Carbon and nitrogen cycling by Red Sea coral reef sponges

A dissertation by

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"Food is brought to them, waste is taken away.
For them in their eternal abyss, with its time-like stream, there is no hurry, there is no return. Such an organism becomes a mere living screen between the used half of the universe and the unused half – a moment of active metabolism between the unknown future and the exhausted past."

Bidder, GP (1923)
The relation of the form of a sponge to its currents.
Q J Microsc Sci 266:293-323

SUMMARY

Sponges are a dominant component of coral reefs where they fulfil a number of important structural and functional roles that make them key ecosystems engineers. In particular, their high filtering capacity and association with diverse microbial communities enables sponges to moderate flows of organic matter and inorganic nutrients, thereby influencing the biogeochemical cycling of carbon (C) and nitrogen (N) on coral reefs (summarized in **Chapter 1**). This cycling of C and N is central to understanding how coral reefs, some of the most productive and diverse ecosystems on Earth, can thrive in such nutrient poor environments. Studying the fluxes of organic matter and nutrients mediated by sponges is therefore essential to understanding coral reef ecosystem functioning. However, many of these fluxes are poorly understood. By investigating the mechanisms and rates at which sponges retain, transform, and transfer organic matter and inorganic nutrients within coral reef ecosystems, this thesis provides new insights into the roles that sponges play in the biogeochemical cycling of C and N on coral reefs.

The first part of the thesis focuses on organic matter cycling by the newly described "sponge loop" in the northern Red Sea. This sponge-mediated pathway for dissolved organic matter (DOM) cycling has major implications for reef ecosystem functioning, but to date has not been investigated on reefs outside of the Caribbean. **Chapter 2** provides the first evidence for the cycling of coral- and algal-derived DOM by coral reef sponges. Importantly, this elucidates a direct trophic link between coral reef benthic primary producers and sponges that enables the large quantities of DOM produced by corals and algae to be transferred to reef fauna that otherwise would not be able to capitalize on this ubiquitous resource. Interestingly, the two DOM sources are processed differently by the sponge-microbe holobiont, with higher uptake and transformation rates for algal- compared to coral-derived DOM. This has direct implications for the magnitude of DOM shunted into the coral reef trophic web by the sponge loop under scenarios of coral reef degradation that lead to phase-shifts from coral to algal dominance. Chapter 2 provides mainly qualitative evidence for the occurrence of a sponge loop in the Red Sea, but does not resolve its quantitative importance to reef ecosystem functioning.

Therefore, in **Chapter 3** an inverse linear trophic food web model is constructed to examine organic C cycling by the water column, benthos, and cryptic cavity sponge community at the ecosystem level. The findings provide quantitative evidence that C cycling by the sponge loop represents a major flux of dissolved and particulate organic carbon (DOC and POC) in the Red Sea. Uptake of DOC by sponges approximates the gross primary production of the entire reef ecosystem, exceeding recycling via the microbial loop. This demonstrates that sponges play a quantitatively important role in the retention and cycling of organic C on oligotrophic warm-water coral reefs.

Coral reefs, however, not only flourish in the warm, shallow, oligotrophic waters of the tropics, but are also found in the cold, deep, and nutrient-rich waters of the deep-sea. **Chapter 4**, therefore, compares the functioning of the sponge loop on a north Atlantic cold-water (CW) coral reef and a warm-water (WW) reef in the Red Sea. Despite vast environmental differences in the two reef ecosystems, both the WW and CW sponges assimilate and transform coral mucus into particulate detritus at remarkably similar rates. The discovery of a sponge loop also on CW reefs, suggests it may be a ubiquitous feature of coral reefs that, by retaining and cycling coral-derived organic matter to higher trophic levels, contributes to the high capacity for biogeochemical element cycling in these N-limited (WW) and C-depleted (CW) ecosystems. However, due to ambient differences in C and N availability on WW and CW reefs, the key function of the sponge loop may differ, with N-cycling on WW and C-cycling on CW reefs disproportionately contributing to ecosystem functioning.

The role of sponge detritus in sponge N cycling is further examined in **Chapter 5** by comparing fluxes of dissolved inorganic nitrogen (DIN), particulate organic nitrogen (PON), and the generation of new N via N_2 fixation by six Red Sea sponge species. Findings reveal that DIN release represents the largest flux of N mediated by sponges with community efflux rates potentially supplying up to 17% of the N required for net primary productivity of the entire reef. Release of PON via the production of sponge detritus accounts for approximately 30 ± 3% of the total sponge N released, providing the first measurements of PON release by reef sponges. The high release of DIN and PON leads to an imbalance in the sponge N budgets that cannot be accounted for by the low rates of N_2 fixation. Dietary stable isotope analysis indicates this missing N is supplied via DON uptake, suggesting these sponges rely on DON uptake to meet their N demands. This highlights the functional importance of sponge DOM uptake at the organism level, complementing the ecosystem processes described in the previous chapters.

Finally, **Chapter 6** addresses the paucity of knowledge regarding potential linkages between C and N cycling by examining the potential for N_2 fixation to support primary productivity (C fixation) in a coral reef sponge and two other dominant reef framework substrates; turf algae and coral rock. The results show that N_2 fixation can provide up to 27% of the N demand for net primary productivity in turf algae and coral rock, while, in contrast, there is no relationship between N_2 fixation and primary productivity in the more heterotrophic sponge. This demonstrates the potential for N_2 fixation to be a key mechanism sustaining primary productivity in coral reef substrates displaying net photosynthesis, and further highlights how interactions between C and N cycling contribute to sustaining the high rates of gross primary productivity characteristic of coral reef ecosystems despite their oligotrophic environments.

Collectively, the findings of this thesis show that coral reef sponges play a critical role in the biogeochemical cycling of C and N on coral reefs by contributing to the efficient retention, transformation and recycling of both organic matter and inorganic nutrients that enables these diverse, productive and unique ecosystems to thrive in the equivalent of a marine desert.

ZUSAMMENFASSUNG

Schwämme stellen eine Hauptkomponente der Biozönose in Korallenriffen dar und übernehmen dort als Ökosystemingenieure eine Reihe von essentiellen, strukturellen und funktionellen Aufgaben. Diese werden insbesondere durch ihr hohes Filtrationsvermögen und ihre Vergesellschaftung mit vielfältigen Mikrobengemeinschaften ermöglicht. Aufgrund dieser Eigenschaften sind Schwämme in der Lage, Stoffflüsse von organischem Material und anorganischen Nährstoffen zu beeinflussen, und somit die biogeochemischen Kohlenstoff (C) und Stickstoff (N) Kreisläufe im Korallenriff mitzusteuern (**Kapitel 1**). Diese Stoffkreisläufe von C und N spielen eine zentrale Rolle in der Erklärung warum Korallenriffe, trotz der sie umgebenden oligotrophen (nährstoffarmen) Umweltbedingungen, zu den produktivsten und artenreichsten Ökosystemen auf diesem Planeten zählen. Um die Funktionsweise von Korallenriffökosystemen besser zu verstehen, ist es daher entscheidend, die von Schwämmen regulierten Stoffflüsse von organischem Material und anorganischen Nährstoffen zu untersuchen, denn bislang sind die meisten dieser Flüsse noch unerforscht. In dieser Dissertation werden daher Funktionen von Schwämmen untersucht, welche zur Retention, Umwandlung und zum Recycling von organischem Material und anorganischen Nährstoffen im Korallenriff beitragen. Durch diese Studien werden wichtige neue Erkenntnisse zur Rolle von Schwämmen in biogeochemischen C und N Kreisläufen des Riffökosystems erlangt.

Der erste Teil dieser Arbeit beschäftigt sich mit dem Kreislauf von organischem Material in einem Korallenriff des nördlichen Roten Meeres im Kontext des kürzlich beschriebenen „Sponge Loop“. Diese von Schwämmen induzierte Umwandlung von gelöstem (DOM) zu partikulärem organischen Material (POM) nimmt womöglich eine funktionelle Schlüsselrolle in Riffökosystemen ein, wurde aber bislang nur in karibischen Riffen untersucht. DOM stellt eine allgegenwärtige Ressource von organischem Material in Korallenriffen dar, die aber physiologisch vom Grossteil der Rifffauna nicht genutzt werden kann. **Kapitel 2** behandelt den ersten Nachweis für Stoffkreisläufe von durch Korallen und Algen produziertem DOM in Schwämmen des Roten Meeres. Von entscheidender Bedeutung ist hier das Aufzeigen einer direkten trophischen Verbindung zwischen benthischen Primärproduzenten (Korallen und Algen) und Schwämmen im Riff, durch die eine große Menge des von Korallen und Algen produzierten DOM der Rifffauna als POM Nahrungsquelle zugänglich gemacht wird. Interessanterweise werden diese zwei DOM Quellen vom Schwamm-Holobiont (Gemeinschaft aus Schwamm und Mikroben) unterschiedlich erschlossen, wobei Algen DOM im Vergleich zu Korallen DOM verstärkt aufgenommen und in POM umgewandelt wird. Die Menge an DOM, welche über den „Sponge Loop“ in biogeochemische C und N Kreisläufe des Korallenriffs eingebracht wird, kann dadurch beeinflusst werden. Dies gilt besonders im Hinblick auf strukturelle Veränderungen im Riffbenthos, die oft eine Verschiebung der Dominanz von Korallen zu Algen mit sich bringen. Kapitel 2 behandelt somit vorrangig den qualitativen Nachweis für das

Vorhandensein des „Sponge Loop“ im Roten Meer, klärt jedoch nicht dessen quantitative Bedeutung für die Funktionsweise des Ökosystems Korallenriff auf.

Daher werden in **Kapitel 3** C Stoffkreisläufe auf Ökosystemebene, in der Wassersäule, im Riffbenthos und durch kryptische Riffschwämme anhand eines inversen linearen Modells untersucht. Die Modell-Ergebnisse liefern einen quantitativen Nachweis dafür, dass C Kreisläufe mittels des „Sponge Loop“ eine Schlüsselfunktion in Stoffflüssen von gelöstem und partikulärem organischen C (DOC und POC) in Korallenriffen des Roten Meeres darstellen. Die DOC Aufnahme durch Schwämme entspricht dabei in etwa der Bruttoprimärproduktion des gesamten Riffökosystems und übertrifft dabei das C Recycling des etablierten „Microbial Loop“. Diese Vergleiche verdeutlichen dabei anschaulich die Schlüsselfunktion der Schwämme in biogeochemischen Kreisläufen von organischem Material in oligotrophen Warmwasserkorallenriffen.

Korallenriffe gedeihen jedoch nicht nur in den warmen, seichten und nährstoffarmen Gewässern der tropischen Meere, sondern auch in den kalten und nährstoffreichen Gewässern der Tiefsee. **Kapitel 4** vergleicht daher die Funktionsweise des „Sponge Loop“ in einem Warmwasser- (WW) Korallenriff des Roten Meeres mit einem Kaltwasser- (KW) Korallenriff des Nordatlantik. Erstaunlicherweise zeigen trotz enorm unterschiedlicher Umweltbedingungen die Raten für die Aufnahme und Umwandlung von durch WW und KW Korallen produzierten organischen Materials in partikulären WW und KW Schwammdetritus ähnliche Werte. Damit wird der „Sponge Loop“ hier erstmals auch für Schwämme aus Kaltwasserkorallenriffen nachgewiesen, und könnte somit eine universelle Funktion in Korallenriffen darstellen. Indem das von Korallen produzierte organische Material höheren trophischen Ebenen zugänglich gemacht wird, trägt der „Sponge Loop“ wesentlich zur Kapazität biogeochemischer C und N Kreisläufe in N-limitierten (WW) und C-armen (KW) Riffökosystemen bei. Aufgrund der unterschiedlichen Limitierung von N (WW) und C (KW) in diesen gegensätzlichen Riffökosystemen, könnte die Funktion des „Sponge Loop“ variieren. Somit wäre der Beitrag zum N-Kreislauf in WW Riffen und der Beitrag zum C-Kreislauf in KW Riffen entsprechend höher.

Die Rolle des von Schwämmen produzierten Detritus im N-Kreislauf von Schwämmen wird weiterführend in **Kapitel 5** behandelt. Hier werden die Stoffflüsse von gelöstem anorganischen Stickstoff (DIN), partikulärem organischen Stickstoff (PON) und der Eintrag von neuem N durch N_2 Fixierung an sechs verschiedenen Schwammarten des Roten Meeres miteinander verglichen. Die Ergebnisse zeigen, dass die Abgabe von DIN den bedeutendsten durch Schwämme vermittelten N-Stofffluss repräsentiert, und dass dieser DIN-Fluss bis zu 17% des N Bedarfs der Nettoprimärproduktion auf Riffökosystemebene entspricht. Die Abgabe von PON als Schwammdetritus, die hier erstmal quantifiziert wurde, kommt dabei ca. 30 ± 3% der gesamten N Abgabe von Schwämmen gleich. Diese hohen DIN und PON Stoffflüsse bewirken ein Ungleichgewicht im Schwamm N Haushalt, welches durch die wiederum niedrigen N_2 Fixierungsraten nicht ausgeglichen werden kann. Analysen der stabilen Isotope der

Schwammnahrung zeigen, dass dieses N Defizit durch die Aufnahme von DON kompensiert wird, und dass Schwämme anscheinend ihren N Bedarf über die Aufnahme von DON decken können. Dies bestätigt die entscheidende Funktion der DOM Aufnahme für Schwämme auf Organismenebene, und ergänzt damit die in den vorhergehenden Kapiteln beschriebenen Prozesse auf Riffökosystemebene.

Abschliessend behandelt **Kapitel 6** die potentielle Kopplung von C und N Fixierung auf Organismenebene und untersucht dabei inwieweit der Prozess der Primärproduktion (C Fixierung) in einem Schwamm und zwei weiteren Riffsubstraten (Aufwuchsalgen und Korallengestein) durch N_2 Fixierung unterstützt werden kann. Die Ergebnisse zeigen, dass N_2 Fixierung bis zu 27% des N Bedarfs der Nettoprimärproduktion von Aufwuchsalgen und Korallengestein decken kann, dennoch nicht den N Bedarf des heterotrophen Schwamms. Die N_2 Fixierung stellt also einen potentiellen Schlüsselprozess für die Aufrechterhaltung der Nettoprimärproduktion von Korallenriffsubstraten dar. Darüber hinaus zeigt sich, dass die Kopplung von C und N_2 Fixierungsprozessen entscheidend zur für oligotrophe Korallenriffökosysteme typischen hohen Bruttoprimärproduktion beiträgt.

Zusammenfassend zeigt diese Arbeit, dass Schwämme in Korallenriffen eine entscheidende Rolle in biogeochemischen C und N Kreisläufen einnehmen, indem sie effizient zur Retention, zur Umwandlung und zum Recycling von organischem Material und anorganischen Nährstoffen beitragen, und damit diese artenreichen, produktiven und einzigartigen Ökosysteme in die Lage versetzen, in einer marinen Nährstoffwüste zu überleben.

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ABBREVIATIONS

$\delta^{13}C$	Expression of the C stable isotope ratio in delta notation calculated relative to the Vienna Pee Dee Belemnite standard as: $\delta^{13}C = (R_{sample}/R_{reference} - 1) \times 1000$, where $R_{sample} = {}^{13}C/{}^{12}C$ and $R_{reference} = 0.01118$
$\delta^{15}N$	Expression of the N stable isotope ratio in delta notation calculated relative to atmospheric N as: $\delta^{15}N = (R_{sample}/R_{reference} - 1) \times 1000$, where $R_{reference} = {}^{15}N/{}^{14}N$ and $R_{reference} = 0.00368$
‰	Parts per mille, the units in which δ values are expressed
BC	Bacterioplankton
C	Carbon
Chl *a*	Chlorophyll *a*
CPC	Cavity particulate organic carbon
CW	Cold-water
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DOM	Dissolved organic matter
FA	Fatty acid
GPP	Gross primary production
HMA	High microbial abundance, referring to sponge species containing dense microbial communities
LIM	Linear inverse food web model
LMA	Low microbial abundance, referring to sponge species containing associated microbial communities with low densities
N	Nitrogen
N_2	Dinitrogen
NCP	Net community production
NH_4^+	Ammonium

NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x^-	Nitrogen oxides (nitrate + nitrite)
O_2	Oxygen
OM	Organic matter
P	Phosphorous
PAR	Photosynthetically active radiation (wavelength 400 – 700 nm)
P_{gross}	Gross photosynthesis
P_{net}	Net photosynthesis
PO_4^{3-}	Phosphate
PLFA	Phospholipid-derived fatty acid
PN	Particulate nitrogen
POC	Particulate organic carbon
POM	Particulate organic matter
PON	Particulate organic nitrogen
PDC	Pelagic dissolved organic carbon
PPC	Pelagic particulate organic carbon
PQ	Photosynthetic quotient
PR	Ratio of gross primary production to respiration
R	Respiration
RQ	Respiratory quotient
SDC	Sediment dissolved organic carbon
SPC	Sediment particulate organic carbon
SPOM	Suspended particulate organic matter
TOC	Total organic carbon
TON	Total organic nitrogen
WW	Warm-water

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CHAPTER 1

GENERAL INTRODUCTION

Coral reefs: Oases in a marine desert

Tropical coral reefs are among the most biologically diverse (Jackson 1991, Sebens 1994, Knowlton et al. 2010) and productive (Odum & Odum 1955, Kinsey 1985, Crossland et al. 1991) ecosystems on Earth. Coral reef gross primary production (GPP) ranges from 100 – 2000 mmol C m^{-2} d^{-1}, far exceeding that of the surrounding plankton-dominated open water and rivaling that of the most productive terrestrial ecosystems (Atkinson 2011). High productivity in marine environments is typically limited to areas of high nutrient concentration, such as coastal upwelling regions, yet coral reefs are typically found in low nutrient (i.e. oligotrophic) tropical waters (Webb et al. 1975, Crossland & Barnes 1983, Kleypas et al. 1999, Atkinson & Falter 2003). This paradox has long generated scientific interest in coral reef nutrient cycling and remains a fundamental question to understanding the complexities of coral reef ecosystem functioning.

Scleractinian corals are the foundation upon which the reef ecosystem is built. They form the reef framework that the rest of the community calls home and supply the energy that sustains the community. Their high primary productivity is made possible by their association with photosynthetic algae of the genus *Symbiodiniun* (i.e. zooxanthellae) that provide the majority of the energetic requirements for the coral host (Muscatine & Porter 1977, Muscatine et al. 1984). The carbon (C) fixed by corals and other reef primary producers is the main source of C supporting the heterotrophic community and thereby places constraints on community biomass and secondary production (Szmant-Froelich 1983, Hatcher 1990). While coral reef GPP is high, community respiration (R) is equally high, such that the net production of the ecosystem (GPP – R) tends to approximate zero (Hatcher 1990, Crossland et al. 1991, Gattuso et al. 1996). There is then little net gain of organic matter within the system, indicating most of the C fixed by coral reef primary producers is consumed within the reef and implying rapid recycling and remineralization of organic matter (Kinsey 1985, Hatcher 1988, Crossland et al. 1991). This tight recycling between the autotrophic and heterotrophic reef compartments plays an essential role in retaining the nutrients required to support the demands of high reef GPP (Muscatine & Porter 1977, Wild et al. 2004, de Goeij et al. 2013). Nevertheless, new nutrients, particularly nitrogen (N) and phosphate (P), are required to sustain net production and growth. The demand for P can be met via uptake from the surrounding water despite its low concentrations, as the volume of water flowing over the reef is high (Atkinson & Falter 2003, Falter et al. 2004). However, the uptake of N is insufficient to meet community demands, making it the key limiting nutrient for primary productivity. The demand for additional N can be met by the capture of allochthonous dissolved and particulate organic matter as well as the generation of new N via dinitrogen (N_2) fixation (Falter et al. 2004, Atkinson 2011). Due to its limiting availability, the biogeochemical cycling of N influences the dominant metabolic processes on the reef: photosynthesis, calcification, and respiration - thereby moderating the flow of C, the reef's energetic

currency. Understanding the relationship between C and N cycling on coral reefs is, therefore, central to understanding the functioning of these diverse, complex, and threatened ecosystems.

Carbon and nitrogen on coral reefs

Much of the C and N on coral reefs is bound in organic matter, and the flow of this matter plays a key role in coral reef trophodynamics (Alongi 1988, Hansen et al. 1992). Organic C in seawater is found in two fractions; dissolved organic carbon (DOC) and particulate organic carbon (POC). DOC is operationally defined as the fraction that passes through a fine filter, typically a GF/F filter with a pore size of ~ 0.7 μm, but represents a heterogeneous mixture of both small colloidal compounds and truly dissolved material (Nebbioso & Piccolo 2013, Carlson & Hansell 2015). POC in oligotrophic coral reef waters is dominated by picoplankton, including eukaryotic phytoplankton (picoeukaryotes) as well as photoautotrophic and heterotrophic bacterioplantkon (Ferrier-Pagès & Gattuso 1998, Charpy & Blanchot 1999, Ferrier-Pagès & Furla 2001). POC is readily incorporated into the food chain primarily by benthic filter feeders (Gili & Coma 1998, Ribes et al. 2003) and planktivorous fish (Hamner et al. 1988, Pinnegar & Polunin 2006, Hamner et al. 2007), and many studies show coral reefs are a sink for POC (Ayukai 1995, Yahel et al. 1998, Richter et al. 2001, Houlbreque et al. 2006, Wyatt et al. 2010, Patten et al. 2011). However, the POC pool accounts for only a fraction (<10%) of the total organic carbon (TOC) pool, while the dissolved pool accounts for the remaining >90%, and therefore represents the largest pool of organic C in the oceans (Carlson & Hansell 2015). Despite its ubiquity, DOC is largely unavailable to most heterotrophic reef fauna. Microbes are the primary consumers of reactive DOC in the ocean (Fenchel 2008, Worden et al. 2015), and via the microbial loop, they mediate the cycling of DOC back into the marine food web through the assimilation of biomass (Azam et al. 1983). Only a small fraction of the DOC in seawater is labile and readily available for uptake and degradation (Hansell & Carlson 1998, Carlson & Hansell 2015), but this labile fraction is higher on coral reefs than the surrounding ocean due to high rates of DOC release by coral reef benthic primary producers (Dinsdale et al. 2008, Tanaka et al. 2011). DOC has been found to influence the metabolism and composition of reef microbial communities, which ultimately affects coral health (Kline et al. 2006, Haas et al. 2013, Nelson et al. 2013). Thus, there is growing evidence that DOC plays a more widespread role in coral reef health, trophodynamics, and biogeochemical cycling than previously recognized (Smith et al. 2006, Barott & Rohwer 2012, de Goeij et al. 2013). However, concentrations of DOC, rates of DOC metabolism, and DOC cycling at the community and ecosystem level are still poorly understood.

Nitrogen is an essential building block for life and yet most N in the oceans (>95%) is bound in the form of N_2, and therefore largely inaccessible for most marine organisms (Voss et al. 2013). Only specialized N-fixing microbes, termed diazotrophs, are capable of breaking its strong triple bond and the N they fix plays an essential role in supplying reefs with new N (Wiebe et al. 1975,

Larkum et al. 1988, O'Neil & Capone 1989, Charpy et al. 2007, Bednarz et al. 2015). Most bioavailable N on coral reefs is bound into dissolved organic nitrogen (DON) and to a lesser extent particulate organic nitrogen (PON), while concentrations of dissolved inorganic nitrogen (DIN) are typically extremely low (Kleypas et al. 1999, Atkinson 2011). Nitrogen cycling on coral reefs, therefore, involves the fixation of inorganic N into organic N for use by reef heterotrophs and the remineralization of organic N back into the inorganic forms that fuel reef primary production. This N cycling is strongly mediated by processes including nitrogen fixation, nitrification and denitrification (Zehr & Ward 2002, Gruber et al. 2008, Hewson et al. 2008) that are carried out exclusively by microbes and the animals that host them (Fiore et al. 2010).

Sponges

Sponges (phylum Porifera) represent the oldest extant animal phylum (Wörheide et al. 2012). These early metazoans have been immensely successful, evolving to form a highly diverse group of more than 8000 species (Cardenas et al. 2012) that occupy marine and freshwater benthic habitats from the tropics to the poles (Dayton 1989, Diaz 2005, McClintock et al. 2005, Bell 2008). On coral reefs, sponge biomass and diversity can exceed that of reef-building corals (Diaz & Rutzler 2001, Richter et al. 2001, Lesser et al. 2009, McMurray et al. 2010), and sponge abundances have been increasing on many coral reefs worldwide (McMurray et al. 2010, Bell et al. 2013). Sponges are a particularly important component of the cryptic reef habitat; the hidden cavities and crevices that account for over two-thirds of the total reef volume and exceed the surface area of the exposed reef by a factor of eight (Jackson et al. 1971, Ginsberg 1983, Richter et al. 2001, Scheffers et al. 2004). Here, sponges dominate the thin living layer of primarily encrusting organisms that cover more than 90% of the available hard substratum (Buss & Jackson 1979, Richter et al. 2001, Wunsch et al. 2000).

Sponges perform a variety of important functional roles such as habitat provision, reef cementation and bio-erosion that make them key coral reef ecosystem engineers (Diaz & Rutzler 2001, Wulff 2001, Bell 2008). However, perhaps most importantly is their ability to influence C and N biogeochemical cycling through the mediation of flows of organic matter and inorganic nutrients (Maldonado et al. 2012). Unlike other metazoans, sponges lack organ systems and instead are sessile filter feeders that rely on a constant flow of water through their highly vascularized internal canals in order to obtain food and oxygen and to remove metabolic end products. Sponges pump vast quantities of water through their bodies per day, up to 50,000 times their own volume (Reiswig 1971, 1974, Weisz et al. 2008), and it is this exceptional capacity for processing water that allows them to influence the availability and composition of C and N in the water column.

Microbial symbionts in sponges

Sponges host a remarkable diversity of inter- and intracellular symbionts, including photosynthetic and heterotrophic bacteria, archaea, fungi, and unicellular algae that together form the sponge holobiont. Microbes can account for up to 35% of the sponge biomass (Vacelet 1975, Wilkinson 1978, Hentschel et al. 2006), representing at least 32 bacterial and archaeal phyla ranging from generalists found in the surrounding seawater to specialists found only in sponges (Taylor et al. 2007, Schmitt et al. 2012, Simister et al. 2012, Webster & Taylor 2012, Taylor et al. 2013). Sponges can be classified based on their microbial abundances. High-microbial abundance (HMA) sponges contain 10^6-10^8 bacteria per gram of sponge wet weight, exceeding the microbial abundance of seawater by 2-4 orders of magnitude, while low-microbial abundance (LMA) sponges have microbial abundances in the range of the seawater, between 10^5-10^6 bacteria per gram of sponge wet weight (Hentschel et al. 2006, Gloeckner et al. 2014). These microbial symbionts equip the sponge host with a variety of complex metabolic pathways, including C and N fixation (Taylor et al. 2007), thereby contributing to the ability of the sponge holobiont to transform C and N.

Sponge and benthic-pelagic coupling

Sponges are exceptionally efficient suspension feeders that can filter particles varying in size from small virioplankton (Hadas et al. 2006) up to larger zooplankton (Vacelet & Bouryesnault 1995), but they are most efficient at removing small particles in the size range of 0.2 to 2.0 μm (Pile et al. 1997, Ribes et al. 1999, Kötter & Pernthaler 2002). Retention rates for nano- and picoplankton can reach up to 99% (Pile et al. 1997, Ribes et al. 1999, Hadas et al. 2009), and these are also the most abundant plankton types available in coral reef waters (Ferrier-Pagès & Gattuso 1998, Gast et al. 1998, Charpy & Blanchot 1999, Ferrier-Pagès & Furla 2001). Due to their high biomass and large filtering capacity, sponges play an important role in benthic-pelagic coupling by mediating the flow of pelagic POM to the benthos (Gili & Coma 1998, Lesser 2006, Pile & Young 2006, Perea-Blazquez et al. 2012a). This influx of POM represents a significant flow of C and N to the benthos (Richter et al. 2001, Ribes et al. 2003, 2005). Nevertheless, the majority of organic matter in coral reef waters is found in dissolved form, and sponges have also recently been found to play a major role in the cycling of dissolved organic matter (DOM) on coral reefs via the so-called "sponge loop" (de Goeij et al. 2013).

The "Sponge Loop"

While sponges are traditionally considered particle feeders (Reiswig 1971, Pile et al. 1997), it has recently been discovered that many sponges also take up DOM (Yahel et al. 2003, de Goeij et al.

2008, van Duyl et al. 2008, Ribes et al. 2012, Mueller et al. 2014a). For the coral reef sponges investigated, DOM accounts for the majority (>90%) of their total diet (Yahel et al. 2003, de Goeij et al. 2008, Mueller et al. 2014a), suggesting these sponges are actually "DOM feeders". In addition to taking up DOM, sponges produce large quantities of detritus (POM) via massive cell shedding of their filtering (choanocyte) cells. These choanocyte cells have the shortest cell cycle of any known animal and as a result are rapidly turned over and shed by the sponge (de Goeij et al. 2009, Alexander et al. 2014, Alexander et al. 2015, Maldonado 2015). Thus, sponges take up DOM and transform into POM, effectively turning over up to 35% of their body C per day (de Goeij et al. 2013). Sponge detritus is then fed on by motile and filter-feeding detritivores, enabling the energy stored in DOM that is otherwise unavailable to most reef heterotrophs to be utilized by higher trophic levels (de Goeij et al. 2013). This "sponge loop" thereby functions in a way analogous to the established microbial loop (Azam et al. 1983). DOM uptake by cryptic sponges in the Caribbean is estimated to approach the same order of magnitude as the total GPP of the entire reef ecosystem (de Goeij & van Duyl 2007, de Goeij et al. 2013). The sponge loop, therefore, is suspected to play a major role in the retention and cycling of nutrients and organic matter on Caribbean reefs but has not yet been investigated in other oceanic regions. Sponges are a ubiquitous component of coral reef ecosystems worldwide from the tropics to the cold-water reefs of the deep-sea suggesting the potential for this process to be widespread. Moreover, to date the sponge loop has only been demonstrated using laboratory-produced diatom DOM, which may not be representative of the DOM naturally produced on the reef. DOM uptake by cavity sponges is orders of magnitude higher than the supply of pelagic primary production to reefs (de Goeij & van Duyl 2007), indicating that pelagic primary production is unable to support the carbon requirements of cavity sponges. Further, stable isotope analysis indicates a substantial portion of the sponge diet originates from organic matter produced on the reef by corals and crustose coralline algae (van Duyl et al. 2011), suggesting a direct trophic link between sponges and coral reef benthic primary producers.

Linking sponges and coral reef benthic primary producers

Many aquatic primary producers release a fraction of their excess photosynthates into the water column as DOM (Khailov & Burlakov 1969, Moebus & Johnson 1974, Mague et al. 1980, Zlotnik & Dubinsky 1989, Brocke et al. 2015). Scleractinian corals and benthic algae are the dominant primary producers on coral reefs (Hatcher 1990) and, through high organic matter release, contribute substantially to the labile DOM that may potentially fuel the sponge loop (Crossland 1987, Ferrier-Pagès et al. 1998, Haas et al. 2010b, Naumann et al. 2010). Thus, a direct trophic link may exist between benthic primary producers and reef sponges, but the uptake of coral- or macroalgal-derived DOM by reef sponges has not been demonstrated. On coral-dominated reefs, coral mucus can dominate the water column organic matter pool, with up to 80% of the released mucus dissolving in

the surrounding reef water (Johannes 1967, Marshall 1968, Wild et al. 2004). However, coral reef degradation is leading to decreased hard coral cover, frequently in combination with increases in macro- and turf algae abundances, on many reefs worldwide (Done 1992, Hughes 1994, McCook et al. 2001, Hughes et al. 2007, Sandin et al. 2008). Macroalgae typically release larger quantities of DOM compared to corals (Haas et al. 2010a, Haas et al. 2011, Haas et al. 2013, Mueller et al. 2014b), which release organic matter primarily as coral mucus with a comparatively larger particulate fraction (Naumann et al. 2010, Wild et al. 2010b). Further, the quality and composition of coral- and algal-derived DOM differs (Haas & Wild 2010, Wild et al. 2010a, Nelson et al. 2013). Algal-derived DOM promotes more rapid microbial growth and respiration in the water column and reef sediments (Wild et al. 2010b, Haas et al. 2011), leading to localized anoxia and the proliferation of pathogenic bacteria (Nelson et al. 2013). As a result, algal-exudates can have negative consequences for coral health (Kline et al. 2006, Smith et al. 2006, Barott & Rohwer 2012, Gregg et al. 2013). Changes in the relative benthic cover of corals and algae can, therefore, alter the quality and quantity of organic matter on coral reefs, which may affect biogeochemical cycles and overall ecosystem functioning (Wild et al. 2011). However, the potential impacts of different DOM sources on the functioning of the sponge loop are still unknown.

Biogeochemical N cycling processes hosted by sponges

The remineralization of ingested POM is an important metabolic pathway for all marine sponges, leading to the excretion of ammonia and phosphate (Maldonado et al. 2012, Perea-Blazquez et al. 2012b, Ribes et al. 2012). Additionally, due to their diverse microbial symbionts, the microbial-mediated processes of N_2 fixation, nitrification, denitrification, and ANAMMOX (anaerobic ammonium oxidation) are all known to occur in marine sponges; further contributing to their role as significant players in coral reef N cycling. Coral reef sponges are a major source of nitrate via nitrification, the biological conversion of ammonia and nitrite to nitrate (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008a). Sponge-mediated nitrate fluxes in the Caribbean are high, reaching up to 15 mmol m^{-2} d^{-1} and exceeding the highest benthic nitrification rates from sediments (Diaz & Ward 1997, Southwell et al. 2008b). Nitrification appears to be a particularly common feature of HMA sponges (Jimenez & Ribes 2007, Southwell et al. 2008b, Ribes et al. 2012). Some marine sponges also host the opposing processes of denitrification or ANAMMOX (Hoffmann et al. 2009, Schläppy et al. 2010), but these processes have not yet been confirmed in tropical coral reef sponges. In addition to recycling N, sponges may also be a source of new N via dinitrogen (N_2) fixation. Many sponges are known to host microbes capable of fixing N_2 (Mohamed et al. 2008, Fiore et al. 2015, Ribes et al. 2015), suggesting this process may be widespread, particularly on coral reefs where low nitrate and ammonium concentrations may favour the energetically costly process of N_2 fixation. However, measurements of N_2 fixation rates in sponges remain scarce (Wilkinson & Fay

1979, Shashar et al. 1994, Shieh & Lin 1994, Wilkinson et al. 1999). The discovery of rapid cell shedding and detritus production in sponges offers a potential new N flux – the production of PON. Yet, detritus production in sponges has not been quantified. N cycling in marine sponges is thus complex, representing a variety of metabolic pathways conducted by the sponge host and a diverse array of microbes (Hentschel et al. 2006, Taylor et al. 2007, Hoffmann et al. 2009). As sponges are a significant source of N on coral reefs (Diaz & Ward 1997, Southwell et al. 2008b), quantifying these fluxes and understanding sponge N metabolism is important for understanding the availability of N on coral reefs.

Research Gaps

As scleractinian corals are the primary ecosystem engineers on coral reefs, it is not surprising that coral reef research has focused disproportionately on the corals themselves. However, given their widespread distribution, high biomass, and potential to exert major influence on the overall functioning of the ecosystems they occupy, sponges are key ecosystem engineers in their own right. Nevertheless, they remain an understudied component of coral reefs (Wulff 2001, Bell 2008, Wulff 2012). Despite recent advances in knowledge on sponge C and N metabolism, more research is required to fully understand the role of sponges in coral reef C and N cycling, particularly regarding the newly described sponge loop. Many sponge feeding studies fail to consider DOM as a potential food source (Koopmans et al. 2011, Perea-Blazquez et al. 2013), even when evaluating the role of food limitation in governing sponge populations (Lesser & Slattery 2013). Coral mucus and algal exudates represent the largest source of labile DOM available for sponge consumption, and yet the uptake of these DOM sources has not been investigated. The role of sponge-associated microbes in DOM uptake as well as the exchange of C and N between sponges and their symbionts is poorly understood (Taylor et al. 2007, Webster & Blackall 2009, Thacker & Freeman 2012). The mechanisms by which sponges produce detritus and the role of this detritus in reef trophodynamics are only recently beginning to be unraveled (de Goeij et al. 2009, 2013; Alexander et al. 2014, 2015; Maldonado et al. 2015). Only one study has attempted to quantify sponge detritus production but considered exclusively bulk detritus, thus the labile organic fraction (POC and PON) remains unknown (Alexander et al. 2014). Detrital PON production may contribute to the complex N cycling exhibited by sponges but has not previously been considered. Additionally, the quantification of other aspects of coral reef sponge N cycling such as nitrification and N_2 fixation remain scarce (Corredor et al. 1988, Diaz & Ward 1997, Southland 2007, 2008a). Further, the contribution of sponges to C and N cycling at the community and ecosystem is still relatively unknown. The sponge loop may represent a major flux of C and N on coral reefs, but it has not been investigated or quantified outside of the Caribbean (de Goeij et al. 2013). Given the abundance of sponges in other aquatic ecosystems, including temperate and cold-water deep-sea coral reefs, there is high potential for the sponge loop to

function in ecosystems other than tropical coral reefs. Coral reefs are increasingly under threat from a multitude of anthropogenic stressors that are altering coral reef community structure and impairing the ability of coral reefs to maintain their ecosystem functions and services, upon which millions of people worldwide depend (Moberg & Folke 1999). Understanding how coral reefs will respond to these impacts requires comprehensive understanding of the key factors that control reef productivity and metabolism, such as organic matter and inorganic nutrient cycling, and this cannot be accomplished without improved understanding of the role sponges play in coral reef biogeochemical cycles and trophic webs.

AIMS AND SPECIFIC RESEARCH QUESTIONS:

This thesis aims to address the above mentioned research gaps by studying the role of sponges in C and N flows on coral reefs. The overall goal is to further our understanding of the biogeochemical organic matter and nutrient cycles within coral reef ecosystems. In order to achieve this, the following specific questions were addressed:

1. Is there evidence for a "sponge loop" functioning in organic matter cycling on coral reefs in oceanic regions other than the Caribbean Sea? Are there differences in the functioning of the sponge loop between shallow, warm-water and deep-sea, cold-water coral reef ecosystems?

2. Is there a direct trophic link between the key benthic primary producers (corals and algae) and the sponge community on coral reefs? How do different DOM sources influence the uptake and transformation of organic matter by reef sponges?

3. What role do sponge-associated microbes play in the uptake of reef-derived DOM by the sponge holobiont? Are there differences in the processing of DOM by high- and low microbial abundance sponge species?

4. How important is organic C cycling by the sponge loop at the ecosystem level?

5. How do PON production and N_2 fixation quantitatively compare to other sponge-mediated N fluxes, and how important are these N fluxes at the ecosystem level?

6. Does N_2 fixation support C fixation (primary productivity) in sponges and other key reef framework substrates?

THESIS OUTLINE

This thesis consists of a general introduction (Chapter 1), five chapters presenting the core research of the PhD, and a general discussion of the key findings (Chapter 7). Each chapter is intended for publication as an independent research article. Additional publications that were completed during the PhD, and are related to the work presented in thesis, but not included in it, are listed below and the abstracts presented at the end of the thesis.

Chapter 2:

Coral reef sponges transfer coral- and algal-derived dissolved organic matter (DOM) as particulate organic matter (POM) to higher trophic levels

Rix L, Wild C, de Goeij JM, van Oevelen D, Struck U, Al-Horani FA, Naumann MS

This chapter experimentally investigates the functioning of a sponge loop in the Red Sea using naturally produced sources of coral reef dissolved organic matter: coral and algal exudates. The aim is to demonstrate a direct trophic link between coral reef benthic primary producers and the primarily heterotrophic sponge community and elucidate a novel pathway by which the DOM produced on the reef can be transferred to higher trophic levels. Stable isotope pulse-chase experiments were conducted to demonstrate the uptake of coral- and algal-derived DOM and its subsequent transformation in sponge detritus (POM) by three encrusting sponge species. Additional longer-term isotope tracer experiments using flow-through set-ups were used to show the uptake of sponge detritus by two sponge-associated detritivores. Lastly, we compare how DOM produced by different coral reef primary producers (corals and algae) is processed by reef sponges and generate hypotheses about how shifts in coral reef benthic communities may impact organic matter cycling by the sponge loop.

This study was initiated by L. Rix, C. Wild, J. M. de Goeij, and M. S. Naumann. Sampling and experimental work was conducted by L. Rix with support from M. S. Naumann. U. Stuck analyzed the samples. F. A. Al-Horani provided field work support. L. Rix analyzed the data and wrote the manuscript with input from all authors. This chapter is in preparation for *Functional Ecology*.

Chapter 3:

Ecosystem-engineered seasonal carbon cycling in a Red Sea coral reef

van Hoytema N, van Oevelen D, **Rix L**, Cardini U, Bednarz VN, Naumann MS, Al- Horani FA, Wild C

This chapter investigates carbon cycling at the coral reef ecosystem level using a linear inverse trophic food web model. Measurements of photosynthetic primary production and organic carbon fluxes in the water column, benthos, and cryptic cavity sponge communities are combined to provide a quantitative overview of carbon cycling on a northern Red Sea fringing coral reef. The model specifically investigates the role of carbon cycling by epi-reefal and cryptic cavity sponge communities in order to quantify the importance of the sponge loop for coral reef trophic webs and reef biogeochemical cycling.

This study was designed by N. van Hoytema, L. Rix, U. Cardini, V. N. Bednarz, and C. Wild. Fieldwork and sample analyses were performed by N. van Hoytema, L. Rix, U. Cardini, and V. N. Bednarz with support from M. S. Naumann and F. A. Al-Horani. The linear inverse trophic food web model was designed by N. van Hoytema and D. van Oevelen with input from L. Rix and M. S. Naumann. The manuscript was written by N. van Hoytema with critical revision by all authors. This manuscript is in preparation for Coral Reefs.

Chapter 4:

Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems

Rix L, Naumann MS, Mueller CE, de Goeij JM, Struck U, Middelburg JJ, van Duyl FC, Al-Horani FA, Wild C, van Oevelen D

This chapter explores the potential for the sponge loop to function in different coral reef ecosystems in the Red Sea (Indo-Pacific) and in cold-water reefs of the deep-sea (Tisler Reef, northeast Atlantic). We hypothesize that a direct trophic link between corals and sponges, two key reef ecosystem engineers, enables the recycling of coral mucus via the sponge loop on both warm-water (WW) and cold-water (CW) reefs. Stable isotope tracer experiments were conducted to investigate the uptake of naturally produced coral mucus from ^{13}C- and ^{15}N-labeled corals (WW: Fungiidae; CW: *Lophelia pertusa*) by the sponges *Mycale fistulifera* (WW) and *Hymedesmia coriacea* (CW). Additional incubations were conducted to demonstrate the transfer of coral mucus into sponge-produced detritus. Assimilation of coral mucus C into sponge phospholipid-derived fatty acid (PLFA) biomarkers was measured to evaluate the potential role of sponge-associated bacteria in its uptake. Finally we compare differences in the functioning of the warm-water and cold-water sponge loops and generate hypotheses regarding the importance of C versus N cycling in these vastly different coral reef ecosystems.

This study was initiated by L. Rix, M. S. Naumann, C. Mueller, J. M. de Goeij, J. J. Middelburg, C. Wild, and D. van Oevelen. Fieldwork was conducted by L. Rix, M. S. Naumann, C. Mueller, and F. C. van Duyl. U. Stuck contributed to sample analyses. F. A. Al-Horani provided field work support. L. Rix analyzed the data and wrote the manuscript with critical input from all authors. This manuscript is in revision at *Scientific Reports*.

Chapter 5:

Organic and inorganic nitrogen release by Red Sea coral reef sponges

Rix L, Wild C, Cardini U, Bednarz VN, Struck U, Al-Horani FA, Naumann MS.

Coral reef sponges are known to be a significant source of DIN in coral reef ecosystems, but the importance of the production of PON, generated as sponge detritus, has not been quantified. Further, many sponges host symbiotic microbes capable of N_2 fixation, but actual measured rates of N_2 fixation in coral reef sponges are scarce. This chapter therefore uses incubation experiments to compare inorganic and organic N fluxes (DIN production, PON production, and N_2 fixation) in six dominant Red Sea coral reef sponges and evaluates their relative contributions to overall sponge N cycling. These fluxes are combined with sponge benthic cover data to determine community N sponge fluxes and in order to evaluate their importance at the ecosystem level.

This study was initiated by L. Rix, M. S. Naumann, and C. Wild. Fieldwork and sample analyses were performed by L. Rix, U. Cardini, and V. N. Bednarz with support from FA Al-Horani. L Rix analyzed the data and wrote the manuscript with critical revision from all authors. This chapter is in preparation for *Marine Ecology Progress Series*.

Chapter 6:

Seasonality in dinitrogen fixation and primary productivity by coral reef framework substrates from the northern Red Sea

Rix L, Bednarz VN, Cardini U, van Hoytema N, Al-Horani FA, Wild C, Naumann MS

This chapter measures N_2 fixation (via acetylene reduction) and photosynthetic primary productivity (via oxygen fluxes) in three common reef framework substrates: turf algae, coral rock, and an encrusting sponge in order to investigate a potential link between C and N fixation. N_2 fixation, photosynthesis, and respiration are quantified on a seasonal resolution (winter, spring, summer, fall) on a high latitude Red Sea fringing reef experiencing seasonally variable environmental conditions in order to evaluate the effect of environmental parameters (irradiance, temperature, and inorganic nutrient availability) on these key metabolic processes. The contribution of N_2 fixation to the N requirement for primary productivity is estimated and correlations between the two processes are investigated in order to evaluate the potential importance of N_2 fixation for sustaining net primary productivity. Finally we calculate the respective contributions of turf algae, coral rock, and the non-cryptic sponge community to benthic N_2 fixation to assess the importance of each substrate to fixed N generation on a northern Red Sea fringing reef.

This study was initiated by V. N. Bednarz, U. Cardini, N. van Hoytema, and C. Wild. Fieldwork and sample analyses were performed by L. Rix, V. N. Bednarz, U. Cardini, and N. van Hoytema with support from F. A. Al-Horani and M. S. Naumann. L. Rix analyzed the data and wrote the manuscript with input from all authors. This manuscript has been accepted for publication in *Marine Ecology Progress Series.*

RELATED PUBLICATIONS

Naumann MS, **Rix L**, Al-Horani FA, Wild C Polydorid polychaetes as frequent associates of the toxic reef sponge *Negombata magnifica* – evidence for a trophic relation? In preparation for *Marine Biodiversity*.

Bednarz VN, **Rix L**, Cardini U, van Hoytema N, Naumann MS, Al-Rshaidat MMD, Wild C Dinitrogen fixation and nitrogen fractionation in scleractinian corals along a depth- gradient in the northern Red Sea. In preparation for *The Journal of Experimental Biology*.

Cardini U, van Hoytema N, Bednarz VN, **Rix L**, Foster RA, Al-Rshaidat MMD, Wild C Diazotrophs contribute towards the resilience of the coral holobiont to ocean warming. In preparation for *Environmental Microbiology*.

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CHAPTER 2

CORAL REEF SPONGES TRANSFER CORAL- AND ALGAL-DERIVED DISSOLVED ORGANIC MATTER (DOM) AS PARTICULATE ORGANIC MATTER (POM) TO HIGHER TROPHIC LEVELS

Rix L, Wild C, de Goeij JM, van Oevelen D, Struck U, Al-Horani FA, Naumann MS. Coral reef sponges transfer coral- and algal-derived dissolved organic matter (DOM) as particulate organic matter (POM) to higher trophic levels. This chapter is in preparation for submission to *Functional Ecology*.

ABSTRACT

Recently the so-called sponge loop has been proposed to play a key role in the retention and recycling of nutrients on coral reefs via sponge uptake of dissolved organic matter (DOM). Excess photosynthates released by corals and macroalgae contribute to the reef water pool of labile DOM that may be available for sponges, but differ in their quality and composition. However, the influence of different reef DOM sources on recycling via the sponge loop has not been investigated. Here we applied stable isotope pulse-chase experiments to compare the processing of coral- and algal-derived DOM by three Red Sea reef sponge species: *Chondrilla sacciformis*, *Hemimycale arabica*, and *Mycale fistulifera*. All three species assimilated both coral- and algal-derived DOM into their tissues, but incorporation rates were significantly higher for algal-derived DOM. The two DOM sources were also differentially utilized by the sponge holobiont as algal-derived DOM was preferentially incorporated into bacteria-specific PLFAs, but coral-derived DOM was incorporated at a higher rate into sponge-specific phospholipid fatty acids (PLFAs). A substantial fraction of the dissolved organic carbon (C) and nitrogen (N) assimilated by the sponges was subsequently released as particulate detritus (15 – 24% C and 27 – 49% N), with higher DOM to POM transformation rates for algal-derived DOM. Additional isotope tracer experiments revealed that sponge detritus was transferred up the food web into sponge-associated detritivores: ophiuroids (*Ophiothrix savignyi, Ophiocoma scolopendrina*) and a polychaete (*Polydorella smurovi*). The observed higher uptake and transformation rates of algal- compared with coral-derived DOM suggests that reef community phase-shifts from coral to algal dominance, with their corresponding increases in DOM quantity and quality, may stimulate DOM cycling through the sponge loop with potential consequences for coral reef biogeochemical cycles and trophic food webs.

INTRODUCTION

Sponges are key components of coral reefs and other aquatic ecosystems where their high filtering capacity and association with diverse microbial communities enables them to influence major biogeochemical cycles (Taylor et al. 2007, Weisz et al. 2008, Maldonado et al. 2012). By efficiently removing particulate organic matter (POM) from the water column (Reiswig 1971, Pile et al. 1997, Ribes et al. 1999), sponges contribute to benthic-pelagic coupling (Gili & Coma 1998, Richter et al. 2001, Perea-Blazquez et al. 2012). More recently, sponges have also been found to play a major role in the cycling of dissolved organic matter (DOM) on coral reefs via the so-called "sponge loop" (de Goeij et al. 2013).

Sponges have long been hypothesized to be capable of retaining DOM (Reiswig 1974), a food source largely unavailable to most reef heterotrophs, and recently DOM uptake has been confirmed in sponges from tropical (Yahel et al. 2003, de Goeij et al. 2008b, Mueller et al. 2014a), temperate (Ribes et al. 2012), to deep-sea habitats (van Duyl et al. 2008). For the coral reef sponges investigated, DOM constitutes the majority (up to ~90%) of their total carbon (C) uptake (Yahel et al. 2003, de Goeij et al. 2008b, Mueller et al. 2014a), and reef framework cavities hosting dense sponge populations act as a major sink for DOM on coral reefs (de Goeij & van Duyl 2007). The mechanisms that enable sponges to take up and assimilate DOM are poorly understood. However, many sponges host dense communities of associated microbes that can account for up to 35% of their biomass (Hentschel et al. 2006, Gloeckner et al. 2014). As microbes are the main DOM consumers in the ocean (Azam et al. 1983), they are suspected to play a role in sponge DOM uptake, with higher uptake rates expected in high-microbial abundance (HMA) compared to low-microbial abundance (LMA) sponges (Reiswig 1974, Weisz et al. 2007, Maldonado et al. 2012).

