography alters carbon flux for a dominant benthic suspension feeder, the giant barrel sponge, on Conch Reef, Florida Keys. *Functional Ecology* **31(11)**:2188–2198 DOI 10.1111/1365-2435.12908.

McMurray SE, Stubler AD, Erwin PM, Finelli CM, Pawlik JR. 2018. A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. *Marine Ecology Progress Series* **588**:1–14 DOI 10.3354/meps12466.

Mohamed NM, Colman AS, Tal Y, Hill RT. 2008. Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. *Environmental Microbiology* **10(11)**:2910–2921 DOI 10.1111/j.1462-2920.2008.01704.x.

Mohamed NM, Saito K, Tal Y, Hill RT. 2010. Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. *ISME Journal* **4(1)**:38–48 DOI 10.1038/ismej.2009.84.

Mueller B, De Goeij JM, Vermeij MJA, Mulders Y, Van Der Ent E, Ribes M, Van Duyl FC. 2014a. Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). *PLOS ONE* **92(2)**:e90152 DOI 10.1371/journal.pone.0090152.

Mueller B, Den Haan J, Visser PM, Vermeij MJA, Van Duyl FC. 2016. Effect of light and nutrient availability on the release of dissolved organic carbon (DOC) by Caribbean turf algae. *Scientific Reports* **6(1)**:23248 DOI 10.1038/srep23248.

Mueller B, Meesters EH, Van Duyl FC. 2017. DOC concentrations across a depth gradient on a Caribbean coral reef. *PeerJ* **5**:e3456 DOI 10.7287/peerj.preprints.2610.

Mueller B, Van Der Zande R, Van Leent P, Meesters E, Vermeij M, Van Duyl FC. 2014b. Effect of light availability on dissolved organic carbon (DOC) release by Caribbean reef algae and corals. *Bulletin of Marine Science* **90(3)**:875–893 DOI 10.5343/bms.2013.1062.

Naumann M, Haas AF, Struck U, Mayr C, El-Zibdah M, Wild C. 2010a. Organic matter release by dominant hermatypic corals of the Northern Red Sea. *Coral Reefs* **29(3)**:649–659 DOI 10.1007/s00338-010-0612-7.

Naumann MS, Mayr C, Struck U, Wild C. 2010b. Coral mucus stable isotope composition and labeling: experimental evidence for mucus uptake by epizoic acoelomorph worms. *Marine Biology* **157(11)**:2521–2531 DOI 10.1007/s00227-010-1516-3.

Parnell A, Jackson A. 2013. *SIAR: stable isotope analysis in R package*. Version 4.2. *Available at* http://CRAN.R-project.org/package=siar.

Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ, Inger R. 2013. Bayesian stable isotope mixing models. *Environmetrics* **24**:387–399 DOI 10.1002/env.2221.

Pawlik JR, Burkepile DE, Thurber RV. 2016. A vicious circle? Altered carbon and nutrient cycling may explain the low resilience of Caribbean coral reefs. *Bioscience* **66(6)**:470–476 DOI 10.1093/biosci/biw047.

Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18(1)**:293–320 DOI 10.1146/annurev.ecolsys.18.1.293.

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ. 2014. Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* **92(10)**:823–835 DOI 10.1139/cjz-2014-0127.

R Core Team. 2017. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. *Available at* https://www.R-project.org/.

Raven J, Walker D, Johnston A, Handley L, Kubler J. 1995. Implications of 13C natural abundance measurements for photosynthetic performance by marine macrophytes in their natural environment. *Marine Ecology Progress Series* **123**:193–205 DOI 10.3354/meps123193.

Ribes M, Dziallas C, Coma R, Riemann L. 2015. Microbial diversity and putative diazotrophy in High- and Low-Microbial-Abundance Mediterranean sponges. *Applied and Environmental Microbiology* **81(17)**:5683–5693 DOI 10.1128/aem.01320-15.

Risk MJ, Lapointe BE, Sherwood OA, Bedford BJ. 2009. The use of δ15N in assessing sewage stress on coral reefs. *Marine Pollution Bulletin* **58(6)**:793–802 DOI 10.1016/j.marpolbul.2009.02.008.

Risk MJ, Sammarco PW, Schwarcz HP. 1994. Cross-continental shelf trends in d^{13}C in coral on the Great Barrier Reef. *Marine Ecology Progress Series* **106**:121–130 DOI 10.3354/meps106121.

Rix L, De Goeij JM, Mueller CE, Struck U, Middelburg JJ, Van Duyl FC, Al-Horani FA, Wild C, Naumann MS, Van Oevelen D. 2016a. Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. *Scientific Reports* **6(1)**:18715 DOI 10.1038/srep18715.

Rix L, De Goeij JM, Van Oevelen D, Struck U, Al-Horani FA, Wild C, Naumann MS. 2016b. Differential recycling of coral and algal dissolved organic matter via the sponge loop. *Functional Ecology* **31(3)**:778–789 DOI 10.1111/1365-2435.12758.

Rue H, Martino S, Chopin N. 2009. Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **71(2)**:319–392 DOI 10.1111/j.1467-9868.2008.00700.x.

Schläppy M-L, Schottner SI, Lavik G, Kuypers MMM, De Beer D, Hoffmann F. 2010. Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Marine Biology* **157(3)**:593–602 DOI 10.1007/s00227-009-1344-5.

Simister RL, Taylor MW, Rogers KM, Schupp PJ, Deines P. 2013. Temporal molecular and isotopic analysis of active bacterial communities in two New Zealand sponges. *FEMS Microbiology Ecology* **85(1)**:195–205 DOI 10.1111/1574-6941.12109.