A substantial fraction of the DOM assimilated by coral reef sponges (up to 35%) is subsequently released as POM via the production of sponge detritus(de Goeij et al. 2009, Alexander et al. 2014, Maldonado 2015), which is readily consumed by other reef fauna (de Goeij et al. 2013). Therefore, this sponge-mediated pathway for DOM recycling functions similarly to the established microbial loop (Azam et al. 1983), facilitating the transfer of the energy and nutrients stored in DOM to higher trophic levels that otherwise would be unable to utilize it. Sponge detritus may then provide a food source for the various reef fauna commonly associated with reef sponges (Wulff 2006), but such trophic interactions have not been documented. DOM uptake by cryptic sponges in Caribbean reefs approximates the gross primary production of the entire ecosystem, indicating sponges play a key role in the cycling of energy and nutrients within coral reefs (de Goeij et al. 2013). However, to date the sponge loop has only been demonstrated with laboratory-produced diatom DOM, which is unlikely representative of the main DOM sources produced on coral reefs.

Coral reef primary producers, such as scleractinian corals and macroalgae, release a substantial fraction of excess photosynthates into the water column, thereby fueling the labile DOM

pool on coral reefs available for recycling by the sponge loop (Crossland 1987, Ferrier-Pagès et al. 1998, Haas et al. 2010b, Brocke et al. 2015). Macroalgae typically release larger quantities of DOM compared to corals (Haas et al. 2010a, Haas et al. 2011, Haas et al. 2013, Mueller et al. 2014b), which release organic matter primarily as coral mucus with a comparatively larger particulate fraction (Naumann et al. 2010a, Wild et al. 2010b). Further, the quality and composition of coral- and algal-derived DOM varies with corresponding different impacts on the respiration, growth and composition of microbial communities in the water column and reef sediments (Haas & Wild 2010, Wild et al. 2010a, Nelson et al. 2013). Algal exudates induce accelerated microbial growth and respiration leading to localized hypoxia and shifts towards more pathogenic microbial communities (Haas et al. 2010a, Haas et al. 2011, Haas et al. 2013, Nelson et al. 2013). Consequently, macroalgal exudates have been implicated in microbial-mediated coral mortality (Smith et al. 2006, Gregg et al. 2013) and are suspected to initiate a feedback loop favouring the growth of macroalgae at the expense of corals (Barott & Rohwer 2012). Changes in the benthic cover of corals and algae can therefore alter the quality and quantity of organic matter on coral reefs, which may affect biogeochemical cycles and overall ecosystem functioning (Wild et al. 2011). In this context, potential impacts on the functioning of the sponge loop are not known. Reef community shifts from coral to algal dominance are hypothesized to benefit sponges by increasing the availability of DOM (de Goeij et al. 2013, Mueller et al. 2014a, Pawlik et al. 2015), but differences in the uptake and processing of coral- vs. algal-derived DOM by sponges have not been investigated.

This study, therefore, compared the uptake and transformation of coral- and algal-derived DOM by Red Sea reef sponges. Stable isotope pulse-chase experiments using dissolved coral and algal exudates enriched in ^{13}C and ^{15}N were conducted to trace coral- and algal-derived DOM into the tissues and detritus of three species of encrusting Red Sea sponges: *Chondrilla sacciformis*, *Hemimycale arabica*, and *Mycale fistulifera*. Phospholipid fatty acid analysis was applied to differentiate uptake and assimilation by the sponge host versus its associated microbes. Additional longer-term tracer experiments using ^{13}C and ^{15}N labeled corals (Fungiidae) were conducted to demonstrate the transfer of detritus from the sponges *M. fistulifera* and *Negombata magnifica,* into the tissues of sponge-associated detritivores: the brittle stars (ophiuroids) *Ophiothrix savignyi* and *Ophiocoma scolopendrina*, and the polychaete *Polydorella smurovi*.

MATERIALS AND METHODS

Study site and organism collection:

Sampling and experimental work was conducted at the Marine Science Station (MSS), Aqaba, Jordan, at the northern Gulf of Aqaba, Red Sea (29°27' N, 34°58' E) during 2013. Free-living fungiid

corals (family: Fungiidae, genera: *Fungia, Ctenactis,* and *Herpolitha*, n = 30) and the macroalgae *Caulerpa serrulata* (n = 6) were collected from the MSS reef between 8 – 12 m by SCUBA. Fungiid corals can be removed from the reef without physical damage, are dominant coral genera on reefs in the region, and produce large quantities of coral mucus (Naumann et al. 2010a). *C. serrulata* is a dominant macroalgal species on reefs in the Gulf of Aqaba that exhibits high DOM release (Haas et al. 2010b). Corals and algae were collected using SCUBA and immediately transported without air exposure to the MSS aquarium facilities for maintenance in 1000 L flow-through aquaria (flow rate ~100 L min^{-1}) supplied with seawater pumped directly from the reef at 10 m water depth. Natural light levels were adjusted using layers of black mesh and parallel measurements with a quantum sensor (Model LI-192SA; Li-Cor) in aquaria and *in situ* at 10 m water depth to ensure photosynthetically active radiation (PAR µmol photons m^{-2} s^{-1}, wavelength 400 – 700 nm) levels in the aquaria corresponded to *in situ* conditions. Corals and algae were acclimated for at least 72 h prior to the start of experiments.

For the stable isotope pulse-chase incubations, three species of common encrusting sponge were collected using SCUBA: *Chondrilla sacciformis, Hemimycale arabica,* and *Mycale fistulifera. H. arabica* and *M. fistulifera* represent abundant non-cryptic species, while *C. sacciformis* is a dominant species in the cryptic reef habitat (Kötter 2002, Wunsch et al. 2000). Both *H. arabica* and *M. fistulifera* belong to the Poecilosclerida, an order of LMA species (Gloeckner et al. 2014), while *C. sacciformis* by contrast, contains high densities of associated bacteria (Kötter 2002). Sponge specimens (3 – 12 cm^{-3}) were chiseled from their respective substrates at 10 – 20 m water depth and immediately transferred without air exposure to the MSS aquarium facilities where they were cleared of epibionts. Each sponge species was maintained in a separate 100 L flow-through aquarium (flow rate ~6 L min^{-1}) under controlled irradiance levels corresponding to their respective *in situ* habitats (shaded for *C. sacciformis* and light for *M. fistulifera, H. Arabica*). Sponges were allowed to heal and acclimate for at least 1 week prior to incubations and only visually healthy specimens (no tissue damage, open oscula, actively pumping) were used. For the longer-term stable isotope labeling experiment, *M. fistulifera* fragments (20 ± 8 cm^{-3}) were collected from dead branching corals as previously described, and fragments were cut from the branching sponge *Negombata magnifica* (10 – 20 m water depth; 67 ± 22 cm^3). The brittle stars *Ophiothrix savignyi* and *Ophiocoma scolopendrina* (Echinodermata, Ophiuroidea) were collected from the surface of sponges (5 – 15 m water depth) and supplemented with *O. scolopendrina* specimens collected from the shallow reef flat. Polychaetes of the species *Polydorella smurovi* (Polychaeta, Spionidae) were collected attached to branches of densely infested *N. magnifica* specimens (10 – 20 m water depth) where they are found living attached to the sponge tissue. Sponges and their associated detritivores were maintained in the MSS aquarium facilities as described above for 1 wk and 48 h, respectively, prior to experimentation.

Coral and algae stable isotope labeling:

Coral (Fungiidae) and algae (*C. serrulata*) stable isotope labeling was conducted over 6 (January 2013) and 8 (September 2013) consecutive days. Each morning at 8:00 the inflow to the coral and algae aquaria were stopped and 36 mg L^{-1} $NaH^{13}CO_3$ and 1 mg L^{-1} $Na^{15}NO_3$ (Cambridge Isotope Laboratories, 98%) were added. Both inorganic labeled compounds are rapidly taken up via photosynthesis (Naumann et al. 2010b). Aquaria pumps maintained water circulation and gas exchange while seawater flow-through was stopped (8 h). Oxygen concentration and saturation, temperature, and pH of the aquaria were continuously monitored with a multi-probe (Hach HQ40d). Water temperature was maintained within ± 1°C of ambient flow-through by placing aquaria in a raceway water bath (flow rate ~1000 L h^{-1}).

Collection of labeled coral- and algal-derived DOM:

On labeling days 2, 4, 6, and 8 coral mucus was collected from each fungiid coral 1 h after aquarium flow-through was resumed. Corals were rinsed thoroughly with fresh-pumped label-free flowing seawater and mucus was collected via air exposure, a naturally occurring phenomenon at the study site during low tides (Loya 1976) that stimulates mucus production (Wild et al. 2005b). The first 30 s of mucus production was discarded to prevent contamination or dilution by adhered seawater. The corals were then transferred into clean zip-lock bags and the released mucus was collected for 5 min before corals were returned to flow-through aquaria overnight.

Algal-derived organic matter was collected using the beaker incubation method (Herndl & Velimirov 1986) as described by Haas et al. (2010b). On algae labeling days 2, 4, 6, and 8 *C. serrulata* specimens (n = 6) were removed from labeling aquaria at 14:00 and rinsed for 10 min in fresh-pumped label-free flowing seawater. Algae were transferred to individual 1 L chambers (n = 6) filled with fresh seawater and placed in a flow-through water bath to ensure *in situ* temperature during the 2 h incubation period. Oxygen concentration and saturation, temperature and pH were monitored with a multi-probe (HACH HQ40d). After 2 h incubation, the algae were returned to the labeling aquaria and flow-through was resumed overnight.

The collected mucus and algae incubation medium were each pooled and immediately refrigerated prior to same-day vacuum filtration through pre-combusted (450°C, 5 h) GF/F filters (VWR: 0.7 µm pore size). The filtrate representing the dissolved fraction of coral- and algae-derived organic matter was collected and stored frozen at -20°C. On the last day of coral and algae labeling all previously collected coral- and algal-derived DOM was defrosted, homogenized, and sub-samples (5 ml, n = 5) were frozen at -80°C for stable isotope analysis. The final mixtures of labeled coral- and algal-derived DOM were divided into aliquots and refrozen at -20°C until use in sponge stable

isotope pulse-chase incubations. The relatively low recovery of coral- and algal-derived DOM meant that we could not analyze fatty acid content of the DOM sources.

Sponge stable isotope pulse-chase incubations:

The sponges *C. sacciformis, H. arabica*, and *M. fistulifera* were incubated independently with each of the two labeled substrates; coral- and algal-derived DOM (n = 3 per species and substrate). Immediately prior to the start of the incubations aliquots of the labeled substrates were defrosted, homogenized, measured into glass cylinders and adjusted to ambient seawater temperature. The incubations were conducted in 2 L gas-tight chambers filled with fresh natural seawater for a total duration of 12 h. Every 3 h, the water in the chambers was refreshed and new, labeled substrate (coral- or algal-derived DOM) was added at approximately 1.5 times the local dissolved organic carbon (DOC) concentration measured *in situ* at 10 m water depth (range: 65 - 90 µmol L^{-1}; Table 2.1). Control incubations were conducted in parallel with the addition of labeled substrate but no sponge (n = 3 per substrate) and sponge but no labeled substrate (n = 3 per species). Incubations were conducted in the dark to ensure any potential photosynthetic activity by the sponge or seawater would not affect dissolved oxygen concentrations, which were monitored continuously using multi-probes (Hach HQ40d) inserted into an airtight fitting in the chamber lid. Chambers were stirred at ~400 rpm using magnetic stirring plates (Cimarec™ i Telesystem Multipoint Stirrers, Thermo Scientific) and placed in a flow-through water bath to ensure near *in situ* temperatures.

For each 3 h interval, POM samples for particulate organic carbon and nitrogen (POC and PON) were taken to determine the production of sponge detritus. Seawater POM samples were taken from the inflow used to fill the chambers (t_0, n = 3) and after 3 h incubation (t_3) from each chamber. The sample volume (~1800 ml) was recorded, vacuum filtered onto pre-combusted GF/F filters, and the filter dried at 40°C for at least 48 h. After the final 3 h interval (12 h total), sponge fragments were removed from the chambers and rinsed in label-free flowing seawater (10 min) followed by 0.2 µm filtered seawater (10 min). Sponge surface area and thickness were measured with calipers before the sponge tissue was removed from the attached substrate using a sterile scalpel blade. Tissue samples were collected in 4 mL pre-weighed, pre-combusted glass vials and stored frozen at -80°C for stable isotope and phospholipid fatty acid analysis.

Transfer of sponge detritus to detritivores:

The transfer of sponge detritus to sponge-associated detritivores was investigated in two separate long-term tracer experiments. Experiment 1 examined the transfer of detritus produced by the sponge *N. magnifica* to its associated polychaete *P. smurovi*. Experiment 2 tested the consumption of detritus released from *M. fistulifera* by the two ophiuroids *O. savignyi* and *O. scolopendrina*. A two-

tiered flow-through aquaria set-up, consisting of six paired upper and lower aquaria (100 L each) connected via constant flow-through was used in both experiments. The six upper aquaria were each supplied with fresh-pumped reef water at a rate of ~10 L min^{-1}, and from there seawater flowed into the lower aquaria. Fungiid corals (10 individuals per aquarium) were labeled with ^{13}C and ^{15}N as previously described and maintained in three of the upper aquaria. The remaining three served as controls. The lower aquaria contained the sponges (each $n = 4$), ensuring the treatment sponges were continuously supplied with water exposed to the labeled corals. Artificial aquaria lights provided the corals with ~120 µmol quanta m^{-2} s^{-1}. After five days exposure to the labeled corals, one sponge per tank was removed from the experimental set-up, rinsed in label-free seawater, and the tissue collected for stable isotope analysis as described above. The labeled corals were removed from the upper aquaria and the tanks cleaned to remove any labeled organic matter originating from the corals. Detritivores were then introduced to the lower aquaria holding the remaining sponges. In Experiment 1, *P. smurovi* was introduced by transferring the polychaetes with a pipette onto the surface of *N. magnifica*. The polychaetes quickly re-established themselves on the surface of the labelled sponges, resumed normal behaviour, and remained attached for the duration of the experiment. In Experiment 2, the ophiuroids *O. savignyi* and *O. scolopendrina* ($n = 4$ per aquaria) were introduced to the aquaria with *M. fistulifera,* where they immediately took refuge in crevices in the sponge. After being associated with the labeled sponges for five days; the polychaetes, ophiuroids, and remaining sponges were collected for stable isotope analysis as previously described. Due to their minute size, all polychaetes from each aquarium were pooled onto a single GF/F filter for stable isotope analysis ($n = 3$).

Sample treatment and stable isotope analysis:

Sponge tissue samples for stable isotope analysis were lyophilized, weighed, and homogenized with mortar and pestle. Subsamples were weighed and transferred to silver cups for bulk $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. Tissue samples for $\delta^{13}C$ analysis were decalcified by acidification with 0.4 M HCl. POC filters for $\delta^{13}C$ analysis were decalcified with fuming HCL, re-dried at 40°C for 24 h and folded into silver cups. Isotopic ratios and C/N content were measured simultaneously using a THERMO NA 2500 elemental analyzer coupled to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer (IRMS) via a THERMO/Finnigan Conflo III- interface. Standard deviations of C and N content are < 3% of the concentrations analyzed and < 0.15‰ for repeated $\delta^{13}C$ and $\delta^{15}N$ measurements of standard material (peptone).

C and nitrogen (N) stable isotope ratios are expressed in delta notation as: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $(R_{sample} / R_{ref} - 1) \times 1000$, where R_{sample} is the ratio of heavy/light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and R_{ref} is the heavy/light isotope ratio of the reference material, the Vienna Pee Dee Belemnite standard for C ($R_{ref} = 0.01118$) and atmospheric nitrogen for N ($R_{ref} = 0.00368$). The

atomic % (atm%) heavy isotope in the sample ($^{13}C/[^{13}C + ^{12}C]$ or $^{15}N/[^{15}N + ^{14}N]$) was calculated as $F_{sample} = R_{sample}/ R_{sample} + 1$. The excess (above background) atm % (E) is the difference between the F of the samples and the background atm % in a control sample: $E = F_{sample} – F_{background}$. To quantify the total uptake of coral- and algal-derived DOM, the excess incorporation was divided by the atm% of the labeled substrates. Stable isotope data were then expressed as the total elemental uptake and reported as μmol C_{tracer} mmol C_{sponge}^{-1} and μmol N_{tracer} mmol N_{sponge}^{-1} (mean ± SD), with tracer indicating the C or N deriving from the coral- or algal-derived DOM. Sponge detritus production rates were corrected for any labelled POM produced in the seawater controls to which labeled coral- and algal-derived DOM was added but no sponge, to ensure only activity by the sponge was considered. Relative enrichments, expressed as $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values, were calculated by subtracting the $\delta^{13}C$ and $\delta^{15}N$ values of the control sponges from the values of the sponges exposed to labeled coral- or algal-derived DOM.

Phospholipid fatty acid analysis:

Ground and lyophilized sponge tissue samples (~0.018 g) were prepared for analysis of the composition and isotope enrichment of phospholipid-derived fatty acids (PLFAs) according to Boschker et al. (2002). Total fatty acids were extracted using a modification of the Bligh Dyer method. PLFAs were separated on a silicic-acid column (Merck Kieselgel 60) and derivatized by mild alkaline transmethylation to generate fatty acid methyl esters (FAMEs). Concentration and C isotopic composition of individual FAMEs were determined with a gas-chromatograph combustion interface isotope ratio mass spectrometer (GC-c-IRMS). Identification of individual FAMEs was based on the comparison of retention times with known standards using columns with different polarity and use of GC-MS when required.

Data analysis:

Statistical analyses were conducted in PRIMER-E version 6 (Clarke & Gorley 2006) with the PERMANOVA+ add-on (Anderson 2001). Due to the low sample size (n = 3), differences between coral- and algal-derived DOM treatments were analysed by non-parametric PERMANOVA with Monte Carlo tests. One-factor PERMANOVAs with type 111 (partial) sum of squares and unrestricted permutation of raw data (9999 permutations) were used to test for differences in DOC and dissolved organic nitrogen (DON) incorporation, POC and PON production, and PLFA incorporation between coral- and algal-derived treatments and between species within treatments. Pairwise tests were carried out when species was identified as a significant factor to determine the pairs of species exhibiting differences.

RESULTS

Coral- and algal-derived DOM labeling:

The coral- and algal-derived DOM was substantially enriched in both ^{13}C and ^{15}N, demonstrating that both stable isotope tracer compounds ($NaH^{13}CO_3$ and $Na^{15}NO_3$) were taken up and rapidly incorporated into the released coral mucus and algae exudates (Table 2.1). Overall, the coral-derived DOM was more highly enriched in both ^{13}C and ^{15}N compared to the algal-derived DOM, therefore these differences were accounted for when calculating the rates of incorporation of coral- and algal-derived DOM. The total amount of C and N added to the incubations over 12 h were comparable between the coral- and algal-derived DOM treatments (Table 2.1), and the C:N ratios of the coral- (12.8 ± 1.2) and algal-derived DOM (12.1 ± 2.2) were not significantly different.

Sponge tissue incorporation:

The three investigated sponge species assimilated both coral- and algal-derived DOM into their tissue, but algal-derived DOC and DON was incorporated at a higher rate than coral-derived DOC and DON (Fig. 2.1a, b). This was significant in all cases except for DON uptake by *C. sacciformis* and DOC uptake by *M. fistulifera* (Table 2.2), possibly due to the larger standard deviations (Fig. 2.1a, b). Differences between coral- and algal-derived DOC and DON incorporation by *H. arabica* and *M. fistulifera* were relatively small (1.4 – 1.7 times), but for *C. sacciformis* the uptake of algal-derived DOC was 4-times higher (DON: 3.5-times). Over the 12 h incubation period 19 – 35% of the coral-derived DOC and 42 – 62% of the added coral-derived DON was assimilated into the tissue of the three sponges, while higher percentages of algal-derived DOC and DON were assimilated (32 - 42% and 79 – 91%, respectively). For both substrates the percentage of DON incorporated was higher than for DOC; however it should be noted that the assimilated DOC lost due to respiration was not measured here, and therefore the total DOC assimilation is underestimated.

Incorporation rates of coral- and algal-derived DOM were species-specific. *M. fistulifera* and *H. arabica* incorporated coral-derived DOC at a significantly higher rate (2.4-times higher) than *C. sacciformis*, while incorporation of coral-derived DON was significantly higher for *M. fistulifera* compared to *C. sacciformis* and *H. arabica* (Fig. 2.1a, Table 2.3). Incorporation rates of algal-derived DOC were similar among the three species, but *H. arabica* assimilated algal-derived DON at a comparably lower rate, although this difference was significant only compared to *M. fistulifera* (Fig. 2.1b; Table 2.3). *C. sacciformis* and *M. fistulifera* incorporated DOC and DON at a similar rate, while *H. arabica* assimilated DOC into its tissue at twice the rate of DON (Fig. 2.1a, b).

Table 2.1 Coral- and algal-derived DOM labeling. Total amount of tracer DOC and DON added over the 12 h labeling period, delta $\delta^{13}C$ and $\delta^{15}N$ enrichment of the coral- and algal-derived DOC and DON, and the percentage of ^{13}C and ^{15}N labeling of the coral- and algal-derived DOM.

	C added (µmol)	$\Delta\delta^{13}C$	$\%^{13}C$ labeling	N added (µmol)	$\Delta\delta^{15}N$	$\%^{15}N$ labeling
Coral-derived DOM						
C. sacciformis	256	407 ± 59	0.46	20	1293 ± 251	0.34
H. arabica	236	128 ± 28	0.15	18	408 ± 32	0.15
M. fistulifera	256	407 ± 59	0.46	20	1293 ± 251	0.34
Algal-derived DOM						
C. sacciformis	272	74 ± 16	0.10	19	457 ± 84	0.17
H. arabica	272	74 ± 16	0.10	19	457 ± 84	0.17
M. fistulifera	272	74 ± 16	0.10	19	457 ± 84	0.17

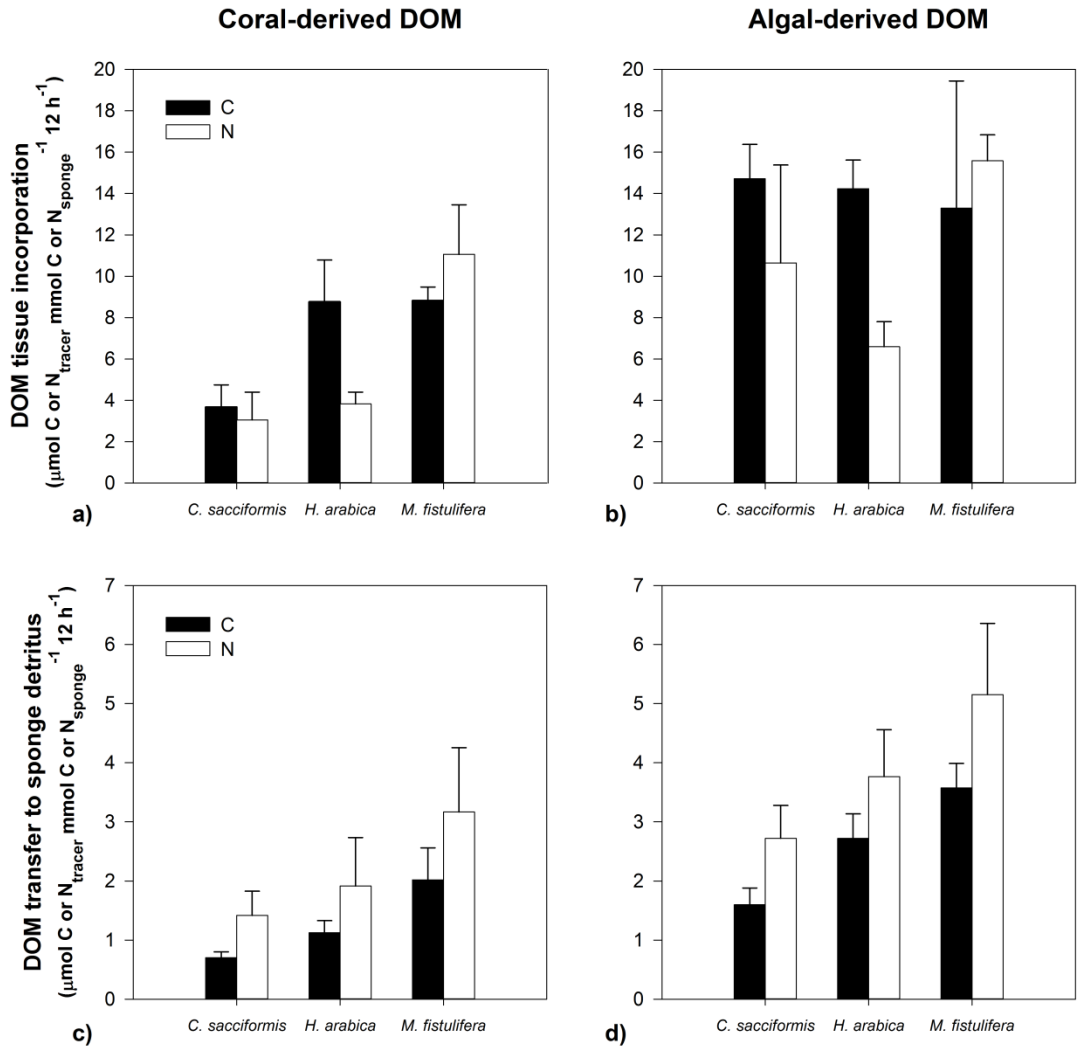

Figure 2.1. Processing of coral- and algal-derived DOC and DON by the three sponge species; *C. sacciformis*, *H. arabica*, and *M. fistulifera*. Shown are the rates of incorporation of a) coral- and b) algal-derived DOC and DON assimilated into the sponge tissue (µmol C or N_{tracer} mmol C or N_{sponge}^{-1} 12 h^{-1}) and the release rate of c) coral- and d) algal-derived DOC and DON as sponge detritus (µmol C or N_{tracer} mmol C or N_{sponge}^{-1} 12 h^{-1}). Data presented as mean ± SD (n = 3).

Transformation into sponge detritus:

All three sponge-species produced detritus enriched in ^{13}C and ^{15}N after exposure to ^{13}C- and ^{15}N-enriched coral- and algal DOM, demonstrating the rapid transfer of coral- and algal-derived DOC and DON into sponge detritus (Fig. 2.1c, d). Control experiments with labeled coral- and algal-derived DOM but no sponge yielded low amounts of labeled POM, indicating the production of ^{13}C- and ^{15}N-enriched POM could be attributed to sponge activity. Similar to tissue incorporation rates, algal-derived DOM was transferred into sponge detritus at higher rates compared to coral-derived DOM (Fig. 2.1c, d). This was significant for all comparisons except the transfer of DON to sponge detritus by *M. fistulifera* (Table 2.2). Across all species coral- and algal-derived DON was released as detritus at a higher rate than DOC (PERMANOVA: df = 1, SS = 3, MS = 3, $F = 7.8$, $p = 0.018$ and df = 1, SS = 8, MS = 8, $F = 13.4$, $p = 0.004$ for coral- and algal-derived DOM, respectively). Among species, *C. sacciformis* transferred both coral- and algal-derived DOC and DON to detritus at lower rates compared to the other two species (Table 2.3). Overall, 15 – 24% of the coral- and algal-derived DOC assimilated by the three sponges was transformed into detritus compared to a significantly higher fraction of DON (27 – 49%; PERMANOVA: df = 1, SS = 3408, MS = 3408, $F = 33.8$, $p < 0.001$). Equivalent percentages of coral- and algal-derived DOM were transferred into detritus with no significant differences between species.

Table 2.2. Results of one-factor Monte Carlo PERMANOVAs testing for differences in the processing of coral- and algal-derived DOM by the three sponge species; *Chondrilla sacciformis*, *Hemimycale arabica*, and *Mycale fistulifera*. Factors tested were incorporation of coral-vs algal-derived DOC and DON and transfer of coral-vs algal-derived DOC and DON into sponge detritus. PERMANOVAs were based on Euclidian distance and Type III (partial) sums of squares were used with unrestricted permutations of raw data (9999 permutations). Significant Monte Carlo (MC) *p*-values are in **bold**.

Coral- vs. algal-derived DOM	df	SS	MS	*F*	*P* (MC)
DOC incorporation					
C. sacciformis	1	183	183	93.5	**0.001**
H. arabica	1	45	45	15.0	**0.02**
M. fistulifera	1	61	61	2.8	0.17
DON incorporation					
C. sacciformis	1	86	86	7.1	0.055
H. arabica	1	11	11	12.8	**0.024**
M. fistulifera	1	31	31	8.4	**0.048**
Detritus C transfer					
C. sacciformis	1	1	1	26.5	**0.006**
H. arabica	1	4	4	35.7	**0.005**
M. fistulifera	1	4	4	15.6	**0.019**
Detritus N transfer					
C. sacciformis	1	5	5	25.9	**0.006**
H. arabica	1	5	5	7.9	**0.046**
M. fistulifera	1	6	6	4.5	0.093

Transfer of sponge detritus to detritivores:

The long-term labeling experiment showed transfer of coral-derived C and N into the tissue and detritus of the two sponges *M. fistulifer*a (Fig. 2.2a) and *N. magnifica* (Fig. 2.2b). Sponge tissue incorporation rates were higher in *M. fistulifera* (3.0 ± 0.9 µmol C_{tracer} mmol C_{sponge} d^{-1} and 3.2 ± 1.6 µmol N_{tracer} mmol N_{sponge} d^{-1}) compared to *N. magnifica* (1.1 ± 0.1 µmol C_{tracer} mmol C_{sponge} d^{-1} and 0.9 ± 0.2 µmol N_{tracer} mmol N_{sponge} d^{-1}). Subsequently, after five days exposure to the labeled sponges, enrichment of both ^{13}C and ^{15}N was detectable in the tissues of the investigated sponge detritus consumers; the ophiuroids *O. savignyi* and *O. scolopendrina* (Fig. 2.2a) and the polychaete *P. smurovi* (Fig. 2.2b). The brittle stars incorporated sponge detritus at rates of 7.6 ± 6.5 µmol $C_{detritus}$ mmol $C_{detritivore}$ d^{-1} and 6.8 ± 4.1 µmol $N_{detritus}$ mmol $N_{detritivore}$ d^{-1}, while the polychaetes assimilated sponge detritus at higher rates of 32.3 ± 13.0 µmol $C_{detritus}$ mmol $C_{detritivore}$ d^{-1} and 24.4 ± 11.3 µmol $N_{detritus}$ mmol $N_{detritivore}$ d^{-1} (Fig. 2.3).

Table 2.3. Results of one-factor Monte Carlo PERMANOVAs testing for species-specific differences in the processing of coral- and algal-derived DOM between the three sponge species: *Chondrilla sacciformis* (CS), *Hemimycale arabica* (HA), and *Mycale fistulifera* (MF). Factors tested were incorporation of coral-vs algal-derived DOC and DON and transfer of coral-vs algal-derived DOC and DON into sponge detritus. PERMANOVAs were based on Euclidian distance and Type III (partial) sums of squares were used with unrestricted permutations of raw data (9999 permutations) and Monte Carlo tests. PERMANOVA reported as *F*-statistic, degrees of freedom, and *p*-value. Pairwise tests are shown with *t*-value and *p*-value. Significant *p*-values are in **bold**.

DOM source	Species	CS – HA	CS - MF	HA - MF
Tissue incorporation	**PERMANOVA**		**Pairwise tests**	
Coral – DOC	14.5, 2, **0.004**	3.8, **0.02**	7.4, **0.001**	0.2, 0.8
Coral - DON	22.3, 2, **0.002**	0.9, 0.4	5.0, **0.006**	5.1, **0.006**
Algal - DOC	0.1, 2, 0.939	-	-	-
Algal - DON	7.2, 2, **0.025**	1.4, 0.2	1.7, 0.1	8.9, **0.001**
Detritus transfer	**PERMANOVA**		**Pairwise tests**	
Coral – DOC	11.8, 2, **0.0083**	3.2, **0.03**	1.2, **0.01**	2.7, 0.05
Coral - DON	5.7, 2, **0.0425**	1.9, 0.13	3.4, **0.03**	1.6, 0.19
Algal - DOC	20.8, 2, **0.0014**	3.9, **0.02**	6.8, **0.002**	2.5, 0.06
Algal - DON	5.6, 2, **0.0454**	1.9, 0.14	3.2, **0.03**	1.7, 0.17

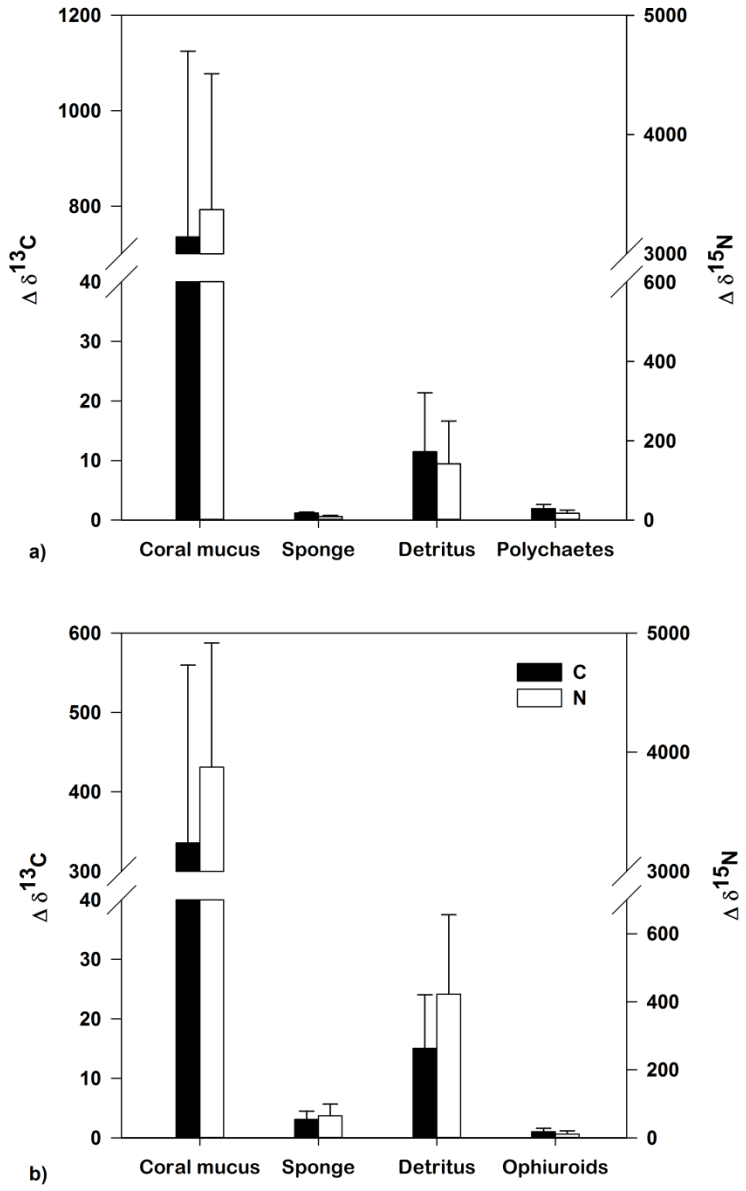

Figure 2.2. Stable isotope (^{13}C and ^{15}N) enrichment in coral mucus, sponge tissue, sponge detritus, and sponge detritus-consumers. Values are presented as mean above-background tracer incorporation $\Delta\delta^{13}C$ (‰) (black bars) and $\Delta\delta^{15}N$ (‰) (white bars) in: a) coral-derived C and N, sponge tissue and detritus of *Negombata magnifica*, and the polychaete *Polydorella smurovi* with sponge tissue and detritus (n = 3) sampled after five days exposure to ^{13}C and ^{15}N labeled corals and polychaete tissue (n = 3) sampled after five days exposure to ^{13}C and ^{15}N labeled sponges, and b) coral-derived C and N, sponge tissue and detritus of *Mycale fistulifera*, and tissue of the detritus feeding ophiuroids *Ophiotrhix savignyi Ophiocoma scolopendrina* with sponge tissue and detritus (n = 3) sampled after five days exposure to ^{13}C and ^{15}N labeled corals and ophiuroid tissue samples (n = 12) after five days exposure to ^{13}C and ^{15}N labeled sponges. Data presented as mean ± SD.

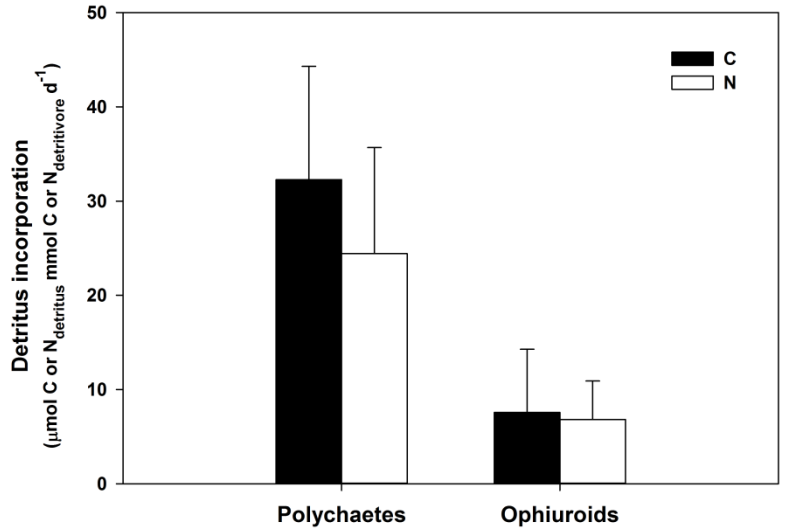

Figure 2.3. Incorporation of sponge detritus by epizoic detritivores. Shown are incorporation rates of sponge detritus POC (black bars) and PON (white bars) by the brittle stars *Ophiothrix savignyi* and *Ophiocoma scolopendrina* (n = 12) and the polychaete *Polydorella smurovi* (n = 3) presented as (µmol $C_{detritus}$ or $N_{detritus}$ mmol $C_{detritivore}$ or $N_{detritivore}^{-1}$ 12 h^{-1}). Data presented as mean ± SD.

Incorporation into sponge- vs. bacteria-specific fatty acids:

The three investigated sponge species exhibited distinct PLFA profiles (Appendix 1 - Fig. 1). Besides common unspecific PLFAs (e.g. C14:0, C16:0), all three species contained PLFAs that could be identified as bacterial or sponge biomarkers (Appendix 1 - Fig. 1, 2). Typical bacterial biomarkers included iso-, anteiso-, methyl-branched, and odd numbered branching PLFAs (Boschker & Middelburg 2002; Appendix 1 - Fig. 1). Sponge-specific PLFAs consisted of known sponge biomarkers such as C25:2(5,9), 22MeC28:(2(5,9), and C30:3(5,9,23); as well as several unidentified long-chain fatty acids (> C:24) characteristic of demosponges (Carballeira & Reyes 1990, Koopmans et al. 2015). These long-chain sponge-specific PLFAs accounted for the 59.4 – 63.2% of the total PLFA composition of the three species with C26:2(5,9) as the most abundant PLFA in *H. arabica* and *M. fistulifera* and C30:3(5,9,23) dominating the PLFA composition of *C. sacciformis* (Appendix 1 - Fig. 1, 2). The abundances of bacterial PLFAs present were significantly different between species (PERMANOVA: df = 2, SS = 576, MS = 288, F = 699, p = 0.0001). The PLFA composition of *C. sacciformis* consisted of 21.2% bacterial PLFAs; significantly higher than for both *H. arabica* (7.4%) and *M. fistulifera* (2.5%) (PERMANOVA pairwise test: t = 24.2, p = 0.0001 and t = 34.5, p = 0.0001, for *H. arabica* and *M. fistulifera* respectively; Appendix 1 - Fig. 2).

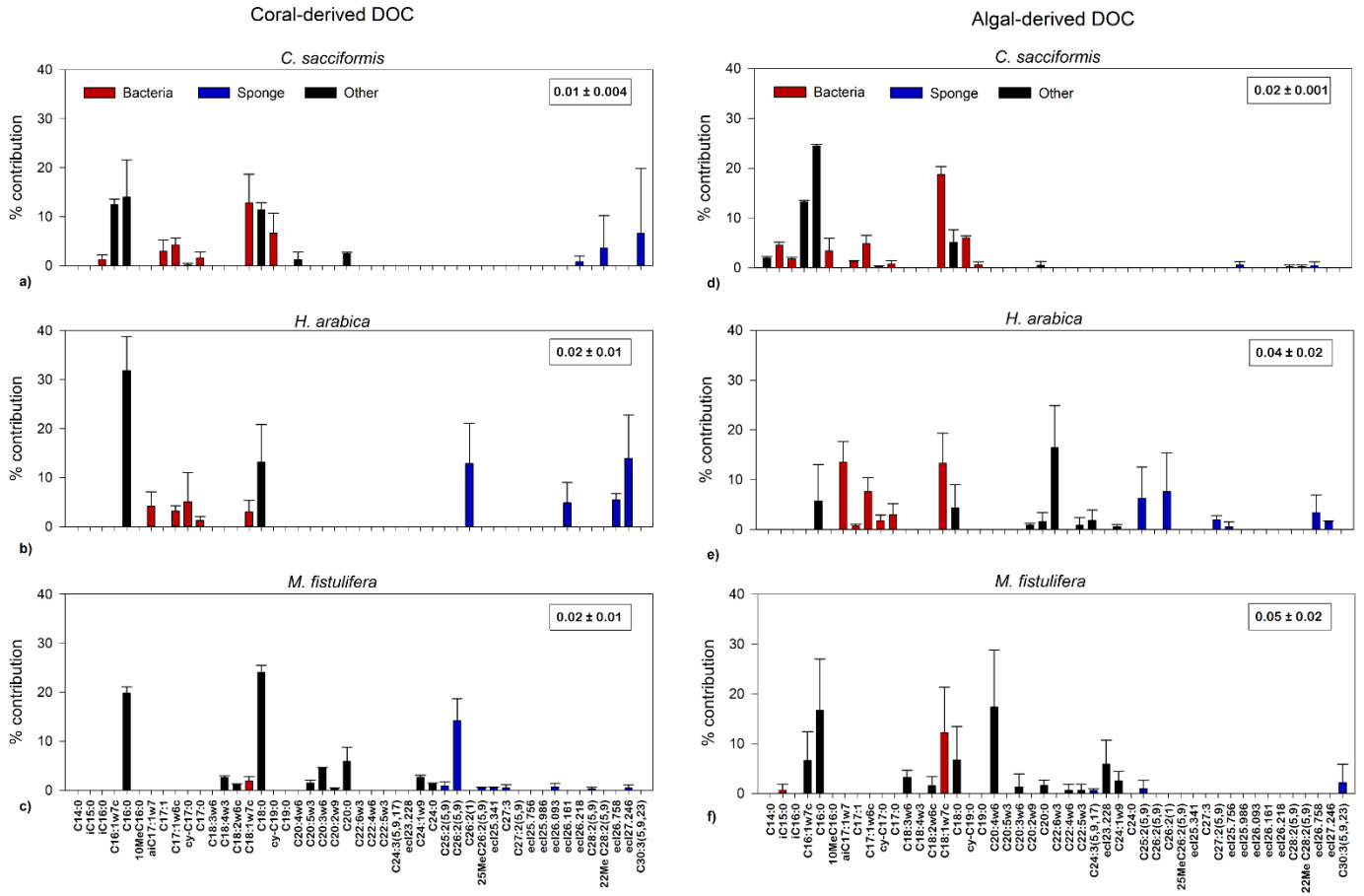

Figure 2.4. Distribution of coral- and algal-derived DOC in sponge phospholipid fatty acids (PLFAs). Data presented as % of total coral-derived C assimilated into PLFAs (mean ± SD) in a) *C. sacciformis*, b) *H. arabica*, and c) *M. fistulifera* and % of total algal-derived C assimilated into PLFAs in d) *C. sacciformis*, e) *H. arabica*, and f) *M. fistulifera*. Depicted are PLFAs exhibiting ≥ 0.5% of total coral- or algal-derived DOC incorporation. Bacterial-specific PLFAs are shown in red, sponge-specific PLFAs in blue, and all other PLFAs in black. Total PLFA incorporation rates are presented in the box in the upper right of each panel (μmol C_{tracer} mmol C_{sponge}^{-1} 12 h^{-1}). Data presented as mean ± SD (n = 3).

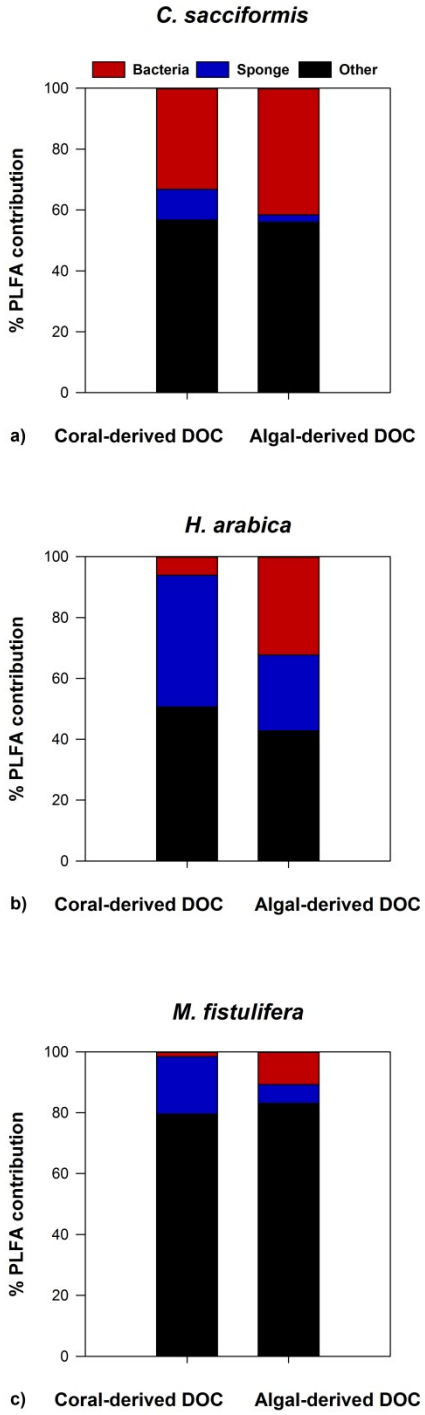

Figure 2.5. Percent distribution of coral- and algal-derived DOC assimilated into bacterial, sponge, and all other phospholipid fatty acids (PLFAs). Data shown for a) *C. sacciformis*, b) *H. arabica*, and C) *M. fistulifera* (each $n = 3$).

All three species assimilated coral- and algal-derived DOC into PLFAs (Fig. 2.4). As for bulk DOC tissue incorporation, PLFA incorporation rates were higher for algal-derived DOC compared to coral-derived DOC, although this difference was significant only for *C. sacciformis* (Fig. 2.4, Appendix 1 - Table 1). The individual PLFAs exhibiting incorporation of coral- and algal-derived DOC differed between the three sponge species, although all species exhibited high uptake into the unspecific PLFAs C16:0 and C18:0 (Fig. 2.4). Since C16:0 is the first fatty acid (FA) produced during FA synthesis, this demonstrates *de novo* synthesis of PLFAs from non-PLFA C sources (eg. carbohydrates). For both the coral and algae treatments, label was only incorporated into PLFAs present in the control sponges, further suggesting *de novo* synthesis of PLFAs rather than dietary uptake of PLFAs from the dissolved food sources (Fig. 2.4, Appendix 1 Fig. 1). Overall, *C. sacciformis* showed significantly higher bacterial-PLFA uptake compared to *H. arabica* and *M. fistulifera* (PERMANOVA pairwise test; $t = 2.6$, $p = 0.0277$ and $t = 7.3$, $p = 0.0001$, respectively; Fig. 2.4, 2.5). When the two DOC sources were compared, algal-derived DOC was incorporated into bacterial-specific PLFAs at a higher percentage and this difference was significant for *H. arabica* and *M. fistulifera* (Fig. 2.4, 2.5; Appendix 1 - Table 1). By contrast, a higher percentage of coral-derived DOC was incorporated into sponge-specific PLFAs (Fig. 2.5), although this difference was significant only for *C. sacciformis* and *M. fistulifera* (Table 1). When the specific rates were examined, algal-derived DOC was incorporated into bacterial PLFAs at twice the rate of coral-derived DOC in *C. sacciformis* and 7 – 8 times in *H. arabica* and *M. fistulifera*. Coral-derived DOC by contrast was incorporated into sponge-specific PLFAs at 1.5 – 4 times higher the rate of algal-derived DOC, despite the lower overall PLFA uptake rates for coral-derived DOC.

DISCUSSION

Uptake and transformation of reef-derived DOM into POM by coral reef sponges:

Here we show the uptake of naturally produced coral- and algal-derived DOM by coral reef sponges, providing the first direct evidence that sponges utilize DOM originating from a variety of primary producers as a food source. Tracer incorporation into sponge phospholipid fatty acids (PLFAs) also indicates that both substrates are actively processed by the sponges and used for cellular components. Sponge tissue incorporation rates of coral- and algal-derived DOM (3.4 – 14.7 µmol C_{tracer} mmol C_{sponge} 12 h^{-1} and 3.1 – 15.6 µmol N_{tracer} mmol N_{sponge} 12 h^{-1}) are in the range, but up to twice as high, compared to diatom-derived DOM assimilation by four Caribbean sponges species (5.2 – 7.9 µmol C_{tracer} mmol C_{sponge} 12 h^{-1} and 4.6 – 8.1 µmol N_{tracer} mmol N_{sponge} 12 h^{-1}; de Goeij et al. 2013). The similar DOM incorporation rates for Red Sea and Caribbean sponges suggests that utilizing reef-derived DOM may be a widely employed and advantageous strategy for sponges on

oligotrophic coral reefs where DOM represents the largest pool of available organic matter (Benner 2002, Yahel et al. 2003) and low POM concentrations may limit sponge growth (Wilkinson & Cheshire 1990). Given the high benthic cover and DOM release rates of corals and algae (Haas et al. 2010a; 2011; 2013, Naumann et al. 2010, Mueller et al. 2014), their exudates likely represent a readily available food source for reef sponges. However, research on DOM feeding by coral reef sponges has focused disproportionately on small encrusting and excavating species (de Goeij et al 2008a,b; 2013, Mueller et al. 2014, this study). These sponges may be better able to capitalize on the DOM released by corals and algae due to their close proximity to such "leaky" benthic primary producers (Pawlik et al. 2015, Slattery & Lesser 2015) that locally elevate labile DOC concentrations (van Duyl & Gast 2001). Interestingly, however, our findings of coral mucus uptake and detritus production also by the branching sponge *N. magnifica* suggests the sponge loop may not be limited to cryptic encrusting sponges as recently suggested (Slattery & Lesser 2015).

A substantial portion of the incorporated coral- and algal-derived DOC and DON was subsequently released as particulate sponge detritus (15 – 24% C and 27 – 49% N). This is remarkably consistent with the turnover of diatom DOM by Caribbean sponges (11 – 24% C and 18 – 36%; de Goeij at al. 2013). DOM uptake by cryptic Caribbean sponges is estimated to approximate reef gross primary production. Given the similarly high uptake and transformation rates, as well as the high sponge coverage in the abundant cryptic reef habitat in the Red Sea (Richter et al. 2001, Wunsch et al. 2000), the sponge loop may also represent a substantial biogeochemical pathway for DOM cycling in also Red Sea reef ecosystems. However, our findings show for the first time that different DOM sources may influence the amount of DOM cycled via the sponge loop. The higher incorporation rate of algal-derived C and N resulted in algal-derived DOM being transferred into sponge detritus at a higher rate, indicating that algal-derived DOM may enhance the functioning of the sponge loop. Regardless of the DOM source, N was transferred into sponge detritus at a higher rate than C. This resulted in the production of detritus with a lower C:N ratio (6.5 ± 1.5) than the ambient POM (8.6 ± 1.2), suggesting sponge detritus is a higher quality food source. However, the quality of sponge detritus as a food source also depends on its composition. Sponge detritus is composed of relatively labile shed cells as well as undigested food and metabolic waste products of a more refractory nature, and the relative proportions of each appear to differ between species (de Goeij et al. 2009, Alexander et al. 2014, Maldonado 2015). Additionally, while high detritus release rates have been observed *ex situ* by this and other studies (de Goeij et al. 2013, Alexander et al. 2014), cell proliferation and shedding is significantly reduced in wounded sponges (Alexander et al. 2015). Since sponges *in situ* can be subject to high predation pressure (Pawlik et al. 1995, Wulff 2006, Pawlik et al. 2013), this may have implications for the amount of coral- and algal-derived C and N made available to other fauna via the sponge loop on reefs experiencing different predation levels.

Uptake of sponge detritus by associated detritivores – closing the loop:

The transfer of coral-derived C and N into the tissues of the ophiuroids (*O. scolopendrina* and *O. savignyi*) and polychaete (*P. smurovi*) confirms the last step of a Red Sea sponge loop – the sponge-mediated transfer of DOM to higher trophic levels. There are two possible pathways for this transfer: 1) the direct uptake of living sponge tissue or 2) the uptake of sponge detritus. *O. scolopendrina* and the genera *Ophiothrix* are known suspension or deposit feeders (Oak & Scheibling 2006, Tamura & Tsuchiya 2011). In fact, both ophiuroids were observed feeding on detritus on the sponge surface (Rix pers. obs.) as reported for the brittle star *O. lineata* associated with the sponge *Callyspongia vaginalis* (Hendler 1984). The feeding ecology of *Polydorella* polychaetes is poorly studied, but all species are believed to exhibit suspension and deposit feeding (Dauer et al. 1981, Williams & McDermott 1997, Williams 2004); suggesting predatory feeding on sponge tissue is unlikely.

While detritus incorporation rates were ~ 4-times higher in the polychaetes compared to the ophiuroids, the rates for both are within the same order of magnitude as those for sponge detritus consumers in the Caribbean (16 – 760 μmol $C_{detritus}$ mmol $C_{detritivore}$ d^{-1} and 16 – 144 μmol $N_{detritus}$ mmol $N_{detritivore}$ d^{-1}; de Goeij et al. 2013). In contrast to the detritivores investigated in the Caribbean, which included motile crustaceans and molluscs as well as fauna residing in the reef sediments, the organisms investigated here are sponge associates. Brittle stars are commonly found living in or on reef sponges (Duarte & Nalesso 1996, Wulff 2006), sometimes forming obligate species-specific associations (Henkel & Pawlik 2005, Henkel & Pawlik 2011), and all known species of *Polydorella* are associated with sponges (Tzetlin & Britayev 1985, Radashevsky 1996, Williams, 2004). Sponges provide associated fauna with a refuge against predation, increased mating success, as well as access to a greater particle supply for suspension feeders and a feeding surface for deposit feeders (Hendler 1984). Our findings show that sponge detritus provides an additional food source for sponge-dwelling detritivores as an added benefit. Brittle stars further experience heavy predation, particularly by reef fish (Hendler 1984), providing a short conduit for the trophic transfer of coral- and algal-derived DOM up the reef food web.

DOM processing within the sponge holobiont

HMA sponges are suspected to be better adapted for and more reliant on DOM uptake than their LMA counterparts (Reiswig 1974, Weisz et al. 2007, Maldonado et al. 2012). Despite this, several LMA sponges also take up DOM (de Goeij et al. 2008b, 2013; Mueller et al. 2014). Interestingly, incorporation rates of algal-derived DOM were similar across all species regardless of their microbial abundances. *C. sacciformis* actually displayed the lowest incorporation rate for coral-derived DOM, providing further evidence that high microbial abundance is not a prerequisite for high DOM uptake. However, the relative contribution of DOM to the overall diets of the

investigated sponges remains to be determined. Due to their lower pumping rates, and therefore lower filtering capacity (Weisz et al. 2008), HMA sponges may depend more on DOM to meet their energetic demands. Despite similar bulk tissue incorporation rates, we detected differences in the specific PLFA processing by the LMA and HMA sponges. The HMA sponge *C. sacciformis* incorporated significantly more coral- and algal-derived DOM into bacteria-specific PLFAs (33 – 42%); indicating bacteria were more active in the uptake and processing of DOM compared to the two LMA sponges, particularly *M. fistulifera*, which showed minimal uptake into bacterial PLFAs (2 – 10%). *C. sacciformis* may then be more reliant on its associated microbes for DOM uptake compared to the LMA sponges. Similar to the Caribbean sponge *Halisarca caerulea* (de Goeij et al. 2008a), all three sponges exhibited uptake of both coral- and algal-derived DOM into sponge-specific PLFAs. This suggests sponge cells are directly involved in DOM-uptake, which could explain the high incorporation rates in the two LMA sponges. DOM is a poorly characterized and heterogeneous mixture of compounds ranging from small colloidal to truly dissolved material (Carlson 2002, Nebbioso & Piccolo 2013). Bacterial and sponge cells may take up different fractions of DOM. Although sponge uptake of dissolved material cannot be excluded, sponge cells may predominately take up larger colloidal material, while their associated bacteria assimilate the truly dissolved fraction (de Goeij et al. 2008). These differing strategies may enable both LMA and HMA sponges to exploit this ubiquitous resource.

Algal-derived DOM was consistently incorporated into both sponge tissue and total PLFAs at a significantly higher rate compared to coral-derived DOM, suggesting it is more readily available to the sponge-microbe association. However, this appeared to be largely due to higher bacterial incorporation, as sponges incubated with algal-derived DOM showed 2 to 8-times higher incorporation of tracer C into bacterial-specific PLFAs compared with sponges fed coral-derived DOM. This suggests bacteria were more active in the uptake of algal-compared to coral-derived DOM, which is consistent with findings that compared to coral exudates, algal exudates stimulate higher respiration and growth in microbes in the water column and reef sediments (Wild et al. 2008, Haas et al. 2011, Haas et al. 2013, Nelson et al. 2013). Coral-derived DOM by contrast promotes slower microbial growth and respiration (Haas et al. 2011, Haas et al. 2013, Nelson et al. 2013), suggesting it is less labile for microbes. Despite exhibiting lower total incorporation into PLFAs, sponges fed coral-derived DOM actually showed higher (1.5 – 4 times) incorporation into sponge-specific PLFAs (synthesized *de novo* or by modification of dietary PLFAs) suggesting coral-derived DOM is more readily available or of higher quality to the sponge host. Coral-derived DOM, particularly from fungiid corals, may contain a higher proportion of proteins and lipids compared to algal-derived DOM (Ducklow & Mitchell 1979, Meikle et al. 1988, Haas & Wild 2010), while algal-derived DOM is relatively enriched in neutral sugars that promote rapid microbial metabolism (Haas & Wild 2010, Wild et al. 2010a, Nelson et al. 2013). DOM originating from coral mucus may also contain more colloidal material compared to algal-derived DOM due to its gel-like nature (Crossland

1987, Brown & Bythell 2005, Bythell & Wild 2011), potentially increasing its availability for sponge cells. Co-uptake of DOM components by sponge and bacterial cells would allow the sponge-microbe holobiont access to a wider range of dissolved compounds (de Goeij et al. 2008a). While this may increase the resilience of the holobiont to fluctuations in POM and DOM availability in the water column, it also implies that coral- and algal-derived DOM differentially affect the metabolism and nutrition of the sponge and its associated microbes. If algal-derived DOM preferentially enhances the metabolism of the sponge-associated microbes, the increase in the bacterial food supply could result in competition between the symbiotic microbes and sponge host for limiting nutrients or increase the costs for the host in regulating its microbial populations (Thacker & Freeman 2012).

Implications of coral-algal phase-shifts for reef biogeochemical cycles:

Coral reef degradation has led to coral decline and increased algal cover on coral reefs worldwide (Hughes 1994, McCook et al. 2001, Sandin et al. 2008). Macro- and turf algae typically release higher quantities of DOM than corals (Haas et al. 2010a, Wild et al. 2010b, Haas et al. 2011, Mueller et al. 2014b) and produce labile DOM that accelerates microbial growth in the water column (Barott & Rohwer 2012, Haas et al. 2013, Nelson et al. 2013). The enhanced microbial respiration, and resulting decrease in oxygen concentrations, combined with the proliferation of pathogenic microbes has negative consequences for scleractinian corals (Kline et al. 2006, Smith et al.. 2006, Gregg et al. 2013). By contrast, sponges may benefit from higher algal cover due to the corresponding increase in dissolved and particulate food supply (de Goeij et al. 2013, Mueller et al. 2014a). While the role of food availability in structuring sponge communities is uncertain (Lesser & Slattery 2013, Pawlik et al. 2015), there is evidence for increased sponge abundances on reefs in the Caribbean and Indo-Pacific (McMurray et al. 2010, Bell et al. 2013, Powell et al. 2014), which may increase the magnitude of recycling by the sponge loop. Our findings also reveal that similarly to the microbial loop (Haas et al. 2013, Nelson et al. 2013), recycling via the sponge loop may increase if the reef DOM pool is dominated by algal-derived DOM. By retaining and cycling reef nutrients, the sponge loop serves an essential function in supporting primary productivity on oligotrophic reefs (de Goeij et al. 2013). However, enhanced DON retention and PON production by the sponge loop on already degraded reefs could exacerbate N enrichment and further promote algal growth. Thus, the interaction between sponges and algae may function in a feedback loop promoting their continued growth at the expense of scleractinian corals, and this may compound the negative effects of algal-induced enhancement of the microbial loop (Barrot & Rohwer 2012, Nelson et al. 2013). Given its potential capacity to influence coral reef trophic structure and biogeochemical cycling, future studies should quantify the magnitude of DOM recycling by the sponge loop in order to evaluate its current and potential future impacts on coral reef functioning.

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CHAPTER 3

ECOSYSTEM-ENGINEERED SEASONAL CARBON CYCLING IN A RED SEA CORAL REEF

van Hoytema N, van Oevelen D, **Rix L**, Cardini U, Bednarz VN, de Goeij JM, Naumann MS, Al-Horani FA, Wild C. Ecosystem-engineered seasonal carbon cycling in a Red Sea coral reef. This chapter is in preparation for submission to *Coral Reefs*:

ABSTRACT

Coral reefs in the northern Red Sea experience oligotrophic conditions, particularly in summer due to water column stratification. These conditions may limit reef carbon (C) fixation via gross primary production (GPP). Recent research revealed that reef cavity sponges in the Pacific and Caribbean potentially take up dissolved organic carbon (DOC) at rates comparable to GPP. This sponge mediated C uptake may exceed pelagic microbial DOC uptake via the microbial loop. Coral reefs in the northern Red Sea harbor high densities of cavity sponges that could play a major role in local reef C cycling, but related knowledge is lacking. Therefore, this study investigated the contributions of GPP and sponge DOC uptake to the coral reef C cycle over all seasons of the year 2013 in a fringing reef close to Aqaba, Jordan. C fluxes of the benthic, pelagic, and cavity communities were empirically quantified using a series of incubation experiments conducted in seasonal resolution. These data were then combined into inverse trophic models to quantify the contributions by the different processes to overall reef C uptake. Findings revealed that DOC uptake by cavity sponges and primary production by hard and soft corals dominated the C flows through the reef in all seasons. Primary production was highest during spring with its relatively high light and nutrient availability (spring GPP: 308 mmol C m^{-2} d^{-1}). Sponge DOC uptake was more than 3-fold higher during summer and fall compared to winter and spring (summer DOC uptake: 535 mmol C m^{-2} d^{-1}), and between 3-fold (winter) and 10-fold (summer) higher than pelagic microbial DOC uptake (seasonal means: 43 – 48 mmol C m^{-2} d^{-1}). These results indicate that the sponge loop is a vital process in C cycling in this high latitude fringing reef, especially so during strongly oligotrophic summer and fall when GPP struggled to meet the metabolic demand of the system.

INTRODUCTION

Coral reefs thrive under oligotrophic conditions (Kleypas et al. 1999). They maintain high gross primary production (GPP) due to efficient utilization and recycling of scarcely available dissolved and particulate nutritious organic material (Wild et al. 2004, de Goeij et al. 2013). Coral reef primary production is characterized by the symbiosis of coral hosts with photosynthetic dinoflagellates of the genus *Symbiodinium* (i.e. zooxanthellae; Wooldridge 2010, Stambler 2011). This symbiosis thrives in oligotrophic surroundings because the coral host supplements the zooxanthellae with nutrients from heterotrophic feeding or nitrogen fixation by symbiotic microbes, while the zooxanthellae provide carbohydrates to the host (Yellowlees et al. 2008, Cardini et al. 2014). Part of the zooxanthellate photosynthates is released as mucus by the coral and enters the pelagic particulate and dissolved organic carbon (POC and DOC respectively) pools in the coral reef (Naumann et al. 2010). POC released as mucus may function as a pelagic particle trap, forming highly enriched aggregates with suspended materials that then sink rapidly to the coral reef where they are remineralized, thereby providing vital nutrients and organic matter to the reef system (Wild et al. 2004, Mayer & Wild 2010). Besides corals, algae also release substantial DOC (Haas et al. 2010). Up to 80% of carbon (C) released by reef primary producers dissolves immediately in seawater (Wild et al. 2004). The primary pathway in which this DOC is made re-available to higher trophic levels in oceanic waters is through the microbial loop (Azam & Malfatti 2007). However, bacterioplankton uptake rates were insufficient to explain DOC removal from coral reef waters (de Goeij & van Duyl 2007). Another pathway of DOC recycling to higher trophic levels is mediated by sponges (Yahel et al. 2003, de Goeij et al. 2008a). They take up vast quantities of DOC and simultaneously expel large amounts of choanocytes and mesohyl cells which are available for consumption by higher trophic levels (de Goeij et al. 2013, Alexander et al. 2014, Maldonado 2015). Sponges can be very abundant in cavities riddling coral reefs (Richter et al. 2001). Their total C uptake and release may therefore have a substantial impact on total reef C cycling. To gain a holistic understanding of coral reef C cycling, it is important to assess all C cycling processes described above together.

The coral reefs of the northern Red Sea experience relatively strong variation in light availability and water temperature due to their high latitude location. The annual fluctuation in sea surface temperature (21 – 29°C) combined with relatively warm deeper water layers (year round ~21°C, water depth > 200 m) result in an annual cycle of deep water mixing from December until May and stratification down to 200 m water depth from June until November (Carlson et al. 2014). Inorganic nutrients are brought up to surface water layers during deep water mixing, while they are trapped in deeper waters during stratification, creating extremely oligotrophic conditions in coral reef surrounding surface waters (Rasheed et al. 2002, Silverman et al. 2007, Rasheed et al. 2012). These local conditions offer the rare opportunity to study the effects of seasonal variation in key environmental factors on C cycling within the coral reefs.

Several studies have investigated coral reef primary production and organic C cycling (e.g. Charpy & Charpy-Roubaud 1991, Hata et al. 2002, Alldredge et al. 2013). However none have done so, while also taking the sponge C cycling loop into account. The vast majority of previous studies have been performed on platform reefs, or reef flats of fringing reefs, leaving the deeper reef slopes of fringing reefs relatively unexamined (e.g. Gattuso et al. 1993, Chisholm & Barnes 1998, Silverman et al. 2007), while reef slopes can comprise up to 85% of total coral reef area (Smith 1978). In addition, many coral reef primary production studies have investigated the reef community as a whole through methods such as flow respirometry, or eddy correlation, making it impossible to determine the relative contributions by functional groups (Long et al. 2013, Koweek et al. 2015). Finally, the Red Sea has received less research effort than the Caribbean and Pacific regions (Berumen et al. 2013, Loya et al. 2014), while its seasonal variation in environmental conditions makes it an ideal natural laboratory to investigate the effect of that variation on coral reef functioning.

This study combines measurements of benthic and pelagic primary production and C cycling in a seasonal resolution to study the effect of changing environmental conditions on these processes in a northern Red Sea reef. Thereby, it differentiates dominant groups of benthic primary producers, enabling the assessment of their individual contributions to C cycling. To combine the various data, four seasonal trophic food web models were developed. Such models have been developed for coral reefs (e.g. Johnson et al. 1995, Opitz 1996, Niquil et al. 1998, Varkey et al. 2012, Heymans et al. 2014), but to our knowledge only one model exists for the Red Sea with a focus on the Eritrean coast (Tsehaye & Nagelkerke 2008). It, like most other coral reef food web models, focuses on fisheries aspects and therefore the top of the food web. The models presented here incorporate the seasonal aspect of the northern Red Sea and studies the foundation of the food web for the less researched reef fore-slope and its overlying water column.

MATERIALS AND METHODS

Study site

The field work for this study was conducted in the Gulf of Aqaba, at the Marine Science Station (MSS) of the University of Jordan and Yarmouk University, 10 km south of Aqaba. Just off the MSS is a ca. 1 km long fringing reef in which all monitoring data and samples were collected at 29° 27' 31" N, 34° 58' 26" E and 10 m water depth. This location on the fore-reef slope, known as "U7", has been studied over the last 40 years (e.g. Mergner & Schuhmacher 1974, Bednarz et al. 2015). All sampling and monitoring was performed at 10 m water depth within 100 m of "U7" during four 4-week periods in 2013 in February, April, September, and November. The timing of these periods (hereafter called winter, spring, summer, and fall) was previously determined from

literature to best cover the annual cycle of environmental conditions in the Gulf of Aqaba (Silverman et al. 2007, Carlson et al. 2014).

Benthic cover

The "U7" area contains a 5 x 5 m^2 rope grid. Vertical pictures were taken of the 25 grid cells on one day in each season and the benthic composition of the research area was quantified using digital image analysis (CPCe image analysis software; Kohler & Gill 2006). 100 random points were placed on every photo and the benthic group underneath was determined down to the lowest possible taxa or substrate type. These data were then grouped into functional groups (Table 3.1). Sediment and coral rock were defined as "bare" reef sand and solid reef structure which were not overgrown by any of the other categories. The functional group data were recalculated to percentage benthic cover in each season. Surface sponge benthic cover was determined by in-situ measurements of sponge surface area within a 0.25 m^2 planar quadrat which was placed every 10 m along three serial 50 m transect lines through the research area at 10 m water depth (n = 15). This was done to improve the estimate of sponge cover as many surface sponges are relatively small and found within cracks and crevices, making them hard to quantify from planar photographs. The benthic cover of sponges in reef cavities within the reef structure was taken from literature at 0.82 m^2 sponge cover per m^2 planar reef surface (Richter et al. 2001). The water column above the reef was assumed to include 8 m^3. Current measurements throughout the water column indicated that the top 2 m were more wind-driven than the 8 m below, reaching substantially higher flow rates than the bottom 8 m (annual mean 25 ± 13 cm s^{-1} and 5 ± 1 cm s^{-1} respectively (mean ± SD); N. van Hoytema unpublished data). It was therefore assumed that there was little exchange between the top layer and the deeper water column.

Environmental monitoring and metabolic incubations

The following describes the methods used for monitoring of environmental conditions in-situ, measurements of heterotrophic bacterial abundance in-situ, metabolic incubations of benthic functional groups and plankton, and sampling and maintenance of specimens for those incubations.

Light (lux) and water temperature at 10 m water depth were measured during all seasons. The lux measurements were converted to photosynthetically active radiation (PAR) by a conversion factor calculated from a simultaneous minute-by-minute measurement of lux and PAR (08:00 to 14:00 on one day, n = 353) using a HOBO pendant logger and a LI-COR LI192SA underwater quantum sensor: lux = PAR x 52.0, R^2 = 0.83 (Long et al. 2012). Water samples were collected on a weekly basis in the four weeks encompassing each study period for measurements of water column status parameters and planktonic net photosynthesis (Pn) and respiration (R), as briefly described below, (n

week^{-1} = 4 - 6). Samples were collected 1 m above the seafloor in clean high density poly-ethylene (HDPE) containers (volume: 5 L) using SCUBA. Subsamples were taken from the containers within 20 min of collection to measure concentrations of inorganic nutrients (NH_4^+, PO_4^{3-}, and NO_x; NO_x = NO_2^- + NO_3^-), particulate organic carbon (POC), dissolved organic carbon (DOC), and chlorophyll a (Chl a). Nutrient concentrations were measured according to Murphy and Riley (1962), Strickland and Parsons (1968), and Holmes et al. (1999) using a Trilogy Fluorometer (Turner Designs) for NH_4^+, and a JASCO-V630 photometer for PO_4^{3-} and NO_x. Subsamples for POC (1L), filtered onto pre-combusted (450 °C, 5 h) GF/F filters (nominal pore size 0.7 μm), were dried (40 °C for 48 h) pending analysis. Filters were acidified (0.1 N HCl) prior to measurement to remove any inorganic C. Measurements were performed on a EuroVector elemental analyzer (EURO EA 3000; analytical precision ≤ 0.1 %). Subsamples for DOC (50 mL) were vacuum filtered (max. pressure 20kPa) through pre-combusted GF/F filters straight into pre-acid-washed (0.4 mol L^{-1} HCl) 30 mL HDPE sample bottles, directly acidified with 80 μL of 18.5 % HCl, and stored at 4 °C in the dark until analysis by high-temperature catalytic oxidation on a Shimadzu TOC-V_{CPH} total organic C analyser using reference water samples (CRM program, Hansell Research Lab, USA, DA Hansell and W Chan; Batch 13, Lot #08-13, 41-45 μmol C L^{-1}) as a positive control after every 10 samples. Chl a subsamples (1L) were filtered onto pre-combusted GF/F filters and stored in the dark at -80 °C. Chl a was later extracted in 90 % acetone for 12 h at 4 °C in the dark and measured on a Trilogy fluorometer using the non-acidification module (CHL NA #046, Turner Designs).

Specimens of benthic functional groups hard and soft corals, macroalgae, turf algae, coral rock, and sediment as well as surface and cavity sponges were collected for quantification of metabolic fluxes (n = 8). The hard corals were represented by the four genera *Acropora*, *Stylophora*, *Goniastrea*, and *Pocillopora*, the two main groups of soft corals were the family Xeniidae, and genus *Sarcophyton*, and macroalgae benthic cover was dominated by the genera *Lobophora* and *Caulerpa*. Turf algae, sediment, and coral rock were treated as bulk groups containing communities of various biotas (Larkum et al. 2003, Schottner et al. 2011). Surface sponges were represented by the genera *Mycale*, *Hemimycale*, *Amphimedon*, *Callyspongia*, and *Negombata*, and cavity sponges by *Chondrilla*. Fragments of hard corals, soft corals, macroalgae, turf algae covered dead coral branches, coral rock, and sponges were collected with hammer and chisel or tweezers. Sediment samples were collected with corers. Sediment cores were extruded from their corers and the top 1 cm was sliced off and placed in a Petri dish with the same surface area as the core (14.52 cm^2). All specimens were maintained in an outside flow through aquarium (800 L, 4000 L h^{-1} flow rate) with water coming from the coral reef at 10 m water depth, thus resembling ambient in-situ conditions of temperature and inorganic nutrients. Light conditions were adjusted to in-situ conditions at 10 m water depth using layers of plastic mesh and monitored with data loggers (Onset HOBO Pendant UA-002-64). The same specimens (or a subset thereof) were used for all metabolic incubations.

Closed cell respirometric incubations under in-situ-like conditions for measurements of net photosynthesis (Pn) and respiration (R) took place in the flow-through aquarium. Planktonic metabolic rates were measured with 0.5L glass chambers filled with water from the weekly environmental monitoring. Start O_2 measurements were taken with an O_2 optode and a conductivity probe (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany, accuracy: ± 0.5% of measured value). The chambers were then closed while removing all air. Chambers for R were placed in dense plastic bags for incubation in the dark and all chambers were moved to the flow through aquarium. Pn incubations under in-situ like conditions lasted from 10:00 until sunset. R incubations lasted for 24 h. After the incubations, end O_2 concentrations were immediately measured. Difference between start and end measurements were normalized for time and volume resulting in rates of nmol O_2 L^{-1} h^{-1}. Benthic specimen incubations were performed using water from the flow through aquarium in 1 L glass chambers for hard corals, soft corals, macroalgae, turf algae, and coral rock, 2 L chambers for sponges (see below), and 0.5 L chambers for sediment (n = 6 - 8). In addition, seawater controls (n = 8) were run in parallel to correct for metabolic activity in the incubation seawater. Chambers were stirred with magnetic stirrer plates. R measurements took place in the dark at least 1 h after sunset to ensure full darkness, while Pn measurements were performed the following day between 12:00 and 14:00 (10:00 and 16:00 for sediment incubations), which represents the most stable and maximum light conditions of the day as determined by light measurements. All specimens, except for sediments and sponges, were incubated for 60 - 90 min for Pn, and 90 - 120 min for R. Sediments were incubated for 120 - 330 min for Pn and 180 - 360 min for R due to lower metabolic activity, and sponges were incubated for 180 min for R due to the larger chamber volume. The measurement procedure was identical to that of the water column incubations. Pn and R rates were calculated by subtracting start from end O_2 concentrations and relating to incubation duration (mg O_2 production/consumption L^{-1} $hour^{-1}$). These O_2 rates were then corrected for the seawater control O_2 fluxes before being normalized to chamber volume and organism surface area (see below). Fluxes were recalculated to nmol O_2 cm^{-2} specimen surface area h^{-1}. Photosynthetic and respiratory quotients were assumed to be 1 for all measured specimens, leading to a direct conversion to nmol C cm^{-2} h^{-1} (Gattuso et al. 1996, Carpenter & Williams 2007).

Organic matter uptake and release by corals, macroalgae, turf algae, coral rock, and sponges were quantified using beaker incubations (Herndl & Velimirov 1986, Naumann et al. 2010). A selection of specimens used for the O_2 flux incubations (n = 6) were incubated from 10:00 until 16:00 h in open 1L glass chambers in the flow through aquarium with simultaneous seawater control incubations. Sponge methodology was different, see below. The setup was covered with transparent plastic foil to prevent contamination with airborne particles while leaving small openings on the sides for air exchange. Chambers were not stirred to prevent water currents from modifying the structural composition through e.g. POC dissolution to DOC. DOC and POC samples were collected at the start and end of the incubation and measured as described in the environmental monitoring section.

Differences in DOC and POC between start and end samples were corrected for the control incubations and then normalized to time, volume, and specimen surface area. Incubations of cavity and surface sponges were conducted in chambers containing 2 L of seawater and over 3 h to take into account the fast filtration activity of sponges. These incubations were stirred to keep POC suspended and thereby available to the sponges for filtration. All organic matter fluxes were recalculated to nmol C cm^{-2} h^{-1}.

The surface area of all specimens used in all metabolic incubations was quantified to normalize metabolic fluxes. Specimen surface areas were calculated using advanced geometry and digital image analysis (Naumann et al. 2009).

Heterotrophic bacterial abundance in the water column was measured to constrain flows pertaining to the microbial loop. Samples (2 ml) were collected in March (n = 4; used for winter and spring) and November (n = 53; used for summer and fall) and fixed with 0.1 % paraformaldehyde (final concentration), frozen with liquid nitrogen, and then stored at -80°C until analysis. The analysis was performed with a flow cytometer (FACSCalibur, Becton Dickinson, 488 nm excitation laser). Samples were stained with SYBR Green 1 (conc. 1 per 1000) for 30 min prior to analysis, sorted at a flowrate of ca. 0.06 µl min^{-1} for 1 min and subsequently gated on a side scatter versus green fluorescence density plot.

Food web model

To comparatively study C flows through the coral community, linear inverse food web models (LIMs) were developed for the four seasons. A LIM consists of matrix equations with equalities and inequalities (Soetaert & van Oevelen 2009). Model constructions and solutions were run in R (version 3.0.2, R Development Core Team, 2013) using the R-package LIM (http://lib.stat.cmu.edu/R/CRAN/web/packages/LIM/index.html). The equalities contain the topology of the food web and single value real world data from measurements on the flows such as respiration and primary production. The inequalities place constraints on the food web flows such as a minimal fraction of assimilated C must go to respiration or respiration must fall between two values. The LIM is the same for all four seasons and consists of three major areas: the water column, the coral reef benthos, and the cavity sponge community within the coral reef. The water column food web is based on a LIM for the water column in a Pacific Atoll (Niquil et al. 1998). It consists of phytoplankton, zooplankton, bacterioplankton, protozoa, POC and DOC. These C compartments are termed pelagic particulate organic carbon (PPC) and pelagic dissolved organic carbon (PDC) from hereon to differentiate them from organic C in sediment and cavities. It is assumed that all biotic compartments respire. In addition to this, phytoplankton performs photosynthesis and is grazed by protozoa and zooplankton. It also releases PPC and PDC to the water column. Zooplankton excretes PPC and PDC and grazes on phytoplankton, protozoa and PPC. Protozoa excrete PPC and PDC,

and feed on PPC, PDC, bacterioplankton, and phytoplankton. Bacterioplankton feeds on PDC and releases PDC. Finally, PPC dissolves into PDC, and there is an export flow from zooplankton to higher trophic levels not described in the model. As the modeled fringing reef is relatively hydrodynamically open, the water residence time is measured in hours, compared to years for a Pacific atoll lagoon (Niquil et al. 1998, Naumann et al. 2012), inflows of C are allowed for all water column compartments. The consistency of the imported C has to reflect the relative biomasses of C for the water column compartments as calculated from the environmental monitoring and from literature on the research area (see below). The coral reef benthos consists of hard corals, soft corals, macroalgae, turf algae, coral rock, sediment, and surface sponges. All these compartments respire and all photosynthesize except for surface sponges (see discussion). Hard and soft corals feed heterotrophically on zooplankton, protozoa, and bacteria. Coral rock with its filter feeding community does the same, but also feeds on phytoplankton (Yahel et al. 2006). In addition, all these compartments can take up, and release, PDC and PPC. Sediment consists of the following sub-compartments: sediment biota, sediment dissolved organic carbon (SDC) and sediment particulate organic carbon (SPC). The sediment biota both takes up and releases SPC and SDC. PPC settles out of the water column to the SPC, and SDC diffuses out to PDC (Chipman et al. 2012). There is also a direct flow from hard corals to SPC based on the release of mucus strings which quickly settle to the sediment (Mayer & Wild 2010, Naumann et al. 2012). All benthic compartments have an export flow which simulates removal of C by processes such as feeding by organisms not included in the model. The surface and cavity sponges respire and it was assumed for the sponges that any C fluxes into and out of the organisms are through assimilation. C which flows through the sponge without being assimilated was ignored. Both sponge compartments assimilate PPC, PDC, phytoplankton, protozoa, and bacteria. The release of sponge cells as part of the sponge loop was simulated as release to PPC for surface sponges. Cavity sponges release cells to cavity particulate organic carbon (CPC). It was assumed that CPC was separate from PPC and only available to detritivores not included in the model. Additionally, both surface and cavity sponges have an export flow to spongivores not included in the model.

The LIM in each season consists of 104 flows, 16 compartments, and 133 equalities, or inequalities. All data introduced to the model was added as ranges (inequalities) to incorporate variability in measurements, or uncertainty of literature values, except for when a flow was 0. This occurred only for certain PPC or PDC uptake rates for the benthos when functional groups in certain seasons only showed release of either PPC or PDC, the uptake rate was then set to 0 (max. 4 flows per model). The models were solved for the flow values by a Monte-Carlo sampling method (Soetaert & van Oevelen 2009). Briefly, 10.000 food web structures were sequentially sampled resulting in 10.000 estimates for each C flow. All estimates were different, but consistent with the matrix equations constructed from the food web topology and entered values. The mean and standard deviation of the sample collection for each flow as presented in the results section represent the best

estimate and a measure of its uncertainty respectively. The model unit for all flows is mmol C m^{-2} reef surface d^{-1}; see below for respective conversions of rates to this unit.

Data treatment for model input

Benthic cover

The two dimensional (2D) benthic cover percentages for the benthic compartments measured using the photo-quadrats were recalculated to three dimensional (3D) surface areas per planar m^2 of reef surface. This was done by 3D/2D conversion factors which were calculated from two measurements of specimens used for the primary production incubations (Table 3.1). First, the 3D surface area of specimens was determined using the same methodologies as used in the metabolic incubations. Second, vertical photographs were taken from all specimens under natural conditions. The vertical pictures were then digitally analyzed with Image J (Schneider et al. 2012) to quantify specimen 2D planar surface area. The 3D surface areas were divided by corresponding 2D planar surface areas to obtain conversion factors. The soft coral conversion was based on measurements of Xeniidae since this group dominated soft coral benthic cover. Branching hard coral conversion was based on the average of measurements on *Acropora* and *Stylophora* by Naumann et al. (2009). Their measurements, which were done on larger fragments and coral colonies than the fragments used here for the primary production incubations, better resembled the size of colonies in-situ, therefore resulting in more accurate conversion factors. Other hard coral and coral rock conversion factors were based on incubated specimens of the genus *Goniastrea* and coral rock respectively. The benthic cover analysis from the "U7" photographs included more categories than incubated. Conversion factors for these categories were derived from conversion factors that were available from direct measurements. Dead coral (no live tissue, but skeletal structure more clear than in coral rock) was assumed to be the average of branching and other corals. The macroalgae *Caulerpa*, growing in loose bundles, was directly measured for its 3D/2D conversion, while *Lobophora* and other macroalgae were assumed to adhere to their substrate. Their conversion was therefore based on an average of coral rock and dead coral. Rubble (small fragments of reef structure lying on sediment areas) was assumed to have the same conversion factor as coral rock. Carbonate sediment was given a conversion factor of 1. The turf algae conversion factor was calculated as the average of its underlying substrates (coral rock, rubble, dead coral, and sediment) weighed by the relative cover of turf algae on each of these substrates. All these conversions resulted in cm^2 3D functional group surface per 2D planar m^2 reef area (Table 3.1) which were then combined with the measured C fluxes from the incubations to produce values per group per m^2 reef area (Appendix 2 - Tables 2-3). The 8 m^3 water column was integrated to the planar reef surface. Surface sponges benthic cover was already measured three

dimensionally in the planar quadrat measurements and could be used directly while the 3D cavity sponge surface area was already available per 2D m^2 reef area from Richter et al. (2001).

Biomasses

To specify the consistency of the C entering the reef from offshore, estimates were made in each season for biomasses of the water column compartments (Appendix 2 - Table 1). PDC and total PPC (including all plankton) concentrations were measured in the environmental monitoring (Table 3.2). Phytoplankton biomass was based on Chl a concentration assuming μg C = 60 x μg Chl a (Yahel et al. 1998). Bacterioplankton biomass was based on measured heterotrophic bacteria abundances; assuming 20 femtogram C bacterial $cell^{-1}$ (Lee & Fuhrman 1987). The protozoa compartment was assumed to consist of ciliates and heterotrophic flagellates. Ciliate biomass was taken as minimum and maximum values for Gulf of Aqaba surface waters from Claessens et al. (2008). Heterotrophic flagellate biomass was calculated from cell abundances ranging from 5 x 10^5 to 1 x 10^6 L^{-1} (Berninger & Wickham 2005), a flagellate cell volume of 15.09 μm^3 (Borsheim and Bratbak 1987), and a mass of 200 femtogram C μm^3 flagellate $cell^{-1}$ (Van Duyl et al. 1990). Zooplankton biomass in Jordanian fringing reefs ranges between 10 and 20 mg dry weight m^{-3} (Al-Najjar & El-Sherbiny 2008). C content was assumed to be 40% of dry weight (Parsons et al. 1984). The detrital component of total PPC (described in other sections as PPC) was determined by subtracting plankton biomasses from total PPC. Ranges were used for the biomasses based on seasonal mean ± SD of the weekly environmental monitoring to incorporate variation within each season. The fractions that pelagic compartments comprised of total water column C were used to constrain the consistency of C entering the reef (Appendix 2 - Table 1). In addition, a range of 2 - 12 mmol C m^{-2} d^{-1} PPC sedimentation to SPC was entered (Naumann et al. 2012), as well as a direct hard coral to SPC mucus string flow of 1.0 - 2.6 mmol C m^{-2} d^{-1} (Mayer & Wild 2010, Naumann et al. 2012).

Table 3.1. Surface areas of dominant benthic functional groups. Benthic cover quantified from 25 1 m^2 photoquadrats per season (100 random points per photoquadrat). 3D/2D conversion factors used to recalculate benthic cover % to 3D cm^2 for each functional group per planar 2D m^2 reef area. Surface sponge surface area determined by in-situ 3D measurements with a quadrat placed every 10 m along 150 m line transect. Cavity sponge surface area based on Richter et al. (2001). NA = not applicable.

	Benthic cover (%)				3D/2D	Surface area (cm^2 m^2 planar $reef^{-1}$)			
	Winter	Spring	Summer	Fall		Winter	Spring	Summer	Fall
Branching corals	2.71	2.99	2.72	3.97	7.58	2051	2265	2060	3007
Other corals	12.17	12.32	12.94	13.96	2.89	3516	3558	3739	4035
Xeniidae	32.04	28.61	17.96	19.21	6.45	20676	18461	11588	12395
Other soft coral	0.98	2.19	1.85	2.30	6.45	635	1415	1195	1487
Lobophora	0.49	0.86	0.86	0.86	3.88	191	332	335	335
Caulerpa	0.50	2.72	0.24	0.12	1.84	92	500	45	23
Other macroalgae	0.00	0.45	0.12	0.17	3.88	0	175	48	64
Turf algae	3.33	3.28	3.38	3.30	2.77	921	910	936	914
Coral Rock	13.96	13.16	18.84	17.56	2.53	3539	3337	4776	4451
Sediment	28.21	25.22	31.10	30.16	1.00	2821	2522	3110	3016
Surface sponges					NA	400	400	400	400
Total	94.39	91.80	90.02	91.62		34842	33875	28231	28326
Cavity sponges					NA	8200	8200	8200	8200

Metabolic rates

The hourly benthic and water column Pn and R rates were extrapolated to daily fluxes by the following calculations. Pn and corresponding R were summed and then multiplied by average seasonal hours of daylight (Table 3.2) to calculate daily gross primary production (GPP). R was multiplied by 24 h to obtain daily community respiration (Rday). Net community production (NCP) was calculated by subtracting Rday from GPP. The PR ratio was finally calculated by dividing GPP by Rday. PPC and PDC uptake/release rates of all benthos compartments were extrapolated to d^{-1} by multiplying by 24 h while taking into account relative differences between day and night. Night PPC fluxes are 0.67 and 0.55 times those during the day for corals and algae respectively (Haas et al. 2010, Naumann et al. 2010). The algae factor was applied to all non-coral groups. It was assumed that PDC fluxes behaved similarly to PPC fluxes. All these extrapolations were performed on the original rates measured for all replicates incubated. Flows to and from compartments were constrained by the

minimum and maximum measured rates from the incubations to incorporate variation between replicate specimens or samples (Appendix 2 - Tables 2-3). For the PPC and PDC fluxes, this meant that groups might both take up and release C as some replicates showed net uptake while others showed release. Planktonic GPP resulted in negative values for some replicates in each season due to higher O_2 consumption in Pn incubations than in R incubations. Because of this, minimum Planktonic GPP in all seasons was set to 0. Planktonic Rday was used as the sum of respiration by all planktonic compartments. Seasonal sponge Rday and PPC or CPC release rates were based on values measured during October. These values were assumed to be applicable to summer and fall, but were adjusted for winter and spring. The sponge genus *Mycale* was incubated in all seasons (chapter 6). Relative differences in summer-fall vs. winter and spring in Rday and PPC or CPC flows in those incubations were used to adjust the overall surface and cavity sponge flows to winter and spring conditions.

Metabolic constraints

Metabolic constraints were placed on compartments which flows were less defined by measurement data to regulate the balance between uptake (i.e. ingestion), assimilation, and production of biomass. Sediment, coral rock, zooplankton, and protozoa all were constrained by assimilation efficiencies: assimilation is 40 - 80% of uptake (Banse 1979, Hendriks 1999), and production efficiencies: production is 30 - 60% of assimilation (Calow 1977, Banse 1979, Hendriks 1999). In addition, coral rock grazing on phytoplankton was constrained specifically (Yahel et al. 2006). Rates in ng Chl a cm^{-2} rock surface h^{-1} for March and September - November were converted for winter-spring and summer-fall respectively to daily fluxes in the reef assuming the Chl a to C conversion mentioned above and the benthic cover of coral rock in the reef in each season. Total zooplankton uptake was constrained by an upper limit using the maximum biomass estimate and water temperature with the following formula: uptake = 0.49 x biomass x $e^{0.0693T}$ (Vezina & Platt 1988). Zooplankton and protozoa grazing on other biota were constrained by grazing rates: zooplankton: 0.007 to 0.013 d^{-1}, protozoa: 0.15 to 1.1 d^{-1} for bacteria and 0.15 to 1.3 d^{-1} for phytoplankton (Grossart & Simon 2002, Sommer et al. 2002). Protozoan production was also constrained by a growth rate of 0.15 d^{-1}: range implemented in the models: 0.05 d^{-1}* min biomass to 0.25 d^{-1}* max biomass (Sakka et al. 2000). In addition, zooplankton and protozoa excretion (excretion = uptake - assimilation) was constrained as 0.33 to 1 x Rday (Niquil et al. 1998). Bacterioplankton flows were constrained by production = 0.1 to 0.6 x uptake (del Giorgio & Cole 1998) and growth = 0.15 to 1.3 d^{-1} x biomass (Grossart & Simon 2002, Sommer et al. 2002). Phytoplankton flows were constrained by Rday = 0.05 to 0.3 x GPP, and excretion = 0.05 to 0.5 x GPP (Niquil et al. 1998). Heterotrophic feeding by hard corals was constrained as 0.2 to 0.6 x Rday (Grottoli et al. 2006). The same constraint was placed on the soft corals, but heterotrophic feeding was only assumed for the

fraction not consisting of Xeniidae since this family has an almost completely autotrophic lifestyle (Schlichter et al. 1983). Sponge flows not directly measured were constrained by Rday and POC release data from incubations, as well as a C assimilation (PPC + PDC) to POC release efficiency of 11 to 24% (de Goeij et al. 2013).

Statistical analyses

The first 1000 solutions sets of every season were analyzed using Primer-E v6 (Clarke & Gorley 2006) with PERMANOVA extension (Anderson 2001) for univariate distance-based permutational nonparametric analyses of variance (PERMANOVA). Solution sets were square root transformed, after which a Bray-Curtis similarity matrix was constructed. A PERMANOVA with Type III sums of squares was used with 999 permutations and residuals under a reduced model due to the size of the dataset. A main test and subsequent pair-wise tests were performed for the factor season assuming a significant difference at $p < 0.05$. The comparison between reduced solution sets was visualized with a multi-dimensional scaling plot (MDS).

RESULTS

The environmental monitoring revealed a clear separation between seasons in light, temperature, and inorganic nutrient concentrations (Table 3.2). Chl a concentrations were lower during low nutrient summer, while PDC concentrations were higher in that season.

All seasonal models were solvable when allochthonous C was allowed to flow into the reef. However, when this inflow was turned off, the summer and fall models were no longer solvable. The models in those seasons required inflow of external organic C to balance their loss terms, while the winter and spring models could rely solely on internal GPP. Significant differences were found between all seasons overall, and between each two seasons separately for the reduced solution sets used in the PERMANOVA (Appendix 2 - Table 4). The MDS visualized a strong difference between the flow estimate collections of winter and spring vs. summer and fall. Additionally, summer and fall were more comparable to each other than winter and spring (Fig. 3.1).

Table 3.2. Seasonal measurements of environmental parameters in the research area. Temperature and irradiance values based on 1 min frequency measurements between 12:00 and 14:00 on all days encompassing each respective sampling period (*n* = 18-27 days). Inorganic nutrient, Chl a, PPC, and PDC concentrations based on weekly measurements in the four weeks encompassing each sampling period. Values are given as mean ± SD. Feb = February, Apr = April, Sep = September, Nov = November. PAR = photosynthetically active radiation. Nitrogen oxides = nitrate + nitrite concentrations. Chl a = chlorophyll a, PPC = pelagic particulate organic carbon, PDC = pelagic dissolved organic carbon.

	Winter (Feb)	Spring (Apr)	Summer (Sep)	Fall (Nov)
Temperature (°C)	22.7 ± 0.2	23.0 ± 0.2	27.2 ± 0.3	25.3 ± 0.2
PAR (µmol photons m^{-2} $s^{-1)}$	147 ± 60	281 ± 61	320 ± 63	162 ± 63
Hours of daylight	11.22	12.82	12.38	10.65
Ammonium (µmol L^{-1})	0.32 ± 0.09	0.46 ± 0.07	0.11 ± 0.01	0.28 ± 0.12
Phosphate (µmol L^{-1})	0.11 ± 0.01	0.10 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Nitrogen oxides (µmol L^{-1})	0.71 ± 0.15	0.56 ± 0.12	0.06 ± 0.02	0.22 ± 0.23
Chl a (µg L^{-1})	0.21 ± 0.03	0.21 ± 0.01	0.10 ± 0.01	0.19 ± 0.05
PPC (µmol L^{-1})	6.16 ± 1.29	10.25 ± 2.95	7.96 ± 2.46	8.81 ± 0.98
PDC (µmol L^{-1})	72.92 ± 5.21	71.95 ± 7.54	90.49 ± 1.03	86.24 ± 2.50

Benthic cover

The combination of planar benthic cover estimates and 3D/2D conversion factors resulted in a benthic cover dominated by soft corals (Table 3.1). Soft coral benthic cover declined from spring to summer, coinciding with an overall reduction in total 3D surface area per planar m^2. The decline of soft coral cover led to an increase in coral rock as this substrate was uncovered. However, the lower 3D/2D conversion factor of coral rock compared to soft corals resulted in the overall decline in 3D surface area. Macroalgae benthic cover peaked in spring, while turf algae cover was stable over the seasons.

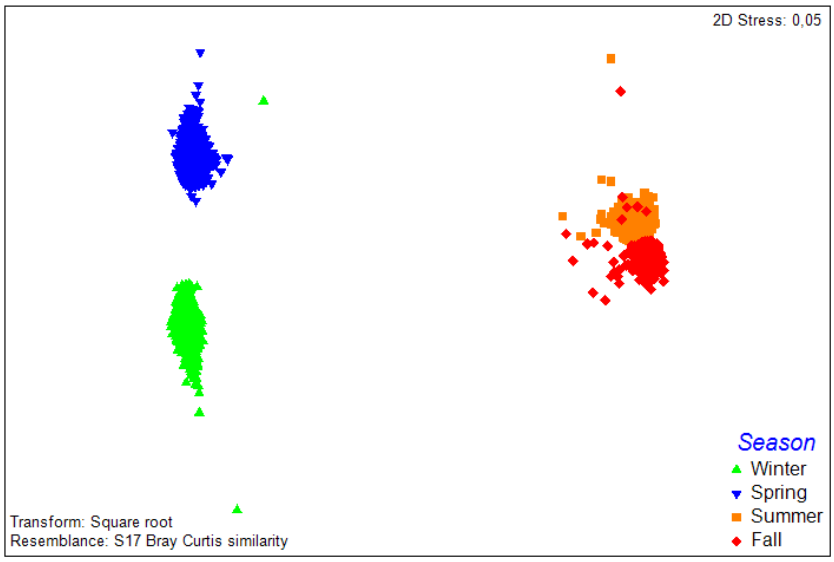

Fig. 3.1. Multi-Dimensional Scaling plot of first 1000 solution sets of every season. Based on Bray-Curtis similarity matrix after solution sets were square root transformed.

Model output

The mean flow values returned by the four seasonal models cover five orders of magnitude (Fig. 3.2), the one exception being DOC uptake by macroalgae in spring (mean: 6.2 x 10^{-4} mmol C m^{-2} d^{-1}, not depicted in Fig. 3.2 and Appendix 2 - Fig 2). The largest flows in all seasons were cavity sponge DOC uptake, hard and soft coral GPP, and bacterioplankton DOC uptake, ranging from ca. 40 to 540 mmol C m^{-2} d^{-1} (Fig. 3.2, Appendix 2 - Fig. 1-4). The smallest flows in all seasons were grazing by zooplankton and soft corals, as well as organic C uptake and release by macroalgae: all flows < 1 mmol C m^{-2} d^{-1}. The standard deviations of the flows are a measure of how well they are constrained. Overall, the flows in each season appear well constrained, especially so for the larger and therefore more important flows (Appendix 2 - Fig. 1-4). The coefficient of variation (CoV, i.e. standard deviation normalized to its corresponding mean) for the flows in each season also reveals good constraint. CoV < 0.5 for 51 to 56% of the flows and < 0.7 for 73 to 75% of the flows over the seasons. Total reef GPP was highest in spring, while Rday was highest in summer and fall (Fig. 3.3). Mean NCP was positive in winter and spring, close to 0 in summer, and negative in fall. Correspondingly, mean PR ratio was 1.3 and 1.4 for winter and spring respectively. Summer mean PR was 1.0 and fall mean PR was 0.83, indicating that the reef C balance was more heterotrophic in summer and fall than winter and spring. This is also visible in the relative balance between GPP and the required inflow of external organic C (Fig. 3.3; Table 3.3). GPP/inflow was 1.3 and 1.5 in winter and spring, but declined strongly to 0.4 and 0.3 in summer and fall. Organic C inflow was dominated by DOC; it constituted 88 - 92% of total inflow (Table 3.3).

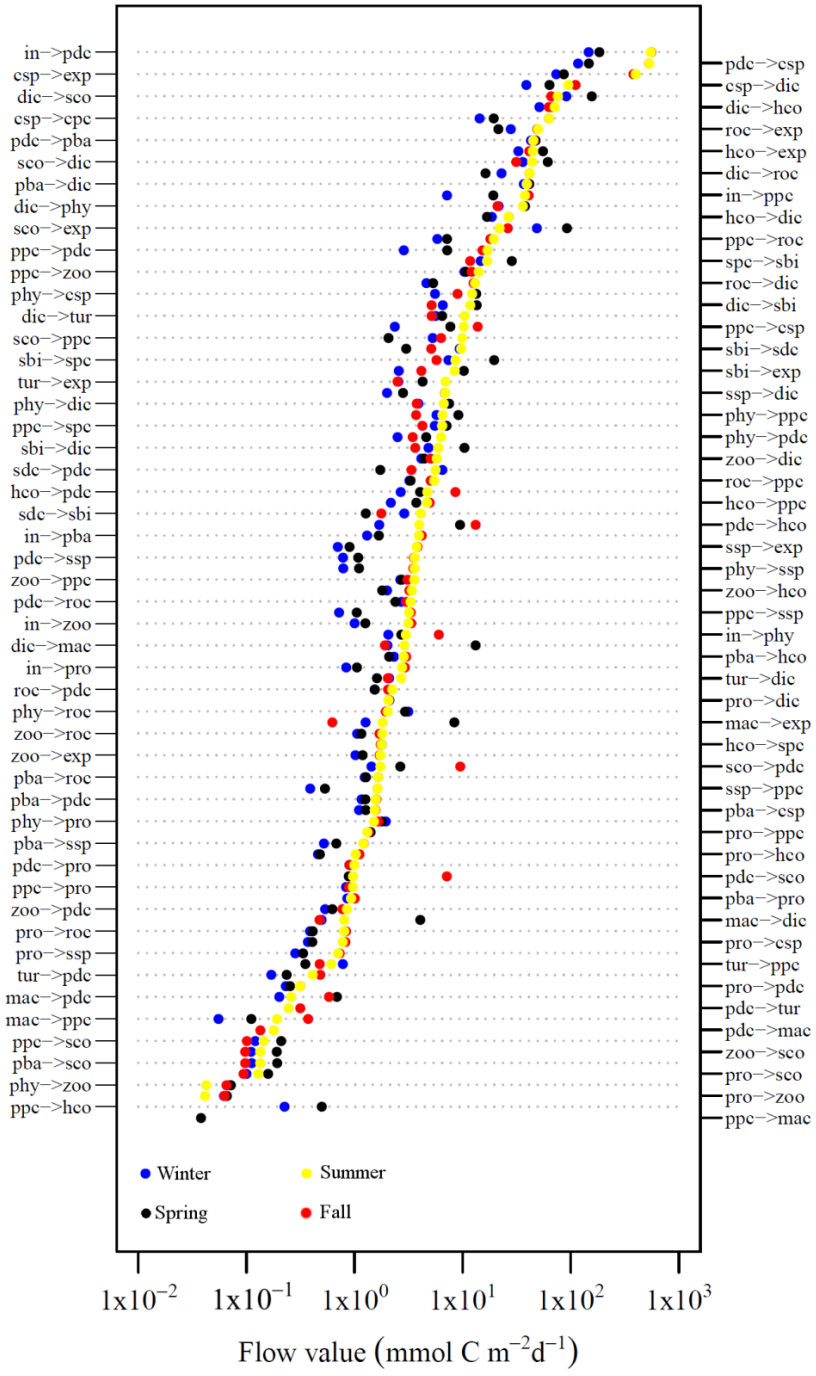

Fig. 3.2. Means of all flows in the seasonal food web models. Ordered for flows in the summer model (high to low). cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.