Southwell MW. 2007. Sponge impacts on coral reef nitrogen cycling, Key Largo, Florida. PhD thesis. University of North Carolina, Chapel Hill.

Southwell MW, Popp BN, Martens CS. 2008. Nitrification controls on fluxes and isotopic composition of nitrate from Florida Keys sponges. *Marine Chemistry* **108(1–2)**:96–108 DOI 10.1016/j.marchem.2007.10.005.

Southwell MW, Weisz JB, Martens CS, Lindquist N. 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnology and Oceanography* **53(3)**:986–996 DOI 10.4319/lo.2008.53.3.0986.

Taylor MW, Radax R, Steger D, Wagner M. 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* **71(2)**:295–347 DOI 10.1128/mmbr.00040-06.

Thacker RW, Diaz MC, De Voogd NJ, Van Soest RWM, Freeman CJ, Mobley AS, LaPietra J, Cope K, McKenna S. 2010. Preliminary assessment of sponge biodiversity on Saba Bank, Netherlands Antilles. *PLOS ONE* **5(5)**:e9622 DOI 10.1371/journal.pone.0009622.

Thacker RW, Freeman CJ. 2012. Sponge-microbe symbioses: recent advances and new directions. *Advances in Marine Biology* **62**:57–111 DOI 10.1016/B978-0-12-394283-8.00002-3.

Thibodeau B, Miyajima T, Tayasu I, Wyatt ASJ, Watanabe A, Morimoto N, Yoshimizu C, Nagata T. 2013. Heterogeneous dissolved organic nitrogen supply over a coral reef: first evidence from nitrogen stable isotope ratios. *Coral Reefs* **32(4)**:1103–1110 DOI 10.1007/s00338-013-1070-9.

Van Beek IJM, Meesters EHWG. 2014. Saba Bank Research expedition 2013-Progress Report. Report nr CO86/14. Wageningen: Wageningen University and Research. *Available at http://edepot.wur.nl/305508.*

Vander Zanden MJ, Rasmussen JB. 2001. Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* **46(8)**:2061–2066 DOI 10.4319/lo.2001.46.8.2061.

Van Duyl FC. 2016. Net coral community calcification and hydrodynamics (Saba Bank/Saba). NIOZ-Cruise report 17/24–31 October 2015. *Available at http://www.dcbd.nl/document/nioz-saba-banksaba-cruise-report-net-coral-community-calcification-and-hydrodynamics-saba.*

Van Duyl FC, Gast GJ. 2001. Linkage of small-scale spatial variations in DOC, inorganic nutrients and bacterioplankton growth with different coral reef water types. *Aquatic Microbial Ecology* **24**:17–26 DOI 10.3354/ame024017.

Van Duyl FC, Hegeman J, Hoogstraten A, Maier C. 2008. Dissolved carbon fixation by sponge-microbe consortia of deep water coral mounds in the northeastern Atlantic Ocean. *Marine Ecology Progress Series* **358**:137–150 DOI 10.3354/meps07370.

Van Duyl FC, Moodley L, Nieuwland G, Van Ijzerloo L, Van Soest RWM, Houtekamer M, Meesters EH, Middelburg JJ. 2011. Coral cavity sponges depend on reef-derived food resources: stable isotope and fatty acid constraints. *Marine Biology* **158(7)**:1653–1666 DOI 10.1007/s00227-011-1681-z.

Wafar M, Wafar S, David JJ. 1990. Nitrification in reef corals. *Limnology and Oceanography* **35(3)**:725–730 DOI 10.4319/lo.1990.35.3.0725.

Webb KL, DuPaul WD, Wiebe W, Sottile W, Johannes RE. 1975. Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. *Limnology and Oceanography* **20(2)**:198–210 DOI 10.4319/lo.1975.20.2.0198.

Weisz JB, Hentschel U, Lindquist N, Martens CS. 2007. Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Marine Biology* **152(2)**:475–483 DOI 10.1007/s00227-007-0708-y.

Wilkinson CR. 1983. Net primary productivity in coral reef sponges. *Science* **219(4583)**:410–412 DOI 10.1126/science.219.4583.410.

Wilkinson C, Fay P. 1979. Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. *Nature* **279(5713)**:527–529 DOI 10.1038/279527a0.

Williams PM, Gordon LI. 1970. Carbon-13:Carbon-12 ratios in dissolved and particulate organic matter in the sea. *Deep Sea Research and Oceanographic Abstracts* **17(1)**:19–27 DOI 10.1016/0011-7471(70)90085-9.

Wiltink M. 2016. Changing benthic communities on Saba Bank. Is Saba Bank becoming a 'Sponge reef'? Master Thesis, University of Amsterdam.

Wyatt ASJ, Lowe RJ, Humphries S, Waite AM. 2013. Particulate nutrient fluxes over a fringing coral reef: source-sink dynamics inferred from carbon to nitrogen ratios and stable isotopes. *Limnology and Oceanography* **58(1)**:409–427 DOI 10.4319/lo.2013.58.1.0409.

Yahel G, Sharp JH, Marie D, Hase C, Genin A. 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: bulk DOC is the major source for carbon. *Limnology and Oceanography* **48(1)**:141–149 DOI 10.4319/lo.2003.48.1.0141.

Zuur AF, Hilbe JM, Ieno EN. 2013. *A beginner's guide to GLM and GLMM with R: a frequentist and Bayesian perspective for ecologists.* Newburgh: Highland Statistics Ltd.

Zuur AF, Ieno EN, Saveliev AA. 2017. *Beginner's guide to spatial, temporal and spatial-temporal Ecological data analysis with R-INLA statistics. Vol. I. Using GLM and GLMM.* Newburgh: Highland Statistics Ltd., 357.