Hard and soft corals were the dominant contributors to GPP (Fig. 3.4). Their combined contribution ranged from 59% in summer to 71% in spring. Planktonic GPP was relatively stable and contributed between 11 and 14% to total GPP. The benthos was most important for Rday during winter (26, 30, 43% contribution to Rday for sponges, water column, and benthos respectively). However, sponge respiration increased strongly from spring to summer and fall, causing higher overall Rday, and a relative contribution of sponges up to 48%, while benthos contribution declined to 31% (Fig. 3.5). Combined surface and cavity sponge DOC uptake is one of the largest processes in the reef, outranking system GPP in summer and fall (Fig. 3.6). Sponge DOC uptake was also up to more than 10-fold higher than either benthic or pelagic DOC uptake in summer and fall (Fig. 3.7).

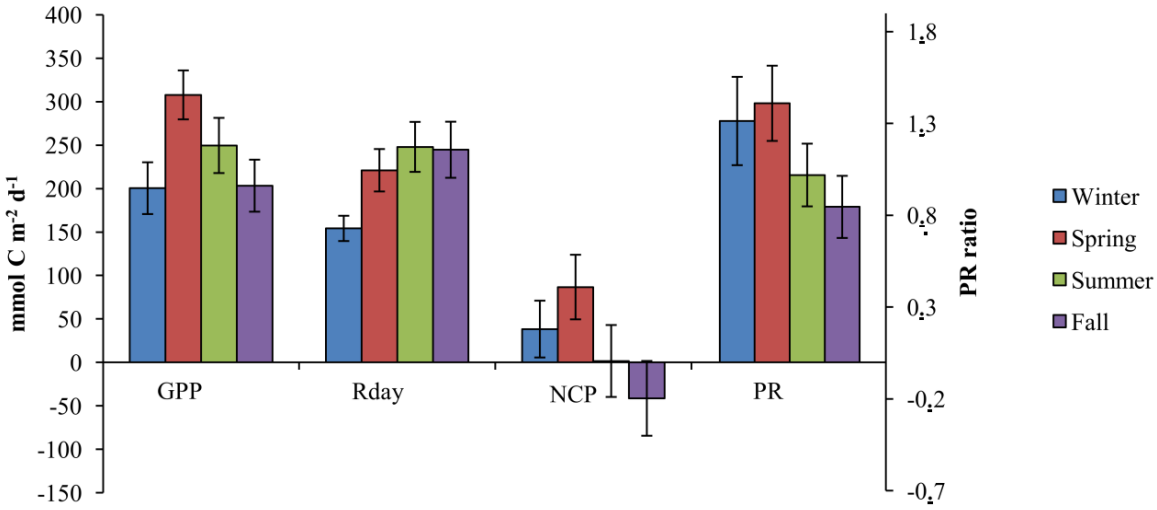

Fig. 3.3. Metabolic parameters of the coral reef community over the seasons. Error bars indicate ± SD. GPP = gross primary production, Rday = community respiration, NCP = net community production, PR = gross primary production to community respiration ratio. GPP, Rday, and NCP are related to the left y-axis, PR ratio is related to the right y-axis.

Table 3.3. Inflow of C into the model over the seasons. Composition of inflow was constrained by water column biomasses from environmental monitoring and literature (Appendix 2 - Table 1). Water column integrated to reef surface: all values in mmol C m^{-2} d^{-1}, values given as mean ± SD.

	Winter	Spring	Summer	Fall
Protozoa	0.84 ± 0.18	1.05 ± 0.20	2.74 ± 0.53	2.92 ± 0.57
Zooplankton	1.00 ± 0.15	1.26 ± 0.17	3.13 ± 0.52	3.34 ± 0.54
Bacteria	1.30 ± 0.06	1.68 ± 0.13	3.93 ± 0.09	4.17 ± 0.10
Phytoplankton	2.06 ± 0.10	2.71 ± 0.17	2.98 ± 0.16	6.02 ± 0.70
PPC	7.17 ± 0.64	19.23 ± 2.34	37.55 ± 6.18	40.82 ± 1.14
PDC	146.08 ± 5.23	183.73 ± 8.29	548.50 ± 12.74	556.41 ± 12.87
Total	158.44 ± 5.70	209.66 ± 9.63	598.85 ± 14.01	613.67 ± 14.24

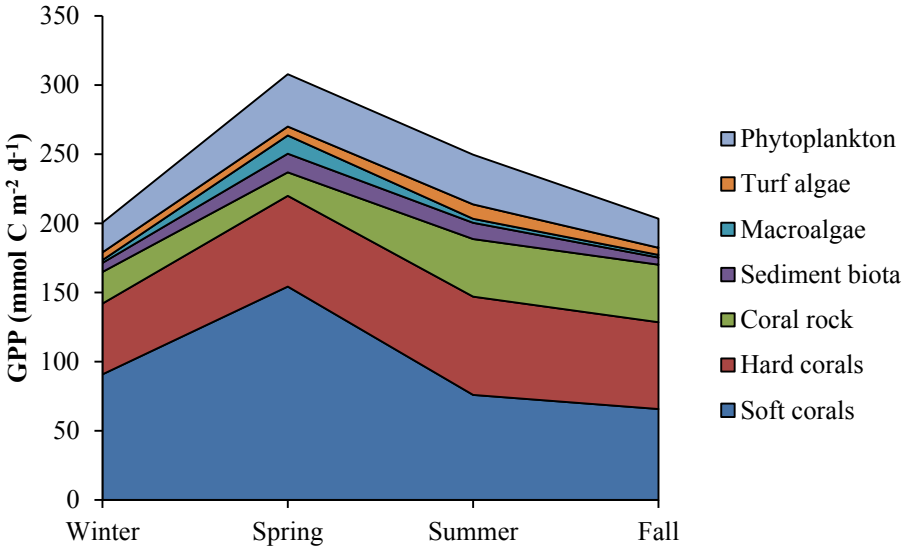

Fig. 3.4. Gross primary production (GPP) over the seasons for the photosynthetic functional groups.

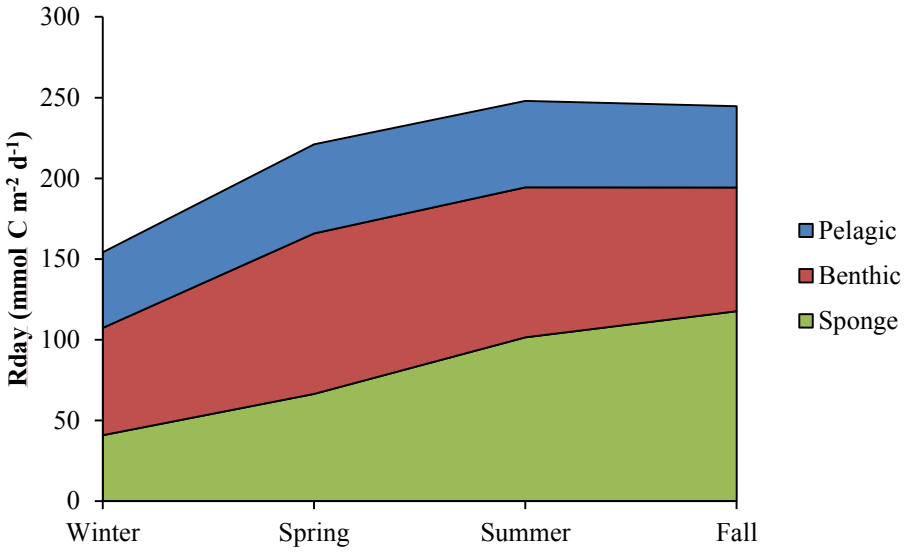

Fig. 3.5. Community respiration (Rday) for the main coral reef compartments over the seasons. Sponge = surface sponges and cavity sponges.

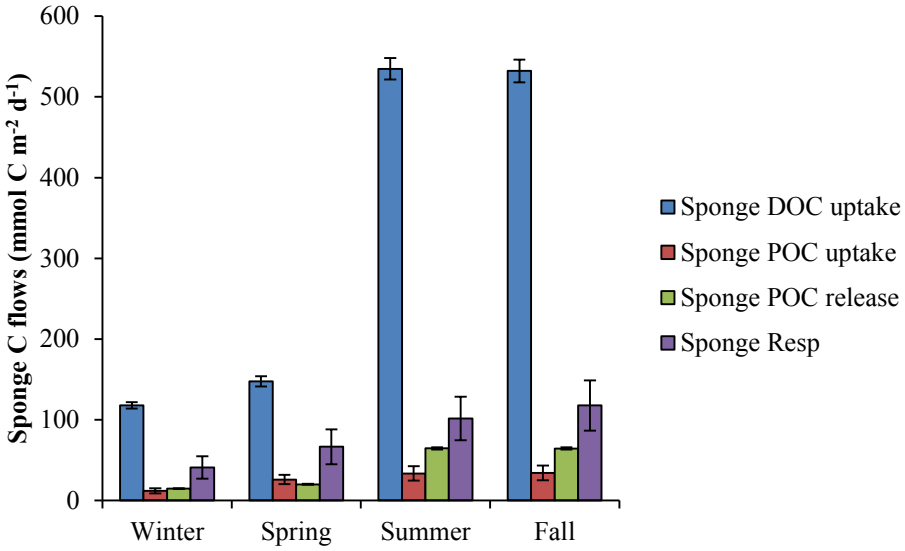

Fig. 3.6. Summed carbon flows of the cavity and surface sponges over the seasons, values given as mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, Resp = respiration.

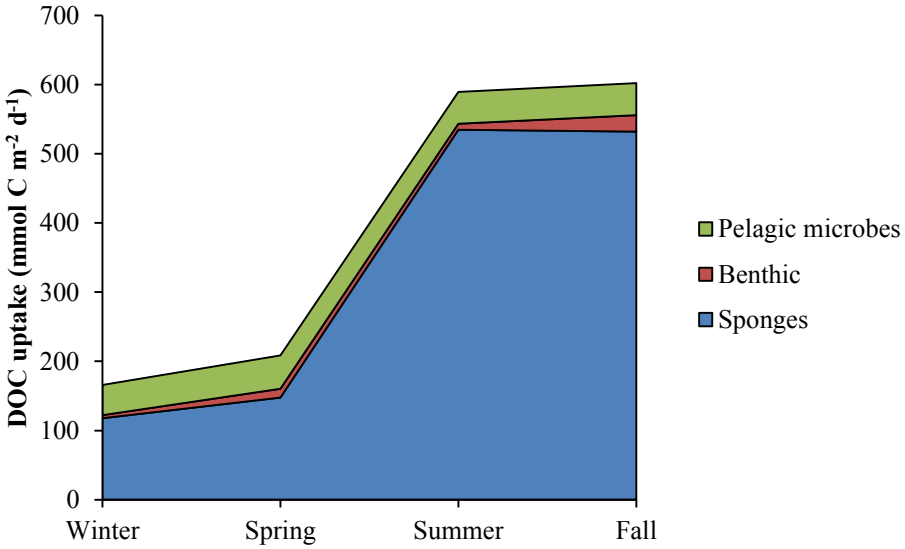

Fig. 3.7. Net dissolved organic carbon (DOC) uptake by the main coral reef compartments over the seasons. Sponges = surface sponges + cavity sponges.

Table 3.4. Export of C from model compartments over the seasons. Export flows simulate processes not included in the model. All values in mmol C m^{-2} d^{-1}, values given as mean ± SD.

	Winter	Spring	Summer	Fall
Zooplankton	1.02 ± 0.87	1.19 ± 0.96	1.75 ± 1.46	1.70 ± 1.39
Macroalgae	1.26 ± 0.59	8.38 ± 3.90	1.82 ± 0.59	0.62 ± 0.42
Turf algae	2.55 ± 0.72	4.25 ± 1.14	6.95 ± 1.09	2.49 ± 0.51
Sediment	2.57 ± 1.83	3.00 ± 1.68	8.42 ± 3.12	4.14 ± 2.07
Coral rock	27.84 ± 8.41	21.42 ± 9.97	49.88 ± 11.83	49.09 ± 15.02
Hard corals	32.66 ± 17.71	55.26 ± 21.94	44.58 ± 27.67	41.40 ± 25.37
Soft corals	48.59 ± 25.65	92.12 ± 19.26	21.87 ± 13.79	26.10 ± 14.93
Surface sponges	0.70 ± 0.49	0.90 ± 0.62	3.74 ± 2.33	3.81 ± 2.36
Cavity sponges	73.23 ± 13.82	86.27 ± 21.50	398.63 ± 28.26	380.23 ± 32.18

DISCUSSION

Modeled community C cycling

The annual ranges of mean GPP, Rday, NCP, and PR are comparable to values found for other reefs (Table 3.5). In general, metabolic rates were more comparable to other reef slopes than reef crests and flats. Those shallower reef areas receive substantially more light, and may have increased rugosity covered by primary producers, leading to increased levels of GPP and corresponding Rday (Long et al. 2013). NCP and PR show that the reef community is net autotrophic in winter and spring, relatively balanced in summer, and net heterotrophic in fall. This is similar to measurements from a reef flat across the Gulf of Aqaba in Eilat, Israel (Silverman et al. 2007). GPP was highest in spring when light levels and inorganic nutrients were relatively high. The reduced nutrient concentrations in summer may have limited GPP while light was readily available (Larned 1998). Rday was highest in summer and fall, coinciding with highest temperatures. Metabolism of organisms in general increases with increasing temperature (Gillooly et al. 2001). These parameters together with the autotrophic and heterotrophic character of the community during the winter-spring and summer-fall seasons respectively indicate that community metabolism in this reef is strongly affected by seasonal changes in environmental conditions.

Table 3.5. Comparison of metabolic parameters with literature. All values in mmol C m^{-2} d^{-1} and given as means, or ranges. GPP = gross primary production, Rday = community respiration, NCP = net community production, PR = gross primary production to community respiration ratio. An O_2:C factor of 1 was assumed when O_2 fluxes were transformed to C fluxes (Gattuso et al. 1996; Carpenter & Williams 2007).

Site	GPP	Rday	NCP	PR	Reference
Aqaba, Jordan, Reef slope, seasonal range	200 - 308	154 - 248	-41 - 87	0.8 - 1.4	This study
Eilat, Israel, Reef crest, winter	260	180	80	1.4	Silverman et al. (2007)
Eilat, Israel, Reef crest, summer	400	390	8	1.0	Silverman et al. (2007)
French Frigate Shoals, Reef flat, winter	356	213	142	1.67	Atkinson and Grigg (1984)
French Frigate shoals, Reef flat, summer	710	405	305	1.75	Atkinson and Grigg (1984)
Kaneohe bay, Hawaii, Reef flat	400	465	-65	0.86	Falter et al. (2008)
Florida Keys, Reef crest	944	566	378	1.7	Long et al. (2013)
Florida Keys, Reef slope	193	199	-6	0.97	Long et al. (2013)
Colombian Caribbean, Reef slope, seasonal range	250 - 305	136 - 147	103 - 169	1.7 - 2.2	Eidens et al. (2014)
Various Caribbean/Pacific, Reef slope	167 - 583	158 - 250	-83 - 425	0.5 - 5.5	Hatcher (1988)

Organic matter release by corals has been identified as an essential process in coral reef dynamics since this matter becomes available to other reef organisms and functions as a particle trap for planktonic matter, which is then brought into the reef C and nutrient cycles (Wild et al. 2004, Mayer & Wild 2010). Total annual mean net organic C release by hard and soft corals (2.7 and 7.3 mmol C m^{-2} d^{-1} respectively) and their relation to total benthic GPP (1.1 and 3.1% respectively) are comparable to values previously estimated for the studied reef (Naumann et al. 2012). The organic matter flows through the sponge compartments were based solely on POC release measured during incubations and a (POC+DOCin) / POCout turnover efficiency of 11-24% (de Goeij et al. 2013). Model estimations of sponge-related flows will therefore be compared to measurements from literature. The sponge POC release rates were lower, but comparable to those found for three sponge genera from other locations when assuming cavity sponge biomass of 1.76 mol C m^{-2} planar reef (Table 3.6; Richter et al. 2001, Alexander et al. 2014). DOC uptake rates during summer and fall were comparable to measurements in the Caribbean (de Goeij et al. 2008b). Temperatures during those seasons were similar to those in the Caribbean research site on the island of Curacao (Alexander et al. 2014). The contribution of DOC to total TOC (TOC = POC + DOC) uptake was comparable to measurements of another sponge from the Gulf of Aqaba (Yahel et al. 2003). Summer and fall respiration rates measured for the sponges were also comparable to those measured by de Goeij et al. (2008b). Richter et al. (2001) estimated cavity community phytoplankton grazing in the studied reef. Seasonal mean phytoplankton grazing by cavity sponges as estimated by the model was lower, but did not include the remaining cavity community of filter feeders. All these data indicate that the flows

estimated by the model fall within natural ranges measured in other sponges. Rates of DOC to POC transformation by the sponge loop were comparable to those estimated for Caribbean and Indo-Pacific reefs (de Goeij et al. 2013). Pelagic microbial DOC uptake and subsequent C availability to higher trophic levels through the microbial loop were also comparable to estimates from that study, and the sponge loop activity was up to tenfold higher than that of the microbial loop (Table 3.7).

Table 3.6. Comparison of sponge flows to literature. All values given as mmol C m^{-2} d^{-1} and ranges or mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, TOC = total organic carbon = DOC + POC.

	This study	Literature	Location	Reference
DOC uptake	118 - 535	645 ± 123	Curacao	de Goeij et al. (2008b)
DOC/TOC uptake	85 - 94%	>90%	Eilat, Israel	Yahel et al. (2003)
POC production	15 - 65	44 - 316	Curacao	Alexander et al. (2014)
Phytoplankton uptake	6 - 13	74 ± 4	Aqaba, Jordan	Richter et al. (2001)
Respiration	41 - 118	137 ± 37	Curacao	de Goeij et al. (2008b)

Table 3.7. Comparison of carbon cycling processes over the seasons as estimated by the models. All values in mmol C m^{-2} d^{-1} and given as mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, micr. = microbial. Pelagic micr. available = carbon from the microbial loop available to higher trophic levels = DOC uptake by bacteria and protozoa - excretion and respiration. The external POC trapping estimate is based on an 8-fold increase in carbon content by released coral mucus (Wild et al. 2004), it was calculated from coral POC release, not produced by the model.

	Winter	Spring	Summer	Fall
GPP	200 ± 30	308 ± 28	250 ± 32	203 ± 30
Rday	154 ± 15	221 ± 24	248 ± 29	245 ± 32
Sponge DOC uptake	166 ± 5	209 ± 9	590 ± 14	602 ± 15
Sponge net POC release	15 ± 0	20 ± 1	64 ± 1	64 ± 1
Pelagic micr. DOC uptake	44 ± 3	48 ± 10	46 ± 6	46 ± 6
Pelagic micr. available	3 ± 1	3 ± 1	3 ± 2	3 ± 2
Benthic heterotrophic feeding	17 ± 3	19 ± 3	33 ± 2	32 ± 2
Coral POC release	8 ± 3	5 ± 5	14 ± 5	15 ± 8
External POC trapping	61 ± 23	43 ± 27	116 ± 42	118 ± 60

Data quality

Food web flows estimated by a model are only as good as the data that were entered into it. Many of the data entered into the model were based on metabolic incubations performed ex-situ. Incubations such as these will always have an effect on measured rates since it is impossible to fully simulate in-situ conditions. Care was taken to create in-situ like conditions by using flow through water which was pumped straight from the coral reef at the correct water depth and screens to mimic in-situ light conditions. Photosynthesis and respiration incubations were kept to a minimum duration to prevent hyper- and hypo-oxic conditions. These incubations were stirred to minimize buildup of a boundary layer around specimens which may influence metabolic rates (Dennison & Barnes 1988, Shashar et al. 1993). Organic matter incubations (except for sponges) were not stirred to prevent water currents from modifying the structural composition through e.g. POC dissolution to DOC. However, organic matter release is stimulated by water movement (Wild et al. 2012); organic matter flow rates given here should therefore be interpreted as conservative estimates. The midday incubations for Pn were performed under the highest and most stable light conditions of the day as measured during the environmental monitoring. It is however possible that photosynthetic organisms experience photoinhibition during midday when peak light levels can damage their photosynthetic apparatus (Long et al. 1994, Franklin et al. 1996). However, coral reef primary producers in the Gulf of Aqaba were not found to show signs of photoinhibition, even at light levels far exceeding those found in this study (Levy et al. 2004, Schneider et al. 2009). The use of night time R as an estimate to calculate GPP is common practice (Falter et al. 2008, Long et al. 2013). However, R of organisms and substrates during daylight can be substantially higher than at night (Al-Horani et al. 2003, Glud 2008). Values given for GPP may therefore be underestimated. As incubations did not cover the entire daily cycle, rates were extrapolated to d^{-1}. Especially GPP may be overestimated by this since highest photosynthetic rates are reached during midday, assuming no photoinhibition (Levy et al. 2004, Schneider et al. 2009). The extrapolation of incubation rates to planar m^2 reef area may introduce substantial errors. However, metabolic rates for the reef community are within the range of those found in coral reefs using in-situ whole community methods as further discussed below, see Table 3.5 (e.g. Hatcher 1988, Silverman et al. 2007). Sponges can have photosynthetic symbionts (Erwin & Thacker 2007). However, the dominant surface sponge in the study site displayed minimal levels of photosynthesis (chapter 6), and surface sponge 3D benthic surface area per planar m^2 reef area was 0.01 % of total 3D surface area. The potential surface sponge contribution to GPP was therefore assumed to be minimal and sponge photosynthesis was ignored. Uncertainty due to extrapolations and simulated in-situ conditions during measurements was introduced into the model by constraining flows where possible only to their minimum and maximum measured values.

The LIM as used in this study was solved under the assumption of steady state for each season. This assumption may be in error for a highly active community such as a coral reef. However,

coral reefs generally display a balanced PR ratio, indicating that net growth of the system as a whole is minimal (Hatcher 1988). PR in the Aqaba reef varied over the seasons, but fluctuated around unity and was comparable to other reef communities. Additionally, Vezina and Pahlow (2003) investigated the effect of the steady state assumption in LIMs on simulated food webs which were in steady and transient state. They found that the steady state assumption did not significantly alter the accuracy of the ecosystem flow reconstructions.

The modeled reef community received allochthonous C in every season ranging from 158 to 614 mmol C m^{-2} d^{-1}. The mean water flow speed over the seasons was very stable at ca. 5 cm s^{-1} (van Hoytema unpublished data). This results in a water flow speed of 4.3 km d^{-1}. Total water moving through the model's 8 m^3 water column was therefore 34.6 x 10^3 m^3 d^{-1}. Total water column C ranged from 71.8 to 95 mmol C m^{-3}, resulting in a potential C flow of 2.4 x 10^6 to 3.3 x 10^6 mmol C m^{-2} d^{-1}. Offshore waters most likely have reduced total organic C due to a lack of benthic activity, but the massive amounts of water moving through the reef per day indicate that the inflows of C as modeled are possible, and the vast majority of C flowing through would not be used by the model.

Ecological implications

The community of this relatively high latitude reef revealed a clear response to the strong seasonality in environmental factors. The metabolic balance shifted from an autotrophic period during relatively high nutrient availability to a heterotrophic period during strongly oligotrophic conditions due to stratification, and increased temperatures. Concurrently, the amount of DOC taken up by the sponge loop increased more than 3-fold between these periods, reaching levels 10-fold higher than the pelagic microbial loop. This primarily cavity sponge-mediated pathway therefore may play an essential role in fulfilling this seasonally increased energy demand of the community. Trapping planktonic particles may increase the C concentration of coral released POM up to eightfold (Wild et al. 2004). This factor which was measured in the Great Barrier Reef and corrected for compaction of the aggregates as applied there, is unavailable for the studied reef. However, comparable gross enrichment of POM was found for the studied reef by Mayer and Wild (2010). A particle trapping capacity of seven times its own C content with subsequent introduction of that allochthonous C into the reef C cycle would place this pathway among the top processes bringing C into the reef community (Table 3.7). Potentially increased particle trapping rates in summer and fall as estimated in Table 3.7 may additionally support the coral reef community during more heterotrophic seasons.

The cavities that riddle the coral reef framework have been described as the largest but least researched habitat in coral reefs (Richter et al. 2001). The high sponge DOC uptake values should be seen as conservative since the assumed cavity sponge benthic cover per planar reef m^2 was an average of several Gulf of Aqaba reefs; mean cavity surface area was higher for the studied reef specifically (Richter et al. 2001). In addition, cavities in the reef structure extended deeper than could be

measured by the experimental setup of Richter et al. (2001), indicating that rates of processes mediated by cavity-dwelling organisms may be higher per planar reef surface area than presently assumed. Further research into these cavities is warranted since the sponges and their microbial symbionts that inhabit them may additionally play a presently unquantified role in the nitrogen cycle which could provide essential nutrients to the wider reef community in its oligotrophic surroundings (Wilkinson & Fay 1979, Webster & Taylor 2012).

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CHAPTER 4

CORAL MUCUS FUELS THE SPONGE LOOP IN WARM- AND COLD-WATER CORAL REEF ECOSYSTEMS

Rix L, Naumann MS, Mueller CE, de Goeij JM, Stuck U, Middelburg J, van Duyl FC, Al-Horani FA, Wild C, van Oevelen D. Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. This chapter is under review in Scientific Reports.

ABSTRACT

Shallow warm-water and deep-sea cold-water corals engineer the coral reef framework and fertilize reef communities by releasing coral mucus, a source of reef dissolved organic matter (DOM). By transforming DOM into particulate detritus, sponges play a key role in transferring the energy and nutrients in DOM to higher trophic levels on Caribbean reefs via the so-called sponge loop. Coral mucus may be a major DOM source for the sponge loop, but mucus uptake by sponges has not been demonstrated. Here we show the transfer of coral mucus into the bulk tissue and phospholipid fatty acids of the warm-water sponge *Mycale fistulifera* and cold-water sponge *Hymedesmia coriacea*, demonstrating a direct trophic link between corals and reef sponges. Further, 21 – 40% of the mucus carbon (C) and 32 – 39% of the nitrogen (N) assimilated by the sponges was subsequently released as detritus, confirming a sponge loop on Indo-Pacific warm-water and Atlantic cold-water coral reefs. Preferential uptake of mucus N by *M. fistulifera* resulted in enhanced N recycling, indicating the importance of the sponge loop in nutrient retention on oligotrophic warm-water reefs. Higher mucus C uptake by *H. coriacea* suggests a key role of the sponge loop in energy conservation on cold-water reefs.

INTRODUCTION

Scleractinian corals act as ecosystem engineers on warm-water (WW) and cold-water (CW) coral reefs by forming the complex 3D-reef framework and driving reef biogeochemical cycles (Freiwald et al. 2004, Wild et al. 2011). While WW coral reefs thrive in the warm, shallow and oligotrophic waters of the tropics, CW reefs are globally distributed along continental shelves, slopes and seamounts in the cold, deep, nutrient-rich waters below the photic zone (Roberts et al. 2006). On shallow WW reefs, corals form endosymbiotic associations with photosynthetic dinoflagellates (zooxanthellae), enabling them to contribute to high benthic autotrophic productivity (Muscatine et al. 1984, Hatcher 1990). Deep-sea CW corals by contrast lack zooxanthellae and instead rely on heterotrophic feeding to meet their energetic requirements (Mortensen 2001, Naumann et al. 2011, Mueller et al. 2014b). Inorganic nutrient availability may limit the autotrophic primary productivity of WW coral reefs in oligotrophic waters (Hatcher 1990). Conversely, the metabolism of CW reefs relies on secondary production and is therefore limited by the quality and quantity of external organic carbon (C) input (Roberts et al. 2006). Consequently, CW reefs are typically restricted to oceanic regions with high surface primary production and enhanced vertical transport due to elevated currents (Duineveld et al. 2004, Mienis et al. 2007, Davies et al. 2009). Despite these pronounced environmental differences (Appendix 1 - Table 1) both WW and CW reefs are considered hotspots of marine biodiversity and biological activity (Roberts et al. 2006). The mechanisms by which they manage inorganic nutrient (WW) and organic carbon (CW) limitation are under debate, but efficient pathways of energy and nutrient cycling are essential for maintaining the high productivity and biodiversity of these contrasting reef ecosystems. Here, we propose that a trophic link between two reef ecosystem engineers, corals and sponges, contributes to sustaining WW and CW reef ecosystems through the recycling of coral mucus, a key organic resource on shallow and deep-sea coral reefs (Wild et al. 2004a, Wild et al. 2008, Wild et al. 2009).

Scleractinian corals secrete a surface mucus layer that is continuously released into the water column in particulate and dissolved forms, thereby substantially contributing to reef organic matter pools (Johannes 1967, Wild et al. 2004a, Wild et al. 2008). Despite environmental and metabolic differences, WW and CW corals release mucus at comparable rates (Wild et al. 2008, Naumann et al. 2010a, Naumann et al. 2014) and devote substantial energy into mucus production; up to 40% of the net C fixed by WW corals (Crossland et al. 1980, Naumann et al. 2011, Tremblay et al. 2012). Composed of a complex mixture of carbohydrates, lipids, and proteins (Ducklow & Mitchell 1979, Meikle et al. 1988, Wild et al. 2010), coral mucus is an energy-rich substrate that acts as an important energy and nutrient carrier on coral reefs (Wild et al. 2004a). The particulate fraction of released mucus functions as a particle trap (Wild et al. 2004a, Mayer & Wild 2010) facilitating the formation of aggregates that can act as a substrate for various reef organisms (Bythell & Wild 2011). However, the majority of released mucus (56 – 80%) dissolves in surrounding reef waters (Wild et al. 2004a, Wild et

al. 2008), making it largely unavailable for most reef fauna. Research on mucus recycling has primarily focused on microbial degradation in the water column and reef sediments where it rapidly stimulates bacterial growth and respiration enabling remineralization and recycling via the microbial loop (Wild et al. 2004b, Wild et al. 2009, Maier et al. 2011).

Recently, the so-called "sponge loop" has been identified as an alternative pathway for transferring dissolved organic matter (DOM) to higher trophic levels on Caribbean WW reefs (de Goeij et al. 2013). Despite largely being considered particle feeders (Reiswig 1971, Pile et al. 1997) a number of sponges have been found to feed on DOM, with DOM accounting for up to 90% of the sponge diet (Yahel et al. 2003, de Goeij et al. 2008b, van Duyl et al. 2008, Ribes et al. 2012, Mueller et al. 2014a). Sponges subsequently transform a substantial fraction of this DOM into particulate organic matter (POM) via detritus production, effectively turning over up to 35% of their body C per day (de Goeij et al. 2008a, de Goeij et al. 2009, Alexander et al. 2014). Sponge detritus is fed on by a variety of motile and filter-feeding detritivores, enabling the energy bound in DOM that is otherwise unavailable to most reef heterotrophs to be utilized by higher trophic levels (de Goeij et al. 2013). In the Caribbean, DOM turnover through sponges amounts to the same order of magnitude as the total gross primary production rates of the entire reef ecosystem (de Goeij et al. 2013). The sponge loop therefore plays a major role in organic matter cycling on Caribbean reefs, but has not yet been investigated in other oceanic regions. Moreover, de Goeij et al. (2013) were only able to show sponge uptake of laboratory-produced diatom DOM, which may not be representative of natural reef DOM. Since coral mucus contributes to reef DOM pools, the sponge loop may play a role in its recycling, but uptake of coral mucus by reef sponges has not been demonstrated.

Here we hypothesize that a direct trophic link between corals and sponges enables the recycling of coral mucus via the sponge loop on WW and CW reefs. Stable isotope tracer experiments using flow-through and incubation set-ups were conducted to investigate the uptake of naturally produced coral mucus from ^{13}C- and ^{15}N-labeled corals (WW: Fungiidae; CW: *Lophelia pertusa*) by the sponges *Mycale fistulifera* (WW) and *Hymedesmia coriacea* (CW). Additional incubations were conducted to demonstrate the transfer of coral mucus into the sponge-produced detritus. Assimilation of coral mucus C into sponge phospholipid-derived fatty acids (PLFAs) was measured to further examine the processing of coral mucus by the sponges and evaluate the potential role of sponge-associated bacteria in its uptake.

MATERIALS AND METHODS

Sample collection and maintenance.

WW corals and sponges were collected from the Marine Science Station (MSS) reef, Aqaba, Jordan (29°27' N, 34°58' E) located in the northern Gulf of Aqaba, Red Sea. Free-living Fungiidae corals from the genera *Fungia, Ctenactis,* and *Herpolitha* were collected from the reef between 8 – 20 m water depth by SCUBA. These genera can be removed from the reef without causing physical damage, are locally abundant, and produce large quantities of mucus (Naumann et al. 2010b). The encrusting sponge *Mycale fistulifera* was selected for experimentation as it is locally abundant and is found in close proximity to living corals. Fragments of *M. fistulifera* were collected between 8 – 12 m water depth by chiseling fragments of dead coral skeleton overgrown by the sponge. Corals and sponges were immediately transferred to the aquarium facilities at the MSS without air exposure. Sponge specimens were trimmed to approximately the same size (0.08 ± 0.04 g DW sponge^{-1}), cleaned of epibionts, and attached to ceramic tiles with coral glue. Corals and sponges were maintained in flow-through aquaria supplied with seawater pumped directly from the reef at 10 m water depth at a rate of ~10 L min^{-1}. Natural light levels were adjusted to *in situ* levels at ~15 m depth (~120 µmol photons m^{-2} s^{-1} PAR) using layers of black mesh and parallel *in situ* and aquarium measurements of photosynthetically active radiation (PAR µmol photons m^{-2} s^{-1}, wavelength 400 - 700 nm) using a quantum sensor (Model LI-192SA; Li-Cor). Sponges were acclimated for 1 week prior to the start of experiments and only healthy individuals that were actively pumping were used. Corals were acclimated for at least 72 h.

The CW coral *Lophelia pertusa* and cold-water encrusting sponge *Hymedesmia coriacea* were collected from Tisler Reef, located at 70 – 155 m depth in the Skagerrak at the border between Norway and Sweden (58°59' N 10°58' E). *L. pertusa* is the main reef-building coral on Tisler Reef, while the sponge *H. coriacea* is locally abundant and commonly found in close contact with *L. pertusa* (Mortensen 2001). Specimens were collected from a depth of 110 m using the remotely operated vehicle Sperre Subfighter 7500 DC and transported in cooling boxes filled with cold seawater (7 – 8°C) within a few hours to the laboratory facilities at the Sven Lovén Centre in Tjärnö, Sweden. Sponge specimens were trimmed to approximately the same size (0.04 ± 0.02 g DW sponge^{-1}) and specimens were cleared of epibionts. All specimens were maintained in flow-through aquaria (~20 L) with sand-filtered water pumped from 45 m depth from the Koster-fjord at a rate of ~1 L min^{-1}. Aquaria were kept in a dark climate-controlled room at 7°C corresponding to *in situ* temperatures on Tisler reef that range from 6 – 9°C. Coral specimens were acclimated for up to 6 weeks, while sponge specimens were acclimated for 1 week prior to experimentation.

Coral labeling.

WW corals were enriched with ^{13}C and ^{15}N by addition of ^{13}C-$NaHCO_3$ and ^{15}N-$NaNO_3$ label compounds (Cambridge Isotope Laboratories, 99% ^{13}C and 98% ^{15}N), which are taken up and transferred to the coral host via photosynthetic endosymbionts (i.e. zooxanthellae) (Naumann et al. 2010b). For 8 days, each morning at 08:00 the inflows to the coral aquaria were stopped and 36 mg L^{-1} $NaH^{13}CO_3$ and 1 mg L^{-1} $Na^{15}NO_3$ were added to each aquaria. Aquaria pumps maintained water circulation and air exchange for the 8 h labeling period and the flow-through was resumed over-night. Water temperature was maintained with a flow-through water bath. Ambient water temperature over the day ranged from 26.7 – 27.5 °C and temperatures in coral aquaria were always within ± 1 °C of ambient.

CW corals were enriched with ^{13}C and ^{15}N by repeated feeding with ^{13}C and ^{15}N-enriched diatoms, a food source readily assimilated by *L. pertusa* (Mueller et al. 2014b). Isotopically labeled diatoms were produced by injecting a sterile inoculum of the diatom *Thalassiosira pseudonana* into an f/2 culture medium composed of 80% ^{13}C-$NaHCO_3$ and 70% ^{15}N-$NaNO_3$ (Cambridge Isotope Laboratories, 99% ^{13}C, 99% ^{15}N). The diatoms were axenically (i.e. bacteria-free) cultured for three weeks at a 12 h light-dark cycle and then concentrated by centrifugation at 450 g, rinsed three times with 0.2 µm filtered seawater to remove residual label, and stored frozen until use. Corals were incubated in 10 L incubations chambers and fed the enriched diatoms at a concentration of 1.6 mg C L^{-1} d^{-1} and 0.3 mg N L^{-1} d^{-1} for three weeks. Water in the incubation chambers was exchanged every 12 h to prevent accumulation of waste products.

Transfer of coral mucus to sponges.

For the WW experiment, the transfer of coral mucus to *M. fistulifera* was investigated using 6 two-tiered flow-through aquaria set-ups, each consisting of a paired upper and lower aquarium connected via flow-through. The six upper aquaria were supplied with fresh flowing seawater pumped directly from the reef (10 m water depth) at ~10 L min^{-1}. Water from the upper aquaria flowed into the lower aquaria below. Isotopically labeled fungiid corals (10 individuals per aquaria) were maintained in three of the upper aquaria, while the additional three upper aquaria without labeled corals served as controls. The lower aquaria each contained 6 replicate sponges (n = 18 per treatment). Thus, the sponges were continuously supplied with water exposed to the labeled corals. Artificial aquaria lights provided the corals with ~120 µmol quanta m^{-2} s^{-1} PAR and aquaria pumps (~150 L h^{-1}) enhanced water-flow. To investigate the incorporation of mucus into sponge tissue over time, three of the six replicate sponges from each of tank (n = 9) were collected after three days exposure to the labeled corals and the remaining three sponges were collected after five days exposure (n = 9). On days three and five the collected sponges were removed from the labeling set-

up and rinsed in label-free flowing seawater for 10 min. Each sponge specimen was then transferred to individual 2 L incubation chambers filled with fresh label-free seawater, and incubated for three hours to determine the production of sponge detritus. At the end of the incubation, the sponges were removed and the incubation water (~ 1.8 L) was filtered onto pre-combusted (450°C, 4 h) GF/F filters to collect the produced particulate organic matter (POM). Filters were then dried at 40 °C for 48 h. Sponge tissue was removed from the attached substrate with a sterile scalpel blade and stored frozen in pre-combusted glass vials at -80°C until further processing. On days one and five, three corals per tank were removed from the experimental set-up, rinsed in label-free seawater and air exposed for mucus production (2 min). The collected mucus was frozen at -80°C for $\delta^{13}C$ and $\delta^{15}N$ determination.

For the CW experiment, the transfer of coral mucus to *H. coriacea* was investigated using a set-up of paired cylindrical incubation chambers filled with GF/F filtered water pumped from 45 m in the Koster-fjord. Isotopically labeled *L. pertusa* fragments (75 g DW coral) were placed in the first chamber while the second chamber contained the sponges (n = 3). The coral chamber was connected to the sponge chamber by a set of two tubes and water was re-circulated between the two chambers at a rate of 200 ml min^{-1} via a pump system. The coral chamber was equipped with an additional pump (150 L h^{-1}) to enhance water circulation. Every 24 h half the water in the set-up was replaced with fresh filtered seawater to prevent the accumulation of metabolic waste products. Control sponges (n = 6) were incubated in parallel but without labeled corals. At the beginning and end of the experiment, water samples were taken from the coral chamber and filtered onto pre-combusted GF/F filters for mucus $\delta^{13}C$ and $\delta^{15}N$ determination. After four days, the sponge fragments were transferred to individual 1 L incubation chambers containing fresh filtered (GF/F) label-free seawater and incubated for 24 h to determine the production of sponge detritus. At the end of the incubation the sponge fragments were removed and the water was filtered onto pre-combusted GF/F filters to collect the produced POM. All POM filters, coral tissue, and sponge tissue samples were frozen at -20°C until further processing.

Sample treatment and analysis.

WW and CW sponge tissues were lyophilized. Dried sponge tissue samples were weighed and homogenized by mortar and pestle. Subsamples of sponge tissue and dried POM filters were weighed and transferred to silver boats for bulk $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. Samples for $\delta^{13}C$ were decalcified by acidification with HCl for analysis of the organic carbon content. Isotopic ratios and C/N content were measured simultaneously using a THERMO NA 2500 elemental analyzer coupled to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer (IRMS) via a THERMO/Finnigan Conflo III- interface (WW) and a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V IRMS (CW).

Carbon and nitrogen stable isotope ratios are expressed in delta notation as: $\delta^{13}C$ or $\delta^{15}N$ (‰) = (R_{sample} / R_{ref} - 1) × 1000, where R_{sample} is the ratio of heavy/light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and R_{ref} is the heavy/light isotope ratio of the reference material, the Vienna Pee Dee Belemnite standard for C (R_{ref} = 0.01118) and atmospheric nitrogen for N (R_{ref} = 0.00368 N). The atomic % (atm %) heavy isotope in the sample ($^{13}C/[^{13}C + ^{12}C]$ or $^{15}N/[^{15}N + ^{14}N]$) was calculated as $F_{sample} = R_{sample}/ R_{sample} + 1$. The excess (above background) atm % (E) was calculated as the difference between the F of the samples and the background atm % in a control sample: $E = F_{sample} - F_{background}$. To correct for the differing enrichment of the WW and CW coral mucus, the excess incorporation was divided by the atm% of the coral mucus supplied to the sponges. Stable isotope data were then expressed as the total elemental uptake and reported as μmol C_{mucus} mmol C_{sponge}^{-1} and μmol N_{mucus} mmol N_{sponge}^{-1} (mean ± SD). Specific enrichment or $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values were calculated by subtracting the $\delta^{13}C$ and $\delta^{15}N$ values of the control sponges from the values of the sponges exposed to labeled corals to present the increase in $\delta^{13}C$ and $\delta^{15}N$ of the labeled sponges relative to the controls.

Phospholipid fatty acid analysis.

Phospholipid-derived fatty acids (PLFAs) of the sponge samples (~0.018 g) were extracted according to Boschker et al. (Boschker et al. 1999). Total fatty acids were extracted using a modified Bligh Dyer method and then separated on a silicic-acid column (Merck Kieselgel 60) to obtain the PLFAs, which were then derivatized by mild alkaline transmethylation to generate fatty acid methyl esters (FAMEs). Concentration and C isotopic composition of individual FAMEs were determined with a gas-chromatograph combustion interface isotope ratio mass spectrometer (GC-c-IRMS). Identification of individual FAMEs was based on the comparison of retention times with known standards using columns with different polarity and use of GC-MS, if needed.

Data analysis.

For the WW data (n = 9), statistical differences were tested using analysis of variance (ANOVA). To confirm assumptions of normally distributed and homogenous residuals, qqplots and scatter plots of residuals against fitted values were visually inspected, and data were log-transformed where necessary. Due to the low sample size of the CW data (n = 3) statistical differences were tested with the Mann-Whitney U test. All statistical tests were carried out in R version 3.1.1 (R Development Core Team, 2014).

RESULTS

Coral mucus labeling.

The WW and CW corals produced and released mucus that was enriched in both ^{13}C and ^{15}N (Fig. 4.1). The WW coral mucus was enriched by 913 ± 250‰ for $\delta^{13}C$ and 3518 ± 1360‰ for $\delta^{15}N$, while the CW mucus was enriched by 492 ± 212‰ for $\delta^{13}C$ and 3219 ± 536‰ for $\delta^{15}N$ (Fig 4.1). Data are presented as mean ± SD. The C:N ratio of the WW mucus (12.7 ± 1.1) was twice as for high as the CW mucus (6.1 ± 0.4) (ANOVA: $F_{1,19} = 196.5$, $p < 0.001$, reflecting the higher C but lower N availability on the WW reef.

Incorporation of coral mucus C and N into sponge tissue.

Both *M. fistulifera* and *H. coriacea* displayed enrichment of $\delta^{13}C$ and $\delta^{15}N$ in their tissue, indicating uptake of coral mucus (Fig. 4.1). On average *M. fistulifera* assimilated 3.6 ± 1.7 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1} and 3.7 ± 1.2 μmol N_{mucus} mmol N_{sponge}^{-1} d^{-1}. The CW sponge *H. coriacea*, incorporated coral mucus at lower but comparable rates of 1.7 ± 1.6 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1} and 2.0 ± 2.0 μmol N_{mucus} mmol N_{sponge}^{-1} d^{-1}, although the variability was higher, likely due to the lower sample size ($n = 3$; Fig. 4.2). The WW sponge *M. fistulifera*, exhibited an increase in $\delta^{13}C$ and $\delta^{15}N$ values compared to background values (i.e. the $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) from day 3 to day 5, indicating the accumulation of mucus-derived C and N over time (Fig. 4.1). However, this increase was significant only for mucus N ($F_{1,16} = 7.3$, $p = 0.02$). There was no significant difference in the actual incorporation rate of mucus-derived C and N by *M. fistulifera* on day 3 compared to day 5 indicating that the sponge accumulated mucus C and N at a constant daily rate (Fig. 4.2).

Despite being supplied with coral mucus with a high C:N ratio of 12.7 ± 1.1, the WW sponge, *M. fistulifera,* assimilated coral mucus into its tissue at a lower C:N ratio of 5.5 ± 0.7 (Fig. 4.3). This was also significantly lower than the C:N ratio of the bulk tissue of *M. fistulifera* (6.2 ± 0.3) ($F_{1,31} = 9.6$, $p = 0.004$), suggesting a higher demand for N by the WW sponge. However, it should be noted that potential respiration of mucus C was not quantified here. By contrast, the CW sponge *H. coriacea* had a lower tissue C:N ratio (5.4 ± 0.2) than *M. fistulifera* but assimilated coral mucus at a higher C:N ratio of 6.4 ± 0.3 (Fig. 4.3). On average this was similar to or higher than the C:N ratio of the coral mucus supplied (6.1 ± 0.4), suggesting a relatively higher preference for C incorporation in *H. coriacea* compared to *M. fistulifera.*

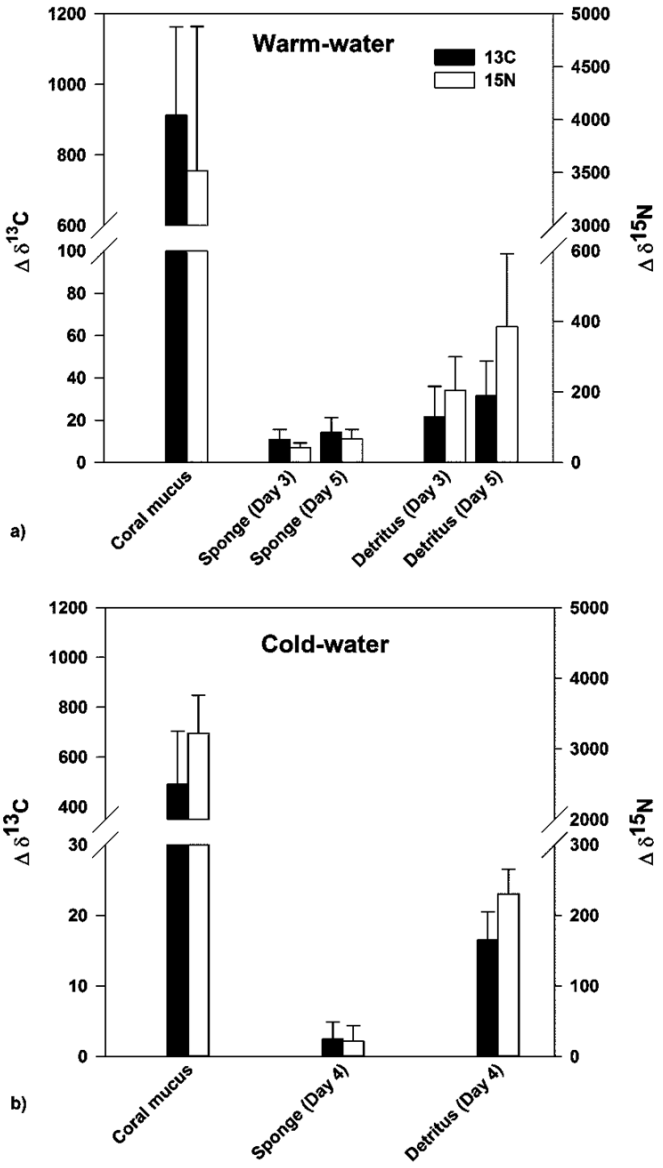

Figure 4.1. Stable isotope enrichment of ^{13}C and ^{15}N in coral mucus, sponge tissue and sponge detritus. Values are presented as the mean above-background isotope tracer incorporation $\Delta\delta^{13}C$ (‰) (dark bars) and $\Delta\delta^{15}N$ (‰) (light bars) in: a) coral mucus, sponge tissue, and sponge detritus from the warm-water sponge *Mycale fistulifera*; with tissue and detritus sampled after 3 and 5 days exposure to ^{13}C and ^{15}N labeled warm-water coral mucus (n = 9), and b) coral mucus, sponge tissue, and sponge detritus from the cold-water sponge *Hymedesmia coriacea* sampled after 4 days exposure to ^{13}C and ^{15}N labeled cold-water coral mucus (n = 3). Data presented as mean ± SD.

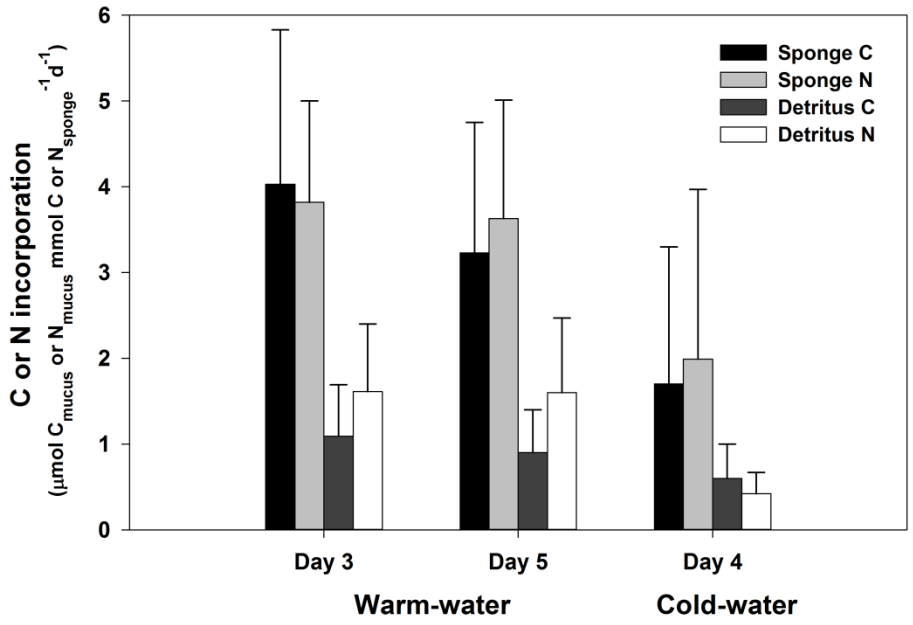

Figure 4.2. Processing of coral mucus C and N by the warm-water sponge *Mycale fistulifera* and the cold-water sponge *Hymedesmia coriacea*. Data presented as daily incorporation rates (mean ± SD) of coral mucus C and N assimilated into sponge tissue (µmol C_{mucus} or N_{mucus} mmol C_{sponge} or N_{sponge}^{-1} d^{-1}), and daily release rates of coral mucus C and N in sponge detritus (µmol C_{mucus} or N_{mucus} mmol C_{sponge} or N_{sponge}^{-1} d^{-1}). Rates shown for *M. fistulifera* after 3 and 5 days exposure to labeled warm-water coral mucus (n = 9) and for *H. coriacea* after 4 days exposure to labeled cold-water coral mucus (n = 3).

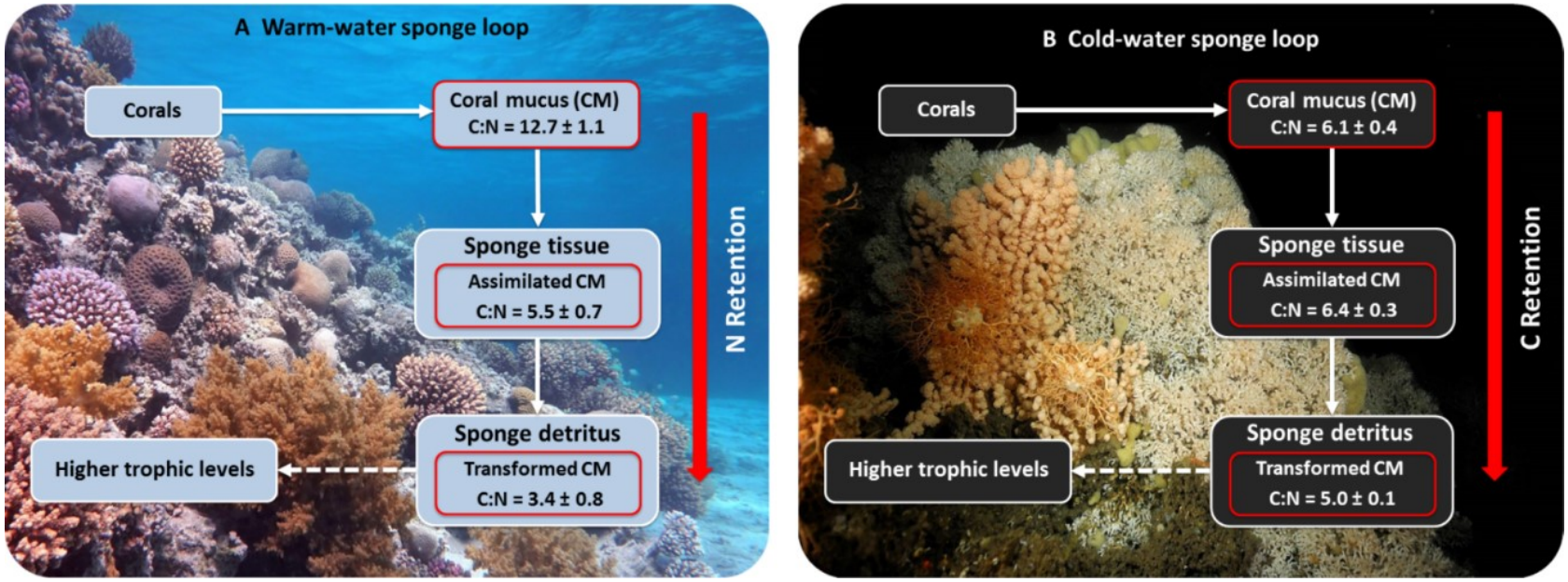

Figure 4.3. Recycling of coral mucus carbon (C) and coral mucus nitrogen (N) in a) the warm-water sponge loop b) the cold-water sponge loop. Numbers inside red boxes indicate the C:N ratio of coral mucus C and N that is transferred at each step of the sponge loop. Solid lines indicate trophic transfer of coral mucus confirmed in the current study and dotted lines indicate trophic transfers inferred from literature. Downward red arrow indicates overall higher N retention by the warm-water sponge loop (a) and higher C retention by the cold-water sponge loop (b). Photograph in (a) © Malik Naumann and (b) © Solvin Zankl.

Transfer of coral mucus C and N into sponge detritus.

The transfer of coral mucus C and N through sponges into sponge detritus is shown by the release of ^{13}C- and ^{15}N-enriched detritus after exposure to labeled coral mucus (Fig. 4.1). The WW sponge, *M. fistulifera* transformed coral mucus C and N into detritus at rates of 0.9 ± 0.5 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1} and 1.5 ± 0.8 μmol N_{mucus} mmol N_{sponge}^{-1} d^{-1}, with no significant differences between day 3 and day 5 (Fig. 4.2). The CW sponge *H. coriacea*, released coral mucus C and N as detritus at rates of 0.6 ± 0.4 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1} and 0.4 ± 0.3 μmol N_{mucus} mmol N_{sponge}^{-1} d^{-1} (Fig. 4.2). While *M. fistulifera* released mucus-derived N at a significantly higher rate than C ($F_{1,16}$ = 13.3, p = 0.002), there was no significant difference in the release of mucus-derived C and N by *H. coriacea*.

Both sponges produced detritus with a lower C:N ratio of mucus-derived C and N compared to tissue values (Fig. 4.3), suggesting differential processing of the C and N. Similar to the tissue results, however, the C:N ratio of mucus-derived C and N in the detritus of *M. fistulifera* (3.4 ± 0.8) was significantly lower compared to *H. coriacea* (5.0 ± 0.1; $F_{1,19}$ = 4.9, p = 0.04). Thus at each step, *M. fistulifera* preferentially took up and transferred mucus N, while *H. coriacea* recycled comparatively more mucus C (Fig. 4.3).

Overall, the WW sponge, *M. fistulifera* released as detritus 21 ± 11% of the mucus C and 32 ± 10 % of the mucus N it assimilated, turning over a significantly higher percentage of mucus N ($F_{1,33}$ = 4.548, p = 0.04). The CW sponge *H. coriacea* transformed a similar percentage of assimilated mucus N (39 ± 10%) into detritus as *M. fistulifera,* but a higher amount of mucus C (40 ± 29%). However, this difference was not significant and when one high outlying value was removed from the CW data, mucus C turnover by the CW sponge was even more comparable at 23 ± 0.5%.

Sponge *versus* associated bacterial specific incorporation of coral mucus C.

In addition to bulk uptake, the WW and CW sponges assimilated coral mucus C into phospholipid derived fatty acids (PLFAs), demonstrating active processing of coral mucus (Fig. 4.4). Incorporation into PLFAs was the fate of 1.8 ± 0.3% of the total mucus C assimilated by the WW sponge *M. fistulifera.* There was no significant difference in the daily PLFA incorporation rates on day 3 and day 5 (0.07 ± 0.03 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1} and 0.06 ± 0.02 μmol C_{mucus} mmol C_{sponge}^{-1} d^{-1}, respectively). The CW sponge *H. coriacea* transferred a similar percentage (1.9 ± 0.4%) of the total assimilated mucus C into PLFAs at a comparable rate of 0.05 ± 0.03 μmol C mmol C_{sponge}^{-1} d^{-1}.

In both species, mucus-derived C could be traced into PLFAs identified as bacterial, coral, algal or sponge biomarkers (Fig. 4.4). In addition to known sponge PLFAs, such as C26:2(5,9), both

species contained a number of unidentified long chain (> C:24) PLFAs characteristic of demosponges (Koopmans et al. 2015), that were therefore considered sponge biomarkers (Fig. 4.4). In the WW sponge, *M. fistulifera,* the amount of mucus-derived C traced into these sponge biomarkers increased from 6% on day 3 to 11% on day 5, although this increase was not significant (Fig. 4.5). A similar percentage (10%) was traced into sponge biomarkers in the CW sponge*, H. coriacea* (Fig. 4.5). Typical bacterial PLFAs, including iso-, anteiso-, methyl-branched, and odd numbered branching PLFAs (Boschker & Middelburg 2002; Fig. 4.3), accounted for only 2% of the total coral mucus C assimilated into PLFAs by *M. fistulifera* (Fig. 4.5), but 24% for *H. coriacea* (Fig. 4.5), suggesting higher uptake of coral mucus by sponge-associated bacteria in the CW sponge. Additionally, mucus C was traced into PLFAs likely originating from the mucus-producing coral hosts, including typical coral biomarkers C20:3ω6, C20:4ω6 and C22:4ω6 (Papina et al. 2003, Treignier et al. 2008, Mueller et al. 2014b), but also including common algal biomarkers C18:4ω3, C20:5ω3, and C22:6ω3 (Fig. 4.4). For the WW coral, these algal biomarkers likely originated from their symbiotic zooxanthellae (Papina et al. 2003, Treignier et al. 2008), as the zooxanthellae were responsible for the photosynthetic uptake of ^{13}C -$NaHCO_3$ and ^{15}N -$NaNO_3$, which are then transferred to their coral host. The heterotrophic CW corals were initially fed with ^{13}C- and ^{15}N-labeled diatoms, explaining the presence of algal biomarkers. PLFAs of coral-host origin accounted for 42 - 46% of the coral-derived C traced into the PLFA fraction in the WW sponge, *M. fistulifera*, higher than the 22% for the CW sponge *H. coriacea* (Fig. 5). In *M. fistulifera*, typical coral PLFAs also accounted for 10 – 17% of the total PLFAs in the natural unlabeled control sponges, suggesting that coral mucus could be a key source of dietary PLFAs in *M. fistulifera*. In *H. coriacea*, the majority (80 ± 12%) of the PLFAs in the unlabeled control sponges were identified as sponge biomarkers resulting from *de novo* synthesis and modification of dietary PLFAs by the sponge. However, coral biomarkers were also present and accounted for 4 ± 3% of the total PLFA content.

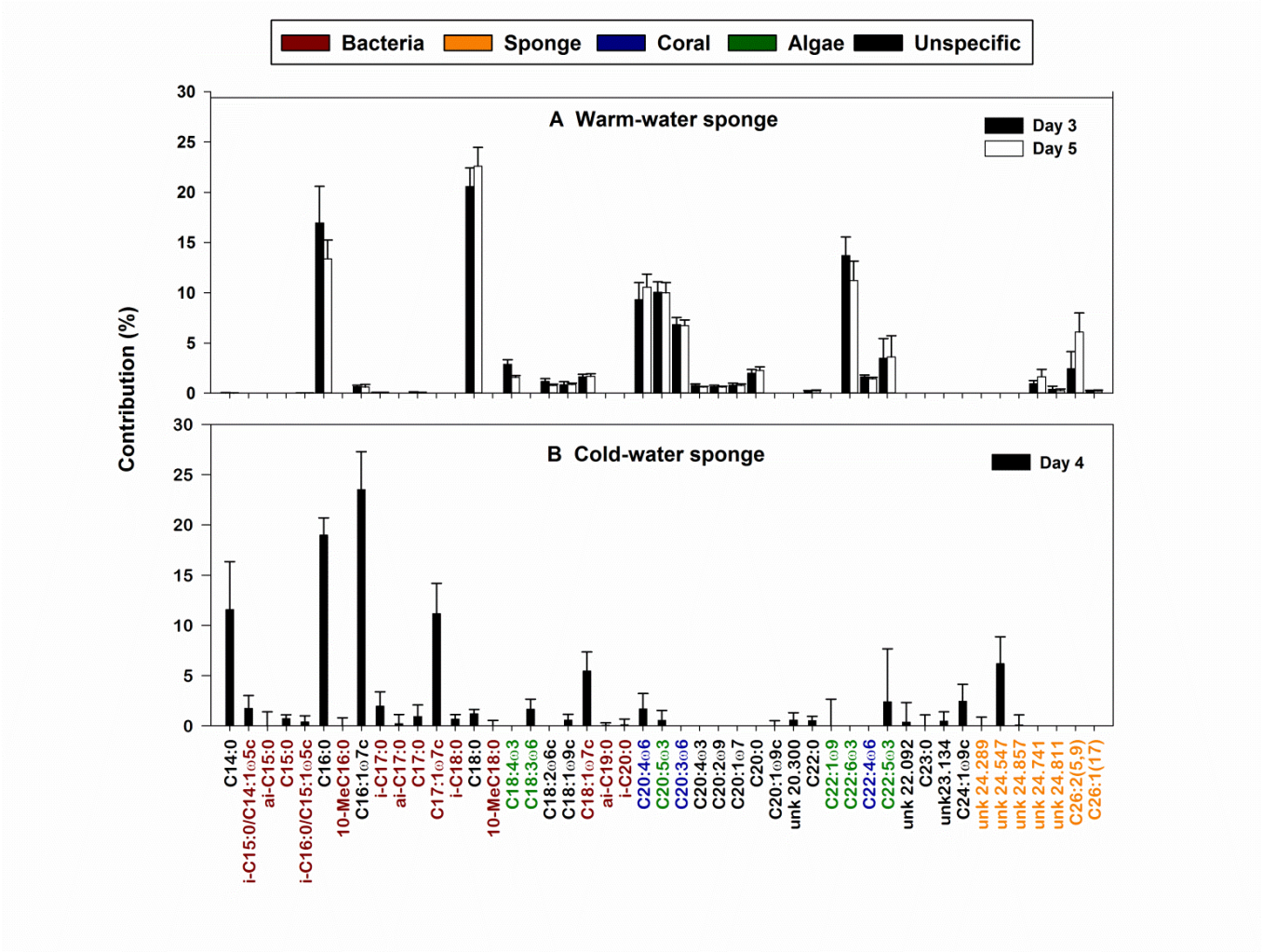

Figure 4.4. Distribution of coral mucus carbon (C) in sponge phospholipid fatty acids (PLFAs). Data presented as % of total coral mucus C assimilated into PLFAs (mean ± SD) in a) the warm-water sponge *Mycale fistulifera,* after 3 and 5 days exposure to labeled warm-water coral mucus (n = 9), and b) the cold-water sponge *Hymedesmia coriacea,* after 4 days exposure to labeled cold-water coral mucus (n = 3).

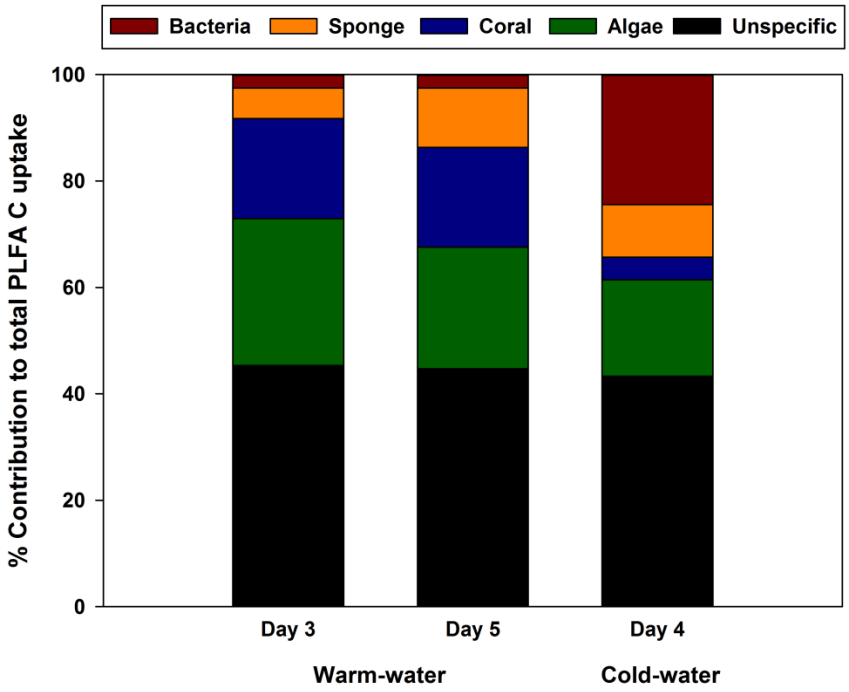

Figure 5.4 Percent distribution of coral mucus carbon (C) assimilation into bacterial, sponge, coral, and algal phospholipid fatty acids (PLFAs). Data shown for warm-water sponge *M. fistulifera* after 3 and 5 days exposure to labeled coral mucus (n = 9), and for the cold-water sponge *H. coriacea* after 4 days exposure to labeled coral mucus (n = 3).

DISCUSSION

By demonstrating the uptake of coral mucus by warm- and cold-water reef sponges and its subsequent transformation into sponge detritus, we provide the first evidence that the sponge loop recently identified in the Caribbean (de Goeij et al. 2013) also functions within shallow coastal reefs of other oceanic regions (i.e. Red Sea, Indo-Pacific) and even in cold-water reefs of the deep sea. Despite pronounced differences in the environmental characteristics of warm-water (WW) and cold-water (CW) reef ecosystems, both sponges assimilated and transformed coral mucus C and N into particulate detritus at remarkably similar rates. Previously, the sponge loop has only been demonstrated using laboratory-produced diatom DOM. Therefore, we not only extend the spatial validity of the sponge loop, but also demonstrate its functioning using a resource naturally produced on the reef: coral mucus. Importantly, this elucidates a direct trophic link between two fundamental reef ecosystem engineers, scleractinian corals and sponges, on both shallow WW and deep-sea CW reefs, hinting at an alternative mechanism for coral mucus recycling in reef ecosystems.

The similar rate of assimilation of coral mucus into the bulk tissue of the two sponges indicates that WW and CW sponges have a similar capacity for mucus uptake. Based on natural stable isotope analysis, coral mucus has been suspected to be a major component (48 – 73%) of the diet of Caribbean cavity sponges (van Duyl et al. 2011), but our findings provide unequivocal and quantitative evidence for the uptake and assimilation of coral mucus by sponges originating from shallow coastal and deep-sea habitats. Furthermore, the assimilation of coral mucus C into sponge phospholipid fatty acids (PLFAs) synthesized *de novo* or by modification of coral-derived PLFAs demonstrates that sponges actively process coral mucus similarly to algal and bacterial food sources, confirming its nutritional value (de Goeij et al. 2008a, Koopmans et al. 2011). Given the dominant coral cover and high release rates of coral mucus on Tisler Reef (Norwegian Shelf) (Wild et al. 2009) and in the Red Sea (Naumann et al. 2010a), as well as the close proximity of the two sponge species to corals in their respective habitats (Mortensen 2001, L. Rix pers. obs.), coral mucus likely represents a readily available food source for the two investigated sponges. Considering the extremely oligotrophic conditions in the Red Sea (Silverman et al. 2007) and the spatially and temporally variable input of organic matter to CW reefs (Duineveld et al. 2004, Wagner et al. 2011), the ability to utilize coral mucus as a reef-produced resource may be an advantageous strategy for reef sponges. However, it remains to be determined how much coral mucus contributes to the overall diet of the two investigated sponges.

In addition to generating an alternative food source for sponges, this trophic link between corals and sponges also has ecosystem level implications. As coral mucus represents an important resource and energy carrier on WW and CW coral reefs, its uptake and recycling by the sponge loop provides a key ecosystem function by preventing the loss of the energy and nutrients bound in coral mucus (Wild et al. 2004a, Wild et al. 2011). Previously, bacteria were believed to be the primary consumers of coral mucus on WW and CW reefs, contributing to its rapid degradation and remineralization in the water column and reef sediments (Wild et al. 2004b, Wild et al. 2009). In combination with microbial degradation processes, uptake by sponges may ensure high retention of coral mucus C and N within WW and CW reef ecosystems. Further, since corals release up 40% of their net photosynthetically fixed C (Crossland et al. 1980, Tremblay et al. 2012), the uptake and transformation of coral mucus by sponges may be a key mechanism by which the energy and nutrients harvested by corals can be transferred to other reef fauna – first to sponges and subsequently to detritivores via the production of sponge detritus. Both the WW and CW sponge released a substantial fraction of assimilated coral mucus C and N: 21% C and 32% N for *M. fistulifera* and 40% C and 39% N for *H. coriacea*. These rates are comparable to detritus conversion rates of Caribbean sponges that released 11 – 24% (C) and 18 – 36% (N) of assimilated diatom DOM (de Goeij et al. 2013), demonstrating that release as detritus is the fate of a substantial fraction of organic matter assimilated by sponges in both WW and CW reef ecosystems and may represent a significant flow of energy and nutrients. In the Caribbean, recycling via the sponge loop approaches reef gross

primary production, exceeding recycling by the microbial loop by one order of magnitude (de Goeij et al. 2013). Mucus release rates by WW and CW corals are comparable (Wild et al. 2008, Naumann et al. 2010a, Naumann et al. 2014) and sponges are also highly abundant on CW reefs (Mortensen et al. 1995, Van Soest & Lavaleye 2005). Given the similar coral mucus uptake and transformation rates by the two sponges, the sponge loop may be similarly important for the recycling of energy and nutrients on CW reefs.

Although their overall mucus uptake and transformation rates were similar, there were differences in the specific processing of coral mucus C and N between the two studied sponges. The WW sponge *M. fistulifera* seemed to exhibit preferential incorporation of N as compared with its CW counterpart. *M. fistulifera* assimilated coral mucus C and N at a lower (i.e. more N) ratio (5.5) than *H. coriacea* (6.4), even though the Red Sea mucus C:N ratio was twice as high (12.7) as for the mucus provided to the CW sponge (6.1; Fig. 4.3). As a consequence, the WW sponge effectively reduced the C:N ratio of coral mucus by half compared to the original mucus ratio. This strongly suggests a high demand for N in the WW sponge through selective uptake of N from its mucus food source (Fig. 4.3). In contrast, *H. coriacea* assimilated coral mucus C and N at a C:N ratio similar to or higher than the supplied coral mucus, which could indicate a relatively higher demand for C in the CW sponge (Fig. 4.3). This may be explained by the differences of the respective sponge environments (Table S1). Low N availability on oligotrophic WW reefs, such as in the Red Sea (Silverman et al. 2007, Bednarz et al. 2015, Chapter 6), likely renders efficient nutrient uptake a key strategy for WW sponges. CW reefs, by contrast, are located in nutrient-rich waters and receive organic matter rich in N, but the input of organic C is limited (Kiriakoulakis et al. 2007, Wagner et al. 2011), possibly necessitating efficient C uptake. The preferential uptake of either coral mucus C or N was also translated into the detritus produced by the two sponges, with the WW sponge producing detritus displaying a significantly lower ratio of mucus-derived C and N (ie. relatively enriched in mucus N) compared to the detritus of the CW sponge (Fig. 4.3). This enrichment of coral mucus N at each step of the WW sponge loop resulted in particularly efficient retention of mucus N, while the CW sponge loop conserved comparatively more mucus C (Fig. 4.3). Overall this suggests the key function of the sponge loop may differ in the two contrasting reef ecosystems. On oligotrophic WW reefs, the retention of coral mucus N may contribute to the efficient N cycling necessary for sustaining high benthic primary productivity. By contrast, retention of organic C (energy) may be the key functional role of the sponge loop on C-limited deep-sea CW reef ecosystems.

Interestingly, both species produced detritus that was relatively enriched in coral mucus N compared to the tissue incorporation (Fig. 4.3), a phenomenon also observed for Caribbean sponges fed with diatom DOM (de Goeij et al. 2013). This suggests a common decoupling of C and N processing by marine sponges. Consequently, sponges not only transform mucus C and N into detrital C and N, enabling its transfer to reef fauna otherwise unable to directly utilize it, but they also

modify the relative availability of this C and N for higher trophic levels thereby providing a high quality food source.

Sponges host diverse microbial populations and can be classified into high microbial abundance (HMA) and low microbial abundance (LMA) species based on the number of associated microbes in their tissue (Gloeckner et al. 2014). Since bacteria are the main consumers of DOM in the ocean, it has been suspected that sponge-associated bacteria play an important role in DOM uptake by sponges with higher uptake predicted for HMA compared to LMA sponges (Maldonado et al. 2012, Ribes et al. 2012). Higher incorporation into bacterial PLFAs in *H. coriacea* (24%) compared to *M. fistulifera* (2%) suggests bacteria were more active in coral mucus uptake in the CW sponge. However, both sponge species belong to the order Poecilosclerida, which appears to exclusively consist of LMA species (Gloeckner et al. 2014). There was also no significant difference in the overall coral mucus incorporation between the two sponges, and mucus-derived C was also incorporated into sponge-specific PLFAs (5 − 10%). This suggests the involvement of sponge cells in coral mucus uptake, particularly in *M. fistulifera* where associated bacteria appeared to play a minor role. Moreover, high DOM uptake, also into sponge-specific PLFAs, is known for other LMA species (de Goeij et al. 2008a, de Goeij et al. 2008b, Mueller et al. 2014a) reviewed by Pawlik et al. (Pawlik et al. 2015). This suggests DOM uptake is not limited to HMA sponges. Alternatively, the variation in specific PLFA incorporation may point to compositional differences in the mucus of *L. pertusa* (CW source) and fungiid corals (WW source). Several studies have found differences in the lipid, protein, and carbohydrate composition of mucus between coral species (Ducklow & Mitchell 1979, Meikle et al. 1988, Wild et al. 2010). *L. pertusa* produces mucus with high DOC:POC ratios (Wild et al. 2008) and a high percentage of labile monosaccharides (Wild et al. 2010), which may promote high uptake by sponge-associated bacteria. Fungiid corals release mucus with a larger particulate fraction (Naumann et al. 2010a), high lipid content (Ducklow & Mitchell 1979, Meikle et al. 1988), and a higher percentage of carbohydrates with low microbial degradability (Wild et al. 2010), possibly favouring sponge cell uptake. Higher lipid content could explain the higher proportion of mucus-derived C traced into PLFAs of coral origin in *M. fistulifera* (42 - 44%) compared to *H. coriacea* (22%). Sponges and their associated microbes may, therefore, be able to utilize a wider range of coral mucus components than free-living bacteria, further contributing to high retention of coral mucus on the reef (de Goeij et al. 2008a).

Detritus production by sponges is a phenomenon that has been observed in tropical (Ribes et al. 1999, Alexander et al. 2014) and deep-sea sponges (Witte et al. 1997); but the mechanisms by which sponges produce detritus are not fully understood. Rapid cell-turnover and shedding is believed to be a key source of sponge detritus, although excretory byproducts are also a component (de Goeij et al. 2009, Alexander et al. 2014, Maldonado 2015). On Caribbean reefs, sponge detritus is rapidly utilized by a variety of filter-feeding and motile detritivores (de Goeij et al. 2013). On CW coral reefs, the consumption of sponge detritus has not been established, but detritivores are an

important component of CW benthic communities (Mortensen et al. 1995, Duineveld et al. 2007). Furthermore, trophic models indicate that detritus can account for 51% of the total C ingested by CW reef communities (van Oevelen et al. 2009), suggesting sponge detritus may be widely utilized by CW reef fauna.

While the transfer of coral mucus C and N to higher trophic levels via sponge detritus remains to be confirmed, our findings strongly suggest coral mucus contributes to fueling a sponge loop in both WW and CW reef ecosystems. This trophic pathway has the potential to substantially impact our understanding of food web dynamics and biogeochemical cycles on reefs. A recent study suggests that phase-shifts from coral to macroalgal dominance will impact fish productivity on WW reefs by altering DOM availability for the sponge loop (Silveira et al. 2015), demonstrating the potential for the sponge loop to impact the entire coral reef food web. Given the potential importance of the sponge loop to ecosystem function on WW and CW reefs, future studies should quantify the magnitude of recycling by the sponge loop to evaluate its role in trophodynamics and biogeochemical cycling in these two complex reef ecosystems. In this context, our present findings suggest that due to differences in ambient C and N availability on WW and CW reefs, the functional role of the sponge loop may differ in the two ecosystems, with N cycling in WW and C cycling in CW reefs disproportionately contributing to ecosystem functioning.

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CHAPTER 5

ORGANIC AND INORGANIC NITROGEN RELEASE BY RED SEA CORAL REEF SPONGES

Rix L, Wild C, Cardini U, Bednarz VN, Stuck, U, Al-Horani FA, Naumann MS. Organic and inorganic nitrogen release by Red Sea coral reef sponges. This chapter is intended for publication in *Marine Ecology Progress Series*.

ABSTRACT

Sponges exhibit complex nitrogen (N) cycling, but many aspects of the related N fluxes are poorly understood. The recent discovery of massive cell shedding and detritus production in coral reef sponges suggests that in addition to dissolved inorganic N (DIN), particulate organic N (PON) may play a significant role in the sponge N budget. However, to date PON production by sponges has not been quantified. Therefore, we investigated N cycling mediated by six species of common Red Sea sponges using incubation experiments to measure fluxes of DIN and PON as well as the generation of new N via N_2 fixation. Our results confirm DIN as the largest efflux of N mediated by coral reef sponges (120 ± 47 μmol cm^{-3} h^{-1}; mean ± SD), but for the first time highlight their substantial production of PON (52 ± 23 μmol cm^{-3} h^{-1}), which accounted for approximately 30 ± 3% of the total N released by the six sponges. Upscaling these rates to the community scale resulted in efflux rates of 19.6 ± 7.7 μmol DIN m^{-2} reef h^{-1} and 8.5 ± 1.3 μmol PON m^{-2} reef h^{-1}, representing a substantial flux of N. In contrast, only two of the investigated sponge species displayed significant N_2 fixation activity with rates 3 – 4 orders of magnitude lower than for the other N fluxes. Generation of new N by N_2 fixation accounted for only 0.006 ± 0.005 μmol N m^{-2} reef h^{-1}, indicating it cannot balance the N loss via PON and DIN release or contribute substantially to N cycling at the ecosystem scale. Dietary stable isotope analysis suggests the imbalance in the sponge N budget due to net PON and DIN release may be resolved by the uptake of DON.

INTRODUCTION

Sponges represent the oldest extant animal phylum (Wörheide et al. 2012), but despite being considered the simplest metazoans, sponges exhibit very complex nitrogen (N) cycling that enables them to contribute to N fluxes in the ecosystems they inhabit. Sponges possess a remarkable capacity for filtering seawater, pumping up to 50 000 times their own volume in a day (Reiswig 1974, Weisz et al. 2008) while effectively removing small particulate organic matter (POM), in particular picoplankton in the size range of 0.2 – 2.0 μm (Pile et al. 1997, Ribes et al. 1999). The remineralization of this ingested POM leads to the release of dissolved inorganic nitrogen (DIN) compounds, particularly ammonium (NH_4^+), and therefore sponges can act as a net source of DIN (Southwell et al. 2008b, Perea-Blazquez et al. 2012).

Sponges also form symbiotic associations with diverse communities of microbes (Schmitt et al. 2012, Simister et al. 2012) that provide them with a varied suite of additional metabolic N pathways (Taylor et al. 2007). Based on their microbial densities, sponges can be classified as either high-microbial abundance (HMA) or low-microbial abundance (LMA) species, with HMA sponges hosting more dense and diverse communities than LMA sponges (Hentschel et al. 2006, Gloeckner et al. 2014). To date, all microbial-mediated biogeochemical N cycling pathways occurring in the marine environment have also been reported to occur in sponges including nitrification (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008a), denitrification (Schläppy et al. 2010), ANAMMOX (Hoffmann et al. 2009), and N_2 fixation (Wilkinson & Fay 1979). Nitrification, the two-step oxidation of ammonium to nitrite and nitrate, appears to be particularly common in HMA sponges, and is responsible for the high rates of nitrate released by many sponges in temperate and tropical coral reef habitats (Diaz & Ward 1997, Jimenez & Ribes 2007, Southwell et al. 2008a, Fiore et al. 2013a). Only a few studies have measured denitrification or ANNAMOX in sponges (Hoffmann et al. 2009, Schläppy et al. 2010), but such competing assimilatory and dissimilatory N pathways are suspected to account for the variation in DIN fluxes observed for some sponges that enable them to act as both a source and sink for DIN (Fiore et al. 2013a). In addition to recycling N, sponges can generate new bioavailable N via dinitrogen (N_2) fixation. Many sponges are known to host microbes capable of fixing N_2 (Taylor et al. 2007, Mohamed et al. 2008, Fiore et al. 2015), suggesting this process may be widespread. However, measurements of N_2 fixation rates for sponges remain scarce (Wilkinson & Fay 1979, Shashar et al. 1994, Shieh & Lin 1994, Wilkinson et al. 1999).

In addition to transforming inorganic nutrients, it has recently been demonstrated that sponges also effectively transform organic matter by taking up DOM and producing POM as sponge detritus via the so-called sponge loop (de Goeij et al. 2013). Sponge detritus is still poorly characterized but is believed to originate largely from the rapid turnover and shedding of cells recently discovered in many tropical and temperate sponges (de Goeij et al. 2009, Alexander et al. 2014, Maldonado 2015). Detritus production may represent a potential new sponge N flux, but so far

detrital PON production by sponges has not been quantified, and therefore its role in sponge N cycling is unknown. Understanding N cycling in sponges is important as they have the capacity to substantially influence the availability of N in the habitats where they are abundant. The contribution of sponges to the biogeochemical cycling of N may be particularly important within oligotrophic environments such as tropical coral reefs where low concentrations of N make it a key limiting nutrient for production processes. Next to scleractinian corals, sponges are the second most abundant benthic functional group on many coral reefs, and indeed, coral reef sponges are known to release substantial quantities of DIN (Corredor et al. 1988, Diaz & Ward 1997, Fiore et al. 2013a), reaching fluxes of up to 640 ± 130 μmol m^{-2} reef h^{-1} (Southwell et al. 2008b).

Despite the fact that sponges are widely acknowledged as significant players in N biogeochemical cycling, more studies have focused on characterizing sponge microbial communities and identifying the microbes potentially involved in mediating specific N metabolic pathways (Taylor et al. 2007, Mohamed et al. 2008, Lopez-Legentil et al. 2010, Mohamed et al. 2010, Fiore et al. 2013b, Zhang et al. 2014, Fiore et al. 2015). By contrast, fewer studies have actually attempted to quantify sponge-mediated N fluxes (Jimenez & Ribes 2007, Schläppy et al. 2010, Perea-Blazquez et al. 2012, Ribes et al. 2012), particularly for coral reef sponges (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008b, Fiore et al. 2013a) where all studies have focused on Caribbean sponges. In additional to measuring N fluxes, stable isotope analysis is a useful tool that has been used extensively in coral reef and other marine ecosystems to investigate trophic relationships (Thurber 2007, Topcu et al. 2010, Freeman & Thacker 2011, van Duyl et al. 2011), and biogeochemical N processes (Southwell 2007, Weisz et al. 2007, Mohamed et al. 2008) in marine sponges.

The objectives of this study were first to quantify and compare DIN and PON flux rates in six species of common Red Sea sponges and measure their generation of new N via N_2 fixation. We then combined these physiological rates with sponge biomass data for a Red Sea benthic reef community, in order to estimate the total rates of the respective N fluxes by the entire benthic sponge community and to evaluate their importance at the ecosystem scale. Finally, ^{13}C and ^{15}N stable isotope analysis was conducted to provide additional insight to the dominant N sources for the different sponges and examine differences in N metabolism between the different sponge species.

MATERIALS AND METHODS

Study site and sponge benthic cover

This study was conducted in the northern Gulf of Aqaba at the Marine Science Station (MSS) Aqaba, Jordan (29°27' N, 34°58' E) during November 2013. Sampling was carried out on the 1 km long fringing reef in front of the MSS, and all experimental work was carried out in the MSS

laboratories. As determined by replicated line point intercept transects, the benthic reef community was dominated by hard and soft corals (58.1 ± 13.8%). Percent sponge cover was determined using quadrat surveys. Six 50 m transects were laid out at the sampling site at 10 m water depth. Every 10 m along each transect a 0.25 m^2 quadrat was laid out (n = 36 total). All sponges found inside the quadrat were counted and their dimensions measured using a tape measure. Sponge surface area and volume were then determined using geometric formulas.

Organism collection and maintenance

Six species of common reef sponges were collected for the incubation experiments: *Amphimedon chloros, Callyspongia (Euplacella) densa, Chondrilla sacciformis, Hemimycale arabica, Mycale fistulifera* and *Negombata magnifica.* Tissue samples for all species were deposited at the Naturalis Biodiversity Center, RMNH.POR.9146. These six species accounted for ~70% of the sponge cover at 10 m water depth. *H. arabica, M. fistulifera*, and *N. magnifica* exhibit low densities of associated microbes (Gillor et al. 2000, Kötter 2002) and belong to the Poecilosclerida, an order of exclusively LMA sponges (Gloeckner et al. 2014). *C. sacciformis* contains high densities of microbes in its tissues (Kötter 2002). The microbial abundances of *A. chloros* and *C. densa* have not been quantified but other species of these genera are LMA (Gloeckner et al. 2014). Sponge specimens were collected from the reef between 10 – 15 m water depth by SCUBA and immediately transferred without air exposure to the MSS aquarium facilities. Encrusting sponges were collected by chiselling small fragments from the reef framework and clearing the attached substrate of epibionts. The two branching sponges (*A. chloros, N. magnifica*) were sampled by clipping small branches from larger specimens and were maintained in flow-through aquaria by hanging branches upside down on fishing line. For each sponge, 6 replicates were collected with an overall mean volume of 11.7 ± 6.2 cm^3. Specimens were maintained in 100 L flow-through tanks supplied with seawater pumped directly from the reef at 10 m water depth at approximately ~6 L min^{-1}, ensuring key environmental parameters (e.g. temperature, inorganic and organic nutrient concentrations, food availability) corresponded to seasonal *in situ* conditions. Each species was maintained in a separate aquarium and irradiance (PAR) was adjusted according to the *in situ* habitats of the respective sponges (shaded for the cryptic cavity sponge *C. sacciformis,* partial shading for *H. arabica* and *C. densa* and full *in situ* light levels at 15 m water depth for the remaining sponges) using black mesh and a quantum sensor (Model LI-192SA; Li-Cor). Sponge specimens were allowed to heal and acclimate for at least 1 wk prior to experiments and only healthy sponges with no damaged tissue that were actively pumping seawater (visualized with fluorescein dye) were used for incubations.

Determination of DIN and PON fluxes

Sponges were incubated individually in 2 L gas-tight acrylic chambers filled with fresh, unfiltered seawater for 3 h (n = 6 specimens per species). Seawater control incubations were conducted in parallel without sponges (n = 3). Additional control incubations were run with bare coral rock to account for any potential activity caused by the attached substrate of the encrusting species (n = 6). Incubations were conducted from ~10:00 – 13:00. Sponges were transferred to chambers without air exposure and incubations were conducted under the conditions described above for the maintenance aquaria. Chambers were stirred at ~400 rpm using magnetic stirring plates (Cimarec™ i Telesystem Multipoint Stirrers, Thermo Scientific) and placed in a flow-through water bath to ensure maintenance of *in situ* temperature. Dissolved oxygen concentrations were monitored continuously using multi-probes (Hach HQ40d) inserted into airtight fittings in the chamber lids.

Inorganic nutrient samples (60 ml) were collected from each chamber at the start and end of the 3 h incubation period. Samples were collected with an acid-washed 60 ml syringe from a sampling port in the chamber lid. Syringes were rinsed once with chamber water before samples were collected. After the initial sample was taken, chambers were refilled with ambient water to ensure there was no headspace. All samples were stored at 4°C and processed within 1 h of collection.

Samples for particulate organic carbon (POC) and nitrogen (PON) were taken to determine the production of sponge detritus. Seawater control samples were taken at the start of the incubation experiment from the inflow water used to fill the chambers (n = 3). At the end of the incubation, the water remaining after sampling for inorganic nutrients was collected from each chamber. The sample volume (~1800 ml) was recorded, divided equally, and vacuum filtered separately onto two separate pre-combusted (450 °C, 4 h) GF/F filters (VWR; nominal pore size 0.7 μm). One filter was used for POC determination and the other for PON. Filters were dried at 40°C for at least 48 h and stored dry until CN elemental analysis. Sponge surface area and thickness were measured with callipers to determine the sponge volume using geometric formulas. Sponge physiological fluxes of DIN, POC, and PON were corrected for differences in seawater controls (or rock controls for encrusting species), normalized to sponge volume, and rates presented as μmol cm^{-3} h^{-1}. Community scale fluxes were estimated by multiplying the rate of each physiological flux (nmol cm^{-3} h^{-1}) for each sponge species by its estimated total volume on the reef (cm^{-3} m^{-2}) in order to determine the respective contribution to the total community flux (μmol m^{-2} reef h^{-1}). To estimate total sponge community DIN, POC, and PON fluxes, the results for the six investigated sponge species were summed and added to the flux for the uninvestigated species derived from multiplication of their total volume and the respective mean flux rates.

Quantification of dinitrogen fixation

N_2 fixation rates were quantified by the acetylene (C_2H_2) reduction assay method (Capone 1993, Wilson et al. 2012) in a separate incubation experiment using the same sponge specimens. Sponges (n = 6 specimens per species) were transferred without air exposure to individual 1000 mL transparent glass chambers containing 800 mL of natural seawater and 200 ml headspace. Immediately prior to the start of the incubations 10% of the seawater was replaced with C_2H_2-saturated seawater. Chambers were then sealed gas-tight with a spring-loaded glass lid and 10% of the 200 mL headspace was replaced with freshly generated C_2H_2 gas via a needle injection port in the glass lid. Sealed chambers were stirred with magnetic stirrers (400 rpm) and placed in a flow-through water bath as describes above to maintain temperature throughout the 6 h incubation period. Each species was incubated once during the day and once at night to determine differences in light and dark N_2 fixation. Daytime incubations were conducted from ~10:00 – 16:00 and nighttime incubations from 22:00 – 04:00. Gas samples (1 ml) were taken at 0, 1 and 6 h from the headspace of each chamber with a gas-tight syringe and transferred into gas-tight 2 mL glass vials that were fitted with butyl septa and previously filled with distilled water. Vials were stored frozen upside down until analysis.

Ethylene (C_2H_4) concentrations in the gas samples were measured using a reducing compound photometer (RCP) (Peak Laboratories) with a detection limit of 100 ppb. Calibration of the RCP was routinely conducted using serial dilutions of a 200 ppm (± 2%) C_2H_4 standard in air (Restek, USA). Differences in C_2H_4 concentration between the 1h and 6 h incubation time intervals were converted into C_2H_4 evolution rates according to Breitbarth et al. (2004). Since preliminary incubations conducted with bare coral rock exhibited substantially higher C_2H_4 evolution than incubations with sponges, only branching sponges and encrusting sponges that could be separated from the substratum without damaging the sponge tissue or that completely covered the substrate were used (ie. *A. chloros, C. densa, M. fistulifera, N. magnifica*). Measured C_2H_4 concentrations were corrected for the signal detected in unfiltered seawater controls (n = 6) and normalized to incubation time and sponge volume in order to calculate C_2H_4 evolution rates (nmol C_2H_4 cm^{-3} h^{-1}). Additional controls were conducted using 0.2 µm filtered seawater (n = 6) and unfiltered seawater with sponges but no addition of C_2H_2 (i.e. natural C_2H_4 production, n = 6). However, these showed negligible C_2H_4 evolution. Sponge volume was determined as described above. To convert C_2H_4 evolution rates to N_2 fixation rates, a conservative theoretical ratio of 4:1 (C_2H_4:N_2) was used, which assumes that 4 mol of C_2H_4 are reduced per 1 mol of N_2 (Capone 1993, Mulholland et al. 2004). Community N_2 fixation rates were calculated as described above for DIN and PON.

Stable isotope sampling

Sponge tissue samples were collected for ^{13}C and ^{15}N stable isotope analysis along a 50 m transect at 10 m water depth. Samples were collected by chiselling or cutting fragments of the six sponge species described above (n = 10 per species). Samples were rinsed in 0.2 μm filtered seawater and encrusting sponges were removed from the underlying substrate with a sterile scalpel blade. Tissue samples were transferred to pre-combusted glass vials and stored at -80°C until further processing. Seawater samples for ^{13}C and ^{15}N stable isotope analysis of suspended particulate organic matter (SPOM) and water column bacterioplankton (BP) were collected from the same sampling location on the reef at 10 m water depth (~1 m above the benthos). Replicate samples were collected on 4 days (n = 2 per day) over 10 days prior to sampling the sponges. Seawater samples were collected in 10 L acid-washed HDPE canisters and transferred to the laboratory for immediate processing. Subsamples from each canister were filtered for $PO^{13}C$ (1 L) and $PO^{15}N$ (2 L) analyses onto pre-combusted GF/F filters. Filters were dried at 40°C for at least 48 h and stored dry until analysis. Subsamples (6 – 8 L) for BP were first filtered through pre-combusted (450 °C, 4 h) GF/F filters (0.7 μm nominal pore-size) before the filtrate was filtered onto aluminum oxide membrane filters (0.2 μm pore size; Whatman Anodisc). BP filters were stored frozen at -80°C until further processing.

Sample processing

Inorganic nutrient water samples were syringe-filtered through cellulose acetate filters (nominal pore size 0.45 μm) for determination of ammonium (NH_4^+), nitrate + nitrite (NO_x^-), and phosphate (PO_4^{3-}) concentrations using standard methods (Murphy & Riley 1962, Strickland & Parsons 1972, Holmes et al. 1999). NH_4^+ samples were immediately processed and measured fluorometrically on the day of collection using a Trilogy Fluorometer (Turner Designs). All other nutrients were frozen for later photometric determination with a continuous flow analyzer. The detection limits for NH_4^+, PO_4^{3-}, and NO_x^- were 0.09, 0.01, and 0.02 μM, respectively.

Filters for POC and PON analysis were folded into silver and tin cups, respectively. POC filters were decalcified with 0.1 N HCl and then re-dried at 40°C. C and N content were determined with a EuroVector elemental analyzer (EURO EA 3000). Analytical precision was ≤0.1% (C) and ≤0.03% (N) using Acetanilide OAS (certificate 187560) as the elemental standard.

Sponge tissue samples, SPOM, and BP samples for ^{13}C and ^{15}N stable isotope analysis were lyophilized and sponge tissues were homogenized with a mortar and pestle. SPOM samples on GF/F filters and sponge tissue samples were transferred to silver (POC) or tin (PON) cups. For the BP samples, the plastic ring was removed from the aluminum oxide filters and the filters were crumbled into silver cups. Sponge tissue samples and GF/F filters were decalcified with 0.4 M HCl. C and N

stable isotope ratios and elemental content of sponge tissue samples, SPOM and BP samples were measured simultaneously using a THERMO NA 2500 elemental analyzer coupled to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer (IRMS) via a THERMO/Finnigan Conflo III- interface. Standard deviations of C and N content were < 3% of the concentrations analyzed and < 0.15‰ for repeated $\delta^{13}C$ and $\delta^{15}N$ measurements of standard material (peptone). C and N stable isotope ratios are expressed using the delta (δ) notation in units per mil (‰) and calculated as: $\delta^{13}C$ or $\delta^{15}N = (R_{sample} / R_{ref} - 1) \times 1000$, where R_{sample} is the ratio of heavy/light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample, and R_{ref} is the heavy/light isotope ratio of the reference material (the Vienna Pee Dee Belemnite for C: $R_{ref} = 0.01118$ and atmospheric nitrogen for N: $R_{ref} = 0.00368$ N).

RESULTS

Sponge benthic cover

Overall sponge cover accounted for 1.2 ± 0.9% of the non-cryptic benthic reef community at 10 m water depth. In total 18 sponge species were identified in the quadrats. *M. fistulifera* was the most abundant sponge accounting for 65% of the total sponge surface area and 20% of the total sponge volume on the reef. The volume of the entire sponge community per m^2 reef area amounted to 163 ± 31 cm^3. The six investigated species accounted for 30% of the total volume of sponges in the study site.

Sponge DIN fluxes

All six sponge species released DIN, but the composition of DIN released differed by species (Fig. 5.1a). There was no significant difference in start and end concentration of the seawater and rock controls, indicating DIN production was due to sponge activity. Only *C. sacciformis* released DIN entirely (100%) as NO_x^-, while exhibiting uptake of NH_4^+. All other sponges released DIN primarily as NH_4^+ (>65%). *C. densa* and *H. arabica* also produced moderate amounts of NO_x^- (~30% of their total DIN release), while NO_x^- production accounted for <20% of the total DIN released by the remaining sponge species (Fig. 5.1a). Total DIN fluxes ranged from 64 ± 29 nmol cm^{-3} h^{-1} in *A. chloros* up to 203 ± 28 nmol cm^{-3} h^{-1} in *C. sacciformis* (Fig. 5.1c), averaging 120 ± 47 nmol cm^{-3} h^{-1} over all species. DIN release represented the largest N flux in all six sponge species. The total DIN release by the non-cryptic reef sponge community amounted to 19.6 ± 2.5 μmol N m^{-2} reef h^{-1}.

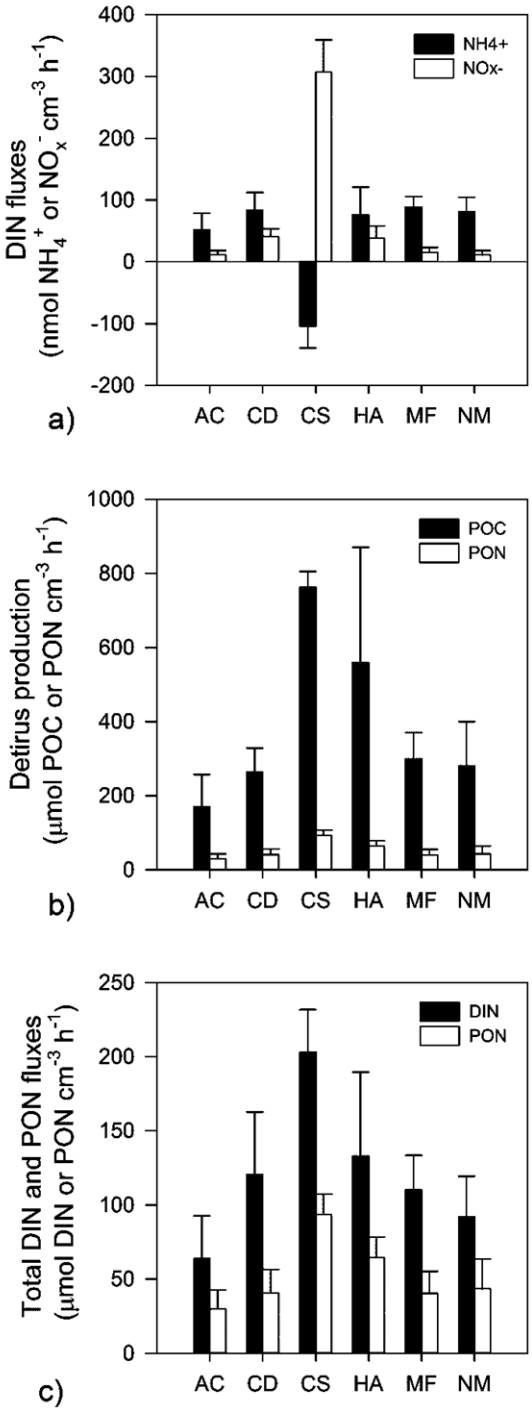

Figure 5.1: Nitrogen fluxes in the six investigated sponge species: *Amphimedon chloros* (AC), *Callyspongia densa* (CD), *Chondrilla sacciformis* (CS), *Hemimycale arabica* (HA), *Mycale fistulifera* (MF), and *Negombata magnifica* (NM). Shown are A) Fluxes of ammonia (NH_4^+), nitrate + nitrite (NO_x^-), B) Detritus production of particulate organic carbon (POC) and particulate organic nitrogen (PON), and C) Total fluxes of dissolved inorganic nitrogen (DON) and particulate organic nitrogen (PON). Data are normalized to sponge volume and rates are presented as nmol cm^{-3} h^{-1} (mean ± SD; n = 6).

Sponge detritus production

All six investigated sponge species released detritus as POC and PON in detectable quantities (Fig. 5.1b). POC release rates exceeded the release of PON, but all six sponges produced detritus with relatively low C:N ratios ranging from 6.5 – 8.7. On average, the C:N ratios of sponge detritus were substantially lower than the C:N ratio of ambient reef water POM (10.3 ± 1.6). PON fluxes ranged from 29.8 nmol cm^{-3} h^{-1} in *A. chloros* and up to 93.5 nmol cm^{-3} h^{-1} in *C. sacciformis*. Thus, the sponge species with the highest and lowest DIN release also exhibited the highest and lowest PON release (Fig. 5.1a, b). Overall, PON production averaged 52 ± 23 nmol cm^{-3} h^{-1} and accounted for a substantial fraction (30 ± 3%) of the total organic and inorganic N released. This percentage was remarkably consistent across all six species, ranging from 25 – 33% (Fig. 5.1c). Based on the measured release rates, the total PON production by the sponge community was estimated at 8.5 ± 1.3 µmol N m^{-2} reef h^{-1}. The estimated community flux of POC was 63.5 ± 18.9 µmol C m^{-2} reef h^{-1}.

Sponge dinitrogen fixation

Only *M. fistulifera* (day and night) and *A. chloros* (day only) exhibited significant N_2 fixation activity (Fig. 5.2). For both of these sponges, N_2 fixation was up to 5-times higher in the day compared to at night (Fig. 5.2). The N_2 fixation activity of *C. euplacella* and *N. magnifica* did not exceed the background activity in the unfiltered seawater controls, indicating N_2 fixation by these two species was negligible. Overall, sponge N_2 fixation rates were several orders of magnitude lower than their DIN and PON fluxes, resulting in comparatively low scale community N_2 fixation rates (0.006 ± 0.005 µmol N m^{-2} reef h^{-1}).

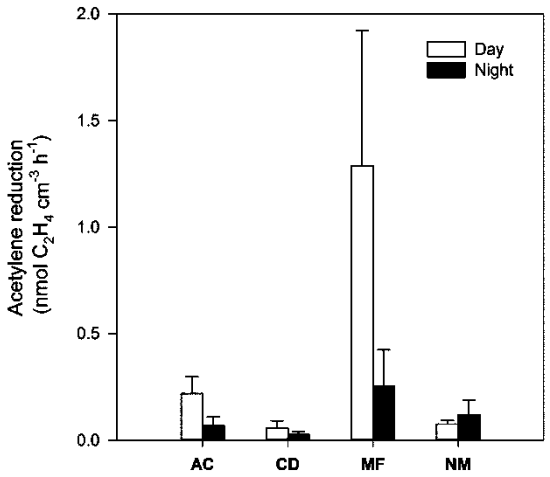

Figure 5.2: N_2 fixation measured as acetylene reduction rates of the four investigated sponge species: *Amphimedon chloros* (AC), *Callyspongia densa* (CD), *Mycale fistulifera* (MF), and *Negombata magnifica* (NM). Shown are the mean light (white bars) and dark (black bars) acetylene reduction rates presented nmol C_2H_4 cm^{-2} h^{-1} (mean ± SD; n = 6).

Stable isotope analysis

The C and N stable isotope analysis of the six sponge species showed considerable inter-species variation, with no overlap in $\delta^{13}C$ and $\delta^{15}N$ signatures between species (Fig. 5.3). The sponges clustered into two groups with group A (*A. chloros, H. arabica, M. fistulifera*) showing lower $\delta^{15}N$ values (< 2‰) and lighter $\delta^{13}C$ values (< -20‰). By comparison, group B (*C. densa, C. sacciformis, N. magnifica*) exhibited higher $\delta^{15}N$ values (> 2‰) and relatively enriched $\delta^{13}C$ values (> -20‰; Fig. 5.3). All six sponge species had $\delta^{13}C$ values that were more enriched in ^{13}C compared to both the ambient SPOM and water column BP (Fig. 5.3), with no overlap between the sponges and either of their potential particulate food sources. Furthermore, all sponges exhibited $\delta^{15}N$ values that were either lower or overlapped with both the SPOM and BP. Thus, none of the sponge showed evidence for an expected trophic increase of 3.4‰ compared to either food source. *M. fistulifera* exhibited the lowest $\delta^{15}N$ values (approaching 0‰), while *C. sacciformis* showed the most enriched $\delta^{13}C$ values (Fig 5.3).

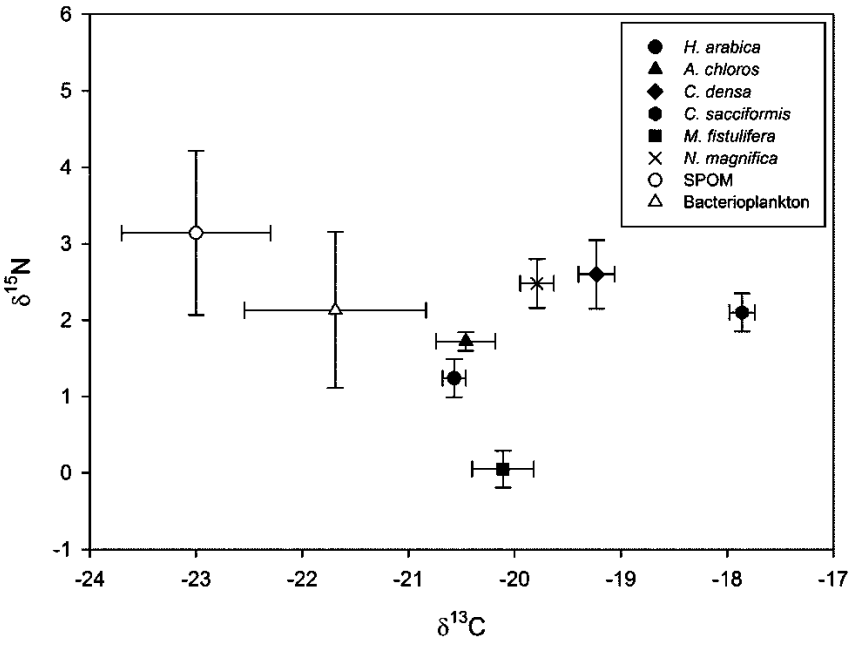

Figure 5.3: Stable isotope signatures of the six investigated sponge species, suspended particulate organic matter (SPOM), and water column bacterioplankton. Plotted are the $\delta^{13}C$ vs $\delta^{15}N$ values of sponges collected *in situ* at 10 – 15 m water depth and SPOM and bacterioplankton sampled from the water column at 10 m water depth, 1 m above the substrate. Values presented as mean ± SD (n = 10).

DISCUSSION

Sponge N fluxes

Our findings reveal that DIN release represents the major physiological N flux quantified for all six sponge species, exceeding rates for both PON release and N_2 fixation. Mean DIN release rates by Red Sea sponges (120 ± 47 nmol cm^{-3} h^{-1}) appear directly comparable to rates determined for 14 species of Caribbean sponges (200 ± 77 nmol cm^{-3} h^{-1}; Southwell et al. 2008b). Eight of these 14 Caribbean species released the majority of their DIN as NO_x^- (Southwell et al. 2008b). By contrast only one of the six Red Sea sponges, *C. sacciformis*, released DIN primarily as NO_x^- while the remaining sponges predominately released NH_4^+. Release of NO_x^- provides evidence for microbial-mediated nitrification as only ammonium oxidizing bacteria and archaea can oxidize ammonium to nitrate (Zehr & Ward 2002, Wuchter et al. 2006). Several studies have found that nitrification is widespread only in HMA sponges (Jimenez & Ribes 2007, Bayer et al. 2008, Southwell et al. 2008a, Southwell et al. 2008b, Ribes et al. 2012, Fiore et al. 2013a). As *C. sacciformis* was the only HMA sponge investigated, our findings provide further support that high nitrifying activity is common only in HMA sponges and implies that the relative composition of HMA and LMA species in the sponge community will influence the dominant type of DIN released. Nitrification in HMA sponges may function in removing toxic NH_4^+ that could build up due to the lower pumping rates of HMA compared to LMA sponges (Weisz et al. 2008, Ribes et al. 2012). The uptake of NH_4^+ combined with NO_x release by *C. sacciformis* may suggest that in addition to utilizing NH_4^+ remineralized by the sponge host, its nitrifying microbes also directly took up ambient NH_4^+. However, NH_4^+ uptake can also be mediated by other autotrophic and heterotrophic microbes (Kirchman et al. 1990, Hoch & Kirchman 1995, Kowalchuk & Stephen 2001). Although the presence of photoautotrophic microbes is unlikely given *C. sacciformis* is a strictly found in shaded habitats, additional heterotrophic pathways cannot be excluded.

Despite numerous reports of sponges producing detrital material (Reiswig 1971, Witte et al. 1997, Ribes et al. 1999, Yahel et al. 2003), detritus production has so far only been demonstrated in a limited number of sponges (de Goeij et al. 2013, Alexander et al. 2014). Alexander et al. (2014) found that eight species of tropical and temperate sponges turned over 2.5 – 18% of their bodyweight in detritus per day, but did not determine the organic C and N content of the produced detritus. Our findings present the first rates of POC and PON release by sponges, demonstrating that detrital PON can account for approximately one third of the total N released by coral reef sponges. Interestingly, the percentage of total N released as PON was remarkably consistent across all six sponges investigated (30 ± 3%), regardless of their microbial abundance (HMA vs. LMA) or growth form (encrusting vs. branching). Thus, species exhibiting higher DIN release also released higher quantities

of PON. However, other studies measuring sponge POM fluxes have found net POM uptake (Jimenez & Ribes 2007), including for *N. magnifica* (Hadas et al. 2009). This may be due to methodological differences in measuring POM. Jimenez & Ribes (2007) sampled SPOM rather than total incubation POM possibly leading to the exclusion of larger sinking particles. Hadas et al. (2009) pre-filtered their POM samples to remove particles considered too large to be digested by the sponge, which may have removed larger detrital particles such as sponge cells. Massive cell shedding of choanocyte cells is proposed to be the key mechanism by which sponges produce detritus (de Goeij et al. 2009, Alexander et al. 2014). However, it has not been possible to directly link detritus production to choanocyte shedding either due to rapid cellular degradation (Alexander et al. 2014) or due to the contribution of other cell types and metabolic waste products to sponge detritus (Maldonado 2015). Sponge detritus is consumed by detritus feeding reef fauna (de Goeij et al. 2013), but the relative contribution of metabolic waste products *vs.* shed cells has been suggested to the quality of sponge detritus as a food source (Maldonado 2015). Our findings, however, show that sponge detritus is relatively enriched in N (i.e. has a lower C:N ratio) compared to ambient seawater SPOM, suggesting that it may be a relatively high quality food source for detritus feeding organisms.

Unlike for the fluxes of DIN and PON, only two of the four sponges investigated, *A. chloros* and *M. fistulifera*, exhibited detectable N_2 fixation activity, although at low rates. This is consistent with the low sponge N_2 fixation activity measured by the few studies quantifying sponge N_2 fixation rates (Wilkinson et al. 1999, Southwell 2007, Ribes et al. 2015, Rix et al. 2015). *M. fistulifera* showed the highest N_2 fixation activity of the four species investigated and also the lowest $\delta^{15}N$ values ($0.1 \pm 0.2‰$). Depleted $\delta^{15}N$ values are consistent with the incorporation of newly fixed N (Mariotti 1983, Montoya et al. 2002), suggesting *M. fistulifera* may have been the only sponge receiving a significant input of N from N_2 fixation. However, isotope fractionation during other microbial-mediated N processes could also contribute to its depleted $\delta^{15}N$ values (e.g. Altabet 2001). The lack of NO_x^- production by *M. fistulifera* suggests it does not host nitrification, but there is evidence for low rates of photosynthesis (Rix et al. 2015) and evidence that photosynthetic microbes can transfer N to their sponge hosts (Freeman & Thacker 2011, Freeman et al. 2013). Interestingly, *M. fistulifera* belongs to the LMA sponge order Poecilosclerida (Gloeckner et al. 2014), indicating N_2 fixation may not be limited to HMA sponges. This is supported by recent evidence for nitrogenase genes (*nifH*) in three LMA, but not the three HMA sponges investigated by Ribes et al. (2015). N_2 fixation has been hypothesized to be a source of reduced inorganic N required for the high nitrification rates observed in some coral reef sponges (Corredor et al. 1988, Diaz & Ward 1997). However, our N_2 fixation rates were orders of magnitude lower than respective NO_x^- production, suggesting this linkage is unlikely. Sponge nitrification is more likely supported by metabolic ammonium released by the sponge host or by uptake from the ambient seawater. Overall, N_2 fixation appears not to play a major role in sponge N cycling and is unable to account for the apparent loss of N due to the net release of DIN and PON.

Sponge N budgets

Importantly, the net release of PON and DIN by the six sponge species implies that they must be utilizing an additional source of N in order to balance the efflux of both DIN and PON. Sponges feed preferentially on particles within the size range of 0.2 – 2.0 μm, with highest retention efficiencies for particles of 1.0 μm (Hadas et al. 2009). Since we used GF/F filters to measure POM, some heterotrophic bacteria falling beneath the 0.7 μm pore size may have been excluded. Thus, some of this imbalance between N ingestion and excretion may be accounted for by the uptake of small bacteria. However, previous studies measuring picoplankton feeding have found similar imbalances between PON ingestion and DIN excretion in sponges and have attributed this to the uptake of DON (Jimenez & Ribes 2007, Hadas et al. 2009). Indeed, de Goeij et al. showed that 18 – 36% of the DON assimilated by four encrusting Caribbean sponges was converted into detrital PON (de Goeij et al. 2013), and this may explain the net PON efflux in the investigated Red Sea sponges. This is further supported by our stable isotope data as consumers typically reflect the $\delta^{13}C$ signatures of their food sources, exhibiting at most a small $\delta^{13}C$ increase of 0.5 – 1.0‰ (Peterson & Fry 1987, Michener et al. 1994); yet none of the sponges displayed $\delta^{13}C$ signatures within a 0 – 1.0‰ range of the ambient SPOM, and only Group A sponges were within range of the water column BP. Moreover, the $\delta^{15}N$ values of the sponges either did not differ significantly or were lower than those of SPOM and BP. Since consumers typically show a $\delta^{15}N$ increase of 3.4‰ per trophic level (Peterson & Fry 1987, Michener et al. 1994, Vander Zanden & Rasmussen 2001, McCutchan et al. 2003), this indicates that other metabolic N processes may have influenced $\delta^{15}N$ signatures of the six sponges. Isotopic fractionation during microbial-mediated N processes was hypothesized to account for lower $\delta^{15}N$ values in HMA compared to LMA sponges in the Florida Keys (Southwell 2007, Weisz et al. 2007). However, we found the lowest $\delta^{15}N$ values in the LMA sponges *H. arabica* and *M. fistulifera* and no evident relationship between $\delta^{15}N$ values and nitrification. N_2 fixation may have contributed to the depleted $\delta^{15}N$ values in *M. fistulifera*, but fixation rates for the other sponges were low. Therefore, our results suggest that SPOM and BP are not the only dietary sources of C and N for the investigated sponges. We hypothesize that DON uptake may account for this imbalance as DOM has recently been found to contribute ~80 - 90% of the total carbon uptake by several coral reef sponges (Yahel et al. 2003, de Goeij et al. 2008, Mueller et al. 2014a). On coral reefs, DOM predominately originates from the high release of photosynthates by benthic primary producers (Wild et al. 2004, Haas et al. 2010, Naumann et al. 2010a, Mueller et al. 2014b, Brocke et al. 2015). These exudates are typically relatively depleted in ^{15}N and enriched in ^{13}C compared to SPOM and BP (Naumann et al. 2010b, van Duyl et al. 2011), which could explain the isotopic signatures of the six sponges (van Duyl et al. 2011). Thus our findings are consistent with the transformation of DOM to POM by the newly described sponge loop (de Goeij et al. 2013).

Ecological implications

There are few estimates of sponge-mediated community N fluxes. Our sponge-mediated benthic N efflux rates in a Red Sea reef are much lower compared to previous findings from Key Largo, Florida (19 ± 7 vs. 640 ± 130 μmol N m^{-2} reef h^{-1}), despite the fact that Red Sea and Caribbean sponges exhibited similar DIN efflux rates (Southwell et al. 2008b). This was due to lower sponge biomass in the Red Sea compared to Key Largo, which is dominated by the massive barrel sponge *Xestospongia muta* that accounts for 73% of the DIN efflux (Southwell et al. 2008b). By contrast, the majority of sponges in our study site were small encrusting species. However, neither study considered the abundant cryptic cavity sponge community. In the Red Sea, cavity sponge biomass far outweighs that of the surface sponge community (Richter et al. 2001). If the estimated cover of cavity sponges is considered (82 ± 55% per unit reef area; Richter et al. 2001), and their average DIN release rates are assumed to be equal to the rates measured for the cavity sponge *C. sacciformis*, this amounts to an additional DIN efflux of 83 ± 23 μmol N m^{-2} reef h^{-1} and PON efflux of 38 ± 6 μmol N m^{-2} reef h^{-1}. Although the generation of new N fixed by the sponge community remains negligible even when cavity sponges are considered (0.006 ± 0.005 μmol N m^{-2} h^{-1}), overall this demonstrates the importance of the cryptic reef habitat for N cycling. Indeed, cavity sponges have been linked to the increased concentrations of DIN observed in coral reef cavities in the Red Sea (Richter et al. 2001) and in the Caribbean (Scheffers et al. 2004, van Duyl et al. 2006). Furthermore, coral reef cavities in the Caribbean and Indo-Pacific have also been shown to be sinks of DOM (de Goeij & van Duyl 2007). Due to the oligotrophic conditions in the Gulf of Aqaba (Silverman et al. 2007, Bednarz et al. 2015), the N fluxes estimated for our sponge community may represent an ecologically important flux of N. DIN released by sponges has been shown to facilitate coral growth (Slattery et al. 2013) and support nearby algae (Davy et al. 2002, Easson et al. 2014). Sponge detritus represents an additional but poorly investigated N flux that may contribute to coral reef detrital food webs but requires further investigation. Collectively our findings hint at the potential functioning of a sponge loop in the Red Sea as we hypothesize that the high N demand resulting from net DIN and PON release in the six sponge species must be met by DON uptake to balance their N budgets.

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CHAPTER 6

SEASONALITY IN DINITROGEN FIXATION AND PRIMARY PRODUCTIVITY BY CORAL REEF FRAMEWORK SUBSTRATES FROM THE NORTHERN RED SEA

Rix L, Bednarz VN, Cardini U, van Hoytema N, Al-Horani FA, Wild C, Naumann MS (In Press) Seasonality in dinitrogen fixation and primary productivity by coral reef framework substrates from the northern Red Sea. This chapter is published in *Marine Ecology Progress Series* doi: 10.3354/meps11383

ABSTRACT

N_2 fixation by coral reef benthic substrates may support primary productivity on oligotrophic coral reefs. However, little is known regarding the influence of environmental parameters on coral reef benthic N_2 fixation. This study quantified N_2 fixation and photosynthesis by three common reef framework substrates: turf algae, coral rock, and the abundant encrusting sponge *Mycale fistulifera* over four seasons in the northern Gulf of Aqaba. N_2 fixation activity was detected during day and night for all substrates, but on an annual average was significantly higher for turf algae (4.4 ± 3.9 nmol C_2H_4 cm^{-2} h^{-1}) and coral rock (3.5 ± 2.8 nmol C_2H_4 cm^{-2} h^{-1}) compared to *M. fistulifera* (0.2 ± 0.2 nmol C_2H_4 cm^{-2} h^{-1}). There was strong seasonality in N_2 fixation, with rates one order of magnitude higher in summer when temperature and irradiance were highest but inorganic nutrient concentrations lowest. During summer and fall, when nutrients were low, we found a significant positive linear relationship between gross photosynthesis (P_{gross}) and N_2 fixation in turf algae and coral rock. Further, we estimate N_2 fixation can supply up to 20 and 27% of the N demand for net photosynthesis (P_{net}) in coral rock and turf algae, respectively. By contrast there was no significant relationship between N_2 fixation and P_{gross} in *M. fistulifera,* which displayed negative P_{net} and heterotrophic metabolism (P_{gross}:Respiration <1). These findings highlight the role of environmental parameters in regulating benthic substrate-associated N_2 fixation and the potential importance of fixed N for supporting primary production, particularly during nutrient-depleted conditions.

INTRODUCTION

Coral reefs are characterized by high productivity but are typically surrounded by oligotrophic waters (Odum & Odum 1955, Hatcher 1988), where nitrogen (N) is a key limiting nutrient for growth (Delgado & Lapointe 1994, Eyre et al. 2008). Efficient internal nutrient recycling in the benthos contributes to this high productivity, but input of new N is essential to sustain net ecosystem production and growth. Though energetically costly, numerous studies demonstrate that dinitrogen (N_2) fixation represents a substantial source of new N on coral reefs (Webb et al. 1975, Larkum et al. 1988, O'Neil & Capone 1989, Charpy et al. 2007).

Biological N_2 fixation is carried out by a diverse group of heterotrophic and photoautotrophic bacteria (Zehr et al. 2003), but cyanobacteria are a key contributor to benthic N_2 fixation on coral reefs (Casareto et al. 2008, Charpy et al. 2012). Cyanobacterial mats have attracted much research focus due to their high N_2 fixation rates (e.g. Charpy et al. 2007, Bauer et al. 2008, Casareto et al. 2008). However, cyanobacteria are also important components of the various reef framework substrates that are ubiquitous on coral reefs; including algal turfs, endolithic algal communities associated with calcium carbonate structures, and endosymbiotic communities of sponges (Charpy et al. 2012). In coral reefs in the northern Gulf of Aqaba, these reef framework substrates are a dominant component of the benthos. Turf algae make up 72% of the benthic community on reefs in Eilat (Israel), while on the Jordanian side of the Gulf, biogenic reef framework with only sparse epilithic overgrowth (hereafter: coral rock) can account for up to 58% of the benthic cover (Bahartan et al. 2010). Sponges meanwhile dominate the cryptic reef habitat (Richter et al. 2001). High rates of N_2 fixation have been measured in both turf algae and coral rock (eg. Larkum et al. 1988, Williams & Carpenter 1998), therefore these reef framework substrates may contribute importantly to fixed N on reefs in the Gulf of Aqaba. Evidence for active N_2 fixation in sponges is scarce (Wilkinson & Fay, 1979, Shashar et al. 1994a, Shieh & Lin 1994), but many species harbor microbial symbionts capable of fixing N (Taylor et al. 2007, Mohamed et al. 2008, Fiore et al. 2015) suggesting N_2 fixation in sponges may be widespread.

Due to their association with photosynthetic cyanobacteria and algae, reef framework substrates also contribute to reef photoautotrophic primary production. Turf algae are dominant primary producers on many reefs (Adey & Goertemiller 1987, Carpenter & Williams 2007) and more than one third of sponges in the Caribbean, Great Barrier Reef (GBR) and West Indian Ocean harbor photosynthetic symbionts (Wilkinson 1987, Steindler et al. 2002, Erwin & Thacker 2007). Due to the oxygen (O_2) sensitivity of nitrogenase, the enzyme responsible for N_2 fixation, photosynthesizing diazotrophs have evolved strategies to allow photosynthesis and N_2 fixation to co-occur (Berman-Frank et al. 2003). Spatial separation in heterocystous cyanobacteria allows the fixation of N_2 during the day (Gallon 2001), while non-heterocystous cyanobacteria typically fix N_2 at night, relying on energy derived from the carbon (C) fixed during the previous daylight period (Bergman et al. 1997,

Charpy et al. 2007). In marine sponges, hypoxic zones may facilitate O_2-sensitive processes such as N_2 fixation (Hoffmann et al. 2005). N_2 fixation may provide an additional source of N to support benthic primary production, however, little is known regarding the interaction between N_2 fixation and photosynthesis in benthic substrates, and few studies have quantified both processes in parallel (Williams & Carpenter 1997, Lesser et al. 2007, Charpy et al. 2007, Casareto et al. 2008).

Fringing reefs in the Gulf of Aqaba experience strong seasonal variation in key environmental parameters as a result of the annual stratification cycle in the water column that occurs due to their northern latitude. Winter and early spring are characterized by low temperature and irradiance but high inorganic nutrient concentrations as deep convective mixing of the water column transports nutrient-enriched deep water into the photic zone (Carlson et al. 2014). Increasing irradiance followed by warming sea surface temperatures throughout spring and summer lead to the development of a thermocline >100 m deep (Carlson et al. 2014) with a nutrient-depleted surface layer (Silverman et al. 2007). This results in summer conditions of high temperature and irradiance but low inorganic nutrient concentrations. Near-surface temperatures range from 21 – 28 °C throughout the year while inorganic nutrient concentrations can vary by an order of magnitude (Silverman et al. 2007, Carlson et al. 2014). Such environmental parameters are known to influence planktonic N_2 fixation (Sohm et al. 2011), but their effect on N_2 fixation by benthic reef diazotrophs is largely unknown (Cardini et al. 2014).

The objectives of this study, therefore, were 1) to quantify rates of N_2 fixation and primary productivity (i.e. photosynthesis) in three dominant reef framework substrates; turf algae, coral rock, and an abundant encrusting sponge in seasonal resolution in order to evaluate the effect of seasonally variable key environmental parameters on these processes, and 2) to estimate the contribution of N_2 fixation to the N requirements for primary production in the three investigated substrates.

MATERIALS AND METHODS

Study site

This study was conducted in the northern Gulf of Aqaba at the Marine Science Station (MSS) Aqaba, Jordan (29°27' N, 34°58' E). Sampling was carried out on the 1 km long fringing reef in front of the MSS, which is designated as a marine reserve, and experimental work was carried out in the MSS laboratories. In order to examine the effect of seasonality, all experiments were repeated over four seasonal periods in 2013: winter (February), spring (April), summer (September), and fall (November).

The benthic reef community was dominated by hard and soft corals (58.1 ± 13.8%), while coral rock represented the third most abundant benthic substrate type at 10 m water depth, covering

on an annual average 14.2 ± 5.0% of the available substrate. The percent cover of turf algae (annual average: 4.4 ± 4.5%) was seasonally variable reaching a maximum of 10.3 ± 4.2% in winter and decreasing to a minimum of 1.0 ± 1.0% in fall. Sponge cover was constant throughout the year averaging 1.2 ± 0.9%. The non-cryptic sponge community was dominated by the abundant encrusting sponge *Mycale fistulifera*, which accounted for 65% of the visible sponge cover at 10 m water depth. Together the three investigated substrates accounted for 19.8 ± 10.3% of the total benthic coverage.

Environmental monitoring

During each season *in situ* water temperature and irradiance were continuously monitored over four weeks at one minute intervals at the sampling site using a data logger (Onset HOBO Pendant UA-002-64; temperature accuracy: ± 0.53 °C, spectral detection range: 150 – 1200 nm) placed in an unshaded position on the reef at 10 m water depth. Parallel irradiance measurements with a quantum sensor (Model LI-192SA; Li-Cor) allowed the conversion of lux measurements to photosynthetically active radiation (PAR µmol photons m^{-2} s^{-1}, wavelength 400 – 700 nm) using a conversion factor of 1 µmol quanta m^{-2} s^{-1} = 52 lux. Irradiance data are presented as seasonal means (± SD) of daily maximum values (µmol quanta m^{-2} s^{-1}) and integrated diurnal values (mol quanta m^{-2} d^{-1}; Table 1.1). Weekly seawater samples (n = 4 per season) were collected by SCUBA using acid-washed high-density polyethylene canisters (n = 4, 5 L) at 10 m water depth (~1 m above the bottom) and immediately transferred to the laboratory for further processing. Subsamples (n = 4) were taken for quantification of inorganic nutrients, particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll *a* (Chl *a*). Inorganic nutrient subsamples were syringe-filtered through cellulose acetate filters (nominal pore size 0.45 µm) for determination of ammonium (NH_4^+), nitrate (NO_3^{2-}), nitrite (NO_2^-), and phosphate (PO_4^{3-}) concentrations using standard methods, although a modification of the cadmium column method for NO_3^{2-} determination using a cadmium sponge was used to enable field analysis (Murphy & Riley 1962, Strickland & Parsons 1972, Holmes et al. 1999). NH_4^+ was determined fluorometrically using a Trilogy Fluorometer (Turner Designs), while all other nutrients were measured photometrically with a JASCO-V630 spectrophotometer (Jasco Analytical Instruments). The detection limits for NH_4^+, PO_4^{3-}, and NO_3^{2-} + NO_2^- were 0.09, 0.01, and 0.02 µM, respectively. Subsamples for Chl *a* determination (n = 4, 1 L) were filtered onto pre-combusted (450 °C, 4 h) GF/F filters (VWR: nominal pore size 0.7 µm) and stored frozen at -80 °C in the dark until further processing. Chl *a* was extracted with 90% acetone (12 h in the dark at 4°C) and analysed fluorometrically using a Trilogy fluorometer fitted with the non-acidification module (CHL NA #046, Turner Designs). Subsamples for POC (1 L) and PN (2 L) were filtered onto pre-combusted GF/F filters and dried in the oven (40 °C, 48 h). Prior to analysis POC filters were decalcified with 0.1 N HCl. POC and PN filter contents were measured on a EuroVector elemental analyzer (EURO

EA 3000) with analytical precision of ≤0.1% (C) and ≤0.03% (N) using the elemental standard Acetanilide OAS (certificate 187560).

Substrate collection and maintenance

Samples of the three investigated reef framework substrates; turf algae, coral rock, and the encrusting sponge *M. fistulifera*, were collected from the reef at 10 m water depth by SCUBA and immediately transferred to the aquarium facility without air exposure. Turf algae were defined as a thick mat consisting of a heterogeneous assemblage of filamentous algae, crustose coralline algae (CCA), and filamentous cyanobacteria. In the Gulf of Aqaba, turf algae are composed predominately of Phaeophyta and Rhodophyta of the order Ceramiales as well as green algae of the genus C*ladophora* and cyanobacteria (Bahartan et al. 2010, Haas et al. 2010). Coral rock was considered biogenic reef framework lacking coverage by a single dominant visible epilithic group with the carbonate structure clearly visible and open for settling organisms. In the Gulf of Aqaba this hard substrate can cover large areas of the reef and is commonly referred to as "bare rock" or "bare substrate" (eg. Shashar et al. 1994a, Bahartan et al. 2010), although it is associated with endolithic algae, epilithic microbial biofilms, and sparse patches of CCA, cyanobacteria and filamentous algae (Charpy et al. 2012, Bahartan et al. 2010). *M. fistulifera* is an encrusting sponge approximately 0.2 – 0.5 cm thick, typically found encrusting coral skeletons. Turf algae and *M. fistulifera* were collected by chiseling small pieces of dead branching corals overgrown by either turf or *M. fistulifera*, and were attached to ceramic tiles with coral glue (Reef Construct, Aqua Medic®) to minimize stress during experimental handling. Coral rock was sampled by chiseling pieces of reef framework. For each substrate, 8 replicates per season were collected with a mean height of 5-6 cm and mean surface area of 33.5 ± 18.9 cm^2. Specimens were maintained in an outdoor 1000 L flow-through tank supplied with seawater pumped directly from the reef at 10 m water depth at approximately 4000 L h^{-1}, ensuring key environmental parameters (e.g. temperature and inorganic nutrient concentrations) corresponded to seasonal *in situ* conditions. Irradiance (PAR) was adjusted to *in situ* levels at 10 m water depth with layers of black mesh. Parallel irradiance measurements with a quantum PAR sensor (Model LI-192SA; Li-Cor) *in situ* at 10 m and in the maintenance tank ensured irradiance corresponded to seasonal *in situ* conditions as presented in Table 6.1 with the following standard deviations: 21.8, 56.4, 26.4, 19.6 µmol photons m^{-2} s^{-1} for winter, spring, summer and fall, respectively. All incubation measurements were conducted in the same maintenance tank to ensure consistent temperature and irradiance values. *M. fistulifera* specimens were allowed to heal and acclimate for 1 wk prior to experiments and only healthy, actively pumping specimens were incubated. Turf algae and coral rock were collected 24 h before incubations were conducted.

Quantification of dinitrogen fixation

N_2 fixation rates were quantified using the acetylene (C_2H_2) reduction assay method (Capone 1993, Wilson et al. 2012). Specimens (n = 8 per substrate) were incubated in individual 1000 mL transparent glass chambers containing 800 mL of natural seawater and 200 ml headspace. Organisms were transferred into chambers without air exposure and positioned to ensure comparable irradiances in all chambers. Immediately prior to the start of the incubations 10% of the seawater was replaced with C_2H_2-saturated seawater. Chambers were then sealed gas-tight with a spring-loaded glass lid and 10% of the 200 mL headspace was replaced with freshly generated C_2H_2 gas via a needle injection port in the glass lid. Sealed chambers were stirred with magnetic stirrers (600 rpm) and positioned in the flow-through tank to ensure *in situ* temperature and irradiance throughout the 24 h incubation period. Parallel measurements of irradiance inside the chambers and in the flow-through tank revealed no significant differences ensuring irradiances inside the chambers corresponded to the seasonal values provided in Table 1. Incubations started and ended just prior to sunset (approximately 17:00) and gas samples were taken at 0, 4, 12, 16 and 24 h, except during spring when samples were taken only at 0 and 24 h. These sampling intervals were selected to capture the periods of dusk, night, dawn, and full daylight as N_2 fixation during low light conditions (dawn and dusk) is a strategy by some diazotrophs to manage the oxygen inhibition of nitrogenase (Lesser et al. 2007). At each time interval 1 mL of gas sample was collected from the headspace of each chamber with a gastight syringe and transferred into gas-tight 2 mL glass vials fitted with butyl septa and filled with distilled water. Vials were stored frozen upside down until analysis.

Ethylene (C_2H_4) concentrations in the gas samples were measured using a reducing compound photometer (RCP) (Peak Laboratories) with a detection limit of 100 ppb. Calibration of the RCP was routinely conducted using serial dilutions of a 200 ppm (± 2%) C_2H_4 standard in air (Restek, USA). Differences in C_2H_4 concentration between the time intervals of the incubation period were converted into C_2H_4 evolution rates according to Breitbarth et al. (2004). The C_2H_4 concentrations of the samples were corrected for the signal of unfiltered seawater controls (n = 8) and normalized to incubation time and surface area of the specimen in order to calculate C_2H_4 evolution rates (nmol C_2H_4 cm^{-2} h^{-1}). Additional controls for 0.2 µm filtered seawater (n = 6), unfiltered seawater and ceramic tile (n = 6), and unfiltered seawater with specimens but no addition of C_2H_2 (natural C_2H_4 production, n = 6), showed negligible C_2H_4 evolution. Surface areas were measured using a standard geometric technique (Advanced Geometry) as described by Naumann et al. (2009). To convert C_2H_4 evolution rates to N_2 fixation rates, a conservative theoretical ratio of 4:1 (C_2H_4:N_2) was used, which assumes that 4 mol of C_2H_4 are reduced per 1 mol of N_2. This is more conservative than the theoretical stoichiometric ratio of 3:1 as it accounts for the inhibition of the hydrogenase reaction of nitrogenase under C_2H_4-reducing conditions (Capone 1993, Mulholland et al. 2004).

Quantification of primary productivity

Primary productivity (i.e. photosynthesis) was quantified via dissolved O_2 fluxes. Substrates and seawater controls (n = 8 replicates each) were incubated in individual 1000 mL airtight transparent glass chambers filled with natural seawater and sealed with a transparent glass lid. The sealed chambers were incubated under identical conditions as described above for N_2 fixation measurements. Incubations for respiration (R) were conducted 1-2 h after sunset in complete darkness for 90 – 120 min. Incubations for net photosynthesis (P_{net}) were carried out at between 12:00 – 14:00 the following day during maximum light intensity for 60 – 90 min. Dissolved O_2 concentrations were measured at the start and end of each incubation period using a salinity and temperature corrected O_2 optode sensor (MultiLine ® IDS 3430, WTW GmbH). Start O_2 concentrations were subtracted from end O_2 concentrations to quantify P_{net} and R. O_2 fluxes were corrected for the mean O_2 difference found in the seawater controls and normalized to incubation time and surface area of the respective specimen. R is presented as a positive rate and gross photosynthesis (P_{gross}) rates were calculated as: $P_{gross} = P_{net} + R$.

To calculate the contribution of fixed N to the N demand for primary production, O_2 fluxes were converted into dissolved inorganic C fluxes using a photosynthetic quotient (PQ) of 1.04 and respiratory quotient (RQ) of 0.96 for turf algae and coral rock (Carpenter & Williams, 2007). Since no literature values were available for marine sponges, a PQ/RQ of 1 was used for *M. fistulifera*. It was assumed that turf algae and *M. fistulifera* assimilate biomass with C:N ratios of 13.7 ± 1.3 and 6.2 ± 0.3, respectively, based on C and N elemental analyses of macroalgae and *M. fistulifera* from the study site (L. Rix unpublished data). Since no data were available for coral rock from the Gulf of Aqaba, C:N ratios of epi- and endolithic algae associated with coral rubble from Le Reunion and Sesoko Islands were used (9.7 ± 1.5; Casareto et al. 2008). These reefs also belong to the Indo-Pacific and display comparable inorganic nutrient concentrations (Casareto et al. 2008), and were therefore deemed representative. However, variations in community assemblages may result in corresponding variations in tissue C:N ratios. Nevertheless, our intention is to provide a mainly qualitative estimate of the importance of N_2 fixation for primary production by demonstrating how much new N is made available by N_2 fixation that could potentially be used to meet the demand for net primary production and biomass generation.

Statistical analysis

The influence of "season" and "substrate" on all physiological parameters was estimated using fully crossed general linear models fitted in R version 3.1.1 (R Development Core Team, 2014). A second model was run examining the effect of "season" and "substrate" and "time of day" (day or night) on N_2 fixation. Season was used as a fixed factor encompassing the combined effects of all

environmental parameters. The influence of individual environmental parameters was further examined using linear regressions. To confirm the assumptions of normally distributed and homogenous residuals, qqplots and scatter plots of residuals against fitted values were visually inspected (Quinn & Keough 2002), and data were log-transformed where necessary. Model stability was checked by examining leverage and Cook's distance as well as dffits and dfbetas, and all values were deemed acceptable. Model significance was tested using likelihood ratio tests, comparing the deviances of full models with those of the null models comprising only the intercept. The significance of individual factors was tested by removing the factor of interest and comparing the deviance to the respective full models. If factors were found to be significant, pairwise post-hoc comparisons (*t* – tests) were used to check the comparisons of interest.

RESULTS

Environmental monitoring

All environmental parameters monitored showed marked seasonal variability over the study period, with the most pronounced differences occurring between winter and summer (Table 6.1). Both the daily maximum irradiance and integrated diurnal irradiance were higher in spring and summer compared to winter and fall (Table 6.1). *In situ* temperature at 10 m water depth ranged from 22.4 to 28.0 °C over the year, remaining low throughout winter and spring then reaching a maximum in summer before decreasing again in fall (Table 6.1). Concentrations of NH_4^+, NO_x and PO_4^{3-} exhibited a negative correlation with temperature (lm: all $p < 0.001$), with concentrations more than twice as high in winter and spring compared to summer and fall, reflecting the deep winter mixing and summer stratification of the water column (Silverman et al. 2007). The ratio of dissolved inorganic nitrogen to phosphate (DIN:PO_4^{3-}) ranged from 3.4 to 15.6 over the year but was consistently lower than the Redfield ratio (16:1), while POC:PN ratios always exceeded the Redfield ratio (106:16), indicating a deficiency of N compared to Redfield proportions. Chl *a* decreased by half in summer compared to all other seasons, while POC and PN were highest in spring during the seasonal plankton bloom (Table 6.1).

Table 6.1 Environmental parameters monitored at 10 m water depth over four seasonal periods in 2013; Winter (February), Spring (April), Summer (September), Fall (November). Parameters include irradiance measured as both the mean daily maximum (μmol photons m^{-2} s^{-2}) and integrated diurnal mean (mol photons m^{-2} d^{-1}), temperature (°C), ammonium (NH_4^+), nitrate + nitrite (NO_3^{2-} + NO_2^-), total dissolved inorganic nitrogen (DIN), phosphate (PO_4^{3-}), particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll a (Chl *a*). Temperature and irradiance were measured continuously during each seasonal period while other parameters were measured once weekly over four weeks (n = 4). Values are presented as mean ± SD.

Parameter	Winter	Spring	Summer	Fall
Daily maximum PAR	180 ± 43	252 ± 38	307 ± 25	171 ± 20
Integrated diurnal PAR	3.43 ± 0.66	5.71 ± 0.32	7.25 ± 0.47	3.51 ± 0.47
Temperature (°C)	22.5 ± 0.1	22.8 ± 0.3	27.5 ± 0.2	25.2 ± 0.2
NH_4^+ (μM)	0.32 ± 0.09	0.46 ± 0.11	0.14 ± 0.07	0.28 ± 0.07
NO_3^{2-} + NO_2^- (μM)	0.79 ± 0.16	0.49 ± 0.19	0.09 ± 0.21	0.18 ± 0.05
DIN (μM)	1.11 ± 0.19	0.96 ± 0.08	0.23 ± 0.07	0.46 ± 0.10
PO_4^{3-} (μM)	0.11 ± 0.01	0.10 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
DIN:PO_4^{3-}	10.50 ± 1.09	9.68 ± 0.43	8.10 ± 3.40	12.93 ± 2.22
POC (μM)	6.33 ± 0.70	10.25 ± 0.72	7.96 ± 1.35	8.81 ± 2.10
PN (μM)	0.85 ± 0.07	1.27 ± 0.05	0.96 ± 0.28	0.87 ± 0.37
POC:PN	7.34 ± 1.15	8.18 ± 1.29	8.34 ± 1.17	10.20 ± 1.62
Chl *a* (μg L^{-1})	0.21 ± 0.02	0.22 ± 0.04	0.10 ± 0.04	0.19 ± 0.04

Dinitrogen fixation

N_2 fixation activity varied significantly by substrate, season, and an interaction between the two factors (Fig. 6.1, Appendix 4 - Table 1). On annual average, N_2 fixation was significantly higher in turf algae (4.4 ± 3.9 nmol C_2H_4 cm^{-2} h^{-1}) and coral rock (3.5 ± 2.8 nmol C_2H_4 cm^{-2} h^{-1}) compared to *M. fistulifera* (0.2 ± 0.2 nmol C_2H_4 cm^{-2} h^{-1}) (post hoc paired *t*-test: both $p < 0.001$). N_2 fixation of turf algae and coral rock were similar on annual average but significantly higher for turf algae in winter and summer, although in summer this was due to higher nighttime N_2 fixation by turf algae (post hoc paired *t*-test: all $p < 0.001$). N_2 fixation rates for all substrates were significantly and up to an order of magnitude higher in summer compared to all other seasons (post hoc paired *t*-test: all $p < 0.001$) (Fig. 6.1). Coral rock and *M. fistulifera* also displayed significantly lower N_2 fixation activity in winter (post hoc paired *t*-test: all $p < 0.05$), with the winter N_2 fixation activity in *M. fistulifera* not significantly different from seawater controls. There were no significant differences in N_2 fixation between spring and fall for any substrate (Fig. 6.1). Irradiance and temperature had a positive effect on N_2 fixation, while inorganic nutrient concentrations had a negative effect (Table 6.2). Irradiance explained more variation in N_2 fixation in turf algae and *M. fistulifera* than in coral rock, and for all substrates DIN explained more variation in N_2 fixation than PO_4^{3-} concentrations (Table 6.2).

N_2 fixation activity also varied significantly by time of day (Fig. 6.2, Appendix – 4 Table 2). However, no significant differences were observed during dawn and dusk, and therefore we present only the day and night rates (Fig. 6.2). *M. fistulifera* exhibited significantly higher N_2 fixation during the day compared to the night in all three seasons examined (i.e. winter, summer, and fall) (post hoc paired *t*-test: all $p < 0.01$). N_2 fixation in turf algae showed a seasonally variable response to time of day, with significantly higher daytime N_2 fixation in winter but significantly higher nighttime N_2 fixation in summer (Fig. 6.2). This was the only instance of significantly higher N_2 fixation at night but it was also the highest N_2 fixation rate measured over all substrates and seasons, with a rate of 17.8 ± 5.5 nmol C_2H_4 cm^{-2} h^{-1}. Coral rock displayed no significant differences in N_2 fixation between day and night (Fig. 6.2).

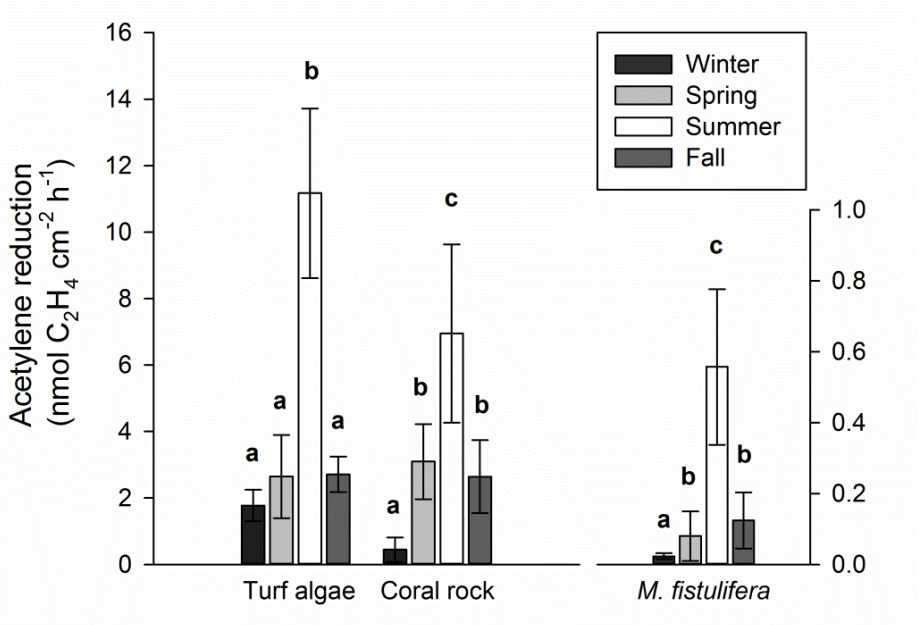

Figure 6.1 Mean N_2 fixation measured as acetylene reduction rates of the three investigated benthic substrates over the four seasonal periods in 2013; winter (February), spring (April), summer (September), fall (November). Values (acetylene reduction nmol C_2H_4 cm^{-2} h^{-1}) are presented as mean (n = 8) ± SD. Different letters indicate statistical differences within each substrate. Note the different y-axis scale for *Mycale fistulifera*.

Table 6.2 Linear regression analysis of the influence of key environmental parameters on the P_{gross} and N_2 fixation rates of the three investigated substrates. Data are presented as R-squared values at significance levels of <0.05*, <0.01**, <0.001***. Bold values indicate a significant positive linear relationship and italicized values indicate a significant negative linear relationship. Abbreviations: P_{gross} = gross photosynthesis, DIN = dissolved inorganic nitrogen, PO_4^{3-} = phosphate.

		Irradiance	Temperature	DIN	PO_4^{3-}
N_2 fixation	Turf algae	**0.542*** **	**0.696*** **	*0.586*** *	*0.399*** *
	Coral rock	**0.415*** **	**0.458*** **	*0.511*** *	*0.396*** *
	Mycale fistulifera	**0.503*** **	**0.714*** **	*0.696*** *	*0.584*** *
P_{gross}	Turf algae	**0.505*** **	**0.424*** **	*0.305***	*0.163 **
	Coral rock	0.028 NS	0.122 NS	0.096 NS	0.088 NS
	Mycale fistulifera	**0.403*** **	**0.212***	*0.244***	*0.162**

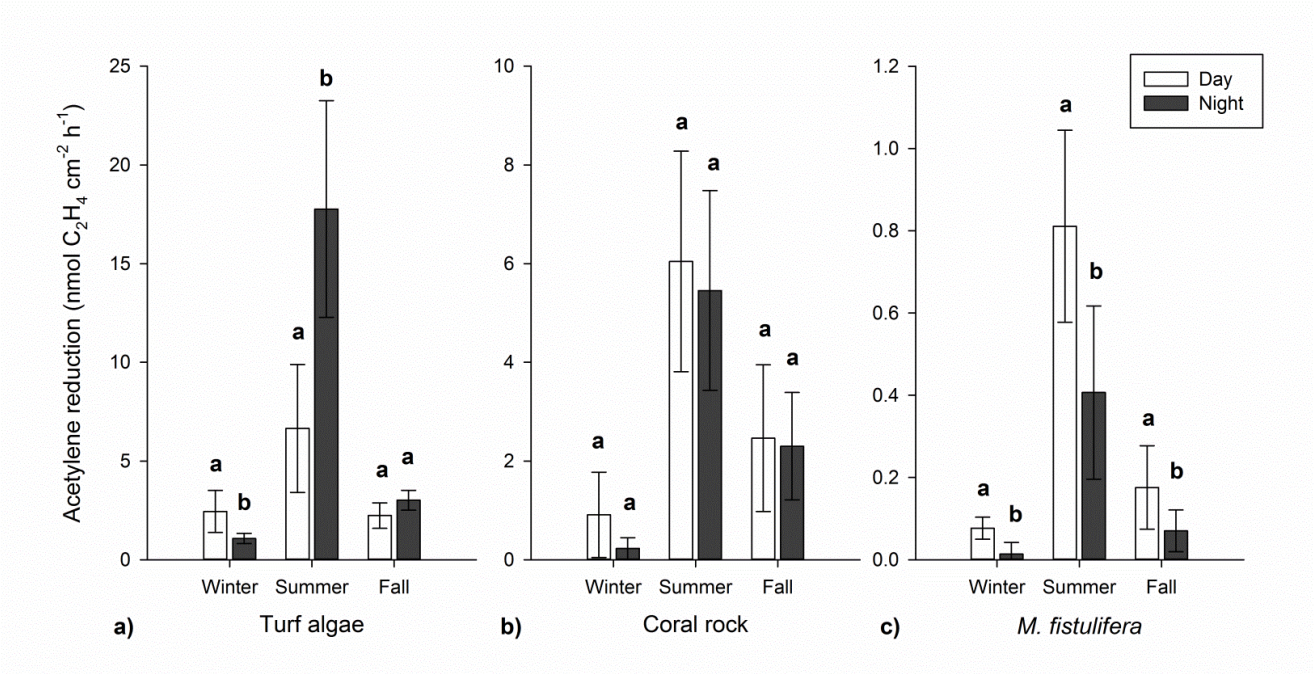

Figure 6.2. Mean day (light bars) and night (dark bars) N_2 fixation measured as acetylene reduction rates of a) turf algae, b) coral rock, and c) *Mycale fistulifera* over three seasons in 2013 (winter, summer, and fall). Values (acetylene reduction nmol C_2H_4 cm^{-2} h^{-1}) are presented as mean (n = 8) ± SD. Different letters indicate statistical differences within each substrate. Note the changes in scale of the y-axis.

Primary productivity

There were significant effects of substrate and season as well as a significant interaction between the two factors for all physiological parameters measured (Appendix 4 - Table 1). Over all seasons, rates of P_{gross} were significantly higher in turf algae compared to coral rock (post hoc paired *t*-test: $p < 0.001$) and significantly lower in *M. fistulifera* compared to both other substrates (post hoc paired *t*-test: both $p < 0.001$). Despite low positive P_{gross} rates, *M. fistulifera* exhibited negative P_{net} rates (Table 6.3). This was due to high R rates, which were significantly higher than for turf algae and coral rock (post hoc paired *t*-test: both $p < 0.001$) and resulted in low P_{gross}:R ratios (< 1) indicating heterotrophic metabolism by the sponge (Wilkinson 1987). Coral rock exhibited significantly higher R and lower P_{gross}:R ratios than turf algae (post hoc paired *t*-test: both $p < 0.001$) (Table 6.3).

Seasonal variations in P_{gross} were less pronounced than for N_2 fixation (Table 6.3). P_{gross} was significantly higher in turf algae in summer (post hoc paired *t*-test: all $p < 0.001$) and significantly lower in coral rock in spring (post hoc paired *t*-test: $p < 0.01$) (Table 6.3). It should be noted that P_{gross} and P_{net} rates for turf algae in spring may represent the lower end of typical spring values due to unseasonably low irradiances during the turf algae photosynthesis incubations (73.8 ± 26.3 µmol photons m^{-2} s^{-1} compared to the seasonal mean of 252 ± 38 µmol photons m^{-2} s^{-1}). P_{gross} in *M. fistulifera* was significantly higher in spring compared to winter and in summer compared at all other seasons (post hoc paired *t*-test: all $p < 0.001$). Irradiance and temperature were positively correlated with P_{gross} in turf algae and *M. fistulifera* and explained more of the seasonal variation in P_{gross} than DIN and PO_4^{3-}, which were negatively correlated (Table 6.2). There was little seasonal variation in P_{gross} of coral rock with no significant effect of any monitored environmental parameter (Table 6.2).

Table 6.3. Metabolic parameters measured in the three investigated substrates over four seasonal periods. Rates are presented as nmol O_2 cm^{-2} h^{-1} (mean ± SD, n = 8). Abbreviations: P_{gross} = gross photosynthesis, R = respiration, P_{net} = net photosynthesis.

Substrate	Season	P_{gross}	R	P_{net}	P_{gross}:R
Turf algae	Winter	528 ± 85	95 ± 12	433 ± 77	5.6 ± 0.7
	Spring	550 ± 107	78 ± 19	473 ± 95	7.2 ± 1.1
	Summer	894 ± 162	119 ± 27	775 ± 155	7.8 ± 2.1
	Fall	509 ± 84	95 ± 19	415 ± 68	5.4 ± 0.6
	Mean	**620 ± 195**	**97 ± 24**	**524 ± 179**	**6.5 ± 1.6**
Coral rock	Winter	472 ± 237	96 ± 55	379 ± 186	4.7 ± 0.4
	Spring	354 ± 165	69 ± 38	271 ± 136	3.4 ± 1.9
	Summer	553 ± 152	179 ± 95	374 ± 124	3.5 ± 1.2
	Fall	446 ± 134	144 ±35	302 ± 105	3.1 ± 0.5
	Mean	**438 ± 189**	**122 ± 72**	**316 ± 158**	**3.7 ± 1.9**
Mycale fistulifera	Winter	64 ± 49	238 ± 38	-174 ± 27	0.3 ± 0.1
	Spring	220 ± 42	348 ± 103	-139 ± 109	0.7 ± 0.2
	Summer	307 ± 108	563 ± 55	-256 ± 80	0.5 ± 0.2
	Fall	139 ± 68	564 ± 106	-393 ± 190	0.3 ± 0.2
	Mean	**190 ± 121**	**431 ± 164**	**-240 ± 149**	**0.5 ± 0.3**

Contribution of dinitrogen fixation to primary productivity

There was a significant positive linear relationship between P_{gross} and N_2 fixation for turf algae and coral rock only during summer and fall (Table 6.4). N_2 fixation explained 65 – 76% of the variation in P_{gross} in turf algae and 62 – 74% in coral rock during these seasons. In contrast there was no significant relationship between N_2 fixation and P_{gross} in *M. fistulifera* during any of the four seasons (Table 6.4). The potential contributions of N_2 fixation to the N demand for P_{net}, which represents the new production available for growth after accounting for respiration, were on average 10.5% for turf algae and 14.5%, for coral rock, but non-calculable for *M. fistulifera,* which displayed negative P_{net} (Table 6.5). This contribution was seasonally variable with N_2 fixation having the potential to supply the highest amounts of N in summer (up to 19.8 and 26.8% of the N required to meet the demand for P_{net} in turf algae and coral rock, respectively), while the contributions in winter were estimated to be less than 6% for all substrates (Table. 6.5).

Table 6.4. Linear regression analysis between the gross photosynthesis (P_{gross}) rates and N_2 fixation rates of each of the three substrates during each of the four seasonal periods. Data are presented as R-squared values at significance levels of <0.05*, <0.01**, <0.001***, and NS which indicates no significance. Bold values indicate a significant positive linear relationship.

	Turf algae	Coral rock	*Mycale fistulifera*
Winter	0.245 NS	0.216 NS	0.022 NS
Spring	0.064 NS	0.318 NS	0.205 NS
Summer	**0.653***	**0.626***	0.199 NS
Fall	**0.741****	**0.704****	0.374 NS

Table 6.5. Dinitrogen (N_2) fixation, net primary production, nitrogen (N) required to meet the demand for net production and the percentage of the N requirement for net production met by N_2 fixation in the three investigated substrates. Values are reported as mean ± SD (n = 8).

Substrate	Season	N_2 fixation (μmol N cm^{-2} d^{-1})	Net production (μmol C cm^{-2} d^{-1})	N requirement for net production (μmol N cm^{-2} d^{-1})	% N requirement met
Turf algae	Winter	0.021 ± 0.006	5.0 ± 0.9	0.36 ± 0.07	5.9 ± 1.4
	Spring	0.032 ± 0.015	5.5 ± 1.1	0.40 ± 0.08	8.1 ± 3.6
	Summer	0.134 ± 0.031	8.9 ± 1.8	0.65 ± 0.12	19.8 ± 3.2
	Fall	0.033 ± 0.006	4.8 ± 0.8	0.35 ± 0.06	9.3 ± 0.9
	Mean	**0.052 ± 0.047**	**6.0 ± 2.1**	**0.44 ± 0.15**	**10.5 ± 5.8**
Coral rock	Winter	0.005 ± 0.004	4.4 ± 2.1	0.32 ± 0.16	2.2 ± 1.9
	Spring	0.037 ± 0.014	3.1 ± 1.6	0.25 ± 0.11	12.6 ± 1.7
	Summer	0.083 ± 0.032	4.3 ± 1.4	0.31 ± 0.10	26.8 ± 6.4
	Fall	0.032 ± 0.013	3.5 ± 1.2	0.25 ± 0.09	12.5 ± 2.8
	Mean	**0.041 ± 0.034**	**3.9 ± 1.6**	**0.28 ± 0.15**	**14.5 ± 10.0**
Mycale fistulifera	Winter	0.007 ± 0.001	-2.0 ± 0.3	-	-
	Spring	0.001 ± 0.001	-1.3 ± 0.8	-	-
	Summer	0.007 ± 0.003	-3.0 ± 0.9	-	-
	Fall	0.001 ± 0.003	-4.5 ± 2.2	-	-
	Mean	**0.002 ± 0.003**	**-2.7 ± 1.7**	-	-

DISCUSSION

Dinitrogen fixation in coral reef framework substrates

N_2 fixation rates presented here are comparable to those reported for turf algae, coral rock, and sponges on coral reefs worldwide (Table 6.6). The relatively high variability in N_2 fixation reported for turf algae likely results from regional differences in turf community composition (Bauer et al. 2008) or responses to local environmental conditions (Williams & Carpenter 1998). While N_2 fixation rates previously reported for turf algae are typically higher than those of coral rock (Table 6.6), our rates for both substrates were similar, with significantly higher N_2 fixation in turf algae only during winter and summer at night. However, studies reporting higher rates of N_2 fixation in "bare" rock compared to rock with epilithic algal, suggest endolithic N_2 fixation can exceed that of some epilithic communities (Wilkinson et al. 1984, Casareto et al. 2008). This demonstrates the importance of apparently "bare" substrate in generating new N on coral reefs.

N_2 fixation rates in *M. fistulifera* were an order of magnitude lower than for turf algae and coral rock, but are in the range typically reported for other animal-microbe symbioses such as scleractinian corals (Shashar et al. 1994b, Davey et al. 2008) and other cnidarians (Shashar et al. 1994a, Bednarz et al. 2015). There are very few reports of active N_2 fixation in marine sponges, but the rates for *M. fistulifera* are consistent with those reported for other Red Sea sponges in the only other study presenting sponge N_2 fixation rates normalized to organism surface area (Shashar et al 1994a, Table 6.6). It should be noted that both studies used the acetylene reduction method, which has reportedly proven problematic for some sponges (Wilkinson 1999). However, all sponges were actively pumping post-incubation and the high pumping rate would ensure rapid flushing of the tissue, excluding the likelihood of acetylene toxicity or insufficient acetylene and ethylene transport. Acetylene may disrupt other N cycling processes, such as nitrification, that occur in some sponges, however the absence of nitrate production by *M. fistulifera* suggests it does not host this process (L. Rix unpublished data). We cannot exclude metabolism of ethylene by microbial symbionts, however this would affect all three substrates, not only the sponge. It may rather be that this apparent difficulty in measuring N_2 fixation in sponges is due to in part to low N_2 fixation activity, as observed here (particularly in winter) and in other studies (Wilkinson & Fay 1979, Shashar et al. 1994a, Shieh & Lin 1994, Wilkinson 1999). Despite the low rates we observed consistent and measureable N_2 fixation with low variability and clear seasonal trends. Further, the low $\delta^{15}N$ tissue values of *M. fistulifera* (< 1‰, L. Rix unpublished data) are consistent with biological ^{15}N fixation (Yamamuro et al. 1995, Montoya et al. 2002). The lower N_2 fixation activity in *M. fistulifera* is likely due to low diazotroph abundances or activity in the sponge-associated microbial community compared to turf algae and coral rock, which can be composed largely of N_2 fixing cyanobacteria (Charpy et al. 2012). Given that

the DIN release rates reported for sponges exceed reported rates of N_2 fixation by orders of magnitude (Diaz & Ward 1997, Southwell 2007), N_2 fixation may not be of high functional importance for the nutrition of the sponge host. As efficient filter feeders sponges may meet their N demand primarily via heterotrophic feeding (Pile et al. 2003).

Table 6.6. Comparison of known N_2 fixation rates of turf algae, coral rock, and sponges reported from coral reefs worldwide. Values are presented as nmol N cm^{-2} h^{-1}. Original C_2H_4:N_2 conversion rates were used to calculate the N_2 fixation rates from acetylene reduction rates if reported in the original study. If no conversion rate was available the conservative ratio of 4:1 was used.

Substrate	N fixation	Region	Reference
Turf algae	0.9 – 5.6	Red Sea	Present study
Turf algae	4.6 ± 0.3	Red Sea	Shashar et al. 1994a
Turf algae	0.3 – 29.7[a]	Great Barrier Reef	Larkum et al. 1988
Turf algae	8.3 – 36.7	Great Barrier Reef	Wilkinson & Sammarco 1983
Turf algae	13.5 ± 5.5	Hawaiian Islands	Williams & Carpenter 1998
Turf algae	3.7 ± 5.4	Caribbean	Williams & Carpenter 1997
Turf algae	6.0 ± 0.9	Caribbean	Den Haan et al. 2014
Coral rock	0.2 – 3.5	Red Sea	Present study
Coral rock	0.3 ± 0.2	Red Sea	Shashar et al. 1994a
Coral rock	0.2 – 1.9	Great Barrier Reef	Wilkinson et al. 1984
Coral rock	0.1 – 6.4	Great Barrier Reef	Davey et al. 2008
Coral rock	6.4 ± 1.8[a]	Great Barrier Reef	Larkum et al. 1988
Coral rock	0.6[b]	French Polynesia	Charpy-Roubaud et al. 2001
Sponge (*M. fistulifera*)	0.01 – 0.3	Red Sea	Present study
Sponges	0.1 ± 0.2	Red Sea	Shashar et al. 1994a

[a] Conversion factor 3.45
[b] Conversion factor 3.3

Diel dinitrogen fixation pattern

While many studies have found substantially higher daytime N_2 fixation activity in coral rock (Wilkinson et al. 1984, Charpy-Roubaud et al. 2001, Holmes & Johnstone 2010) and turf algae (Williams & Carpenter 1997, den Haan et al. 2014), our results show consistent and substantial nighttime N_2 fixation by both substrates. Turf algae exhibited significantly higher daytime N_2 fixation in winter but significantly (3-times) higher nighttime N_2 fixation in summer. This could indicate a shift in the turf community towards more non-heterocystous cyanobacteria or heterotrophic diazotrophs in summer, as typically only heterocystous cyanobacteria can fix N_2 in the presence of O_2 generated by photosynthesis (Bergman et al. 1997). While cyanobacteria have long been considered the primary diazotrophs responsible for benthic marine N_2 fixation, the role of heterotrophic bacteria is increasingly being recognized (Zehr et al. 1995, Bauer et al. 2008). Identification of the diazotroph community would provide further insight into the patterns of N_2 fixation observed here. Similar day and night N_2 fixation rates by coral rock suggest a diazotroph community equally adapted to light and dark conditions. Only *M. fistulifera* consistently exhibited significantly higher N_2 fixation activity in the

day compared to night (2-times), suggesting either the role of phototrophic diazotrophs or heterotrophic diazotrophs energetically dependent on photosynthetic products. Sponges host diverse communities of microbial symbionts and nitrogen fixation *nifH* genes affiliated with a range of cyanobacteria and heterotrophic bacteria have been detected in tropical sponges (Mohamed et al. 2008, Zhang et al. 2014, Fiore et al. 2015). Evidence for active cyanobacterial *nifH* expression dominating during the day and higher proteobacterial *nifH* expression at night in the congeneric *Mycale laxissima* suggests multiple diazotrophs can contribute to sponge-associated N_2 fixation and provides a potential explanation for diel patterns of sponge N_2 fixation activity (Mohamed et al. 2008, Zhang et al. 2014). The presence of cyanobacteria could also explain our findings of P_{gross} in *M. fistulifera* and future studies should investigate the symbionts responsible for photosynthesis and N_2 fixation in the sponge. The lack of peaks in N_2 fixation during low light levels as observed for the coral *M. cavernosa* (Lesser et al. 2007) and the co-occurrence of N_2 fixation during the day with photosynthesis indicate that the diazotroph communities of the three investigated substrates are equipped with other strategies to overcome O_2 inhibition of nitrogenase.

Seasonality in dinitrogen fixation and primary productivity

This is the first study examining seasonal N_2 fixation in a diverse group of reef framework substrates, and our findings highlight the importance of environmental parameters in regulating benthic N_2 fixation activity. Temperature and irradiance positively affected N_2 fixation, while inorganic nutrients had a negative influence, resulting in the highest rates in summer when irradiance and temperature were highest but inorganic nutrients lowest. This seasonal pattern showed remarkable consistency across the three substrates despite their differing trophic strategies, and is consistent with reports of higher summer benthic N_2 fixation in the GBR (Larkum et al. 1988) as well as higher summer N_2 fixation in soft corals (Bednarz et al. 2015) and pelagic communities in the Red Sea (Rahav et al. 2015). High temperatures can directly stimulate the enzymatic activity of nitrogenase and are associated with increased growth and N_2 fixation in free-living cyanobacteria (Breitbarth et al 2007). Conversely, lower temperatures can increase respiratory costs associated with N_2 fixation in unicellular cyanobacteria (Brauer et al. 2013). However, temperature alone likely cannot explain the observed seasonality, as there were no significant differences in N_2 fixation between spring and fall, despite a 2.5 ºC temperature difference. By enhancing photosynthesis, irradiance may stimulate the energetically costly process of N_2 fixation through the provision of larger quantities of energy-rich photosynthates (Bebout et al. 1993), if the responsible diazotrophs are protected from the corresponding increase in O_2 production. Although this largely appears to be the case for the substrates investigated here, given only turf algae and *M. fistulifera* exhibited significantly higher summer photosynthesis but all three substrates displayed an increase in N_2 fixation, irradiance was unlikely the primary driving factor. The effect of decreased DIN concentrations during summer likely

played a key role and can be explained by the higher energetic costs of N_2 fixation compared to DIN assimilation (Gallon 2001), making it a seasonal strategy when external nutrients are scarce. This is supported by observations of increased nitrogenase activity in N-starved cultured filamentous cyanobacteria (Ramos et al. 1985) and inhibition of N_2 fixation in coral skeletons and reef sediments under elevated NH_4^+ concentrations (Koop et al. 2001, Holmes & Johnstone 2010). These findings suggest diazotrophs are capable of altering their N_2 fixation activity to adjust to the availability of external N sources. While elevated N_2 fixation also increases iron (Fe) demand compared to NH_4^+ assimilation (Kustka et al. 2003), Fe limitation is unlikely to be a limiting factor in the Gulf of Aqaba due to high dust inputs (Ying et al. 2007, Foster et al. 2009). Alternatively, seasonal variability in N_2 fixation activity may be influenced by seasonal changes in the diazotroph communities associated with the three substrates. Overall the combination of key environmental parameters in summer (i.e. high irradiance, high temperature, and low inorganic nutrients), appear to interact to cause substantially higher N_2 fixation rates. Importantly, this results in the highest N_2 fixation when the inorganic N supply is lowest.

Contribution of dinitrogen fixation to primary productivity

Interestingly, high photosynthesis rates were sustained in summer when temperature and irradiance were highest, despite low inorganic nutrient availability. This suggests that either primary production was not nutrient (DIN) limited or that additional nutrient sources contributed to supporting photosynthesis. Given the significant positive linear relationship between P_{gross} and N_2 fixation for both turf algae and coral rock only during summer and fall when inorganic nutrient concentrations were low, this suggests fixed N may play a role in supporting primary production, at least when other sources of nutrients are scarce. For turf algae, we estimated that while fixed N could only supply 6% of the N demand for P_{net} in winter, this increased to 20% in summer, suggesting N_2 fixation has the potential to be an important N supply for photosynthesis. Williams & Carpenter (1997) found N_2 fixation contributed less than 2% to the N demand for P_{net} in turf algae in the Caribbean, with the estimated contribution by NH_4^+ assimilation an order of magnitude higher. While comparable to our winter estimates, this is substantially lower than our summer values. However, the N_2 fixation rates measured by Williams & Carpenter (1997) were lower than those measured during our study in summer and NH_4^+ concentrations may have differed. While DIN assimilation would also represent an important process at our study site, increased summer N_2 fixation may compensate for the decrease in DIN concentrations, sustaining high summer P_{net} rates. For coral rock we estimate that N_2 fixation could supply 2% of the N demand for P_{net} in winter and 27% in summer. This is remarkably consistent to the 2 – 28% estimated for coral rock from Sesoko Island (Pacific Ocean) and Le Reunion (Indian Ocean) (Casareto et al. 2008). It should be noted though that these calculations are highly dependent on the C:N of the substrates, which may vary spatially and

temporally. Further, photosynthetic rates change over the day but here were measured only during periods of maximum irradiance.. However, since these measurements represent the maximum P_{net} rates (preliminary photosynthesis-irradiance curves showed no photoinhibition at these irradiances), our estimates of the potential contribution of N_2 fixation to primary production can be considered conservative as they may underestimate the importance of N_2 fixation at lower P_{net} rates occurring over the day. Future studies should investigate the utilization of fixed N by the turf algae and coral rock consortia to confirm a relationship between N_2 fixation and primary production. In contrast to turf algae and coral rock, we found no significant relationship between N_2 fixation and P_{gross} in *M. fistulifera*. Sponges release large quantities of DIN (eg. Southwell et al. 2008), which may provide another source of N for their photosynthetic symbionts and could explain the lack of correlation between the two processes found here. While photosynthesis supplies a significant portion of the energy demand of some sponge species (Wilkinson 1987, Erwin & Thacker 2007), given *M. fistulifera* exhibited negative P_{net} and overall heterotrophic metabolism (P_{gross}:R < 1), photosynthesis is unlikely to be important to its overall nutrition. Nevertheless, these findings highlight the potential for N_2 fixation to contribute to sustaining high rates of P_{net} in coral rock and turf algae during the period of water column stratification in the Gulf of Aqaba.

Ecological implications

In addition to directly supporting their own growth, N fixed by turf algae and coral rock may substantially contribute to new N on coral reefs via N release from cyanobacterial cells (Mulholland et al. 2004), mechanical disturbances such as grazing (Williams & Carpenter 1997), or by the recycling of diazotroph biomass. Using the estimated 3D-surface area of each substrate per m^2 of reef, we calculate that N_2 fixation by the three substrates introduces to the reef 47 μmol N_2 m^{-2} d^{-1} of fixed N in winter and up to 185 μmol N m^{-2} d^{-1} in summer. These values are comparable to the benthic reef community N_2 fixation estimated for One Tree Island (GBR) of 78 – 156 μmol N_2 m^{-2} d^{-1} (Larkum 1988), but lower than those calculated for Eilat (Red Sea) of 576 – 960 μmol N_2 m^{-2} d^{-1} (Shashar et al. 1994a). However, it is likely that N_2 fixation by other benthic substrates not accounted for here, such as reef sands (Charpy-Roubaud et al. 2001, Casareto et al. 2008, Bednarz et al. in press), cyanobacterial mats (Charpy et al. 2007), and hard corals (Lesser et al. 2007), also substantially contribute to reef N generation. Due to the low abundance of *M. fistulifera* on the studied reef and the comparatively low rates of N_2 fixation associated with the microbial community of *M. fistulifera*, it contributed < 1% of the total new N fixed by the three investigated substrates. Unless the abundant cryptic sponge community fixes N at higher rates, sponges likely do not contribute substantially to reef N generation, at least via N_2 fixation. They do produce large quantities of inorganic nutrients through remineralization of particulate organic matter and association with nitrifying bacteria (Richter et al. 2001, Southwell et al. 2008). While turf algae are increasingly a dominant component on many

coral reefs (e.g. Bahartan et al. 2010), their low and seasonally variable abundance at the present study site resulted in a substantial contribution to new reef N generation only in winter (72%). Low abundances in other seasons resulted in a contribution of 10 – 31% of the total N fixed by the three investigated substrates during the rest of the year. Given their high potential for N_2 fixation, turf algae are likely an important source of new N on turf-dominated reefs (eg. den Haan et al. 2014). High N_2 fixation and high benthic coverage (11 – 20%) characterize coral rock as the major year-round contributor of newly fixed N among the three investigated substrates, accounting for 28% in winter but 69 – 90% of the N fixed during the other seasons. Overall two- to four-times more N was fixed in summer compared to the other seasons. This fixed N appears to be of greater ecological importance during the low nutrient summer season in the Gulf of Aqaba, particularly for supporting primary production. This highlights the potential significance of N_2 fixation by coral rock and turf algae in coral reef ecosystems with more constant oligotrophic conditions.

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CHAPTER 7

GENERAL DISCUSSION

KEY FINDINGS

The aim of this thesis was to advance the understanding of the roles that sponges play in the biogeochemical cycling of C and N on coral reefs, particularly in regards to the newly described sponge loop. Through a variety of experimental approaches, including stable isotope tracer experiments, fatty acid analysis, C and N flux incubations, and modeling, this thesis was able to provide preliminary answers to each of the six key questions formulated in the general introduction (Chapter 1) with the hope that future research will be able to build and expand upon these findings. The key findings of the thesis are synthesized and summarized in the responses to the six questions below.

1. Is there evidence for a "sponge loop" functioning in organic matter cycling on coral reefs in oceanic regions other than the Caribbean Sea? Are there differences in the functioning of the sponge loop between shallow, warm-water and deep-sea cold-water, coral reef ecosystems?

The findings of this thesis present the first evidence for the functioning of a sponge loop outside the initial observation in the Caribbean by de Goeij and colleagues (2013), demonstrating the sponge loop is not limited to shallow, warm-water (WW) coral reefs but also occurs in cold-water (CW) reefs of the deep sea. Remarkably, rates of organic matter uptake and transformation by sponges were similar on both WW (Red Sea) and CW (north Atlantic) coral reefs (Chapter 2, 4). Moreover, all WW and CW sponge species investigated exhibited DOM uptake, suggesting this may be a widespread strategy employed by coral reef sponges inhabiting environments where the concentrations of POM are low (e.g. Chapter 6). DOM uptake may also explain the imbalance in sponge carbon (C) and nitrogen (N) budgets that have been reported for several sponge species, including *N. magnifica* investigated in Chapter 2 (Reiswig 1974; 1981, Thomassen & Riisgard 1995, Hadas et al. 2009, Maldonado et al. 2012). Furthermore, we observed detritus (POM) production in all species investigated (Chapter 2, 4, 5), and consistently found that 12 – 40% of the DOC and 29 – 49% of the DON assimilated by the sponges was subsequently turned over as sponge detritus (Chapter 2, 4). This demonstrates that a major fraction of the DOM assimilated by sponges is subsequently released in a particulate form available to other organisms. Finally, we confirm that sponge detritus is consumed by two reef-associated detritivores, ophiuroids and polychaetes; thereby demonstrating the transfer of the energy and nutrients bound in DOM to higher trophic levels that completes the final step of the sponge loop (Chapter 2). The demonstration of a sponge loop in Caribbean, Red Sea, and north Atlantic reefs suggests that this sponge-mediated recycling loop may be a ubiquitous pathway for organic matter cycling on coral reefs and may contribute to the high

biogeochemical cycling observed in oligotrophic (WW) carbon-deplete (CW) ecosystems by enhancing the retention and cycling of DOM (Hatcher 1988, Crossland et al. 1991, van Oevelen et al. 2009, White et al. 2012).

Nevertheless, despite overall similarities in uptake and transformation rates, Chapter 4 discovered differences in the specific processing of C and N by the WW and CW sponges. The slightly lower rates of DOM uptake and transformation in the CW sponge may be due to the lower temperatures in the CW environments; as Chapters 3 and 6 demonstrated that sponge metabolism was affected by seasonal changes in temperature. Furthermore, while the WW sponge *M. fistulifera* exhibited preferential uptake of mucus N; the CW sponge *H. coriacea* displayed relatively higher C uptake. This is consistent with the ambient availabilities of C and N in the respective sponge environments. Nitrogen limits primary production on oligotrophic WW reefs (Delgado & Lapointe 1994, Larned 1998, Atkinson 2011, den Haan et al. 2013); whereas C is the limiting resource on energy-depleted CW reefs that are entirely reliant on the input of external organic matter (Duineveld et al. 2004, Kiriakoulakis et al. 2004, Roberts et al. 2006). Therefore, we hypothesize that due to these differences in C and N availability, the functional role of the sponge loop may differ in the two ecosystems with N cycling in WW and C cycling in CW reefs disproportionately contributing to ecosystem functioning. However, additional experiments with more sponge species and larger replication are needed to confirm this hypothesis. Nevertheless, efficient organic matter retention and recycling by sponges may provide key functions in these two coral reef ecosystems, that despite their vast environmental differences, can both be considered as oases in a marine desert. The discovery of functioning sponge loops in a variety of coral reef ecosystems also highlights the potential for a sponge loop in other oligotrophic marine ecosystems where sponges are abundant.

2. Is there a direct trophic link between the key benthic primary producers (corals and algae) and the sponge community on coral reefs? How do different DOM sources influence the uptake and transformation of organic matter by reef sponges?

The exudates of scleractinian corals and benthic algae constitute an important resource and a major source of labile DOM on coral reefs (Wild et al. 2004a, Haas et al. 2010, Naumann et al. 2010). Despite potentially representing a key source of DOM fueling the sponge loop, the uptake of coral- and algal-derived DOM by sponges has not previously been investigated. Furthermore, the impact of different DOM sources on recycling via the sponge loop is completely unknown. The findings of this thesis present the first direct evidence for the uptake of coral- and algal-derived DOM by reef sponges, demonstrating that both coral and algal-DOM fuel the sponge loop (Chapter 2, 4). A major strength of this study is the demonstration of the sponge loop with natural sources of coral reef DOM; a difficult feat given the challenges of producing large quantities of coral and algal-exudates

with sufficient labelling to follow the ^{13}C and ^{15}N tracers through multiple trophic steps (eg. zooxanthellae – coral – coral mucus – sponge – sponge detritus – detritivores). As the dominant primary producers on coral reefs, scleractinian corals and algae support the rest of the reef community through the photosynthetic fixation of C. The release of up to 40% of their net C fixed as organic matter (Crossland et al. 1980, Muscatine et al. 1984, Tremblay et al. 2012) presents a mechanism by which the C fixed by corals and algae can be transferred to other reef fauna. However, the majority of this released organic matter immediately dissolves in the surrounding water (Wild et al. 2004a, Haas et al. 2010), and therefore, is largely unavailable to most heterotrophic reef fauna. Previously, this DOM was believed to be consumed primarily by planktonic bacteria and recycled exclusively via the microbial loop (Ferrier-Pagès et al. 2000, Wild et al. 2004b, Tanaka et al. 2011). Therefore, the findings of this thesis demonstrate an alternative pathway by which the C fixed by coral reef benthic primary producers can be transferred to higher trophic levels. Bacteria exhibit high losses of respired carbon, such that the microbial loop is an inefficient route for the transfer of DOM to higher trophic levels (Williams 2000, Fenchel et al. 2008, Worden et al. 2015). Furthermore, the C transferred via bacteria primarily fuels a largely isolated planktonic food web. Coral reef sponges appear to respire only up to ~45% of their assimilated C (de Goeij et al. 2008b). By releasing POM near the benthos, the sponge loop may then play a pivotal role not only in capturing and retaining reef-derived resources on the reef, where they are subject to recycling, but also in enabling them to be transferred to higher trophic levels.

Importantly, the uptake of coral- and algal-derived DOM by reef sponges elucidates a novel trophic link between benthic primary producers and the predominately heterotrophic sponge community. In particular this highlights a key trophic interaction between sponges and scleractinian corals, the primary ecosystem engineers on both WW and CW reefs (Freiwald et al. 2004, Wild et al. 2011). Similarly to scleractinian corals, sponges also modify the availability of resources for other reef fauna by providing habitat; impacting the reef framework through calcification, cementation and bioerosion; and modifying C and N availability in the water column (Diaz & Rutzler 2001, Wulff 2001, Bell 2008, Chapters 2, 3, 4, 5, 6). Therefore, sponges also embody the classic definition of an ecosystem engineer (Jones et al. 1994). Our findings then demonstrate how trophic interactions between ecosystem engineers can exert major influence on ecosystem C and N flows, providing a key example of how interspecific interactions between species can enhance resource use, biogeochemical cycling, and ecosystem functioning; thereby ultimately influencing the efficiency and productivity of the system (Stachowicz 2001, Bruno et al. 2003, Hooper et al. 2005). This highlights how it is not only the ecosystem engineers acting in isolation, but also the competitive and facilitative interactions between them and other organisms, that shape ecosystem structure and function. Such interactions are ubiquitous on coral reefs (e.g. Glynn 1976, Muscatine & Porter 1977, Hill 1998, Easson et al. 2014) and are increasingly recognized as essential for shaping coral reefs and other ecosystems (Bruno & Bertness 2001, Connell et al. 2004, Bronstein 2009).

The findings of this thesis further suggest that the interaction between scleractinian corals and sponges may be mutualistic (i.e. it benefits both organisms; Bronstein 1994b), or at least facultative (i.e. beneficial to one organism; Bronstein 2009). Corals benefit the sponges by providing a food source for the sponge holobiont (Chapter 2, 4); while sponges provide a source of DIN that could in turn be used by the corals for primary production (Chapter 5). Indeed, sponges are suspected to be benefit corals through nutrient provision (Slattery et al. 2013). Still, such interspecific interactions are context dependent, and the costs and benefits of the interaction can shift due to changing environmental factors (Bronstein 1994a). For example, sponges can also compete with and overgrow corals (Vicente 1978, Aerts & vanSoest 1997, Lopez-Victoria et al. 2006, Gonzalez-Rivero et al. 2011). Coral cover is declining on coral reefs worldwide due to a multitude of anthropogenic stressors (Pandolfi et al. 2003, Bellwood et al. 2004, Knowlton & Jackson 2008, De'ath et al. 2012). Algae are replacing corals on many of these reefs (McCook et al. 2001, Hughes et al. 2007, Sandin et al. 2008); although, sponges have also been identified as organisms that may benefit from coral decline and the projected effects of climate change (Norström et al. 2009, Bell et al. 2013). Indeed, sponge abundances are increasing on many coral reefs worldwide (Diaz & Rutzler 2001, Ward-Paige et al. 2005, McMurray et al. 2010, Powell et al. 2010). Thus, benthic algae may replace corals as the key organisms interacting with sponges. Benthic turf- and macroalgae produce DOM in larger quantities than scleractinian corals (Wild et al. 2010, Haas et al. 2011, Mueller et al. 2014b), and sponges appear to be able to take up DOM linearly with increasing availability (Mueller et al. 2014a). In this context, Chapter 2 of this thesis also shows that sponges exhibit higher DOM uptake and transformation rates when provided with algal- compared to coral-derived DOM. Furthermore, while coral-derived DOM was incorporated at higher rates into sponge-specific PLFAs, algal-derived DOM was more rapidly incorporated into bacterial biomarkers confirming that sponges actually process various sources of DOM differently. Since algal-derived DOM appears to be more readily taken up by reef sponges, this highlights the potential for benthic community phase shifts from coral to algal dominance to enhance recycling by the sponge loop. This increased retention and recycling of nutrients may lead to increased nutrient enrichment (de Goeij et al. 2013). Eutrophication is already a symptom of many degraded reefs, linked to both declining coral reef and the promotion of algal growth (Littler & Littler 1984, Lapointe 1997, McCook 1999, Lapointe et al. 2004). Thus, accelerated recycling by the sponge loop under such scenarios of coral-algal phase shifts could fuel a feedback loop that further contributes to coral reef decline. This might compound the negative feedback loop induced by algal exudates through accelerated recycling of the microbial loop; where algal-exudates promote faster microbial growth and higher respiration leading to localized anoxia and the development of more pathogenic microbial communities, in turn causing increased coral mortality (Smith et al. 2006, Barott & Rohwer 2012, Gregg et al. 2013). This further highlights the similar functions and potential impacts of the sponge and microbial loops. Importantly, increased recycling

by the sponge and microbial loops could also influence higher trophic levels with potential wider impacts on trophic structure (Silveira et al. 2015), and therefore warrants more investigation.

3. What role do sponge-associated microbes play in the uptake of reef-derived dissolved organic matter by the sponge holobiont? Are there differences in the processing of DOM by high- and low-microbial abundance sponges?

The role of symbiotic microbes in contributing to the overall health, metabolism, and functioning of their animal hosts is increasingly being recognized in many organisms from corals (Lesser et al. 2007, Rosenberg et al. 2007, Thompson et al. 2015) to humans (Dethlefsen et al. 2007, Turnbaugh et al. 2007). Sponges host diverse and unique communities of microbes that endow them with a range of metabolic pathways (Taylor et al. 2007). The fact that microbes can account for more than 35% of the sponge biomass (Vacelet 1975) and form stable and specific associations (Lee et al. 2011, Schmitt et al. 2012, Simister et al. 2012, Webster et al. 2013), suggests these microbes have helped shape sponge evolution by playing key functional roles (Taylor et al. 2007, Webster et al. 2010, Webster & Taylor 2012). Consequently, at least some of these interactions are suspected to be mutualistic, even though evidence for such mutualism is scarce (Weisz et al. 2007, Webster & Blackall 2009). As microbes are the dominant consumers of DOM in the ocean, DOM uptake in sponges is believed to be at least partially mediated by their symbiotic microbes (Reiswig 1974, Weisz et al. 2007, Maldonado et al. 2012). However, most microbes dwell in the inner mesophyll of the sponge, and therefore, are not in direct contact with DOM in the ambient water, suggesting also the involvement of the sponge host. By demonstrating assimilation of coral- and algal-derived DOM into both sponge and bacterial biomarkers, the results of this thesis were able to show that indeed both sponge cells and sponge-associated microbes play a role in the uptake of DOM (Chapter 2, 4). This is consistent with the only other comparable study examining uptake of diatom-DOM in the Caribbean sponge *Halisarca caerulea* (de Goeij et al. 2008a). As might be expected, microbes were more active in the uptake of DOM in the HMA compared to LMA sponges, as evidenced by higher bacterial PLFA incorporation in the HMA sponge *C. sacciformis*. Interestingly, DOM uptake has been hypothesized as one of the advantages to hosting dense microbial populations, potentially accounting for discrepancies in the sponge C budget observed particularly for HMA sponges (Reiswig 1974, Weisz et al. 2007, Maldonado et al. 2012). Yet, no differences were found in DOM uptake rates between HMA and LMA sponges, suggesting they have an equal capacity for taking up DOM. This implies that microbes confer no additional benefit to the sponge host in terms of the ability to access dissolved food sources. This finding is consistent with other reports of DOM uptake in LMA sponges (de Goeij et al. 2013, Mueller et al. 2014a) and indicates that other benefits such as autotrophic

metabolism, elimination of toxic metabolic by-products, or the production of protective secondary metabolites may have been more influential in the development and co-evolution of sponge-microbe symbioses (Taylor et al. 2007, Webster & Taylor 2012). Indeed, a recent review suggests that, at least on Caribbean reefs, sponges are governed primarily by top-down processes such as predation (Pawlik et al. 2015); while the role of food availability remains controversial (Lesser & Slattery 2013, Pawlik et al. 2013). Nevertheless, due to their lower pumping capacity, and therefore lower capacity for POM filtering (Weisz et al. 2008), DOM may be relatively more important to the diet of HMA sponges as hypothesized by Reiswig (1974) and others (Maldonado et al. 2012). Consequently, the actual contribution of coral- and algal-derived DOM to the total sponge C budget should be determined. Moreover, microbes may increase the proportion of the DOM pool available to the sponge holobiont as sponge cells may primarily take up colloidal DOM, while the truly dissolved fraction may be more readily available to the associated microbes (de Goeij et al. 2008a). This may explain the apparent differences in the contribution of bacteria to total PLFA tracer assimilation between different types of DOM sources (Chapter 2, 4), which demonstrates that DOM quality and composition influences uptake by the sponge holobiont. Finally, while we could not quantitatively determine the contributions of microbes vs. sponges cells in DOM incorporation, HMA sponges appear to be more reliant on their associated microbes for taking up DOM with bacterial PLFA incorporation accounting for 33-42 % of the total tracer PLFA incorporation in *C. sacciformis* compared to 2 - 10% in *M. fistulifera* (Chapter 2). While translocation of C and N between microbes and the sponge host is suspected, there is limited evidence for such transfer from heterotrophic microbes to the sponge host (Webster & Blackall 2009, Thacker & Freeman 2012). Given that the percentages of DOM turned over as POM were similar across all sponges regardless of microbial abundances, and the fact that this POM is derived from the sponge host, this could be taken as evidence of a trophic transfer between the microbes and the sponge host - particularly for the HMA sponge *C. sacciformis* where up to 42% of assimilated DOM was traced into bacterial PLFAs. This study, therefore, provides further support that while there are differences in the specific C and N processing between HMA and LMA sponges, the overall DOM uptake and transformation rates are similar; thus HMA and LMA sponges appear to participate equally in the sponge loop. This supports the recent hypothesis that marine sponges display a high level of functional convergence, where, despite differences in the specific microbial groups present, the functioning of the sponges remains the same (Fan et al. 2012, Ribes et al. 2012, Freeman et al. 2013).

4. How important is organic C cycling by the sponge loop at the ecosystem level?

Carbon is the energetic currency of the reef; thus, understanding C cycling is fundamental to understanding coral reef ecosystem functioning. By combining the fluxes of DOC uptake and POC production by reef sponges with rates of primary production and organic matter release by benthic

reef primary producers into a trophic model, Chapter 3 shows that C cycling by reef sponges plays a quantitatively important role in the overall biogeochemical cycling of C at the ecosystem level in the Red Sea. However, these high C fluxes were due almost entirely to the high activity of the cryptic sponge community, which far outweighed the contribution of the epi-reefal surface sponge community as the high surface area of the cryptic reef habitat and its dominant coverage by sponges exceeds the biomass of the epi-reefal community (Richter et al. 2001, Wunsch et al. 2000). The cryptic reef community is ubiquitous in reef ecosystems, representing both the largest and most poorly explored component of the reef habitat (Jackson et al. 1971, Ginsberg 1983, Scheffers et al. 2004). Sponges dominate the fauna in this cryptic habitat, which acts as a major sink for both POC and DOC (Gast et al. 1998, de Goeij & van Duyl 2007) and as an important source of inorganic nutrients via the remineralization of this organic matter (Richter et al. 2001, Rasheed et al. 2002, van Duyl et al. 2006). Therefore, when building trophic models or considering the role of sponges in C and N biogeochemical cycling in reef ecosystems, it is essential to consider the unseen but abundant cryptic sponge community. Uptake of DOC by the cryptic sponge community represented one of the largest fluxes of organic C on the reef (Chapter 3). In summer and fall, sponge DOC uptake was on the same order of magnitude as the gross primary production of the entire reef ecosystem, exceeding DOC uptake by the microbial loop. These Red Sea rates are comparable to estimates of DOC uptake by cryptic sponges in the Caribbean and Indo-Pacific (de Goeij & van Duyl 2007, de Goeij et al. 2013), suggesting the widespread importance of the sponge loop to coral reef C cycling on reefs worldwide. Sponge metabolism was strongly influenced by the high seasonality in the Gulf of Aqaba with lower rates of respiration, DOC and POC uptake, and POC production in winter and spring. Nevertheless, even in these seasons sponge, DOC uptake equaled uptake by the microbial loop. Thus, these two recycling loops play a major role in C cycling on coral reefs in the Red Sea, and combined, they may be able to retain most of the DOC produced by the reef, as well trap new DOC from the surrounding water. This not only provides a unique function in enabling the energy and nutrients bound in DOC to be shunted to higher trophic levels (Azam et al. 1983, de Goeij et al. 2013, Worden et al. 2015), but also contributes to the efficient recycling between the autotrophic and heterotrophic reef compartments that ensures high retention of the energy and nutrients fixed within the system (Muscatine & Porter 1977, Richter et al. 2001, Wild et al. 2004a). Thus, the high recycling capacity of the sponge loop appears to contribute to enabling the high gross primary productivity of the reef ecosystem in the oligotrophic waters of the Red Sea. However, this should be confirmed by incorporating fluxes on N into future trophic models. Given the magnitude of C fluxes mediated by sponges in the Red Sea, its influence on C cycling should be quantified in other systems in order to determine its potential contribution to trophic webs and ecosystem functioning. In particular, CW coral reefs host abundant sponge populations, and have recently been identified as hotspots of deep-sea C cycling, but the contribution of the sponge loop to this high C cycling is unknown (van Oevelen et al. 2009, White et al. 2012).

5. How do PON production and N_2 fixation quantitatively compare to other sponge-mediated N fluxes, and how important are these N fluxes at the ecosystem level?

Despite widespread acknowledgment that sponges are an important source of dissolved inorganic nitrogen (DIN) on coral reefs, many aspects of N cycling by sponges remain poorly characterized. Chapter 5 provides a comprehensive study on N fluxes in coral reef sponges, presenting the first estimates of detrital PON production by reef sponges and adding to the scarce literature regarding N_2 fixation in sponges. Parallel measurements of DIN, PON, and the generation of new N via N_2 fixation in six sponge species enabled comparisons of the relative magnitude of these fluxes. Findings showed that consistent with previous literature from Caribbean (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008) and temperate reefs (Jimenez & Ribes 2007, Perea-Blazquez et al. 2012), sponges in the Red Sea are a substantial source of DIN. Chapter 5 also showed that Red Sea sponges had very comparable DIN release rates compared to Caribbean sponges (Southwell et al. 2008), but appeared to release a higher percentage of DIN as ammonia compared to most Caribbean sponges (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008). This is likely due to the fact that only one HMA sponge was investigated, as high rates of nitrification appear to typically only in HMA sponges (Southwell et al. 2008, Ribes et al. 2012, Chapter 5). The findings of high DIN release are consistent with reports of elevated DIN concentrations in sponge-dominated coral reef cavities in the Red Sea (Richter et al. 2001). While DIN remained the largest efflux of N generated by the six sponge species, the production of PON was substantial and accounted for approximately 30% of the total N released by all six sponges. This is an important finding given that PON production is not currently considered in sponge N budgets (Maldonado et al. 2012, Ribes et al. 2012, Fiore et al. 2013). This percentage was remarkably consistent across the six species, with the species releasing higher quantities of DIN also releasing the most PON. This indicates that the production of sponge detritus, resulting from the rapid cell renewal and shedding of sponge choanocyte and mesophyll cells (de Goeij et al. 2009, Alexander et al. 2014, Maldonado 2015), represents an important metabolic pathway in coral reef sponges, and the fate of a large portion of the organic matter they assimilate. This is consistent with the findings of Chapter 2 and 4 that 27 – 49% of the DON assimilated by both warm-water and cold-water sponges is subsequently released as sponge detrital PON. Hence, despite feeding on PON (Pile et al. 2003, Ribes et al. 2003, 2005, Hadas et al. 2009), sponges may actually represent a net source, rather than a sink, of PON. If this is the case, the uptake of DON is likely necessary to balance the N budget of the sponge (Jimenez & Ribes 2007, Hadas et al. 2009); particularly since the N generated via N_2 fixation is orders of magnitude lower than the fluxes of DIN and PON, and therefore is unlikely to make a major contribution to the sponge N budget (Chapter 5, 6; Ribes et al. 2015).

Using the benthic cover of sponges on the reef we were further able to estimate that DIN and PON production by the epi-reefal sponge community resulted in efflux rates of 19.6 ± 7.7 μmol DIN m^{-2} h^{-1} and 8.5 ± 1.3 μmol PON m^{-2} h^{-1}, representing a substantial flux of N. These rates are lower than for Caribbean reefs, where the sponge community is dominated by massive sponges with biomasses far exceeding those of the predominately encrusting epi-reefal sponge community in the Red Sea. However, when the estimated biomass of the cryptic cavity sponge community is included, these fluxes increase by over 400% to 83.2 ± 19.2 μmol DIN m^{-2} h^{-1}. The DIN produced by the entire Red Sea sponge community (2468 μmol N m^{-2} d^{-1}), therefore, exceeds the estimated N generated via N_2 fixation by the entire reef communities of One Tree Island in the Great Barrier Reef (145-328 μmol N m^{-2} d^{-1}; (Larkum et al. 1988) and the present study site in the Red Sea (160 – 920 μmol N m^{-2} d^{-1}; Cardini 2015). Moreover, this DIN is estimated to be able to supply 17% of the N demand for the net primary production of the entire reef ecosystem based on the primary production rates determined in Chapter 3 and assuming a C:N ratio of 106:16 (Redfield 1934). This is a conservative estimate as coral reef benthic primary producers typically have much higher C:N ratios than the Redfield ratio characteristic of phytoplankton (Atkinson & Smith 1983). Using the *in situ* DIN concentrations (Chapter 6), and a typical maximum coral reef coefficient value (*S*) of 15 (Atkinson & Falter 2003), DIN uptake at the study site can be calculated to range from 3.5 mmol N m^{-1} d^{-1} in summer to 16.7 mmol N m^{-1} d^{-1} in winter. This indicates that the estimated sponge DIN fluxes (2.5 mmol N m^{-1} d^{-1}) are not insignificant at the ecosystem level. DIN production by reef sponges could, therefore, play an important role in supplying N for primary productivity at the ecosystem scale; particularly when ambient DIN concentrations are low. In fact, cryptic cavity sponges have been shown to facilitate the growth and diversity of nearby coral (Slattery et al. 2013). While the DIN produced by sponges can support benthic primary producers, the particulate fraction of N released by sponges fulfills a different function by providing a food source for various detritus-feeding reef fauna (de Goeij et al. 2013, Chapter 2). Thus, sponges not only influence N availability for primary producers but also play a key role in transferring the DON produced by these same primary producers to higher trophic levels, providing them with a unique functional role. In contrast to the high fluxes of DIN and PON, only two sponge species displayed significant N_2 fixation, and the N generated by N_2 fixation (0.006 ± 0.005 μmol N m^{-2} h^{-1}) was 3 – 4 orders of magnitude lower. This demonstrates that compared to DIN and PON release, sponge N_2 fixation does not contribute substantially at the ecosystem scale. In the Gulf of Aqaba (Red Sea), the DIN released by the sponges is likely to be highly beneficial to the reef community due to the extremely oligotrophic conditions - particularly during the stratified summer season (Chapter 5). However, many reefs worldwide are experiencing increased nutrient input (Burke et al. 2011), and such eutrophication has been linked to coral reef degradation by favouring the growth of benthic turf- and macroalgae at the expense of coral growth and resilience (Littler et al. 2006, Smith et al. 2010, Vermeij et al. 2010, Jessen et al. 2013, Wiedenmann et al. 2013). Under such scenarios, the additional DIN generated by sponges may

have negative consequences on coral reef health by further promoting eutrophication and algal growth. Indeed, sponges have been shown to transfer nutrients to nearby algae (Davy et al. 2002, Easson et al. 2014). Therefore, on top of the effects of accelerated nutrient retention and recycling facilitated by the sponge loop (described in the response to question 2), DIN release provides an additional mechanism by which sponges could contribute to eutrophication and coral reef degradation on phase-shifting coral reefs. This highlights the need to consider the impact of sponges in influencing the trajectories of coral reefs in changing environments.

6. Does N_2 fixation support C fixation (primary productivity) in sponges and other key reef framework substrates?

Nitrogen is a key limiting nutrient on coral reefs, and therefore N availability directly influences the key metabolic process in coral reef ecosystems, placing constraints on primary productivity and community metabolism (Hatcher 1990). For this reason, examining links between C and N biogeochemical cycling is essential to understanding ecosystem function. N_2 fixation has been estimated to supply an important source of N for photosynthesis on coral reefs at both the organism and ecosystem level (Wiebe et al. 1975, Larkum et al. 1988, Charpy et al. 2007, Lesser et al. 2007, Cardini 2015). Chapter 6 confirms the importance of N_2 fixation in contributing to photosynthetic N demand as, despite no evidence for a relationship between photosynthesis and N_2 fixation in the sponge *M. fistulifera*, there was a significant positive linear relationship between gross photosynthesis (P_{gross}) and N_2 fixation in turf algae and coral rock. Furthermore, we estimate that N_2 fixation can provide up to 20 and 27% of the N demand for net photosynthesis (P_{net}) in coral rock and turf algae, respectively. Rates of N_2 fixation were one order of magnitude higher during the stratified summer season when irradiance and temperature were highest and inorganic nutrient concentrations lowest, providing insight into the influence of environmental parameters on coral reef benthic N_2 fixation, which to date has received limited attention (Cardini et al. 2014). Importantly, this demonstrates that N_2 fixation can be an important source of N, particularly when ambient concentrations of inorganic N are low. The absence of a link between C and N_2 fixation may not be surprising in *M. fistulifera;* considering it also exhibited high rates of DIN release (Chapter 5), offering a potential alternative and abundant supply of N for its photosynthetic symbionts. N_2 fixation has been hypothesized to be especially beneficial for sponges which obtain the majority of their energetic requirements via photosynthates that are low in N (Wilkinson et al. 1999). Despite exhibiting low rates of $P_{gross,}$ *M. fistulifera*, displayed negative P_{net} rates and overall heterotrophic metabolism (P_{gross}:R <1). Thus, there may be potential for a link between photosynthesis and N_2 fixation in the many coral reef sponges that do obtain the majority of their energetic demands via photosynthesis (Wilkinson 1983, 1987, Erwin & Thacker 2008). This may be an interesting focus of future research, particularly since N_2 fixation has recently been found to play an important role in supporting photosynthesis in other

animal-microbe symbioses such as corals (Cardini 2015). Finally, while sponge N_2 fixation rates were low, turf algae and coral rock exhibited high rates of N_2 fixation that exceed the rates of both carbonate and silicate sands at the study site (Bednarz et al. 2015). This highlights the importance of reef framework substrates, particularly the deceptively "bare" coral rock framework, in generating new N on coral reefs.

FUTURE PERSPECTIVES

While this thesis has provided many new insights into the role that sponges play in organic matter and inorganic nutrient cycling on coral reefs, the biogeochemical cycling on C and N in sponges is nothing if not complex; and there are many more aspects of sponge metabolism and function that remain to be established. Quantitative budgets of N cycling in sponges remain elusive (Jimenez & Ribes 2007, Hadas et al. 2009, Maldonado et al. 2012) hinting at processes that await to be discovered. There is evidence that sponges can also take up DIN (Jimenez & Ribes 2007, Fiore et al. 2013, Ribes et al. 2015), and recently denitrification has been identified in coral reef sponges (Fiore et al. 2013, Fiore et al. 2015); presenting competing N pathways to the established processes of remineralization and nitrification that generate DIN. These processes appear to vary in space and time (Fiore et al. 2013), with consequences for the amount of DIN released by sponges over larger spatial and temporal scales. The production of PON, which has previously been ignored, needs to be included into future sponge N budgets. The quantity of detritus released by sponges appears to be species specific (Alexander et al. 2014) and is influenced by the physiological state of the sponge (Alexander et al. 2015), indicating that POM fluxes may also vary in time and space. More research is needed to fully elucidate the cellular processes underlying the production of sponge detritus (de Goeij et al. 2009, Alexander et al. 2014, Alexander et al. 2015, Maldonado 2015) as well as the role of sponge detritus in coral reef trophic webs. Importantly, the magnitude of C and N cycling by the sponge loop needs to be quantified to determine its potential importance to the functioning of different coral reef ecosystems. Given the potential magnitude of sponge mediated C and N fluxes (Chapter 3, 5), the trophic interactions identified by this thesis should be considered in future coral reef trophic models. In addition to better quantifying the fluxes of C and N in sponges, there is a need for improved knowledge of the underlying metabolic pathways, and the for identification of the microbes responsible for carrying out these pathways, in order to evaluate their functional significance to the sponge host (Webster & Blackall 2009, Webster & Taylor 2012). Importantly, the quantitative contribution of bacteria in sponge DOM uptake remains to be determined. Moreover, the potential for the translocation of DOM and other metabolites between microbes and the sponge host should be established to evaluate the importance of associated microbes for sponge nutrition (Erwin & Thacker 2008, Thacker & Freeman 2012). Finally, understanding the stability of sponge-

microbe associations to environmental perturbations, as well as the potential role of these microbes in sponge disease (Webster et al. 2002, Webster 2007, Webster et al. 2008), will likely be key to understanding how sponges, and their accompanying fluxes of C and N, may respond to current and future environmental change. Despite their many important functional roles (Bell 2008), sponges evidently remain a critically understudied component of coral reef ecosystems (Wulff 2001), and therefore offer many opportunities for future research.

CONCLUSION

Collectively, the findings of this thesis demonstrate that via multiple complex C and N transformations sponges mediate major fluxes of C and N on coral reefs in the Red Sea; thereby making significant contributions to the biogeochemical cycling of C and N at the ecosystem level. The sponge holobiont retains reef-derived C and N within the coral reef ecosystem through DOM uptake, transforms DOM into POM, traps and remineralizes PON into DIN, fixes C and N_2, and transforms N through nitrification. Sponges provide a key link between benthic primary producers and higher trophic levels on coral reefs by transferring the energy and nutrients bound in primary produced DOM to other reef fauna. Conversely, by efficiently trapping and remineralizing organic matter, sponges generate large quantities of DIN that can again be utilized by primary producers. Sponges, therefore, occupy a unique niche within coral reef trophic food webs mediating multiple links between the autotrophic and heterotrophic reef compartments. On nutrient-limited (WW) and energy-depleted (CW) coral reefs, these C and N transformations and fluxes serve an important function by retaining and recycling the C and N essential to sustaining coral reef production. However, under scenarios of coral reef degradation, sponge-derived N may fuel eutrophication and algal growth potentially favouring community shifts from coral to algal dominance, particularly on reefs where overfishing, excess nutrient input, bleaching and disease have already tipped the balance in favor of competing benthic algae. Ultimately, the findings of this thesis show that considering the role of sponges in coral reef biogeochemical cycles and trophic webs is essential for understanding coral reef ecosystem functioning and the future trajectories of these ecosystems in the face of the rapid environmental changes that await them.

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RELATED PUBLICATIONS

The effect of light availability on dinitrogen fixation associated with scleractinian corals along a depth-gradient in the Northern Red Sea

Bednarz VN, Cardini U, van Hoytema N, **Rix L**, Naumann MS, Al-Rshaidat MMD, Wild C

Abstract

Light availability in coral reefs has a major influence on photosynthesis (PS) and dinitrogen fixation (NF) activity associated with scleractinian corals. Several photo-adaptive mechanisms are known to sustain high primary productivity under variable light regimes, but the light-dependency of NF and its potential physiological relationship to PS has received considerably less attention. Therefore, the present study simultaneously investigated PS and NF of two dominant scleractinian corals (Acropora sp. and Stylophora sp.) along a depth-mediated light gradient from 5 m (680 ± 140 μmol m-2 s-1) down to 20 m (110 ± 10 μmol m-2 s-1) water depth in a northern Red Sea fringing reef. In addition, coral samples were analyzed for δ15N signatures, zooxanthellae density and chlorophyll a content. Findings revealed that PS and NF remained constant along the depth gradient, although NF associated with Stylophora sp. slightly decreased with increasing water depth. However, corals from deeper waters possessed a depleted δ15N signature thus indicating a higher usage of NF products that may in turn support the corals' observed photo-adaptation (increased zooxanthellae density and chlorophyll a content) to low light availability. Overall, this study suggests that the physiological stability in corals along the depth gradient may be found in the mutual interplay and trade-off of PS and NF products between zooxanthellae and diazotrophs leading to an optimal adaptation of the involved symbiotic partners to different environmental light conditions.

In preparation for the *Journal of Experimental Biology*:

Functional significance of dinitrogen fixation in sustaining coral productivity under oligotrophic conditions

Cardini U, Bednarz VN, Naumann MS, van Hoytema N, **Rix L**, Foster RA, Al-Rshaidat MMD, Wild C

Abstract

1. Functional traits define species by their ecological role in the ecosystem. Evidence is accumulating that animals themselves are host-microbe ecosystems (holobionts) and that the application of ecophysiological approaches can help to understand their functioning. Communities of dinitrogen (N_2) fixing prokaryotes (diazotrophs) may equip hard coral holobionts with a functional trait by providing bioavailable nitrogen (N) that could sustain their high productivity under oligotrophic conditions.
2. This year-long study quantified N_2 fixation by diazotrophs associated with four dominant genera of hermatypic corals on a northern Red Sea fringing reef exposed to high seasonality.
3. We found N_2 fixation activity to be 5- to 10-fold higher in summer, when inorganic nutrient concentrations were lowest and water temperature and light availability highest. Concurrently, gross coral productivity remained high despite lower *Symbiodinium* densities and tissue chlorophyll *a* contents. In contrast, chlorophyll *a* content per *Symbiodinium* cell increased, suggesting that algal cells overcame limitation of N, an essential element for chlorophyll synthesis. In fact, N_2 fixation was positively correlated with coral productivity in summer, when its contribution was estimated to meet 11 % of the *Symbiodinium* N requirements.
4. These results provide evidence of an important functional role of diazotrophs in sustaining coral productivity in the northern Red Sea when alternative external N sources are scarce.
5. If these results are valid for other oligotrophic reef systems, the functional trait of N_2 fixation in coral holobionts may become redundant if the worldwide trend of increasing local eutrophication continues.

In preparation for *Ecology*

Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching

Cardini U, van Hoytema N, Bednarz VN, **Rix L**, Foster RA, Al-Rshaidat MMD, Wild C

Abstract

Different coral holobionts (i.e., coral-algal-prokaryote symbioses) exhibit varying thermal sensitivities, which may determine if they will adapt to global warming. However, studies simultaneously investigating the effects of warming on all holobiont members are lacking. Here we show that exposure to higher temperature affects key physiological traits of all members (herein: animal host, *Symbiodinium* and diazotrophs) of both *Acropora hemprichii* and *Stylophora pistillata* during and after thermal stress. *S. pistillata* experienced severe loss of *Symbiodinium* (i.e., bleaching) with no net photosynthesis at the end of the experiment. Conversely, *A. hemprichii* was more resilient to thermal stress. Exposure to increased temperature (+6 °C) resulted in a drastic increase in daylight dinitrogen (N_2) fixation, particularly in *A. hemprichii* (3-fold compared to controls). After the stress event, diazotrophs exhibited a reversed diel pattern of activity, with increased N_2 fixation rates recorded only in the dark, particularly in bleached *S. pistillata* (2-fold compared to controls). Concurrently, both animal hosts, but particularly bleached *S. pistillata*, displayed impaired organic matter release and picoplankton feeding. Our findings indicate that physiological plasticity by coral-associated diazotrophs may play an important role in determining the response of coral holobionts to ocean warming.

In preparation for *Environmental Microbiology*.

Marine Eutrophication – Overview of Indicators

Jessen C, Bednarz VN, **Rix L**, Teichberg M, Wild C

Abstract

Eutrophication is one of the key local stressors for coastal marine ecosystems, particularly in those locations with many estuaries, intense coastal development or agriculture, and a lack of coastal forests or mangroves. The land-derived import of not only inorganic nutrients, such as nitrate and phosphate, but also particulate and dissolved organic matter (POM and DOM) affects the physiology and growth of marine organisms with ensuing effects on pelagic and benthic community structures, as well as cascading effects on ecosystem functioning. Indicators for marine eutrophication are therefore not only key water quality parameters (inorganic and organic nutrient concentrations, oxygen and chlorophyll availability, and biological oxygen demand), but also benthic status and process parameters, such as relative cover and growth rates of indicator algae, invertebrate recruitment, sedimentary oxygen demand, and interactions between indicator organisms. The primary future challenge lies in understanding the interaction between marine eutrophication and the two main marine consequences of climate change, ocean warming, and acidification. Management action should focus on increasing the efficiency of nutrient usage in industry and agriculture, while at the same time minimizing the input of nutrients into marine ecosystems in order to mitigate the negative effects of eutrophication on the marine realm.

Published as a book chapter in Environmental Indicators (2014) Ed: Armon RH, Hänninen O. Springer Netherlands, pp. 177 – 203.

The frequent association of polydorid polychaetes with a Red Sea reef sponge – new insights to a potential trophic relation

Naumann MS, **Rix L**, Al-Horani FA, Wild C

Many polychaetes are commensals or parasitic symbionts of metazoan hosts that primarily provide them with shelter and food. The genus *Polydorella* (Augener, 1914) currently contains five species of minute polydorid polychaetes (sized <2 mm), all described as inconspicuous epibionts of Indo-Pacific sponges (Martín and Britayev 1998, Williams 2004). Potential functional benefits generated by polydorid-sponge associations are still unresolved. Polydorid polychaetes are imagined to feed on sponge tissues or detrital matter released by their hosts but evidence is lacking, as is knowledge on more basic properties such as their employed feeding modes (Williams 2004). Here, we report on a frequent polydorid-sponge association observed in the Red Sea and provide new insights to a potential trophic relation. During a reef survey in the Gulf of Aqaba (29°27'29.93"N, 34°58'27.67"E) in March 2013, a conspicuous red branching sponge covered with a dense population of whitish epibionts (≤10 ind. cm^{-2} sponge) was observed on the reef slope at 15 m water depth (Fig. 1a, b). Microscopic analysis of collected specimens identified the epibionts as *Polydorella smurovi* (Polychaeta, Spionidae; Fig. 1c), while the host was classified as the Red Sea sponge *Negombata magnifica* (Demospongiae, Podospongiidae; sample deposited at Naturalis Biodiversity Center, RMNH.POR.9146). During subsequent surveys, this frequent polydorid-sponge association (~30% of *N. magnifica* encountered) was documented by photo and video recordings. Video analysis revealed a continuous bilateral palp movement performed by *P. smurovi* implying the capture of loose detrital matter on the sponge surface and its subsequent transport towards the pharynx for consumption (ESM 1). Besides providing a food source for *P. smurovi*, this potential capture of detrital matter may benefit *N. magnifica* by clearing its surface of debris, as described for other polychaete-sponge associations (Martín et al. 1992). Particulate matter origin (i.e. sponge-derived detritus or deposited material) and its nutritional value for *P. smurovi* remain to be investigated.

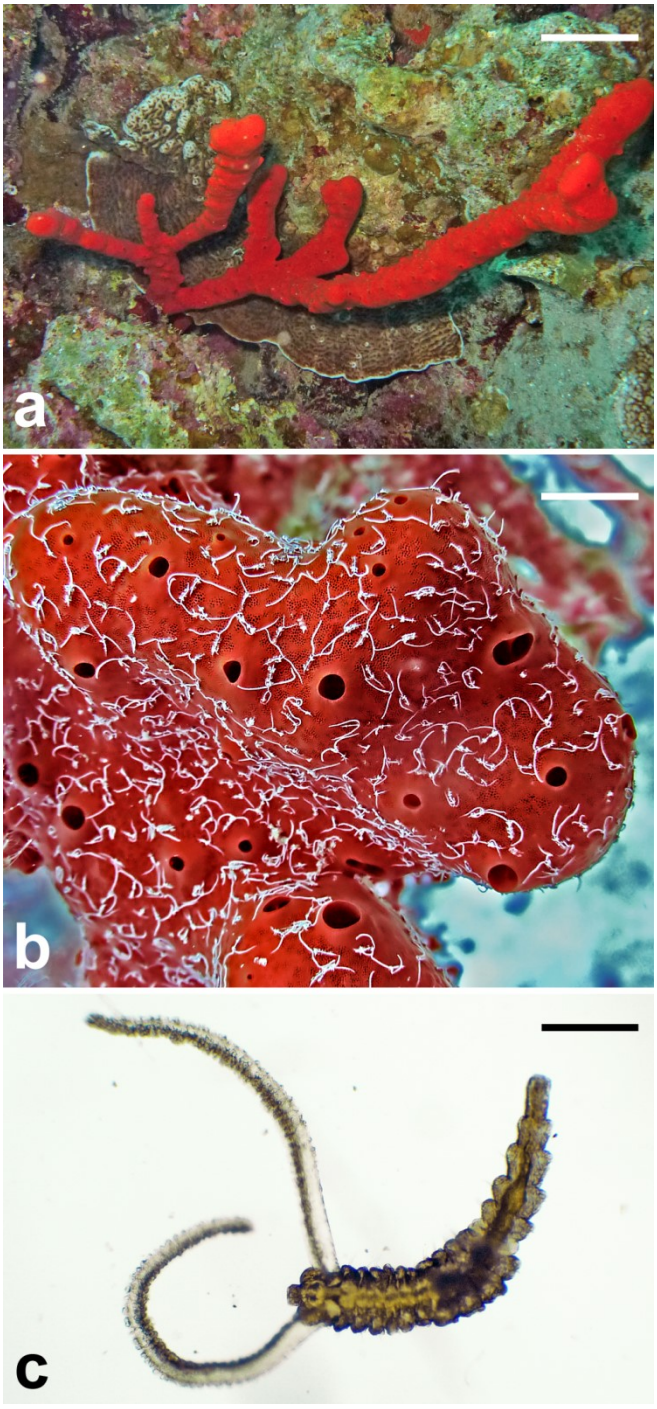


Figure 1. Association of the polydorid polychaete *Polydorella smurovi* with the Red Sea sponge *Negombata magnifica*. **a** the Red Sea sponge *N. magnifica* hosting inconspicuous polydorid epibionts, **b** dense population of *P. smurovi* inhabiting the sponge surface, **c** dorsal view (50x) of a live specimen of *P. smurovi*, scale bars: 10 (a), 0.8 (b), 0.03 cm (c)

In preparation for *Marine Biodiversity*.

Effects of seasonality on planktonic primary production and dinitrogen fixation in a Red Sea coral reef

van Hoytema N, Cardini U, Bednarz VN, **Rix L**, Naumann MS, Al-Horani FA, Wild C

Abstract

The northern Red Sea, because of its relatively high-latitude location, experiences strong seasonality in environmental conditions. This allows the study of regulatory effects by key status parameters (temperature, inorganic nutrient and organic matter concentrations) on process parameters (primary production and dinitrogen (N_2) fixation) and picoplanktonic abundance in the water column above coral reefs. Knowledge on interactions between these parameters is lacking. Therefore, this study, for the first time in high latitude coral reef waters, measured status and process parameters, and picoplankton abundance using a comparative approach between mixed (January – April) and stratified (September – November) water column scenarios in 2013. Findings revealed that inorganic nutrient concentrations were significantly higher in the mixed compared to the stratified season. Concurrently, daily gross primary production decreased 4-fold from the mixed to stratified season, while N_2 fixation did not change significantly. The phytoplanktonic community changed from dominance by picoeukaryotes to that by *Prochlorococcus* sp. and indications were found for a diazotrophic community shift from its autotrophic to its heterotrophic component. Primary production was primarily regulated by inorganic N concentrations, while dissolved organic carbon concentrations affected both primary production and N_2 fixation, emphasizing the importance of the microbial loop in planktonic tropho-dynamics of Red Sea coral reefs. N_2 fixation could potentially contribute 3.4 % of N needed for primary production in the mixed season. However, this contribution increased drastically to 20.8 % in the stratified season, indicating planktonic N_2 fixation as an important potential source of N to phytoplankton during very oligotrophic summer conditions.

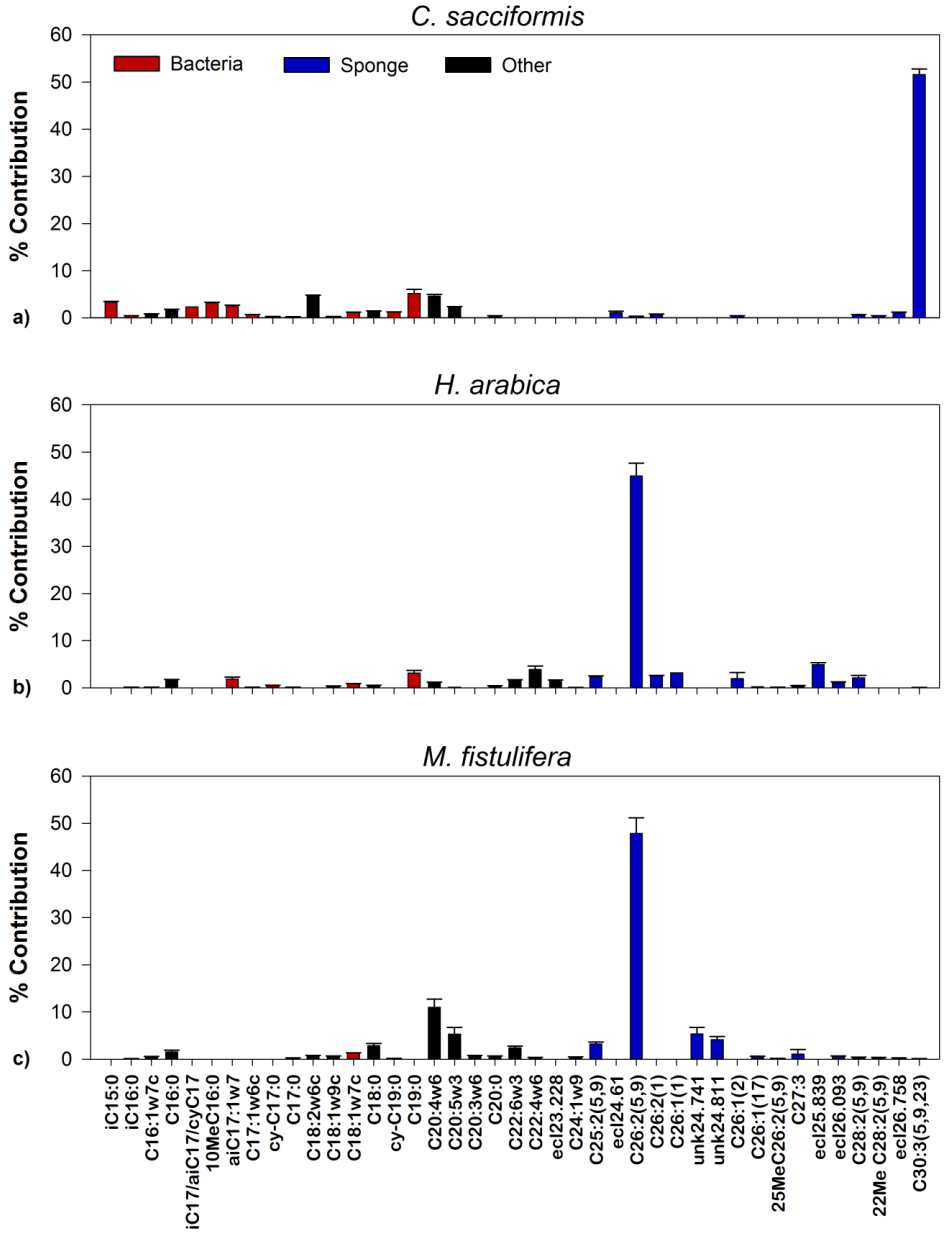
In preparation for *Marine Environmental Research.*

APPENDICES

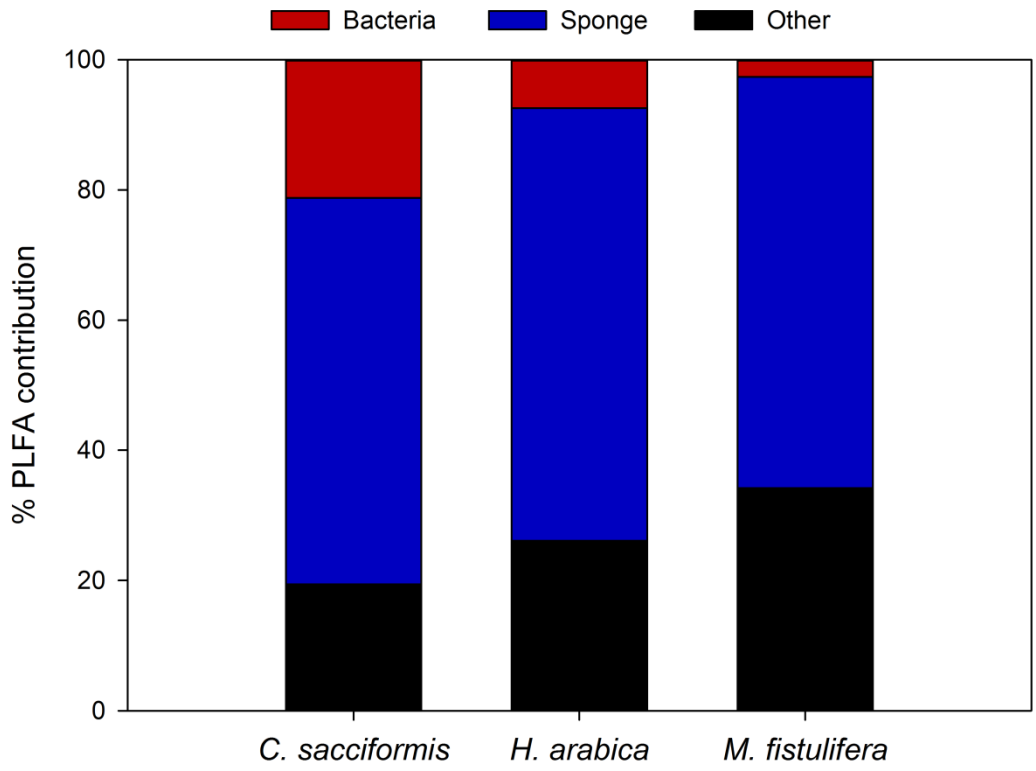
APPENDIX 1

Appendix 1 - Table 1: Results of one-factor Monte Carlo PERMANOVAs testing for differences in phospholipid fatty acid (PLFA) incorporation between coral- and algal-derived DOC by the three sponge species; *Chondrilla sacciformis*, *Hemimycale arabica*, and *Mycale fistulifera*. Differences between coral- and algal-derived DOC incorporation were tested for total PLFA incorporation, bacterial-specific PLFA incorporation, and sponge-specific PLFA incorporation. PERMANOVAs were based on Euclidian distance and Type III (partial) sums of squares were used with unrestricted permutations of raw data (9999 permutations). Significant Monte Carlo (MC) *p*-values are in bold.

Coral- vs. algal-derived DOC	df	SS	MS	*F*	*P* (MC)
Total PLFA incorporation					
C. sacciformis	1	118	118	9.4	**0.0398**
H. arabica	1	878	878	4.0	0.1428
M. fistulifera	1	1226	1226	4.0	0.1151
Bacterial PLFA incorporation					
C. sacciformis	1	105	105	5.5	0.0822
H. arabica	1	696	696	13.0	**0.0353**
M. fistulifera	1	467/10	467/10	19.2	**0.0215**
Sponge PLFA incorporation					
C. sacciformis	1	378	378	12.755	**0.0386**
H. arabica	1	266	266	3.2	0.1684
M. fistulifera	1	240	240	16.3	**0.0155**



Appendix 1 - Figure 1. Phospholipid fatty acids (PLFA) profiles of a) *C. sacciformis*, b) *H. arabica*, and c) *M. fistulifera*. Data presented as % of total PLFAs (mean ± SD, n = 3). Depicted are PLFAs accounting for ≥0.5% of the total PLFA composition. Bacterial-specific PLFAs are shown in red, sponge-specific PLFAs in blue, and other PLFAs in black.



Appendix 1 - Figure 2. Percent distribution of bacterial, sponge, and other phospholipid fatty acids (PLFAs) in the PLFA profiles of a) *C. sacciformis*, b) *H. arabica*, and C) *M. fistulifera.* Data presented as mean ± SD (n = 3).

APPENDIX 2

Appendix 2 - Table 1. Pelagic biomass ranges used for constraining inflow of C and metabolic activity. Water column integrated to the reef surface area, all values in mmol C m^{-2}.

	Winter	Spring	Summer	Fall
Phytoplankton	7.10 - 9.38	6.91 - 10.25	3.48 - 4.39	5.80 - 9.21
Zooplankton	2.66 - 5.33	2.66 - 5.33	2.66 - 5.33	2.66 - 5.33
Protozoa	1.67 - 4.67	1.67 - 4.67	1.67 - 4.67	1.67 - 4.67
Bacteria	5.04 - 5.30	5.04 - 5.30	5.04 - 5.30	5.04 - 5.30
POC	22.47 - 34.93	42.08 - 80.08	31.07 - 63.68	47.47 - 53.76
DOC	541.64 - 625.03	515.25 - 635.94	715.75 - 732.16	669.94 - 709.91
Total	580.58 - 684.65	573.62 - 741.58	759.68 - 815.52	732.58 - 788.18

Appendix 2 - Table 2. Ranges of gross primary production (GPP) and community respiration (Rday) rates entered into the models. All values in mmol C m^{-2} d^{-1}.

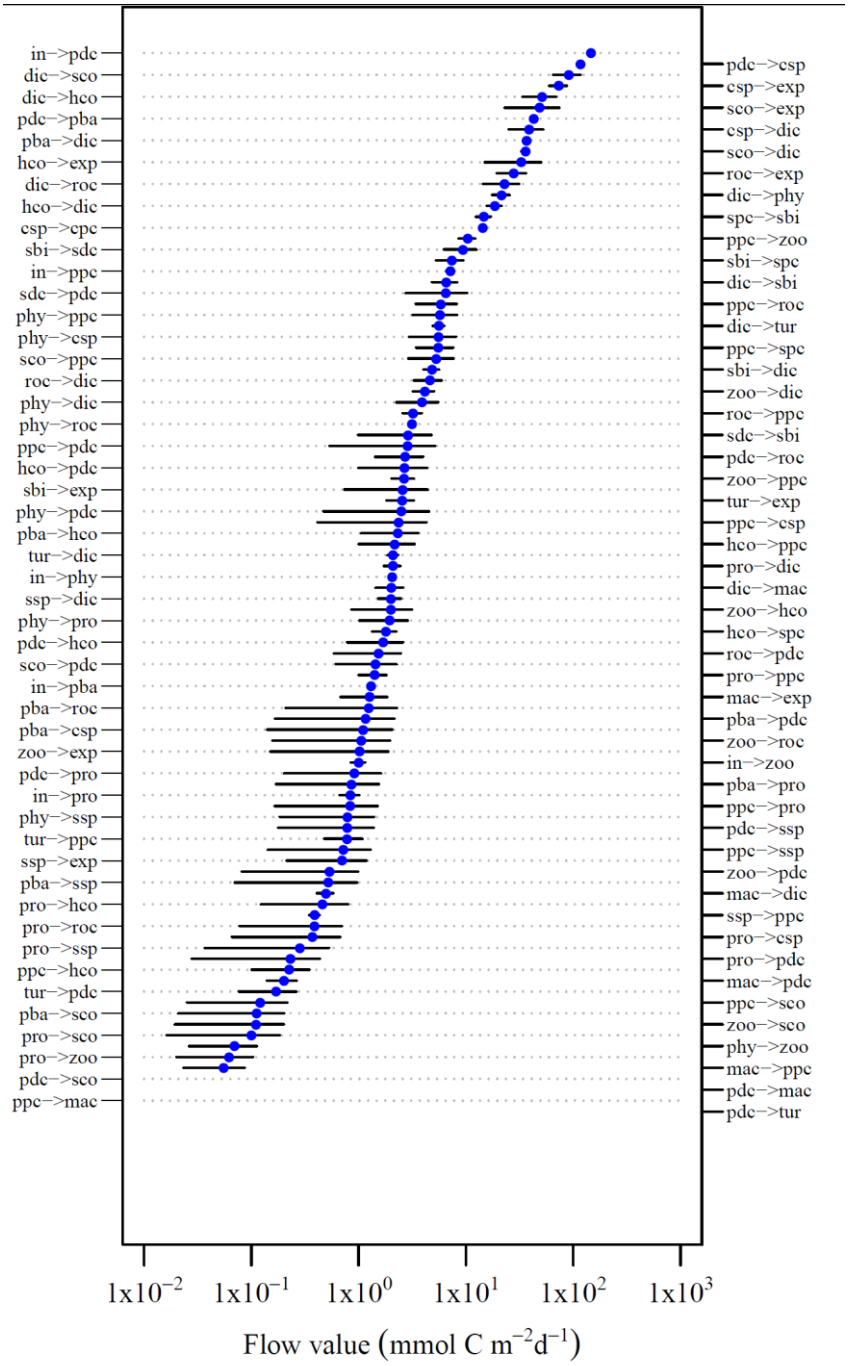
GPP	Winter	Spring	Summer	Fall
Water column	0 - 35.07	0 - 45.35	0 - 46.02	0 - 26.59
Hard corals	18.10 - 82.55	33.78 - 96.69	23.19 - 113.79	17.65 - 95.65
Soft corals	46.18 - 138.95	120.66 - 192.33	51.06 - 97.01	37.86 - 91.22
Macroalgae	0.98 - 3.02	5.24 - 21.34	1.95 - 3.82	1.06 - 2.47
Turf algae	4.18 - 7.01	4.60 - 8.27	7.67 - 13.20	3.93 - 6.41
Coral rock	5.12 - 38.65	0 - 33.45	21.55 - 61.60	15.47 - 67.08
Sediment	0.28 - 9.02	11.91 - 36.67	6.54 - 17.21	2.53 - 7.51
Rday				
Water column	22.36 - 50.68	33.65 - 110.62	35.72 - 101.07	33.28 - 99.98
Hard corals	15.30 - 48.79	13.47 - 52.56	19.66 - 72.40	21.08 - 75.32
Soft corals	25.58 - 39.84	45.67 - 66.12	18.14 - 52.39	22.60 - 36.01
Macroalgae	0.34 - 0.64	2.02 - 6.12	0.56 - 1.04	0.25 - 0.74
Turf algae	1.67 - 2.50	1.03 - 2.18	1.93 - 3.45	1.48 - 2.57
Coral rock	2.30 - 16.34	1.56 - 12.87	11.98 - 47.55	11.81 - 26.02
Sediment	2.12 - 5.94	3.16 - 11.39	3.95 - 7.23	2.45 - 4.24
Surface sponges	1.30 - 6.74	1.90 - 9.87	3.08 - 15.99	3.08 - 15.99
Cavity sponges	17.25 - 69.35	25.25 - 101.52	40.92 - 164.52	40.92 - 164.52

Appendix 2 - Table 3. Ranges of particulate and dissolved organic carbon flow rates (POC and DOC respectively) entered into the models. All values in mmol C m^{-2} d^{-1}. Negative values indicate net uptake, positive values indicate net release.

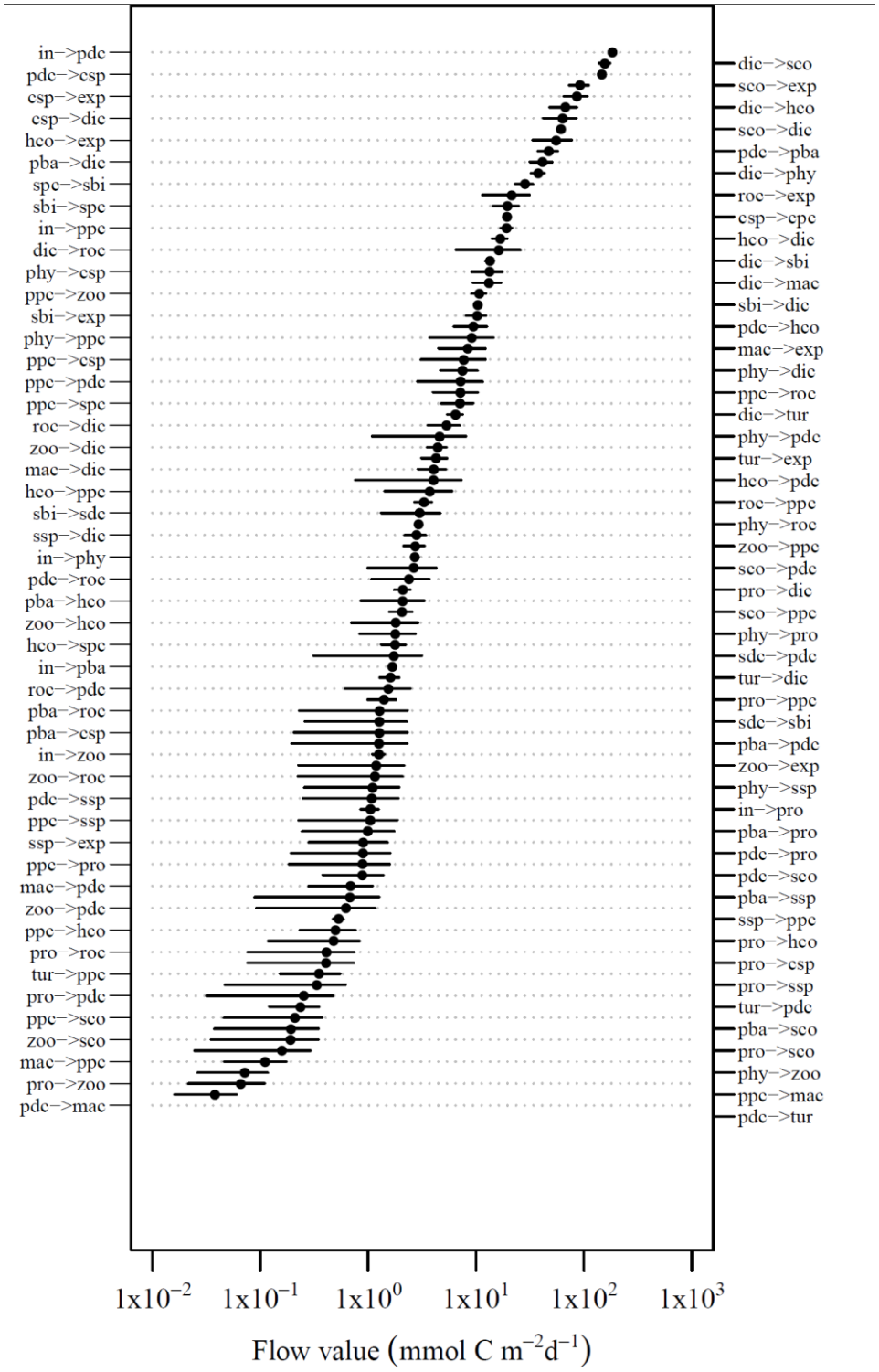
POC	Winter	Spring	Summer	Fall
Hard corals	-0.43 - 4.12	-0.93 - 8.45	0.57 - 8.88	0.92 - 9.46
Soft corals	0.42 - 9.09	1.21 - 2.92	2.31 - 17.82	3.11 - 9.59
Macroalgae	0.001 - 0.11	-0.08 - 0.22	0.16 - 0.22	0.19 - 0.58
Turf algae	0.24 - 1.30	0.004 - 0.69	0.38 - 0.83	0.32 - 0.64
Coral rock	0.57 - 4.23	0.56 - 4.12	0.79 - 5.84	0.72 - 5.34
Surface sponges	0.05 - 0.44	0.06 - 0.60	0.20 - 1.93	0.20 - 1.93
Cavity sponges	5.33 - 14.75	7.30 - 20.20	23.32 - 64.57	23.32 - 64.57
DOC				
Hard corals	-3.18 - 5.88	-13.67 - 14.76	-7.41 - 10.38	-20.13 - 46.16
Soft corals	0.04 - 2.90	-1.74 - 5.91	-1.92 - 3.53	-12.99 - 26.59
Macroalgae	0.09 - 0.31	-0.001 - 1.42	-0.36 - 0.52	-0.26 - 1.56
Turf algae	0.01 - 0.34	0.04 - 0.43	-0.49 - 0.82	-0.63 - 0.99
Coral rock	-4.68 - 3.31	-4.56 - 3.22	-6.46 - 4.56	-5.90 - 4.17

Appendix 2 - Table 4. Results of PERMANOVA main and pair-wise tests for the factor season on the reduced model solution set of the first 1000 solutions.

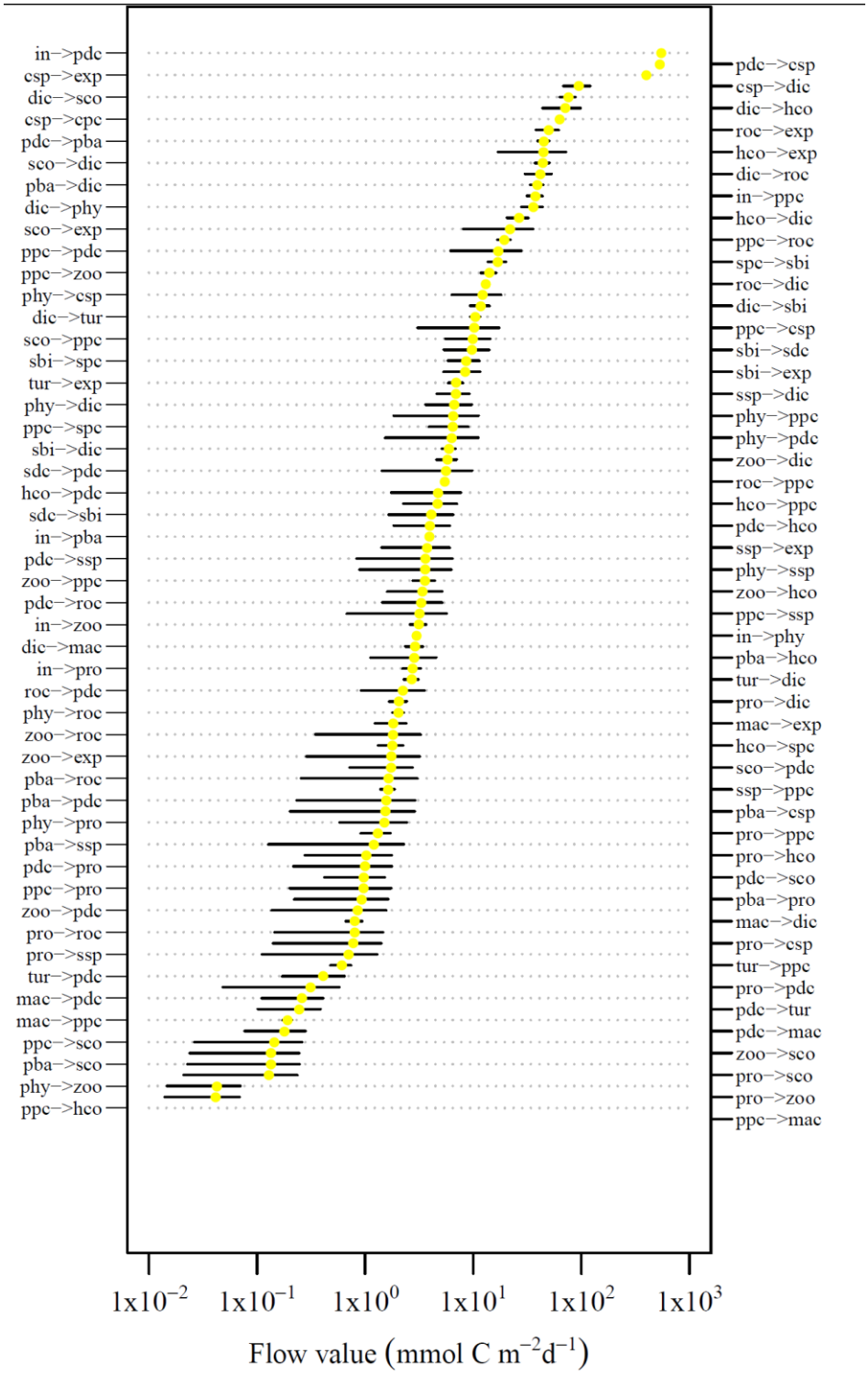
Comparison	Effect	df	SS	MS	Pseudo-F	p-value	Unique perms
Overall	Season	3	4.85×10^5	1.62×10^5	3938	0.001	997
	Residuals	3993	1.64×10^5	41.12			
	Total	3996	6.50×10^5				
					t	p-value	Unique perms
Pair-wise			Winter	Spring	43.61	0.001	999
			Winter	Summer	72.01	0.001	999
			Winter	Fall	74.78	0.001	998
			Spring	Summer	68.32	0.001	999
			Spring	Fall	75.27	0.001	999
			Summer	Fall	23.46	0.001	999



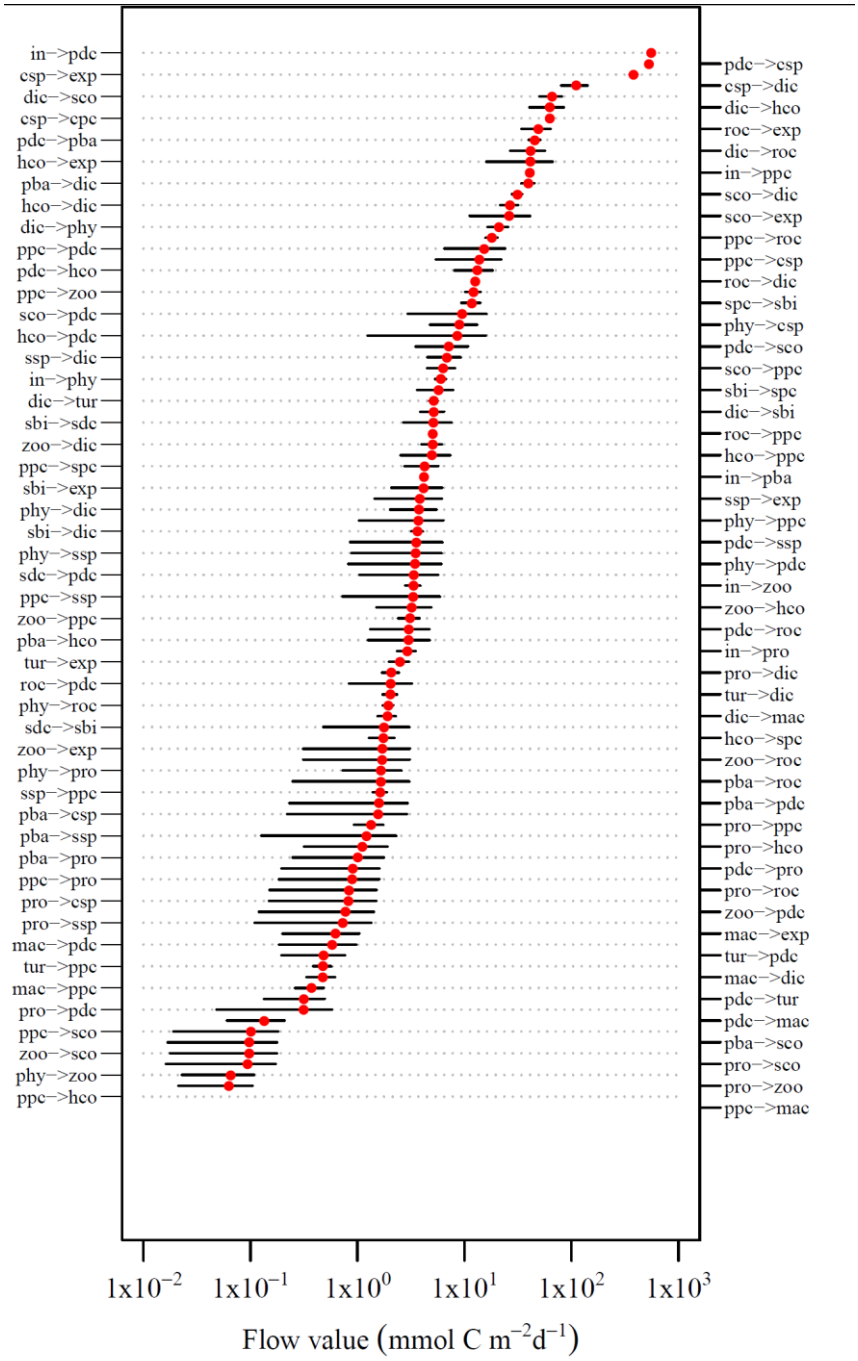
Appendix 2 - Figure 1. Means and standard deviations of all flows of the winter model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 2 - Figure 2. Means and standard deviations of all flows of the spring model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 2 - Figure 3. Means and standard deviations of all flows of the summer model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 2 - Figure 4. Means and standard deviations of all flows of the fall model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.

APPENDIX 3

Appendix 3 - Table 1. Environmental parameters characteristic of warm-water (WW), Red Sea coral reefs and cold-water (CW), north Atlantic *Lophelia pertusa* reefs. Parameters include dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP), dissolved organic carbon (DOC), particulate organic carbon (POC), particulate nitrogen (PON), and chlorophyll a (Chl *a*). [a]Indicates the inorganic nutrient supply limiting WW coral growth and [b]the organic nutrient supply limiting CW coral growth.

Parameter	Warm-water Red Sea coral reefs	Cold-water North Atlantic coral reefs
Depth (m)	1 – > 100[1]	50 – > 1000[2]
Temperature (˚C)	21 – 29[3]	6 – 10[2,4,5]
DIN (µmol L^{-1})[a]	0.2 – 1.0[6]	2.2 – 19.1[2]
DIP (µmol L^{-1})[a]	0.04 – 0.1[6]	0.3 – 3.6[2]
DOC (µmol L^{-1})	76 – 87[7]	51 – 73[8]
POC (µmol L^{-1})[b]	7.2 – 11.5[6]	1.2 – 5.2[4,9,10]
POC:PON	7.3– 10.2[6]	5.8 – 9.0[4,9,10]
Chl a (µg L^{-1})	0.1 – 0.2[6]	0.02 – 1.17[2]
Current (cm s^{-1})	0 – 10[3]	0 – 50[2,4,5]
Aragonite saturation (Ω_{arag})	3.7 – 4.4[3]	1.4 – 2.4[2]
pH	8.2 – 8.3[3]	7.92 – 8.19[2]
Salinity	40.5 – 41.0[3]	34.6 – 35.7[2]

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APPENDIX 4

Appendix 4 - Table 1. Fully crossed two-factor general linear model with N_2 fixation, net photosynthesis (P_{net}), respiration (R), gross photosynthesis (P_{gross}), and the ratio of P_{gross}:R as a function of substrate and season.

Parameter	Factor	*df*, $df_{residual}$	F	*p*
N_2 fixation	Season	6, 83	5.78	<0.001
	Substrate	3, 83	60.09	<0.001
	Season * Substrate	2, 77	299.37	<0.001
P_{gross}	Season	3, 79	12.68	<0.001
	Substrate	2, 79	63.64	<0.001
	Season * Substrate	5, 74	3.96	<0.001
R	Season	3, 89	18.00	<0.001
	Substrate	2, 89	156.27	<0.001
	Season * Substrate	6, 83	15.34	<0.001
P_{net}	Season	3, 80	6.65	<0.001
	Substrate	2, 80	210.64	<0.001
	Season * Substrate	5, 75	7.59	<0.001
P_{gross}:R	Season	3, 80	8.44	<0.001
	Substrate	2, 80	409.47	<0.001
	Season * Substrate	5, 75	6.52	<0.001

Appendix 4 - Table 2. Fully crossed three-factor general linear model with N_2 fixation as a function of substrate, season, and time of day (day vs. night).

	df, $df_{residual}$	F	*p*
Season	2, 122	106.12	<0.001
Substrate	2, 122	224.02	<0.001
Time of day	1, 122	6.86	<0.01
Season*Substrate	4, 114	6.22	<0.001
Season*Time of day	2, 114	9.28	<0.001
Substrate*Time of day	2, 114	10.39	<0.001
Season*Substrate*Time of day	4, 110	3.70	<0.01

Universität Bremen

ERKLÄRUNG

Bremen, 30 Juli 2015

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

Carbon and nitrogen cycling by Red Sea coral reef sponges

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

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Laura Rix